# REPRODUCTIVE TOXICITY INDUCED BY URANYL ACETATE DEHYDRATE

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**Abstract**

Our study looked at the potential adverse effects of uranyl acetate dehydrate (UAD) exposure on skeletal abnormalities in fetuses tried to determine a dose - effect relationship for UAD exposure via three different routes and tried to obtain an overall understanding about the toxic effects during the period of organogenesis. Pregnant female Sprague Dawley rats received uranyl acetate dehydrate in doses of up to 1 mg/kg/day delivered via orogastric gavage, subcutaneous injection or subcutaneous implantable osmotic pumps during gestational days 5-16.Gross inspection and histology examination of fetal organ samples was performed following C-section delivery of pups on E20. Maternal toxicity was indicated by significant reduction of body weight gain during pregnancy, behavior changes and death in some cases. Increased rate of abortion, fetal developmental arrest and decreased fetal viability was noted in females exposed to 1mg/kg/day UAD. Significant reduction in the average size of the litters was noted and size/weight of the newborns in females receiving 0.830 mg/kg/day UAD. No gross fetal malformations were noted however nonspecific target organ changes were recorded.

**Key words**: uranium toxicity, fetal toxicity, congenital malformations, uranyl acetate dehydrate.

## Introduction

Uranium makes up approximately 2–4mg/kg of the earth's crust (ATSDR, 1999). It is more plentiful than silver or tin, with abundance equal to that of molybdenum or arsenic. Although in nature there are more than 100 different uranium ores, uranium typically occurs as the mixed oxide $U_3O_8$, in amorphous (pitchblende) or crystalline forms (uraninite).

Depleted uranium (DU) is a man-made, radioactive, heavy metal derived from uranium ore. It is chemically identical to natural and enriched uranium, although it is approximately 40% less radioactive than the naturally occurring metal (ATSDR, 1999).

It reacts with most non-metallic elements; it has pyrophoric properties and may spontaneously ignite at room temperature in air, oxygen and water. These unique properties make it appealing for use in many civilian and military applications.

DU is used as: radiation shielding, for missile projectiles, as target elements in plutonium production reactors, for gyroscope components, and as counterweights or stabilizers in aircrafts. Additional applications for DU also include X-ray radiation shielding in hospitals, as counter weights for rudders and flaps in commercial aircrafts, in keels of sailing yachts and as ballast in both military and non-military airplanes. (Hindin et al., Environmental Health, 2005) Military applications for DU consist of production of distinctly powerful projectiles (e.g., bullets/ penetrators, missile nose cones) and also as a protective armor for tanks. As a projectile, a DU penetrator ignites on impact under high temperature; it has a low melting point.

In the early days of the Manhattan Project, a very extensive toxicology program on uranium was carried out (Tannenbaum, 1951; Voetglin and Hodge, 1953).

Nowadays, the biokinetics, metabolism, and chemical toxicity of uranium, including the adverse effects on main target tissues, are established (Taylor et al., 1997; Craft et al., 2004; Brugge et al., 2005).

Until recent years little attention was paid to the potential toxic effects of uranium on reproduction and development. Moreover, most experimental studies on uranium-induced developmental toxicity have been performed in a sole species of mammals, mice (Albina et al., 2003)

Potential mechanisms of toxic action of DU alloy include mutagenicity and genotoxicity, disturbances in cell division, changes or inhibition of protein or steroid synthesis, disturbance or inhibition of enzyme systems, and disruption of behavioral patterns involved in normal reproduction. The end product of these mechanisms may be: 1) increased or decreased cell death; 2) disturbed cell-to-cell contact; 3) reduced biosynthesis; 4) increased morphogenetic pattern formation; or 5) disruption of tissue structure that may lead to abnormal pathogenesis in the reproductive system or developing fetus. If the repair processes inherent to fetal tissue become overwhelmed, dysmorphogenesis of the developing fetus may occur resulting in too few cells or cell products being formed to affect structure and functional maturation of the developing individual.

It has been shown that uranium is a developmental toxicant when given orally or subcutaneously to mice. Decreased fertility, embryo/fetal toxicity including teratogenicity, and reduced growth of the offspring have been observed following uranium exposure at different gestation periods (Domingo, 2001).

An increased incidence of cleft palate and dorsal and facial hematomas was found among litters from pregnant Swiss–Webster mice dosed with uranyl acetate dehydrate (UAD) at 1–50 mg/kg per day by gavage on gestational days 6–15.

A dose-related increase in liver weight was found among pups with increasing maternal dose levels of UAD. Brain, heart, lung, kidney, and spleen weights of pups with exposure to uranium during gestation and lactation were not significantly different from the weights of these organs from control animals. Fetotoxicity, characterized by significant decreases in fetal weight and incomplete bone ossification at several sites was observed in offspring born to dams exposed to 1 or 2 mg/kg per day.

