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Evaluation of the effect of implanted depleted uranium on male reproductive success, sperm concentration, and sperm velocity ☆

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Abstract

Depleted uranium (DU) projectiles have been used in battle in Iraq and the Balkans and will continue to be a significant armor-penetrating munition for the US military. As demonstrated in the Persian Gulf War, battle injury from DU projectiles and shrapnel is a possibility, and removal of embedded DU fragments from the body is not always practical because of their location in the body or their small size. Previous studies in rodents have demonstrated that implanted DU mobilizes and translocates to the gonads, and natural uranium may be toxic to spermatazoa and the male reproductive tract. In this study, the effects of implanted DU pellets on sperm concentration, motility, and male reproductive success were evaluated in adult (P1) Sprague–Dawley rats implanted with 0, 12, or 20, DU pellets of 1×2 mm or 12 or 20 tantalum (Ta) steel pellets of 1×2 mm. Twenty DU pellets of 1×2 mm (760 mg) implanted in a 500-g rat are equal to approximately 0.2 pound of DU in a 154-lb (70-kg) person. Urinary analysis found that male rats implanted with DU were excreting uranium at postimplantation days 27 and 117 with the amount dependent on dose. No deaths or evidence of toxicity occurred in P1 males over the 150-day postimplantation study period. When assessed at postimplantation day 150, the concentration, motion, and velocity of sperm isolated from DU-implanted animals were not significantly different from those of sham surgery controls. Velocity and motion of sperm isolated from rats treated with the positive control compound α-chlorohydrin were significantly reduced compared with sham surgery controls. There was no evidence of a detrimental effect of DU implantation on mating success at 30–45 days and 120–145 days postimplantation. The results of this study suggest that implantation of up to 20 DU pellets of 1×2 mm in rats for approximately 21% of their adult lifespan does not have an adverse impact on male reproductive success, sperm concentration, or sperm velocity.

Keywords: Depleted uranium; Reproductive toxicity; Rats; Sperm concentration; Sperm motion

1. Introduction

The impact of embedded depleted uranium (DU) alloy fragments on human health has been an ongoing subject of study since the first use of DU projectiles in combat in the 1991 Persian Gulf War. Exposure to DU has been blamed for reproductive problems and increased cancer rates in military personnel (Drozdiak, 2001; Durakovic, 2001; Ross, 2001; Schoettler, 2001). The potential health hazards associated with exposure to DU alloy are both radiological and chemical

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(Sztajnkrycer and Otten, 2004; Arfsten et al., 2001; McClain et al., 2001; Miller et al., 1998b) and both modes of toxicity would be expected to occur in cases where DU projectiles or fragments become internally deposited (e.g., embedded) in tissue (Arfsten et al., 2001; McClain et al., 2001). DU predominately emits alpha radiation along with small amounts of beta and gamma radiation (Sztajnkrycer and Otten, 2004; AEPI, 1995) and has a radioactivity that is approximately 60% of the radioactivity of natural uranium (0.4 μCi/g versus 0.7 μCi/g, respectively). DU is likely to be chemically toxic based on the findings of toxicity in animals exposed to natural uranium with the kidney being the likely target organ of DU chemical toxicity (Domingo, 2001). Implantation of DU alloy into rat muscle has been shown to increase the frequency of implantation site soft tissue sarcomas (Hahn et al., 2002) while in vitro studies have shown DU to be mutagenic (Miller et al., 1998a, b) and clastogenic (Miller et al., 1998b) suggesting that embedded DU could be carcinogenic in humans.

An ongoing study of Persian Gulf War (1991) veterans involved in DU friendly fire incidents has found evidence to suggest that DU exposure may be associated with changes in reproductive (McDiarmid et al., 2002) and neurocognitive function (McDiarmid et al., 2000) parameters. Several of these veterans were found to be excreting uranium in their urine at levels greater than background when assessed 9–11 years after the end of the Persian Gulf War (McDiarmid et al., 2004), suggesting that these veterans may be exposed to significant levels of uranium in part or wholly as a result of their exposure to DU in the 1991 Persian Gulf War. Hypoxanthine–guanine phosphoribosyl transferase (HPRT) mutation frequency was found to be correlated with high urine uranium levels in this friendly fire cohort (McDiarmid et al., 2004) and suggests that the veteran's continued uranium exposure could be hazardous. However, further follow-up and research is needed to evaluate the impact of increased HPRT mutation frequency on human health.

There are several possible mechanisms by which embedded DU fragments could possibly affect adult reproduction (Arfsten et al., 2001). DU exposure may cause reproductive toxicity by (1) interacting with germ and other cells of the reproductive system, (2) directly interacting with the central nervous system (CNS), resulting in abnormal reproductive behavior or function, and (3) modifying the CNS, leading to alterations in the secretion of hormones or gonadotropins. There have been no animal studies that have examined the effects of DU exposure on the male reproductive system, but studies in rats implanted with DU pellets have found that DU mobilizes and translocates to the reproductive organs (McClain et al., 2001; Pellmar et al., 1999; Benson, 1998; Benson and McBride, 1997). Administration of high doses of uranium has been shown to cause male reproductive toxicity in rodents. Chronic administration of uranium in the diet to male rats for 2 years at concentrations of 0.01–0.25% was found to be associated with aspermia and degenerative changes in the testes and epididymis (Maynard et al., 1953). The number of female mice impregnated successfully was significantly reduced following mating with male Swiss mice administered 10, 20, 40, or 80 mg/kg/day of uranium in their drinking water for 64 days as compared with negative controls (Llobet et al., 1991). Exposure to 80 mg/kg/day of uranium was associated with interstitial alterations and vacuolization of the Leydig cells, and the number of spermatozoa present in the epididymis was consistently lower for uranium exposed animals as compared with negative controls (Llobet et al., 1991). Injection of male rats with uranium compounds was shown to result in significant decreases in average testes weight (Malenchenko et al., 1978). Testicular injection of male mice with uranyl fluoride containing enriched uranium resulted in a dose-dependent increase in chromosomal aberrations in spermatogenia, primary spermatocytes, and mature sperm possibly as a result of both chemical and radiological toxicity (Hu and Zhu, 1990; Zhu et al., 1994).

