23 AUG 1996

Ref: 96-F-1461

Mr. Nemoto Michio
Ohayo-Nippon (Morning News)
NHK
Jinnan, Shibuya-Ku
Tokyo, JAPAN

Dear Mr. Michio:

This letter responds to your July 10, 1996, Freedom of Information Act (FOIA) request.

The Armed Forces Radiobiology Research Institute provided the enclosed documents as responsive to your request. There are no chargeable costs for processing your FOIA request in this instance.

Sincerely,

"Signed"
A. H. Passarella
Director
Freedom of Information
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#838

Establishment of an Animal Model to Evaluate the Biological Effects of Intramuscularly Embedded Depleted Uranium Fragments

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#838

Armed Forces Radiobiology Research Institute Technical Report 96-3

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1996	Technical Report

4. TITLE AND SUBTITLE
Establishment of an Animal Model to Evaluate the Biological Effects of Intramuscularly Embedded Depleted Uranium Fragments

5. FUNDING NUMBERS
PE: NWED QAXM

6. AUTHOR(S)
Castro CA, Benson KA, Bogo V, Daxon EG, Hogan JB, Jacocks HM, Landauer MR, McBride SA, Shehata CW

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Armed Forces Radiobiology Research Institute
8901 Wisconsin Avenue
Bethesda, MD 20889-5603

8. PERFORMING ORGANIZATION REPORT NUMBER
TR96-3

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Uniformed Services University of the Health Sciences
4301 Jones Bridge Road
Bethesda, MD 20814-4799

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

During the Persian Gulf War, 36 U.S. soldiers were wounded by depleted uranium (DU) munitions. Based on medical guidelines for conventional shrapnel injuries (nonradioactive), many DU fragments were left in soldiers. Unfortunately, health risks associated with embedded DU were unknown, and an animal model to investigate this did not exist. The purpose of this study was to develop an animal model to examine the health risks associated with DU shrapnel injuries. Twelve rats were surgically implanted intramuscularly with 8 DU pellets (1 mm in diameter x 2 mm in length) or 8 chemically inert tantalum (Ta) pellets of similar size. Urinary uranium levels were measured on days 1, 3, 7, 14, 28, 60, and 120 after implantation of DU pellets. Physiological and behavioral parameters, including locomotor activity, forelimb and hindlimb grip strength, food and water consumption, and urinary output, were measured 5 and 3 days before surgery and on days 1, 3, 7, 14, 28, 60, and 120 after surgery. Urinary uranium levels for Ta-implanted rats remained at background levels. In contrast, the average urinary uranium level for DU-implanted rats was significantly elevated on day 1 (28.69 µg U/l) after implantation and remained elevated until day 120 (204.56 µg U/l). There was no significant difference between DU- and Ta-implanted rats in any behavioral or physiological measures. Results indicate that the rat is an appropriate animal model for evaluating biological effects of embedded DU fragments.

14. SUBJECT TERMS	15. NUMBER OF PAGES
	21
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

CLASSIFIED BY:

DECLASSIFY ON:

Contents

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Introduction

Natural uranium (U) consists of three isotopes: ^{238}U (99.276%), ^{235}U (0.718%), and ^{234}U (0.0056%). During the uranium enrichment process two isotopic mixtures are produced, "enriched uranium" and "depleted uranium" (DU) with different relative ratios of the three isotopes. Enriched uranium contains a higher percentage of the fissionable isotope ^{235}U and is used for nuclear reactor fuel and nuclear weapons. DU has a lower ^{235}U content. The DU used by the U.S. military for kinetic energy penetrators is alloyed with titanium (0.75% by weight) to increase its tensile strength and to retard oxidation. Current U.S. antitank weapons contain DU penetrators, and most of the Abrams tanks are armored with DU. During Operation Desert Storm, DU munitions were fired by the Army and Air Force. Unfortunately, during this conflict, a number of U.S. military personnel were wounded by DU fragments (Daxon, 1993; Daxon and Musk, 1993; GAO Report, 1993). Many of these fragments were not removed because the surgical procedure would produce excessive tissue damage. Radiographs of injured soldiers show multiple embedded fragments ranging in size from 1 mm to over 5 mm in diameter (see figures 1 and

Fig. 1. Radiograph of the leg of a soldier wounded by a DU munition during the Persian Gulf War. This soldier also had DU fragments in the feet and knees of both legs.

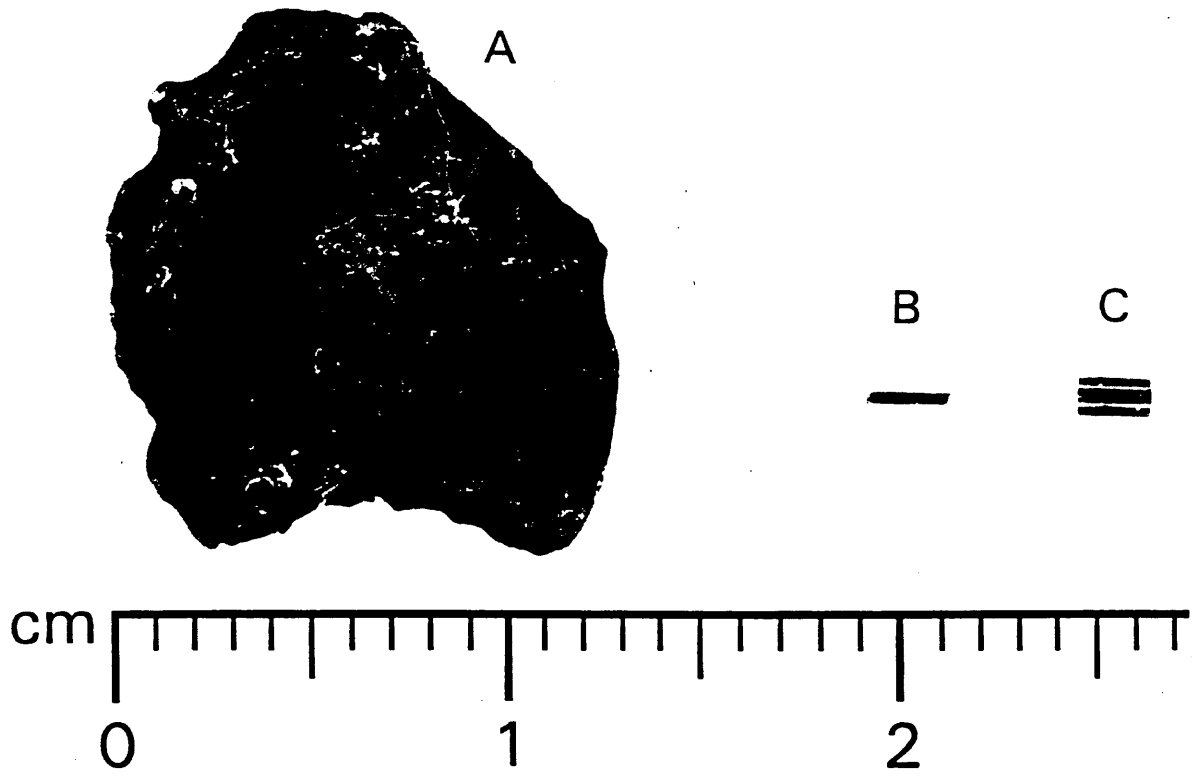

Fig. 2. (A) Photograph of an actual DU fragment removed from a soldier wounded during the Gulf War. (B) Photograph of a Ta pellet implanted in a rat. (C) Photograph of a DU pellet implanted in a rat.

2a). Indeed, fragments as large as 20 mm in diameter have been noted in other patients. Bioassays taken over a year after injury indicate that uranium was present at levels up to 30 μg U/l urine, well in excess of natural background (U.S. Army Environmental Hygiene Agency Memorandum for Office of the Surgeon General, 1994).

Although the toxicity of embedded DU is unknown, numerous studies have addressed the consequences of inhalation, ingestion, and parenteral administration of other forms of uranium (Diamond, 1989; La Touche et al., 1987; Morrow et al., 1982; Ortega et al., 1989a, b; Wrenn et al., 1989). After uranium is absorbed, it circulates in the blood as the uranyl ion, forming uranium-carbonate and uranium-albumin complexes. As the uranium-carbonate complex passes through the kidney, it is filtered rapidly by the glomeruli where 60% to 80% of the absorbed uranium is excreted in the first 24 hours after acute exposure. The uranium that is not excreted is reabsorbed by the proximal tubules where it produces significant toxic effects. Uranium also enters the bone, where it competes with calcium to form complexes with phosphate ions, thus becoming part of the bone matrix (Cabrini et al., 1984; Domingo et al., 1992; Guglielmotti et al., 1989; Neuman, 1950). This bone matrix then serves as both a long- and short-term storage site from which uranium is slowly released back into circulation (Kathren et al., 1989; Wrenn et al., 1985). The liver and muscle are other major sites of uranium deposition, with a possible long-term storage mechanism in the kidney (Kathren et al., 1989; Wrenn et al., 1985).

Acute morphological and biochemical changes of the kidney result from uranium exposure (Diamond, 1989; Kocher, 1989; Leggett, 1989; Neuman, 1950). Changes in the glomerular epithelial architecture (Kobayashi et al., 1984) and cellular necrosis in the proximal tubules near the corticomedullary junction of the kidney have been reported in experimental animals after acute uranium exposure (Brady et al., 1989; Haley et al., 1982; Haley, 1982). In addition,

polyuria, enzymuria, glucosuria, and increased excretion of amino acids have been demonstrated (Diamond, 1989; Diamond et al., 1989; Kocher, 1989; Zalups et al., 1988). Acute renal failure can indeed occur following exposure to high doses of uranium (Neuman, 1950; Ubios et al., 1994). Even acute environmental stressors such as restricted diets or changes in housing conditions have enhanced uranium toxicity significantly (Andrews and Bates, 1987; Damon et al., 1986).

Few studies have addressed the chronic toxicity of uranium, and the results available are conflicting (U.S. Department of Health and Human Services, 1990). Galibin and colleagues (1971) reported severe renal toxicity in rats that inhaled ammonium diuranate (1 or 8 mg/m^3), a slightly soluble uranium compound, for 128 days. Urine protein and blood non-protein nitrogen were elevated. In the proximal tubules, there were sloughed dead cells and abnormal regenerating cells. Although the total number of tubules was reduced and the kidney exhibited an increased amount of connective tissue, all the animals recovered. In contrast, Leach and colleagues (1970; 1973) found no renal toxicity in rats repeatedly exposed to uranium dioxide dust (5 mg/m^3) for a period of 12 months nor in dogs or monkeys exposed for 5 years. Yet uranium concentrations in the kidneys were as high as 1.1 µg U/g kidney wet weight in the rat, 8.3 µg U/g kidney weight in the dog, and 17.0 µg U/g kidney weight in the monkey. Uranium concentrations at these levels have been reported to cause acute renal toxicity (e.g., Kathren et al., 1989). Thus, the chronic effects of uranium exposure remain for the most part unresolved (Diamond, 1989).

The threshold concentration of kidney uranium levels in humans that result in kidney chemical toxicity is in dispute (Diamond, 1989; Kathren and Moore, 1986; Kocher, 1989; Stradling et al., 1988). While the Nuclear Regulatory Commission has set the level at 3.0 µg U/g kidney weight for renal damage in humans, there is evidence from both human and animal reports that this level could be considerably lower. For example, chronically exposed uranium mill workers, whose kidney uranium levels probably did not exceed 1 µg U/g kidney weight (Thun et al., 1985), showed mild renal dysfunction with increased urinary excretion of $ß_2$-microglobulin and various amino acids. In rats exposed subchronically to low doses (cumulative dose: 0.66 or 1.32 mg/kg) of uranyl fluoride, kidney uranium levels as low as 0.7 to 1.4 µg U/g wet weight kidney produced cellular and tubular necrosis of the proximal tubule, proteinuria, and enzymuria (Diamond et al., 1989). These changes in rat renal function, however, were temporary, with complete recovery occurring within 35 days of exposure. These studies are important because they indicate that renal injury can occur at kidney uranium levels well below the 3.0 µg U/g limit.

Currently, no research into the direct toxic effects of embedded DU has been reported. The toxicity data that exist for low-level chronic uranium exposure used other routes of administration, and the results are contradictory. The uranium levels in humans that result in kidney toxicity are in dispute. For these various reasons, it is necessary to determine the health risks to the soldier resulting from long-term exposure to DU fragments. The goal of this pilot study was to establish an animal model that could be used in future research to investigate the biological effects of embedded DU.

Methods

Subjects and Experimental Design

Subjects were 12 naive Sprague-Dawley male rats (8-10 weeks old) obtained from Charles River Breeding Laboratories, Raleigh, N.C. On arrival, rats were quarantined and screened for diseases and were maintained in an AAALAC-accredited facility in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 86-23). Six rats were implanted with eight DU pellets (four in each biceps femoris muscle of the lateral thigh), and six rats were implanted with eight tantalum (Ta) pellets. Rats were individually housed in plastic Micro-Isolator cages with hardwood chips as bedding; during urine collection, rats were placed in metabolic cages. Commercial rodent chow and acidified water (pH 2.5, using concentrated HCl) were provided *ad libitum*. Rats were on a 12-hour light/dark cycle.

DU and Ta Pellets

DU pellets (1 mm in diameter x 2 mm in length) were obtained from Oak Ridge National Laboratories, Oak Ridge, Tenn. (see figure 2c). The cylindrical shape was chosen because it is the geometrical average of fragments left in soldiers wounded by conventional or DU munitions. The size of the pellets was based on two considerations. First, the total DU implanted was approximately 1% of the total biceps femoris muscle volume and did not seem to cause undue discomfort to the animal. Second, the surface area of 8 DU pellets of this size should result in detectable urinary uranium levels. DU pellets consisted of 99.25% DU and 0.75% titanium by weight. The uranium isotopes in DU were ^{238}U (99.75%), ^{235}U (0.25%), and trace amounts of ^{234}U. This is the same DU alloy used in U.S. military munitions.

Ta pellets (1 mm in diameter x 2 mm in length) were obtained from Alfa Products, Ward Hill, Mass., and served as the heavy metal control (see figure 2b). Ta was selected because its density is similar to DU density, 16.6 g/cm^3 for Ta versus 18.8 g/cm^3 for DU (Radiological Health Handbook, 1970), it is relatively inert in a biological medium (Johansson et al., 1990), and it is commonly used in human orthopedic reconstructive surgery (Hockley et al., 1990).

Surgical Procedures for Pellet Implantation

Before implantation surgery, the DU and Ta pellets were cleaned by immersion in an industrial detergent, rinsed in absolute alcohol, sterilized by immersion in a 50% nitric acid solution for 3 minutes, rinsed with sterile water, and then placed in acetone to inhibit oxidation. These sterilization procedures completely remove the oxide formation from the surface of DU metal (Tonry, 1993), and the results of an abbreviated sterility test of 10 Ta pellets using either a thioglycollate medium or soybean-casein digest medium detected no microorganisms.

Rats were administered atropine (0.05 mg/kg i.m.) before being anesthetized. Anesthesia was induced with ketamine hydrochloride (50 mg/kg) in combination with xylazine hydrochloride (10 mg/kg) given i.p. in a 0.5-ml bolus, using a 25-gauge needle. These injections were administered intraperitoneally to prevent irritating the site of implantation. The surgical sites were then shaved and cleansed with Betadine. Four pellets were implanted approximately 15 mm apart in each biceps femoris muscle on the lateral side of each thigh. Using a scalpel blade, incisions were made through the skin and approximately 10 mm deep into the muscle mass. The proximal incisions were 10 mm distal to the iliac crest and were the implantation sites of the first pellets. Pellets were secured in place with absorbable sutures (Dexon 4-0) to prevent movement. Rats were closely monitored following surgery until they were ambulatory. A veterinarian or a veterinary technician examined the surgical sites for signs of inflammation, infection, and local DU toxicity daily for 2 weeks

following surgery and weekly thereafter throughout the study.

Behavioral Measurements

Locomotor activity and grip strength were assessed on days 3 and 5 before surgical implantation and on days 1, 3, 7, 14, 28, 60, and 120 after surgery. Locomotor activity was quantified using computerized Digiscan activity monitors (Omnitech Electronics, Columbus, Ohio). Each monitor used an array of infrared photodetectors spaced 2.5 cm apart to determine horizontal locomotor activity, which was expressed as total distance traveled. Activity was monitored for 1 h with measurements taken every 5 min (Landauer et al., 1988).

Immediately following locomotor activity testing, the strength of both hindlimb and forelimb grips of each animal was measured using a grip strength apparatus (San Diego Instruments, San Diego, Calif.). In this test, the animal was required to grip a rectangular wire mesh surface (12 x 7 cm) with its forepaws and was then gently pulled back along a platform until its grip was broken. The backward motion was continued until the animal's hindpaw gripped another rectangular wire mesh surface (12 x 10 cm). As with the forelimb grip, the animal was gently pulled back until the hindlimb grip was broken. Readings on three push-pull strain gauges were used to record the maximum strain required to break both forelimb and hindlimb grips. This behavioral test is used in many laboratories to assess muscular weakness (Haggerty, 1989; Meyer et al., 1979).

Urinary Sampling and Collection Procedures

Urine samples were collected following behavioral testing on days 1, 3, 7, 14, 28, 60, and 120 after surgery and analyzed for uranium levels. Sampling at these time points was necessary because signs of nephrotoxicity in laboratory animals exposed to low doses of uranium are frequently not detected until 3 to 5 days after exposure and may subside within 7 days (Diamond, 1989). Urine samples were collected from rats in individual metabolic cages (23.5 cm diameter x 12 cm high) where they had continuous access to food and water. Rats were acclimated to the metabolic cages for 5 days before the study began because naive rats exposed to these housing procedures have shown a stress-induced increase in uranium toxicity (Damon et al., 1986).

A 24-h urine sample was obtained from each rat, and the volume was recorded. In addition, each animal's body weight and food and water consumption were recorded. Care was taken to prevent contaminating the urine with food or feces. After collection, urine was filtered to remove any debris and stored in plastic containers at 4° C until analyzed. The metabolic cages were disinfected and decontaminated between each animal use. During animal-handling periods, overt signs of behavioral toxicity and the overall appearance of the rats were recorded.

Determination of Urinary Uranium Levels

Urinary uranium levels were determined by alpha spectrometric techniques (Martin Marietta Energy Systems, Inc., Oak Ridge, Tenn.). An aliquot of the sample was dissolved in nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The sample was then wet ashed, and the uranium coprecipitated with calcium oxalate. After dissolving the precipitate in HCl, the uranium was further separated by ion exchange chromatography. The uranium was then eluted from the column with a solution of dilute HCl to which titanous chloride had been added to reduce actinides that may have been in an elevated oxidation state. The final fraction of the eluate was treated first with ascorbic acid to reduce any iron and then with hydrofluoric acid. The uranium isotopes were next coprecipitated on neodymium fluoride. The neodymium was caught on a 0.1-µm filter, which was rinsed, dried, and then mounted on a planchet for alpha spectrometry. The minimum detectable activities (MDA) for uranium in urine using these procedures were 1.4×10^{-6} µg/l for ^{234}U and 0.03 µg/l for ^{238}U.

Results

Surgical Implantation

Two rats assigned to the DU group and one rat assigned to the Ta group did not survive implantation surgery. One of these rats expired during surgery, and the other two within 6 h after surgery. Necropsies indicated asphyxiation, suggesting that the animals received too much anesthetic. The other nine animals were alert and moving in the metabolic cages within 2 h after surgery. Figure 3 is a radiograph of the left rear leg of a rat implanted with four DU pellets; the right rear leg was also implanted with four DU pellets. The cylindrical shape and size of the pellets are similar to DU fragments observed in wounded soldiers (figure 1).

Locomotor Activity and Grip Strength

The locomotor activity of rats implanted with DU pellets was not significantly different from the activity of rats implanted with Ta, $p > 0.05$ (figure 4).

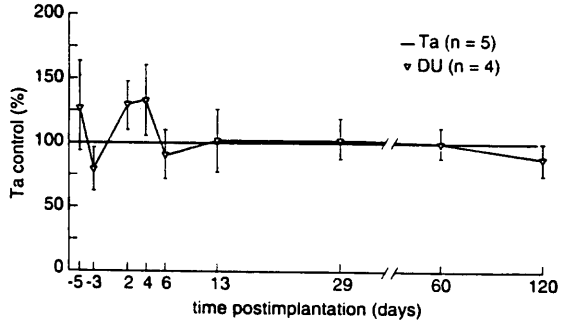

Fig. 4. Locomotor activity of rats surgically implanted with DU pellets expressed as percent of Ta control. Vertical bars represent the SEM (standard error of the mean).

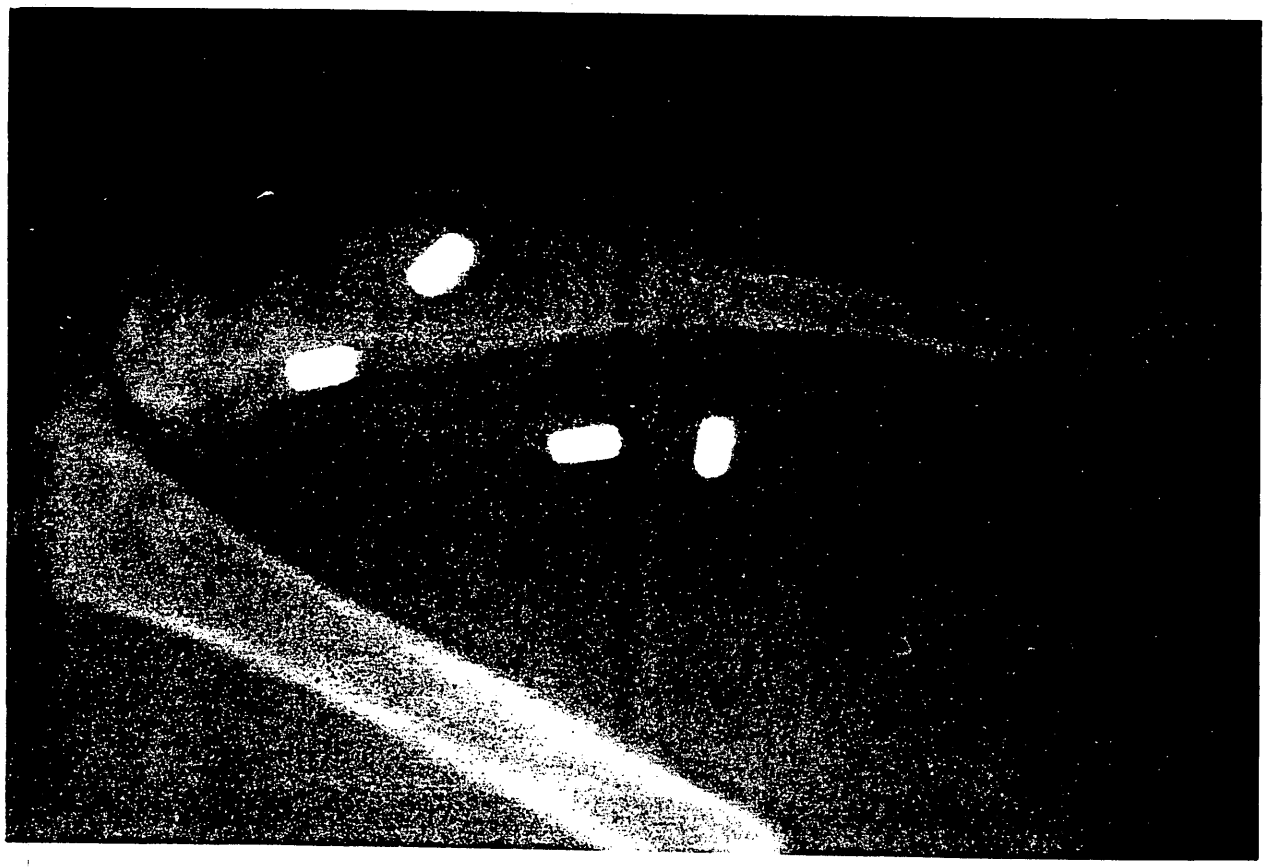

Fig. 3. Radiograph of the left rear leg of a rat surgically implanted with four DU pellets (1 mm in diameter x 2 mm in length).

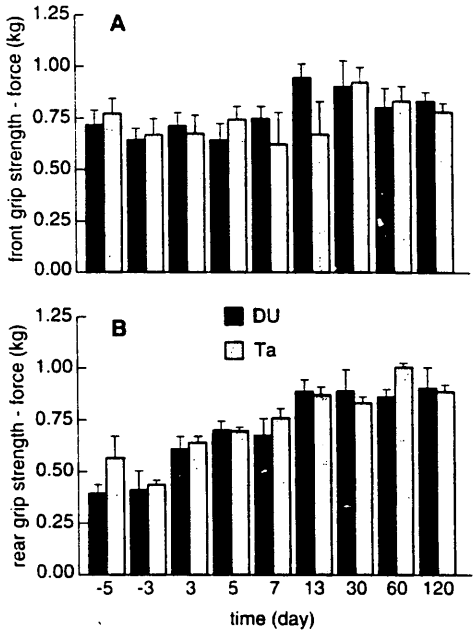

Fig. 5. (A) Forelimb grip strength of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM. (B) Hindlimb grip strength of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

Similarly, neither the forelimb nor the hindlimb grip strength of the two groups was different, $p > 0.05$ (figures 5a and 5b).

Body Weights, Food and Water Consumption, and Urinary Output

The body weights of the rats embedded with DU pellets were not different than the body weights of rats embedded with Ta, $p > 0.05$ (figure 6). In fact,

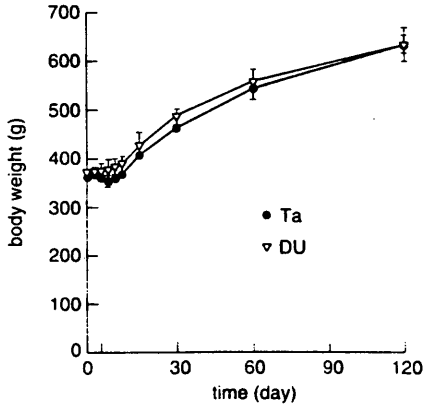

Fig. 6. Body weights of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

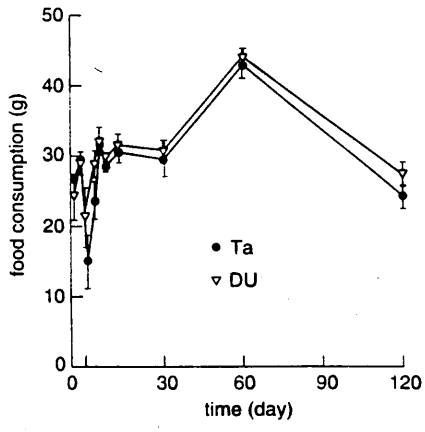

Fig. 7. Food consumption of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

the body weights in both groups remained relatively stable for the first week following surgery and, as expected, increased throughout the study as observed in normal rats.

The food and water consumption for the DU- and Ta-implanted rats did not differ, $p > 0.05$ (figures 7 and 8). There was, however, a trend toward a decrease in water consumption for the Ta group and an increase in water consumption for the DU group.

There was a significant difference in the volume of urinary output between the DU and Ta groups. On the day of surgery, urine output for the Ta group decreased but did not change for the DU group, p

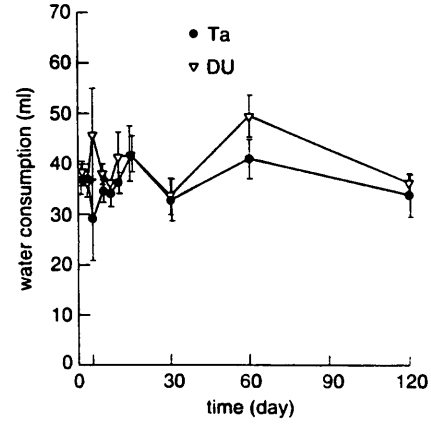

Fig. 8. Water consumption of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

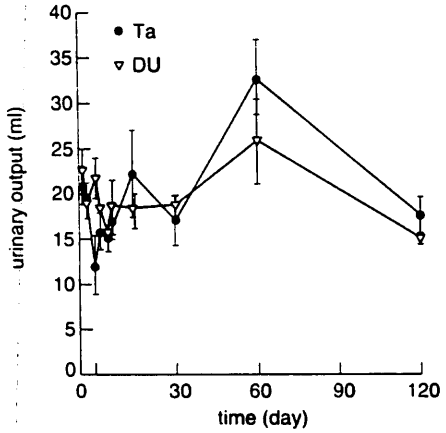

Fig. 9. Urinary output of rats before and after receiving DU or Ta implants. Vertical bars represent the SEM.

<0.05 (figure 9). This decrease in the urinary output for the Ta group, however, was temporary and returned to baseline levels by day 3 after surgery.

Urinary Uranium Levels

Figure 10 illustrates mean uranium levels in the urine of DU-implanted animals and the pooled value of the uranium analysis for Ta-implanted animals after implantation surgery. Figure 11 provides the individual urinary uranium levels of the four DU-implanted rats. As expected, only background levels of uranium were detected in the Ta control group. In contrast, significant levels of uranium were detected within 24 h of DU implantation (mean = 28.69 ± 10.00,

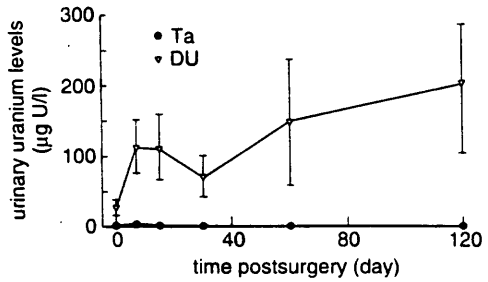

Fig. 10. Time course of uranium levels detected in the urine of rats implanted with either DU or Ta. Uranium concentration detected in the Ta group is at background levels. Vertical bars for the DU group (N = 4) represent the SEM. Urine for the Ta-implanted animals was pooled for uranium analyses.

range = 14.21 to 56.99 µg U/l). By day 7 following surgery, uranium levels had increased nearly fourfold (mean = 111.86 ± 41.05, range = 56.38 to 233.91 µg U/l) and remained elevated at day 120 (mean = 204.56 ± 99.73, range = 35.01 to 458.53 µg U/l).

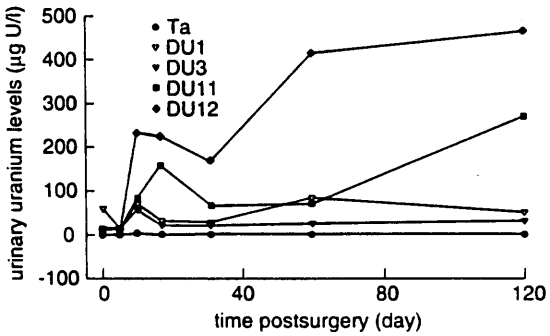

Fig. 11. Individual time courses of uranium levels detected in the urine of each rat implanted with DU. Data on Ta time courses are the same as in figure 10.

Discussion

The purpose of this study was to develop an animal model that could be used in future research to determine the health risks associated with DU fragment injuries. It was especially important to establish procedures in which DU exposure would produce urinary uranium levels comparable to those observed in soldiers wounded by DU munitions during the Persian Gulf War. Measured by these criteria, this initial study was successful. The average urinary uranium level in the rat 24 h after DU implantation was 28.69 µg U/l. This value is very close to the urinary level of 30 µg U/l reported for soldiers wounded during the Persian Gulf War and assayed 1 year after injury. Unfortunately, no bioassays were taken of any of the soldiers within the first year after DU injury so no direct time course comparisons can be made.

It should be emphasized that the urinary uranium levels in the rat did not reach asymptote until day 7 following DU implantation surgery and remained elevated throughout the study (figure 10). Although the data are preliminary, this finding has clinical significance because it indicates that soldiers with suspected DU fragment wounds should be monitored for uranium exposure for at least the first week after injury and perhaps even longer. Certainly a complete pharmacokinetic study should be conducted to definitively address this patient-monitoring issue (Daxon, 1993).

Although numerous studies have assessed the toxic effects of other forms of uranium exposure (Diamond, 1989, and Kocher, 1989, for the latest reviews of the literature), this is the first study that assessed the effects of intramuscularly embedded DU. The rat proved to be an excellent animal model for this purpose. It tolerated the surgical procedures for pellet implantation relatively well, as measured by both locomotor activity and grip strength (figures 4 and 5), both indices of quality of life for humans. Further, the lateral thigh muscle of the adult rat is large enough to implant at least four pellets (1.0 mm diameter x 2 mm length) into each leg (figure 3), with the possibility of as many as ten pellets. Moreover, the rat's lifespan of more than 18 months enables it to be used in chronic toxicity studies (Brady et al., 1989; Lang and White, 1994; Lumley et al., 1992; Lumley and Walker, 1986; Monro, 1993; Nohynek et al., 1993; Rao et al., 1990).

In conclusion, this study was successful in developing a rodent model that can be used to evaluate the biological effects of intramuscularly embedded DU fragments. However, the potential short-term and long-term health risks associated with DU exposure remain to be investigated. Certainly the behavioral, physiological, biochemical, and histological consequences of embedded DU are research areas of immediate concern. Equally important is identification of the health risks to the fetus exposed *in utero* to DU from fragments embedded in the mother before pregnancy (Angleton et al., 1988; Bosque et al., 1993; Domingo et al., 1988a, b, c; Paternain et al., 1989). This latter research area is especially significant considering that the placenta does not prevent cross-placental transfer of uranium (Durbin and Wrenn, 1976; Sikov and Mahlum, 1968). Moreover, fetal toxicity often occurs in the absence of maternal toxicity (e.g., Price et al., 1985). Regardless of the research strategy adopted, a coordinated interdisciplinary health hazard assessment is required to identify the potential medical risks that DU poses to our soldiers wounded by this unconventional munition.

Acknowledgements

We thank Ms. Elizabeth L. Wampler for radiation safety advice, Major Rebecca A. Cockman-Thomas for performing surgical implantations, Dr. G. David Ledney and Dr. Thomas B. Elliott for conducting and interpreting pellet sterility tests, Mr. William E. Jackson III for statistical advice, and Ms. Modeste E. Greenville and Ms. Carolyn Wooden for publication assistance.

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AD_____

MIPR: 95MM5530

TITLE: Health Hazard Assessment of Depleted Uranium: In Vivo and In Vitro Studies

PRINCIPAL INVESTIGATOR: Dr. Terry C. Pellmar

CONTRACTING ORGANIZATION: Armed Forces Radiobiology Research Institute
Bethesda, MD 20889-5603

REPORT DATE: November 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

#838

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	November 1995	Annual (1 Dec 94 - 30 Nov 95)

4. TITLE AND SUBTITLE
Health Hazard Assessment of Depleted Uranium: In Vivo and In Vitro Studies

5. FUNDING NUMBERS
95MM5530

6. AUTHOR(S)
Dr. Terry C. Pellmar

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Armed Forces Radiobiology Research Institute
Bethesda, MD 20889-5603

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, MD 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

This study assesses the health risks associated with embedded depleted uranium (DU) fragments by evaluating the behavioral, physiological and histological consequences of intramuscularly implanted DU pellets in a rodent model. In addition, distribution of uranium is determined and will be used to develop a biokinetic model. In the first year of this study, we established the appropriate doses (5 experimental groups) for subsequent analysis: 1) control (20 1-mmx2-mm chemically inert tantalum (Ta) pellets), 2) high dose (20 1-mmx2-mm DU pellets), 3) medium dose (10 DU and 10 Ta pellets), 4) low dose (4 DU and 16 Ta pellets) and 5) nonsurgical controls. We completed the study of the 30-day time point following pellet implantation, although analysis is still preliminary. Examination of the pellets *in situ* reveals fibrous tissue adhering to the DU but not the Ta pellets. Capsule formation is not yet evident. Uranium levels are high and dose-dependent in kidney, bone and urine and moderately high in muscle, brain (only at the high dose) and spleen. There is no evidence of renal toxicity or behavioral neurotoxicity at this time point. The 6, 12 and 18 month time points will be examined in future experiments.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Depleted Uranium, Toxicology, Behavior, Physiology, Histology, Biokinetic Model	34
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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Tony P. Chen 11/27/95

PI - Signature Date

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ORIGINAL

INTRODUCTION

Natural uranium consists of three isotopes: ^{238}U (99.276%), ^{235}U (0.718%) and ^{234}U (0.0056%). During the uranium enrichment process two products are produced, "enriched uranium" and "depleted uranium" (DU), that contain different relative ratios of these three isotopes. Enriched uranium contains the higher amount of the fissionable isotope ^{235}U and is used for nuclear reactor fuel and nuclear weapons. DU has a lower ^{235}U content and is a highly dense material. The DU used by the US in kinetic energy penetrators is alloyed with titanium (0.75% by weight) to retard oxidation. This DU alloy is of concern because the U.S. military currently uses this metal for munitions and armament. During Operation Desert Storm, a number of U.S. military personnel were wounded by shrapnel fragments consisting of DU[6,7]. Since surgical removal can produce excessive tissue damage, these DU fragments were treated as conventional shrapnel and left in place in the wounded soldiers. The radiographs of injured soldiers show multiple embedded fragments ranging in size from 1 mm to over 5 mm in diameter. Fragments as large as 20 mm have been noted in other patients. Uranium bioassays taken over a year after injury indicate that uranium was present in the urine well in excess of natural background, up to 30 μg U/l of urine. DU fragments present a radiologically and toxicologically unique situation with unknown health risks. Congress has mandated the study of these risks.