Domingo et al., found that if the uranyl acetate dehydrate was given by means of single subcutaneous injections of (4 mg/kg) to the pregnant females, the number of dead and reabsorbed fetuses and percentage of postimplantation loss was greatest on day 10 of gestation . Also, fetal weight was significantly reduced and a higher incidence of skeletal variations occurred among surviving offspring as compared with negative controls.

There are only a few human studies so far that looked at the relationship between depleted uranium and congenital malformations in humans. The present studies were a result of the observations made in military combat area in the postwar period.

The Nuclear Policy Research Institute, USA reports that as early as 1995-96, Iraqi doctors suspected a rise in leukemia and birth defects among children born or treated at the Women and Children's Hospital in central Basrah, Iraq's second largest city.

The Iraqi studies, the only population-based studies available, have their limitations including a lack of independent measures of exposure such as tissue and urine samples, no control city for comparison, mobile population so that some exposed individuals moved from the area while unexposed people moved into the area and, as a retrospective study, a question of assessment bias.

Additional information comes from, Imad Al-Sadoon et al., 1999, who performed an analysis of registered congenital malformation among births in Basrah, Iraq for the period from 1990 to 2000. In general there was an apparent increase in the incidence rate from 1995 upwards. In 2000 such incidence was almost six folds higher than in 1991.To improve statistical efficiency of the data collected and overcome small numbers of cases recorded, the pattern and incidence of congenital malformations are grouped into three periods, 1991 to 1994, 1995 to 1998 and 1999 to 2000.

The incidence rate for the first period was 2.5 congenital malformations per 1000 births while the respective figure for the second period is 4.57 and for the third period was 13.49. Congenital heart diseases and chromosomal aberrations were reported at a higher frequency during the latter years. Such unusual malformations as phocomelia and icthyosis, which were not reported in 1990 have been recorded later though in small numbers. The frequency of cleft lip and palate followed a similar trend. No apparent trends were observed in the remaining malformations.

Our study looked at the potential adverse effects of UAD exposure on skeletal abnormalities in fetuses, tried to determine a dose - effect relationship for UAD exposure via three different routes and tried to obtain an overall understanding about the toxic effects during the period of organogenesis.

**Material and methods**

Sexually mature male and female Sprague- Dawley rats were obtained from the University of Medicine and Pharmacy "Victor Babes",Timisoara in joint collaboration with "Pius Branzeu" Experimental Surgery Research Center in Timisoara, Romania location where all the animals experiments were performed.

Female rats were mated with males (2:1) until copulation was detected. Finding of sperm (plugs) indicated copulation and the day of detection was considered as day 0.5 of gestation. Experiments involving the above mentioned animal species was approved by the Commission of Ethics - University of Medicine and Pharmacy "Victor Babes ", Timisoara, Romania.

Uranyl acetate dehydrate (UAD) was purchased from SPI-ChemTM (West Chester, PA).

Physical, chemical properties as well as handling instructions were obtained and followed as described in the Material Safety Data Sheet (MSDS – UAD).

The chemical was administered by oral-gastric gavage or subcutaneous injections where 0.9% NaCl solution was used as a vehicle. For the oro-gastric administration animals received anesthesia prior the procedure with Isoflurane (Hospira Worldwide Inc.) Animals received 0.830 mg/kg/day or 1 mg/kg/day of UAD by either gastric gavage (10 animals) or subcutaneous injections (20 animals) received the treatment between days 6 through 15 of gestation.

An equal number of animals for all groups, labeled as control animals received the same regime but instead of UAD they received 0.9% NaCl solution throughout the same gestational days as the experimental groups.

An additional group of animals (20 animals ) received UAD through subcutaneous implantable osmotic pumps ( Alzet – 200 microliters, Cupertino, CA ) that contained a UAD&0.9% NaCl (0.830 or 1 mg/kg/day of UAD) mixture that was implanted at day 6 of gestation. The use of osmotic pumps offer the benefit of saving critical time by eliminating the need for frequent animal handling and repetitive injection schedules reducing the stress on the animals as well as since it is a dependable drug delivery systems have proven invaluable in predictably sustaining compounds at therapeutic levels, avoiding potentially toxic or misleading side effects and ensuring accurate research results. The pumps were surgically implanted through a

minimal incision on the back of the animal in the cervical area and the wound closed with two interrupted 4.0 vicryl sutures (Ethicon, Inc.). Sutures were removed 4 days later and the implanted pumps were removed at the time of the caesarian section of the pregnant females. Animals that displayed signs of end point criteria or severe signs of distress were euthanized and excluded from the study.

Animals included in the study were monitored on daily basis and parameters such as food consumption, body weight gain and clinical signs of toxicity were regularly followed.

Cesarean sections were performed on all females, after previous euthanasia with carbon monoxide (CO), on gestation day 20. After median laparotomy and exposure of the gestational uterus the neonate rat pups were extracted, counted, weighed and examined for external variations, visceral malformations and skeletal abnormalities as showed below.

After delivery, rat pup fetuses were carefully dissected and numerous organ samples were harvested. Presumably targeted organs by the UAD toxicity were: heart, lungs, liver, kidney, intestine, muscles and bones. Additional samples of skin, placenta, uterus, umbilical cord were harvested as well.