In 2002 the Naval Health Research Center Detachment Environmental Health Effects Laboratory, formally Detachment Toxicology, applied for and was granted funding by the US Army Medical Research Acquisition Activity (USAMRAA) through the Peer-Reviewed Medical Research Program (PRMRP) to conduct a two-generation reproductive toxicology study of implanted DU pellets in rats. This paper describes the reproductive studies conducted on adult male rats implanted with DU pellets at 30–45 and 120–145 days postimplantation. Each animal was surgically implanted with 0, 12, or 20 DU pellets of 1 × 2 mm. Tantalum (Ta) steel pellets were used as inert control materials to account for potential physical effects of the pellets on reproductive health and behavior. Reproductive endpoints measured in this study included sperm motility and concentration, mating success, and average time to insemination used as an indirect measurement of reproductive behavior relative to that of untreated controls animals.

2. Materials and methods

2.1. Metal pellets

Cylindrical depleted uranium alloy (99.2% DU, 0.8% titanium) pellets, 1 × 2-mm diameter, were obtained from the United States Department of Energy (Y-12 National Security Complex (BWXT Y-12), Oak Ridge, TN) under DOE Project No. 2348-S535-A1. The

1×2-mm pellets used in this study were milled from a single 120-mm APFSDS projectile. As a quality control measure, 2 pellets were randomly selected from every 50 pellets milled and their 1×2-mm dimensions were verified by Y-12 scientists. Cylindrical Ta steel (e.g., "surgical steel") pellets, 1×2 mm, were manufactured by and purchased from Alfa Aesar (Ward Hill, MA).

2.2. Surgical implantations

Male and female Sprague–Dawley rats (4 weeks of age) were obtained from Charles River Laboratories (Raleigh, NC) and housed in an AAALAC-accredited environmental control animal facility at Wright-Patterson AFB (Dayton, OH). At 8 weeks of age, the animals were surgically implanted with DU or Ta pellets using the methods previously described by Pellmar et al. (1999) except that isoflurane vapor was administered as a general anesthetic using an open circuit system with a scavenger/recapture system (IMPAC[6]; VetEquip, Inc., Pleasanton, CA).

Males were implanted with 0 (e.g., sham-surgery controls), 12, or 20 DU alloy pellets or 12 or 20 Ta steel pellets. Females used in the mating tests were implanted with 0, 12, or 20 Ta steel pellets or 4, 8, 12, or 20 DU pellets. Ta steel pellets served as inert controls to account for possible adverse effects on well-being and reproduction associated with implantation of solid, 1×2-mm objects in the rat gastrochnemius. Ta pellets have been used previously in DU implantation studies as inert foreign body controls (e.g., Benson, 1998; Pellmar et al., 1999; Hahn et al., 2002); 2, 4, 6, or 10 pellets were implanted in each gastrochnemius muscle, resulting in a total of 4, 8, 12, or 20 pellets implanted per rat.

The surface area (SA) of 4, 8, 12, and 20 DU pellets of 1×2 mm approximates to 31, 63, 94, and 157 mm^2, respectively, and is 0.1%, 0.3%, 0.4%, and 0.6%, respectively, of the estimated body surface area of an adult rat ($SA = 0.025\,m^2$). Twelve DU pellets of 1×2 mm are equivalent to one 30-mm APFSDS-T DU solid projectile ($\approx$425 mg DU, 28 cm (11 in) in length). Twenty pellets of 1×2-mm DU (760 mg of DU) in a 250-g rat is equal to approximately 0.22 kg (0.5 lb) of DU in a 70-kg (154-lb) person.

The physical well-being of each animal was assessed once daily for signs of debilitating toxicity and disease (OECD Guideline No. 19) by AALAS-accredited veterinary technicians until the end of the P1 adult reproductive segment of the study at postimplantation day 15 (OECD, 2000).

2.3. Animal manipulations

2.3.1. Measurement of uranium excretion of implanted males

Ten male animals from each of the 13 treatment groups listed in Table 1 were placed in Nalgene metabolism chambers for 24 h at 27 and 117 days postimplantation. Urine was collected and analyzed for uranium content. Animals were allowed access to food and water ad libitum before being placed into metabolism chambers and throughout the 24-h collection phase. Urine samples were prepared for analysis and analyzed for uranium content by ICP-MS using EPA Method 6020. The limit of detection (LOD) for uranium in urine was 10 μg/L.