This study evaluates the consequences of both short-term and long-term exposure to DU fragments in the rat model. Using an interdisciplinary approach, we are assessing neurotoxicity, nephrotoxicity, histopathology of the tissue surrounding the fragment and pathology including evaluation of neoplastic changes in several body tissues. In addition, based on our animal data, we will develop a biokinetic model that describes the distribution of uranium from embedded fragments as a function of time.

Uranium toxicity: Although the toxicity of embedded DU is unknown, numerous studies have ad-

dressed the consequences of inhalation, ingestion and parenteral administration of other forms of uranium[27,38,45,62]. After uranium is absorbed, it circulates in the blood as the uranyl ion forming uranium-carbonate and uranium-albumin complexes[8,26,31]. As the uranium-carbonate complex passes through the kidney, it is filtered rapidly at the glomerulus where 60%-80% of absorbed uranium is excreted in the first 24 hours after acute exposure. The uranium not excreted is reabsorbed by the proximal tubules where it produces acute toxic effects. Uranium also enters the bone where it competes with calcium to form complexes with phosphate ions, thus becoming part of the bone matrix[3,10,16,42]. This bone matrix then serves as a storage site from which uranium is slowly released back into circulation [23,61]. The liver, muscle, and kidney are other major sites of uranium disposition, with a possible long-term storage mechanism in the kidney [19,23,27,51,62]. At low doses, uranium may not readily distribute to the central nervous system (CNS)[45]. With higher doses (8 mg/kg/day orally for 4 weeks), however, brain uranium levels are comparable to those in liver and in bone[45], major sites for uranium accumulation.

Acute morphological and biochemical changes of the kidney result from uranium exposure[8,26,31,42]. The glomerular epithelial architecture is altered[25] and cellular necrosis occurs in the proximal tubules near the corticomedullary junction in the kidney[2,17,18]. In addition, polyuria, enzymuria, glucosuria, and increased excretion of amino acids result[8,9,26,63]. Acute renal failure can be the cause of death with exposure to high doses of either soluble or insoluble forms of uranium[43,57]. Environmental stressors such as restricted diets or changes in housing conditions significantly enhance uranium toxicity[1,4].

Few studies have addressed the chronic toxicity of uranium and the results available are conflicting. Galibin and colleagues[14] reported severe renal toxicity in rats that inhaled the slightly soluble uranium compound, ammonium diuranate (1 or 8 mg/m^3) for 128 days. Urine protein and blood, non-protein nitrogen were elevated. In the proximal tubules, there were sloughed dead cells and abnormal regenerating cells. These animals recovered, although the total number of tubules was reduced, with an accompanying increased proportion of connective tissue in the kidney. In contrast, Leach et al.[29,30] found no renal toxicity in rats repeatedly exposed for a period of 12 months to uranium dioxide dust (5 mg/m^3) (or in dogs or monkeys exposed for 5 years). Yet uranium concentrations in the kidney were as high as 1.1 μg U/g kidney wet weight in the rat (8.3 in the dog and 17.0 in the monkey), levels

reported to cause acute renal toxicity (e.g., [23]). Thus the chronic effects of uranium exposure remain, for the most part, unresolved[8].

The threshold concentration of kidney uranium levels in man that results in kidney chemical toxicity is in dispute[8,26,52]. While the Nuclear Regulatory Commission has set the level at 3 μg/g kidney for renal damage in man, there is evidence from both human and animal reports that this level could be much lower. For example, chronically exposed uranium mill workers, whose kidney uranium levels probably did not exceed 1 μg U/g[54], showed mild renal dysfunction with increased urinary excretion of β_2-microglobulin and various amino acids. In rats exposed subchronically to low doses (cumulative dose: 0.66 or 1.32 mg/kg) of uranyl fluoride, kidney uranium levels as low as 0.7 to 1.4 μg U/g wet kidney produced cellular and tubular necrosis of the proximal tubule, proteinuria, and enzymuria[9]. These changes in rat renal function, however, were temporary, with complete recovery within 35 days after exposure. These studies are important because they indicate that renal injury can occur at kidney uranium levels well below the 3.0 μg U/g limit.

Neurological effects have been reported with uranium exposure. In uranium workers excreting up to 200 μg U/l in their urine, normal mental function was disrupted[24]. One case study linked the handling of a uranium bar and a subsequent increase in stool uranium with foot cramps, leg pain and abnormal gait[15]. With oral and subcutaneous administration of relatively high doses of uranyl acetate (210 mg/kg and 10 mg/kg, respectively), rats exhibited tremors[11]. The uranyl ion has been demonstrated to enhance muscle contraction with acute local concentrations of 200-400 μM[13,32]. At the neuromuscular junction in the mouse, multiple sites of action were identified, including increased duration of the muscle action potential, broadening of the compound nerve action potential, increased amplitude and quantal content of the endplate potential and increased frequency of the miniature endplate potentials[32]. These studies indicate that embedded DU fragments could lead to neural damage, affecting both motor and cognitive function. The CNS effects of uranium toxicity can result from secondary mechanisms since hormonal changes, electrolyte disruption and immune responses can all influence nervous system activity [47].

Local Tissue Response and Capsule Formation: Foreign bodies in tissue elicit an immune

response that can result in encapsulation. Even when encapsulated, DU fragments provide a local, chronic source of α-radiation. Within 10-15 cells of the fragment, the dose rate is expected to be approximately 8.5 Gy/yr. This radiation could result in injury or damage to local muscle or nerve tissue (axonal injury, demyelination)[48,58]. In addition, capsule formation around a DU fragment in close proximity to a nerve could increase the risk of compression injury to those nerves.

Encapsulation could limit the chemical toxicity of the DU fragments by decreasing the rate of release of the metal, as has been observed with lead[35]. Encapsulation can also result in the formation of pseudocysts. Pseudocysts were formed that contained fluid with very high concentrations of soluble lead and insoluble lead dioxide particles[33,35] and with "black pigment...firmly adherent..." to portions of the inner wall of the capsule[33]. If these cysts should rupture, the rapid release of this fluid could cause period spikes in circulating lead levels and result in acute lead toxicity 5 to 40 years after the initial injury[33,35,59]. Similar type lesions may form around DU fragments. Intracapsular fluid may contain high concentrations of both soluble and insoluble DU. Tonry[55] demonstrated that DU disks formed both a soluble fraction and black insoluble particulates when emersed in simulated lung fluid. After a large fragment (approx. 20 mm) was removed from a U.S. soldier 17 months after he was wounded, the surgeon[28] noted that the fragment was encased in a fibrous capsule. When the capsule was breached, approximately 1-2 ml of a black fluid "gushed forth" from the cystic space.

DU can cause both local and systemic toxicity through a variety of mechanisms. Our study defines many of the potential sites of pathology that can result from long-term exposure to DU fragments and will provide a rationale for treatment of our wounded soldiers. The first six months of the study established the doses of DU to be used in future experiments (aim 1). This dose ranging study determined the number of DU pellets required to obtain uranium levels in the range of 0.7 to 1.4 μg/g wet weight of kidney. This level of uranium has been reported to produce early signs of renal damage as measured by both biochemical and histopathological changes[9] and would define the high dose in our toxicological studies. The low dose was chosen to produce no measurable acute toxicity. Subsequent experiments use the established doses to evaluate neurotoxicity, nephrotoxicity and histopathology and determine uranium distribution for biokinetic modelling.

Neurotoxicity is assessed by (a) a battery of behavioral tests to assess functional consequences and (b) conduction velocity studies in motor nerves to uncover any peripheral neuropathies. Behavioral tests have frequently been employed to detect and characterize potential neurotoxic effects in rodents and have been used extensively in animal toxicity studies[44]. The neurobehavioral battery consists of (i) a functional observational battery (FOB), which is a series of tests designed to assess the neuromuscular, autonomic, and sensory integrity of the rat[12,36,37,39,40], (ii) an automated test of locomotor activity and (iii) the passive avoidance test used to evaluate memory. Electrophysiological experiments monitor nerve conduction velocity and integrity of the neuromuscular response. Nerve conduction velocity studies have been used clinically for many years to diagnose peripheral neuropathies and can even detect subclinical neuropathy induced by lead exposure[20,41,49].

Markers of renal function in the urine and plasma are used to assess nephrotoxicity. Altered creatinine clearance and proteinuria can indicate glomerular damage although tubular changes can also contribute. Increased urine content of enzymes such as lactate dehydrogenase (LDH) and N-acetyl-β-glucosaminidase (NAG) have been interpreted to reflect tubular damage[46]. In addition, appearance of glucose in the urine, can indicate alterations in tubule reabsorption. These markers have demonstrated sensitivity with acute uranium nephrotoxicity[8,9,31,63] and should indicate any toxicity that might result from long-term exposure to DU fragments.

Capsule formation and the sporadic release of pseudocyst fluid-contents can significantly influence the time course and concentration of uranium distributed through the body. The encapsulation process and pseudocyst formation is characterized at the time of euthanasia (1, 6, 12, 18 months after implantation), surrounding tissues are histologically examined and any capsular fluid is analyzed for its uranium content. In addition, tissues that are known to accumulate soluble uranium or uranium particulates (liver, bone, kidney, spleen)[19,27,29,30,61,62] are histologically evaluated.

Although the distribution of uranium in the rat has been characterized for a variety of routes of internalization (inhalation, ingestion, and parenteral administration of soluble compounds), this information is not available for embedded fragments. We are measuring uranium in urine, plasma, kidney, bone (tibia and skull), liver, spleen, brain, and skeletal muscle that is proximal and distal from the

embedded pellets. Uranium is transported in plasma and urine and is stored in kidney and bone[19,27,61,62]. Uranium has been detected in the liver and spleen of animals[19,29,30] as well as in human subjects[23]. The skeletal muscle is being sampled to determine the local concentrations of uranium. The brain was chosen because of the paucity of data and the need to assess whether any neurological effects observed were due to the direct or indirect interaction of uranium in the body. These data will allow a rat biokinetic model for implanted DU fragments to be developed.

METHODS

Subjects: Sprague-Dawley rats (8-10 weeks of age) are maintained 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, rats are quarantined and screened for diseases. Except during urine collection, all animals are housed in plastic microisolator rat cages with hardwood chips as bedding. Commercial rodent chow and water are provided *ad libitum*. Rats are on a 12-hr light/dark cycle.

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 is ^{238}U (99.75%), ^{235}U (0.20%) and trace levels of ^{234}U. This is the same DU alloy used in U.S. military munitions. Tantalum (Ta) fragments were obtained from Alfa Products, Ward Hill, MA. Ta was chosen as the control substance because it is a biologically inert metal[22] with a similar mass to uranium and is frequently used in human prostheses[21,53]. Each fragment (both DU and Ta) is approximately 1 mm diameter x 2 mm long.

Surgery: The DU and Ta pellets are cleaned and chemically sterilized prior to implantation. The pellets are immersed in industrial detergent, rinsed in absolute alcohol, soaked in 50% nitric acid solution for 3 min and then rinsed with acetone. This procedure completely removes the oxide formation on the surface of the DU pellet[55]. Anesthesia is induced with ketamine hydrochloride (50 mg/kg) in combination with xylazine hydrochloride (10 mg/kg), given i.m. These injections are administered in the lumbar muscles to prevent irritating the site of implantation.

Fragments are implanted within the gastrocnemius muscle spaced approximately 8-10 mm apart on the lateral side of each leg. The surgical sites are shaved and cleansed with betadine, a topical disinfectant, prior to surgery. Scalpel incisions are made through the skin and pellets are inserted into the muscle with a trochar (16 gauge needle with plunger). Incisions are closed with absorbable sutures and surgical cement. Rats are closely monitored following surgery until they are ambulatory and an analgesic (Demerol, 10 mg/kg, i.m.) is administered if needed. A veterinarian regularly examines the surgery sites for signs of inflammation, infection and local DU toxicity.

Behavioral neurotoxicity: The functional observational battery (FOB) consists of behavioral evaluations (home-cage, handling and manipulative) and several physiological measures. The parameters to be recorded are listed below and grouped according to the following functional domains: 1) Autonomic: lacrimation, salivation, palpebral closure, piloerection, defecation, urination, 2) Sensorimotor reactivity: tail pinch response, tactile response, click response, approach response; 3) Neuromuscular: gait, foot splay, forelimb and hindlimb grip strength, righting reflex, and 4) CNS Excitability: arousal, posture, ease of removal from cage, handling reactivity, convulsions, and locomotor activity.

The observer is blind as to the identity of each group. The behavioral battery commences with brief home cage observations during which time the observer describes the posture, and the existence of tremors or convulsions, and palpebral closure. The rats are then removed from their cage and rated for ease of removing and handling. While handling the rat, presence of piloerection and the degree of lacrimation and salivation are observed. The animals are then placed in an open-field with a perimeter barrier on clean absorbent white paper for 3 min. The number of rears, the gait, level of alertness, stereotypy (repetitive movements e.g., head weaving), unusual behaviors (e.g., writhing), and the number of fecal boli and urine pools are recorded.

Sensorimotor responses also are determined and include: approach response to a blunt probe, touch on the rump (tactile response), click response (auditory response), and pinch on the tail using forceps. Next, neuromuscular responses are determined and include: righting reflex, forelimb and hindlimb grip strength using digital strain gauges[37], and landing foot splay[12]. The animals are weighed and rectal temperature determined using a digital thermometer. The FOB is conducted during the light portion

of the light-dark cycle. Details of the FOB tests can be found in Moser et al.[40] and McDaniel and Moser[36].

Approximately, 1 hr after the FOB, the rats are monitored for horizontal and vertical locomotor behavior. Motor activity is recorded for 1 hr using automated photocell activity cages (Digiscan Analyzer, Omnitech Electronics, Columbus, OH). On the day following the FOB and motor activity tests, animals are trained on a passive avoidance test. This test is used to determine whether DU affects memory function. The tests are conducted in a passive avoidance apparatus (San Diego Instruments, San Diego, CA) that consists of 2 chambers (1 lighted, 1 darkened) separated by a sliding door. The animal receives a training trial during which time it is initially placed into the lighted chamber. The natural tendency is for the rat to enter the darkened chamber. When it does, it receives a mild foot shock. During this acquisition phase, the rats are tested for eight trials or until criterion is met. The criterion is 2 consecutive trials during which the rat does not cross into the darkened chamber. Each trial is 3 min in duration with a 1 min intertrial interval. Seventy-two hours later the rat is placed into the lighted chamber and retested. A comparison is made with the initial training session to see if memory of the task has been retained.

Conduction velocities: One week following the behavioral testing, the rats are evaluated electrophysiologically. Rats are anesthetized with ketamine (80 mg/kg) with xylazine hydrochloride (4 mg/kg) i.m. (supplemented as necessary). The right sciatic nerve is exposed and bipolar stimulating electrodes are positioned along the nerve in the thigh close to the sciatic notch and in a second location close to the knee. A recording electrode is inserted into the medial gastrocnemius muscle to monitor the compound muscle action potential. Nerve temperature is monitored and maintained near 37° C with a heat lamp. Nerves are stimulated at a frequency of 0.2 Hz. Stimulus intensity is varied between approximately 10 and 100 V (0.1 ms duration) to determine the input-output relationship and the supramaximal stimulation parameters to use. Five muscle responses are averaged and the latency, duration and amplitude of the potentials are measured. Conduction velocities are calculated by dividing the distance between the stimulating electrodes by the average latency difference between the time of onset of the compound muscle action potentials.

Duration of the muscle action potential reflects the synchrony of discharge. In general, the distal stimulating electrode will produce a faster, larger response than the proximal electrode. Greater dispersion and greater decrease in amplitude than normal would suggest nerve damage. For example, demyelinating disorders cause dispersion of the muscle action potential by slowing the nerve conduction velocities[5,50]. If dispersion occurs over a short segment, compression neuropathy may be indicated[5].

All stimulation and recording are controlled by a 486 PC using standard electrophysiological software (Axon Instruments). Data are analyzed with routines written in AxoBasic (Axon Instruments) and statistical analysis is done with RS/1 (BBN Software Products) routines. Two-way analysis of variance (for time and dose) is used to compare differences among the experimental groups.

Sample collection: Following behavioral testing, blood and urine samples are obtained from all rats for analysis of renal function. To safely collect the blood samples, rats are immobilized by placing them in a Plexiglas restrainer. During each collection, 0.3-0.5 ml of blood is obtained from the tail vein using a 22-gauge needle. The blood is then centrifuged for 5 min at 3,000 X g. The serum is analyzed for uranium levels and/or for biochemical indices of renal function. Serum is stored at -70°C until ready for analysis.

Urine samples are collected by housing the rats in individual metabolism cages (23.5 cm diameter X 12 cm high) where they have continuous access to food and water. However, since these housing procedures have been shown to induce stress and thus increase the toxicity of uranium[4], the rats are acclimated to the metabolic cages for 5 days before the study begins. The metabolic cages are disinfected and decontaminated between each animal use. The 24-hr urine collection sample is obtained from each rat and the volume recorded (10-20 ml). Urine collection at 4°C is unnecessary since enzyme activity has been shown to be stable at room temperature for up to 24 hours[63]. After collection, urine is filtered to remove any debris and stored in plastic containers at 4°C until analyzed (less than 1 wk).

Evaluation of renal function: Measurement of urine volume and osmolarity, urine levels of NAG, LDH, glucose, total protein, creatinine and blood levels of glucose, urea and creatinine are used as

indicators of renal function. In addition, since weight loss may be indicative of nephrotoxicity, all the rats are weighed weekly throughout the study. Osmolarity of the urine is measured with a vapor pressure osmometer (Model 5100B, Wescor, Inc.). A Kodak Ektachem 700 Analyzer is used to determine plasma and urine levels of creatinine, glucose and urea. Total urine protein is measured with a dye-binding assay (Coomassie Blue, BioRad) sensitive down to 1 μg. The activity of NAG is measured by the methods of Tucker et al.[56] using 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide as the fluorescent substrate (excitation wavelength=356 nm; emission wavelength=446 nm). The dilution of the urine for this assay eliminates the effects of any inhibitors present[56]. For LDH measurements, 1 ml of urine is dialyzed for 4 hr at 4°C with 1 liter of deionized water. LDH is quantitated with a colorimetric assay that measures a reaction product which is proportionate to LDH activity (Oxford Biomedical Research Inc). Only 50-100 μl of fluid (urine or plasma) are required for each of these assays.

Although, urine volume and osmolarity can vary greatly with fluid intake, these measures provide physical indicators of renal function. For example, acute kidney failure drastically decreases urine volume, while moderate renal toxicity can increase urine output, as is seen with uranium exposure (e.g., [11]). Osmolarity can reflect the ability of the kidney to concentrate (or dilute) the urine. Plasma urea also changes with renal insufficiency. Since the rate of urea formation is proportionate to the rate of protein metabolism, other factors such as hepatic injury or altered protein intake can affect the measured urea in plasma. A small concentration of protein is normally present in the urine. Increases in total urine protein could result either from glomerular leakage or failure of tubule reabsorption. Urinary enzymes are sensitive, non-invasive markers of toxicity primarily in the kidney tubules[46]. NAG is a lysosomal enzyme found in proximal renal tubule cells. LDH is a cytosolic enzyme of the tubular epithelium.

Creatinine clearance is a commonly used measure of glomerular filtration rate in the rat despite a significant but constant tubular secretion. The use of an intrinsic metabolite has an obvious advantage over inulin or mannitol which (although not secreted) must be infused. Interpretation must be cautious since tubular injury with uranium could cause an underestimate of the glomerular filtration rate regard-

less of the marker used[8]. Creatinine clearance (C_c) is calculated from the equation: $C_c=U_c*V_u/P_c$ where U_c and P_c are the creatinine concentrations in urine and plasma, respectively, and V_u is the rate of urine production (ml/min).

Appearance of glucose in the urine occurs when the tubule reabsorption maximum from the filtrate is exceeded. This can occur with hyperglycemia or with a decrease in tubular reabsorption capacity. Measurement of both urine and plasma glucose help to distinguish between these two possibilities. Changes in reabsorption is reflected in the calculated fractional excretion (FE): $FE = (U_g/P_g)\div(U_c/P_c)$; where U_g and P_g are the glucose concentrations in urine and plasma, respectively.

The proposed assays provide a broad spectrum of measures of kidney toxicity. Many of these substances have been shown to be very sensitive in acute uranium toxicity[8,31]. Glucose is one of the most sensitive indicators[8,9] showing increased urine glucose, without concurrent increases in plasma. LDH and to a lesser extent NAG increase following uranium exposure[8,31]. A transient increase in urine volume and the appearance of protein in the urine also occur with acute uranium toxicity[31]. These measures are used together as indicators of kidney toxicity and carefully interpreted and correlated with histopathology. Two-way ANOVA is used to test the statistical significance of any changes.

Histopathology. Immediately following euthanasia on the day of electrophysiological analysis, tissue samples from bone (tibia, skull), hippocampus, sciatic nerve, kidney, liver, spleen and fragment capsule with associated skeletal muscle is obtained for histological examination or uranium measurement . Based on the literature, these are the most likely tissues to show increased levels of uranium[19,27,29,30,61,62]. Standard procedures for handling biologic specimens are used in the preparation of the samples. Tissues are perfused, embedded, mounted and stained with hematoxylin and eosin stain (H & E)[34]. Specialized stains are used to demonstrate specific lesions or further delineate lesions not well defined by the H & E stain. For example, silver stains are used on neural tissue to delineate nerve fiber disruption or degeneration[34].

The pathologist evaluating the tissue is blind to the experimental group from which the tissue was obtained. The pathologist generates a 0 to 4 scoring system to evaluate the degree of microscopic changes observed; where 0=no change, 1=minimal change, 2=mild change, 3=moderate change, and

4=marked or severe change. All tissue changes observed in the rats implanted with DU are contrasted and compared to the identical tissues taken from the controls. If there are significant changes noted in a particular system, for example the renal system, a detailed statement of criteria for 0-4 scores is stated by the pathologist at the time of interpretation.

Uranium measurement Tissue samples are frozen and shipped by overnight courier on dry ice to Battelle, Pacific Northwest Laboratories for analysis of uranium content. The samples are stored at -70 C until the wet ashing procedure. Wet ashing consists of 12 cycles of treatment of the samples (over 3 days) with 2 ml of 16 N nitric acid followed by several hours of heating, brief cooling, addition of 0.5 ml of 30% hydrogen peroxide and reduction of the volume to approximately 0.5 ml. After this, samples are heated to dryness, dissolved in 2 ml of 4 M nitric acid with warming and filtered through 0.45 μm syringe filter units. For analysis, 0.5 ml of sample or identically handled standards are dissolved in 2 ml of Uraplex reagent. The samples are analyzed with a Kinetic Phosphorescence Analyzer (KPA-11, Chemchek Instruments Inc, Richland WA). Background measurements are made using 4 M nitric acid. Calibration curves are established prior to sample analysis. Measurements include analysis of relative standard deviations and correlation coefficients of the luminescence decay curve.

RESULTS

DOSE RANGING STUDY

The first aim of our study was to determine appropriate doses for the subsequent toxicological analysis. Our pilot studies revealed that 8 DU pellets were well tolerated by the rats despite high urine levels of uranium; biochemical and histopathological damage were not evident. To determine the high dose for the present study we attempted to maximize the implanted number of DU pellets that could be tolerated by the rats and produced kidney uranium levels in the range of 0.7-1.4 μg/g. We implanted 4,6, 16, 18 and 20 pellets into 4 animals each and evaluated urine, plasma and kidney levels after two weeks. The two-week time point was chosen to allow the urine and kidney levels of uranium to stabilize following implantation. Tantalum pellets were implanted in 4 animals for controls. As illustrated in Figure 1, the uranium (U) levels in urine, plasma and kidney were significantly increased in all DU implanted animals. There was a wide variation in the levels from animal to animal but a dose dependence was evident. Animals implanted with 4 DU pellets averaged 0.66 ± .20 μg U/g while 20 pellets resulted in 1.22 ± .31 μg U/g in the kidney. Urine levels in animals with 4 DU pellets were 83.3 ± 37.2 μg U/l and in animals with 20 pellets were 262.0 ± 99.2 μg U/l. In comparison, tantalum (Ta) implanted animals showed 0.002 μg/g U in kidney and 2.66 μg U/l in urine. None of the implanted or control animals demonstrated any obvious health problems. No significant differences were observed in the biochemical analyses of urine and serum: NAG, LDH, protein, osmolarity, glucose, urea, or creatinine. Based on these data, we chose 20 DU pellets as our high dose and 4 DU pellets as our low dose. The intermediate dose was calculated as the approximate logarithmic mean of the high and low doses: 10 DU pellets. All animals always received a total of 20 pellets, 10 in each hindlimb. For example, the low dose of 4 DU pellets consisted of 2 DU pellets and 8 Ta pellets in each rear leg.

THIRTY-DAY TOXICITY STUDY

Neurotoxicity: Animals implanted with 4, 10 or 20 DU pellets, 20 Ta pellets and non-surgical

controls were evaluated for body weight and for changes in the functional observation battery (FOB), locomotor activity, and passive avoidance learning. The rats were weighed weekly and all steadily gained weight. No significant differences in body weigth were observed among the 5 experimental groups at any time point (Figure 2).

There were no significant differences among the 5 experimental groups for their performance on the passive avoidance test. All animals learned to avoid the mild foot shock within 2-3 trials (n=7-10/group). The latency to initial crossover (approximately 60 sec) was also not significantly different among groups (Figure 3). In addition, the FOB did not reveal any significant differences among the experimental groups. Grip strength of the hind- and forelimbs was unaltered with DU exposure (Figure 4). All sensorimotor, neuromotor and autonomic responses appeared normal. Locomotor activity did not show significant differences among the experimental groups. As expected in all groups, the initial activity was high when the animals were first placed in the activity boxes because of exploratory behavior which subsided over time (Figure 5). Conduction velocity measurements from the nerves of the hind limb also did not reveal any significant differences among the experimental groups.

Nephrotoxicity: The urine and serum samples from 6 rats from each of the 5 experimental groups have been analyzed for biochemical markers of kidney toxicity. Osmolarity, 24-hour volume and pH were not significantly altered by experimental treatment. Urine levels of protein, LDH, NAG, glucose, and urea nitrogen were unaffected. Serum levels of urea nitrogen and glucose were also unaffected by experimental procedures. The data for urine glucose, protein and NAG are shown in Figure 6. Creatinine clearance was not different among the experimental groups: Non-surgical 2.9 ± 0.5; Ta controls 2.6 ± 0.7; 4DU 2.7 ± 0.6; 10DU 2.8 ± 0.2; 20DU 2.6 ± 0.6 (n=8-10 per group). Fractional excretion (FE) of glucose (glucose clearance/creatinine clearance) was similarly unaffected by the experimental procedures with all groups showing an FE approximately equal to 0.001 with high variability among all of the animals.

Histopathology: Tissues have been excised and fixed for histopathological analysis. These tissues (bone: tibia and skull, kidney, spleen, liver, brain, and muscle: proximal and distal) have not yet been

processed and evaluated. During excision of the pellets it was observed that the depleted uranium pellets but not the tantalum pellets were associated with adherent tissue. In the 30-day animals, a capsule had not fully formed around the pellets and no dark fluids were localized with the fragments.

Uranium distribution: Two of the 7 sets of tissues and fluids slated for analysis of uranium content have been measured by Batelle Pacific Northwest Laboratories. The remainder of the tissues have been shipped recently to the Batelle laboratories but the data have not yet been received. Tissues from two rats from each of the 5 experimental groups were analyzed and provide some interesting preliminary findings. As expected uranium in the urine and kidney was very dose dependent (Figure 7a,c). Serum uranium, in contrast, was less consistent (Figure 7b). Similarly, uranium clearly distributed to bone, both skull and tibia, in relation to the number of DU pellets (Figure 8). The levels in bone were comparable to the levels in kidney. Muscle from the forelimb also showed a dose-dependent distribution, although at much lower levels. Samples from muscle near the pellets showed extensive scatter. It is our belief that the high levels found in some of the samples resulted directly from fragments of the implanted pellets in the samples. This "contamination" could have occurred during the removal of the pellets at time of necropsy or might have happened by flaking and redistribution *in vivo*. Further analyses are expected to clarify this issue.

Spleen samples showed accumulation of uranium (Figure 9a). However, liver samples only had background levels of uranium (Figure 9b). Brain tissue showed little accumulation of uranium with 4 or 10 embedded pellets. Yet, in animals with 20 DU pellets, brain uranium levels reached levels comparable to those in the spleen of the same animals (Figure 9c).

CONCLUSIONS

The dose ranging study provided the data for establishing the appropriate numbers of DU pellets to be used in the toxicological studies. We found that 20 pellets of DU met our criteria for the high dose while 4 pellets was determined to be appropriate for our low dose. Ten pellets was calculated to be the appropriate intermediate dose for the evaluation of the toxicological effects of depleted uranium. The 30-day study was initiated and almost all of the 30-day experimental animals have been now been euthanized. The biochemical analyses have been completed on approximately two-thirds of the samples. Uranium analysis has been completed on only two animals from each experimental group but the remaining samples from an additional 5 animals per group are currently under evaluation. Histological evaluation of the collected tissues will be initiated shortly. This month, we are initiating the surgical implantation of the depleted uranium pellets for all the remaining time points (6 months, 12 months and 18 months). Although our current findings do not demonstrate significant toxicological effects within the first thirty days of exposure, the levels of uranium in various target tissues suggest the potential for measurable toxicity with chronic exposure. Furthermore, continued analyses of the toxicological endpoints and localization of uranium will allow an improved model of the biokinetic distribution of the metal.

The high dose of DU used in our study (20 DU pellets) produced kidney uranium levels of approximately 1.2 μg U/g kidney wet weight within 2 weeks. These levels were found to be sustained for at least 30 days. Although these levels of uranium have been reported by others to cause renal toxicity, our data do not demonstrate any significant signs of nephrotoxicity. Chemical form, route of administration, and the dose of uranium exposure can all affect the toxicological consequences and distribution of uranium. The uranium levels that result in kidney toxicity are a matter of debate in the literature. The Nuclear Regulatory Commission has set 3 μg/g as an lower limit for toxicity. However, several studies reflect damage at lower levels. For example, Diamond et al.[9] observed acute, but reversible, renal toxicity in rats at levels as low as 0.7 μg/g following i.v. injection of uranyl fluoride. In contrast are the studies of Leach et al.[29,30] demonstrating no renal toxicity in rats following chronic inhalation

exposure to uranium dioxide producing kidney levels up to 1.1 μg/g. The absence of effects in our present study does not preclude the possibility that with longer exposures to the uranium, toxicity will develop.

While bone and kidney are well accepted as primary reservoirs of uranium, other organs accumulate the metal to varying degrees. With oral administration of 8 mg/kg/day uranyl acetate for 4 weeks, Ortega et al.[45] found kidney, liver and thyroid as primary sites. A single intravenous injection of sodium uranyl tricarbonate distributed in 24 hours to kidney, liver, spleen and bone but at 30 days was detected predominantly in spleen and bone[60]. As expected, our preliminary data reveal that 30 days after implantation of DU pellets, levels of uranium in bone and kidney are high. Both marrow bones (tibia) and non-marrow bones (skull) accumulated uranium. Concentrations in the liver were not above background while concentrations in the spleen and muscle were significantly higher. Muscle levels raise the possibility that neuromuscular deficits will develop through heavy metal effects. Spleen levels cause concern that immunological consequences could arise. Future studies are planned to address this possibility. In agreement with the literature[1,45] uranium did not accumulate in the brain at the lower doses of DU. However, at the high dose, the levels were comparable to those in the muscle and spleen. This raises the concern that central nervous system consequences will occur with continued high levels of DU exposure. Our later time points planned for this study will address these concerns with behavioral and electrophysiological analyses. Levels of uranium excreted in the urine remained high throughout the 30 days. This suggests that uranium continues to leech out of the DU pellets, although serum levels are relatively low. This is also reflected in the Desert Storm veterans with embedded DU shrapnel who continue to excrete uranium in their urine even years after injury.

