Influence of Maternal Stress on Uranium-Induced Developmental Toxicity in Rats

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It has been demonstrated that uranium is an embryo/fetal toxicant when given orally or subcutaneously to pregnant mice. On the other hand, maternal stress has been shown to enhance the developmental toxicity of a number of metals. In this study, maternal toxicity and developmental effects of a concurrent exposure to uranyl acetate dihydrate (UAD) and restraint stress were evaluated in rats. Four groups of pregnant animals were given subcutaneous injections of UAD at 0.415 and 0.830 mg/kg/day on Days 6 to 15 of gestation. Animals in two of these groups were also subjected to restraint for 2 hr/day during the same gestational days. Control groups included restrained and unrestrained pregnant rats not exposed to UAD. Cesarean sections were performed on gestation Day 20, and the fetuses were weighed and examined for malformations and variations. Maternal toxicity and embryotoxicity were noted at 0.830 mg/kg/day of UAD, while fetotoxicity was evidenced at 0.415 and 0.830 mg/kg/day of UAD by significant reductions in fetal body weight and increases in the total number of skeletally affected fetuses. No teratogenic effects were noted in any group. Maternal restraint enhanced uranium-induced embryo/fetal toxicity only at 0.830 mg/kg/day, a dose that was also significantly toxic to the dams. As in previous studies with other metals, maternal stress enhances uranium-induced developmental toxicity at uranium doses that are highly toxic to the dams; however, at doses that are less acutely toxic the role of maternal stress would not be significant. Exp Biol Med 228:1072–1077, 2003

Key words: rats; uranyl acetate dihydrate; maternal restraint; maternal toxicity; embryo/fetal toxicity; interactive effects

Although uranium (U) exposure can result in both chemical and radiological toxicity, in general, chemical toxic effects from uranium compounds occur at lower exposure levels than radiological toxicity (1). Uranium from the environment enters the human body by ingestion with food and drink, and by inhalation of airborne uranium-containing dust particles or aerosols (2). Moreover, in recent years the use of depleted uranium (DU) in munitions has given rise to a new exposure route for this hazardous heavy metal (3–5).

There is an extensive literature on the general toxicity of uranium from inhalation, ingestion, and injection exposures (6–9). However, until recent years little attention was paid to the potential toxic effects of uranium on reproduction and development (10, 11). Moreover, most experimental studies on uranium-induced developmental toxicity have been performed in a sole species of mammals, mice (11). Information concerning developmental toxic effects of DU in mammals is even more meager. It is limited to one study in rats implanted with DU pellets, in which it was shown that DU crossed the placental barrier and entered fetal tissue (12).

Therefore, investigations on uranium toxicity during pregnancy would be of interest. Consequently, the present study was designed to obtain an overall understanding about the toxic effects of uranium during the period of organogenesis. The maternal and developmental toxicity of subcutaneous exposure to uranyl acetate was assessed in pregnant rats. On the other hand, various studies have shown that maternal stress during gestation can enhance the adverse effects on the development of fetuses and neonates induced by elements such as aluminum, arsenic, and mercury (13–16). While pregnant women can be potentially exposed to uranium through the diet, inhalation, or can experience contamination through wounds (6), they can be also concurrently subjected to various types of stress. Therefore, in this investigation we also examined whether maternal restraint could enhance the potential adverse effects of uranium in rats. Among the animal models to examine the effects of maternal stress on the developmental toxicity of a chemical, restraint has been widely used (13–19).

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Materials and Methods

Animals. Sexually mature male and female Sprague-Dawley rats (220–240 g) were obtained from Criffa (Barcelona, Spain). Animals were housed in plastic cages in a climate-controlled facility with a constant day-night cycle (light: 0800–2000 hr) at a temperature of 22 ± 2°C, and a relative humidity of 50 ± 10%. After a quarantine period of 7 days, female rats were mated with males (2:1) until copulation was detected. Finding of sperm indicated copulation and the day of detection was designated as Day 0 of gestation. Food (Panlab rodent chow, Barcelona, Spain) and tap water were available *ad libitum*. The use of animals and the experimental protocol were approved by the Animal Care and Use Committee of the "Rovira i Virgili" University.

