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# Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets



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**During the 1991 Persian Gulf War several US military personnel were wounded by shrapnel fragments consisting of depleted uranium. These fragments were treated as conventional shrapnel and were not surgically removed to spare excessive tissue damage. Uranium bioassays conducted over a year after the initial uranium injury indicated a significant increase in urine uranium levels above natural background levels. The potential mutagenic effects of depleted uranium are unknown. To assess the potential mutagenic effects of long-term exposure to internalized depleted uranium, Sprague–Dawley rats were implanted with depleted uranium and their urine and serum were evaluated for mutagenic potential at various times after pellet implantation using the Ames *Salmonella* reversion assay. Tantalum, an inert metal widely used in prosthetic devices was used for comparison. Enhancement of mutagenic activity in *Salmonella typhiurium* strain TA98 and the Ames II$^{TM}$ mixed strains (TA7001–7006) was observed in urine samples from animals implanted with depleted uranium pellets. In contrast, urine samples from animals implanted with tantalum did not show a significant enhancement of mutagenic activity in these strains. In depleted uranium-implanted animals, urine mutagenicity increased in a dose- and time-dependent manner demonstrating a strong positive correlation with urine uranium levels ($r = 0.995$, $P < 0.001$). There was no mutagenic enhancement of any bacterial strain detected in the sera of animals implanted with either depleted uranium or tantalum pellets. The results suggest that uranium content in the urine is correlated with urine mutagenicity and that urinary mutagenicity might be used as a biomarker to detect exposure to internalized uranium.**

## Introduction

Several US military personnel participating in Operation Desert Storm were wounded in friendly fire accidents and have retained large fragments of the radioactive metal depleted uranium (DU) in their bodies. DU, used as an effective material in kinetic energy penetrators, is ~1.7-fold as dense as lead (19 versus 11.35 g/cm) and is chemically similar to natural uranium (National Research Council Committee on the Biological Effects of Ionizing Radiation, 1988). While the chemical and physical properties of DU and natural uranium are essentially identical, their radiological properties are different. Natural uranium consists of three isotopes, $^{238}U$ (99.276%), $^{235}U$ (0.718%) and $^{234}U$ (0.0056%), and during the enrichment process two products are produced, enriched uranium and DU, which contain different relative ratios of the three isotopes. The resultant DU contains $^{238}U$ (99.8%), $^{235}U$ (0.20%) and $^{234}U$ (0.001%). DU is therefore ~60% as radioactive as naturally occurring uranium, since it has essentially been depleted of $^{235}U$ and $^{234}U$. As a result, the specific activity of DU is significantly less than natural uranium (0.4 versus 0.7 μCi/g, respectively).

DU internalization, in contrast to occupational uranium exposure, presents a unique toxicological problem, because it combines chemical toxicity with radiological exposure. While reports have linked occupational uranium exposure to lung carcinogenesis, it is believed that this is due to inhalation of the uranium decay product radon gas and the chemical toxicity of uranium is not involved (National Research Council Committee on the Biological Effects of Ionizing Radiation, 1988; Vahakangas *et al.*, 1992). Therefore, limited data exist to permit an accurate assessment of health risks from embedded DU fragments. Furthermore, the chemical toxicity of acute, short-term exposures to uranium has clearly been demonstrated in animals and humans (Wrenn *et al.*, 1985, 1990), while the long-term health risks associated with chronic internal exposure are not as well defined (National Research Council Committee on the Biological Effects of Ionizing Radiation, 1988). In view of medical management questions relevant to current and possible future incidents of DU internalization, examination of potential mutagenic effects of DU internalization are critical and will assist in an understanding of the etiology of potential disease development.

The goal of the current study was to conduct mutagenicity testing of the urine and serum samples obtained from rats implanted with either DU or tantalum pellets using the Ames reversion assay. We wanted to determine if there was a time- or metal dose-dependent relationship to urine mutagenicity. Additionally, we wanted to evaluate the relationship between urine uranium levels and mutagenic potential. The data strongly indicate that urine mutagenicity is correlated with increased urine uranium content. The implications of these results to the development of a monitoring program designed to assess the health effects of chronic exposure to internalized DU in humans is discussed.