2.3.2. Mating of P1 generation and monitoring of pregnancy and offspring

Surgically implanted adults (P1 generation) were mated at 30 days and 120 days postsurgical implantation as summarized in Table 1. Male/female pairs were placed in open metal grid mating cages for 7 days. The bottoms of the mating cages were checked twice daily by two trained technicians for evidence of mating (e.g., seminal plugs). Appearance of a seminal plug was considered evidence of a mating success and recorded for the male. The average time to insemination was calculated by subtracting the day of the appearance of a seminal plug from the date that the male and female

Table 1
P1 experimental groups

Group 1	Negative controls-sham implantation surgery		
Group 2	Males: 12 Ta steel pellets	X	Females: 12 Ta steel pellets
Group 3	Males: 12 DU pellets	X	Females: 12 Ta steel pellets
Group 4	Males: 12 DU pellets	X	Females: 4 DU, 8 Ta steel pellets
Group 5	Males: 12 DU pellets	X	Females: 8 DU, 4 Ta steel pellets
Group 6	Males: 12 DU pellets	X	Females: 12 DU pellets
Group 7	Males: 12 Ta steel pellets	X	Females: 4 DU, 8 Ta steel pellets
Group 8	Males: 12 Ta steel pellets	X	Females: 8 DU, 4 Ta steel pellets
Group 9	Males: 12 Ta steel pellets	X	Females: 12 DU pellets
Group 10	Males: 20 DU pellets	X	Females: 20 Ta steel pellets
Group 11	Males: 20 Ta steel pellets	X	Females: 20 DU pellets
Group 12	Males: 20 DU pellets	X	Females: 20 DU pellets
Group 13	Males: 20 Ta steel pellets	X	Females: 20 Ta steel pellets

were placed together in a mating cage. If no evidence of mating was found for the mated pair by day 8, the male was returned to its home cage and the mating recorded as a "failure." The female was then paired for 7 days with a male from the same treatment group that had a first-time mating success. The cage was checked twice daily by two trained technicians for seminal plugs and the average time to insemination calculated. If no evidence of mating was found for the mated pair, the male was returned to its home cage on mating day 8 and the mating was recorded as a mating "failure."

In the interest of achieving adequate statistical power for detecting decrements in mating success, it was decided to pool mating success data for male rats implanted with 12 or 20 DU or Ta steel pellets regardless of the implantation status of their female mates. Male reproductive success data were pooled for the following groups: Groups 3–6, Groups 2 and 7–9, Groups 10 and 12, and Groups 11 and 13. Previous evaluations did not find any evidence of an adverse effect of implanted DU or Ta steel pellets on P1 female weight gain, mortality, or reproductive success (Arfsten et al., 2005). Testing of P1 females implanted with up to 20 DU or Ta steel pellets found no evidence of an adverse effect on neurobehavior when evaluated between postimplantation days 145–150 (results to be published in a future article). Neurobehavioral evaluations conducted for P1 females were acoustic startle, acclimation/watermaze, conspecific, and open field tests (Rossi et al., 2000).

Surviving P1 male animals were euthanized by rapid decapitation on postimplantation day 150. Both distal cauda epididymis were rapidly removed. One cauda was used for assessing sperm motility, and the other cauda was used for determining caudal sperm concentration. A gross examination of the thoracic and abdominal organs was performed on each animal by making a ventral midline incision extending from the level of the mandible to the pelvis and the skin reflected laterally. The thoracic cavity was further exposed by removing the ribcage along the cartilaginous junction. The gross appearance of the brain was also evaluated. The implantation site (e.g., gastocnemius) was evaluated visually for evidence of tumors, infections, and other abnormalities.

2.3.3. *Measurement of sperm motility and concentration*

An additional 20 male Sprague–Dawley rats (6 weeks of age) were obtained from Charles River Laboratories and assigned to the α-chlorohydrin (ACH)-positive control group. At 8 weeks of age, each of the 20 positive control males was given a single oral dose of α-chlorohydrin (CAS: 96-24-2) at 100 mg/kg dissolved in deionized water. The animals were euthanized by rapid decapitation 3 h postdose. A single oral gavage dose of ACH (100 mg/kg) has previously been shown to significantly reduce rat sperm motility within hours of exposure (Vetter et al., 1998; Wier and Rumberger, 1995).

The sperm motility of P1 males from Groups 1–13 (Table 1) was measured following methods established by Slott et al. (1994). Each excised cauda epididymis was clamped longitudinally with hemostats and the distal end punctured three times with an 18-gauge needle. The cauda was then placed in a petri dish with 500 mL M199 solution (buffered Earles salts, sodium bicarbonate, L-glutamine, and Hepes buffer; Invitrogen Life Technologies, Carlsbad, CA) and incubated at 37 °C for 5 min to allow sperm swim-out. A 100-μL portion of the sperm sample was diluted with 200 μL of M199/bovine serum albumin solution in a prewarmed microcentrifuge tube. A 15-μL portion of the diluted sample was transferred to a Hamilton Thorne sperm analysis chamber (Beverly, MA). Percentage of motile sperm, percentage of progressively motile sperm, curvilinear velocity (VCL), straight line velocity (VSL), average path velocity (VAP), and amplitude of lateral sperm head displacement (ALH) were measured using a Hamilton Thorne IVOS-12 sperm analyzer. The "standard rat" was selected in the IVOS-12 subroutines for sperm motility and sperm motion analysis. Motility and motion parameters were measured from sperm present in a minimum of six standard viewing fields. A minimum of 200 sperm were analyzed per animal. Percentage progressive sperm is the ratio of the number of motile sperm with path velocity (VAP) $> V_0$ and straightness (STR) $> S_0$ to the total number of sperm (Kato et al., 2001). Percentage progressive sperm and the sperm motion parameters VCL, VSL, VAP, and ALH have all been shown to be sensitive measures for detecting adverse effects on sperm motion (Kato et al., 2001).