The collected samples were placed in histology recipients, stored overnight in Formalin 10% and then sent to the Histology Core at the University of Medicine and Pharmacy "Victor Babes ",Timisoara were analysis of the samples was performed . Slides were prepared from embedded samples in blocks that were cut at 3µm thickness and stained with haematoxylin and eosin (HE).Resulting slides were read in a double blinded fashion for accuracy by two independent investigators.

The Student t test was used for comparing differences between the groups of animals.

Measurements within each experiment were averaged, and mean values and SD were derived from the averaged values. Results are presented as mean with statistical significance set at $p<0.05$. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) 14.0 for Windows.

**Results**

The UAD administration either by oro-gastric gavage, subcutaneous injection or osmotic pumps resulted in maternal toxicity in all treated animals.

At 0.830 mg/kg/day administered through oro-gastric gavage on gestational days 6-15, maternal toxicity was indicated by a significant reduction in body weight gain during gestation and a decrease in body weight at termination. On the average, the weight gain in the UAD group was about 8 grams compared to the control group were an average of 24 grams was recorded. (Figure 1)

When given by means of subcutaneous injection or osmotic pump the same amount of UAD (0.830 mg/kg/day) produced significant maternal and fetal toxicity. Almost all rat pups at the time of the C-section were either dead or extremely small for their gestational age. In one case we noticed the uterus filled with pus like collection with lack of pups. We concluded that all pups experienced developmental arrest that occurred between or around gestational age 6-15. At this dose 2 pregnant females died on gestation day 7.

Animals exposed to UAD at 0.830 mg/kg/day by all 3 routes experienced a weight loss during the pregnancy in 100% of cases in some cases severe.

Administered by subcutaneous injection or osmotic pump of 1 mg/kg/day UAD on gestational days 6-15 produced maternal toxicity, death in many cases and fetal death in all cases. Two pregnant females died on day 7 of gestation and we only recorded fetuses in 2 cases out of 10 females. In the event of fetus presence the pups were in low numbers (2 in the mentioned cases) and they were extremely small for their gestational age when compared to the age matched control. At gross inspection the pups appeared to have developed and then experienced developmental arrest at an early gestational date.

In one case that received 1 mg/kg/day UAD through subcutaneous osmotic pump we noticed a local infection that we concluded stopped the normal functioning of the pump thus not allowing the UAD to produce a systemic effect. In this particular case the female at the time of the C-section produced 13 normal looking pups with a 4.5 gram average weight.

With 0.830 mg/kg/day administered through oro-gastric gavage, we did not notice any maternal death at any stage of the gestation. Females in the UAD exhibited a decrease in appetite, food consumption and a decreased level of activity noted by the animal caregiver staff after attentive monitoring of animals 3 times a day.

A decrease in the number of pups was noted among the UAD group animals compared with the control group. A normal litter can carry anywhere from 7 to 16 pups fact confirmed by the number of neonates in the control group. Females in the UAD group had significantly smaller litters. The number of fetal deaths and the sex ratio for live fetuses were unaffected by treatment.

When given by means of subcutaneous injection or osmotic pump the same amount of UAD (0.830 mg/kg/day) proved to produce more effective maternal and fetal toxicity then the gavage route and we recorded a decreased number of fetuses on average 12 in the females that did present with pups at the time of the C-section.

When looking at the birth weight of the pups we noted that there was a statistically significant difference between animals in the UAD groups and control group originated pups. (Figure 1)

Examination of the live fetuses revealed no dose-dependent or statistically significant increases in the incidence of fetal gross external alterations.

Additional experiments with lower amounts of UAD helped us conclude that the no-observable-adverse-effect level (NOAEL) for maternal toxicity was $< 0.5$ mg/kg/day and the NOAEL for embryofetotoxicity was also $<0.5$ mg/kg/day as no significant increases in the incidence or type of malformations were observed at this dose.

Samples of intestine harvested from pups originated from mothers treated with UAD by means of gavage , 0.830

mg/kg/day, had signs of minor to severe damage consisting of epithelial cell lifting and/or separation and necrosis to mid villus level. The crypts had a normal architecture. However, the results indicated that UAD was not toxic for the intestine, as measured by histological appearance at 0.830 mg/kg/day. (Figure 1)