## Materials and methods

### *Experimental animals*

Studies were conducted using male Sprague–Dawley inbred rats, weighing 200–250 g, ~10 weeks old at the time of pellet implantation. The animals were housed in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication no. 86-23). Upon arrival animals were quarantined and screened for diseases. Except during urine collection, all animals were housed in plastic microisolator rat cages with hardwood chips as bedding and were on a 12 h light/dark cycle. Commercial rodent chow and water were provided *ad libitum*.

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*Metal fragments*

DU fragments consisting of 99.25% DU and 0.75% titanium by weight were obtained from Oak Ridge National Laboratories (Oak Ridge, TN). The uranium isotopes present are $^{238}U$ (99.75%), $^{235}U$ (0.20%) and trace levels of $^{234}U$. This is the same DU alloy used in US military munitions. Tantalum (Ta) fragments were obtained from Alfa Products (Ward Hill, MA). Ta was chosen as the control metal because it is biologically inert (Johansson *et al.*, 1990) with a similar mass to uranium and is frequently used in human prostheses (Hockley *et al.*, 1990; Strecker *et al.*, 1993). Each fragment is ~1 mm diameter×2 mm long.

*Experimental groups*

Rats were divided into five experimental groups: (i) non-surgical controls; (ii) Ta controls; (iii) DU low dose; (iv) DU medium dose; (v) DU high dose. Four time periods for assessment were chosen: (i) 0 days; (ii) 6 months; (iii) 12 months; (iv) 18 months. All surgically implanted animals had a total of 20 pellets of either Ta or DU or a mixture. Low dose DU rats were implanted with 4 DU pellets and 16 Ta pellets. Medium dose DU rats were implanted with 10 DU and 10 Ta pellets. The high dose DU rats were implanted with 20 DU pellets. Initially 10–12 animals were implanted per group/time period, however, for most analyses eight animals were used. The 0 time point included eight animals and these animals had no implants of any type. For the purposes of comparison, the treatment groups were compared with the animals with no implants at the same time period unless otherwise noted.

*Pellet implantation surgery*

The DU and Ta pellets were cleaned and chemically sterilized prior to implantation. Pellets were immersed in industrial detergent, rinsed in absolute alcohol, soaked in 50% nitric acid solution for 3 min and then rinsed with sterile water. Animals were anesthetized with ketamine hydrochloride (80 mg/kg) in combination with xylazine hydrochloride (4 mg/kg), given i.p. Fragments were implanted within the gastrocnemius muscle and were spaced ~8–10 mm apart on the lateral side of each leg. Prior to surgery, the surgical sites were shaved and cleansed with betadine. Scalpel incisions were made through the skin and pellets were inserted into the muscle with a 16 gauge needle with plunger. Incisions were closed with absorbable sutures and surgical cement. Rats were closely monitored following surgery until they were ambulatory. The surgical sites were regularly examined by a veterinarian for signs of inflammation, infection and local DU toxicity.

*Urine and serum sample preparation*

Urine and serum samples were collected from different populations of rats at 6, 12 and 18 months after pellet implantation. Rats were placed individually in metabolic cages for 24 h to collect the urine. The 24 h urine collection sample was obtained from each rat and the volume recorded (10–20 ml). Four milliliters of the urine collected from each individual animal were set aside for urine mutagenicity testing; the reminder was used for uranium and chemical analysis. Urine uranium content was analyzed by Batelle (Pacific Northwest Laboratory). Urine was filtered to remove any debris and frozen until analysis

Frozen urine for mutagenicity testing was thawed and the urine from 8 animals/treatment group was combined to make a total volume of 32 ml urine/treatment group. Filtered urine was passed over chromatographic columns containing Amberlite XAD-4 resin. The effluents were collected and passed through a column packed with Amberlite XAD-8 resin. Resins were purified by Soxhlet extraction before use. Use of these columns allowed the collection of a hyrdrophobic urine fraction (XAD-4) and a hydrophilic fraction (XAD-8). Furthermore, preliminary tests indicated that use of either C18 resin or cyanopropyl columns resulted in an 18 or 15% reduction in mass recovery, respectively (data not shown). The column was successively washed with 10 ml distilled water and eluted with acetone. The dry extracts were dissolved in DMSO (320 µl). This process resulted in a 100× concentrate.