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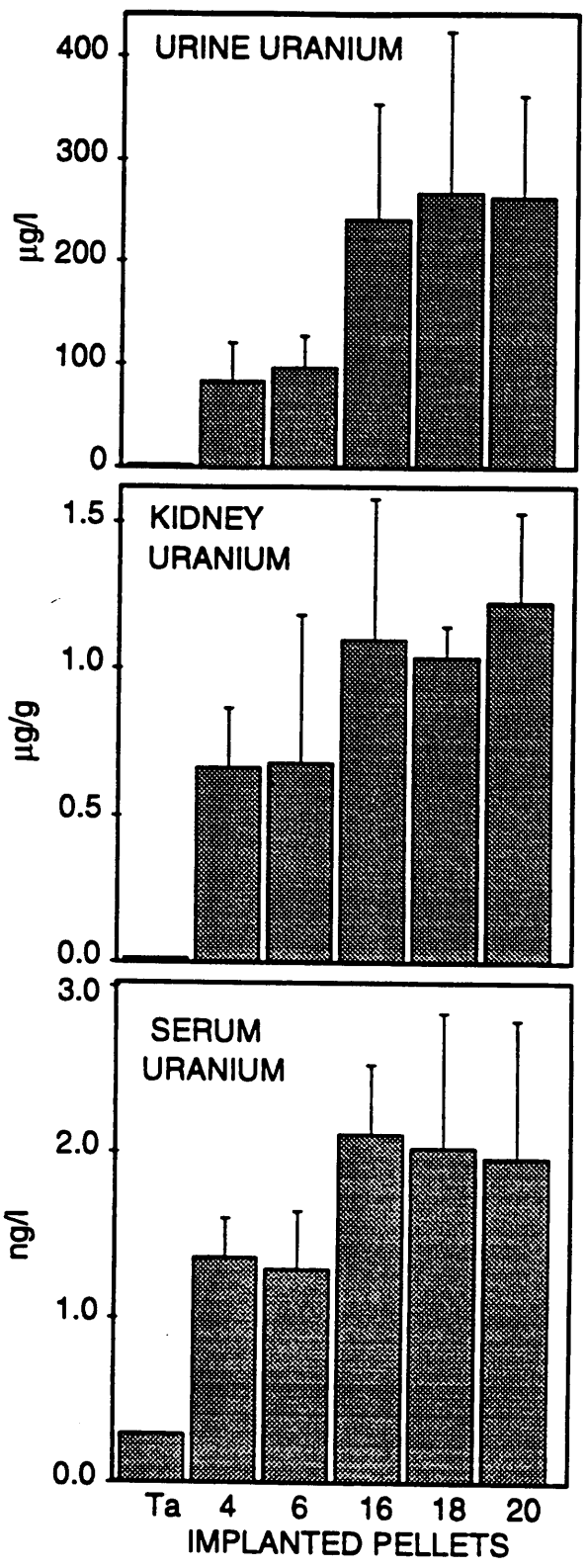

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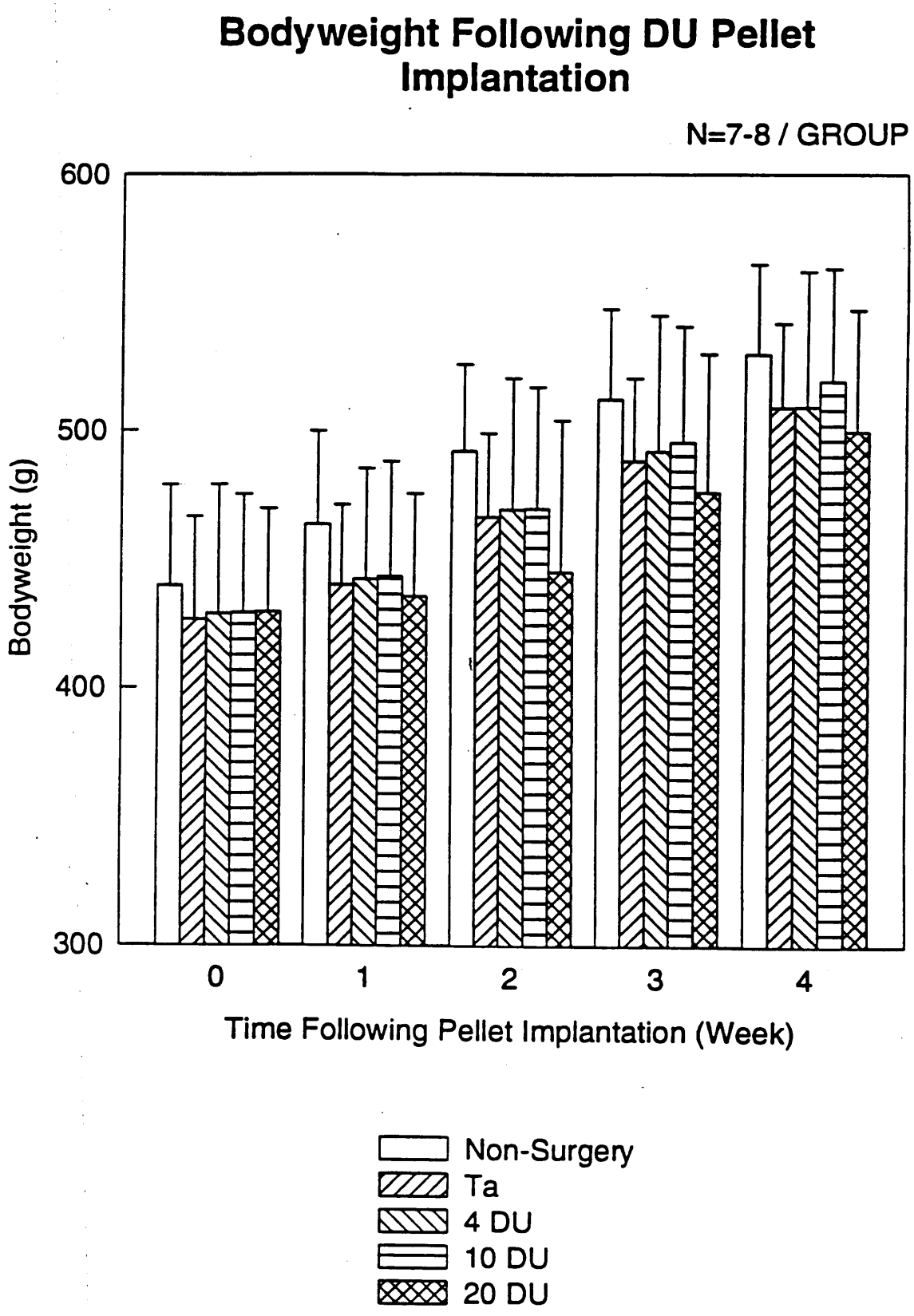

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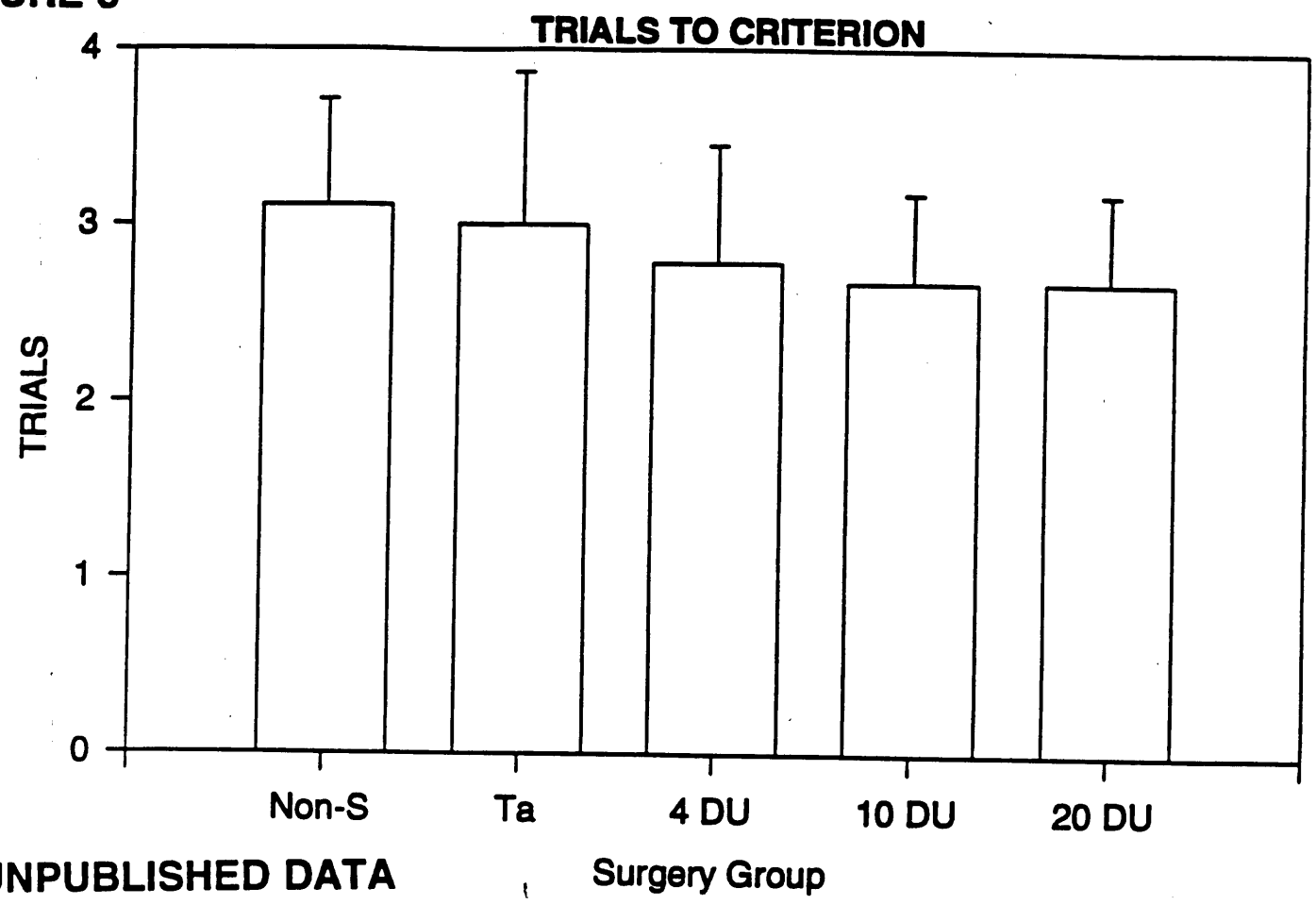

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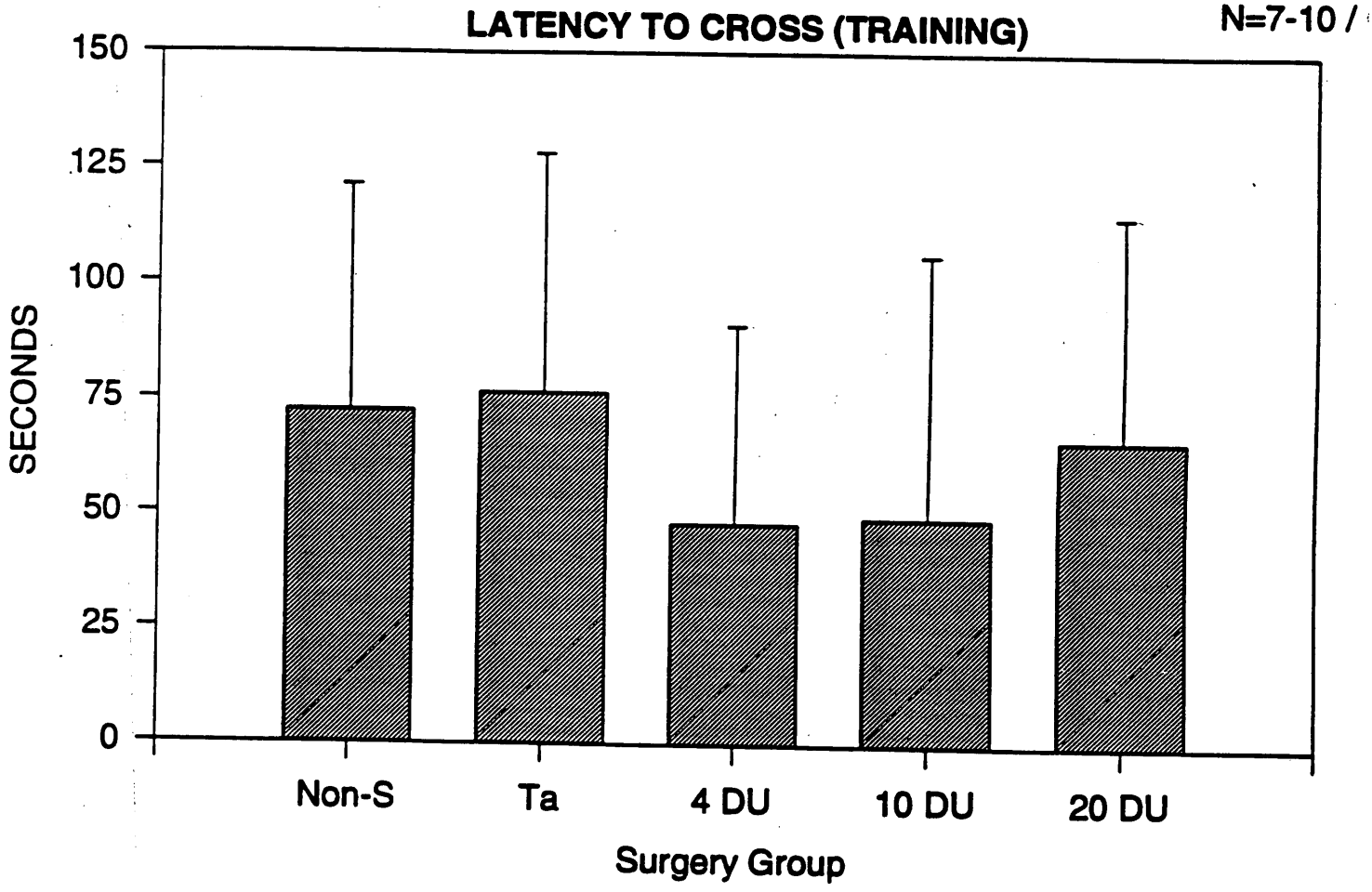

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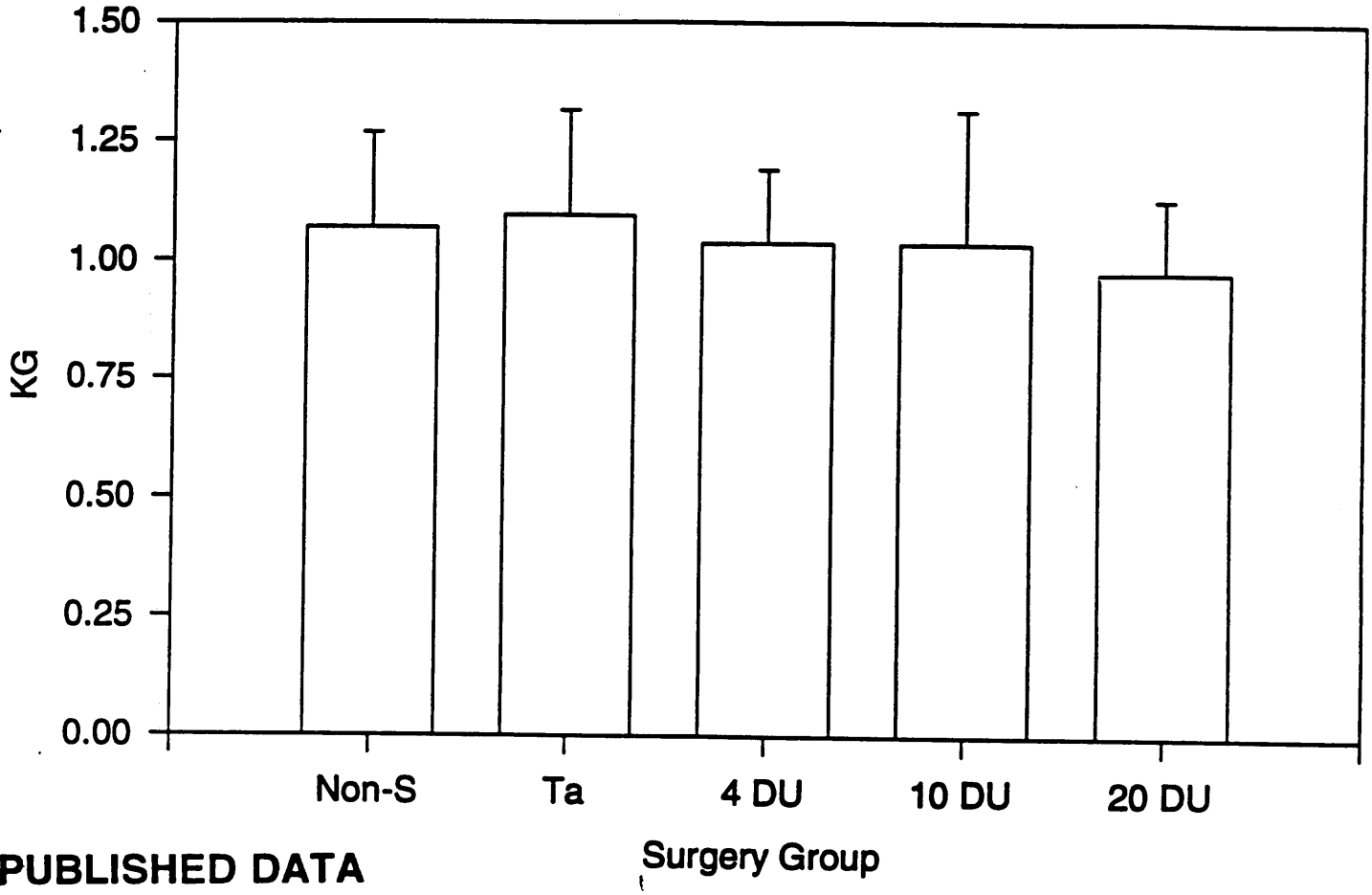

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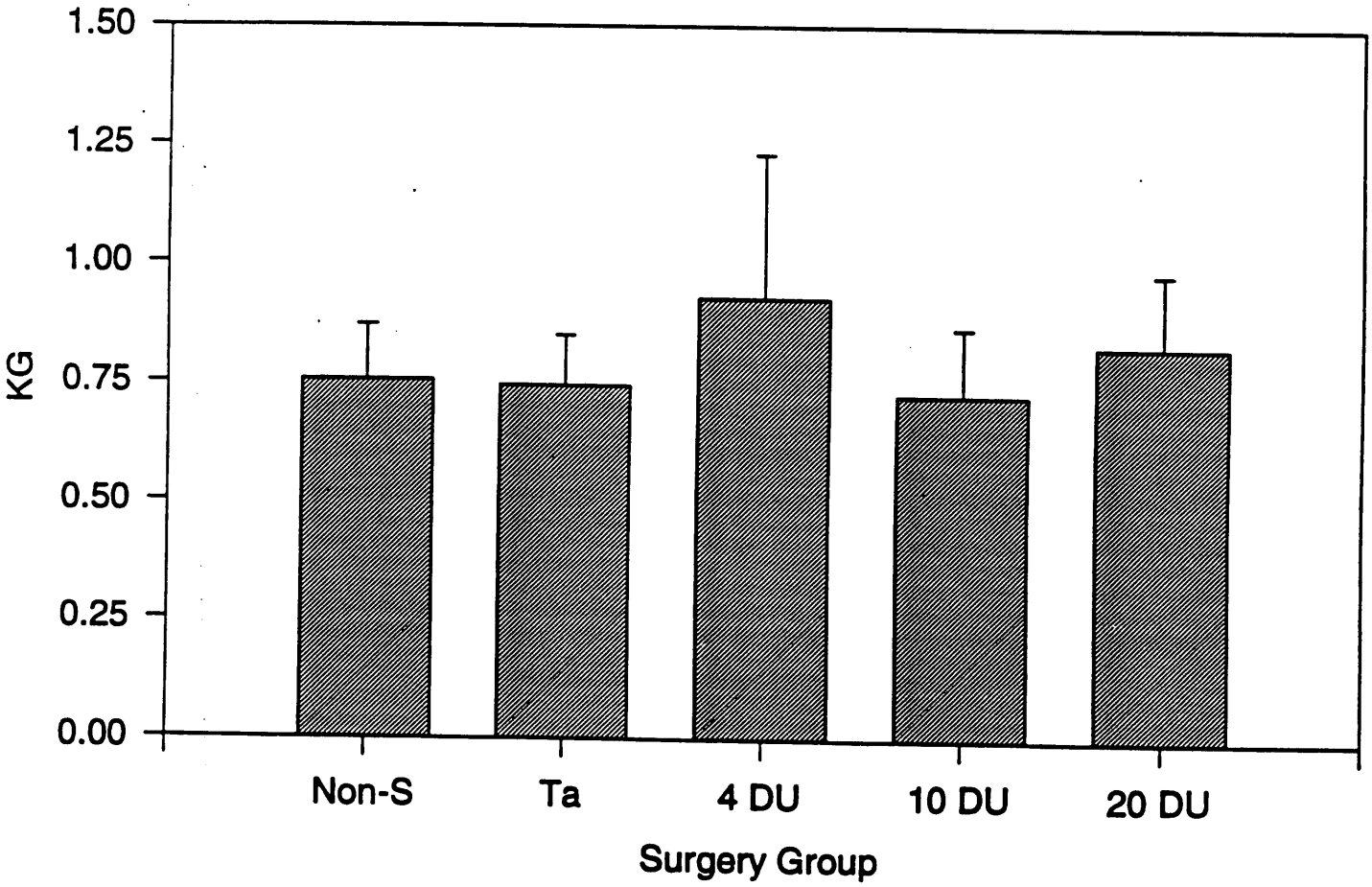
FIGURE 4

HINDLIMB GRIP STRENGTH

N=7-10 / GR



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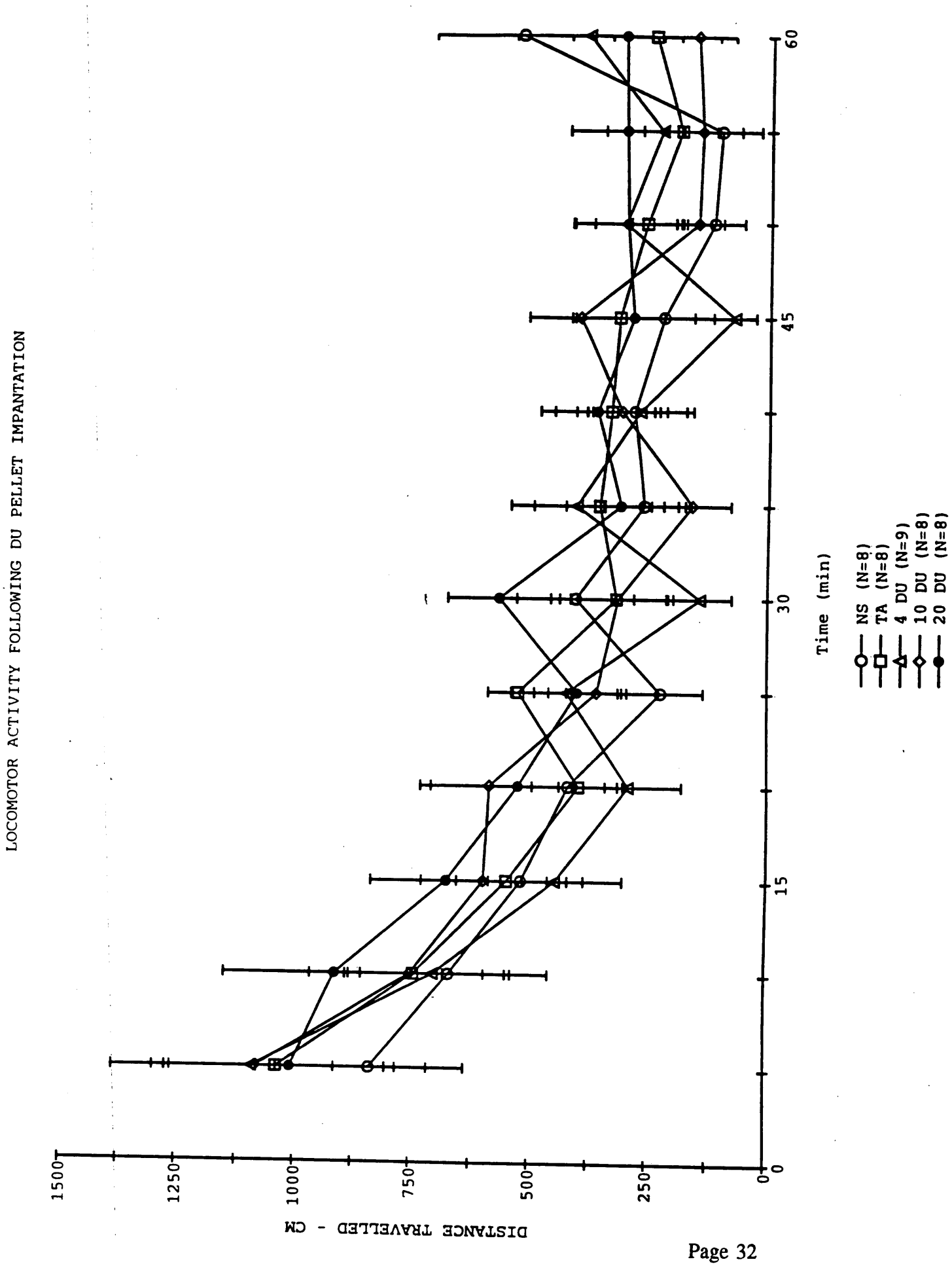

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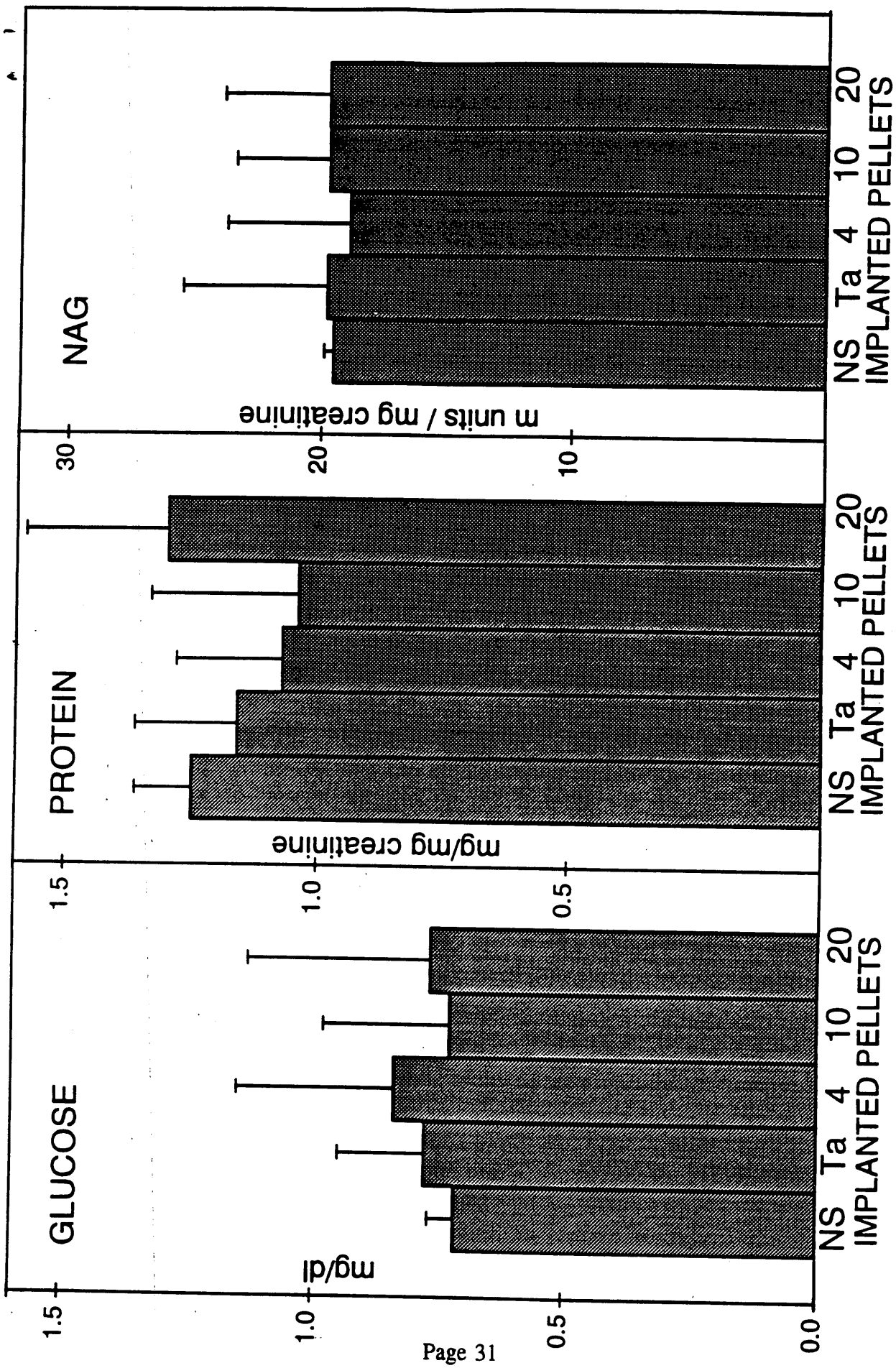

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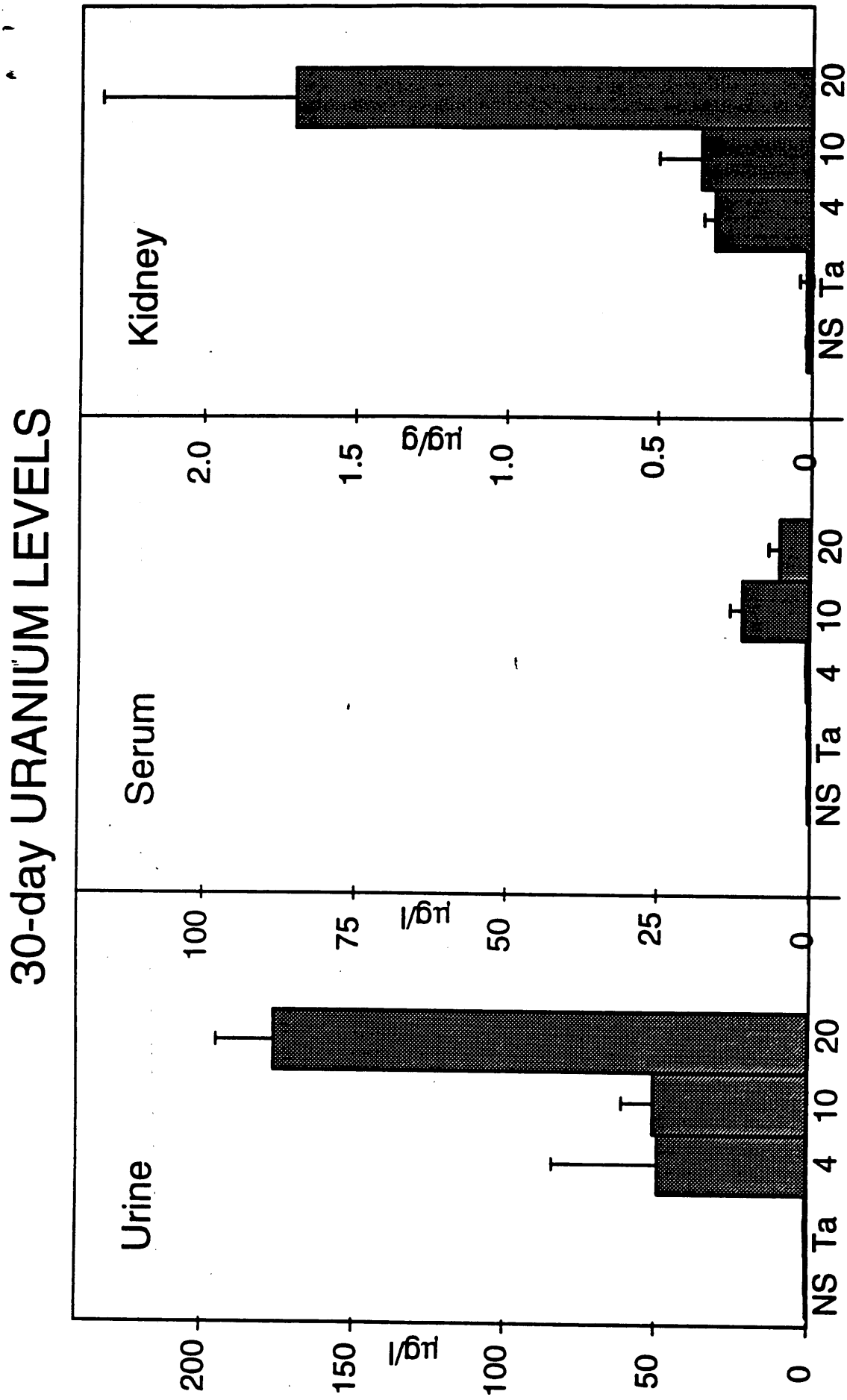

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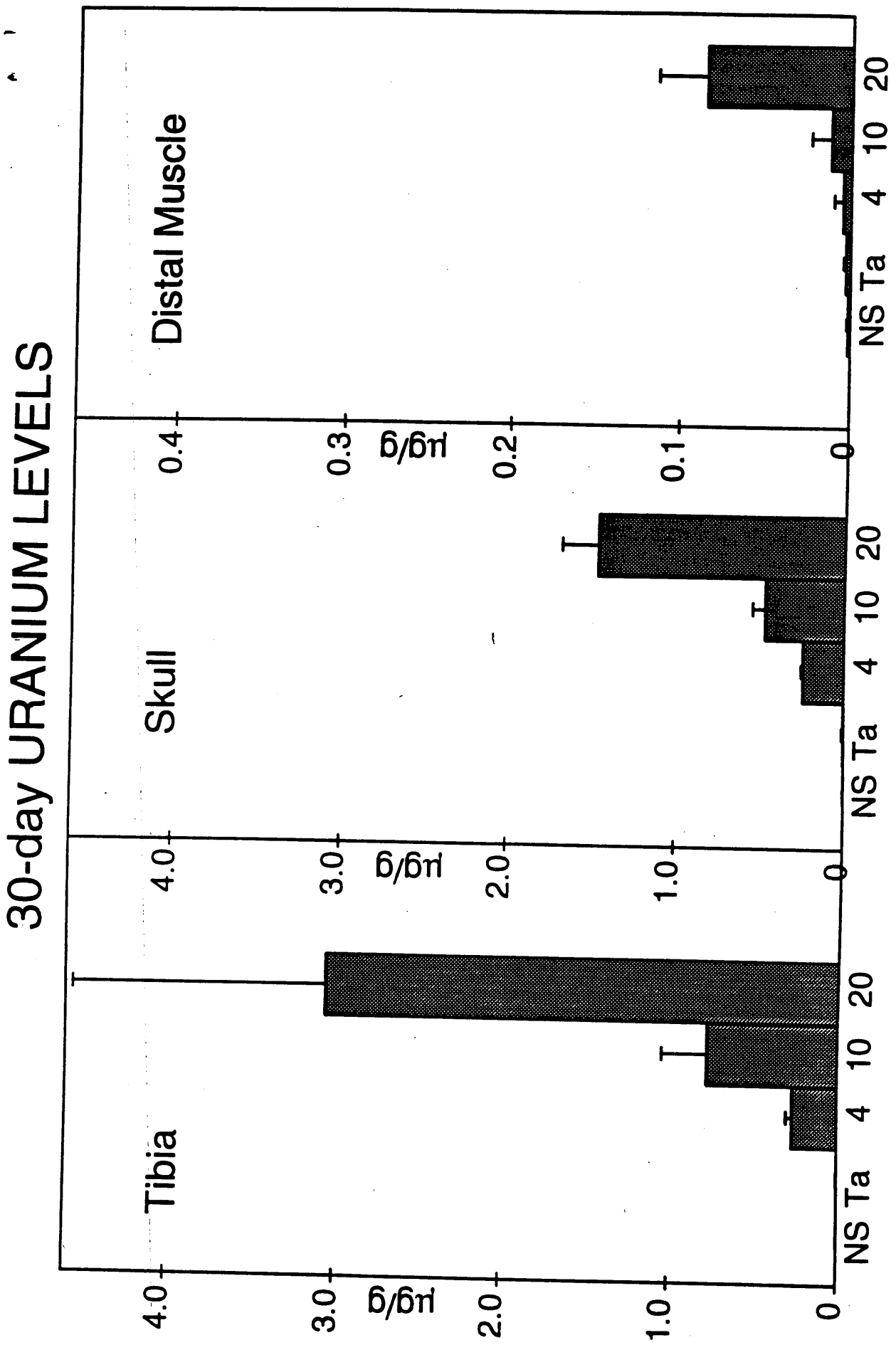

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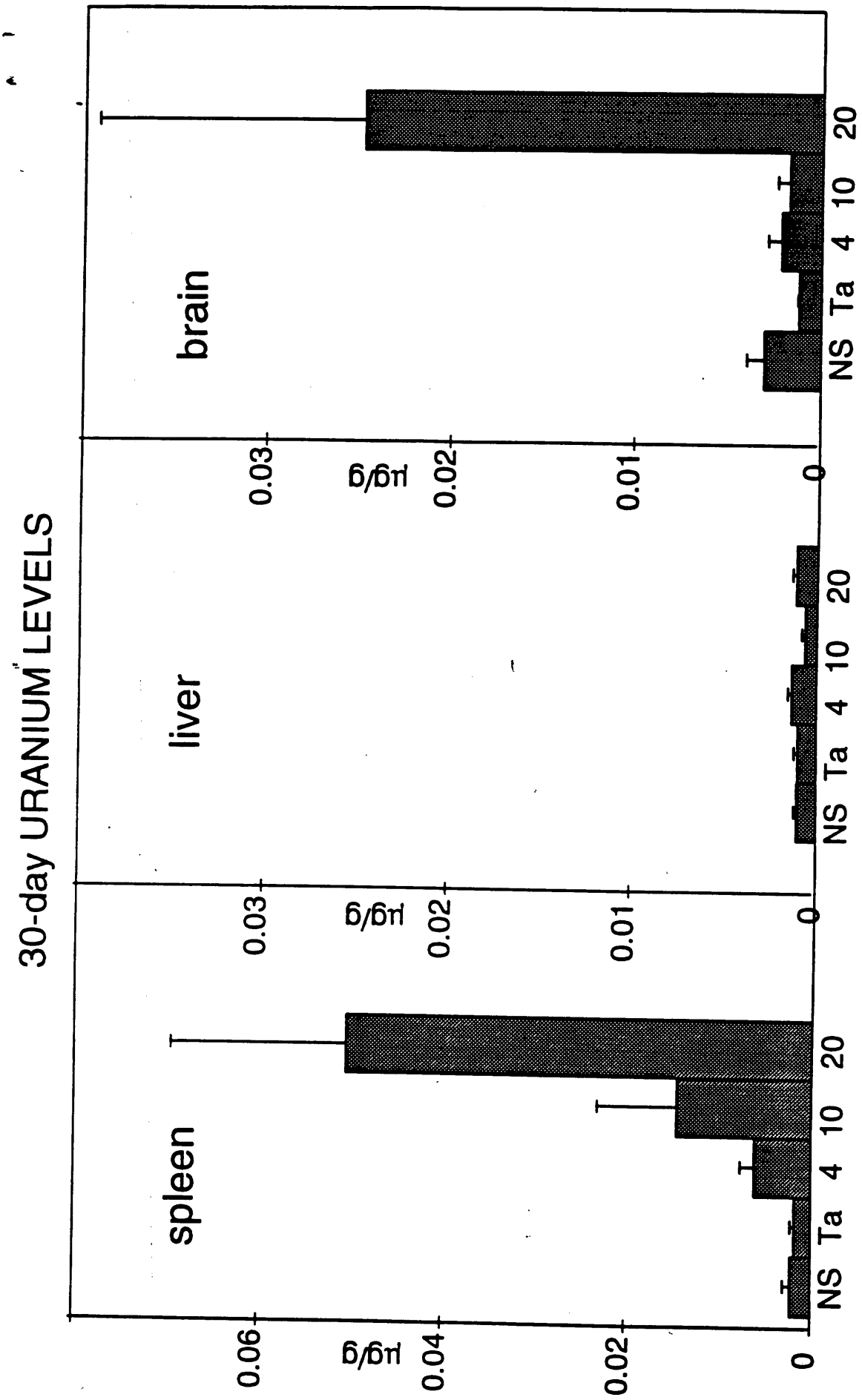

Evaluation of the Health Risks of Embedded Depleted Uranium (DU) Shrapnel on Pregnancy and Offspring Development

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Title of Study: Evaluation of the health risks of embedded depleted uranium (DU) shrapnel on pregnancy and offspring development

Keywords: Depleted Uranium (DU) Fragments, Maternal and Fetal Toxicity, Development, Learning and Memory, Functional Observation Battery, Behavioral Teratology

Abstract: The use of depleted uranium (DU) munitions in Operation Desert Storm resulted in 36 patients with embedded DU fragments. Standard medical guidelines for fragment injuries (based upon non-radioactive, non-DU fragments) were used to determine which fragments should be removed, resulting in many of the fragments being left in place. Although all of the current patients are male, women soldiers will be injured by DU munitions in future conflicts because of their increased presence in combat units and the increased production of DU munitions by other nations. Current assessments and research are focused on the risks to the patient from embedded DU fragments; the prospect of women being injured by DU shrapnel forces a change to include consideration of the fetus. Recent analyses of animal data indicates that the level of systemic uranium in personnel with retained fragments is potentially high enough to adversely affect the developing fetus. This proposal addresses this issue by developing an animal model to examine toxicological and behavioral effects of DU on rats exposed *in utero*. Specifically, we propose 1) to establish a dose-response profile for the chronic effects of embedded DU pellets on kidney function in the female rat, 2) to determine the effects of *in utero* exposure to DU from pellets embedded in the mother on prenatal and postnatal development and behavior and 3) to determine the level of uranium in the fetus exposed *in utero* to DU from pellets embedded in the mother. The results of this study will determine the course of treatment for the female soldier wounded by DU shrapnel.