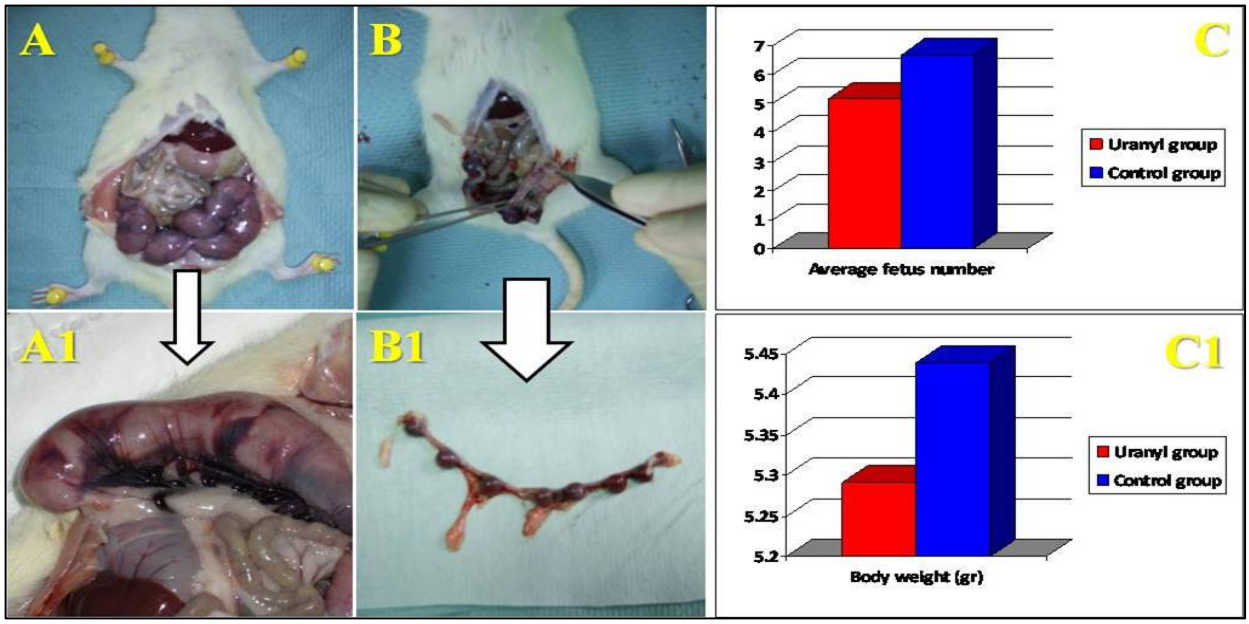


Figure 1 – A, A1 – Normal pregnancy vs. pregnancy in rats that received UAD ; lack of fetuses and small size uterus (B,B1).Decreased number of pups with a significantly lower body weight in females that received 0.830 mg/kg/day UAD.

The alveolar structure of the lungs of UAD rat pups originated from females that received, 0.830 mg/kg/day, was altered, the thin histological weft disappeared and large spaces were formed by the damage to the interalveolar septa. In the other regions of the lung, the amount of fibrous connective tissue was slightly increased. (Figure 2)

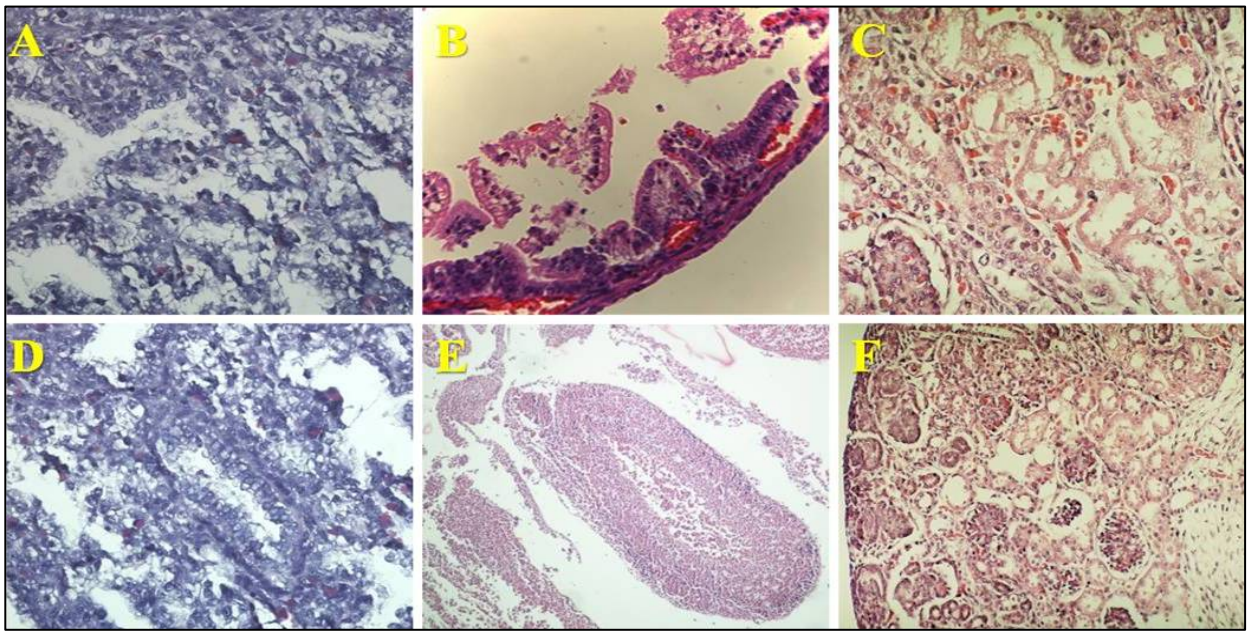


Figure 2 – A;D – Lung H&E staining 40X – organ aspect altered, the thin histological weft disappeared and large spaces were formed by the damage to the interalveolar septa with slightly increased amount of fibrous connective tissue. B;E – Intestine H&E staining 40X - minor to more severe damage consisting of epithelial cell lifting or separation and sometimes necrosis to mid villus level. C;F – Kidney H&E staining 40X - signs of immaturity and marked hyperemia changes.