For the serum mutagenicity studies, 8 rats/treatment group were used. At the designated time intervals (6, 12 and 18 months), the animals were killed and blood was obtained from the aorta. Serum was separated by centrifugation (5 min at 3000 *g*) and was vacuum dialyzed through a dialysis tube with a cut-off value of 30,000 (Miller *et al.*, 1993; Szucs *et al.*, 1994).

*Ames Salmonella reversion assay*

To assess urine and serum mutagenicity, the *Salmonella* bacterial reversion assay, developed by Maron and Ames (1983), was used. Specifically, the Ames II™ mutagenicity assay from Xenometrix allowed rapid assessment of the mutagenic potential of the urine and serum samples. The two strains used were the Ames II™ mixed strains and the frameshift tester strain TA98. AmesII™ mixed strains contains equal numbers of the cells of each of six strains (TA7001–7006). Individually, these strains revert by only one specific base pair substitution out of six possible changes. Therefore, when mixed, all six base substitution mutations can be represented in one culture (Gee *et al.*, 1994). A complete description of genotypes can be found elsewhere (Gee *et al.*, 1994).

Aliquots of 10 µl urine extracts or 1000 µl dialyzed sera were incorporated into three plates inoculated with either *Salmonella* strain TA98 or the Ames II™ TA7001–7006 strains without metabolic activation. The dose of the urine tested was 1 ml equivalent/plate. This experiment was repeated three times on different days. Sensitivity of the strains was assessed with positive and negative controls as previously done (Gee *et al.*, 1994) and several revertant colonies were randomly selected for verification according to Maron and Ames (1983). The results are expressed as revertants/µmol creatinine after adjustment for urinary creatinine concentration. Statistical analysis included the Student's unpaired *t*-test to determine the level of significance. Correlation between biomarker values were calculated using Pearson's correlation test.

*Uranium measurements*

Frozen urine and serum samples were shipped overnight on dry ice to Batelle Pacific Northwest Laboratories for analysis of uranium content. Samples were prepared by wet ashing and were analyzed with a Kinetic Phosphorescence analyzer (KPA-11; Chemchek Instruments Inc., Richland, WA) (Brina and Miller, 1992). Calibration curves were established prior to sample analysis; measurements included analysis of relative standard deviations and correlation coefficients of the luminescence decay curve.

## Results

A significant elevation in mutagenic potential was observed with the XAD-4 and XAD-8 fractions of urine that was dependent on both the length of time and number of DU pellets implanted. Figure 1A shows that the XAD-4 fraction of urine collected from rats implanted with DU pellets induced a significant increase in the revertant number of the TA98 strain at each assessment point (6, 12 and 18 months). The mutagenic potential showed a steady increase through the first 12 months after DU pellet implantation for animals with 4, 10 or 20 DU pellets. At 18 months, however, the XAD-4 urine fraction from animals with the highest dose of DU implanted (20 DU pellets) resulted in a revertant number that was not statistically different from the number seen at 12 months in animals with 20 DU pellets (12 months, 287 ± 42 revertants/µmol creatinine versus 18 months, 390 ± 91 revertants/µmol creatinine). In contrast to the DU-implanted rats, the animals that had no or tantalum implants did not demonstrate any increase in the mutation yield of strain TA98. Results with the XAD-8 urine concentrate indicated that animals implanted with either 10 or 20 DU pellets exhibited a statistically significant increase ($P < 0.01$) in mutagenic potential at 12 and 18 months after pellet implantation in comparison with animals with no or tantalum implants at those same time periods. No significant increase in mutagenicity of the XAD-8 fraction was observed in animals with the lowest number of DU pellets (4) or in those with tantalum implants using strain TA98 at any of the time periods. Statitical analysis revealed that there was no significant difference between the number of revertants at 12 and 18 months in animals with either 10 or 20 DU pellets.

