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The Effects of Uranium on Reproduction, Gestation, and Postnatal Survival in Mice

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Uranyl acetate dihydrate was tested for its effects on reproduction, gestation, and postnatal survival in Swiss mice. Four groups of animals, each of which consisted of 25 males and 25 females, were administered 0, 5, 10, and 25 mg/kg/day of uranyl acetate dihydrate. Mature male mice were treated orally for 60 days prior to mating with mature virgin female mice treated orally for 14 days prior to mating. Treatment of the females continued throughout mating, gestation, parturition, and nursing of the litters. One-half of the dams in each group were sacrificed on Day 13 of gestation and the remaining dams were allowed to deliver and wean their offspring. Postnatal development was monitored after 0, 4, and 21 days of lactation. No adverse effects on fertility were evident at the doses employed in this study. Nevertheless, embryolethality could be observed in the 25 mg/kg/day group. Significant increases in the number of dead young per litter were seen at birth and at Day 4 of lactation in the 25 mg/kg/day group. The growth of the offspring was always significantly lower for the uranium-treated animals. However, the present results suggest that uranium does not cause any adverse effects on fertility, general reproductive parameters, or offspring survival at the concentrations usually ingested by man. © 1989 Academic Press, Inc.

INTRODUCTION

Uranium is a ubiquitous constituent of man's natural environment. In some cases, its presence is due to human activities such as the mining and milling of uranium (Cothern and Lappenbusch, 1983). Uranium is found in both food and drinking water. The uranium content of most foods is in the range 10–100 ng/g and the average daily intake of uranium is about 1.0 μg/day (Cothern *et al.*, 1983; Hamilton, 1972) although average daily intakes of 1.3–1.4 μg/day have also been reported (Welford and Baird, 1967). However, the possibility of ingesting uranium through drinking water is usually greater than that through food. Thus, on the average, food contributes about 15% of ingested uranium and drinking water contributes about 85%. People living near a uranium mine or mill may inhale or ingest abnormal amounts of the metal (Cothern and Lappenbusch, 1983) giving rise to internal contamination. This contamination will result in radiation doses to the organs of the body and, if the intake of uranium is large enough, chemical toxic effects. In some circumstances the toxic chemical effects of uranium are more important than the radiation dose received (Johnson, 1980).

The spectrum of acute toxicity has been extensively studied in animals, and to some extent in man (Clarkson and Kench, 1952; Cross *et al.*, 1981; Domingo *et al.*, 1987; Harvey *et al.*, 1986; Thun *et al.*, 1985). Nephritis is the primary chemically

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induced health effect of uranium in animals and humans. Enzymuria, glycosuria, aminoaciduria, proteinuria, and acute renal failure have been reported to occur after acute uranium poisoning (Bentley *et al.*, 1985; Blantz, 1975; Damon *et al.*, 1986). Much less is known about the chronic effects caused by the ingestion of uranium. Moreover, in recent years very little attention has been paid to the possible effects of uranium exposure on reproduction. In a review on the chemical toxicity of uranium, Stopps and Todd (1982) have mentioned that during the Second World War, two studies were carried out, one of which examined high levels of uranium and the other only a brief 24-hr exposure, but both demonstrated statistically significant effects on reproduction. However, these studies have not been corroborated by other investigators and so questions remain about the effects of uranium on reproduction, gestation, and postnatal survival.

The purpose of the present study was to investigate the effects of uranium on reproduction, gestation, parturition, and lactation in mice upon oral administration. Because uranium is commonly found as the uranyl ion UO_2^{2+} in nature, and this is the form in which the 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

Male and female Swiss mice weighing between 26 and 30 g were purchased from Interfauna Ibérica (Barcelona, Spain). The animals were freely supplied with pelleted dry chow (Panlab, Barcelona, Spain) and were housed in a fully air-conditioned facility where the temperature was regulated at 22 ± 2°C, and the humidity at 55 ± 5%. After a taming period of 1 week, four groups of 25 male mice were orally administered uranyl acetate dihydrate (E. Merck, Darmstadt, FRG) at dose levels of 0, 5, 10, and 25 mg/kg/day for 60 days before mating with female mice (25 animals per group) which had received orally the same doses of uranyl acetate dihydrate for 14 days prior to mating. Oral doses were given intragastrically. Males and females were mated according to the respective dose levels. Daily vaginal smears were taken. The day on which sperm was found in the vaginal smear was designated Day 0 of gestation. Pregnant females were also treated during the periods of gestation, delivery, and lactation.