Sperm concentrations were measured with the Hamilton Thorne IVOS-12 sperm analyzer using the HTM-IDENT option as described by Strader et al. (1996). Epididymides were removed at necropsy and frozen at −20 °C until analyzed. Frozen epididymides were thawed at room temperature for 2 h. Each epididymis was weighed and homogenized in 15 mL of deionized H_2O. Then 100 μL of the diluted homogenate and 100 μL of deionized H_2O were placed into a Hamilton Thorne IDENT stain reaction vial containing a pellet of dehydrated *bis*-benzimide trihydrochloride. The mixture was vortexed and allowed to incubate at room temperature for 5 min. The tube was occasionally revortexed for 10–20 s during the incubation period to promote staining of the sperm DNA with *bis*-benzimide trihydrochloride. Immediately following the incubation period, 8 μL of the sperm solution was transferred to a 20-μm Cell-VU chamber (Fertility Technologies, Natick, MA). The standard setting in the HTM-IDENT program for rat cauda sperm (Set D) was used for sperm count analysis. The settings used were as follows: Frame

Rate, 60 Hz; Frames Acquired, 12; Minimum Contrast, 53; Minimum Cell Size, 2; Threshold Straightness, 80; Medium VAP Cut-off, 25; Low VAP Cut-off, 5; Low VSL Cut-off, 30; Static Size Limits, 0.35–2.00; Static Intensity Limits, 0.83–2.00; Static Elongation Limits, 15–84; and Field Selection, Automatic. The 10 × UV objective was used for analysis; the magnification setting obtained was 1.95. Ten fields were analyzed per sample. Results for each of the 10 fields were summed and an average sample concentration was calculated for each sample.

2.3.4. Serum chemistries

Serum chemistries were measured using a VetTest Snap Reader (IDEXX Laboratories, Inc., Westbrooke, ME). A 100-μL sample of serum from each animal was analyzed for total protein (g/dl), alkaline phosphatase (ALKP) activity, alanine aminotransferse (ALT) activity, urea/BUN concentration (mg/dl), creatinine (CREA) concentration (mg/dl), glucose concentration (mg/dl), phosphatase (PHOS) concentration (mg/dl), and total bilirubin (TBIL) concentration (mg/dl). Serum chemistries were not measured for P1 rats implanted with 20 Ta steel pellets.

2.4. Statistical analysis

Systat 10.2 (Systat Sofware Inc., Richmond, CA) was used to determine the normality of the distributions of continuous variables (e.g., body weight) using probability plots, skew and kurtosis, and one-sample Kolmogorov–Smirnov test. Many of the distributions for mean body weight, mean body weight gain, and mean tissue weights at 120 and 150 days were not normal. Because the distributions either could not be transformed to normal or required different power transformations for each variable, separate rank transformations were carried out on each combination of time and tissue weights or weight gains. The ranks were analyzed using a one-way ANOVA (Conover and Iman, 1981). When the data were normally distributed or could be appropriately transformed, the parametric data were analyzed using a one-way ANOVA and compared with the results from the rank transformation. Following each one-way ANOVA, Dunnett's test was used to compare all treatments against the control. Tukey's HSD test was used to compare all possible pairs. The distribution for average gestation length was multinomial (i.e., 21, 22, 23, or 24 mm) and was compared between treatment groups using an exact Kruskal–Wallace test (StatXact 5.0.3; Cytel, Cambridge, MA). Analysis results were considered statistically significant if the probability of a difference was $P \leqslant 0.05$.

Each sperm motility parameter (% motile, VAP, VSL, VCL, ALH, BCF) was transformed to a normal distribution using the Box–Cox power transformation. Treatment group differences were determined using analysis of variance (Systat 10.2). Analysis results were considered statistically significant if the probability of a difference was $P \leqslant 0.05$. The square roots (Shapiro–Wilks $P > 0.2$) and ranks (Conover and Iman, 1981) of sperm concentrations were analyzed using a one-way ANOVA (Systat 10.2). Dunnett's test was used to compare all treatments against the control. Tukey's HSD test was used to compare all possible pairs. Analysis results were considered statistically significant if the probability of a difference was $P \leqslant 0.05$.

3. Results

3.1. Urine uranium concentrations

When measured on postimplantation day 27, no uranium was detected (LOD: 10 μg/L) in the 24-h urine output from P1 male sham-surgery controls or the inert-control males (12 or 20 Ta pellets). Uranium was present in the 24-h urine output of P1 males implanted with 12 DU pellets at an average concentration of (mean ± 95% CI) 116 ± 27 μg/L. Uranium was also detected in the 24-h urine output of P1 males implanted with 20 DU pellets at an average concentration of (mean ± 95% CI) 448 ± 272 μg/L.

When measured on postimplantation day 117, no uranium was detected in the 24-h urine output from P1 male sham-surgery controls or the inert-control males (12 or 20 Ta pellets) (LOD: 10 μg/L). Uranium was present in the 24-h urine output of P1 males implanted with 12 DU pellets at an average concentration of (mean ± 95% CI) 200 ± 98 μg/L. Uranium was also present in the 24-h urine output of P1 males implanted with 20 DU pellets at an average concentration of (mean ± 95% CI) 369 ± 84 μg/L.

No uranium was detected in the rodent chow (LOD: 450–550 μg/kg) or drinking water (LOD: 10.0 μg/L) fed to rats throughout the study.