Chemical. Uranyl acetate dihydrate (UAD) was purchased from E. Merck (Darmstadt, Germany). It was injected subcutaneously in solutions of 0.9% saline at doses of 0.415 and 0.830 mg/kg/day on gestation Days 6 to 15. These doses are approximately equal to 1/20 and 1/10 of the acute subcutaneous LD_{50} of UAD in adult rats (20).

Treatment. Pregnant rats were randomly divided into 6 groups and received the following treatments. A control group was unrestrained and subcutaneously injected with 0.9% saline. A second group of animals (restraint only group) was immobilized for 2 hr/day on gestation Days 6 to 15 by placing the animals in metacrilate cylindrical holders from Letica Scientific Instruments (Panlab, Barcelona). The restrained rats were held in a prone position with paws immobilized with elastic adhesive tape. According to previous studies in mammals, this procedure produces stress in pregnant animals (14, 16, 19). Rats in the third and fourth groups (uranium-treated groups) were given subcutaneous injections of 0.415 and 0.830 mg/kg/day of UAD solutions. Finally, rats in the fifth and sixth groups (combined uranium and restraint groups) received subcutaneous injections of 0.415 and 0.830 mg/kg/day of UAD on gestation Days 6 to 15, concurrently with restraint stress for 2 hr/day during the same days.

Maternal and Fetal Observations. Rats were observed daily for food consumption, body weight gain, and clinical signs of toxicity. On gestation Day 20, all animals were euthanized with diethyl ether. Maternal body, liver, kidney, and uterine weights were recorded. Dams were evaluated for the status of uterine implantation sites (i.e., number of sites, resorptions, and dead and live fetuses). Live fetuses were weighed, sexed, and examined for external malformations and variations. To examine internal and skeletal abnormalities, animals were randomly distributed in 2 groups. Approximately one-half of the available fetuses were fixed in 95% ethanol, cleared with 1% KOH, stained with Alizarin red S, and examined for skeletal malformations and variations (21). The remaining fetuses were fixed in Bouin's fluid, sectioned, and evaluated for internal abnormalities (22). All fetuses were examined by observers who were blind to the treatment conditions.

Uranium Analysis. Samples of kidney, spleen, liver, and placenta were taken from all dams in each group. In turn, 1 (whole) fetus per litter was randomly selected for uranium analysis. Uranium concentrations were determined by inductively coupled plasma-mass spectrometry (Perkin Elmer, Elan 6000) according to previously described methods (23, 24).

Data Analysis. The unit of comparison was the dam, the fetus, or the litter. Results of the quantitative continuous variables (e.g., maternal body weight gain, organ weights, and fetal weights) were compared using 2-way analysis of variance followed by the Bonferroni method for multiple comparisons. Maternal deaths, the number of dams with completely resorbed litters, and the incidence of fetal anomalies were evaluated using the Kruskal-Wallis test and the Mann-Whitney U-test. Significance was set at $P \leq 0.05$.

Results

Maternal Toxicity. There were no deaths, abortions, early deliveries, or completely resorbed litters due to uranium only, restraint alone, or combined UAD (0.415 mg/kg/day) and immobilization. However, 25% (3 of 12) of dams in the group concurrently exposed to 0.830 mg/kg/day of UAD and restraint during organogenesis carried completely resorbed litters. One dam (9%, 1/12) also died in this group. On the other hand, in comparison with the results of the control group no significant differences in maternal food consumption and body weight gain, body weight at termination, corrected body weight, corrected body weight change, and absolute and relative liver and kidney weight were found in the groups exposed to restraint only and 0.415 mg/kg/day of uranium alone, as well as in the group given uranium at 0.415 mg/kg/day plus maternal restraint (excepting a significant decrease in corrected body weight change and an increase in absolute and relative kidney weight) (Table I). By contrast, uranium exposure at 0.830 mg/kg/day caused significant reductions in food consumption on gestation Days 0 to 20 (only in the uranium plus restraint group), body weight gain on gestation Days 0 to 20, body weight at termination, gravid uterine weight, corrected body weight change, and absolute and relative kidney weight. Notwithstanding, maternal restraint did not cause significant differences in these parameters between the groups exposed to 0.415 and 0.830 mg/kg/day of UAD alone and those in which UAD was given at these doses combined with restraint (Table I).