The mutagenic potential of both the XAD-4 and XAD-8 fractions was also examined using the Ames II™ mixed strains (TA7001–TA7006). Both urine fractions showed a significant increase in mutagenic activity that was dependent on time and DU pellet number (Table I). The mutagenic activity of the XAD-4 fraction from DU-implanted animals exhibited variable elevation at 6 months after pellet implantation. However, there was a significant increase in the mutagenicity of the XAD-4 fraction at 12 and 18 months that was both time- and DU dose-dependent in comparison with animals with no or tantalum implants. Statistical analysis demonstrated that at 18 months, however, the XAD-4 urine fraction from animals with any dose of DU implanted (4, 10 or 20 DU pellets) resulted in a revertant number that was not statstically different from the

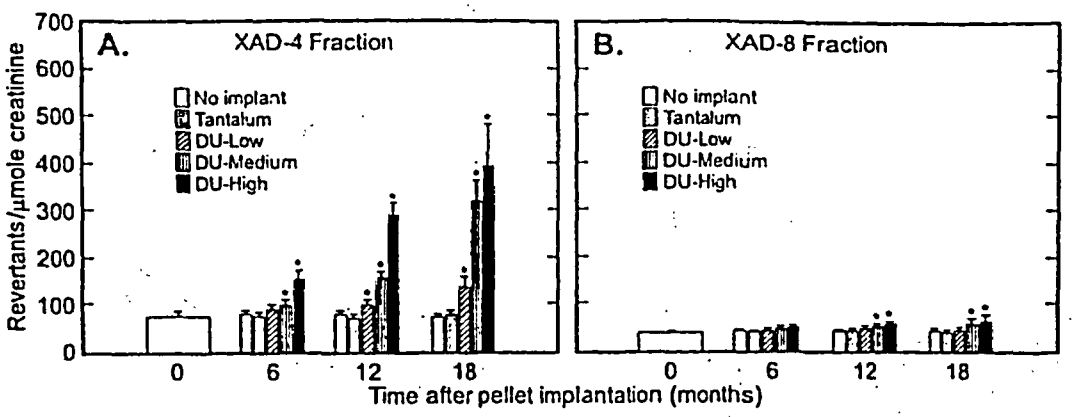


Fig. 1. Number of revertant clones in strain TA98/µmol creatinine after treatment with the urinary fractions of animal implanted with either no metal (□), tantalum (▩) or 4 (▨), 10 (▥) or 20 (■) pellets of DU at increasing times after pellet implantation: (A) XAD-4 fraction; (B) XAD-8 fraction. Using a urine extract dose of 1 ml equivalent/plate, the number of revertants/plate was determined. Mutagenicity data were calculated after adjustment for urinary creatinine concentrations. At each time point, the bars that are statistically different from the no implant controls at that time point are marked with an asterisk ($P < 0.05$ using Student's $t$-test).

Table I. Mutagenicity data of urinary fractions from DU- or tantalum-implanted animals using the Ames II™ mixed strains: comparison with animals with no implants[a]