3.2. Toxicological endpoints in P1 rats

Three P1 males died of unknown causes during the 150-day postimplantation period. Of the expired males, one was implanted with 12 DU pellets, one was implanted with 20 DU pellets, and one was implanted with 20 Ta steel pellets. Four P1 females died over the 150-day postimplantation period. One sham surgery female died from accidental drowning and another sham surgery female died of unknown causes. One female implanted with 20 DU pellets developed a palpable mammary tumor prior to mating on test day 120 and was euthanized. Gross necropsy confirmed the presence of a mammary gland tumor with the characteristics of a mammary gland carcinoma. One female implanted with

20 DU pellets died during the birthing process on gestation day 22 after mating at 120 days postimplantation (e.g., study day 142). Five underdeveloped fetuses were present in the uterine horn at necropsy. No animals were identified at any time during the 150-day post-surgery period as having outward clinical signs suggestive of toxicity or illness listed in OECD Guideline No. 19 (OECD, 2000).

There were no statistically significant differences in mean body weights and body weight gains of male rats implanted with DU pellets as compared with the mean body weights and body weight gains of male rats implanted with the same number of Ta steel pellets (Table 2). Postimplantation day 150 mean body weights and body weight gains were not available for male rats implanted with 20 DU or 20 Ta steel pellets. None of the means for the eight serum chemistries measured in P1 male rats fell outside the normal range for rats (Table 2).

Mean heart weight was significantly higher for animals implanted with 20 Ta steel pellets as compared to the mean heart weight for sham-surgery control animals (Table 3). Inspection of the hearts from animals implanted with 20 Ta pellets did not identify any cardiac abnormalities.

3.3. Sperm motion and concentration

There were no significant differences ($P \leqslant 0.05$) between the mean percentage of motile sperm or the mean percentage of progressively motile sperm for sham-surgery control animals and those for animals implanted with Ta steel or DU pellets (Table 4). The mean percentage of motile sperm was significantly lower for animals treated by oral gavage with the positive control compound ACH than for all other treatment groups. The mean percentage of progressive sperm did not differ for animals treated with ACH as compared with all other treatment groups.

Mean sperm cell tract speed velocity (VCL) was significantly higher ($P \leqslant 0.001$) for sperm isolated from animals implanted with 20 Ta or 20 DU pellets than for sperm isolated from sham-surgery control animals (Table 4). Mean sperm cell average path velocity (VAP), straight line velocity (VSL), VCL, and lateral sperm head displacement (ALH) were all significantly lower for sperm isolated from animals treated with ACH than for sperm isolated from all other treatment groups.

Average caudal sperm concentration for each of the treatment groups is summarized in Fig. 1. There was no statistically significant ($P \leqslant 0.05$) difference in average caudal sperm concentration between treatment groups.

3.4. Mating success and average insemination times

The collective mating success rate for both mating periods for the 13 treatment groups listed in Table 1 was 89%, with 446 pairings producing 396 litters. Comparing mating periods, the success rate at 30–45 days postimplantation for the 13 treatment groups was lower than the mating success rate for the 120–145 days postimplantation mating period (86% versus 91%). However, this difference was not statistically significant.

Collective statistical analysis of mating success and insemination time by ANOVA for the 13 treatment groups listed in Table 1 found no statistical differences for these parameters for both 30 and 120 days postimplantation mating. For the 30–45 day postimplantation group, the ANOVA P values were mating success $P = 0.43$ and insemination time $P = 0.46$. For the 120–145 day postimplantation group, the ANOVA P values were mating success $P = 0.91$ and insemination time $P = 0.59$. Also, there was no evidence of a dose-response for mating success or insemination time for either the 30–45 or the 120–145 days postimplantation mating (data not shown). As a result of these analyses, it was concluded that the treatment groups could be collapsed into sham-surgery controls and 12- and

Table 2
Toxicological endpoints measured in adult male rats at 120 and 150 days postimplantation (dpi) surgery (95% confidence interval)

Endpoint	Sham-surgery (n)	12 Ta steel pellets (n)	12 DU pellets (n)	20 Ta steel pellets (n)	20 DU pellets (n)
Mean body weight, 120 d pi (g)	613±55 (15)	615±67 (42)	578±33 (40)	574±40 (17)	567±44 (17)
Mean body weight, 150 d pi (g)	619±81 (11)	620±37 (43)	603±24 (43)	—	—
Mean weight gain, 0–120 d pi (g)	339±80 (15)	271±35 (42)	265±29 (40)	301±31 (17)	300±37 (17)
Mean weight gain, 0–150 d pi (g)	287±95 (11)	297±28 (43)	289±21 (43)	—	—
Total protein (5.3–6.9 g/dl)	7±0.2 (10)	6±0.2 (7)	7±0.2 (10)	—	6±0.5 (5)
ALKP (16–302 U/L)	10±0 (10)	11±2 (7)	13±5 (10)	—	10±0 (5)
ALT (20–61 U/L)	50±10 (10)	41±4 (7)	44±5 (10)	—	50±14 (5)
AST (39–111 U/L)	97±26 (10)	73±22 (7)	58±8 (10)	—	96±16 (5)
PHOS (5.8–11.2 mg/dl)	6±0.1 (10)	7±1 (7)	8±1 (10)	—	5±1 (5)
CREA (0.05–0.65 mg/dl)	0.3±0.1 (10)	0.4±0.1 (7)	0.4±0.1 (10)	—	0.3±0.1 (5)
TBIL (0.1–0.7 mg/dl)	0.3±0.1 (10)	0.2±0.2 (7)	0.3±0.2 (10)	—	0.3±0.2 (5)
BUN (9–21 mg/dl)	17±2 (10)	18±2 (7)	18±2 (10)	—	17±5 (5)