Proposal Relevance

Approximately 50% of current U.S. antitank weapons contain DU penetrators, and most of the Abrams main battle tanks are armored with DU. During Operation Desert Storm, at least 40 tons of DU munitions were fired by the U.S. Army and Air Force (Daxon, personal communication). Unfortunately, during this conflict, a number of U.S. military personnel were wounded by DU shrapnel. Many of these fragments were not removed because the removal procedure would produce excessive tissue damage. The effect that fragments such as these would have on a female soldier's future offspring is unknown. The need to determine if embedded DU fragments in a female soldier will one day adversely affect her ability to conceive or carry to term a healthy offspring is imperative because women are playing an ever increasing role in military operations. Although there is no human data concerning the prenatal effects of uranium exposure, experiments with laboratory animals have shown teratological effects of acute uranium exposure before and during gestation. We intend to determine if a constant exposure to uranium throughout gestation, via maternally embedded DU pellets, will also be detrimental to the developing fetus. Unlike previous studies, we intend to examine the neurological development of the offspring, which can be affected by many toxicants including other heavy metals such as lead and mercury. Determining the health risks to the fetus exposed *in utero* to DU is vital, considering that fetal toxicity can occur without maternal toxicity. It is especially important to assess the impact of embedded DU fragments on physical and neurobehavioral development because these are the most sensitive indices of fetal toxicity. Neurobehavioral alterations would most likely be seen at DU levels lower than would yield congenital malformations. This proposal intends to investigate the behavioral teratology, not the structural malformations, as these would most likely be seen at higher uranium doses than will be tested for behavioral alterations.

Body of Proposal

1. Background and Significance

Natural uranium (U) consists of three isotopes: ^{238}U (99.276%), ^{235}U (0.718%), and ^{234}U (0.0056%). During the uranium enrichment process two products are produced, "enriched uranium" and "depleted uranium" (DU), that contain different relative ratios of these three isotopes. Enriched uranium contains the higher amount of the fissionable isotope ^{235}U and is used for nuclear reactor fuel and nuclear weapons. DU has a lower ^{235}U content. The DU used by the United States in kinetic energy penetrators is alloyed with titanium (0.75% by weight) to increase its tensile strength and to retard oxidation[27]. Approximately 50% of current U.S. antitank weapons contain DU penetrators, and most of the Abrams main battle tanks are armored with DU. During Operation Desert Storm, at least 40 tons of DU munitions were fired by the U.S. Army and Air Force (Daxon, personal communication). Unfortunately, during this conflict, a number of U.S. military personnel were wounded by DU shrapnel[9, 10, 21]. Many of these fragments were not removed because the removal procedure would produce excessive tissue damage. A radiograph of an injured soldier shows multiple embedded fragments ranging in size from 1 mm to over 5 mm in diameter. Shrapnel fragments as large as 20 mm have been noted in other patients. Uranium bioassays taken over a year after injury indicate that uranium was present in the urine well in excess of natural background, up to 30 •g U/l of urine[41].

The long-term health impact of leaving these radioactive and chemically toxic fragments in place is unknown. Further, military roles are changing significantly and the female soldier now plays a vital part in many combat scenarios. It is therefore important to include female soldiers among those that might be injured by DU shrapnel. Consideration also must be given to the potential harmful effects of *in utero* exposure to embedded DU shrapnel fragments on fetal and offspring development. This is important because animal research has shown that females are less sensitive to the effects of uranium than are males[59]. Thus, while the female may tolerate a greater dose of DU with no adverse effects, the dose may still lead to detrimental effects on the offspring. The fetus is generally more susceptible to central nervous system (CNS) damage by toxicological agents than the adult; consequently, it is especially important to determine the effects of embedded DU on neurobehavioral development[32].

Uranium toxicity. Although the toxicity of embedded DU is unknown, numerous studies have addressed the consequences of inhalation, ingestion and parenteral administration of other forms of uranium[11, 15, 23, 28, 33, 34, 35, 37, 42, 45, 46, 47, 55, 57, 68]. After uranium is absorbed, it circulates in the blood as the uranyl ion forming uranium-carbonate and uranium-albumin complexes. As the uranium-carbonate complex passes through the kidney, it is filtered rapidly by the glomeruli where 60%-80% of absorbed uranium is excreted in the first 24 hours after acute exposure. The uranium that is not excreted is reabsorbed by the proximal tubules where it produces significant toxic effects. Uranium also enters the bone, where it competes with calcium to form complexes with phosphate ions, thus becoming part of the bone matrix[6, 18, 22, 44]. This bone matrix then serves as both a long- and short-term storage site from which uranium has been shown to be slowly released back into circulation[29, 67]. The liver, muscle, and kidney are other major sites of uranium deposition, with a possible long-term storage mechanism in the kidney[29, 67].

Acute morphological and biochemical changes of the kidney result from uranium exposure[11, 31, 36, 44]. Changes in the glomerular epithelial architecture[30], and cellular necrosis in the proximal tubules near the corticomedullary junction of the kidney have been reported in experimental animals after acute uranium exposure[5, 23, 24]. In addition, polyuria, enzymuria, glucosuria, and increased excretion of amino acids have been reported[11, 12, 31, 70]. Acute renal failure can occur following exposure to high doses of uranium[44, 61]. Environmental stressors such as restricted diets or changes in housing conditions have been shown to significantly enhance uranium toxicity[1, 8].

Uranium-induced Fetal and Developmental Toxicity. *In utero* exposure to uranium has recently been shown to produce both fetal and developmental toxicity. For example, administration (s.c.) of uranium in the form of uranyl acetate dihydrate (0.5-2.0 mg/kg/d) to gravid (pregnant) mice from gestational days (GD) 6-15 leads to significant decreases in both maternal weight gain and fetal body weights at GD 18[4]. Soft tissue and skeletal examination of the fetuses also revealed a significant increase in the occurrence of renal hypoplasia in all uranium-treated groups. Skeletal anomalies in these mice included bipartite sternebrae, dorsal

hyperkiphosis, and incomplete ossification of several bo[illegible]milar skeletal malformations were also seen following daily oral administration of uranyl acetate dihydra[illegible]-50 mg/kg/d) in gravid mice during the same period of gestation[17].

While the above results examined the effects of uranium on prenatal development, several studies have been conducted to evaluate the effects of uranium on postnatal development (from birth to age 21 days)[16, 48]. Significant decreases in body weight and body length in the offspring of mice treated with 25 mg/kg/d for 14 days prior to mating have been reported[48]. There were also significantly more dead young per litter at this uranium dose at both birth and day 4. Uranyl acetate given orally to gravid mice from GD 13 to 21 days following parturition led to a significant increase in offspring liver weights in all the uranium treated groups (5.0-50.0 mg/kg/d), and decreased mean litter size on day 21 in the highest dose group (50 mg/kg/d). However, developmental parameters such as pinna detachment, incisor eruption and eye opening were unaffected[16].

Unfortunately, uranium levels in the dam, fetus, or placentae were not measured in any of these fetal and developmental toxicity studies. In order to determine the effects of embedded DU on a developing fetus, it is important to know the *in utero* uranium exposure level, though little work has been done to examine the cross-placental transfer of uranium[3, 19]. While there are distinct anatomical differences between the rodent placenta and the human placenta, little correlation has been shown between the anatomic classification of the placenta and the transfer of xenobiotics between mother and fetus[63]. In rodents and primates, the placenta may act as a barrier, limiting or preventing many toxicological insults to the fetus. This does not appear to be the case with uranium. When ^{233}U was administered intravenously to pregnant rats, almost identical levels of uranium were found in the placenta and fetus[54], indicating little discrimination for uranium by the placenta. The soft tissue levels of uranium in 19- to 20-day-old fetuses were equal to or greater than the maternal liver concentrations. Immature bone also exhibited a greater deposition of uranium than did the adult bone[19].

The effects of DU exposure on behavioral and neural development are unknown[62]. However, *in utero* exposure to other heavy metals such as lead and mercury have been shown to adversely affect postnatal neurological development. For instance, increased distractibility and deficits in perceptual-motor integration occur in children exposed to lead *in utero*[66]. Studies examining IQ performance in children exposed to lead during pregnancy have produced variable results[43, 64, 66]. However, studies examining fetal exposure to mercury in children have reported deficits that include mental retardation and delayed cognitive development[52]. Although the primary sites of action for lead and mercury are different than that for DU, these findings underscore the importance of investigating the impact of heavy metals such as DU on postnatal behavioral and neural development[13].

It is important to make the distinction between the study of the teratological effects of uranium, to which most of the literature refers, and the study of the behavioral teratology of uranium, to which this proposed experiment refers. Behavioral teratology is the appearance of behavioral alterations in the absence of gross structural deformity. Also, these congenital behavioral changes occur after exposure to levels below the teratogenic levels and the critical gestational period extends into late pregnancy, a time not considered susceptible to teratological malformations[69].

2. Hypotheses

This proposal is designed to address three experimental hypotheses.

a. *In utero* exposure of the rat fetus to DU at doses that do not produce maternal toxicity will alter prenatal and postnatal physical and neurobehavioral development.

b. The placentas of rats embedded with DU do not act as a protective barrier for the fetus against uranium toxicity.

3. Technical Objectives (Specific Aims)

Specific Aim I. Establish a dose response profile (by surface area) for the acute effects of embedded DU pellets on kidney function.

Specific Aim II. Determine the effects of *in utero* exposure to embedded DU pellets on prenatal and postnatal development and behavior.

Specific Aim III. Determine the level of uranium that enters the rat fetus exposed *in utero* to DU implanted in the mother.

4. General Research Design and Methods

Subjects. Rats will be maintained 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, rats will be quarantined and screened for diseases. Except during urine collection, all animals will be housed in plastic microisolator rat cages with hardwood chips as bedding. Commercial rodent chow and acidified water (Ph 2.5, using concentrated HCl) will be provided *ad libitum*. Rats will be on a 12-hour light/dark cycle.

DU and Ta Pellets. DU pellets (1 mm diameter x 2 mm long) will be obtained from the Oak Ridge National Laboratories, Oak Ridge, TN. This cylindrical shape was chosen because it is the geometrical average of the shrapnel left in soldiers wounded by either conventional or DU munitions (see appendix 1). DU pellets will consist of 99.25% DU and 0.75% titanium by weight. The uranium isotopes present in DU will be ^{238}U (99.75%), ^{235}U (0.25%), and trace amounts of ^{234}U. This is the same DU alloy used in U.S. military munitions with titanium.

Tantalum (Ta) pellets (1 mm diameter x 2 mm long) will be obtained from Alfa Products, Ward Hill, MA and will be the heavy metal control. Tantalum was selected because (a) it has a similar mass to DU (16.6 g/cm^3 for Ta versus 18.8 g/cm^3 for DU[50], (b) it is relatively inert in a biological medium[26], and © it is commonly used in human orthopedic reconstructive surgery[25].

Surgical Procedures for Pellet Implantation. Before the implantation surgery, the DU and Ta pellets will be cleaned and sterilized. Pellets will be cleaned by immersing them in an industrial detergent and rinsing them in 70% alcohol. They will be sterilized by placing them in a 50% nitric acid solution for 3 minutes, rinsing them with sterile water, and then placing them in 70% ethanol until implantation. These sterilization procedures completely remove the oxide formation from the surface of DU metal[58]. The efficacy of these procedures were verified in the preliminary study in that no infections were seen in any of the rats after pellet implantation.

Anesthesia will be induced with ketamine hydrochloride (50 mg/kg) in combination with xylazine hydrochloride (10 mg/kg) and given i.p. in a 0.5-ml bolus, using a 25-gauge needle. The surgical sites will then be shaved and cleansed with betadine. Pellets will be implanted in each biceps femoris muscle spaced approximately 15 mm apart on the lateral side of each thigh. Using a scalpel blade, incisions will be made through the skin exposing the biceps femoris muscle. The pellets will then be inserted into the muscle by polacing the pellet in a 16 ga needle, inserting the needle into the muscle and then a metal plunger pushes the pellet out of the needle and into the muscle. This procedure proved very effective in the preliminary study.

Rats will be closely monitored following surgery until they are ambulatory, and an analgesic (Demerol®, 10 mg/kg, i.m.) will be administered if needed. A veterinarian and/or a veterinary technician will examine the surgery sites for signs of inflammation, infection, and local DU toxicity. Rats will be treated by the attending veterinarian as required.

Determination of Uranium Levels in the Urine. Uranium levels of the blood and urine will be measured by Quanterra Environmental Services, Richland Washington. The basic procedures are briefly described in Part C.1. The complete procedures are proprietary.

Data Analyses. For all continuous data, two-way ANOVA procedures will be conducted using a between-subject factors of dose and time[65]. If the ANOVA results in an overall effect of dose, or dose x time interaction, post hoc comparisons will be made to control values using Dunnett's t test. Descriptive and rank data (e.g., gait scores and sensorimotor responses) will be analyzed using nonparametric statistics[53]. For all tests, $p < .05$ will be considered significant. Additionally, the litter will be considered the statistical unit of measure.

5. Specific Research Design and Methods

Specific Aim I. Establish a dose response profile (by surface area) for the acute effects of embedded DU pellets on kidney function.

In order to identify the doses (by surface area) of DU to be used in the fetal toxicity studies described in Specific Aims II and III, a dose ranging study will be conducted. This study will determine the number (by surface area) of DU pellets required to reach a nephrotoxic level in female rats. A kidney level of 0.70 μg U/g wet kidney has been shown to produce early signs of kidney damage in male rats, as measured by both biochemical and histopathological changes[12], but our preliminary data indicates that we may need to achieve levels higher than this in our female rats. In addition, it has been shown that female rats and rabbits are more resistant to the toxic effects of uranium[59].

Subjects. Fifty-six female Sprague-Dawley rats (8-10 weeks of age) will be used.

Dose Ranging Procedures. All subjects will undergo implantation surgery and have either DU or Ta pellets embedded intramuscularly in the biceps femoris. The number of pellets (by surface area) implanted will be determined using results we obtained in the pilot study. Based on these results of our preliminary study, there will be 7 dose groups: Ta control, 16 DU pellets, 20 DU, 24 DU, 28 DU, 32 DU, and 32 DU-impregnated (see below) with 8 rats per dose group. The goal is to identify the number of DU pellets (by surface area) required to produce early signs of kidney toxicity, which has been seen with uranium kidney levels of 0.70 μg U/g wet kidney (Diamond, 1989). The only control group used in this design will be the Ta pellet group, because our objective is to determine kidney uranium levels and early signs of kidney toxicity. Urine and plasma samples will be collected at 3, 5, 7, 15, 30, 45 and 60 days following surgery and analyzed for biochemical changes in kidney function. Uranium levels in the urine will also be determined on these days. On day 60, the animals will be euthanized, and the uranium levels in the plasma and kidneys determined. There will also be an additional group of rats that will be implanted with the highest dose of DU, then impregnated. They will be monitored for the same parameters as the main group of rats, but will be used to determine if the physical and hormonal changes associated with pregnancy alter the toxicity of uranium. Due to the 21 day gestational period, the time course of study for these rats will be shortened to allow enough data to be collected prior to parturition. Data from this study will also provide us with a time course of the urinary uranium levels, allowing us to see at what point the levels detected in the urine stabilize. This will give us insight as to the best time post-implantation to begin breeding the rats in Specific Aims II and III.

Sample Time Points and Collection Procedures. Blood and urine samples will be collected and analyzed for uranium levels and biological markers of kidney function. Baseline samples will be collected on 1 and 3 days prior to implantation surgery. To assess whether embedded DU pellets result in acute kidney toxicity, blood and urine samples will be taken on 3, 5, 7, 15, 30, 45 and 60 days after implantation surgery and assayed for uranium levels and indices of nephrotoxicity.

To safely collect blood samples, rats will be immobilized by placing them in a Plexiglas® restrainer. During each collection, approximately 0.3 ml of blood will be obtained from the tail vein using a 22-gauge needle. Plasma and the red blood cells will be separated by centrifuging for 5 min at 3,000 X g. The plasma will be analyzed for uranium levels and biochemical indices of kidney toxicity.

Urine samples will then be collected by housing the rats in individual metabolism cages (23.5 cm diameter x 12 cm high) where they will have continuous access to food and water. The rats will be acclimated to the metabolic cages for 5 days before the study begins because naive exposure to these housing procedures has been shown to induce stress in the animals and to increase the toxicity of uranium[8].

A 24-hour urine collection sample will be obtained from each rat and the volume recorded. Rats in the preliminary study produced 10-20 ml urine in a 24 hour sampling period. Care will be taken to prevent contamination of the urine with food or feces. After collection, urine will be filtered to remove any debris and stored in plastic containers at 4°C until analyzed. The metabolic cages will be disinfected and decontaminated between each animal use. During the animal-handling periods, overt signs of behavioral toxicity and the overall appearance of the rats will be noted.

Assessment of Uranium on Kidney Function. Measurement of urine volume and osmolarity, urine levels of NAG, LDH, glucose, total protein, creatinine, and blood levels of glucose, urea, and creatinine will

be used as indicators of kidney function. Osmolarity of the urine will be measured with a vapor pressure osmometer (model 5100-B, Wescor Inc., Logan, UT). A Kodak Ektachem 700 Analyzer will be used to determine serum and urine levels of creatinine, glucose, and urea. Total urine protein will be measured with a dye-binding assay (Coomassie Blue, BioRad) sensitive down to 1 •g. The activity of NAG will be measured by the methods of Tucker et al.[60], using 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide as the fluorescent substrate (excitation wavelength = 356 nm; emission wavelength = 446 nm). The dilution of the urine for this assay eliminates the effects of any inhibitors present[60]. For LDH measurements, 1 ml of urine will be dialyzed for 4 hours at 4°C with 1 liter of deionized water. LDH will be quantitated with a colorimetric assay that measures a reaction product proportionate to LDH activity (Oxford Biomedical Research Inc.). Only 50-100 •l of fluid (urine or serum) are required for each assay.

Although urine volume and osmolarity can vary greatly with fluid intake, these measures provide physical indicators of renal function. For example, kidney failure drastically decreases urine volume, while moderate renal insufficiency can increase urine output. Osmolarity can reflect the ability of the kidney to concentrate (or dilute) the urine. Plasma urea also changes with renal insufficiency. Because the rate of urea formation is proportionate to the rate of protein metabolism, other factors such as hepatic injury or altered protein intake can affect the measured urea in plasma. A small concentration of protein is normally present in the urine. Increases in total urine protein could result either from glomerular leakage or failure of tubule reabsorption. Urinary enzymes are sensitive noninvasive markers of toxicity primarily in the kidney tubules[49]. NAG is a lysosomal enzyme found in proximal renal tubule cells. LDH is a cytosolic enzyme of the tubular epithelium.

Creatinine clearance is a commonly used measure of the glomerular filtration rate in the rat, despite a significant but constant tubular secretion. The use of an intrinsic metabolite has an obvious advantage over inulin or mannitol which (although not secreted) must be infused. Interpretation must be cautious since tubular injury with uranium could cause an underestimate of the glomerular filtration rate regardless of the marker used[11]. Creatinine clearance (C_c) will be calculated from the equation: $C_c = U_c * V/P_c$, where U_c and P_c are the creatinine concentrations in urine and plasma, respectively, and V is the rate of urine production as ml/min.

Appearance of glucose in the urine occurs when the tubule reabsorption maximum from the filtrate is exceeded. This can occur with hyperglycemia or with a decrease in tubular reabsorption capacity. Measurement of both urine and plasma glucose will help to distinguish between these two possibilities. Changes in reabsorption will be reflected in the calculated fractional excretion (FE): $FE = (U_g/P_g) \cdot (U_c/P_c)$, where U_g and P_g are the glucose concentrations in urine and plasma, respectively.

The proposed assays will provide a broad spectrum of measures of kidney toxicity. Many of these substances have been shown to be very sensitive biomarkers of acute uranium toxicity[11, 36]. Glucose is one of the most sensitive indicators of nephrotoxicity[11, 12] (and data from the preliminary study) with increased glucose detected in the urine but no concurrent increases found in the plasma. LDH, and to a lesser extent NAG, increased following uranium exposure[11, 36]. A transient increase in urine volume and the appearance of protein in the urine also occur with acute uranium toxicity[36] (and preliminary data). These measures will be used together as indicators of kidney toxicity and carefully interpreted and correlated with histopathology.

Histopathology. Histopathology will be conducted by AFRRI's Veterinary Sciences Pathology Department to determine if acute exposure to DU produces renal changes. Sixty days after implanting DU pellets, all rats will be euthanized, and the kidneys dissected out. Animals not surviving prior to the scheduled euthanization will be necropsied to determine the cause of death. At the time of necropsy or sacrifice, all DU pellets will be retrieved. Standard procedures for handling biologic specimens will be used in the preparation of the samples. Tissues will be perfused, embedded and stained with hematoxylin and eosin stain (H & E)[39]. Specialized stains will be used, as warranted, to demonstrate specific lesions or further delineate lesions not well defined with H & E stain.

The histopathological evaluations will be done blind such that the technician preparing the tissue and the pathologist evaluating the tissue will not know the experimental group in which each rat belongs. A histopathology worksheet will be prepared for each animal. Any unusual gross tissue alterations noted during necropsy (i.e., neoplasia) will be collected and added to the histopathology worksheet. The pathologist will generate a 0 to 4 scoring system to evaluate the degree of microscopic changes observed where 0 = no change,

1 = minimal change, 2 = mild change, 3 = moderate cha[illegible], and 4 = marked or severe change. All tissue changes observed in the rats implanted with DU will be contrasted and compared to the tissues taken from the controls. If there are significant changes noted in the renal syst[illegible] a detailed statement of criteria for 0-4 scores will be made by the pathologist at the time of interpretation.

Specific Aim II. Determine the effects of *in utero* exposure to embedded DU pellets on prenatal and postnatal development and behavior.

Previous research shows that uranium exposure to the fetus can produce both prenatal and postnatal toxicity in the form of physical abnormalities[4]. The effects of uranium exposure on behavioral development, however, remains unknown. Specific Aim II is designed to address this issue by assessing the developmental toxicity of rats exposed *in utero* to embedded DU from birth to adulthood. A battery of behavioral tests designed to assess functional toxicity will be used. Behavioral measurements of cognitive functioning will assess the development of the rat's learning and short-term memory capacity.

Subjects and Experimental Groups. Subjects will be 240 male and 240 female Sprague-Dawley rat pups obtained from the litters of 60 females bred at the Armed Forces Radiobiology Research Institute, Bethesda, MD. There will be six experimental groups with 10 female rats in each group. Dams in three of the experimental groups, 30 females, will be surgically implanted with DU pellets. The number of DU pellets implanted will differ to represent a low, intermediate, and high dose of DU and will be determined based on the results of the DU dose ranging studies in Specific Aim I. The goal will be to select a high dose of DU that produces some overt sign of maternal toxicity, but does not result in a maternal death rate greater than 10%. The low dose will be selected so that it induces no observable effects attributable to the DU pellets, while the intermediate dose will be located logarithmically between the high and low. The three remaining experimental groups will be controls, with 10 dams per experimental group. One group will be implanted with tantalum pellets to control for the invasive presence of the pellets. Another group will control for the surgical procedures but will receive no implants, and the final group will receive no surgical treatment. In order to ensure that we obtain at least 10 pregnant female rats per group, 12 female rats will initially be assigned to each experimental group.

Surgical Procedures for Pellet Implantation. The surgical procedures for pellet implantation will be the same as those described in General Research Design and Methods.

Mating Procedures. Two female rats will be housed with a single male rat once urine uranium levels are stable in the females, but no sooner than 7 days postsurgery. The male rats will be approximately the same age as the female rats and will have been individually housed for at least one week in cages 46 cm long x 23 cm wide x 20 cm deep. Male rats will not receive any DU treatment and will serve only as breeders. Vaginal washings will be performed each morning and examined microscopically for the presence of sperm, which will define GD 0. If sperm is not detected in the vaginal washings after 15 breeding days, then the female rat will be housed with another male breeder for 15 days. This procedure will be repeated at least three times before the female rat is removed from the study. Male rats will also be removed from the study if they fail to impregnate female rats after two such rotations. A total of 50 male rats will be used as breeders. On GD 0, dams will be removed from the males' cages and housed individually. Nesting material will be provided for each dam on GD 18. At parturition, the litters will be examined and records made of litter size, number of males and females, pup weights, and any overt teratological signs. On PND 1, the litters will be culled to 8 pups per litter consisting of 4 males and 4 females. All pups used in this study will be permanently marked by tattooing their hind and/or forepaws for identification.

Subject Assignment. On PND 4, one male and one female pup from each litter will be assigned to one of four testing conditions. In the first test condition, the rats will be assessed at 30 and 90 days of age using the Functional Observational Battery (FOB), a locomotor activity test, and an active avoidance learning and retention test. In the second test condition, the short-term memory capacity of 30-day-old rats will be determined using a passive avoidance test, while in the third testing condition, a passive avoidance task will be used to assess short-term memory capacity of 90-day-old rats. Animals assigned to the fourth testing condition

will serve as an experimental group for determination of [illegible]n levels in the fetuses and dams described in Specific Aim III.

Indices of Maternal Toxicity. To separate maternal [illegible]city from fetal and/or developmental toxicity, the following measures of maternal toxicity will be recorded: gestation length, litter size, body weight changes (during gestation and after gestation), and food and water intake throughout the study.

Indices of Developmental Toxicity. Throughout the study, any behavioral changes and signs of toxicity in the rat pups will be recorded. In addition, physical maturation features and reflex behavior, as ontogenic markers of CNS maturation, will be monitored. The maturation signs to be observed will include pinna detachment, upper and lower incisor eruption, ear canal and eye opening and fur development[38]. Surface righting will also be tested. Each pup will be placed on its back and given 60 sec to right itself to a normal position with all 4 paws on the surface. The number of pups per litter that right themselves will be recorded each testing period, until the entire litter meets the criterion, at which time the litter will no longer be tested for righting reflex. If a pup fails to meet the criterion at a given test period, that pup will be retested after the entire litter has been tested. This is an attempt to control for the fact that the pups' attention and arousal states tend to fluctuate, and failure to right itself may be due to a problem with arousal and not to a delay in development. These assessments will be made twice a day from PND 1 until PND 21.

Functional Observation Battery and Locomotor Activity. The functional observation battery (FOB) is commonly used in toxicity testing to characterize alterations in sensorimotor, neuromuscular, and autonomic function, and CNS excitability[40]. This test battery consists of home-cage, handling, open-field, and manipulative measures, as well as physiological measures. See McDaniel and Moser, 1993, Moser et al., 1989 and Part C.2 for details of the specific testing and scoring procedures. An automated assessment of activity will be made to quantify locomotor behavior (Digiscan Animal Activity Monitor, Omnitech Electronics, Columbus, OH). The Digiscan Monitor uses an array of infrared photodetectors to determine horizontal ambulation expressed as the distance moved and rearing activity expressed as the number of vertical movements. On testing days, locomotor activity will be assessed for 1 hour. The FOB and locomotor activity tests are noninvasive, and therefore they can be administered throughout development. Subjects assigned to this experimental condition will be tested at least at 30 and 90 days of age. Rats at these ages are considered adolescents and young adults, respectively.

Evaluation of Learning and Memory Function. Learning and short-term memory capacity will be measured using active and passive avoidance paradigms to assess the potential affects of DU on cognitive functioning. Learning and memory functioning are routinely measured to evaluate the developmental toxicity of a number of chemical compounds[38]. These assessments will be made in a computer-controlled two-way shuttle box (San Diego Instruments, San Diego, CA). The shuttle box is a two-compartment chamber separated by a vertically driven door. Each chamber consists of lights mounted on the far wall, speakers, and a grid floor. Shuttle boxes will be located in sound-attenuating cubicles.

The same animals tested on the FOB and the locomotor activity test will be tested on the active avoidance test beginning at PND 30[20,56]. On PND 90, these animals will receive an active avoidance retention test. Before training begins on the active avoidance test, animals will receive a single 15-minute habituation trial during which they will be placed in the shuttle box where they will be free to explore both chambers of the box. Training begins the following day when an animal will be placed in the shuttle box for 2 minutes with the lights off and the vertical door closed. At that time, the door will be opened and both the light and tone will be activated. If the rat fails to move to the opposite compartment within 10 sec, it will receive a low-level foot stimulation (0.5 mA at PND 30 and 1.2 mA at PND 90) for 10 seconds, or until it moves to the opposite chamber. Trials will be separated by 10-second intertrial intervals. The rat's performance on each trial will be scored in one of three ways. If the rat moves to the opposite chamber *before* the stimulation occurs, the trial will be scored as an avoidance response. If, however, the rat does not move to the opposite chamber until *after* the stimulation occurs, then the trial will be scored as an escape response. If the rat does not move to the opposite chamber during the entire 10 second stimulation period, then the trial will be scored as a failure. Each rat will receive 50 trials/day, until it avoids the foot stimulation on 80% of the trials or until it has received 300 training trials. The number of training trials required to reach this criterion is an indicator of learning. At 90 days of age, the retention capacity of these animals will be tested. The retention test will be identical to the

initial training paradigm. A retention index will be determ[illegible]y comparing the number of trials required to reach criterion at 30 days of age with the number of trials [illegible]red to reach criterion at 90 days of age.

Short-term memory function will be assessed using a passive avoidance test[51]. Animals tested on the passive avoidance test will be naive and will only be tested at either 30 or 90 days of age. In the passive avoidance paradigm, rats will be placed in one of the darkened compartments with the door closed. After 15 sec, the light and tone will be activated only in the chamber where the rat is located and the door will be raised. The natural tendency of the rat is to avoid the light and noise, so it will cross into the darkened chamber. When the rat moves to the dark chamber, the door will be closed, and the animal will receive a mild, 1-second electrical stimulation (0.5 mA at PND 30 and 1.2 mA at PND 90). Rats will then be removed from the apparatus and returned to their home cages for 1 minute until the start of the next trial. This procedure will be repeated a maximum of 8 times or until the rat learns to stay in the lighted chamber for 60 sec for 2 consecutive trials. The same start compartment will be used on each trial. The latency to cross from one chamber to the other will be recorded on each trial.

Memory capacity will be assessed 72 hours later for both the 30- and 90- day-old rats. For these tests, the rat will again be placed in a darkened chamber. After 10 seconds, the light and tone will be presented in the compartment holding the rat and the door raised. However, the rat will not receive a mild foot stimulation if it crosses into the opposite darkened chamber. The latency for the rat to cross to the other compartment 72 hours later will be compared to its initial crossing latency. Increased latencies will be considered as evidence that the rat remembers the foot stimulation and is avoiding it. Similarly, no change or a reduced latency will indicate that the rat has no memory of the mild foot stimulation.

Specific Aim III. Determine the level of uranium that enters the rat fetus exposed *in utero* to implanted DU.

While previous research has demonstrated that the placenta does not act as a barrier to prevent the transfer of uranium from the mother to the fetus[54], the degree of fetal exposure from maternal implanted DU is unknown. Specific Aim III will address this question by comparing the blood uranium level of the fetus with the blood uranium level of the mother. The uranium level of the placentae will also be measured. Bone, kidney, spleen, liver and muscle tissue of the mother and fetuses will be removed and frozen for potential determination of specific distribution of uranium at a later date.

Subjects. Subjects will be 50 female Sprague-Dawley rats and their litters. Because all female rats will not become impregnated, an additional 10 rats will be bred (N = 60 female rats). Male Sprague-Dawley rats described in Specific Aim II will be used for breeding. All rats will be housed according to the procedures outlined in Specific Aim I.

Experimental Groups. Female rats will be equally divided into 5 treatment groups (10 rats per group): 3 DU dosage groups (low, medium, and high dosages to be determined in the dose response study in Specific Aim I), a Ta pellet control group, and a nontreated control group. Sham surgery and anesthesia control groups will not be used in this experiment because there is no reason to believe that either of these treatments will alter uranium levels. At GD 20, dams and their litters from each group will be sacrificed, and their tissues analyzed for uranium levels. Uranium levels will also be analyzed at PND 21 using pups and dams from litters in Specific Aim II.

Surgical Procedures for Pellet Implantation. Procedures will be the same as described in General Research Design and Methods.

Surgical Recovery and Stable Level of Uranium in Urine. Procedures will be the same as described in Specific Aim II.

Mating Procedures. Breeding procedures will be the same as described in Specific Aim II.

Indices of Maternal Toxicity. Maternal weights and food and water intake will be monitored throughout gestation in all dams in this study.

Prenatal Tissue Collection. Unlike the procedures described in Specific Aim II, these dams will not deliver their litters, but will be euthanized on GD 20 via decapitation. Maternal trunk blood will be collected at this time. Dams will be immediately cesarean sectioned, and the uterine horns removed. Fetuses will be dissected out, and all the placentae for that litter collected. The uterine horns will be examined for any

resorption sites. Litters will be examined, and a record made of (1) total number of fetuses, (2) number of viable fetuses, (3) fetal weights, (4) sex ratio, and (5) any overt signs of teratological effects. Any nonviable offspring will be eliminated from further study. Any obviously malformed fetuses will be set aside and analyzed separately from the viable, nonmalformed offspring. All viable offspring of the litter will be analyzed for uranium levels. The placentae from all pups will be collected and pooled for uranium analysis for each litter. The viable offspring will be used for determining uranium tissue levels. Immediately following dissection of the brains from these pups, hind-limb muscle and bone tissue will be removed, and the liver and kidneys dissected out. These tissues will be pooled for the entire litter, homogenized, and frozen for potential further analysis of uranium content. For each of the five treatment groups there will be 10 samples of maternal blood for a total of 50 maternal samples. There will also be, for each of the five treatment group, 10 placentae and 10 brain, for a total of 100 samples.

Postnatal Tissue Collection. This phase of the experiment is designed to assess the degree of uranium exposure in nursing pups as sampled on PND 21. Twenty PND-21 pups, ten male and ten female, and their dams will be obtained from each of the five treatment groups in the experiment outlined in Specific Aim II (in order to maximize the use of animals). On the day of analysis, dams will be euthanized by decapitation and trunk blood obtained for uranium level determination. Pups will also be euthanized, and trunk blood collected and pooled for uranium level analysis. The brains will also be analyzed individually for uranium. Muscle, bone, kidney and liver tissues will also be removed and frozen for possible future analysis of uranium levels.

Tissue Preparation and Uranium Analysis. On site tissue preparation will consist of centrifugation of blood for serum collection, homogenization of placentae using a polytron homogenizer, and homogenization of euthanized fetuses. The homogenate will be frozen and stored for shipment for analysis of uranium levels to Quanterra whose procedures are briefly outlined in Part C.1.

6. Preliminary Data

Several DU studies have been or are currently being conducted at AFRRI. An initial study to determine the ability to implant the pellets into the rat biceps femoris and measure the uranium in the urine and kidney was successful. Illustration 1 depicts a time course of the measurable urinary uranium for a period of 120 days, at which time the rats were euthanized and the kidneys analyzed for uranium content.

Another pilot study ongoing in this laboratory is examining the ability to breed DU embedded rats and to detect uranium in the fetus.. This pilot study involved the implantation of several doses of DU pellets (No pellets, Tantalum pellets, 4, 8 and 12 DU pellets) in mature female rats. Fourteen days post-implantation, the rats were placed in cages with male rats for breeding. On average, the rats were impregnated within 4 days of being exposed to the male rat, although there were several rats that were not pregnant until much later post-implantation. The food and water intake, as well as maternal weight gain, were monitored throughout gestation. On day 20 of gestation, one day prior to parturition, the rats were sacrificed and various maternal and fetal tissues removed, including maternal blood. Whole fetus, placenta and maternal kidney homogenates, along with maternal serum, were sent to Quanterra labs for analysis of uranium content. The results are not completely received, although a picture can be made from what has been received.