We found similar changes in the few surviving rat pups that originated from females that received 1 mg/kg/day by subcutaneous injection or osmotic pump delivery system.

There was no gross external alteration to the structure of the lung noted in either of the two groups examined. Since the caesarian section produced some degree of prematurity in the rat pups examined in both the control and the experimental group we conclude that the above mentioned changes were not significantly different between the groups.

Several samples of bone and muscle were taken from each fetus including femur, upper and extremity, ribs and in cases whole body sections were examined.

There was no gross external difference between femurs originated from fetuses of females that received, 0.830 mg/kg/day. The histological aspect of femur bone sections from UAD rat did not reveal any pathological changes.

Bone samples from animals belonging to both the control and the UAD groups showed presence of cartilage cells and immature reticular cells without any significant changes.

In isolated cases we noticed areas of necrosis when looking at sections of ribs but since this was a sporadic observation we concluded that this can't be generalized. We concluded however that the UAD fetuses bone sample slides depicted decreased ossification when compared to their control counterparts. (Figure 3)

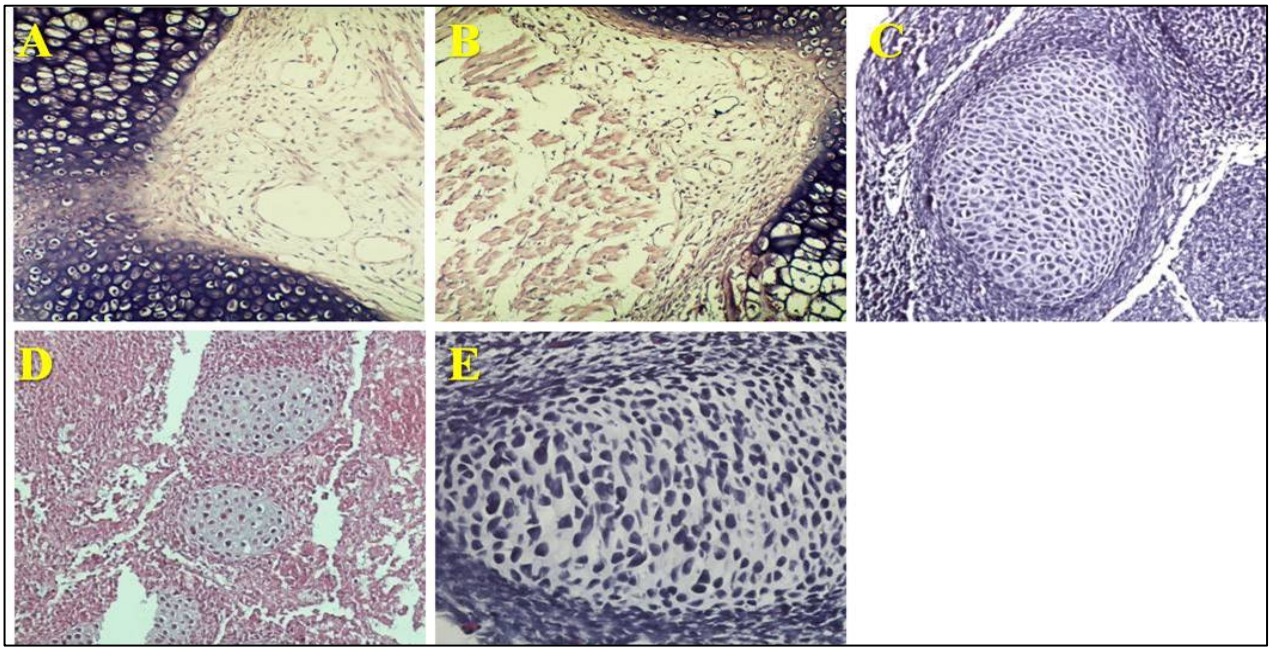


Figure 3 - Bone sections – A;B – femur – without pathological changes , normal appearance cartilage cells and immature reticular cells – C;E – humerus – normal appearance – D- ribs and intercostals muscle section – decreased ossification when compared to the control group animals.

The analyzed muscle samples in the case of UAD pups showed signs ranging from normal aspect to atrophy and to inflammatory infiltrate.