Developmental Toxicity. Data on pregnancy outcome measures are shown in Table II. There were no significant differences among groups in the number of total implants per litter, the number of viable and nonviable implants per litter (with the exception of an increase in the number of nonviable implants in the group concurrently exposed to 0.830 mg/kg/day of UAD and restraint), or in the sex ratio. However, the percentage of postimplantation loss in the group given 0.830 mg/kg/day of UAD plus restraint was significantly higher than those in the control group and

Table I. Effects of Uranium, Maternal Restraint, and Combined Uranium and Restraint on Maternal Toxicity in Pregnant Rats

Uranium (mg/kg/day) Type of stress	0 None	0 Restraint	0.415 None	0.830 None	0.415 Restraint	0.830 Restraint
Data at termination						
No. of dams	11	10	12	11	11	12
Food consumption (g/dam) on gestation Days 0–20	479.00 ± 66.29^{a}	462.90 ± 74.47^{a}	411.90 ± 65.90^{ab}	400.83 ± 52.11^{ab}	414.75 ± 36.78^{ab}	364.67 ± 54.55^{b}
Body weight gain (g) on gestation Days 0–20	270.44 ± 29.57^{a}	246.90 ± 25.04^{a}	241.50 ± 21.94^{a}	187.17 ± 20.76^{b}	227.15 ± 18.94^{a}	181.50 ± 55.28^{b}
Body weight at termination (g)	420.64 ± 19.41^{a}	410.30 ± 32.82^{a}	378.36 ± 18.11^{ab}	331.91 ± 64.91^{bc}	389.80 ± 21.59^{a}	312.81 ± 54.38^{c}
Gravid uterine weight (g)	89.29 ± 16.47^{a}	85.35 ± 13.47^{ab}	80.24 ± 10.42^{ab}	61.39 ± 12.50^{bcd}	78.73 ± 12.95^{ad}	50.51 ± 31.89^{c}
Corrected body weight (g)[1]	304.07 ± 90.37^{ab}	327.57 ± 27.13^{a}	292.00 ± 22.88^{ab}	259.90 ± 48.20^{b}	303.81 ± 26.64^{ab}	274.17 ± 28.53^{ab}
Corrected body weight change (g)[2]	65.71 ± 14.39^{a}	61.04 ± 11.71^{ab}	56.01 ± 23.06^{ab}	40.36 ± 18.99^{bc}	39.97 ± 12.89^{bc}	24.85 ± 15.39^{c}
Liver weight (g)	14.74 ± 1.45	14.12 ± 1.68	14.25 ± 1.68	14.24 ± 2.95	14.67 ± 1.99	13.02 ± 1.87
Relative liver weight (%)[3]	4.44 ± 0.38	4.31 ± 0.43	4.88 ± 0.50	5.40 ± 1.79	4.83 ± 0.55	4.67 ± 0.49
Kidney weight (g)	1.87 ± 0.17^{a}	1.79 ± 0.18^{a}	2.36 ± 0.50^{ab}	3.90 ± 1.06^{c}	2.75 ± 0.51^{b}	3.84 ± 0.94^{c}
Relative kidney weight (%)[3]	0.56 ± 0.04^{a}	0.54 ± 0.04^{a}	0.93 ± 0.36^{ab}	1.38 ± 0.52^{c}	0.97 ± 0.20^{b}	1.35 ± 0.26^{c}

Results are expressed as mean values ± SD. [1] Corrected body weight = (Body weight at sacrifice)–(Gravid uterine weight). [2] Corrected body weight change = (Corrected body weight)–(Body weight on gestation Day 0). [3] Relative liver and kidney weight were calculated as percentages of corrected body weight. Values in the same row showing a common superscript (a,b,c,d) are not significantly different at $P < 0.05$.