| Time (months) | Dose[b] | Mutagenicity (revertants/µmol creatinine) | |
|---|---|---|---|
| | | XAD-4 fraction[c] | XAD-8 fraction[c] |
| 0 | None | 10.3 ± 1.0 | 16.3 ± 1.4 |
| 6 | None | 11.3 ± 1.0 | 16.5 ± 1.8 |
| | Tantalum | 11.0 ± 1.2 | 14.0 ± 1.2 |
| | DU low | 12.0 ± 1.0 | 27.0 ± 2.8[d] |
| | DU medium | 21.5 ± 1.9[d] | 50.1 ± 5.1[d] |
| | DU high | 14.0 ± 1.7 | 89.9 ± 8.8[d] |
| 12 | None | 11.3 ± 1.2 | 14.9 ± 1.7 |
| | Tantalum | 12.6 ± 2.1 | 16.1 ± 1.6 |
| | DU low | 16.5 ± 1.5[d] | 52.8 ± 5.8[d] |
| | DU medium | 24.9 ± 2.3[d] | 79.9 ± 7.9[d] |
| | DU high | 36.7 ± 3.5[d] | 184.4 ± 18.8[d] |
| 18 | None | 12.0 ± 1.3 | 16.1 ± 1.6 |
| | Tantalum | 11.4 ± 1.6 | 14.2 ± 1.4 |
| | DU low | 18.4 ± 2.2[d] | 49.9 ± 9.3[d] |
| | DU medium | 33.2 ± 4.7[d] | 99.9 ± 23.0[d] |
| | DU high | 39.2 ± 8.7[d] | 170 ± 40.1[d] |

[a]Ames II™ mixed strains contains an equimolar mixture of strains TA7001–TA7006. For a complete description of strain genotype see Gee *et al.* (1994).
[b]Dose of metal: *DU low*, 4 pellets; *DU medium*, 10 pellets; *DU high*, 20 pellets; tantalum, 20 pellets.
[c]Using a urine extract dose of 1 ml equivalent/plate, the number of revertants/plate was determined. Mutagenicity data were calculated after adjustment for urinary creatinine concentrations.
[d]Statistically significant from animals with no implants assessed at the same time period ($P < 0.05$).

revertant number seen at 12 months (Table I). For example, with 20 DU pellets (12 months, 36.7 ± 3.5 revertants/µmol creatinine versus 18 months, 39.2 ± 8.7 revertants/µmol creatinine). In contrast, the XAD-8 fraction demonstrated a rapid increase in mutagenic potential, with animals implanted with the lowest number of DU pellets (4) even showing a significant increase in revertant colonies 6 months after pellet implantation. Similar to the results with the TA98 strain, urine from non-surgical control animals or from those with tantalum implants did not exhibit any significant increase in mutagenicity. Again, the number of revertants appeared to plateau at 12 months, since the number of revertants at 18 months showed no statistically significant increase from the number observed at 12 months for all doses of DU.

In contrast to the results obtained with urine from DU-implanted animals, the sera from these animals did not demonstrate any enhancement of the mutation yield in the TA98 strain nor in the mixed strains (TA7001–7006) (Table II). Similarly, the sera from non-surgical control animals and from those with tantalum implants did not exhibit any alteration in mutagenic potential.

Urine and serum were analyzed for uranium content. The data in Table III illustrate that animals implanted with DU pellets exhibited both a time- and dose-dependent increase in urine uranium levels up to 12 months after pellet implantation. At 18 months, however, the urine uranium levels either decreased (4 and 20 pellet groups) in comparison with the levels at 12 months or remained the same (10 pellet group). Non-surgical controls or tantalum-implanted animals did not demonstrate any significant change in their urine uranium levels at the same time points (Table III). To determine what, if any, effect the urine fractionation/chromatographic process had on the urine uranium content, the eluates were analyzed for uranium content. Data demonstrated that the hydrophobic (XAD-4) and hydrophilic (XAD-8) fractions contained 42 ± 4% and 58 ± 6% of the total unfractionated urine uranium, respectively. These data suggest that very little uranium was lost during the urine fractionation process.

In contrast to the urine analysis, serum uranium analysis demonstrated that the content of uranium in the serum of the DU-implanted animals was not significantly altered from controls at 6, 12 or 18 months after pellet implantation (data not shown).