Table 3
Mean weights (±95% confidence interval) in grams of major organs and organ systems of male adult rats. $n = (x)$

Organ or organ system	Sham surgery (n)	12 Ta steel pellets (n)	12 DU pellets (n)	20 Ta steel pellets (n)	20 DU pellets (n)
Sex organs[a]	8.9±2 (23)	8.8±1.4 (35)	9.1±1.0 (63)	9.7±2.0 (13)	9.5±2.0 (18)
Liver	21.5±1.9 (23)	21.7±1.2 (35)	21.5±1.0 (63)	20.2±1.9 (13)	20.4±1.8 (18)
Kidneys	4.9±0.4 (23)	4.5±0.4 (35)	4.6±0.3 (63)	5.0±0.7 (13)	5.1±0.4 (18)
Heart	1.9±0.2 (19)	2.2±0.2 (16)	2.2±0.3 (41)	2.3±0.3* (13)	2.2±0.2 (18)
Spleen	0.9±0.2 (19)	0.9±0.2 (16)	1.0±0.2 (41)	1.0±0.1 (13)	1.0±0.1 (18)
Brain	1.9±0.2 (23)	2.1±0.1 (35)	2.0±0.1 (63)	2.0±0.2 (13)	2.2±0.2 (18)

*$P \leqslant 0.05$ compared to sham-surgery control animals.
[a]Testis (2), corpus epididymous (2), caput epididymous (2), vas deferens (2), coagulating glands (2), vesicular glands (2), ampullary gland.

Table 4
Sperm motion parameters (95% confidence interval)

Treatment group	n	% Motile	% Progressive	VAP[a] (μm/s)	VSL[b] (μm/s)	VCL[c] (μm/s)	ALH[d] (μm)
Sham surgery	14	69±7	22±4	184±12	128±9	351±15	15±1
α-Chlorohydrin	20	60±4[e]	20±1	137±5[f]	95±4[f]	211±7[f]	10±1[f]
12 Ta steel pellets	21	74±4	21±2	190±8	130±6	352±13	15±1
12 DU pellets	35	74±4	21±2	186±8	126±6	355±12	16±1
20 Ta steel pellets	16	73±7	23±4	196±15	135±11	380±22[f]	16±1
20 DU pellets	16	73±5	24±2	203±15	141±11	384±25[f]	16±1

[a]VAP, average path velocity.
[b]VSL, progressive straight line velocity.
[c]VCL, tract speed velocity.
[d]ALH, lateral sperm head displacement.
[e]$P \leqslant 0.05$ in Dunnett two-sided test versus sham surgery.
[f]$P \leqslant 0.001$ in Dunnett two-sided test versus sham surgery.

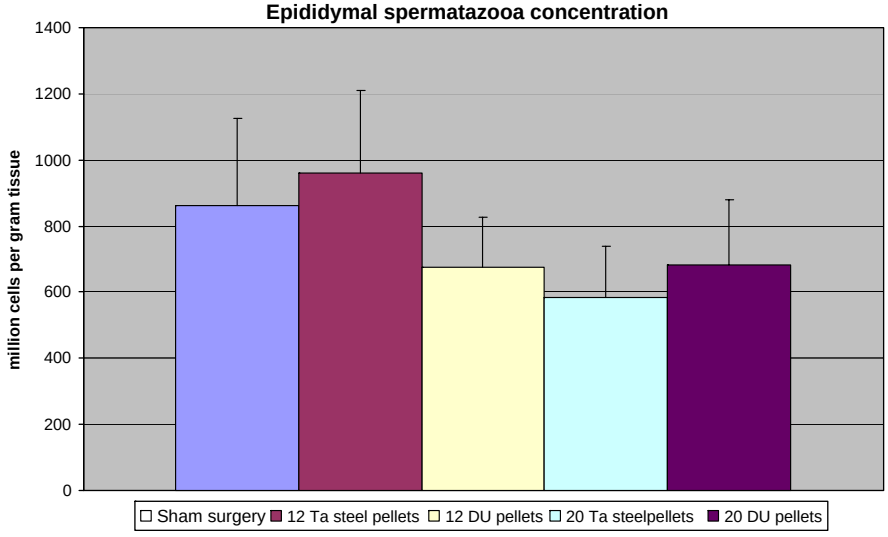

Fig. 1. Mean sperm concentrations (±95% CI) (95% confidence interval).

20-pellet treatment groups to increase statistical power for comparing reproductive success and insemination times.

The mating success rates and average insemination times of the sham-surgery controls and the 12- and 20-pellet treatment groups at 30 and 120 days postimplantation are summarized in Tables 5 and 6, respectively. There were no statistically significant differences in mating success rates or average insemination times between the five treatment groups at 30–45 days postimplantation mating (Table 5). Percentage mating

Table 5
Male reproductive success at 30–45 days postimplantation

Treatment group	Mating success	Failure	Percentage mating success	Average insemination time (days)[a]	95% CI
Sham surgery	17	4	81	1.9	0.5
12 Ta steel pellets	65	7	90	2.2	0.5
12 DU pellets	67	5	93	1.9	0.2
20 Ta steel pellets	20	2	90	1.9	0.5
20 DU pellets	22	3	88	2.0	0.5

[a]Number of days between introduction to female and appearance of vaginal plug in cage.