Table II. Effects of Uranium, Maternal Restraint, and Combined Uranium and Restraint on Gestational Parameters in Pregnant Rats

Uranium (mg/kg/day) Type of stress	0 None	0 Restraint	0.415 None	0.830 None	0.415 Restraint	0.830 Restraint
No. of dams	11	10	12	11	11	12
No. of implants/litter	14.82 ± 2.40	14.60 ± 2.59	15.17 ± 1.80	16.54 ± 2.25	15.27 ± 1.85	15.42 ± 3.37
No. of viable implants/litter	14.45 ± 2.39	14.20 ± 2.78	14.50 ± 1.58	13.45 ± 4.32	14.18 ± 2.89	9.08 ± 8.23
Dead fetuses/litter	0.00 ± 00	0.00 ± 0.00	0.08 ± 0.29	0.54 ± 1.51	0.45 ± 1.21	1.83 ± 4.69
Resorbed fetuses/litter	0.45 ± 1.21^{a}	0.40 ± 0.97^{a}	0.08 ± 0.29^{a}	2.54 ± 2.84^{ab}	0.63 ± 1.03^{a}	4.50 ± 5.14^{b}
No. of non-viable implants/litter	0.45 ± 1.21^{a}	0.40 ± 0.97^{a}	0.17 ± 0.38^{a}	3.09 ± 4.20^{ab}	1.00 ± 1.67^{a}	6.33 ± 5.98^{b}
Postimplantation loss/litter (%)	2.84 ± 7.24^{a}	2.76 ± 6.84^{a}	1.16 ± 2.76^{a}	18.27 ± 23.73^{a}	7.67 ± 13.77^{a}	47.76 ± 45.15^{b}
Sex ratio (M/F)	0.42 ± 0.13	0.61 ± 0.31	0.44 ± 0.14	0.42 ± 0.12	0.40 ± 0.06	0.44 ± 0.16
Average fetal body weight/litter (g)	4.12 ± 0.62^{a}	3.67 ± 0.27^{ac}	3.34 ± 0.53^{bc}	2.56 ± 0.61^{d}	3.35 ± 0.73^{bc}	2.68 ± 0.62^{bd}

Results are expressed as mean values ± SD. Values in the same row showing a common superscript (a,b,c,d) are not significantly different at $P < 0.05$.

the group given 0.830 mg/kg/day of UAD only. Fetuses in the groups whose mothers were exposed to uranium, either alone or combined with restraint, showed a lower mean body weight than those in the control group. Although this reduction was dependent on the uranium dose, it was not significantly modified by maternal stress.

No external, internal, or skeletal malformations, as well as external and internal variations, which could be attributed to uranium exposure or maternal immobilization were noted. The types and frequencies of skeletal anomalies (most delayed or reduced ossification) are summarized in Table III. Although in relation to the control group the total number of fetuses with skeletal defects was significantly increased by uranium exposure, this increase was not dose-related. Again maternal restraint did not enhance the number of fetuses with skeletal variations. Moreover, when the

Table III. Effects of Uranium, Maternal Restraint, and Combined Uranium and Restraint on Skeletal Defects in Rat Fetuses