With regards to the kidney, on gross inspection there was no significant difference between the experimental and control animals however in one case the most remarkable abnormality detected was kidney hypoplasia. Histology did not reveal any major abnormalities in the kidneys of UAD fetuses except signs of immaturity and marked hyperemia, changes that were also seen in the control animals. (Figure 2)

Macroscopic examination of the lives did not pointed at any significant changes, we didn't notice any structural changes at 0.830 mg/kg/day of UAD. The average weight of livers from animals in both groups did not differ. There weren't any signs of fatty liver or lobular necrosis. Histology revealed that there wasn't any degree of injury to the liver cells. Cell had a normal aspect but an inflammatory cell infiltration in portal areas and sinusoids was noted in the rat pups originated from females that received, 0.830 mg/kg/day. Extensive congestion and inflammatory cells aggregating in hepatic sinusoid lumen was noted as well in the UAD fetuses. (Figure 4)

Where there were no fetuses the uterus was harvested and histological analysis showed only signs of pregnancy, like (increased mucosa thickness) and from gross observations ''resorbing sites'' - locations where presumably fetuses were located.

There were also area of degeneration and micro calcifications noted in the placenta of females exposed 0.830 or 1 mg/kg/day of UAD. (Figure 5)

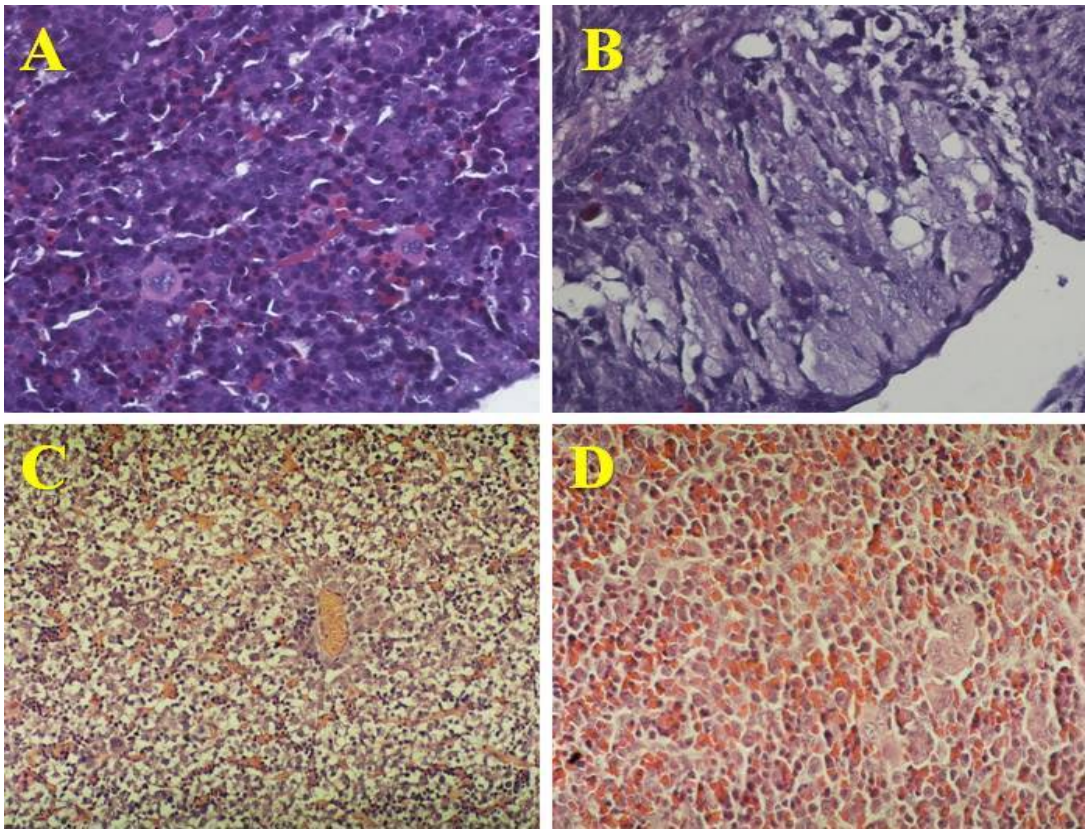


Figure 4 – Liver sections H&E staining - congestion and inflammatory cell infiltration in the liver.