Table 6
Male reproductive success at 120–145 days postimplantation

Treatment group	Mating success	Failure	Percentage mating success	Average insemination time (days)[a]	95% CI
Sham surgery	19	2	91	2.2	0.6
12 Ta steel pellets	63	7	90	3.0	0.8
12 DU pellets	64	7	90	2.2	0.3
20 Ta steel pellets	21	1	96	3.6	1.5
20 DU pellets	21	3	87	2.5	0.8

[a]Number of days between introduction to female and appearance of vaginal plug in cage.

success was lower for sham-surgery controls than for the other four treatment groups but this difference was not statistically significant ($P <= 0.05$) among themselves in mating success rates.

4. Discussion

This study addressed the hypothesis that implanted DU may cause genetic damage in developing sperm in the testes and/or mature sperm in the cauda epididymis resulting in decreased fertility or decreased litter sizes. This hypothesis was based on findings that implanted solid DU mobilizes in the rat and is taken up by the testes (Pellmar et al., 1999) and that DU may be mutagenic (Miller et al., 1998a), clastogenic (Miller et al., 1998b), and possibly carcinogenic (Hahn et al., 2002). Additionally, administration of high doses of natural uranium compounds to adult male rodents by injection or by the oral route has been shown to produce adverse effects on the male reproductive system (e.g., testicular atrophy, decrease in spermatozoa) (Maynard et al., 1953; Malenchenko et al., 1978; Llobet et al., 1991). This study found no evidence of male reproductive toxicity associated with the implantation of DU hard metal implants manufactured from a standard US 120-mm APFSDS armor penetrator. Although the mating success rate at 30–45 days postimplantation was lower at 86% for all treatment groups than the mating success rate of 91% at 120–145 days postimplantation, the average mating success rate for all treatment groups for the study was 89%. The mating success of rats of breeding age is 90–94% per pairing (Barrow, 1990; Baker, 1979). Additionally, preliminary analysis has not found evidence of a negative impact of parental DU implantation on F1 survival and development through 90 days after birth (Arfsten et al., 2005).

The finding of no difference in the mating success rates in different treatment groups suggests that DU implantation did not have an adverse impact on mating behavior in this study. Although not discussed in detail here, extensive neurobehavior testing has been completed on a portion of the P1 adults in this study. Aspects of neurobehavior that were evaluated and methods used for neurobehavioral testing are the same as those used to evaluate adult F1 offspring (see Arfsten et al., 2005). Preliminary analyses suggest that DU implantation has no adverse effect on the aspects of neurobehavior evaluated in these tests (e.g., motor coordination, memory, habituation, socialization). Findings from a previous study gave indications that neurobehavior could be altered following DU implantation. Pellmar et al. (1999b) found that hippocampus electrophysiology was altered among adult SD rats implanted with 20 DU pellets of 1×2 mm for 12 months. No significant difference in neurobehavioral test scores occurred among rats implanted with 10 or 20 DU versus Ta steel pellets for 30 days or 6 months; gross examination of the brain tissue of these rats indicated no obvious lesions (Pellmar, 1996).

In this study, uranium was present in urine of male P1 rats implanted with DU in a dose-dependent manner

when assessed 3 days prior to mating on study days 30 and 120. No uranium was present in the urine of P1 sham-surgery control or males implanted with Ta steel, and no uranium was found to be present in study food or water sources (Arfsten et al., 2005) at the LOD for uranium of 450–550 μg/kg for food and 10.0 μg/L for water. This suggests that the implanted DU was the most likely source of uranium in urine. Additionally, the dose-dependent increase of uranium in urine further supports the hypothesis that DU can solubilize in the body and enters general circulation. Results reported previously by Pellmar et al. (1999) support this conclusion. At 6 months postimplantation, uranium was present in 24-h urine output at concentrations ranging from 0.01 to 0.65 μg U/mL for rats implanted with 4–20 DU pellets of 1 × 2 mm in the gastocnemius. At 12 months postimplantation, uranium was present in the 24-h urine output of rats implanted with DU at concentrations ranging from 0.224 to 1.01 μg U/mL (Pellmar et al., 1999). Uranium was not present in the 24-h urine output from rats implanted with 20 Ta steel pellets. The methods used for uranium analysis by Pellmar et al. (1999) were approximately 2000 times more sensitive than the methods used in this study for detecting uranium in tissues. In the latter study, uranium was detected by kinetic phosphorimetric analysis with a limit of detection of 0.005 μg/L and recovery of uranium from spiked tissue matrices was greater than 90%. The limit of detection for uranium in urine in this study was 10 μg/L with an acceptance limit of 70–130% for recovery of uranium from a spiked sample.

A threshold for toxicity was not achieved in this study as indicated by the findings for the various indicators of toxicity evaluated in this study. Therefore, it is not known whether implantation of a greater mass of DU or possibly longer implantation times would result in effects consistent with toxicity in the Sprague–Dawley rat. The results of previous studies suggest that the threshold of toxicity for implanted DU in rats may be greater than 32 1 × 2-mm pellets (≈1216 mg DU). No increases in mortality were reported in studies of Sprague–Dawley rats implanted with up to 32 DU pellets of 1 × 2 mm for 84 days (Benson, 1998) or up to 20 DU pellets of 1 × 2 mm for 18 months (Pellmar et al., 1999). In a cancer bioassay conducted by Hahn et al. (2002), median survival times of Wistar rats implanted in the thigh with 4 DU pellets of 1 × 2 mm, four squares of DU (2.5 × 2.5 × 1.5 mm, 175 mg), or four squares of DU (5.0 × 5.0 × 1.5 mm, 698 mg) were not significantly different from those of sham-surgery control rats, suggesting that DU was not toxic per se. However, there were significant increases in localized proliferative reactions and soft tissue sarcomas around the 5.0 × 5.0-mm squares of DU and in rats injected with the positive control material Thorotrast (radioactive thorium dioxide). A slightly increased incidence occurred in rats implanted with the 2.5 × 2.5-mm DU squares and with the 5.0 × 5.0-mm squares of Ta. No tumors were seen in rats with 2.0 × 1.0-mm DU pellets or in the surgical controls. In this study, no evidence of frank tumors was found in P1 males or females following 150 days of DU implantation. The implantation sites of P1 animals are currently being characterized histopathologically and the results of the analysis will be presented in follow-on reports.