Uranium (mg/kg/day) Type of stress	0 None	0 Restraint	0.415 None	0.830 None	0.415 Restraint	0.830 Restraint
No. of fetuses examined skeletally (litters)	77 (11)	70 (10)	86 (12)	68 (11)	75 (11)	50 (8)
Total fetuses with skeletal defects (litters)	12^{a} (4)	21^{ac} (9)	47^{bc} (12)	60^{b} (11)	61^{b} (11)	50^{bc} (8)
Parietal bone, reduced ossification	0 (0)	1 (1)	19 (5)	12 (6)	10 (3)	3 (1)
Occipital bone, reduced ossification	0 (0)	1 (1)	5 (2)	10 (6)	6 (2)	3 (1)
Caudal, delayed ossification	8^{a} (3)	11^{a} (5)	39^{ac} (9)	52^{bc} (10)	29^{ac} (10)	37^{bc} (8)
Sacro, delayed ossification	0 (0)	0 (0)	1 (1)	10 (3)	0 (0)	1 (1)
Sternum, delayed ossification	5^{a} (2)^{a}	19^{ab} (8)^{ab}	42^{b} (10)^{b}	55^{b} (11)^{b}	41^{b} (9)^{ab}	39^{b} (8)^{b}
Assymetrical sternum	0 (0)	3 (2)	0 (0)	0 (0)	0 (0)	6 (1)
Xyphoid, delayed ossification	0^{a} (0)^{a}	1^{ac} (1)^{a}	29^{ab} (6)^{ab}	45^{b} (10)^{b}	23^{ab} (6)^{ab}	32^{b} (7)^{b}
Assymetrical ribs	0 (0)	3 (2)	0 (0)	0 (0)	0 (0)	0 (0)

Values in the same row (fetuses/litters) showing a common superscript (a,b,c) are not significantly different at $P < 0.003$.

litter was used as the unit of comparison for the total number of skeletal defects, there were no significant differences among groups.

The concentrations of uranium in kidney, spleen, liver, and placenta of rats exposed to UAD, restraint, or UAD plus restraint on gestation Days 6 to 15, as well as in whole fetuses are shown in Table IV. While uranium was not detected in the control and the restraint only groups, it significantly accumulated in kidney, spleen, and liver of UAD-treated dams. However, uranium could not be detected in placenta and whole fetuses.

Discussion

Until recent years, the potential adverse effects of uranium exposure during pregnancy had been scantly investigated, with an evident lack of published observations. In a review on the chemical toxicity of uranium, Stopps and Todd (9) mentioned that during WWII two studies were carried out, one of which featured exposure to high levels of the metal and another involved only a brief 24-hr exposure. Although it was reported that in both studies significant effects on reproduction were observed (9), the results were not repeated or extended by other investigators

Because of the scarce information on uranium-induced reproductive and developmental toxicity, in 1987 a series of investigations focused on these issues was started in our laboratory. We demonstrated that uranium is a reproductive, maternal, and developmental toxicant when given orally to mice at relatively low doses (25–28). Thus, the no-observed-adverse-effect level (NOAEL) for maternal toxicity was found below 5 mg/kg/day when UAD was given by gavage to mice on gestation days 6 to 15 at 0, 5, 10, 25, and 50 mg/kg/day (25). In turn, the NOAEL for fetotoxicity (including teratogenicity) was also below 5 mg/kg/day, as some anomalies were still observed at this dose (25). The effects of multiple maternal subcutaneous injections of UAD (0, 0.5, 1, and 2 mg/kg/day) given from Day 6 through 15 of gestation were also evaluated in mice (29). Maternal toxicity occurred in all uranium-treated groups as evidenced primarily by some deaths and decreases in body weight gain and body weight at termination. Although it was not dose-related, embryotoxicity also occurred in all uranium-treated groups, while fetotoxicity (reduced fetal body weight and morphological defects) was evident at 1 and 2 mg/kg/day. These doses were approximately equal to 1/20 and 1/10 of the acute subcutaneous LD_{50} of UAD in adult mice (20).