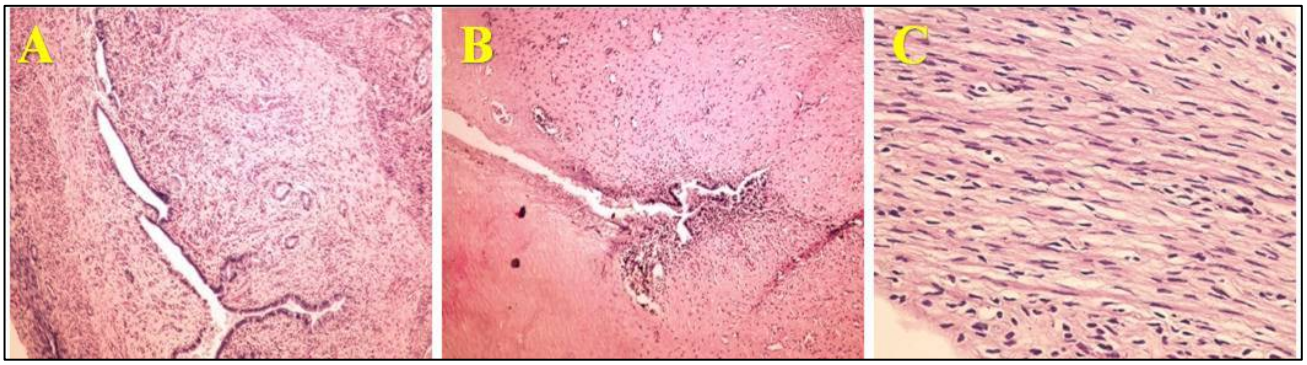


Figure 5 – Uterus H&E 40X sections – myometrium ( C ) and endometrium (A&B) – specific pregnancy changes with increased width of mucosa layer. Depth and number of glands are increased appreciably. Glands are straighter and are lined by columnar epithelium. Myometrium of normal appearance.

**Discussions**

In the last decade, many parts of the world suffered substantial demographic, social, cultural, economic, and ecological turbulences as direct or indirect consequences of the war scenarios. Public attention worldwide has been drawn to an apparently increased incidence of malignant diseases in the areas of military activities.

This increase was associated with alleged radioactive and/or chemical contamination by military equipment and weapons.

Our study looked at the embryotoxicity and teratogenicity in rats following different routes of administration of UAD.

In general terms, there is a good agreement between the dose and the effect obtained though as our previous hypothesis; it appears that the route of exposure (oral vs. subcutaneous administration vs. gradual delivery via osmotic pump) can determine the degree of toxicity of UAD. The results of our study are similar to other studies where uranium was administered orally or subcutaneously to mice. Effects such as, decreased fertility demonstrated by

the reduced number of fetuses, reduced size of the pups, embryo/fetal toxicity following uranium exposure at different gestation periods (Domingo et al., 2001), were also encountered in our study.

Arfsten et al., found irregular estrous cycles identified in uranium-exposed females as compared with satellite control females over a three-month interval.

Females in the uranium-exposed group that did not have litters over the first seven months of the experiment did not have any litters over the last five months of the experiment. The authors concluded that the decrease in reproductive success in uranium-exposed animals may have been an indirect effect resulting from decreased food intake as evidenced by depressed body weights and irregular estrous cycles. However, it is possible that there was a direct chemical interaction on the reproductivity of uranium-exposed breeders given the fact that reproductivity continued to be poor once uranium was reduced to background levels in their diets.

In a study by Maynard and Hodge (1949), rats (50/sex) were exposed to dietary levels of uranyl nitrate of 2% (about 460 mg/kg) for one day. Males and females were then paired, over a period of 7 months. Declines in total number of pups born (1959 vs. 1725; 12% decrease) and litter size (8.6 vs. 7.6; 7% decrease) were observed with treatment, but the actual number of litter bearing females increased from 43/50 to 44/50 with treatment.

Other investigators found that maternal toxicity was apparent at a dose level of

5 mg/kg/day indicated by decreased body weights as compared with controls, suggesting that the observed developmental variations may have resulted from a maternal toxic response. However, it was concluded that some of the fetal effects were independent of maternal toxicity. A significant reduction in body weight gain during pregnancy, treatment-related signs in behavior, abortion, and death have been reported to be general criteria for the existence of maternal toxicity in rodents. We found that females in the UAD exhibited a decrease in appetite, food consumption and a decreased level of activity.

Several studies looking into the changes of behavior patterns in animals exposed to uranium have showed that there is an accumulation of the chemical in the brain of animals exposed by various routes to the element.

According to Houpert et al. following chronic ingestion of DU, uranium accumulated mainly in the hippocampus. The hippocampus is known to be involved in the spatial working memory processes and previous experiments have shown that this kind of memory was altered in rats after repeated DU inhalation or after chronic ingestion.

Some other behavioral changes were observed after uranium exposure, such as sleep–wake cycle modification, increased anxiety-like behavior, or changes in exploratory activities. We found very similar changes in behavior in exposed females throughout pregnancy. However, the mechanism by which uranium induces such effects still remains to be elucidated. The most probable hypothesis is a direct chemical or radiological effect on one or more cerebral areas, although an indirect effect can't be entirely excluded.

Since our results did not provide any concrete evidence of lung pathology as a result of exposure of pregnant females to UAD during gestation we hypothise that the lung can only be affected when directly exposed to particles of uranium via air. One study found that "uranium dust" caused lipid peroxidation and micronucleiformation; however, chemical analysis of the dust revealed that there was no uranium component in the dust, and thus, these results are likely due to the other chemical components of the dust or to the particles themselves. (Ohshima S. et al., 1998) The other study found that insoluble DU induced neoplastic transformation of human bronchial cells consistent with the possibility that exposure to particulate DU may cause lung cancer, although that study did not consider specific genotoxic events that may have led to the transformation. (Yang, Z. H et al., 2002). Investigators have provided evidence that miners exposed to uranium particles have an increased incidence of lung cancer compared to other exposure routes.

Since this route of exposure, inhalation is nonexistent in case of the fetuses this could explain in part the normal appearance of the lungs in these neonates.