About one-half of the fertilized animals were sacrificed on Day 13 of gestation and the following examinations made: determination of the number of total implantations, early and late resorptions, as well as the number of living and dead fetuses. The remaining females were allowed to deliver and to nurse their young to 21 days. The offspring were observed for mortality, body weight, body length, and general symptomatology at birth (Day 0) and after 4 and 21 days of nursing. After 21 days, the offspring were killed and the following organs removed and weighed: heart, lungs, spleen, liver, kidneys, and brain. The ratios "organ weight/body weight" were calculated.

For statistical evaluation of results, means and estimated standard deviations were calculated. Significant variance by either analysis of variance or covariance was further evaluated by Student's t test or the Kruskal–Wallis statistical test to locate the source of variance. Significance was set at the 0.05 probability level.

RESULTS

Table 1 summarizes the effects of uranium on reproduction parameters in the mice killed on Day 13 of gestation. The average numbers of total implantations in the

TABLE 1

THE EFFECT OF ORAL ADMINISTRATION OF URANYL ACETATE DIHYDRATE IN MICE ON GESTATION DAY 13[a]

No. of	Dose (mg/kg/day)			
	0	5	10	25
Pregnant mice	12	11	10	12
Total implants	13.8 ± 2.0	16.4 ± 2.7*	14.8 ± 3.4	13.7 ± 2.1
Total resorptions	1.0 ± 1.1	0.9 ± 0.9	1.3 ± 1.5	2.3 ± 1.7*
Early resorptions	0.5 ± 0.8	0.4 ± 0.5	0.5 ± 0.8	0.7 ± 0.8
Late resorptions	0.0 ± 0.5	0.5 ± 0.5	0.8 ± 1.0	1.6 ± 1.1**
Live fetuses	12.6 ± 0.9	14.8 ± 1.8	13.0 ± 3.1	10.3 ± 1.6*
Dead fetuses	0.2 ± 0.4	0.6 ± 0.9	0.5 ± 1.2	1.1 ± 1.1*

[a] The results are expressed as mean values per dam ± SD.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

uranium-treated groups were not significantly different from controls except for an increase in the 5 mg/kg/day group ($P < 0.05$). Nevertheless, the numbers of late resorptions and dead fetuses were significantly increased for the 25 mg/kg/day group. Moreover, the number of live fetuses was significantly decreased in that group.

Table 2 shows data from mouse pups nursed by uranium-treated dams during the period of nursing. Results are presented at birth and on Days 4 and 21 after birth. The number of litters decreased with time (neonatal period) in the 10 and 25 mg/kg/day groups. The litters from these four dams were cannibalized at birth or died within a few days of birth. Treatment with uranyl acetate dihydrate had no effect on either litter size at birth or sex ratios, but close inspection of data revealed a significant increase in the average number of dead young per litter at birth in the 10 and 25 mg/kg/day groups. A significant increase in the number of dead young per litter was also observed at Day 4 of lactation in the 25 mg/kg/day group. The viability indices were lower for the uranium-treated animals than for the controls. From these results, a dose–response relationship can be induced. No significant differences could be observed for the lactation indices.

With regard to the growth of the offspring as a measure of body weight and body length, it was always significantly lower for the uranium-treated animals. This decrease should be imputed to the treatment, and a significant dose–response relationship is observed.

The relative organ weights of mouse pups sacrificed after 21 days of lactation are given in Table 3. No significant hypertrophy or hypotrophy of the organs removed after uranium treatment could be seen.

DISCUSSION

The doses of uranyl acetate dihydrate administered in this study correspond approximately with 1/50, 1/25, and 1/10 of the acute oral LD_{50} for adult Swiss mice previously determined (Domingo *et al.*, 1987). At these doses, uranium did not produce significant adverse effects on the fertility in the treated mice. Nevertheless, the number of resorptions and the number of dead fetuses were significantly increased