In the present study, UAD was administered subcutaneously to rats on gestation Days 6 to 15 at 0.415 and 0.830 mg/kg/day, doses that are also approximately equal to 1/20 and 1/10 of the acute subcutaneous LD_{50} of this chemical in rats (20). Similar to the effects observed in mice (29), maternal toxicity and embryotoxicity could be noted at 1/10 of the LD_{50} (0.830 mg/kg/day), while at 1/20 (0.415 mg/kg/day) and 1/10 (0.830 mg/kg/day) of the LD_{50} fetotoxicity was evidenced by significant reductions in fetal body weight and increases in the total number of skeletally affected fetuses. However, in contrast to the results in mice no significant maternal toxicity was noted in rats at 1/20 of the LD_{50} (0.415 mg/kg/day). The embryo/fetal toxic effects observed in the current study might be, at least in principle, the result of the direct contact of uranium with the fetal tissues and/or a direct consequence of maternal toxicity. Although the fact that uranium was not found in placenta and whole fetuses at detectable levels might suggest that the adverse developmental effects seen in the current investigation would be the result of uranium-induced maternal toxicity, there is no clear evidence that uranium did not cross the placenta and reached the fetus. In our previous study in mice (29), as well as in the present investigation, UAD was subcutaneously injected at doses approximately equal to 1/20 and 1/10 of the respective LD_{50}. A comparison of the results of both studies indicates that mice are more sensitive than rats to the toxic effects of uranium during gestation. No maternal deaths were found in rats at 0.415 mg/kg/day, while in contrast to mice embryo/fetal toxicity was not found in the group of rats exposed to 1/20 of the LD_{50} (0.415 mg/kg/day) of UAD on gestation Days 6 to 15. At 1/20 of the LD_{50}, significant maternal and embryotoxicity had been observed in mice (29).

With respect to the potential influence of stress on uranium-induced maternal and developmental toxic effects, there were no significant differences in maternal toxicity, the number of nonviable implants, fetal body weight per litter, or the total number of fetuses with skeletal defects between animals in the group given 0.415 mg/kg/day of UAD only, and those exposed to 0.415 mg/kg/day of UAD

Table IV. Uranium Concentrations (μg/g Tissue) After Exposure of Rats to Uranium, Maternal Restraint, and Combined Uranium and Restraint on Gestation Days 6–15

Uranium (mg/kg/day) Type of stress	0 None	0 Restraint	0.415 None	0.830 None	0.415 Restraint	0.830 Restraint
Kidney	ND	ND	253.63 ± 71.73[a]	661.92 ± 221.50[b]	374.29 ± 103.41[a]	686.29 ± 120.35[b]
Spleen	ND	ND	11.03 ± 6.70[a]	43.84 ± 18.00[b]	18.34 ± 6.84[a]	48.67 ± 19.79[b]
Liver	ND	ND	8.57 ± 6.55[a]	37.47 ± 18.76[b]	18.26 ± 3.84[a]	34.94 ± 15.51[b]
Placenta	ND	ND	ND	ND	ND	ND
Fetus	ND	ND	ND	ND	ND	ND

Results are expressed as mean values ± SD. ND: not detected; detection limit: 0.002 μg/g.
Values in the same row showing a common superscript (a,b) are not significantly different at $P < 0.05$.

and concurrently subjected to restraint. However, in the group exposed to 0.830 mg/kg/day of uranium plus restraint, the percentage of postimplantation loss was significantly higher than that seen at 0.830 mg/kg/day of uranium only. Although at 0.830 mg/kg/day of UAD restraint apparently enhanced uranium-induced maternal toxicity as evidenced by 1 death and 3 dams carrying totally resorbed fetuses, the level of statistical significance was not reached ($P > 0.05$).