Bosque et al., (1992), found that fetotoxicity was evidenced by a significant decrease in fetal body weight and significant increases in the incidence of several skeletal districts unossified or with decreased ossification in the 1 and 2 mg/kg/day UAD groups.

Our study found that skeletal defects were not present at 0.5 mg/kg/day, whereas internal or skeletal malformations were only evident in the 1 mg/kg/day UAD when fetuses were found to be present in the uterus at the time of the c-section.

Tasat et al., (2007), notice ultra structural alterations in the nucleus and the cytoplasm of osteoblasts after in vivo exposure to uranium of adult rats (2 mg/kg; 1x) demonstrating a clear toxic effect.

Uranium exposed flat cells covering bone exhibited fragmented and swollen rough endoplasmic reticulum cisternae, scattered free ribosomes, and few coated vesicles with a fuzzy content. Uranium-treated osteoblasts showed signs of severe alterations, exhibiting absence of cell membrane and Golgi complex, swollen and fragmented RER cisternae presenting flocular content, and puffy nuclei with fine granular content when compared to non treated animals. Cell, (osteoblasts) viability was determined after 24 h in culture. None of the assayed doses of uranyl nitrate (0.1–100 μM) affected cell viability, which always remained close to control values. After 24 and 48 h in culture, cells exposed to 0.1–100 μM uranyl nitrate failed to show the typical morphologic features resembling those of apoptotic cells, such as pyknosis and nuclear fragmentation.

In our study gavage administration of UAD, 0.830 mg/kg/day to pregnant females failed to produce any significant alterations of osteoblasts structure in the fetuses in all segments of bones analyzed.

It is well documented that metals induce imbalance in oxidative metabolism, mainly through the increase in

reactive oxygen species (ROS), triggering apoptosis in many cell types including bone cells. It is well known that radiation, certain toxic drugs, chemical agents, and metal traces, can increase the physiological production of ROS. The balance between the production and detoxification rates of ROS determines their intracellular steady-state concentrations, which under pregnancy conditions might be kept in balance.

Up to a certain unknown concentration of uranium we think that there is a compensative mechanism for the increased ROS that protects the newborns from increased cell apoptosis and indirectly skeletal malformations.

Uranium is a classic nephrotoxin, and it's use at high doses for experimental induction of nephrotoxicity is well established with renal failure being reported. (Bosque et al., 1993)

Several studies have looked at the chronic exposure of adult animals to uranium but little is known about the effect on fetus kidneys in females exposed to the element.

In our study at the gavage dose of UAD 0.830 mg/kg/day that we used there was no damage to the kidney detected and in the case of 1 mg/kg/day we weren't able to obtain evidence of kidney damage because of the low number of specimens available.

Donnadieu-Claraz et al. investigated the effect of chronic exposure to uranium in rat kidneys that received uranium in the drinking water (40 mg uranium liter$^{-1}$).

Microscopic analysis showed that proximal tubule cells from contaminated rats had increased numbers of vesicles containing dense granular inclusions. These inclusions were composed of clusters of small granules and increased in number with the exposure duration. The authors identified these characteristic granules as iron oxides. Uranium was found to be present as a trace element but was never associated with the iron granules. These results suggest that the mechanisms of iron homeostasis in kidney could be affected by chronic uranium exposure.

Other animal studies, like the one described by Novikov and Yudina (1970) where they administered female rabbits (6 to 8/group) oral doses of uranyl nitrate of 0, 0.02, 0.2, and 1 mg U/kg/day for 12 months noted no differences when compared with controls with respect to serum urea, creatinine or chlorides. (U.S. Environmental Protective agency 1989)

The lack of signs of liver toxicity after oral gavage of the substance is similar to results reported by other authors such as Domingo et al. (2003) that investigated the Influence of maternal stress on uranium induced developmental toxicity in rats. They found that there were no changed in the liver associated with UAD exposure with or without stress up to 0.830mg/kg/day administered either by oro-gastric gavage or subcutaneous route.

The investigators however noted that while uranium was not detected in the control and the restraint only groups and it not produced any major changes to the liver in the experimental animals it significantly accumulated in kidney, spleen, and liver of UAD treated dams.

There are no studies to our knowledge that looked at the effects of uranium on the fetus intestine, however Dublineau et al. addressed the biological consequences of a contamination with depleted uranium on intestinal properties such as the barrier function and/or the immune status of this tissue. Their study concluded that depleted uranium is not toxic for the intestine after acute exposure (204 mg/kg). Nevertheless, depleted uranium seems to modulate the expression and/or production of cytokines (IFN gamma) and chemokines (MCP-1) in the intestine. The amount that these rats received, 204 mg/kg, far exceeds the amount that the fetuses were exposed to in our study so we consider our findings regarding the intestine as collaborative with the dose.

The current investigation might be the basis for further studies on the potential role of uranium exposure on maternal and fetal toxicity and it's role in the genesis of congenital malformations following other ways of exposure such as inhalation or through wounds.

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