It has been shown that uranium accumulates in the kidney (ATSDR, 1999; ICRP, 1995, 1996) and causes reversible nephrotoxicity in humans (ATSDR, 1999; Pavlakis et al., 1996) and laboratory animals (ATSDR, 1999; Bentley et al., 1985; Morrow et al., 1982; Maynard and Hodge, 1949; Roberts, 1949). However, all studies completed thus far have not found evidence of kidney toxicity in rodents implanted with large amounts of DU. The present study did not find evidence of kidney toxicity in male rats implanted with up to 20 DU pellets of 1 × 2 mm for 150 days. Mean kidney weights were similar across treatment groups and serum biomarkers for abnormal kidney function (BUN, TP, PHOS, CREA) were all within reference ranges for the rat. Also, no evidence of general toxicity or kidney function abnormalities was found for adult female rats implanted with up to 20 DU pellets of 1 × 2 mm for 150 days (data not shown) toxicity. Benson (1998) reports that implantation of female SD rats with up to 32 DU pellets of 1 × 2 mm for up to 84 days did not result in evidence of kidney toxicity. Similarly, no evidence of kidney toxicity was found for rats implanted with up to 20 DU pellets of 1 × 2 mm for up to 6 months (Pellmar, 1996). Hahn et al. (2002) found that implantation of Wistar rats with DU was possibly associated with an increase in the incidence of renal tumors, but the increase was not statistically significant and was not associated with a dose-response for kidney uranium concentration.

The results of this study differ from the findings of the health and well-being of the 120-member cohort exposed to DU during the Persian Gulf War (1991). This group of US Persian Gulf War veterans was involved in friendly fire incidents involving depleted uranium weapons and several were struck with DU shrapnel that became embedded in muscle and soft tissue (McDiarmid et al., 2001). In most cases, the shrapnel was not surgically removed (McDiarmid et al., 2001) and a number of the cohort members were found to be excreting greater-than-background levels of uranium in urine when assessed 9–11 years after the end of the Persian Gulf War (McDiarmid et al., 2004). A 1997 follow-up of 30 friendly fire cohort members found evidence of subtle perturbations in the reproductive and nervous systems of DU-exposed veterans as compared with nonexposed Persian Gulf War veterans (McDiarmid et al., 2000). DU-exposed veterans had elevated

prolactin levels suggesting that their DU exposure had a possible effect on reproductive function (McDiarmid et al., 2000). Neurocognitive tests showed a statistical relationship between urine uranium levels and lowered performance on computerized tests designed to assess performance efficiency (McDiarmid et al., 2000). Also, a 1999 follow-up of 50 members of the friendly fire cohort found significantly elevated sperm counts, higher percentages of progressive spermatozoa, and a higher percentage of rapid progressive spermatozoa among veterans excreting high levels of uranium in urine ($>0.10\,\mu g/g$ creatinine) as compared with veterans excreting low levels of uranium in their urine (McDiarmid et al., 2001).

The current study found no evidence of adverse effects on reproductive function or neurobehavior in male rats. Possible reasons for these differences are (1) differences in DU dose levels in the rats embedded with DU in this study and the members of the friendly fire cohort, (2) differences in the length of residence time of DU alloy in the rats in this study versus the friendly fire cohort members, (3) differences in toxico/pharmacokinetics of embedded DU in rats versus humans, and (4) differences between rats versus humans in sensitivity and response to DU/uranium exposure. In this study, rats were implanted with 12 or 20 DU pellets of 1×2 mm. Twelve DU pellets of 1×2 mm is equivalent to one 30-mm APFSDS-T DU solid projectile ($\approx$425 mg DU, 28 cm (11 in) in length). Twenty DU pellets of 1×2 mm (760 mg DU) in a 250-g rat is equal to approximately 0.22 kg (0.5 lb) of DU in a 70-kg (154-lb) person. It is doubtful that DU fragments equal to the mass of more than one 30-mm APFSDS-T DU solid projectile remain embedded in any of the members of the friendly fire cohort, but at this time there are no published studies to support this opinion. The length of time that male rats were implanted with DU pellets in this study does not differ significantly from the current length of follow-up for the 1991 Persian Gulf friendly fire cohort. Male rats were implanted with DU pellets for up to 150 days which approximates 20% of a rats average lifespan of 2 years. Comparatively, some members of the 1991 Persian Gulf friendly fire cohort have been followed medically since shortly after returning from the Persian Gulf War to 2001 ($=10$ years) which approximates 13% of a human lifespan of 75 years. The perceived differences between the results of this study and the findings for the friendly fire cohort could be explained by species differences in DU dissolution, translocation, and tissue distribution pharmacokinetics or differences between rats and humans in sensitivity and response to DU/uranium exposure. However, at this time there are no data to suggest that there are differences between rats and humans with regard to embedded DU pharmacokinetics or sensitivity and response to DU/uranium exposure.

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