A number of human studies have examined the potential association between stressful events or experiences and anomalies in offspring. Most investigations concluded that women who experienced stressful life events during pregnancy might be at increased risk of delivering infants with certain congenital anomalies (30–33). Moreover, a number of experimental investigations have also shown that maternal stress might have a notable influence on the adverse maternal and embryo/fetal effects of some developmental toxicants. Interactive effects in developmental toxicity have been reported to occur in pregnant animals exposed to salicylate and restraint (34), all-*trans*-retinoic acid and restraint (18), caffeine and restraint (35), and caffeine, aspirin, and restraint (36). In turn, a number of experimental studies performed in our laboratory showed also the presence of interactive effects in maternal and developmental toxicity in pregnant animals concurrently exposed to metals such as aluminum, arsenic and mercury, and restraint (14–16). Notwithstanding, as in the current investigation maternal restraint enhanced the metal-induced developmental toxicity only at high doses of the metals, which were also toxic to the dams.

In summary, while the results of the present study in rats corroborate only partly some of the adverse effects of uranium exposure during gestation reported in mice (11, 29), according to the overall data in both species, the results indicated that uranium is a developmental toxicant in mammals. Although most pregnant women are not currently exposed to uranium levels that could cause adverse effects on health, higher rates of uranium exposure have been reported for some populations (e.g., individuals who consume foods grown in areas with elevated concentrations of uranium in the soil, or subjects with elevated uranium levels in their drinking water) (6). In turn, the military use of DU might be of concern as an additional source of exposure. On the other hand, if uranium exposures occur at levels that may provoke maternal toxicity, the potential adverse developmental effects could be enhanced by stress.

1. Hartmann HM, Monette A, Avci HL. Overview of toxicity data and risk assessment methods for evaluating the chemical effects of depleted uranium compounds. Hum Ecol Risk Assess **6**:851–874, 2000.
2. Taylor DM, Taylor SK. Environmental uranium and human health. Rev Environ Health **12**:147–157, 1997.
3. McDiarmid MA, Keogh JP, Hooper FJ, McPhaul K, Squibb K, Kane R, DiPino R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Hamilton M, Jacobson-Kram D, Burrows B, Walsh M. Health effects of depleted uranium on exposed Gulf War veterans. Environ Res **82**:168–180, 2000.
4. McDiarmid MA. Depleted uranium and public health. BMJ **322**:123–124, 2001.
5. Abu-Qare AW, Abou-Donia MB. Depleted uranium–the growing concern. J Appl Toxicol **22**:149–152, 2002.
6. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for uranium. Atlanta, GA: US Department of Health and Human Services, Public Health Service; 1999.
7. Voetglin C, Hodge HC. *The Pharmacology and Toxicology of Uranium Compounds*. New York: McGraw-Hill; 1949.
8. Tannenbaum A. *Toxicology of Uranium*. New York: McGraw-Hill; 1951.
9. Stopps GJ, Todd M. The Chemical Toxicity of Uranium with Special Reference to Effects on the Kidney and the Use for Biological Monitoring. Research Report, Atomic Energy Control Board. Ottawa, Canada; 1982.
10. Corbella J, Domingo JL. Developmental and reproductive effects of aluminum, manganese, uranium, and vanadium. In: Chang LW, ed. *Toxicology of Metals*. Boca Raton: CRC Lewis Publ; 1083–1096, 1996.
11. Domingo JL. Reproductive and developmental toxicity of natural and depleted uranium: A review. Reprod Toxicol **15**:603–609, 2001.
12. Benson KA, McBride SA. Uranium levels in the fetus and placenta of female rats implanted with depleted uranium pellets prior to breeding. Toxicologist **36**:258, 1997.
13. Rasco JF, Hood RD. Effects of maternal restraint stress and sodium arsenate in mice. Reprod Toxicol **8**:49–54, 1994.
14. Colomina MT, Albina ML, Domingo JL, Corbella J. Effects of maternal stress on methylmercury-induced developmental toxicity in mice. Physiol Behav **58**:979–984, 1995.
15. Colomina MT, Albina ML, Domingo JL, Corbella J. Influence of maternal restraint stress on arsenic-induced pre- and postnatal alterations in mice. Psychobiology **24**:227–234, 1996.
16. Colomina MT, Esparza JL, Corbella J, Domingo JL. The effect of maternal stress on developmental toxicity of aluminum in mice. Neurotoxicol Teratol **20**:651–656, 1998.
17. Paré WP, Glavin GB. Restraint stress in biomedical research: a review. Neurosci Biobehav Rev **10**:339–370, 1986.
18. Rasco JF, Hood RD. Maternal restraint stress-enhanced teratogenicity of all- *trans*-retinoic acid in CD-1 mice. Teratology **51**:57–62, 1995.
19. Torrente M, Colomina M, Domingo JL. Effects of prenatal exposure to manganese on postnatal development and behavior in mice: Influence of maternal stress. Neurotoxicol Teratol **24**:219–225, 2002.
20. Domingo JL, Llobet JM, Tomas JM, Corbella J. Acute toxicity of uranium in rats and mice. Bull Environ Contam Toxicol **39**:168–174, 1987.
21. Staples RE, Schnell VL. Refinements in rapid clearing technique in the KOH- alizarin red S method for fetal bone. Stain Technol **43**:61–63, 1968.
22. Wilson JG. Embryological considerations in teratology. In: Wilson JG, Warkany J, eds. *Teratology: Principles and Techniques*. Chicago: University of Chicago Press; 251–277, 1965.
23. Ortega A, Domingo JL, Gomez M, Corbella J. Treatment of experimental acute uranium poisoning by chelating agents. Pharmacol Toxicol **64**:247–251, 1989.
24. Sanchez DJ, Belles M, Albina ML, Sirvent JJ, Domingo JL. Nephrotoxicity of simultaneous exposure to mercury and uranium in comparison to individual effects of these metals in rats. Biol Trace Elem Res **84**:139–154, 2001.
25. Domingo JL, Paternain JL, Llobet JM, Corbella J. Developmental toxicity of uranium in mice. Toxicology **55**:143–152, 1989.
26. Domingo JL, Ortega A, Paternain JL, Corbella J. Evaluation of the perinatal and postnatal effects of uranium in mice upon oral administration. Arch Environ Health **44**:395–398, 1989.