The correlation between the urine uranium levels and urine mutagenicity was evaluated using urine (XAD-8 fraction, tested with the Ames II mixed strains) obtained from animals implanted with DU (4, 10 or 20 pellets) or tantalum for 12 months. A strong positive correlation was obtained between urinary mutagenicity and urine uranium content, as seen in Figure 2 (Pearson $r = 0.995$, $P < 0.001$) for measurements made using urine obtained from animals 12 months after pellet implantation. A similar correlation analysis was done with urine uranium levels and urine mutagenicity from animals at 6 and 18 months after pellet implantation (data not shown).

Table II. Mutagenicity of serum samples from DU- or tantalum-implanted animals: number of revertant colonies/ml serum using strain TA98 or Ames II™ mixed strains[a]

| Time (months) | Dose[b] | Mutagenicity (revertants/ml serum) | |
|---|---|---|---|
| | | Strain TA98[c] | Mixed strains[d] |
| 0 | None | 45 ± 4 | 11.2 ± 1.2 |
| | Tantalum | 43 ± 4 | 11.5 ± 1.0 |
| | DU low | 46 ± 5 | 13.2 ± 1.4 |
| | DU medium | 47 ± 4 | 11.2 ± 1.2 |
| | DU high | 44 ± 5 | 11.9 ± 1.0 |
| 6 | None | 43 ± 5 | 11.5 ± 1.1 |
| | Tantalum | 52 ± 6 | 10.5 ± 1.2 |
| | DU low | 49 ± 5 | 10.7 ± 1.0 |
| | DU medium | 60 ± 13 | 12.9 ± 1.2 |
| | DU high | 43 ± 4 | 11.6 ± 9.0 |
| 12 | None | 49 ± 5 | 13.4 ± 1.5 |
| | Tantalum | 51 ± 6 | 12.0 ± 1.2 |
| | DU low | 55 ± 6 | 10.9 ± 1.5 |
| | DU medium | 49 ± 4 | 11.1 ± 9.0 |
| | DU high | 60 ± 9 | 13.5 ± 2.0 |
| 18 | None | 55 ± 6 | 11.8 ± 1.4 |
| | Tantalum | 59 ± 8 | 11.9 ± 1.4 |
| | DU low | 57 ± 7 | 12.2 ± 1.0 |
| | DU medium | 56 ± 6 | 14.2 ± 1.9 |
| | DU high | 42 ± 8 | 12.1 ± 1.1 |

[a]Ames II™ mixed strains contains an equimolar mixture of strains TA7001–TA7006. For a complete description of strain genotype see Gee *et al.* (1994).
[b]Dose of metal: DU low, 4 pellets; DU medium, 10 pellets; DU high, 20 pellets; tantalum, 20 pellets.
[c]Using a serum dose of .1 ml/plate, the number of strain TA98 revertants/plate/ml serum was determined.
[d]Using a serum dose of 1 ml/plate, the number of Ames II™ mixed strain revertants/plate/ml serum was determined.

Table III. Urine uranium level of DU- or tantalum-implanted animals: comparison with animals with no implants

| Time (months) | Implant[a] | Uranium level[b] (ng/µmol creatinine) |
|---|---|---|
| 0 | None | 1.91 ± 0.31 |
| 6 | None | 1.79 ± 0.34 |
| | Tantalum | 2.64 ± 0.74 |
| | DU low | 48.01 ± 14.02[c] |
| | DU medium | 251.60 ± 53.80[c] |
| | DU high | 670.80 ± 152.58[c] |
| 12 | None | 1.55 ± 0.37 |
| | Tantalum | 2.99 ± 0.92 |
| | DU low | 220.20 ± 30.01[c] |
| | DU medium | 340.10 ± 49.75[c] |
| | DU high | 1002.21 ± 80.22[c] |
| 18 | None | 1.38 ± 0.54 |
| | Tantalum | 3.13 ± 1.02 |
| | DU low | 151.27 ± 58.00[c] |
| | DU medium | 533.05 ± 290.15[c] |
| | DU high | 500.01 ± 127.43[c] |

[a]Dose of metal: DU low, 4 pellets; DU medium, 10 pellets; DU high, 20 pellets; tantalum, 20 pellets.
[b]Urine uranium content in ng/µmol creatinine.
[c]Statistically significant from control levels in animals with no implants ($P < 0.05$).