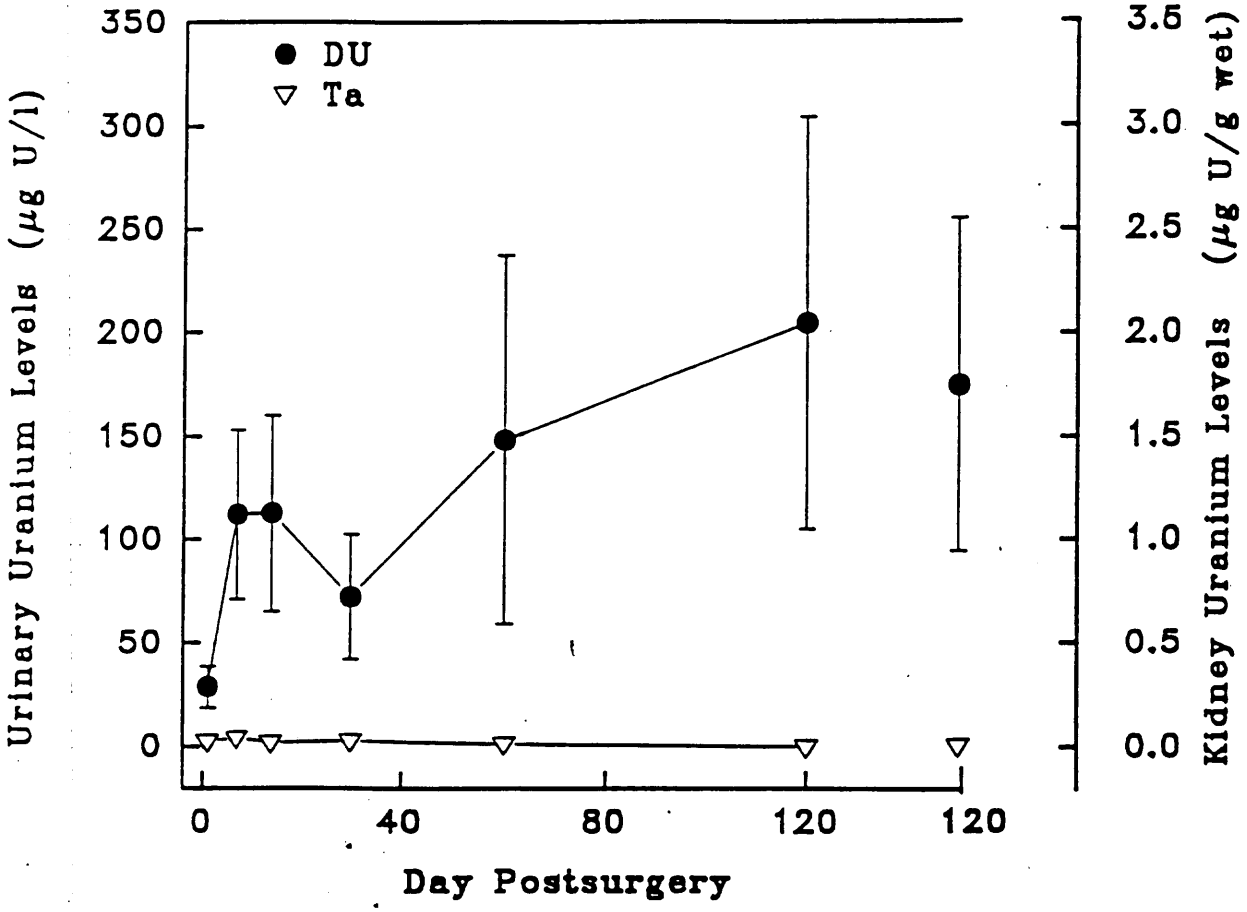
From the results it would seem that (a) serum levels of uranium do not exhibit a dose response relationship, (b) no dose response relationship can be seen in the whole fetus levels of uranium either, (3) there does appear to be a dose response effect on uranium levels in the placenta, (d) a dose response relationship is also evident in the uranium levels found in the dams kidneys. The kidney levels, however, did not achieve the level we had anticipated being necessary for reproducing the effects seen by previous researchers, that being a kidney level of a minimum of 0.7 ug/g. Our highest DU level only averaged approximately 0.5 ug/g U in the maternal kidney. It is also important to note that the DU pellets did not adversely affect the ability to breed these rats, or for them to maintain the pregnancy until the day of sacrificing. The maternal toxicity parameters of maternal weight gain, food intake and water intake were also not adversely affected by the DU dose levels. All litters were examined for any very obvious overt signs of teratogology, and none were noted. The litter sizes and sex distributions were not altered by the DU dose levels. There were also 15 dams that delivered their litters, due to an error in calculating their gestational day 0. These litters were maintained in the lab, weighed

and monitored periodically until post-weaning. The DU dose appeared to have no obvious adverse affect on these litters.

The results of this preliminary study has opened up more questions than were answered. It is important that we now attempt to reach a level of DU exposure that is maternally toxic. The DU level achieved in the kidneys of our highest dose was not even at the minimum level that is known to be nephrotoxic. Future attempts will be made to achieve and possibly exceed this minimum of level of 0.7 ug/g. This may be done by increasing the DU dose via increased numbers of implanted pellets, or by allowing the pellets to remain longer before the rats are bred. It is possible that a longer time period is needed for the uranium levels to stabilize and our attempts to breed the rats soon after surgery in our preliminary study may have actually hindered our ability to achieve and equilibrium. A lack of uranium in the whole fetus is to be expected if the uranium is sequestering in specific tissues in the fetus, such as the kidney or bone. Then homogenizing the whole fetus will dilute the uranium to levels that may not be detectable with our assay. Analysis of individual tissues should answer this problem.

Additional data from an ongoing DU study at this institute also answers the question of whether the DU can accumulate in the rat brain. Rats that were implanted with 20 DU pellets had a mean uranium level of 176.2 ng/ml in the urine, mean kidney level of 1706.0 ng/g of tissue and a mean brain level of 24.9 ng/g of cortex. This is significantly different from the control rats as well as from lower doses of DU (4 and 10 pellets). If DU is able to penetrate to the brain of these adult animals with intact blood brain barriers, it is possible also that the fetus brain is being exposed to uranium during the vital developmental periods, making it extremely susceptible to damage from the DU insult.

Illustration 1



Time course of uranium levels detected in the urine of rats implanted with either DU or tantalum (Ta). The uranium concentration detected in the Ta group is at background levels. Vertical bars for the DU group are the standard error of the means. The standard error bars for the Ta group are smaller than the symbol. The levels of uranium in the DU group were elevated on day 1 and increased four fold on days 7 and 14 after surgery.

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March 1993

AFRRI

93-1 TECHNICAL REPORT

Assessment of the Risks from Imbedded Fragments of Depleted Uranium

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#838

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 1993	Technical Report

4. TITLE AND SUBTITLE
Assessment of the Risks From Imbedded Depleted Uranium Fragments

5. FUNDING NUMBERS
PE: NWED QAXM

6. AUTHOR(S)
Daxon, E. G., and Musk, J. H.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Armed Forces Radiobiology Research Institute
8901 Wisconsin Ave.
Bethesda, Md. 20889-5603

8. PERFORMING ORGANIZATION REPORT NUMBER
TR-93-1

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Nuclear Agency
6801 Telegraph Road
Alexandria, Va. 22310-3398

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS

15. NUMBER OF PAGES
15

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	SAR

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BRP

27 March 1992

MEMORANDUM FOR DIRECTOR, PROFESSIONAL SERVICES, OFFICE OF THE SURGEON GENERAL (SGPS-PSP)

SUBJECT: Research Request; Health Effects of Depleted Uranium Imbedded in Tissue

Reference: Brigadier General Ronald R. Blanck (SGPS-PSP) letter of 26 February 1992

In response to your letter of 26 February 1992, subject as above, AFRRI has conducted a detailed review of the pertinent scientific literature regarding the health effects of depleted uranium (DU) fragments which are imbedded in tissue. In addition, we have consulted with a wide range of scientists with expertise in this area. A summary of our findings is attached.

It is clear from our analysis that there are several areas in which there is little or no scientific data which would enable more definitive risk assessments to be made. Nevertheless, in order to meet your operational requirements, attachment (1) addresses each of the issues raised in your letter. To address areas in which there remains substantial scientific uncertainty, attachment (1) also identifies specific research needs.

Based on available data, in almost all cases, we recommend that standard medical criteria should be used to determine the advisability of the removal of imbedded DU fragments without regard to the radiological characteristics of the fragment. More specific guidance is provided in attachment (1).

Point of contact is Lieutenant Colonel Eric G. Daxon, Chief, Operational Dosimetry Division, Radiation Biophysics Department, 301-295-2299.

Robert L. Bumgarner

ROBERT L. BUMGARNER
Captain, MC, USN
Director

Attachment:
as stated

AFRRI Technical Report 93-1

ASSESSMENT OF THE RISKS FROM IMBEDDED DEPLETED URANIUM FRAGMENTS

Prepared by:

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and
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March 1993

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Assessment of the Risks From Imbedded Depleted Uranium Fragments

1. General

a. Authority

Letter, Office of The Army Surgeon General, (SGPS-PSP). Subject: Research Request; Health Effects of Depleted Uranium Imbedded in Tissue. 28 February 1992.

b. Mission

Assess the health risks associated with implanted DU fragments in the body to provide medical guidance for current and future patients with these fragments, and provide recommendations for future research.

2. Background

The primary conclusion of the review of the uranium literature[1-16] and discussions with others[17-26] in the field is that this situation is radiologically and toxicologically unique. The health risks of allowing depleted uranium (DU) fragments or any other radioactive heavy metal to remain imbedded in an organ have not been studied. The uranium literature reviewed is focused on inhaled or ingested uranium compounds. There is only one reported instance of a DU fragment accidentally injected subcutaneously in a patient. This case, as reported by Cole,[22,27] provides little information for long-term effects because the fragment was surgically removed after 8 months.[22,27]

3. Chemical Toxicity

The toxicological effects of uranium are well known. The target organ for uranium heavy metal toxicity is the kidney. The literature concerning the acute effects of uranium heavy-metal poisoning on the kidney is extensive and is summarized in recent articles by Leggett[12], Diamond[13] and Kocher.[14] While the generally accepted threshold level for kidney toxicity is from 1-3 µg of uranium per gram of kidney mass,[12,13,14] there is considerable discussion in recent literature concerning this limit.[12,13,14]

A review of uranium toxicology conducted by USAEHA[11] concluded that, while there was substantial toxicologic data for inhaled and ingested uranium compounds, there was little or no data for the metabolic behavior of implanted DU fragments. Key uncertainties include organ specific solubilities; organ specific retention functions; the metabolic impact of a source term other than the lung or GI tract; the potential for chronic kidney toxicity; the impact of fibrotic encapsulation, if it occurs; and the chemical form of the imbedded fragment.

The potential for wound contamination (the injection of small sub-millimeter fragments) and for spallation of small fragments from large fragments introduces two additional dispersal mechanisms - macrophage transport and the physical movement of intact particles by the blood stream. The impact of both is an increase in the rate at which uranium is deposited in the kidney and other organs. De Rey et al.[2] found insoluble UO_2 particles in the kidney 6 to 48 hours after injection of 4 to 40 micrometer diameter UO_2 micro-spheres into the subcutaneous tissue of the dorsal skin of female rats. This is significantly quicker than predicted by standard metabolic transfer models for insoluble compounds of uranium.

These limitations and uncertainties preclude a definitive assessment of the toxicologic risks of allowing DU fragments to remain in the body for extended periods of time.

4. Radiological Effects

The literature is extensive concerning the deterministic and stochastic effects of acute and chronic exposure to inhaled and ingested uranium compounds.[1,28-32] The lack of data for imbedded uranium fragments precluded a direct determination of the potential long-term radiological effects of these fragments. An estimate of the potential effects was obtained by reviewing the literature available for plutonium, Thorotrast, and hot-particles.

The plutonium (Pu) literature[33-44] reviewed also focused on inhalation and ingestion, but there were several studies that dealt with injected plutonium compounds. Lushbaugh[19,34,42] and Langham et al.[42] summarize the findings of studies of eight patients with injected plutonium. Lagerquist et al.[43] and Carbaugh et al.[44] discuss patients with plutonium contamination of puncture wounds. While these studies are somewhat useful, their usefulness is limited because the exposure duration was relatively short (the longest was 5-8 years), the particle sizes were small, and in each case the wounds were debrided to removed the injected plutonium. For both the animal and human studies, the plutonium injected was in the form of the fine particulates expected from injection wounds caused by contaminated, sharp tools.

The Thorotrast literature is extensive[1,34,47-62] and important because of the radiological similarities with the situation under study. Thorotrast is a colloidal suspension of thorium dioxide (ThO_2) that was used as an intravenously-injected contrast agent for radiographic imaging from the late 1920's until the late 1950's when its long-term radiologic health effects became apparent.[45,46] The Thorotrast literature provides the most definitive evidence that both clinically-significant deterministic and stochastic effects are possible from long-term irradiation of low dose-rate α and β emitting radionuclides.

However, the differences in particle size and chemical properties between Thorotrast and DU are significant enough to preclude a direct application of the data. The ThO_2 particles in Thorotrast were small enough (nanometers in size) to be engulfed by both the mobile and fixed macrophages in the reticuloendothelial (RE) system which led to a time dependent, selective concentration in the liver and spleen. This time dependence makes dose-dependent extrapolations from Thorotrast data to a DU fragment difficult. In addition, the selective retention by the RE system limited the exposure to the organs in this system.

Although directed specifically at the radiation effects on the skin of a highly radioactive, beta-emitting particle, the hot-particle research literature[63-70] provides valuable information concerning the differences between the highly nonuniform irradiation that results from an imbedded fragment and the results of the uniform organ irradiation upon which assessments of radiation risk are based. Specifically, the hot particle research sheds light on the relationship between the fraction of an organ system irradiated and the dose required to produce both deterministic and stochastic effects. The primary conclusion of this work is that the radiation risk of both endpoints is dependent upon dose and the number of cells irradiated.

Based upon this review, the following radiobiological effects are possible from imbedded DU fragments.

a. Granuloma Production

Cole's[22,27] experience and Lushbaugh's[19,34,42] work indicate that granuloma production in the muscle and fatty tissue will probably occur and will occur in all other tissue types that elicit similar cellular responses to foreign bodies. It is still questionable whether this encapsulation is permanent or will undergo the degradation-regeneration cycle suggested by Lushbaugh for the plutonium cases he studied.

The data to date are insufficient to allow a determination of whether Thorotrastoma-like growths are possible. A Thorotrastoma is a large growth that appears at the sites of extravascular Thorotrast with a latent period of from 5-35 years postinjection.[17,47,48,53,60] These granulomas grow to large sizes; in a few cases, clinically significant blood vessels and/or nerves were enveloped, resulting in fatal conditions.[24] While a strictly chemical causation cannot be dismissed, there is sufficient evidence to suggest a radiogenic mechanism.

b. Local Tissue Necrosis

The results of the Thorotrast, lung inhalation studies, and animal studies showed that local tissue necrosis followed by fibrosis was possible from the long-term irradiation of tissues by a low dose-rate, α and β emitting radionuclide.

Dose estimates made at AFRRI based upon published data[71,72,73,74] indicated that the probability of deterministic effects at distances greater than 1-3 mm from the surface of any fragment is negligibly small. Depth-dose calculations indicated that at the distances from the surface of all particle sizes studied (1-4 mm in diameter) the dose-rates were less than the repopulation dose-rate for non-proliferative cells provided by the ICRP[28] (1-5 mGy/d). The assumption in this analysis is that at distances greater than this, deterministic effects will not occur because cell repopulation will compensate for cell death for most tissue types. The most notable exception to this assumption is mature neural tissue, the neurons of which do not usually have a proliferative potential.

The clinical significance of necrosis at distances closer to the fragment is dependent upon the location of the fragment and the body's response to the fragment. Lushbaugh,[34] in his analysis of cases of injected plutonium, found that "...metallic plutonium implanted in the skin in minute amounts elicits a foreign-body reaction of the granulomatous type, which after subsiding in cellular activity becomes fibromatous." As time progressed, the collagen in the vicinity of the fragment liquified.

Lushbaugh speculated that the "pointed" nature of the granulomas he found and the fact that the granulomas became more superficial, suggested that the altered collagen might induce a cycle of inflammatory reaction followed by a reorganization and re-liquefaction of the collagen.

c. Whole-Organ Deterministic Effects

The potential for multiple fragments in a single organ led to the examination of the potential for whole-organ deterministic effects. A whole-organ deterministic effect is defined as one in which there is a clinically significant compromise of organ function due to the ionizing radiations emitted by one or more DU fragments.

The appearance of whole-organ deterministic effects from acute, high dose rate exposure is well documented. Mettler and Mosely,[75] Conklin and Walker,[76] and ICRP 41[28] provide excellent summaries with extensive bibliographies for whole-organ deterministic effects based primarily on examination of the Japanese atomic bomb survivors, radiation accident victims, and radiation therapy patients. Direct extrapolation from high dose rate, acute exposure to low dose rate protracted exposure is difficult because of the dose rate dependence of the threshold dose required to produce a deterministic effect.[28]

The results of inhalation studies with uranium and plutonium summarized in ICRP 31[30] and in other references[6,7,33,39] show that whole organ deterministic effects are possible from inhaled particulates. The Thorotrast studies[1,34,47-62] provide the clearest evidence that deterministic effects are possible from protracted exposures to low dose rate internal alpha emitting isotopes. These studies showed that both fibrosis of the spleen and cirrhosis of the liver could be related to the radiation emitted by the thorium dioxide (ThO_2) in the Thorotrast. The latent period for the onset of clinically significant liver cirrhosis was on the order of 20 years after Thorotrast administration.[57] The latent period for significant spleen fibrosis was not reported but is assumed to be comparable.

A dose calculation, made using similar methodology as described above, showed that the risk of whole-organ stochastic effects do not become significant until the fragment density in the organ exceeds one fragment per cm^3 of organ volume for the fragment sizes considered (1-4 mm diameter). At particle densities greater than this, the average dose rate in the organ will exceed the repopulation dose rate for non-proliferative cells.

d. Stochastic Effects

The standard ICRP stochastic-risk-estimation methodology[77] is directly applicable for systemic DU but can be used only with caution when assessing the risks of imbedded DU fragments. There are several unknowns that could cause this and similar procedures to either overestimate or underestimate the stochastic risks. Included are these specifics:

(1) The hot-particle research indicates that the risk from an imbedded fragment could be significantly less because fewer cells are irradiated. ICRP methodology assumes that the dose is uniformly distributed over all of the cells in the organ while a DU fragment will irradiate only the cells within a finite range of the fragment.

(2) The Thorotrast experience showed evidence that the constant irradiation of the same cell population could increase the risk by adding necrosis-regeneration as an additional cancer induction mechanism. This mechanism is not considered in the ICRP models or cancer risk estimates.

An estimate of the stochastic risk posed by an implanted, insoluble fragment was made by calculating the effective dose equivalent (H_E) for a range of fragments (1-4 mm) for each organ listed in ICRP 60.[31] The actual organ weights were used to calculate the dose as were the actual weighting factors (w_T). The calculation was performed assuming that alpha dose could be ignored because the energy of these particles will be expended producing lethal damage to the cells adjacent to the fragment and thus contribute nothing to the stochastic risk.

For the largest fragment size evaluated (4 mm), the highest H_E is in the thyroid because of its relatively small mass. In this case, H_E is 1 mSv/y (100 mRem/y). Using current risk estimates,[32] these values represent an increase in lifetime risk of fatal cancer of 0.3%. The value for other organs will be substantially lower because of their larger masses.

At this point in the discussion, it is important to recognize that this risk estimate is based upon a single, insoluble fragment imbedded in an organ and does not include the risk from systemic DU.

5. Conclusions

a. Chronic kidney toxicity is a potentially clinically significant health effect from imbedded DU fragments. While the toxicology of uranium in the kidney is well known, little is known about the toxico-kinetic behavior of imbedded uranium. This information is required to make definitive estimates of both the toxicological and radiological risk.

b. Based upon the literature reviewed, the potential exists for both stochastic and deterministic radiation effects from the long-term exposure to imbedded DU fragments.

(1) The most clinically significant, radiogenic effect is the potential for a Thorotrastoma-like growth to form at the site of single or multiple imbedded-fragments. The risk, if any, of this growth formation cannot be estimated. It is still uncertain whether this is a radiation effect or an effect due to the chemical nature of the Thorotrast colloid.

(2) The risks of fragments near neural tissues should be carefully assessed because of the nonproliferative nature of these cells.

(3) The potential does exist for whole-organ deterministic effects but only for organs with a large number of imbedded fragments. The point at which this effect is likely to occur requires a detailed estimate of the dose to the organ from all sources of DU. First order, dose estimates indicate that particle densities greater than one fragment per cm^3 of organ volume are required as long as the fragments are insoluble and there are no other sources of DU in the body. Fragment sizes considered in this calculation range in diameter from 1-4 mm.

(4) Using the best risk estimation procedures available, the estimated increased lifetime risk of fatal cancer from a single, insoluble, DU fragment in any organ is at most 0.3%. Scaling this risk for multiple fragments or fragments with systemic DU is difficult and should be done on a case-by-case basis after assessing the total DU content in the patient.

c. The toxicological and radiological unknowns are significant enough to warrant both follow-up of current patients and research to more clearly define the long-term risks associated with these fragments. This is especially important in light of the latent periods noted for both deterministic and stochastic radiogenic effects.

6. Clinical Recommendations

a. The primary clinical recommendation is to continue to use standard medical criteria for fragment removal. Include consideration of the potential impact of a granuloma or a Thorotrastoma-like growth as a part of the decision making process for fragment removal as well as the potential for tissue necrosis for fragments lodged in or within 1-3 mm of neural tissue.

b. Determine the total amount of DU in the patient and continue to monitor patients with confirmed DU fragments for signs of kidney toxicity and any of the radiological endpoints discussed. Monitoring is required primarily because of the toxicological but also because of the radiological uncertainties.

c. If fragments are excised based upon accepted clinical criteria, save the fragment and surrounding tissue for further analysis.

7. Research Recommendations

a. Epidemiology

Establish a registry that will allow for the efficient acquisition, cataloging, and analysis of the results of patient monitoring. This effort should include

(1) periodic examinations to watch for and catalogue signs of chronic kidney toxicity, granuloma induction, and cancer;

(2) periodic bioassay and whole-body counting to determine the metabolic behavior of the internalized DU and to provide information concerning the solubility of the DU; and

(3) a program for tissue analysis if fragments are subsequently removed for medical reasons.

b. Animal Model Experimentation

The primary objective of animal model experimentation is to allow a detailed observation and study of the pathology of these fragments under controlled conditions. The specific objectives of this experimentation should include the following steps:

(1) Accurately assess the toxico-kinetic properties of the various chemical forms of DU that could be imbedded in patients.

(2) Investigate whether there are DU specific cancer induction mechanisms similar those observed in Thorotrast-specific liver cancers.

(3) Determine whether the radiogenic deterministic effects noted above occur and, if they do, at what fragment densities and latent periods.

(4) Assess the impact of long-term, low-dose-rate irradiation of specific tissues such as those of the nervous system.

(5) Determine the potential for chronic nephrotoxicity as a function of organ in which the DU is implanted

(6) Conduct pathological studies of the tissue surrounding the fragment.

c. Dosimetry

Perform definitive absorbed dose calculations using advanced techniques to determine the significance of particle size and shape.

Acknowledgements

This document could not have been prepared in the time frame allotted without the expert assistance the authors received from scientists at AFRRI and from the scientific community at large. The expert critique and many fruitful discussions with CDR E. Kearsley, AFRRI, are gratefully acknowledged and were keys to the successful completion of this document. The keen radiobiological insights provided by Dr. E. J. Ainsworth, AFRRI, and Dr. P. Durbin, Lawrence Berkeley Laboratory, showed the way for many of the approaches taken in this work. A special note of thanks is due to Dr. Durbin for her excellent discussion of the applicability of the Thorotrast data to this work.

The medical advice of Dr. R. Bumgarner, AFRRI, and Dr. D. Browne, AFRRI, was crucial in forming the clinical recommendations of this work. The review and advice given by CAPT W. Flor and Dr. R. Young, RARP, DNA, are gratefully appreciated. The experience of Mr. L. Cole (Aerospace Ordnance) with imbedded DU fragments was instrumental in the formation of one of the major conclusions of this report.

The efforts of Mr. M. Weeks, USAEHA, in reviewing the uranium toxicology literature were superb. His expertise and keen insights in this area were indispensable. Mr. R. Swatski, USAEHA, deserves a note of thanks for his assistance in bioassay techniques.

The insights and expertise of Dr. N. Wald and Mr. J. Rosen, University of Pittsburgh, concerning the need and methodology for quantifying DU in the body contributed significantly to the direction of this project.

As always, the personnel of REAC/TS were exceptionally helpful and shared both their time and expertise. A special note of thanks is due to Dr. Fry, Dr. Lushbaugh, and Dr. Ricks.

The scientists at the Inhalation Toxicology Research Institute (ITRI) and the Pacific Northwest Laboratories (PNL) Hanford Laboratories were especially helpful in sharing both their data and insights into this problem. A special note of thanks is due to Dr. B Scott, ITRI, Dr. R. Guilmette, ITRI, Dr. E. H. Carbaugh, PNL, Dr. G. Dagle, PNL, and Dr. M. Swint, PNL.

The contributions of the Army personnel from the Walter Reed Army Medical Center (WRAMC) and Uniformed Services University of the Health Sciences (USUHS) are gratefully acknowledged. The work of CPT M. Melanson, WRAMC, Dr. Philips, WRAMC, and Dr. C. Ferguson, USUHS contributed to this effort.

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March 1993

AFRRI

93-2 TECHNICAL REPORT

Protocol for Monitoring Gulf War Veterans with Imbedded Fragments of Depleted Uranium

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AFRRI TR 93-2

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 1993	Technical Report

4. TITLE AND SUBTITLE
Protocol for Monitoring Gulf War Veterans With Imbedded Depleted Uranium Fragments

5. FUNDING NUMBERS
PE: NWED QAXM
WU: 4610

6. AUTHOR(S)
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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8901 Wisconsin Ave.
Bethesda, Md. 20889-5603

8. PERFORMING ORGANIZATION REPORT NUMBER
TR93-2

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Defense Nuclear Agency
6801 Telegraph Road
Alexandria, Va. 22310-3398

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS

15. NUMBER OF PAGES
17

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	SAR

CLASSIFIED BY:

DECLASSIFY ON:

AFRRI Technical Report 93-2

PROTOCOL FOR MONITORING GULF WAR VETERANS WITH IMBEDDED DEPLETED URANIUM FRAGMENTS

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March 1993

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The research requirements of this protocol will be approved by the appropriate DoD or VA Institutional Review Board for the Protection of Human Subjects.

Preface

The Army's Office of The Surgeon General (OTSG) initiated this effort to care for Desert Storm veterans with imbedded depleted uranium (DU) shrapnel. In February 1992, OTSG requested that the Armed Forces Radiobiology Research Institute (AFRRI) conduct a review of the potential health hazards (radiological and toxicological) of allowing DU shrapnel to remain imbedded throughout the lifetime of the soldier. Specifically, OTSG wanted to know if there was any reason to change the current surgical practice for fragment removal. No compelling evidence was found in the literature review[1] to change current surgical criteria for fragment removal. There were, however, significant uncertainties about the impact of DU fragments on the health of these patients that warranted long-term follow-up.

OTSG concurred with this finding and initiated action to implement this follow-up in the Army. The Department of Veterans Affairs (DVA) agreed to perform the follow-up for personnel discharged from the service. Both the DVA and OTSG requested AFRRI's assistance in drafting the protocol to be used in the follow-up effort.

A group of DoD physicians and scientists met at AFRRI to draft the protocol. At a subsequent meeting on 10 September 1992, a panel of experts reviewed and revised the draft protocol; representatives of the DVA and OTSG also attended this meeting. The protocol was once again reviewed and approved by the panel of experts.

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Protocol for Monitoring Gulf War Veterans With Imbedded Depleted Uranium Fragments

1. Objectives

This protocol will implement two separate but complementary efforts. The first is the clinical follow-up of Desert Storm patients with known or suspected imbedded depleted uranium (DU) fragments, DU contaminated wounds or significant amounts of inhaled DU. The second is the conduct of research into the toxicological and radiological effects of this unique exposure modality. Specifically, this protocol will provide the following:

a. Early detection of abnormalities related to the presence of DU so that prompt, efficacious treatment is effected if required. The study will also provide the scientific data required to fairly settle claims for compensation.

b. Treatment recommendations that will provide a firm clinical basis for fragment removal decisions and for decisions concerning the need for efforts to reduce the uranium in the body.

c. Quantification and documentation of the toxicological (heavy metal toxicity) and radiological (cancer and tissue necrosis) risks of imbedded uranium fragments by

(1) measuring and documenting uranium levels in each soldier using *in vivo* and *in vitro* measurement techniques,

(2) determining the parameters and models needed to translate uranium levels in the body into estimates of the increased cancer risk from this exposure,

(3) comparing the clinical course of the body's response to the DU fragments with that for other non-DU fragments to determine whether clinically significant differences exist due to either the chemical or radiological properties of depleted uranium, and

(4) determining the risk of chronic kidney toxicity due to the long-term chronic exposure to elevated levels of uranium.

2. Approach

The comparison of the clinical course of DU fragments with non-DU fragments will be made using a prospective study approach. The data from patients with internalized DU (the exposed population) will be compared to that from two unexposed populations: patients with fragments that are not DU and soldiers who were not wounded and not exposed to DU.

a. Exposed Population

Each crew member of the attacked vehicles is a candidate for inclusion in the exposed group. An initial check has revealed that there are approximately 22 soldiers whose records indicate that they have imbedded fragments that might be DU. There are an additional 13 soldiers who were wounded and hospitalized but were not specifically identified as having shrapnel. The remaining crew members (besides the 35 already discussed) were either not wounded during the incident or had minor wounds that were treated in the field. The latter two sets of soldiers might have inhaled uranium or experienced DU contamination of wounds or minor fragmentation wounds that were either not noticed or did not require extensive treatment.

The small size of the exposed population limits the study's ability to detect differences to only those effects where the differences between DU and non-DU imbedded fragments are large. For example, it is highly unlikely that definitive conclusions concerning cancer induction will be obtainable from the study. However, this approach will allow a direct comparison of differences that may exist in deterministic effects. Examples of such effects include differences in the body's propensity to encapsulate a DU fragment, the onset of local or whole-organ tissue necrosis, thorotrastoma-like growth induction, or the onset of chronic kidney toxicity. In addition, following a nonexposed group will provide information concerning nominal values for each metabolic value studied in protocol (e.g., normal concentrations of uranium in the body and body fluids as well as kidney function variations with age).

There are two criteria for including a soldier in the exposed group. First, the soldier must have been in or on the vehicle when the vehicle was struck by DU munitions. Second, the soldier must have internalized DU at levels that are high enough to cause the uranium in the urine either to exceed background levels of uranium excretion by a factor of four or to be detected by whole-body or partial-body counting. Uranium in urine measurements from the control group will establish the background levels for these two measurements. The exposed group has two subgroups.

(1) The first consists of those soldiers with internalized DU not from imbedded fragments. This group will consist of personnel who have DU from inhalation or through wound contamination. The data from this group will be used in both the metabolic modelling and chronic kidney toxicity studies.

(2) The second consists of soldiers with imbedded DU fragments. The data from this group will be used to compare the clinical course of DU fragments with non-DU shrapnel as well as to provide information for the metabolic modelling and kidney toxicity studies.

Determining the presence of DU shrapnel is not as straightforward as verification of the presence of internalized DU. The analysis is complicated because the penetration of an armored vehicle by a DU penetrator generates DU fragments, non-DU fragments, and fragments that are a mixture of DU and the other components of the vehicle. In addition, the size of these fragments will vary dramatically. In the two cases that have been studied so far, the fragment sizes ranged from just at the resolution limit of film radiography (approximately 0.5 mm) to 15 mm in diameter. Until more experience is gained, a patient is assumed to have DU shrapnel if shrapnel is detected radiographically and internalized DU is detected.

b. Special Study Group

A subset of the exposed group will be selected for inclusion in the special study group. This group will receive the more intensive testing required to determine uranium metabolism accurately, identify early signs of toxicity, monitor fragment dissolution rates, and determine how the uranium is partitioned in the body as a function of time. Evaluation of these variables will provide the information required to construct the metabolic models needed to assess the risks associated with internalized DU.

Criteria for selection include the presence of DU fragments in the body, uranium in urine levels that exceed 14 μg/d (10 μg/l[a]) and the soldier's availability for the intensive monitoring envisioned. Recognizing that participation in the special study group will require a significant commitment, soldiers will be selected who are highly motivated to participate and are located near testing facilities.

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[a]*All conversions were calculated based on an assumed urinary excretion rate of 1.4 l/d.*

c. Nonexposed Groups

The data from the exposed population will be compared with that from two unexposed populations, which will serve as control groups for the study. The first control group, patients with non-DU fragments, is needed to determine whether the body's response to DU fragments differs from the body's foreign body normal response to shrapnel. The second group, unwounded and nonexposed, is needed to compare normal changes in kidney function with changes that might be due to the presence of uranium. The need for the second control group is based upon the assumption that non-DU fragments might cause changes in the parameters being measured.

Members of the non-DU fragment control population (the first control population) will be selected from veterans wounded in incidents not involving DU munitions. This will eliminate the possibility of a control group member having a small undetected DU fragment. Members of the unwounded and nonexposed control group will be selected from any unwounded population that does not meet the criteria for inclusion in the exposed population. In each case, groups will be appropriately matched (age, sex, smoking habits, similar Desert Storm experiences, etc.) with the exposed population.

d. Study Duration

At this point, it is difficult to determine the study duration, but the long latent periods for some effects[1] require that the study last at least 5 years. The study could extend for the lifetime of the members of the study groups.

3. Program Management

The program management group will supervise the initiation and conduct of the measurements, analysis, and documentation required by this project. The group will exercise oversight of each phase of the study and will control its overall direction. The group will consist of four representatives, at most, from the Department of Veterans Affairs, the Department of the Army and/or the Armed Forces Radiobiology Research Institute (AFRRI). The group has the following responsibilities:

a. Fiscal Management

The group will establish yearly budgetary requirements and maintain the records required to track the expenditure of funds during the fiscal year.

b. Patient Management

The group will identify the patients in each study group and establish mechanisms for patient tracking.

c. Data Gathering

The group will serve as the central repository for the data gathered in all phases of this study, including selecting the laboratories that will perform the required tests and developing and supervising the quality assurance program for these laboratories.

d. Data Analysis

The results of each required test will be submitted to this group for analysis and study. This group will be responsible for calculating and documenting dose estimates for each patient as well as determining if clinically significant changes had occurred.

e. Protocol Changes

The group will direct any changes required to meet the objectives of this study.

f. Treatment Recommendations

The group will be responsible for evaluating the data received to determine if an alteration in treatment is required and will make its recommendations to the attending physician.

g. Research Recommendations

The group will make recommendations for further research based upon their findings as appropriate.

h. Subject Matter Experts

To ensure the availability of the expertise required for this effort, the program management group will be augmented by a panel of subject matter experts. This panel will consist of physicians and scientists with expertise in radiation injury, epidemiology, health physics, uranium toxicology, and the laboratory procedures required by this protocol.

4. Patient Briefing

This briefing will be in sufficient detail to meet the requirements for informed-consent for participation in a human research project. Since long-term patient participation is key to the success of this study, it is recommended that this briefing be given by someone who will be with the project for an extended period of time and who has experience with this type of long-term study. This briefing will include a discussion of

a. the scope of the program and how the data will be used;

b. the tests and the frequency of testing, along with the risks entailed with participation and nonparticipation in the program;

c. the benefits and requirements of participation in the U.S. Uranium Registry;

d. procedures to follow for fragment removal. Standard medical guidelines should be used for decisions concerning fragment removal.[1] Once the removal decision is made, surgeons should use the procedures listed in paragraph 6.d. below for the removal of DU and non-DU fragments.

5. Protocol Test Requirements

a. Tests Required

(1) Table 1 outlines the required tests and test frequencies for each of the study populations. The specifics for each of the tests are explained in paragraph 6.

(2) The increased frequency of testing for soldiers with uranium concentrations in their urine at levels greater than 14 μg/d (10 μg/l) of urine (see Table 2) is based on the clinical need to monitor for signs of long-term kidney toxicity.

b. Modifications of the Test Protocol

(1) The program management group (see paragraph 3) will make modifications to the protocol as a whole or for an individual patient based upon its analysis of the results received. This re-evaluation should take place at least annually.

(2) The presence of symptoms in a patient (e.g., indications of toxicity, unusual growths, or other abnormalities) will trigger an immediate re-evaluation of the required tests and their frequency, and the need for medical/surgical intervention.