TABLE 2

SUMMARY OF DATA FROM MOUSE PUPS NURSED BY URANYL ACETATE DIHYDRATE-TREATED DAMS DURING THE PERIOD OF LACTATION

	Day	Dose (mg/kg/day) 0	5	10	25
No. of litters	0	9	11	11	12
	4	9	11	10	9
	21	9	11	10	9
No. of living young	0	110	146	121	127
	4	106	135	108	87
	21	101	114	97	82
No. of dead young	0	0	2	11	15
	4	4	11	13	40
	21	5	21	11	5
Dead/living ratio (×100)	0	0	1.3	9.0	11.8
	4	3.7	8.1	12.0	45.9
	21	4.9	18.4	11.3	6.1
Male/female ratio	0	0.79	0.64	0.98	0.86
	4	0.89	0.66	0.96	0.71
	21	0.90	0.69	0.92	0.73
Living young/litter[a]	0	12.2 ± 1.5	13.3 ± 2.6	11.2 ± 4.6	10.7 ± 3.8
	4	11.8 ± 1.3	12.2 ± 2.1	10.8 ± 4.2	9.7 ± 5.9
	21	11.2 ± 1.3	10.4 ± 2.5	9.8 ± 4.8	9.1 ± 4.1
Dead young/litter[a]	0	0.0 ± 0.0	0.2 ± 0.6	1.0 ± 2.2**	1.3 ± 2.4**
	4	0.4 ± 0.5	1.0 ± 1.5	1.3 ± 1.8	4.0 ± 3.1**
	21	0.5 ± 1.0	1.9 ± 2.2	1.1 ± 2.1	0.5 ± 0.9
Viability index	4/0	0.96	0.92	0.88	0.68
	21/0	0.91	0.78	0.79	0.64
Lactation index	21/4	0.95	0.84	0.89	0.94
Body weight[a] (g)	0	1.86 ± 0.65	1.76 ± 0.46	1.66 ± 0.28	1.59 ± 0.15**
	4	2.60 ± 0.87	2.48 ± 0.68	2.33 ± 0.50*	2.20 ± 0.33**
	21	9.74 ± 2.75	8.98 ± 1.31*	8.85 ± 1.65*	8.32 ± 1.25**
Body length[a] (cm)	0	3.43 ± 0.30	3.42 ± 0.20	3.39 ± 0.27	3.30 ± 0.23*
	4	3.91 ± 0.38	3.90 ± 0.42	3.83 ± 0.28	3.80 ± 0.19*
	21	7.26 ± 1.41	7.23 ± 0.51	7.11 ± 0.49	6.93 ± 0.37*

[a] The results are expressed as mean values ± SD.
* Significantly different from the control at $P < 0.05$.
** Significantly different from the control at $P < 0.01$.

in the 25 mg/kg/day group. Therefore, embryolethality of uranium can be induced at this dose. On the other hand, the development of the offspring always significantly decreased from birth and during the entire lactation period for the animals receiving the highest dose of uranyl acetate.

The results of the present study suggest that uranium, as uranyl acetate dihydrate, does not adversely affect mating or fertility of mature mice at dose levels up to 25 mg/kg/day. However, fetal and neonatal development appeared to be adversely affected at the 25 mg/kg/day dose level. In addition, significant differences could also be observed in the 10 mg/kg/day group.

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

TABLE 3

RELATIVE ORGAN WEIGHTS[a] OF MOUSE PUPS NURSED BY URANYL ACETATE DIHYDRATE-TREATED MOTHERS

	Dose (mg/kg/day)			
	0	5	10	25
Heart	0.58 ± 0.16	0.67 ± 0.15	0.76 ± 0.19*	0.65 ± 0.27
Lungs	1.17 ± 0.32	1.16 ± 0.24	1.25 ± 0.40	1.22 ± 0.32
Spleen	0.62 ± 0.20	0.68 ± 0.16	0.72 ± 0.15	0.61 ± 0.20
Liver	4.98 ± 1.03	4.86 ± 1.01	4.74 ± 0.77	4.21 ± 1.09
Kidneys	1.73 ± 0.26	1.75 ± 0.58	1.60 ± 0.19	1.54 ± 0.19
Brain	3.13 ± 0.64	2.96 ± 0.80	3.59 ± 1.54	2.71 ± 0.70

[a] The results are expressed in grams per 100 g body weight ± SD.
* Significantly different from the control at $P < 0.05$.

of 100 μg/liter in order to limit toxic effects in the kidney (Wrenn *et al.*, 1985). Thus, consuming 2 liters/day the average person (70 kg body wt) should 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). In the present investigation, the lowest uranyl acetate dihydrate dose administered was 5 mg/kg/day, and no adverse findings were noted at this dose level. Taking into account that the concentrations of uranium in the diet suggest a daily intake of approximately 1–1.4 μg U (Hamilton, 1972; Welford and Baird, 1967), it seems clear that these additional amounts of uranium would not increase the risk of adverse reproductive effects.

With regard to the drinking water, it has been reported that of the 59,812 community water supplies in the U.S.A., it is estimated that between 25 and 650 would exceed a uranium concentration of 29.9 μg/liter, 100–2000 would exceed 14.9 μg/liter, and 2500–5000 would exceed 7.46 μg/liter (Cothern and Lappenbusch, 1983; Cothern *et al.*, 1983).

CONCLUSION

In conclusion, the present results indicate that uranium would not cause any adverse effect on fertility, general reproductive parameters, or offspring survival at the concentrations usually ingested in the diet and drinking water, with a safety factor of more than 1000.

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