Similar to that observed at 12 months, a positive correlation was found between urinary mutagenicity and urine uranium content for 6 and 18 month data (6 months, Pearson $r$ = 0.934, $P < 0.001$; 18 months, Pearson $r$ = 0.83, $P < 0.001$). This analysis strongly argues that the uranium-associated urinary mutagenesis was due to the presence of uranium in the urine.

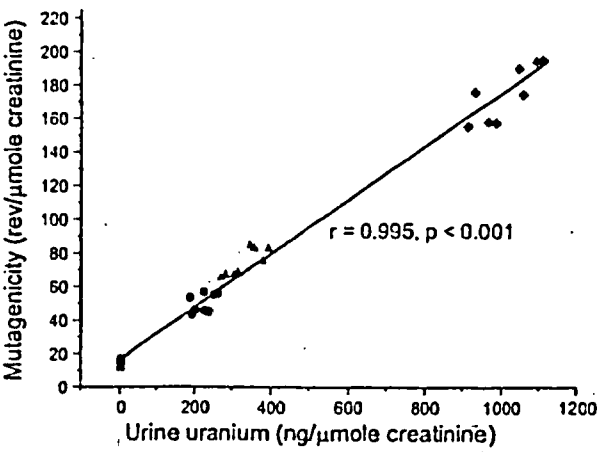


Fig. 2. Correlation (Pearson $r$) between urinary mutagenicity (revertants/µmol creatinine) and urinary content of uranium (ng/µmol creatinine) of animals implanted with either low, medium or high doses of DU, tantalum or no implant ($n$ = 40, 8 per group) at 12 months. Mutagenicity data were calculated after adjustment for urinary creatinine levels. Correlation between uranium and mutagenicity values was calculated using Pearson's correlation test.

## Discussion

Several US soldiers were wounded by shrapnel fragments consisting of DU during the 1991 Gulf War. Uranium bioassays conducted over a year after the initial uranium injury indicated the presence of uranium in the urine in excess of natural background at up to 50 µg U/l urine; the natural background uranium urine excretion rate is typically 0.6 µg/day (US Army Environmental Policy Institute, 1995). Although the soldiers with the embedded DU fragments had urine uranium levels well above background a year after the initial uranium injury, threshold toxic uranium urine excretion rates are typically 1000 µg/day (National Research Council Committee on the Biological Effects of Ionizing Radiation, 1989), which is significantly higher than the injured soldiers' 1 year levels. The Department of Veterans Affairs is monitoring these soldiers in a follow-up study to assess their current urine uranium levels.

Studies of the long-term health consequences of other forms of uranium, e.g. natural uranium, and types of exposure, e.g. inhalation, have demonstrated that uranium exposure is linked to lung carcinogenesis (Vahakangas *et al.*, 1992; Jostes, 1996). However, embedded DU fragments present a radiologically and toxicologically unique situation with unknown health risks and there is limited information regarding its mutagenic or carcinogenic potential. Furthermore, urinary mutagenicity has been widely used as a general biomarker of exposure to genotoxic agents and may be an effective tool to complement human population monitoring of individuals exposed to DU.

This study was conducted to determine if the urine and/or serum of rats implanted with DU pellets was mutagenic. The data demonstrate that with increasing urine uranium content, urine mutagenicity increased. Urine from rats implanted with a greater number of DU pellets had greater amounts of uranium and greater mutagenic potential, suggesting that uranium in the urine was at least one of the potential mutagenic components of the urine tested. Both the uranium levels and mutagenicity steadily increased between 0 and 12 months, indicating the time dependence of this increase in urine mutagenic potential.