Table 1. *Recommended Tests and Test Frequencies.*

TEST	Exposed Population Urinary Excretion <14 µg/d	Exposed Population Urinary Excretion ≥14 µg/d	Special Study Group	Control Groups
Uranium in Urine	Annually	Table 2	Twice Weekly	Annually
Urine Chemistry	Annually	Table 2	Table 2	Annually
Uranium in Feces[1]			As Needed	As Needed
Tissue Analysis[2]	As Needed	As Needed	As Needed	As Needed
Whole Body and Regional Counting[3]	Initially	Initially	Biennially	Initially
Uranium in the Skeleton			Annually	Annually
Uranium in Blood			Quarterly	Annually
Blood Chemistry	Annually	Table 2	Table 2	Annually
Clinical Evaluation	Annually	Annually	Quarterly	Annually
Diagnostic Imaging[4]	Annually	Annually	Annually	Annually

[1]*Fecal samples will be performed whenever inhalation exposure is suspected. Control group fecal analysis will be used to provide estimates of normal uranium levels in feces.*
[2]*Tissue analysis will be performed on tissue samples taken as a result of a fragment removal procedure for both DU and non-DU fragments.*
[3]*Repeat after fragment removal or as required by the program management group.*
[4]*Radiographs are only required for personnel with imbedded fragments. This is not required for exposed or control group patients who do not have fragments.*

6. Test Specifications

This section describes the purpose and specifications for each of the tests required in the protocol. The specifications are designed to provide minimum test standards required to meet the objectives of the protocol. Selection of the laboratories where these tests are done will be made by the program management group based upon the guidance in this section and an assessment of the site's capabilities. The laboratories must meet the quality assurance requirements in paragraph 7 below. It is highly recommended that the same laboratory be used for each test whenever possible.

References 2 and 3 contain a partial listing of commercial and government laboratories with the capability for whole-body counting and for radiobioassay. While DoD laboratories are not specifically listed, the Army (U.S. Army Environmental Hygiene Agency) and the Air Force (Armstrong Laboratory) have the technology required to perform some of the radiobioassay procedures listed.

Table 2. Test Frequency for Selected Tests as a Function of Initial Urine Uranium Concentration.

Uranium Excretion Rate in Urine (μg/d)	*Test Frequency**	*Remarks*
14-50	*Quarterly*	
50-250	*Monthly*	*Potential for the onset of kidney toxicity.*
> 250	*At Least Weekly*	*Potential for kidney toxicity.*

**Tests include uranium in urine, urine chemistry, and blood chemistry.*

a. Uranium Concentration in Urine

(1) Purpose. This test will provide a direct determination of the uranium excretion rate which will be used for metabolic model construction and risk assessment.

(2) Specifications

(a) While a 24-hour urine sample is desirable, timed urine samples are acceptable. For 24-hour urine samples, it is important that all voids be collected. For timed urine samples, accurate accounting of the time period is a requirement and time periods of not less than 12 hours are recommended.

(b) Urine samples must be processed in a laboratory where the uranium measurement methods have a minimum detection limit of 0.4 μg of uranium per liter of urine or better. The laboratory must meet the quality assurance requirements listed in paragraph 7.

(c) Detailed sample collection and preservation procedures will be established by the laboratory performing the analysis.

(d) The nonexposed group will provide urine samples that will be used to establish background urinary excretion levels for uranium.

b. Urine Chemistry

(1) Purpose. The primary purpose of this test is to monitor the urine for signs of kidney toxicity or other abnormal changes in kidney function.

(2) Specifications. Urine chemistry to include a quantitative analysis of gamma-glutamyltransferase, beta-2-microglobulinuria, protein, amino acids, creatinine, phosphorus, and urinalysis (specific gravity, albumin, glucose, and microscopic sediment analysis) is required. Serum creatinine and creatinine clearance studies are needed to assess glomerular function and tubular integrity. It should be noted that these tests might underestimate filtration rate if tubular injury is present.

c. Uranium in Feces

(1) Purpose. This test is designed to give an indirect assessment of the uranium content in the lung and to assist in establishing lung clearance rates for metabolic modeling. This test should be administered only if significant lung contamination is suspected.

(2) Specifications. The specifications for fecal samples will be determined based upon the requirements for each test. As a general rule, the minimum detection limits for laboratories should be less than 3 μg of uranium per sample (less than 1 pCi per sample). Preservation and shipment requirements will be determined by the laboratory doing the analysis.

d. Tissue Analysis

(1) Purpose. This series of tests will be performed on tissues removed from a patient as a result of the patient's decision to have a fragment removed. The purpose of these examinations will be to determine

(a) the uranium content of the tissue (information will be useful in both metabolic modelling and risk assessment) and

(b) whether significant changes have occurred in the tissues surrounding the fragment. Thorotrast data indicate that long-term exposure to low-dose-rate alpha emitters can cause tissue fibrosis and necrosis with latent periods in excess of 5 years.[1]

(2) Specifications

(a) Surgical Removal of the Fragment. In addition to standard procedures, the following steps should be accomplished.

- Photograph the procedure. Of particular interest is evidence of total or partial fibrotic encapsulation; local tissue necrosis; growing granuloma; or if there is evidence of a breakdown, a formed fibrotic capsule.

- If the fragment is encapsulated, remove and save the intact capsule (with the fragment still inside) if possible. If the fragment must be removed from the capsule or if the capsule breaks during removal, document the capsular fluid appearance and volume. The capsule, capsular fluid, and any other tissue removed should be saved for histopathology and radioassay. Take careful note of the physical characteristics of the fragment upon removal. Specifics include color, shape, and any evidence that the fragment is breaking up. Color photographs of the fragment with a means of measuring its size are desirable. Seal the fragment in a plastic bag. Contact the program management group for instructions concerning the disposition of the fragment.

(b) Histopathology. The objective of this series of experiments is to determine if there are any unusual changes in cell structure of the surrounding tissue.

(c) Uranium in Tissue and Fluids. The uranium in retained tissue or fluids should be determined using techniques with the capability of detecting uranium levels on the order of 0.2 μg (0.06) pCi per tissue sample submitted. It should be noted that there are ultrasensitive fission-track counting techniques that can be used to detect 10^{-14} grams of uranium. At this level, the same tissue samples could be used for both histopathology and uranium concentration determinations.

(d) Sample Preservation Techniques. The sample preservation techniques used will depend upon which of the two procedures will be performed. At this point, it is uncertain if the same tissue sample can be used for both uranium concentration and histopathologic procedures. Once notified that a fragment will be removed, the program management group will decide which of the two procedures will be performed.

e. Whole-Body Counting, Regional-Area Counting, and Skeletal Uranium Determination

(1) Purpose. The combination of whole-body and regional-area counting allows for the quantification of the total amount of uranium in the body and the amounts of uranium in key locations in the body, using external measurement techniques. This information will be used in conjunction with urine and feces uranium contents to determine the metabolic models for uranium retention. The *in vivo* skeletal counting is an attempt to track uranium deposition in the skeletal system.

(2) Specifications

(a) The systems used must be capable of performing both whole-body and regional-area counting of uranium. Current systems can provide minimum detectable activities[b] (MDA) for regional-area counting of the lung on the order of at least 2 nCi (6 mg of DU) of DU in the lung by measuring the ^{234}Th progeny of ^{238}U.[3]

(b) Regional-area counting is required for the lungs, kidneys, liver, all wound or burn sites (regardless of how minor the wound), and of all areas with suspected DU fragments.

(c) Radiographs will be used to determine fragment location(s) so that estimates of tissue absorption and self absorption corrections for each of the areas counted.

(d) The *in vivo* skeletal-counting systems used should have an MDA of 10 nCi (30 mg) with adequate procedures for discriminating sources originating in the bone from those originating in the remainder of the body. The skeletal counting system developed at New York University is a good example of an acceptable skeletal-counting system.[3]

f. Uranium Concentration in the Blood

(1) Purpose. The test will measure the concentration of uranium in the blood by measuring the uranium concentration in the serum and cellular components of the blood.

(2) Specifications. Typical minimum detection limits for systems designed to measure the uranium content of the blood are on the order of 1 nano-gram of uranium per ml of blood. The laboratory selected to perform this test should have comparable efficiencies.

g. Blood Chemistry Evaluation

(1) Purpose. These tests are aimed at determining whether or not heavy metal toxicity and/or bone-marrow suppression has occurred.

(2) Specifications. SMA -12/20 or equivalent with complete blood count with differentials and platelet count.

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[b]*The referenced work defined MDA as 4.65σ where σ is the standard deviation of the background count.*

h. Clinical Evaluation

(1) Purpose. Clinical evaluation will determine the presence of any abnormalities such as nodules or unknown growths in the vicinity of fragmentation wounds or a degradation in the viability of the tissues. Reference 1 contains a discussion of potential abnormalities and estimates of the latent periods associated with each.

(2) Specifications. Emphasis will be placed on organ/structure dysfunction related to the location of the fragment(s) and to the consequences of the potential radiological and chemical effects. Specific tests are determined by the location of the fragment(s). Particular attention will be given to detecting thorotrastoma-like growths at the site of fragment implantation. A thorotrastoma is a growth that appears at the sites of extravascular Thorotrast with a latent period of 5-35 years post injection.[4-8] In some instances, these granulomas grew to enveloped clinically significant blood vessels and nerves and, in some cases, proved fatal.

i. Diagnostic Imaging

(1) Purpose

(a) Determine the composition of the fragments in an attempt to differentiate between solid DU and aluminum DU mixtures.

(b) Determine the approximate size and anatomic location of the fragment(s) in the body with sufficient detail to make absorption and self absorption corrections for whole-body counting data.

(c) Detect or confirm the presence of the formation of the fibrous encapsulation or of a thorotrastoma-like growth.

(d) Determine if there have been any gross changes in the location or size of the fragment.

(2) Specifications

(a) Both magnetic resonance imaging (MRI) and radiographic imaging are required for patients with imbedded fragments. MRI will be used to detect soft tissue abnormalities (granulomas, thorotrastomas) in the tissues surrounding imbedded fragments. MRI will only be performed after determining that there are no ferromagnetic fragments or objects in the patient.

(b) Radiographic imaging will be used to determine the size and position of imbedded fragments with sufficient accuracy to detect changes in the location of the fragment and to make the tissue absorption and self-shielding corrections required for whole-body counting. It is anticipated that at least two projections will be required.

7. Quality Assurance

The long-term nature of this protocol mandates the implementation of a stringent quality assurance program to ensure the accuracy and precision of the data collected. The program management group will develop the details of the quality assurance program. The program must incorporate the following provisions:

a. The use of accredited laboratories when possible. The laboratory performing each of these tests must be accredited by an appropriate accrediting agency to perform the required test. The laboratory must have a viable quality assurance program that is in accordance with the guidance provided in References 9 and 10. The program management group will establish standards based upon the guidance in this protocol when such accreditation is not available.

b. The use of the same laboratory to perform each type of test when possible. Adoption of this strategy will ensure the consistency of the data and enhance the program management group's ability to monitor the quality of the data collected. When the same laboratory cannot be used, the program management group must develop procedures to ensure the comparability of the data generated.

c. The use of intercomparisons by the specific laboratories chosen. The quality assurance program must include either a program of intercomparisons with other laboratories or, ideally, comparisons with a national standard.

Standard records management quality control procedures will be implemented to ensure the accuracy of the records maintained by the program management group.

Glossary of Terms

Exposed population. There are two criteria for including a soldier in the exposed group. First, the soldier must have been in or on the vehicle when the vehicle was struck by DU munitions. Second, the soldier must have internalized DU at levels that are high enough to cause the uranium in the urine either to exceed background levels of uranium excretion by a factor of four or to be detected by whole-body or partial-body counting. Uranium in urine measurements from the control group will establish the background levels for these two measurements.

Nonexposed population. The nonexposed population is composed of two subgroups: The first subgroup includes those soldiers with fragment wounds that are known not to be DU. The second consists of those soldiers who were not wounded and do not have internalized DU. DU is considered not to be present in significant amounts if the uranium concentrations in the urine are less than four times the background level and the results of whole-body or partial-body counting are negative.

Minimum detectable amount (MDA). The smallest amount of a substance that can be detected with a probability β of nondetection (Type II error) while accepting a probability α of erroneously deciding that a positive (non-zero) quantity is present in an appropriate blank sample (Type I error).[9] For this protocol both α and β are set at 0.05.

Program management group. A multi-disciplinary team that will oversee the implementation of the protocol and evaluate the results and direct changes in the protocol as required.

Radiobioassay procedure. For the purposes of this protocol, a radiobioassay procedure is any procedure used to measure the uranium in the body (whole-body counting) or in biologic material excreted or removed from the body for the purposes of estimating the uranium content in the body.[9]

Special study group. A subset of the exposed group that will receive the more intensive testing required to accurately determine uranium metabolism, to identify early signs of toxicity, to monitor fragment dissolution rates, and to determine how the uranium is partitioned in the body as a function of time.

Thorotrastoma. A large growth that appeared at the sites of extravascular thorotrast in patients injected with thorotrast, a thorium containing radiographic contrast agent. The growth appeared with a latent period of 5-35 years postinjection. These granulomas grew to large sizes and some enveloped clinically significant blood vessels and nerves and, in some cases, proved fatal. Thorotrastomas are discussed in the references.

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2. Type of Organization:

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3. Title:

Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology and Biokinetic Modelling

4. Proposal Also Being Submitted To:

5. USMRDCALC Log No.: _____ **6. Proposed Amount:** $721,635

7. Requested Start Date: 1 October 1994 **8. Duration:** 3 years

9. Proposal Valid Until (*minimum 6 months*): 1 November 1994

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Glennel K. Cooper 5/27/94

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Typed Name: Dr. E. John Ainsworth

Typed Title: Scientific Director, AFRRI

Signature: E.J. Ainsworth **Date Signed:** 27 May 94

#838

HEALTH RISK ASSESSMENT OF EMBEDDED DEPLETED URANIUM: BEHAVIOR, PHYSIOLOGY, HISTOLOGY AND BIOKINETIC MODELLING

Keywords: Depleted uranium, neurotoxicity, nephrotoxicity, capsule formation, biokinetic model, histopathology, uranium distribution.

Abstract

During Operation Desert Storm, a number of U.S. soldiers were wounded by depleted uranium (DU) shrapnel which has not been surgically removed. DU presents a unique toxicological problem because it combines potential chemical toxicity and a radiological hazard. Using an interdisciplinary approach, this proposal will evaluate the behavioral, physiological, biochemical and histological consequences of implanted DU in a rodent model. Motor activity and memory will be assessed with behavioral tests. Peripheral nerve function will be evaluated with conduction velocity measurements. Central nervous system excitability will be evaluated in a brain slice preparation. Renal function will be monitored with biochemical measurements of markers in urine and plasma. Local tissue damage and capsule formation will be characterized. A variety of tissues will be histologically examined and measured for uranium content. A biokinetic model will be developed to describe the distribution and quantification of uranium from embedded DU fragments as a function of time. Our findings will provide an integrated analysis of the risks associated with embedded DU and significantly contribute to the health hazard data base on this potentially toxic substance.

Table of Contents for Task I

HEALTH RISK ASSESSMENT OF EMBEDDED DEPLETED URANIUM: BEHAVIOR, PHYSIOLOGY, HISTOLOGY AND BIOKINETIC MODELLING

I. BACKGROUND

Natural uranium consists of three isotopes: ^{238}U (99.276%), ^{235}U (0.718%) and ^{234}U (0.0056%). During the uranium enrichment process two products are produced, "enriched uranium" and "depleted uranium" (DU), that contain different relative ratios of these three isotopes. Enriched uranium contains the higher amount of the fissionable isotope ^{235}U and is used for nuclear reactor fuel and nuclear weapons. DU has a lower ^{235}U content and is a highly dense material. The DU used by the US in kinetic energy penetrators is alloyed with titanium (0.75% by weight) to retard oxidation. This DU alloy is of concern because the U.S. military currently uses this metal for munitions and armament. During Operation Desert Storm, a number of U.S. military personnel were wounded by shrapnel fragments consisting of DU[1,2]. Since surgical removal can produce excessive tissue damage, these DU fragments were treated as conventional shrapnel and left in place in the wounded soldiers. The radiographs of injured soldiers show multiple embedded fragments ranging in size from 1 mm to over 5 mm in diameter (see Fig. 1 of project overview). Fragments as large as 20 mm have been noted in other patients. Uranium bioassays taken over a year after injury indicate that uranium was present in the urine well in excess of natural background, up to 30 µg U/l of urine. DU fragments present a radiologically and toxicologically unique situation with unknown health risks. Congress has mandated the study of these risks.

This proposal evaluates the consequences of both short-term and long-term exposure to DU fragments in the rat model. Using an interdisciplinary approach, we will assess neurotoxicity, nephrotoxicity, histopathology of the tissue surrounding the fragment and pathology including evaluation of neoplastic changes in several body tissues. In addition, based on our animal data, we will develop a biokinetic model that describes the distribution of uranium from embedded fragments as a function of time.

Uranium toxicity: Although the toxicity of embedded DU is unknown, numerous studies have addressed the consequences of inhalation, ingestion and parenteral administration of other forms of uranium[3-6].

After uranium is absorbed, it circulates in the blood as the uranyl ion forming uranium-carbonate and uranium-albumin complexes[7-9]. As the uranium-carbonate complex passes through the kidney, it is filtered rapidly at the glomerulus where 60%-80% of absorbed uranium is excreted in the first 24 hours after acute exposure. The uranium not excreted is reabsorbed by the proximal tubules where it produces acute toxic effects. Uranium also enters the bone where it competes with calcium to form complexes with phosphate ions, thus becoming part of the bone matrix[10-13]. This bone matrix then serves as a storage site from which uranium is slowly released back into circulation[14,15]. The liver, muscle, and kidney are other major sites of uranium disposition, with a possible long-term storage mechanism in the kidney[3,4,14,16,17]. At low doses, uranium may not readily distribute to the central nervous system (CNS)[5]. With higher doses (8 mg/kg/day orally for 4 weeks), however, brain uranium levels are comparable to those in liver and in bone[5], major sites for uranium accumulation.

Acute morphological and biochemical changes of the kidney result from uranium exposure[7-9,11]. The glomerular epithelial architecture is altered[18] and cellular necrosis occurs in the proximal tubules near the corticomedullary junction in the kidney[19-21]. In addition, polyuria, enzymuria, glucosuria, and increased excretion of amino acids result[7,8,22,23]. Acute renal failure can be the cause of death with exposure to high doses of either soluble or insoluble forms of uranium[24,25]. Environmental stressors such as restricted diets or changes in housing conditions significantly enhance uranium toxicity[26,27].

Few studies have addressed the chronic toxicity of uranium and the results available are conflicting. Galibin and colleagues[28] reported severe renal toxicity in rats that inhaled the slightly soluble uranium compound, ammonium diuranate (1 or 8 mg/m^3) for 128 days. Urine protein and blood, non-protein nitrogen were elevated. In the proximal tubules, there were sloughed dead cells and abnormal regenerating cells. These animals recovered, although the total number of tubules was reduced, with an accompanying increased proportion of connective tissue in the kidney. In contrast, Leach et al.[29,30] found no renal toxicity in rats repeatedly exposed for a period of 12 months to uranium dioxide dust (5 mg/m^3) (or in dogs or monkeys exposed for 5 years). Yet uranium concentrations in the kidney were as high as 1.1 μg U/g kidney wet weight in the rat (8.3 in the dog and 17.0 in the monkey), levels reported to cause acute renal toxicity (e.g., see[14]). Thus the chronic effects of uranium exposure remain, for the most part, unresolved[7].

The threshold concentration of kidney uranium levels in man that results in kidney chemical toxicity is in dispute[7,8,31]. While the Nuclear Regulatory Commission has set the level at 3 μg/g kidney for renal damage in man, there is evidence from both human and animal reports that this level could be much lower. For example, chronically exposed uranium mill workers, whose kidney uranium levels probably did not exceed 1 μg U/g[32], showed mild renal dysfunction with increased urinary excretion of β_2-microglobulin and various amino acids. In rats exposed subchronically to low doses (cumulative dose: 0.66 or 1.32 mg/kg) of uranyl fluoride, kidney uranium levels as low as 0.7 to 1.4 μg U/g wet kidney produced cellular and tubular necrosis of the proximal tubule, proteinuria, and enzymuria[22]. These changes in rat renal function, however, were temporary, with complete recovery within 35 days after exposure. These studies are important because they indicate that renal injury can occur at kidney uranium levels well below the 3.0 μg U/g limit.

Neurological effects have been reported with uranium exposure. In uranium workers excreting up to 200 μg U/l in their urine, normal mental function was disrupted[33]. One case study linked the handling of a uranium bar and a subsequent increase in stool uranium with foot cramps, leg pain and abnormal gait[34]. With oral and subcutaneous administration of relatively high doses of uranyl acetate (210 mg/kg and 10 mg/kg, respectively), rats exhibited tremors[35]. The uranyl ion has been demonstrated to enhance muscle contraction with acute local concentrations of 200-400 μM[36,37]. At the neuromuscular junction in the mouse, multiple sites of action were identified, including increased duration of the muscle action potential, broadening of the compound nerve action potential, increased amplitude and quantal content of the endplate potential and increased frequency of the miniature endplate potentials[36]. These studies indicate that embedded DU fragments could lead to neural damage, affecting both motor and cognitive function. The CNS effects of uranium toxicity can result from secondary mechanisms since hormonal changes, electrolyte disruption and immune responses can all influence nervous system activity[38].

Local Tissue Response and Capsule Formation: Foreign bodies in tissue elicit an immune response that can result in encapsulation. Even when encapsulated, DU fragments provide a local, chronic source of α-radiation. Within 10-15 cells of the fragment, the dose rate is expected to be approximately 8.5 Gy/yr (Appendix I-D). This radiation could result in injury or damage to local muscle or nerve tissue (axonal

injury, demyelination)[39,40]. In addition, capsule formation around a DU fragment in close proximity to a nerve could increase the risk of compression injury to those nerves.

Encapsulation could limit the chemical toxicity of the DU fragments by decreasing the rate of release of the metal, as has been observed with lead[41]. Encapsulation can also result in the formation of pseudocysts. Pseudocysts were formed that contained fluid with very high concentrations of soluble lead and insoluble lead dioxide particles[41,42] and with "black pigment...firmly adherent..." to portions of the inner wall of the capsule[42]. If these cysts should rupture, the rapid release of this fluid could cause period spikes in circulating lead levels and result in acute lead toxicity 5 to 40 years after the initial injury[41-43]. Similar type lesions may form around DU fragments. Intracapsular fluid may contain high concentrations of both soluble and insoluble DU. Tonry[44] demonstrated that DU disks formed both a soluble fraction and black insoluble particulates when emersed in simulated lung fluid. After a large fragment (approx. 20 mm) was removed from a U.S. soldier 17 months after he was wounded, the surgeon[45] noted that the fragment was encased in a fibrous capsule. When the capsule was breached, approximately 1-2 ml of a black fluid "gushed forth" from the cystic space.

The radiation emitted by DU might, in fact, affect the long term integrity of the fibrous capsular wall of the pseudocyst and contribute to the potential for a breakdown of the encapsulation process, causing a release of uranium and producing latent toxicity. Analysis of eight patients with injected plutonium-239 fragments[46,47] led to the conclusion that the high dose-rate radiation from ^{239}Pu caused a three stage reaction[46]: a granulomatous inflammatory process, followed by formation of a dense collagen encapsulation, which exhibited a final stage of degeneration and liquefaction. The process may then repeat itself, inducing a cycle of inflammatory reactions followed by a reorganization and re-liquefaction of the collagen. Similarly, Thorotrast which has a low dose rate comparable to DU[41,46] (DU is 4x Thorotrast), produces these types of changes in tissues over a 5-10 year time span[48-55]. Although the duration of this proposal may be too short to observe these changes, we may be able to observe and characterize pseudocyst formation with DU.

In summary, we expect that DU will cause both local and systemic toxicity through a variety of mechanisms. This proposal will define many of the potential sites of pathology that can result from long-term exposure to DU fragments and will provide a rationale for treatment of our wounded soldiers.

II. HYPOTHESES and AIMS

We hypothesize that long-term exposure to embedded DU will cause toxicity. To address this hypothesis, we have four specific aims:

Aim 1. Establish a dose-response profile for the acute effects of embedded DU pellets on renal function. These results will determine the DU doses to be implanted in rats used for Aims 2, 3 and 4.

Aim 2. Assess neural function following long-term exposure to DU fragments. Behavioral, peripheral and central neural damage will be tested.

Aim 3. Evaluate renal function, characterize capsule formation around the fragments and histopathologically characterize tissue responses to long-term exposure to DU.

Aim 4. Develop a biokinetic model to predict the distribution of uranium from embedded DU fragments. Estimate total body burdens of uranium based on measurements of excreta or on fragment surface area.

III. TECHNICAL OBJECTIVES

Aim 1: Dose-Response Profile. In order to identify the doses of DU to be used in Aims 2, 3 and 4, a dose-ranging study using a modified version of Dixon's up-and-down method[56] will be conducted. These experiments will determine the number of DU pellets required to obtain a uranium level of 0.70 μg/g wet weight of kidney. This level of uranium has been shown to produce early signs of renal damage as measured by both biochemical and histopathological changes[22] and will define the high dose of subsequent studies in this proposal. The low dose is that which produces no measurable acute toxicity. Our preliminary data (Appendix I-A) have demonstrated that in DU implanted rats, uranium can be detected in the urine. We anticipate that within 6 months we will be able to define the 3 doses of DU to be used in the long-term studies.

Aim 2: Neurotoxicity We will evaluate 3 manifestations of neural function: (a) a battery of behavioral tests to assess functional consequences; (b) conduction velocity studies in motor nerves to uncover any peripheral neuropathies and (c) electrophysiological analysis of hippocampal slices to assess CNS excitability. With these measurements it should be possible to determine the neurological consequences of long-term exposure to DU.

(a) Behavioral tests have frequently been employed to detect and characterize potential neurotoxic effects in rodents and have been used extensively in animal toxicity studies[57]. The neurobehavioral battery will consist of (i) a functional observational battery (FOB), which is a series of tests designed to assess the neuromuscular, autonomic, and sensory integrity of the rat[58-62], (ii) an automated test of locomotor activity and (iii) the passive avoidance test used to evaluate memory.

(b) Electrophysiological experiments will monitor nerve conduction velocity and integrity of the neuromuscular response. Nerve conduction velocity studies have been used clinically for many years to diagnose peripheral neuropathies and can even detect subclinical neuropathy induced by lead exposure[63-65]. The technique can distinguish between axonal degeneration, nerve demyelination, neuromuscular deficits and muscle disorders, allowing us to distinguish among the possible direct and indirect consequences of DU fragments.

(c) To evaluate CNS effects of DU, we will use the hippocampal brain slice isolated from implanted rats. Electrophysiological analysis of hippocampal brain slices has been used successfully to determine the time course of changes in neuronal excitability following *in vivo* exposure to low doses of ionizing radiation[66] as well as other *in vivo* treatments[67,68]. Because of its roles in memory and learning[69,70] as well as in motor activity[71,72], the hippocampus is a relevant area of the brain to assess. With this preparation, we can evaluate basic neuronal functions and the process of long-term potentiation[73], considered by many to be a physiological mechanism of memory (e.g., see[74-76]).

Aim 3. Nephrotoxicity and histopathology Other signs of toxicity resulting from the chemical and/or radiological effects of DU will be monitored. We will (a) evaluate renal function following long-term exposure to DU fragments using biochemical markers and histopathological evaluation; (b) characterize the early fragment encapsulation process and the potential for pseudocyst formation; and (c) histopathologically evaluate other organs that accumulate uranium.

(a) Markers of renal function in the urine and plasma will be used to assess nephrotoxicity. Altered creatinine clearance and proteinuria can indicate glomerular damage although tubular changes can also contribute. Increased urine content of enzymes such as lactate dehydrogenase (LDH) and N-acetyl-β-glucosaminidase (NAG) have been interpreted to reflect tubular damage[77]. In addition, appearance of

glucose in the urine, can indicate alterations in tubule reabsorption. These markers have demonstrated sensitivity with acute uranium nephrotoxicity[7,9,22,23] and should indicate any toxicity that might result from long-term exposure to DU fragments.

(b) Capsule formation and the sporadic release of pseudocyst fluid-contents can significantly influence the time course and concentration of uranium distributed through the body. Consequently, characterization of the capsule or pseudocyst around the DU fragment and the contents of the surrounding fluid are critical to understanding the long-term consequences of embedded DU. The encapsulation process and pseudocyst formation will be characterized at the time of euthanasia (1, 6, 12, 18 months after implantation), surrounding tissues will be histologically examined and any capsular fluid will be analyzed for its uranium content.

(c) Tissues that are known to accumulate soluble uranium or uranium particulates (liver, bone, kidney, spleen)[3,4,15,17,29,30] will be histologically evaluated for any lesions or neoplastic transformations. These data will complement the *in vivo* carcinogenesis studies in Task II. In addition, neural tissue (hippocampus and sciatic nerve) will be evaluated to provide histological correlates of the behavioral and neurophysiological data. The sciatic nerve will be specifically examined for lesions related to local radiation damage.

Aim 4: Biokinetic model. Although the distribution of uranium in the rat has been characterized for a variety of routes of internalization (inhalation, ingestion, and parenteral administration of soluble compounds), this information is not available for embedded fragments. We propose to develop this model using timed measurements of uranium in urine, plasma, kidney, bone (femur and skull), liver, spleen, brain, and skeletal muscle that is proximal and distal from the embedded pellets. Uranium is transported in plasma and urine and is stored in kidney and bone[3,4,15,17]. Uranium has been detected in the liver and spleen of animals[17,29,30] as well as in human subjects[14]. The skeletal muscle is being sampled to determine the local concentrations of uranium. The brain was chosen because of the paucity of data and the need to assess whether any neurological effects observed were due to the direct or indirect interaction of uranium in the body. These data will allow a rat biokinetic model for implanted DU fragments to be developed. Comparisons of uranium distributions with implantation vs inhalation or ingestion will be made in the rat model and applied to the human metabolic model as well. Appendix I-F contains a complete discussion of these models.

IV. MILITARY SIGNIFICANCE

DU munitions were developed by the military in the late 1970's and used for the first time in combat during the Persian Gulf War. At least 36 U.S. soldiers were wounded by DU shrapnel as a result of friendly fire[78]. The long-term health effects of DU shrapnel are unknown. This proposal will provide data on the physiological consequences of leaving DU fragments in place. Examination of the fragment capsule and surrounding fluid and tissue will provide insights into local tissue damage and systemic dangers of capsular fluid release. In addition, development of the biokinetic model will allow estimates of organ concentrations of uranium as predicted from urine and/or plasma levels. Consequently, the vulnerability of various tissues to embedded uranium can be assessed. These data will permit a rationale for deciding the efficacy of the Army's current fragment removal policy.

V. PRELIMINARY STUDIES

Our pilot studies have established the feasibility of the rat model for the proposed project. The surgeries were successfully accomplished and rat locomotor activity was normal within 48 hrs after surgery. We found that implantation of DU pellets causes the appearance of detectable levels of uranium in the urine. The thigh muscles of 4 rats were implanted with 8 DU pellets (4 on each side). The pellets were 1 mm diameter X 2 mm length. Another group of 5 rats were implanted with tantalum (Ta) pellets as a surgical and implantation control. The distribution of pellets in the left rear leg of one rat is illustrated in an x-ray photograph in Appendix I-A (3x magnification) (Fig. 1). The cylindrical shape and size of the pellets are similar to those DU fragments depicted in the X-ray photograph (1/2 reduction) of the wounded soldier (Fig 1 of project overview). Urine was collected on days 1, 7 and 14 after surgery and uranium levels determined. The time course of uranium in the urine is illustrated in Appendix I-A (Fig. 2). Only background levels of uranium were detected in the Ta control group. In contrast, within 24 hr after DU implantation, significant levels of uranium were detected (28.69 μg U/l). By day 7, uranium levels had increased nearly four-fold (111.86 μg U/l) and remained at this level at day 14 (112.61 μg U/l) following surgery.

VI. METHODS AND EXPERIMENTAL DESIGN

Approach

After defining appropriate doses (by surface area, i.e. number of DU pellets) to be used for analysis

of DU effects (Aim 1), a study of 325 rats will provide toxicity data for 3 DU doses (low, medium, high) at 4 time points (1, 6, 12, 18 months). Each rat will be thoroughly evaluated for changes in behavior, peripheral nerve function, CNS excitability, renal function and tissue histology including capsule formation. In addition, data on tissue uranium levels from a subgroup of rats will be used in aim 4 to develop a biokinetic model to predict uranium distribution.

Rats will be randomly assigned to 5 treatment groups: 1) rats implanted with low-dose DU, 2) rats implanted with medium-dose DU, 3) rats implanted with high-dose DU, 4) rats implanted with tantalum (Ta) to control for fragment implantation, and 5) a non-implanted sham-surgical control group. In the low-dose and medium-dose groups, Ta will be substituted for a fraction of the DU pellets in order to keep the total number of implanted fragments constant. Half of the total number of pellets will be implanted in each thigh with 1 pellet near the sciatic nerve between the sciatic notch and the knee.

Each rat will be evaluated for behavioral effects, changes in conduction velocity and changes in hippocampal activity (Aim 2). In addition, rats will provide tissue and body fluids for histological or biochemical analysis (Aim 3). Five of the rats in each experimental group will provide tissue for uranium quantification (Aim 4). Based on the variance of control data for electrophysiological effects in the CNS, a group size of 15 rats is necessary to see significant changes of 20% or greater at the $p \leq 0.05$ level. Additional animals (20 rather than 15) will be implanted for the 18 month time point with the expectation of a 20-25% natural mortality[79-81]. The DU implantations will be scheduled across a 7 month period to provide 3 animals for neurological evaluation each week at the appropriate time points.

Rats will be neurologically evaluated, euthanized and tissues samples taken at 1, 6, 12 or 18 months following fragment implantation. The testing schedule for each animal is outlined in Appendix I-B. The 1 month time point provides a measure of short-term toxicity. Six month intervals will allow us to monitor the onset and progression of long-term toxicity. The 18 month upper limit was selected for several reasons: 1) It exceeds the current Food and Drug Administration requirement of 12 months for chronic animal toxicity testing[82]. 2) Natural mortality increases dramatically after 18 months[79-81]. 3) Beyond 18 months, normal age-related changes in the renal function could present difficulties in data interpretation[83,84].

General Methods

Subjects: Sprague-Dawley rats (8-10 weeks of age) will be maintained 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, rats will be quarantined and screened for diseases. Except during urine collection, all animals will be housed in plastic microisolator rat cages with hardwood chips as bedding. Commercial rodent chow and acidified water (pH 2.5, using concentrated HCl) will be provided *ad libitum*. Rats will be on a 12-hr light/dark cycle.

Fragments: DU fragments, consisting of 99.25% DU and 0.75% titanium by weight, will be obtained from Oak Ridge National Laboratories, Oak Ridge, TN. The uranium isotopes present will be ^{238}U (99.75%), ^{235}U (0.20%) and trace levels of ^{234}U. This is the same DU alloy used in U.S. military munitions. Tantalum (Ta) fragments will be obtained from Alfa Products, Ward Hill, MA. Ta was chosen as the control substance because it is a biologically inert metal[85] with a similar mass to uranium and is frequently used in human prostheses[86,87]. Each fragment (both DU and Ta) will be approximately 1 mm diameter x 2 mm long.

Surgery: The DU and Ta pellets will be cleaned and chemically sterilized prior to implantation. The pellets will be immersed in industrial detergent, rinsed in absolute alcohol, soaked in 50% nitric acid solution for 3 min and then rinsed with acetone. This procedure completely removes the oxide formation on the surface of the DU pellet[44]. The animals will be administered atropine (0.05 mg/kg i.m.) prior to being anesthetized. Anesthesia will be induced with ketamine hydrochloride (50 mg/kg) in combination with xylazine hydrochloride (10 mg/kg), given i.m. in a 0.5 ml bolus, using a 25 gauge needle. These injections will be administered in the lumbar muscles to prevent irritating the site of implantation.

Fragments will be implanted within the biceps femoris muscle spaced approximately 15 mm apart on the lateral side of each thigh. One fragment in one leg of each rat will be positioned near the sciatic nerve between the sciatic notch and the knee. The surgical sites will be shaved and cleansed with betadine, a topical disinfectant, prior to surgery. Incisions utilizing a scalpel blade will be made through the skin approximately 10 mm deep into the muscle mass. The proximal incision will be 10 mm distal to the iliel crest and will be the site of the first pellet. Each fragment will be secured in place by use of absorbable

sutures (Dexon 3-0) to prevent movement of the implants.

Rats will be closely monitored following surgery until they are ambulatory and an analgesic (Demerol, 10 mg/kg, i.m.) will be administered if needed. A veterinarian will examine the surgery sites for signs of inflammation, infection and local DU toxicity daily for 2 weeks following surgery and weekly thereafter throughout the study. The rats will be treated by the attending veterinarian as required.