27. Paternain JL, Domingo JL, Ortega A, Llobet JM. The effects of uranium on reproduction, gestation and postnatal survival in mice. Ecotoxicol Environ Saf **17**:291–296, 1989.
28. Llobet JM, Sirvent JJ, Ortega A, Domingo JL. Influence of chronic exposure to uranium on male reproduction in mice. Fundam Appl Toxicol **16**:821–829, 1991.
29. Bosque MA, Domingo JL, Llobet JM, Corbella J. Embryotoxicity and teratogenicity of uranium in mice following subcutaneous administration of uranyl acetate. Biol Trace Elem Res **36**:109–118, 1993.
30. Saxen I. Cleft lip and palate in Finland: parental histories, course of pregnancy and selected environmental factors. Int J Epidemiol **3**:263–270, 1974.
31. Adams MM, Mulinare J, Dooley K. Risk factors for conotruncal cardiac defects in Atlanta. J Am Coll Cardiol **14**:432–442.
32. Laumon B, Martin JL, Bertucat I, Verney PM, Robert E. Exposure to organic solvents during pregnancy and oral clefts: a case-control study. Reprod Toxicol **10**:15–19, 1996.
33. Carmichael SL, Shaw GM. Maternal life event stress and congenital anomalies. Epidemiology **11**:30–35, 2000.
34. Goldman AS, Yakovac WC. The enhancement of salicylate teratogenicity by maternal immobilization in the rat. J Pharmacol Exp Ther **142**:351–357, 1963.
35. Albina ML, Colomina MT, Sánchez DJ, Torrente M, Domingo JL. Interactive effects of caffeine and restraint stress during pregnancy in mice. Exp Biol Med **227**:779–785, 2002.
36. Colomina MT, Albina ML, Sanchez DJ, Domingo JL. Interactions in developmental toxicology: combined actions of restraint stress, caffeine and aspirin in pregnant mice. Teratology **63**:144–151, 2001.