Even the lowest number of implanted DU pellets resulted in mutagenic urine as the pellet exposure time increased. At 18 months both uranium content and mutagenicity leveled off or decreased, further arguing for this correlation. This association is additionally supported by the similar analysis of serum samples. They did not contain increased levels of uranium nor did they demonstrate any mutagenic activity, indicating that there were very low serum levels of the mutagenic substance(s).

The mutagenicity pattern indicates that both the hydrophobic (XAD-4) and hydrophilic (XAD-8) fractions from DU-implanted animals contain both frameshift and base pair substitution mutagens to varying extents, depending upon the length of time after implantation and the number of DU pellets implanted. The high chemical reactivity of uranium in biological fluids is well known. It has been shown to complex with a variety of substances such as carbonates, proteins, minerals and phospholipids (Dounce and Flagg, 1949; Neuman and Tishkoff, 1953; Blake *et al.*, 1956; Cooke and Holt, 1974; Schullery and Miller, 1977). The ability of specific bacteria such as *Escherichia coli* and *Citrobacter* spp. to accumulate uranium from solution has also been observed (Macaskie *et al.*, 1992). Therefore, it is possible to speculate that uranium, possibly complexed with substances found in either the hydrophobic and hydrophilic fractions of the urine, causes an increase in mutagenic potential.

The strong positive relationship between urine uranium levels and mutagenicity argues that urine uranium may be the substance primarily responsible for the urine mutagenicity. However, other factors can affect mutagenicity and urine analysis. The measurement of both urine uranium and mutagenic potential, particularly at the 18 month time point, could have been affected by the advanced age of the animals tested (21 months old). Aging has been shown to be causally related to both renal dysfunction (Li *et al.*, 1996) and increased mutagenicity in humans (Scarlett *et al.*, 1990). The urine mutagenicity of control animals (both non-surgical and Ta-implanted), however, did not exhibit significant differences with advanced age. Another consideration is the potential nephrotoxicity of the implanted DU. It is possible that cellular changes in the kidney resulting from DU exposure could affect interpretation of the results. It is important to note, however, that preliminary histological assessment of the kidney revealed no abnormal pathology or precancerous lesions (unpublished observations).

Our report is the first showing that internalized DU can result in a significant enhancement of urinary mutagenicity. This is markedly different from other studies showing that high specific activity uranium exposure *in vitro* is mutagenic [using several mutagenicity assays including the Ames test and the *HPRT* T lymphocyte cloning assay (Roos *et al.*, 1988; Baltschulkat and Horneck, 1991; Metting *et al.*, 1992)]. As discussed previously, occupational exposure of uranium miners to the uranium decay product radon has not only been linked to lung carcinogenesis, but to mutagenicity (Sram *et al.*, 1993; Hussain *et al.*, 1997). While the presence of other potential genotoxic agents in the urine cannot be ruled out, the lack of mutagenic potential of the urine from animals with tantalum implants or those without any metal implants strongly suggests that the uranium is potentially responsible for the urine mutagenicity. Additionally, the low uranium content of the serum, concomitant with the lack of serum mutagenicity, further argues that uranium is one of the mutagenic components in the urine. Other studies are needed to definitively determine what other substances in the urine of the animals with implanted DU are potentially responsible for the urinary mutagenicity observed here.

Urinary mutagenicity has been used as a biomarker as an integral component of human population monitoring and disease epidemiology (DeMarini *et al.*, 1997). Its effectiveness as a biomarker for genotoxic exposure has been demonstrated among people who smoke cigarettes (Yamasaki and Ames, 1977), were exposed to antineoplastic drugs (Falck *et al.*, 1979; Nguyen *et al.*, 1982) or were occupationally exposed to chemicals (Dolara *et al.*, 1981) or to sewage (Scarlett-Kranz *et al.*, 1986). Despite its inability to identify the specific mutagen(s) in the urine, urine mutagenicity testing is a cost-effective, general biomarker of exposure to genotoxic agents. Our data suggest that this type of biomarker analysis might also be an effective tool in a program designed to assess the health effects of chronic exposure to internalized DU in humans.

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