Specific Methods for Aim 1

Dose-response profile: Forty-eight male Sprague-Dawley rats (8-10 weeks of age) will be implanted with either DU or Ta pellets, embedded intramuscularly in the biceps femoris. The number of pellets (by surface area) implanted will be determined using a sequential "up-and-down", or staircase, design. Using this design, 3 animals will be implanted with the same number of DU pellets and 3 animals will be implanted with a similar number of Ta pellets, and the outcome of the kidney toxicity and uranium concentration analyses (see Aims 3 and 4) will be determined before the next animals are implanted. The goal is to identify the number of DU pellets required to produce early signs of nephrotoxicity (i.e, 0.70 μg U/g wet weight of kidney,[22]). Based on the results of our pilot study (Appendix I-A), we will begin this dose ranging study by implanting 3 animals with 8 DU pellets and 3 animals with 8 Ta pellets. No additional control groups will be used since the objective is only to determine kidney uranium levels and early signs of nephrotoxicity.

Baseline urine and plasma samples will be collected 1 and 3 days prior to implantation surgery. At 1, 3, 5, and 7 days following surgery, urine and plasma samples will be collected and analyzed for biochemical changes in renal function (see Aim 3) and uranium levels (see Aim 4). Sampling at these timepoints is necessary because signs of nephrotoxicity in laboratory animals exposed to low doses of uranium are frequently not detected until 3 to 5 days after exposure and may subside within 7 days[22]. On day 7, the animals will be sacrificed and the uranium levels in the plasma and kidneys determined. The kidneys will be histologically examined for any evidence of damage (see Aim 3). The local tissue around the fragment (fragment, capsule and adjacent striated muscle) will also be examined for characterization of the capsular response to the DU fragment (see Aim 3).

If after 7 days the uranium level in the kidney is below 0.70 μg/g, the number of DU pellets implanted

in the next group will be doubled. Conversely, if the uranium level is above 0.70 μg/g then the number of DU pellets implanted will be halved. This up-and-down procedure will be repeated until the mean number of pellets which result in a kidney uranium level of 0.70 μg/g can be estimated to within 0.10 μg/g. We anticipate needing no more than 8 up-and-down experiments to obtain this goal. This approach in determining a dose-response profile for DU on renal function requires 30-40% fewer animals and assays than a classical dose-response approach[88].

Kidney levels of 0.70 U μg/g wet weight produce both biochemical and histopathological changes[22], indicating acute renal toxicity. The number of DU pellets required to produce this level will be defined as the high dose in subsequent studies in this proposal. The low dose will be the largest dose that produces no measurable acute toxicity; the medium dose will be logarithmically intermediate between the high and low levels.

Specific Methods for Aim 2

Behavioral neurotoxicity: The functional observational battery (FOB) consists of behavioral evaluations (home-cage, handling and manipulative) and several physiological measures. The parameters to be recorded are listed below and grouped according to the following functional domains: 1) Autonomic: pupil response, lacrimation, salivation, palpebral closure, piloerection, defecation, urination, 2) Sensorimotor reactivity: tail pinch response, tactile response, click response, approach response; 3) Neuromuscular: gait, foot splay, forelimb and hindlimb grip strength, righting reflex, and 4) CNS Excitability: arousal, posture, ease of removal from cage, handling reactivity, convulsions, and locomotor activity.

The observer will be blind as to the identity of each group. The behavioral battery will commence with brief home cage observations during which time the observer will describe the posture, and the existence of tremors or convulsions, and palpebral closure. The rats will then be removed from their cage and rated for ease of removing and handling. While handling the rat, presence of piloerection and the degree of lacrimation and salivation will be observed. The animals will then be placed in an open-field with a perimeter barrier on clean absorbent white paper for 3 min. The number of rears, the gait, level of alertness, stereotypy (repetitive movements e.g., head weaving), unusual behaviors (e.g., writhing), and the number of fecal boli and urine pools will be recorded.

Sensorimotor responses also will be determined and include: approach response to a pencil eraser, touch on the rump (tactile response), click response (auditory response), pinch on the tail using forceps, and the pupillary response to a penlight stimulus. Next, neuromuscular responses will be determined and include: righting reflex, forelimb and hindlimb grip strength using digital strain gauges[62], and landing foot splay[61]. The animals will be weighed and rectal temperature determined using a digital thermometer. The FOB will be conducted during the light portion of the light-dark cycle. Details of the FOB tests can be found in Moser et al.[58] and McDaniel and Moser[60] and outlined in Appendix I-C.

Approximately, 1 hr after the FOB, the rats will be monitored for horizontal and vertical locomotor behavior. Motor activity will be recorded for 1 hr using automated photocell activity cages (Digiscan Analyzer, Omnitech Electronics, Columbus, OH). On the day following the FOB and motor activity tests, animals will be trained on a passive avoidance test. This test will be used to determine whether DU affects memory function. Results will be compared with the hippocampal electrophysiology experiments described below. The tests will be conducted in a passive avoidance apparatus (San Diego Instruments, San Diego, CA) that consists of 2 chambers (1 lighted, 1 darkened) separated by a guillotine door. The animal will receive a training trial during which time it will be initially placed into the lighted chamber. The natural tendency is for the rat to enter the darkened chamber. When it does, it will receive a mild foot shock. During this acquisition phase, the rats will be tested for eight trials or until criterion is met. The criterion will be 2 consecutive trials during which the rat does not cross into the darkened chamber. Each trial will be 3 min in duration with a 1 min intertrial interval. Seventy-two hours later the rat will be placed into the lighted chamber and retested. A comparison will be made with the initial training session to see if memory of the task has been retained.

Statistical analysis of the functional observation battery will be based on Creason[89]. For all continuous data, two-way ANOVAs will be conducted using a between-subject factors of dose and time. If the ANOVA resulted in a significant overall effect of dose, or dose x time interaction, post hoc comparisons will be made to control values using Dunnett's t test. Descriptive and rank data (e.g., scores such as sensorimotor responses, gait score) will be analyzed using a categorical procedure (CATMOD)[90]. For all tests, $p \leq 0.05$ will be considered significant.

Conduction velocities: One week following the behavioral testing, the rats will be evaluated electrophysiologically. Rats will be anesthetized with pentobarbital 50 mg/kg i.p. (supplemented 5 mg/kg every 3-4 hrs or as necessary). Both the right and left sciatic nerves will be exposed and bipolar stimulating electrodes will be positioned at the sciatic notch and the knee (cathode distal). A bipolar electrode will be inserted into the medial gastrocnemius muscle to monitor the compound muscle action potential. The recording electrode will be positioned over the endplate such that a biphasic response will be recorded. Nerve temperature will be monitored and maintained near 37° C with a heat lamp. Nerves will be stimulated at a frequency of 0.2 Hz. Stimulus intensity will be varied between approximately 10 and 100 V (0.1 ms duration) to determine the input-output relationship and the supramaximal stimulation parameters to use. Five muscle responses will be averaged and the latency, duration and amplitude of the potentials will be measured. Conduction velocities will be calculated by dividing the distance between the stimulating electrodes by the average latency difference between the time of onset of the compound muscle action potentials.

Duration of the muscle action potential reflects the synchrony of discharge. In general, the distal stimulating electrode will produce a faster, larger response than the proximal electrode. Greater dispersion and greater decrease in amplitude than normal would suggest nerve damage. For example, demyelinating disorders cause dispersion of the muscle action potential by slowing the nerve conduction velocities[91,92]. If dispersion occurs over a short segment, compression neuropathy may be indicated[91].

We will also use a variation of Hopf's collision method for determining the conduction velocities of slower fibers[92,93]. The interstimulus interval (ISI) between stimulation of the proximal and distal electrodes will be varied. At short ISIs the antidromic action potentials (AP's) elicited by the distal electrode collide with the orthodromic AP's elicited by the proximal electrodes, thereby preventing them from reaching the muscle. As the ISI is increased, the AP of the faster fibers pass the distal electrode before it is stimulated. Further increase in the ISI allows medium fibers, as well, to transmit their potentials to the muscle. At the longest ISI, all fibers escape the collision with the antidromic AP's. The absolute refractory period of the axon, during which reactivation of an AP is impossible, contributes approximately 1 ms to the ISI required for collision of the AP's. (This refractory period could also be affected by DU exposure.) Conduction

velocities are calculated from the distance between the 2 stimulating electrodes ÷ (ISI -1 ms). A plot of the muscle action potential amplitude elicited by the proximal electrode versus ISI is normally a sigmoid curve with the base of the curve representing the velocity of the largest fibers and the top of the curve representing the smallest slowest motor nerve fibers.

Since frequency response can also change with nerve or neuromuscular junction damage, the stability of the response will be examined at stimulation frequencies of 5 and 20 Hz in trains of 10 pulses, at 30 second intervals. A decrement with stimulation at 5 Hz will indicate neuromuscular junction damage[94]. Anomalies in the response to rapid rates of stimulation will reflect other neuromuscular, nerve and/or muscle damage[94-96]. The distribution of conduction velocities, the frequency response, and the ratio of distal/proximal amplitudes and durations will be compared among groups matched for survival time.

All stimulation and recording will be controlled by a 486 PC using standard electrophysiological software (Axon Instruments). Data will be analyzed with routines written in AxoBasic (Axon Instruments) and statistical analysis will be done with RS/1 (BBN Software Products) routines. Two-way analysis of variance (for time and dose) will be used to compare differences among the experimental groups.

Hippocampal slice electrophysiology: At the termination of the conduction velocity experiment, the rat will be euthanized by cervical dislocation. The brain will be quickly removed from the skull and submerged in iced oxygenated artificial cerebrospinal fluid (ACSF). Both hippocampi will be dissected out and sliced on a McIlwain tissue chopper (425 μm thick). Tissue will be incubated at room temperature in oxygenated ACSF (see below) for 1 hr to allow recovery from the slicing procedure. During this interval, tissue will be isolated from the rats for analysis of pathology and DU content (Aims 3 and 4).

A single slice of rat hippocampus will then be placed in a submerged slice chamber and perfused at a rate of 1-2 ml/min with warmed (30°C) oxygenated ACSF. ACSF has the following composition (in mM) 124 NaCl, 3 KCl, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 1.24 KH_2PO_4, 10 glucose, 26 $NaHCO_3$, pH 7.4, equilibrated with 95% O_2/5% CO_2. Extracellular recordings will be obtained with glass microelectrodes filled with 2 M NaCl placed in s. radiatum and s. pyramidale of field CA1 to record the population synaptic potential (pPSP) and the population spike (PS) respectively. A stainless steel, concentric, bipolar stimulating electrode will be positioned in s. radiatum of field CA1 to activate afferents. Constant current stimuli (0.1 - 1.5 mA, 300

μsec) will be applied at a frequency of 0.2 Hz. Except when generating input/output (I/O) curves, the stimulus current will be held constant at an amplitude that elicits approximately 30% maximal response.

To obtain I/O curves, stimulus intensity will be varied from approximately 0.1 to 1.5 mA in 13 steps. Three responses at each current step will be recorded and averaged. I/O curves will be generated following a 30-min equilibration period. The 3 I/O curves (volley vs PS, volley vs pPSP, pPSP vs PS) will be analyzed with the data analysis software RS1 (BBN Software Products, Cambridge MA). The responses at each stimulus intensity will be averaged for all experiments at each time point. A sigmoid curve will be computer fit to the points. Differences between curves will be tested for significance by comparing the residual sum of squares for the curve fit to the data of each experimental condition with the residual sum of squares for the curve fit to all the data. Significance will be accepted at $p \leq 0.05$.

Following generation of I/O curves, long-term potentiation (LTP) will be elicited using a high frequency stimulation (HFS) (100 Hz) for 1 sec delivered at half maximal stimulus intensity. These stimulation parameters have been shown to produce LTP (defined as at least a 30% increase in size 1 hr post-HFS) in almost all untreated slices[73]. The field potentials will be evaluated for 1 hr after the stimulation.

Specific Methods for Aim 3

Following behavioral testing (schedule in Appendix I-B), blood and urine samples will be obtained from all rats for analysis of renal function (see below). In addition, immediately following euthanasia on the day of electrophysiological analysis, tissue samples from bone (femur, skull), hippocampus, sciatic nerve, kidney, liver, spleen and fragment capsule with associated skeletal muscle will be obtained for histological examination (see below). Based on the literature, these are the most likely tissues to show increased levels of uranium[3,4,15,17,29,30]. In addition, any tissue showing unusual gross tissue alterations noted during necropsy (i.e. neoplasia) also will be collected. The patterns of nephrotoxicity and histopathology will be correlated with any functional deficits we observe in the behavioral and electrophysiological testing.

Sample collection: To safely collect the blood samples, rats will be immobilized by placing them in a Plexiglas restrainer. During each collection, 0.3-0.5 ml of blood will be obtained from the tail vein using a 22-gauge needle. The blood will then be centrifuged for 5 min at 3,000 X g. The plasma will be analyzed for uranium levels and/or for biochemical indices of renal function. Plasma will be stored at -

70°C until ready for analysis.

Urine samples will be collected by housing the rats in individual metabolism cages (23.5 cm diameter X 12 cm high) where they will have continuous access to food and water. However, since these housing procedures have been shown to induce stress and thus increase the toxicity of uranium[27], the rats will be acclimated to the metabolic cages for 5 days before the study begins. The metabolic cages will be disinfected and decontaminated between each animal use. The 24-hr urine collection sample will be obtained from each rat and the volume recorded (10-20 ml in pilot studies). Urine collection at 4°C is unnecessary since enzyme activity has been shown to be stable at room temperature for up to 24 hours[23]. After collection, urine will be filtered to remove any debris and stored in plastic containers at 4°C until analyzed (less than 1 wk). During the animal handling periods, overt signs of behavioral toxicity and the overall appearance of the rats will be noted.

Evaluation of renal function: Measurement of urine volume and osmolarity, urine levels of NAG, LDH, glucose, total protein, creatinine and blood levels of glucose, urea and creatinine will be used as indicators of renal function. In addition, since weight loss may be indicative of nephrotoxicity, all the rats will be weighed weekly throughout the study. Osmolarity of the urine will be measured with a vapor pressure osmometer (Model 5100B, Wescor, Inc.). A Kodak Ektachem 700 Analyzer will be used to determine plasma and urine levels of creatinine, glucose and urea. Total urine protein will be measured with a dye-binding assay (Coomassie Blue, BioRad) sensitive down to 1 μg. The activity of NAG will be measured by the methods of Tucker et al.[97] using 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide as the fluorescent substrate (excitation wavelength=356 nm; emission wavelength=446 nm). The dilution of the urine for this assay eliminates the effects of any inhibitors present[97]. For LDH measurements, 1 ml of urine will be dialyzed for 4 hr at 4°C with 1 liter of deionized water. LDH will be quantitated with a colorimetric assay that measures a reaction product which is proportionate to LDH activity (Oxford Biomedical Research Inc). Only 50-100 μl of fluid (urine or plasma) are required for each of these assays.

Although, urine volume and osmolarity can vary greatly with fluid intake, these measures provide physical indicators of renal function. For example, acute kidney failure drastically decreases urine volume, while moderate renal toxicity can increase urine output, as is seen with uranium exposure (e.g., see[35]).

Osmolarity can reflect the ability of the kidney to concentrate (or dilute) the urine. Plasma urea also changes with renal insufficiency. Since the rate of urea formation is proportionate to the rate of protein metabolism, other factors such as hepatic injury or altered protein intake can affect the measured urea in plasma. A small concentration of protein is normally present in the urine. Increases in total urine protein could result either from glomerular leakage or failure of tubule reabsorption. Urinary enzymes are sensitive, non-invasive markers of toxicity primarily in the kidney tubules[77]. NAG is a lysosomal enzyme found in proximal renal tubule cells. LDH is a cytosolic enzyme of the tubular epithelium.

Creatinine clearance is a commonly used measure of glomerular filtration rate in the rat despite a significant but constant tubular secretion. The use of an intrinsic metabolite has an obvious advantage over inulin or mannitol which (although not secreted) must be infused. Interpretation must be cautious since tubular injury with uranium could cause an underestimate of the glomerular filtration rate regardless of the marker used[7]. Creatinine clearance (C_c) will be calculated from the equation: $C_c=U_c*V_u/P_c$ where U_c and P_c are the creatinine concentrations in urine and plasma, respectively, and V_u is the rate of urine production (ml/min).

Appearance of glucose in the urine occurs when the tubule reabsorption maximum from the filtrate is exceeded. This can occur with hyperglycemia or with a decrease in tubular reabsorption capacity. Measurement of both urine and plasma glucose will help to distinguish between these two possibilities. Changes in reabsorption will be reflected in the calculated fractional excretion (FE): $FE = (U_g/P_g)\div(U_c/P_c)$; where U_g and P_g are the glucose concentrations in urine and plasma, respectively.

The proposed assays will provide a broad spectrum of measures of kidney toxicity. Many of these substances have been shown to be very sensitive in acute uranium toxicity[7,9]. Glucose is one of the most sensitive indicators[7,22] showing increased urine glucose, without concurrent increases in plasma. LDH and to a lesser extent NAG increase following uranium exposure[7,9]. A transient increase in urine volume and the appearance of protein in the urine also occur with acute uranium toxicity[9]. These measures will be used together as indicators of kidney toxicity and carefully interpreted and correlated with histopathology. Two-way ANOVA will be used to test the statistical significance of any changes.

Histopathology. Standard procedures for handling biologic specimens will be used in the preparation

of the samples. Tissues will be perfused, embedded, mounted and stained with hematoxylin and eosin stain (H & E)[98]. Specialized stains will be used to demonstrate specific lesions or further delineate lesions not well defined by the H & E stain. For example, silver stains will be used on neural tissue to delineate nerve fiber disruption or degeneration[98]. Animals not surviving until the scheduled euthanasia will have tissues sampled and analyzed in order to determine cause of death. At the time of necropsy or sacrifice, all DU pellets will be retrieved for appropriate disposal.

The pathologist evaluating the tissue will be blind to the experimental group from which the tissue was obtained. The pathologist will generate a 0 to 4 scoring system to evaluate the degree of microscopic changes observed; where 0=no change, 1=minimal change, 2=mild change, 3=moderate change, and 4=marked or severe change. All tissue changes observed in the rats implanted with DU will be contrasted and compared to the identical tissues taken from the controls. If there are significant changes noted in a particular system, for example the renal system, a detailed statement of criteria for 0-4 scores will be stated by the pathologist at the time of interpretation. Any pre-neoplastic or neoplastic transformations observed in selected tissues will be described and/or diagnosed by the pathologist in accordance with the Bulletin of the World Health Organization[99,100].

The implanted fragments and associated capsular fluid will be removed and evaluated. Thickness of the fibrous capsule, cell types, inflammatory response, and/or neoplastic changes will be noted. Contents of the capsules will be collected, described and assayed for uranium. Changes in kidney glomerular and proximal tubular epithelium will be carefully evaluated. Apoptotic cells, cell sloughing, loss of the brush border membrane are changes that have been described for acute uranium toxicity[22,23]. Neural tissue (hippocampus and sciatic nerve) will be examined for signs of axonal degeneration and demyelination. In the CNS, we will also look for chromatolysis in the neuronal cell bodies. These observations will provide histological correlates for the neurotoxicity data.

Methods for Aim 4

Tissues from 5 of the 15 animals in each experimental group at each time point will be collected for uranium analysis. An additional set of rats (5 per experimental group) will be implanted with DU (3 doses and controls) and euthanized 24 hrs after implantation to provide data on earlier distribution of uranium

which is required for the model. The tissues to be evaluated are urine, plasma, kidney, brain, bone (femur and skull), liver, muscle (both distal and proximal) and spleen. Samples will be frozen and shipped on dry ice to Battelle, Pacific NW. The analysis will be conducted by Dr. Fuciarelli at Battelle, Pacific Northwest Laboratories using kinetic phosphorescence analysis (Appendix I-E). Using this approach we expect to be able to measure down to 10pg U/g tissue which is well below background levels (50-100 pg U/g). The uranium analyses should be complete within a week after receipt at Battelle. The uranium measurements will be provided to our collaborators at Oak Ridge National Laboratories for biokinetic modelling. The model will be developed as outlined in Appendix I-F.

VII. QUALIFICATIONS OF THE INVESTIGATORS

Terry Pellmar, PhD, is a neurophysiologist with considerable experience with electrophysiological techniques, including hippocampal brain slices and motor nerve conduction velocities. She has evaluated the actions of radiation, free radicals and many pharmacological agents in neural preparations.

Michael Landauer, PhD, is a behavioral toxicologist and has extensive experience in conducting neuro-behavioral tests in rodent models. He has performed behavioral tests in rodents following exposure to chemical toxins (pesticides), pharmaceutical agents and ionizing radiation. Dr. Landauer is currently on the executive council of the Association of Government Toxicologists.

Steven M. Stiefel, DVM, Major, US Army, is a veterinary pathologist with 14 years of experience performing both clinical pathology and histopathological diagnoses in support of a wide variety of research studies. He has 6 years experience dealing exclusively with effects of and diagnoses of radiation-induced lesions.

Clifford L. Ferguson, MD, Colonel, U.S. Army, is board-certified in internal medicine with 12 years of experience in Nephrology. He is an expert in human and animal kidney diseases and has research experience in heavy metal toxicity.

Appendix I-A

PRELIMINARY STUDIES: Pilot Data

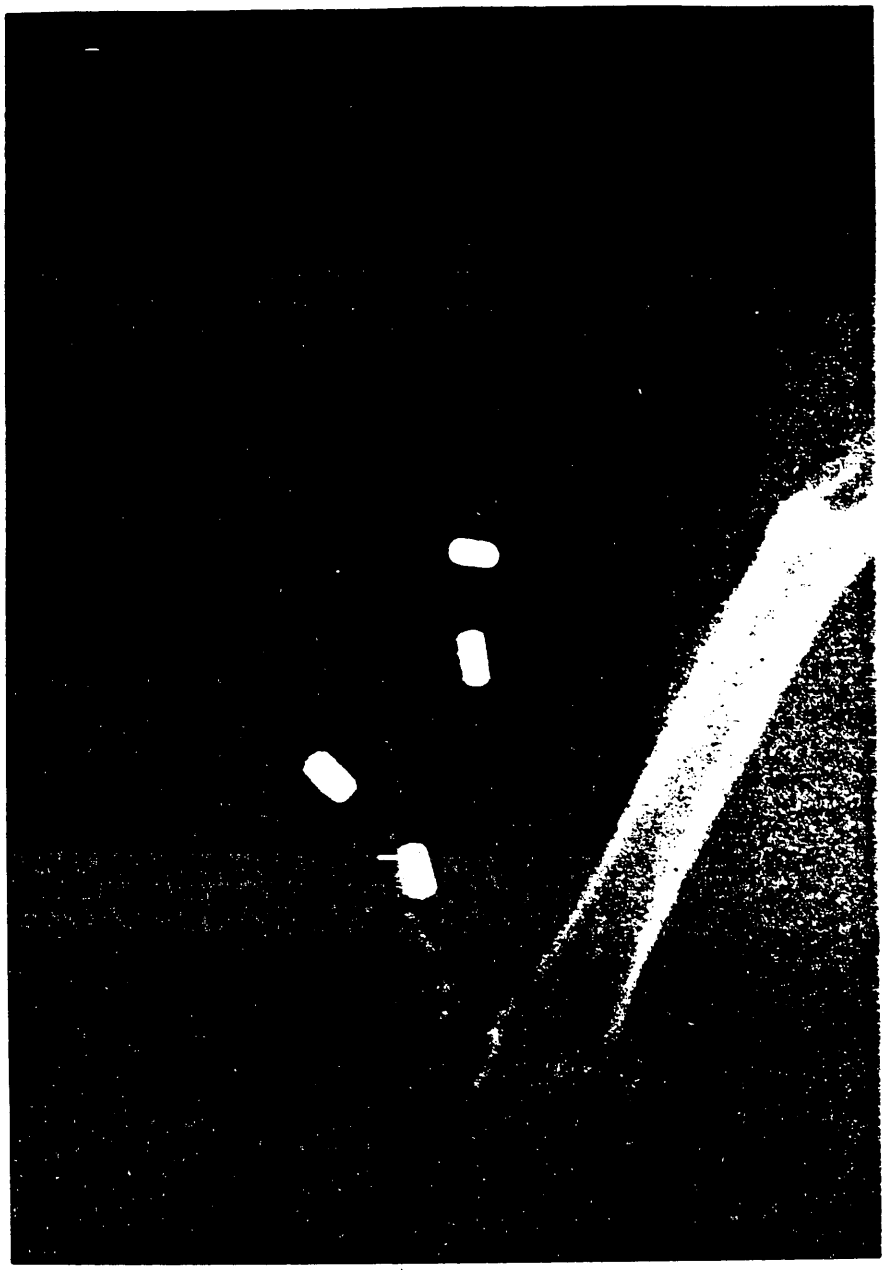

Fig I-A1. An x-ray photograph of the left leg of a rat surgically implanted with 4 depleted uranium (1 mm diameter X 2 mm length) pellets. Scale: 3X magnification.

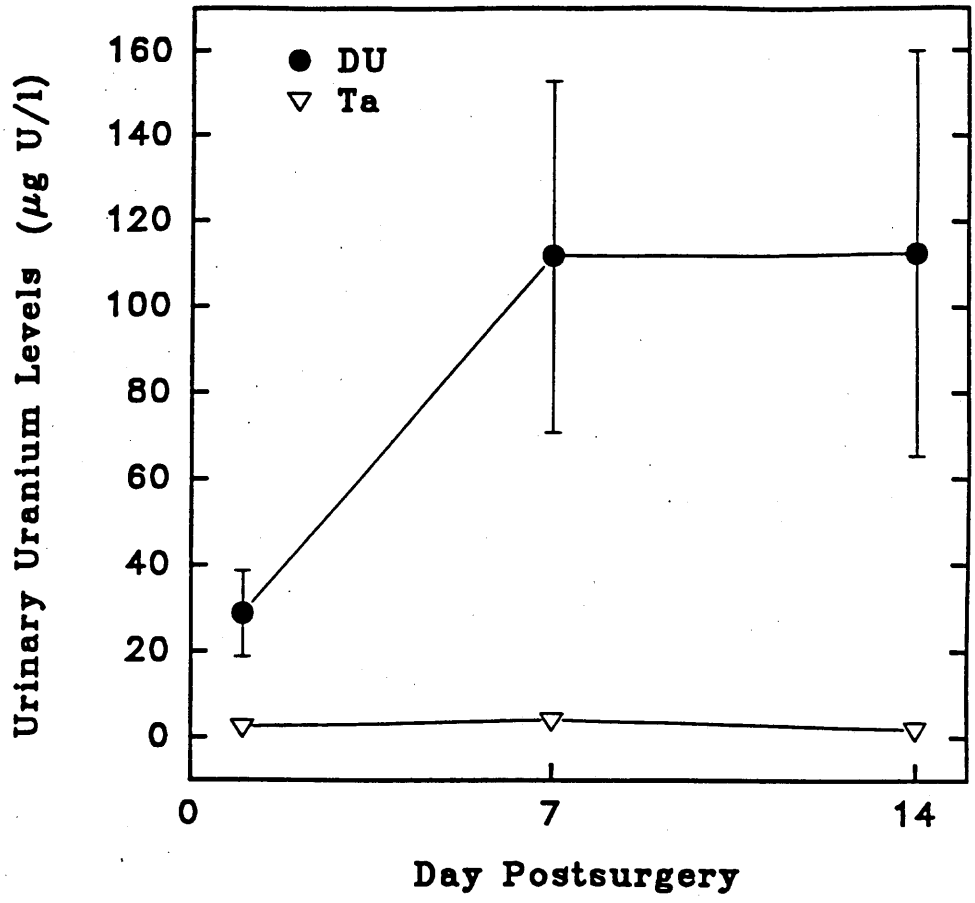

Fig. I-A2. Time course of uranium levels detected in the urine of rats implanted with either DU or tantalum (Ta). The uranium concentration detected in the Ta group is at background levels. Verticle lines are the standard errors of the means. The levels of uranium in the DU group were elevated on day 1 and increased four-fold on days 7 and 14 after surgery.

Appendix I-B
TIME LINE FOR EXPERIMENTAL PROCEDURES FOR RATS IN AIM #2 AND AIM #3

TIME POINTS*:	1 MO	6 MO	12 MO	18 MO
EXPERIMENTAL PROCEDURES	(Day of Measurement)			
Implant Surgery	day 0	day 0	day 0	day 0
Body Weight	weekly	weekly	weekly	weekly
Behavioral Tests:				
Functional Observation Battery (FOB)	30	180	360	540
Motor Activity	30	180	360	540
Memory test:				
acquisition	31	181	361	541
retention	34	184	364	544
Uranium Analysis:				
Blood (plasma)	35	185	365	545
Urine (24 hr sampling)	35-36	185-86	365-66	545-46
Neurophysiology:				
Conduction Velocity of Sciatic Nerve	37	187	367	547
Electrophysiology of Hippocampus	37	187	367	547
Tissue Analysis:				
Histopathology	37	187	367	547
Uranium Analysis of soft tissues (study termination)	37	187	367	547

*At each time sampling point (1, 6, 12, 18 months), 5 treatment groups will be evaluated. Doses of DU (low, medium, high) will be determined from the dose ranging study (Aim #1). Treatment groups: (1) low dose DU implant; (2) medium dose DU implant; (3) high dose DU implant; (4) implant (inert Ta) control; and (5) sham surgical control.

Appendix I-C

Protocol for conducting a functional observational battery (from[60])

HOME-CAGE MEASUREMENTS
CARRIED OUT WHILE RAT IS IN HOME CAGE

Posture (Descriptive)

1. Sitting or standing
2. Rearing
3. Asleep, lying on side or curled up
4. Flattened, limbs may be spread out
5. Lying on side, limbs in air
6. Crouched over
7. Head bobbing

Note: Only 1, 2, or 3 are "typical"

Involuntary Motor Movements (Descriptive)

Clonic

1. Repetitive movements of mouth and jaws
2. Quivers of limbs, ears, head, or skin (sometimes seen in untreated rats)
3. Mild tremors
4. Severe or whole body tremors
5. Myoclonic jerks
6. Clonic convulsions
7. Wet dog shakes

Tonic

1. Contraction of extensors such that limbs are rigid and extended
2. Opisthotonos: head and body rigidly arched backward
3. Emprosthotonos: head and body rigidly extended forward
4. Explosive jumps into the air with all feet leaving the surface
5. Severe clonic and/or tonic convulsions resulting in dyspnea, postictal depression, or death

Vocalizations (Quantal)

Spontaneous, not in reaction to being handled. Also includes spontaneous vocalizations in the open field.

Palpebral Closure (Ranked)

1. Eyelids wide open
2. Eyelids slightly drooping
3. Eyelids drooping approximately half-way

4. Eyelids completely shut

REMOVE RAT FROM CAGE

Ease of Removing Rat From Cage (Ranked)

1. Very easy (rat sits quietly, allows investigator to pick it up)
2. Easy (vocalizations, without much resistance to being picket up)
3. Moderately difficult (rat rears, often following investigator's hand.
4. Rat flinches (with or without vocalizations)
5. Difficult (runs around cage, or is hard to grab, with or without vocalizations)
6. Very difficult (tail and throat rattles, with or without vocalizations)

Reactivity to Being Handled (Ranked)

1. Low (no resistance, rat is easy to handle)
2. Moderately low (slight resistance to being handled, with or without vocalizations)
3. Moderately high (rat may freeze, or be tense, or rigid in hand, with or without vocalizations)
4. High (squirming, or twisting, or attempting to bite, with or without vocalizations)

MEASUREMENTS MADE WHILE HANDLING RAT

Remove from cage and hold in hand. Note (under "other" on data sheet such things as increased or decreased body tone, bite marks, soiled fur appearance, missing toe nails, piloerection, emaciation (shallow stomach, prominent spinal vertebrae), or death. (Observations such as piloerection may also be made while rat is on open field)

Lacrimation (ranked)

1. None
2. Slight
3. Severe

Palpebral Closure

Same as in home-cage measurements.

Salivation (Ranked)

1. None
2. Slight
3. Severe

Piloerection (Quantal)

"+" indicates presence of piloerection (i.e., coat does not lie down after stroking).

OPEN-FIELD MEASUREMENTS

Rat is placed in the center of a flat surface with a perimeter barrier covered with clean absorbent paper for exactly 3 min. During this time, the number of rears is counted and other observations are made. (Suggested size of cart: approximately 60 x 90 cm with a 6.5 cm rim):

Rearing (Count)

Defined as each time the front legs of the rat come completely off the surface, although the rat does not necessarily have to raise itself up (i.e., this is a measure of the ability of the rat to place its weight on its haunches). Includes when the rat uses the side or lip of a cart top as support.

Involuntary Motor Movements

Same as in home-cage measurements.

Gait (Descriptive)

Note, if rat did not move during the 3-min observation period, it may be gently prodded (after the 3 min is over) in order to observe the gait.

1. Ataxia, excessive sway, rocks, or lurches
2. Hindlimbs show exaggerated of overcompensated movements, drag, or are splayed
3. Feet markedly point outward from body
4. Forelimbs drag, are extended, or unable to support weight
5. Walks on tiptoes
6. Hunched or crouched body position
7. Body drags or is flattened against surface

Gait Score (Ranked)

Ranking of gait abnormalities.

1. No abnormal gait
2. Slightly abnormal
3. Moderately abnormal
4. Severely abnormal

Mobility Score (Ranked)

Ability of rat to locomote despite gait abnormalities (different from gait score)

1. No impairment
2. Slightly impaired
3. Somewhat impaired
4. Totally impaired

Arousal (Ranked)

Level of unprovoked activity and alertness in the open field

1. Very low (stupor, coma)
2. Low (somewhat sluggish, some head or body movement)
3. Somewhat low (slightly sluggish, some exploratory movements with periods of immobility)
4. Alert, exploratory movements
5. Somewhat high (slight excitement, tense, excited, sudden darting or freezing)
6. Very High (hyperalert, excited, sudden bouts of running or body movements)

Stereotypy

Record any behaviors that are excessive or repetitive such as circling, stereotypic grooming, pacing, repetitive sniffing, or head weaving.

Bizarre Behavior

Record any unusual behaviors such as self-mutilation, Straub tail, retropulsion, writhing, flopping.

Excretion

At the end of 3 min, measure defecation and urination.

Defecation (Count)

Number of fecal boluses on paper. "D" will be recorded if diarrhea is present

Urination (Count)

Number of pools of urine on the paper. "X" will be recorded if polyuria, or overlapping pools, is present

STIMULUS REACTIVITY

Performed while rat is sitting on cart surface.

Approach Response (Ranked)

Approach rat head-on with the end of a blunt object, such as a pencil, hold approximately 3 cm from face for 4 s

1. No reaction
2. Rat slowly approaches and sniffs or turns away
3. Rat flinches, actual muscle contractions
4. More energetic response than 2) or 3)
5. Exaggerated reaction-jumps, bites, or attacks

Touch Response (Ranked)

Coming in from the side, touch rump gently with blunt object, such as a pencil

1. No reaction
2. Rat may slowly turn or walk away, or vocalizations with little or no movement
3. Rat flinches, actual muscle contractions
4. More energetic response than 2. or 3)
5. Exaggerated reaction: jumps, bites, or attacks

Click Response (Ranked)

Position clicker approximately 5 cm above the back of the rat and make sudden sound.

1. No reaction
2. Slight reaction, some evidence that noise was heard
3. Rat flinches, actual muscle contractions
4. More energetic response than 2) or 3)
5. Exaggerated reaction-jumps, bites, or attacks

Tail Pinch Response (Ranked)

Metal tweezers are used to squeeze the tail approximately 2-3 cm from the tip

1. No reaction
2. Rat may turn or walk forward, or vocalizations with little or no movement
3. Rat flinches, actual muscle contractions
4. More energetic response than 2 or 3
5. Exaggerated reaction: jumps, bites, or attacks

Pupil Response (Quantal)

The beam of a penlight flashlight is brought in from the side of the rat's head. Constriction of the pupil is noted with a "+", and "-" indicates lack of response. (This may be difficult to observe in some strains of rats and may be dependent on ambient lighting conditions)

Righting Reflex (Ranked)

Rat is held supine, then dropped from approximately 30 cm. Score ease of landing. Note, if the rat is paralyzed or severely affected, this test and the landing foot splay will not be carried out so as not to injure the rat.

1. Rat lands on feet
2. Slightly uncoordinated
3. Lands on side
4. Lands on back

Forelimb and Hindlimb Grip Strength (Continuous)

Strain gauges are used with wire mesh screens for the rats to grab. Screen for the forelimb grip measurement is oriented horizontally from the strain gauge, while the hindlimb grip screen is placed at a 45 angle from the gauge to allow full contact with the hind feet when the rat is pulled off the support platform. Two readings are taken and averaged (modified from Meyer et al.[62]).

Body Weight (Continuous)

Body Temperature (Continuous)

Rectal temperature is taken and thermistor allowed to stabilize before reading

Landing Foot Splay (Continuous)

Fourth digit pads of hind feet are dotted with Tempera paint. Rat is dropped twice from prone position 40 cm from paper, and ink spots where he lands are noted. Measure distance between middle of ink blots, and average (modified from Edwards and Parker[61]).

Other

Includes torn toenails, broken teeth, soiled fur, fur discoloration, convulsions at any time other than in the home cage or open field, crustiness around face or eyes, red pigmented excretions from eyes, or any findings which may impact the data.

Appendix I-D

LOCAL FRAGMENT DOSIMETRY

1.0 *In Vivo* Macroscopic Dosimetry for a DU Fragment.

1.1 Objective. The goal of *in vivo* macroscopic dosimetry in the present context is to determine the α- and β-dose distributions around a DU fragment embedded in soft tissue.

1.2 Introduction. The depth-dose estimates from the given DU fragment will be based on the α- and β-particle emissions from the various radionuclides present in the fragment. For α particles the nuclides of interest are ^{238}U and ^{234}U, and for β particles, the nuclides of interest are ^{234}Th and ^{234m}Pa (which are in secular equilibrium with ^{238}U). The dose contribution from ^{235}U as well as that by the γ radiation is negligible and therefore will not be included in the final dose estimates.

1.3 Method. The given DU fragment will be considered to be of spherical shape with a uniform distribution of activity in it. For a rod-shaped fragment of dimensions 1-mm diameter x 2-mm length, the sphere of equivalent mass will have a diameter of 0.144 cm. The sphere model will give dose values approximating that from the actual DU fragment embedded in tissue. The accuracy of these dose estimates will be dependent, among other things, on the validity of the model.

The α-radiation dose-rate at a point at any depth in tissue will be calculated using Coleman's DU depth-dose data in Mylar plastic (Coleman *et al.*, 1983). Coleman had measured the total (α + β) doses from a plane DU source (using an extrapolation chamber) and therefore, in general, his data are not directly applicable for making dose estimates from a spherical DU source. However, in the case of α-particle emission by the given DU sphere a reasonable plane source approximation is possible. This is due to the fact that the range of DU α-particles in tissue (~0.004 cm) is much less than the diameter of the equivalent DU sphere (0.144 cm). For this reason, the unmodified Coleman depth-dose data will be applied as such in the estimation of the α dose-rates from the given spherical DU fragment at various tissue depths. From

the Coleman data, which gives the total ($\alpha + \beta$) doses at various depths in Mylar, the α-dose contribution at any tissue depth within the α-particle range could be easily separated out.

For the estimation of the β-radiation doses from the DU fragment the Coleman plane source data cannot be used. This is because the range of β-particles in tissue (~1 cm) is much larger than the diameter (0.144 cm) of the given DU fragment and due to this the fragment surface cannot be assumed to be a plane surface. Therefore, as an alternative to the use of Coleman's depth-dose data, we will be making use of the VARSKIN MOD2 computer code (Durham, 1992) for estimating the β-dose distribution in tissue from a spherical DU fragment embedded in tissue. Although VARSKIN MOD2 is meant for the calculation of skin dose from skin contamination, we will use it to calculate depth-dose distributions in tissue under a modified interpretation of the skin-dose estimates made at various skin depths.

The total dose at any point in tissue surrounding the spherical DU fragment will be taken to be the sum of the α and β doses at that point (evaluated as above).

1.4 Dose calculations. The tissue dose-rates (mrad/h) around a spherical DU fragment embedded in muscle tissue were computed by following the method described as above. In these computations the α-radiation dose-rates were extracted from Coleman's data and were assumed to have the same constant value (545.5 mrad/h) for all tissue depths, less than and including, 1.78 mg cm^{-2} (this was the minimum depth for which DU dose-rates were measured by Coleman). Table 1 gives the values of α, β and the total ($\alpha + \beta$) dose-rates. In these calculations the various tissue depths (1.78 mg cm^{-2} and above), which were used by Coleman were maintained for comparison purposes. Figure 1 gives a plot of total ($\alpha + \beta$) dose-rate against the tissue depth. Two distinct regions in the depth-dose distribution around the spherical DU fragment are apparent. Whereas for tissue depths greater than ~30 μm (~3 mg cm^{-2}), the total dose is entirely due to the β radiation; for depths below ~20 μm, it is α radiation which dominates. The average dose-rate (mrad/h) at which any tissue volume near a DU fragment is continuously irradiated could be determined by making use of these depth-dose data.

References

Coleman RL, Hudson CG, Plato PA (1983) Depth-dose curves for ^{90}Sr and natural and depleted uranium in Mylar. **Health Physics** 44(4): 395-402.

Durham JS (1992) VARSKIN MOD2 and SADDE MOD2: Computer Codes for Assessing Skin Dose from Skin Contamination, NUREG/CR-5873 and PNL-7913, U.S. Nuclear Regulatory Commission, Washington, DC 20555.

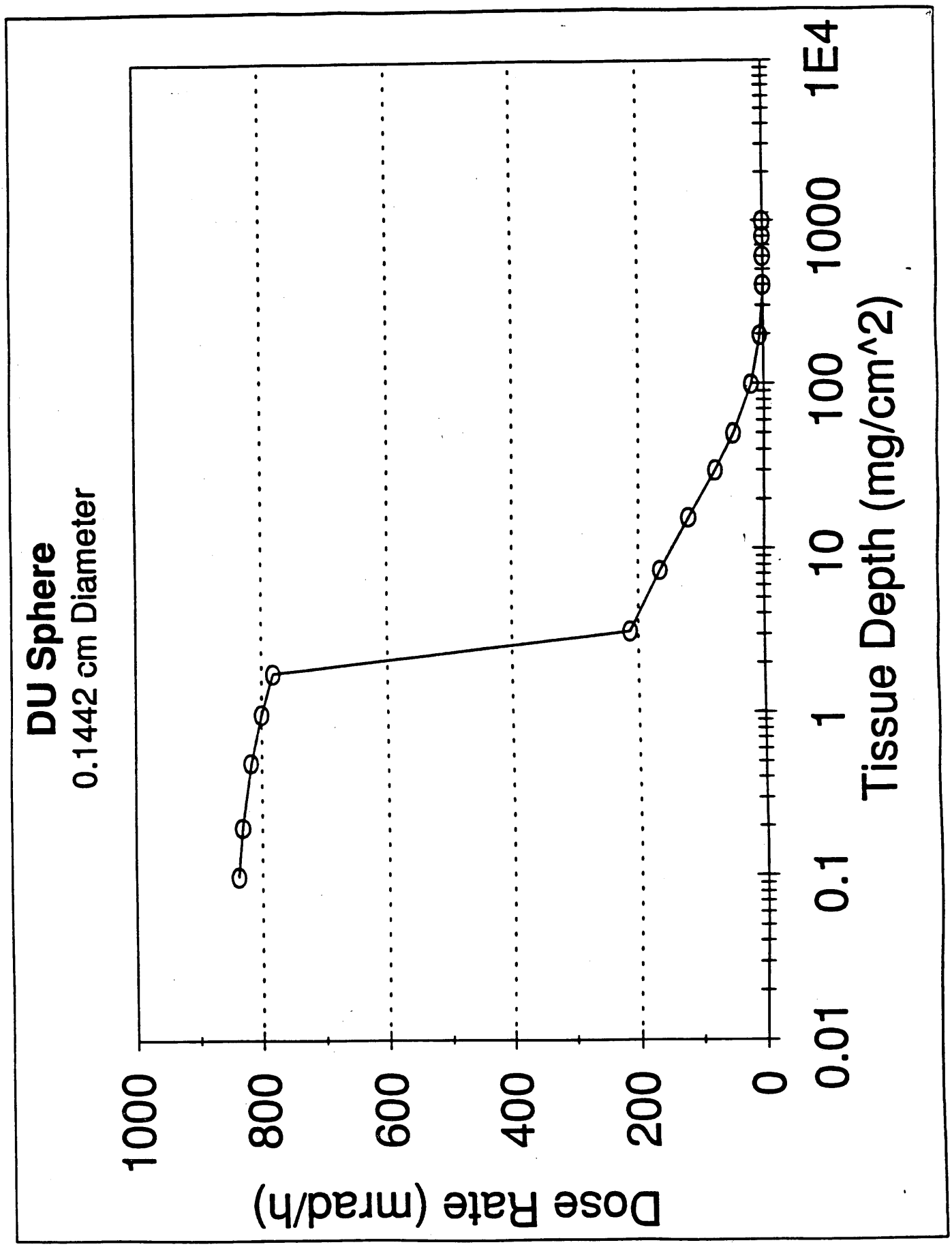
DU Sphere
0.1442 cm Diameter
Dose Rate (mrad/h)
1000
800
600
400
200
0
0.01
0.1
1
10
100
1000
1E4
Tissue Depth (mg/cm^2)

Figure 1

Table 1: Tissue dose rate from 0.1442 cm diameter spherical DU fragment

Tissue Depth (mg/cm^2)	Dose Rate (mrad/hr)		
	Alpha	Beta	Total
0.10	545.30	293.00	838.30
0.20	545.30	286.20	831.50
0.50	545.30	272.40	817.70
1.00	545.30	255.40	800.70
1.78	545.30	237.30	782.60
3.18	0.00	212.80	212.80
7.50	0.00	165.60	165.60
15.73	0.00	120.10	120.10
30.10	0.00	77.90	77.90
50.58	0.00	47.90	47.90
99.40	0.00	20.10	20.10
196.98	0.00	5.40	5.40
399.20	0.00	0.60	0.60
600.00	0.00	0.10	0.10
796.60	0.00	0.00	0.00
1002.00	0.00	0.00	0.00

Appendix I-E

METHODS OF URANIUM ANALYSIS

Item A

Analysis of Rat Tissues for Uranium After Administration of Depleted Uranium Particles

Responsible Investigator: A.F. Fuciarelli

Battelle Pacific Northwest Laboratory proposes to collaborate with the Armed Forces Radiobiology Research Institute in conducting research on the biologic disposition and effects of depleted uranium. The task outlined here will be concerned with the analyses of tissues from rats that have received implanted U_2O_3 particles or fragments. It is expected that the *in vivo* animal studies will be conducted by AFRRI at their facilities. The necropsy of animals will also be performed at AFRRI by AFRRI personnel. Samples of blood, urine, kidney, liver, and bone will be provided to PNL by AFRRI for analysis of depleted uranium. The samples will be frozen and then shipped on dry ice. It is expected that the samples will be received within 48 hours from the time they are shipped.

Uranium analyses will be performed using laser-induced kinetic phosphorimetric analysis (KPA) as described by Brina and Miller (1992). The use of this method involves digestion of samples of tissue (usually 1-2 grams) with concentrated nitric acid and H_2O_2 to produce a colorless ash. If necessary, the samples after wet ashing can be muffled to insure that all colored material is destroyed. The colorless ash is then dissolved in 1 M nitric acid and diluted to the desired volume. A phosphate complexing agent is added and the sample transferred to cuvettes for analysis by the KPA instrument (CHEMCHEK INSTRUMENTS, INC.). The sample is excited at a wavelength of 425 nm and the emission at 515 nm determined with a photomultiplier tube. The timing of the reading can be adjusted to eliminate short-lived luminescence sources, thus increasing the sensitivity of the system.

The instrument is calibrated by measuring the emission intensities from standard solutions and storing the information in the microprocessor. The experimental results are then compared empirically with values obtained from the standard solutions and the best-fit linear curve. The limit of detection of this method for uranium is 0.01 μg/l or 10 ng/l. This corresponds to a detection limit of approximately 10 pg/ml. The method is linear over several orders of magnitude for uranium (from the limit of detection to 5 mg/l).

The translocation of depleted uranium from point of deposition in the animal to other tissues is likely to be comparatively slow, especially for the materials prepared at high temperatures and milled into particles with relatively small surface areas. It is desirable therefore to have the ability to detect levels as low as 0.05 to 0.10 μg/kg of tissue. This is equivalent to 50 to 100 pg/g of tissue, which is well above the limit of the method (10 pg).

The cost estimates associated with tissue sample analyses are based on several assumptions: 1) only a single sample of a tissue will be analyzed; 2) multiple tissue samples will be available for analysis so that time can be efficiently used; and 3) spiking of tissue samples will be done on a limited basis only for methods validation. Multiple samples of the same tissue will be treated as independent samples.

The expectation is that the samples as received at PNL will be coded so that the analyst does not know the treatment of the animal from which the sample was derived. Therefore, the data reported to AFRRI will include: sample identification number, amount of tissue analyzed, amount of uranium in the tissue sample, the amount of uranium per unit weight of tissue, and comments on analytical problems or unusual occurrences.

Reference

Brina, R. and A.G. Miller. 1992. Direct detection of trace levels of uranium by laser-induced kinetic phosphorimetry. Anal. Chem. 64:1413-1418.

Item F **Curriculum Vitae**

NAME: Alfred Frank Fuciarelli **ROLE ON PROJECT:** Analytical Chemist

EDUCATION:

University of Toronto, Toronto, ON, Canada	B.Sc.	1982	Biology
University of Alberta, Edmonton, AB, Canada	M.Sc.	1984	Experimental Radiology
University of Alberta, Edmonton, AB, Canada	Ph.D.	1987	Medical Sciences

National Institute of Standards and Technology, Gaithersburg, MD, USA 1987-1988

Armed Forces Radiobiology Research Institute, Bethesda, MD, USA 1987-1988

RESEARCH AND PROFESSIONAL EXPERIENCE:

A) Staff Appointments:

1989-1990:
Assistant Radiation Biologist, Department of Radiation Medicine, Massachusetts General Hospital and Assistant Professor of Radiation Biophysics, Harvard Medical School, Boston, MA

1991-1993:
Research Scientist, Battelle, Pacific Northwest Laboratory, Richland, WA

1994-Present:
Senior Research Scientist, Battelle, Pacific Northwest Laboratory, Richland, WA

B) Selected List of Honors, Awards, Committees, and Miscellaneous Recognitions:

1985 and 1986: Radiation Research Society Student Travel Award

1987: Young Investigator Travel Grant: International Congress of Radiation Research

1990: Invitation to attend the NATO ARW meeting entitled: "Early Effects of Radiation on DNA", May 7-11, 1990, San Miniato, Italy

1991-Present: Chairperson-Education and Training Committee Radiation Research Society

1993: Member of NIH Special Review Committee (*ad hoc*): "The Regulation, Function and Specificity of Proteins Induced in Mammalian cells Exposed to Ionizing Radiation.

SIGNIFICANT PUBLICATIONS (Selected from a list of 25):

Raleigh JA, Fuciarelli AF. Distribution of damage in irradiated 5'-AMP: 8,5'-cycloAMP, 8-hydroxy-AMP and adenine release. Radiat. Res. 102: 165-175, 1985.

Fuciarelli AF, Miller GG, Raleigh JA. An immunochemical probe for 8,5' -cycloadenosine 5'-monophosphate and its deoxy analog in irradiated nucleic acids. Radiat. Res. 104: 272-283, 1985.

Fuciarelli AF, Shum FY, Raleigh JA. Intramolecular cyclization in irradiated nucleic acids. Correlation between high-performance liquid chromatography and an immunochemical assay for 8,5'-cycloadenosine in irradiated poly A. Radiat. Res. 110: 35-44, 1987.

Fuciarelli AF, Mele FG, Raleigh JA. Interaction of nitroaromatic radiosensitizers with irradiated polyadenylic acid as measured by an indirect immunochemical assay with specificity for the 8,5'-cycloadenosine moiety. Int. J. Radiat. Biol. 51: 629-639, 1987.

Alexander AJ, Fuciarelli AF, Kebarle P, Raleigh JA. Characterization of radiation-induced damage to polyadenylic acid using high-performance liquid chromatography/tandem mass spectrometry. Anal. Chem. 59: 2484-2491, 1987.

Raleigh JA, Miller GG, Franko AJ, Koch CJ, Fuciarelli AF, Kelly DA. Fluorescence immunohistochemical detection of hypoxic cells in spheroids and tumour tissue. Br. J. Cancer 56: 395-400, 1987.

Fuciarelli AF, Koch CJ, Raleigh JA. Oxygen dependence of product formation in irradiated adenosine 5'-monophosphate. Radiat. Res. 113: 447-457, 1988.

Gajewski E, Fuciarelli AF, Dizdaroglu M. Structure of hydroxyl radical-induced DNA-protein crosslinks in calf thymus nucleohistone in vitro. Int. J. Radiat. Biol. 54: 445-459, 1988.

Fuciarelli AF, Wegher BJ, Gajewski E, Dizdaroglu M, Blakely WF. Quantitative measurement of DNA base products using gas chromatography-mass spectrometry. Radiat. Res. 119: 219-231, 1989.

Jackson JH, Gajewski E, Schraufstatter IU, Hyslop P, Fuciarelli AF, Cochrane CG, Dizdaroglu M. Damage to the bases in DNA induced by stimulated human neutrophils. J. Clin. Invest. 84: 1644-1649, 1989.

Blakely WF, Fuciarelli AF, Wegher BJ, Dizdaroglu M. Hydrogen peroxide-induced base damage in deoxyribonucleic acid. Radiat. Res. 121: 338-343, 1990.

Fuciarelli AF, Wegher BJ, Blakely WF, Dizdaroglu M. Yields of radiation-induced base products in DNA: Effects of DNA conformation and gassing conditions. Int. J. Radiat. Biol. 58: 397-415, 1990.

Beach, C, Fuciarelli, AF, Zimbrick, JD. Electron migration along 5-bromouracil-substituted DNA irradiated in solution and in cells. Radiat. Res. 137: 385-393, 1994.

Fuciarelli, AF, Sisk, EC, Zimbrick, JD. Electron migration in oligonucleotides upon gamma-irradiation in solution. Int. J. Radiat. Biol. 63: 409-418.

Weir, MS, Springer, DL, Fuciarelli, AF, Thrall, BD, Edmonds, CG. Characterization of natural and radiation-induced modifications of histones. Techniques in Protein Chemistry, V, (Ed. R.H. Angellette), Academic Press, 115-122, 1994.

INFORMATION ON OTHER COMMITMENTS OF TIME, SUCH AS SABBATICAL OR ANTICIPATED EXTENDED LEAVE:

No sabbatical or anticipated extended leave time.

PROPORTION OF TIME DEVOTED TO THIS AND TO OTHER RESEARCH:

Proportion of time devoted to this project 14%
Proportion of time devoted to other projects 80%

(a) Source: U.S. Department of Energy- Office of Health and Environmental Research
Principal Investigator: Dr. A.F. Fuciarelli
Project Title: Biochemistry of Free Radical-Induced Damage in Nucleic Acids
Description: Utilization of mass spectrometry methodology for the chemical characterization of radiation and chemical damage in nucleic acids.

(b) Source: National Institutes of Health
Principal Investigator: Dr. C.G. Edmonds
Project Title: Structural studies of radiation-induced NA-protein cross-linked species.
Description: Characterization of DNA-protein cross-links using electrospray ionization-mass spectrometry.

(c) Source: Laboratory Directed Research and Development Funds
Principal Investigator: Dr. A.F. Fuciarelli
Project Title: Spectroscopic Analysis of DNA Damage
Description: Development of mass spectrometry methodology for analysis of DNA damage.

NUMBER OF GRADUATE STUDENTS FOR WHOM YOU ARE RESPONSIBLE: 1

Item I

URANIUM TISSUE ANALYSIS

Principal Investigator/Project Director Dr. T.S. Tenforde

TOTAL PROJECT PERIOD COST ESTIMATE

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD

BUDGET CATAGORY TOTALS	INITIAL BUDGET PERIOD	ADDITIONAL YEARS OF SUPPORT REQUESTED			
	1st	2nd	3rd	4th	5th
DIRECT LABOR COSTS Salary & Fringe Benefits	16,052	16,052	0	0	0
MAJOR EQUIPMENT	0	0	0	0	0
SUPPLIES	3,334	3,334	0	0	0
SUBCONTRACTS/SUBGRANTS	750	750	0	0	0
TRAVEL COSTS	937	937	0	0	0
PUBLICATION COSTS	0	0	0	0	0
CONSULTANT COSTS	0	0	0	0	0
OTHER DIRECT COSTS	2,244	2,244	0	0	0
TOTAL DIRECT COSTS	**23,316**	**23,316**	**0**	**0**	**0**
INDIRECT COSTS	**45,934**	**45,934**	**0**	**0**	**0**
TOTAL COSTS	**69,250**	**69,250**	**0**	**0**	**0**

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$46,632**
TOTAL INDIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$91,867**
TOTAL COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$138,499**

Principal Investigator/Project Director Dr. T.S. Tenforde

DETAILED COST ESTIMATE FOR INITIAL BUDGET PERIOD

From 5/10/94 Through 9/30/96

DIRECT LABOR COSTS					DOLLAR AMOUNT REQUESTED			
NAME	ROLE ON PROJECT	TYPE APPT (mo.)	% EFFORT ON PROJECT	BASE ANNUAL SALARY	SALARY REQ.	FRINGE BENEFITS % RATE/	$	TOTALS
Fuciarelli, Alfred	Sr. Rsch. Scientist	2.0	15.8%	$82,808	9,326	31.8%	4,349	13,675
Lauhala, Kathy	Tech. Spec. II	0.9	6.7%	$44,378	2,120	31.8%	989	3,109
Sisk, Ellen	Tech. Spec. II	4.0	31.7%	$44,378	9,998	31.8%	4,662	14,660
Thompson, Linda	Secretary	0.2	1.9%	$33,032	446	31.8%	212	658
SUBTOTALS					21,890		10,212	$32,103
MAJOR EQUIPMENT							0	
							0	
							0	0
MATERIALS,SUPPLIES,CONSUMABLES							0	
							0	
				Supplies (Invoice prices plus 4.3% escalation)			6,668	6,668
SUBCONTRACTS/SUBGRANTS					Graphics		0	
DIRECT COSTS					Maintenance		1,500	
INDIRECT COSTS							0	1,500
TRAVEL COSTS							0	
					Two-day trip to DC for one staff member.		1,874	1,874
PUBLICATION AND REPORT COSTS							0	
					Duplicating (*Labor included above)			0
CONSULTANT COSTS							0	
							0	0
OTHER DIRECT COSTS					Waste Disposal		2,500	
					Purchasing Scv Chrg & Subcontract Svc Chrg		1,987	4,487
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD								$46,632
INDIRECT COSTS FOR INITIAL BUDGET PERIOD (% Rate)							66.3%	$91,867
TOTAL COSTS FOR INITIAL BUDGET PERIOD								$138,499

Appendix I-F

BIOKINETIC MODEL

PROPOSED SCOPE OF WORK

DOSIMETRY FOR URANIUM-CONTAMINATED WOUND CASES

Prepared by

Dr. R. W. Leggett
Dr. K. F. Eckerman

for the

Defense Nuclear Agency
Armed Forces Radiobiology Research Institute

DOE Project Number 0046-H036-A1

OAK RIDGE NATIONAL LABORATORY
managed by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U. S. DEPARTMENT OF ENERGY
under Contract No. DE-AC05-84OR21400

DOSIMETRY FOR URANIUM-CONTAMINATED WOUND CASES

I. BACKGROUND

A. Statement of the problem

The use of depleted uranium (DU) munitions on the battle field in Operation Desert Storm resulted in casualties with imbedded DU fragments. Standard medical guidelines for fragment injuries were used to determine which fragments should be removed, but these guidelines do not address radiological or chemical risks from U in fragments left in the body.

An evaluation of the radiological and chemical risks will require estimates of the U content of the fragments, the rate of mobilization of U from the fragments to blood, and the resulting time-dependent tissue distribution and excretion of the mobilized U. The concentration of U in urine can be measured directly, but the rate of mobilization of U from fragments and the time-dependent distribution of systemic U must be inferred from: (1) the rate of urinary excretion of U, (2) any information on the surface area and U contents of fragments that can be obtained by external scans over the injured tissue, (3) a model of the kinetics of U in the human body, and (4) experimental data on laboratory animals with implanted DU fragments.

B. Best available biokinetic model

The International Commission on Radiological Protection (ICRP) has recently adopted a new, physiologically descriptive biokinetic model for U for use in ICRP Publication 56, Age-dependent doses to members of the public from intake of radionuclides (Part 3, to be published). The model framework is shown in Figure 1. This is a generic framework applied in ICRP Publication 56 to a class of elements that follow the movement of Ca in bone to some extent. Transfer rates for the model were derived from the following sources of information:

(1) Data on human subjects injected with U. There have been three studies in which human subjects were intravenously injected with U and followed for periods varying from a few days to 1.5 y after injection. These studies are referred to as the Boston study, the Bassett study (sometimes called the Rochester study), and the Terepka study.

The Boston study (Bernard and Struxness 1957, Struxness et al. 1956, Luessenhop et al. 1958) involved at least 11 patients, ages 26-63 y, in the terminal phases of diseases of the central nervous system. Data on eight of the subjects have been described in several reports. The original data on the other three subjects were located recently by Drs. Patricia Durbin and Keith Eckerman. Most of the Boston subjects were comatose at the time of injection. Uranyl nitrate solutions enriched with ^{234}U and ^{235}U were administered to nine of the subjects by intravenous injection, and the other two received intravenous injections of tetravalent U as UCl_4. The mass of administered U apparently ranged from about 0.04 to about 1 mg kg^{-1}. Blood and urine were sampled frequently during the first several days after administration, and autopsy samples were obtained from various bones and soft tissues of subjects dying at times from 2.5 d to 4 mo after injection and from one subject dying 566 d after injection. The usefulness of these data for purposes of constructing a biokinetic model for U is limited by the poor physical conditions of the subjects. Struxness et al. (1956) pointed out, for example, that the bed-ridden condition of these subjects indicated a negative Ca balance, which might "hasten the removal of U from the skeleton". Another limitation of these data is that the subjects

were administered relatively high masses of U, which may have altered the kinetics of U to some extent, particularly in the kidneys (Leggett 1989). A third difficulty is that the postmortem data are not sufficiently detailed in some cases to allow a close determination of the total U content of some massive tissues such as the skeleton, muscle, fat, and skin.

The Bassett study (Bassett et al. 1948) involved six subjects, ages 24-61 y. These subjects were hospital patients but were ambulatory. Subject 1 suffered from rheumatoid arthritis, subject 2 from cirrhosis of the liver, subject 3 from chronic undernutrition, subject 4 from alcoholism, subject 5 from unresolved pneumonia, and subject 6 from pulmonary fibrosis and a gastric ulcer. The subjects received intravenous injections of uranyl nitrate solutions enriched with ^{234}U and ^{235}U. Administered masses ranged from 6.3 to 70.9 μg U kg^{-1} body weight. Total urine and fecal collection was made from subjects for periods ranging up to 16 d, and several blood sample were taken.

Terepka and coworkers (Terepka et al. 1964; also see Hursh and Spoor 1973) investigated the possibility of evaluating bone disorders based on the level of retention of intravenously injected uranium. They injected hexavalent U (30 $\mu g\ kg^{-1}$) into three control patients and seven patients with various bone disorders (Paget's disease, hyper- or hypoparathyroidism, osteomalacia, senile osteoporosis). Some patients were investigated before and after estrogen or parathyroid extract treatments. Urinary excretion of U was measured for at least 6 d in each subject.

(2) Data on occupationally and environmentally exposed subjects. Additional information on the biological fate of U in humans is provided by measurements of U in blood, urine, or postmortem tissues of occupationally and environmentally (non-occupationally) exposed subjects (Donoghue et al. 1972; Boback 1975; Campbell 1975; Roberts et al. 1977; Igarashi et al. 1985; Fisenne and Welford 1986; Singh et al. 1986, 1987; Kathren et al. 1989). Occupational exposures were mainly by inhalation, while ingestion appears to be the significant exposure pathway for environmental exposures. These data provide the best available information on the long-term systemic distribution and rate of excretion of U in humans, but the value of the data is limited by the small numbers of subjects examined; uncertainties in the exposure histories of those subjects; uncertainties in estimates of total-organ contents of the subjects based on small samples of tissue, particularly skeletal tissues; and limitations in the measurement techniques, particularly with regard to determination of the typically low concentrations of U in blood, urine, and tissues of environmentally exposed subjects.

(3) Experimental data for non-human primates. The biological behavior of U has been studied in baboons and monkeys exposed to various U compounds by intravenous injection or inhalation (Leach et al. 1973, Neton et al. 1979, Lipsztein 1981, Bhattacharrya et al. 1989, Metivier et al. 1992). Data for monkeys (Leach et al. 1973) are too complicated to be of much use for purposes of biokinetic modeling. Studies on baboons have involved only a few animals, but the limited data indicate that the biokinetics of U in baboons is fairly similar to that in humans, at least over the first few months after exposure. Thus, the baboon data were used to supplement the human data in development of parameter values for the model.

(4) Experimental data for dogs. Dogs, particularly beagles, have proved to be valuable laboratory models for man with regard to the biokinetics of radionuclides. The biological behavior of U has been studied in dogs exposed to different chemical forms of U by injection or inhalation (Tannenbaum 1951, Fish and Bernard 1961, Sanotskii et al. 1966, Bruenger et al. 1976, Stevens et al. 1980, Morrow et al. 1982). Insofar as comparison is possible, the kinetics of U appears to be fairly

similar in dogs and humans, although differences in the rate of clearance of U from blood are evident. Also, species differences in the long-term clearance of U from bone are expected (due to species-dependent bone turnover rates) but have not yet been clearly demonstrated. In the development of the ICRP's new biokinetic model, data on the kinetics of U in dogs were used frequently to supplement information on humans. Sufficient data are available to develop a reasonable biokinetic model for U in dogs.

(5) Experimental data for rats. Several workers have studied the biokinetics of intravenously injected U in rats (Hamilton 1948; Neuman et al. 1948; Neuman 1953; Maynard et al. 1953; Durbin 1960; Muir et al. 1960; Jones 1966; Priest et al. 1982; Sontag 1983, 1984; Bentley et al. 1985, La Touche et al. 1987), and general similarities in the biokinetics of U in rats and humans are indicated. It appears, however, that skeletal uptake of U may be higher in adult male rats than adult humans. Both male and female rats have a higher rate of bone turnover than do adult humans, but bone turnover probably affects only long-term retention of U in the skeleton.

Comparison of available data on kidney retention of U in humans and rats is complicated by an apparent dosage dependence in the behavior of U in the kidneys. For example, Jones and coworkers (Muir et al. 1960, Jones 1966) found that retention of U by the rat kidney was considerably greater at injection levels of 0.1-1.0 mg U kg^{-1} than at levels of 0.001-0.01 mg U kg^{-1} (Figure 2). Results of other authors indicate that U injection levels substantially greater than 1 mg U kg^{-1} can lead to reduced rather than increased kidney retention.

The apparent dosage dependence (and probably also some sex and age dependence) in renal retention of U also complicates the derivation of a relation between urinary U and kidney U in rats. For example, at 4 days after parenteral injection of U nitrate into rats, the percentages of injected U in kidney and cumulative urine were, respectively, about 12% and 73% in animals receiving trace amounts of U, 21% and 56% in animals receiving about 0.2 mg U kg^{-1}, and 7% and 67% in animals receiving 2 mg U kg^{-1} (Durbin 1975).

One of the most detailed descriptions of the distribution and excretion of U in rats as a function of time after administration was provided by Cooper and coworkers (1982), who administered different compounds of ^{233}U to rats by pulmonary intubation. The rats were female and were about 10 weeks old. It appears that fairly low masses of U were administered in this experiment. For these animals, the early retention in the kidneys was several percent greater than predicted by the model for humans indicated in Figure 1 and Table 1, while cumulative urinary excretion was similar to that predicted for humans (Figures 3 and 4).

(6) Experimental data for other species. The biokinetics of U has also been studied in mice (Tannenbaum 1951, Kisieleski et al. 1952, Walinder 1967, Bhattacharrya et al. 1989) and a few other animal species. These data were found to be broadly consistent with information derived for humans, baboons, dogs, and rats, and thus provide support for the model; however, these data did not enter directly into the choice of parameter values.

(7) Other information. Some parameter values for bone were based on quantitative data on physiological processes thought to control long-term removal of U from bone. Also, because of qualitative similarities in the short- and intermediate-term behavior of U and the alkaline earth elements in the skeleton, some age-specific parameter values for bone were based largely on analogy with the alkaline earth elements.

C. Limitations of the ICRP's biokinetic model

Because of its relatively great detail and physiological basis, the ICRP's new biokinetic model for U would serve as a useful starting point for modeling the biological behavior of U in the subjects with imbedded U fragments. Uncertainties arise in application of this model to the Desert Storm veterans, however, because the physicochemical form of U that migrates from the DU fragments may be different from the form of U reaching blood in some or all of the studies on which the biokinetic model was based. For example, it is conceivable that a substantial portion of U migrating from the fragments is accumulated by the reticuloendothelial system, a situation that does not appear to occur for forms of U that have been administered to humans or laboratory animals. Some information on the biological behavior of the pertinent physicochemical form of U may be available soon from simulated wound studies on rats. This information may reveal whether adjustments in transfer rates of the biokinetic model are needed to account for chemical form.

D. Additional tools needed for calculations

In addition to a biokinetic model for U, a set of algorithms is needed to calculate backwards from measurements of U in urine to the total rate of mobilization of U from all imbedded fragments in a given subject. The computer code RBD (Radiological Bioassay and Dosimetry) recently developed by our group for the U. S. Army was designed to perform such calculations for inhalation or ingestion exposures and included provisions for further consideration of wound exposures (Eckerman et al. 1993). Estimates concerning the mobilization of U from individual fragments would require information on the relative surface areas of the different fragments, which may be available in some cases.

E. Frame of reference for radiological and chemical risks

There are two kinds of hazards associated with intake of uranium by humans: (1) chemical toxicity, particularly interference with normal renal function; and (2) radiogenic injury to lungs, bone, and other tissues. Chemical toxicity is expected to be the primary concern for wound contamination with depleted U because of its relatively low specific activity (radioactive decays per unit mass). However, comparisons of chemical and radiological guidelines should be made on the basis of the exposure conditions and biokinetic model appropriate for the Desert Storm cases. New radiological guidance has been issued recently by the ICRP (ICRP Publication 60, 1990) and seems appropriate for consideration of these cases, but it is not apparent that commonly used guidelines based on chemical toxicity are appropriate for these cases. Since the early 1950's a renal U concentration of 3 μg U g^{-1} kidney has served as a bench mark for limiting exposures to uranium when chemical toxicity is the critical endpoint. Recent experimental studies and reanalyses of older data indicate, however, that renal abnormalities of unknown significance may occur at concentrations that are roughly an order of magnitude lower than this longstanding guidance level (Leggett 1989). The apparent discrepancy between the early findings and more recent conclusions are attributable in large part to different definitions of chemical toxicity and more sensitive biological indicators used in recent years. Also, the guideline value of 3 μg g^{-1} was based largely on data for acute intakes. It may be that a given transient renal U concentration following an acute exposure may not produce the same renal damage or dysfunctions as the identical concentration experienced during chronic exposure; for example, the distribution of U in the kidneys could be much different in the two cases even if the total renal burden is the same.

II. PURPOSE

The purposes of this project are to (1) derive estimates of the time-dependent distribution of U in the subjects and resulting radiation doses to tissues if the fragments are not removed, (2) compare computed radiation doses with current radiological guidelines, and (3) interpret computed concentrations of U in the kidneys in terms of current information on the toxic effects of U on the kidneys. These estimates will provide a more complete basis for determining whether removal of additional fragments is warranted for a given subject.

III. SCOPE OF WORK

The work will be divided into four tasks:

Task 1. The ICRP's new biokinetic model for U will be modified insofar as information allows to address the special conditions of the DU exposure cases. For example, modifications may be needed if it is found in new studies (at another laboratory) on rats that the physicochemical form of U that migrates from the DU fragments behaves differently from forms previously used in experimental studies. Also, it may be necessary to modify the model to address the dependence of U kinetics in the kidneys on the mass of U present.

Task 2. Separate parameters for the biokinetic model for U will be developed for rats. The rat model will be based on previously reported experimental data on rats, plus new experimental data generated by wound simulation experiments conducted at another laboratory. The biokinetic model and experimental data for rats will be used to investigate the potential radiological and chemical hazards when the source term is DU fragments.

Task 3. The computer code RBD will be modified to address time-dependent flow rates from one or more imbedded DU fragments. The modified code will be tested and adjusted using the biokinetic model for rats and the wound simulation data developed for these animals.

Task 4. The modified biokinetic model for U in humans and the modified computer code RBD will be applied to bioassay data for the soldiers with imbedded fragments. Lifetime radiation doses to the soldiers will be computed, and the computed radiation doses and time-dependent kidney burden will be compared with current radiological and chemical guidelines for U.

IV. DELIVERABLES

Complete descriptions of the developed models, estimates of radiation doses for the subjects, and comparisons of results with current radiological and chemical guidelines for U will be described in a detailed letter report to Lieutenant Colonel Eric Daxon of AFRRI. It is anticipated that the results of this study will also be submitted for publication in the open literature.

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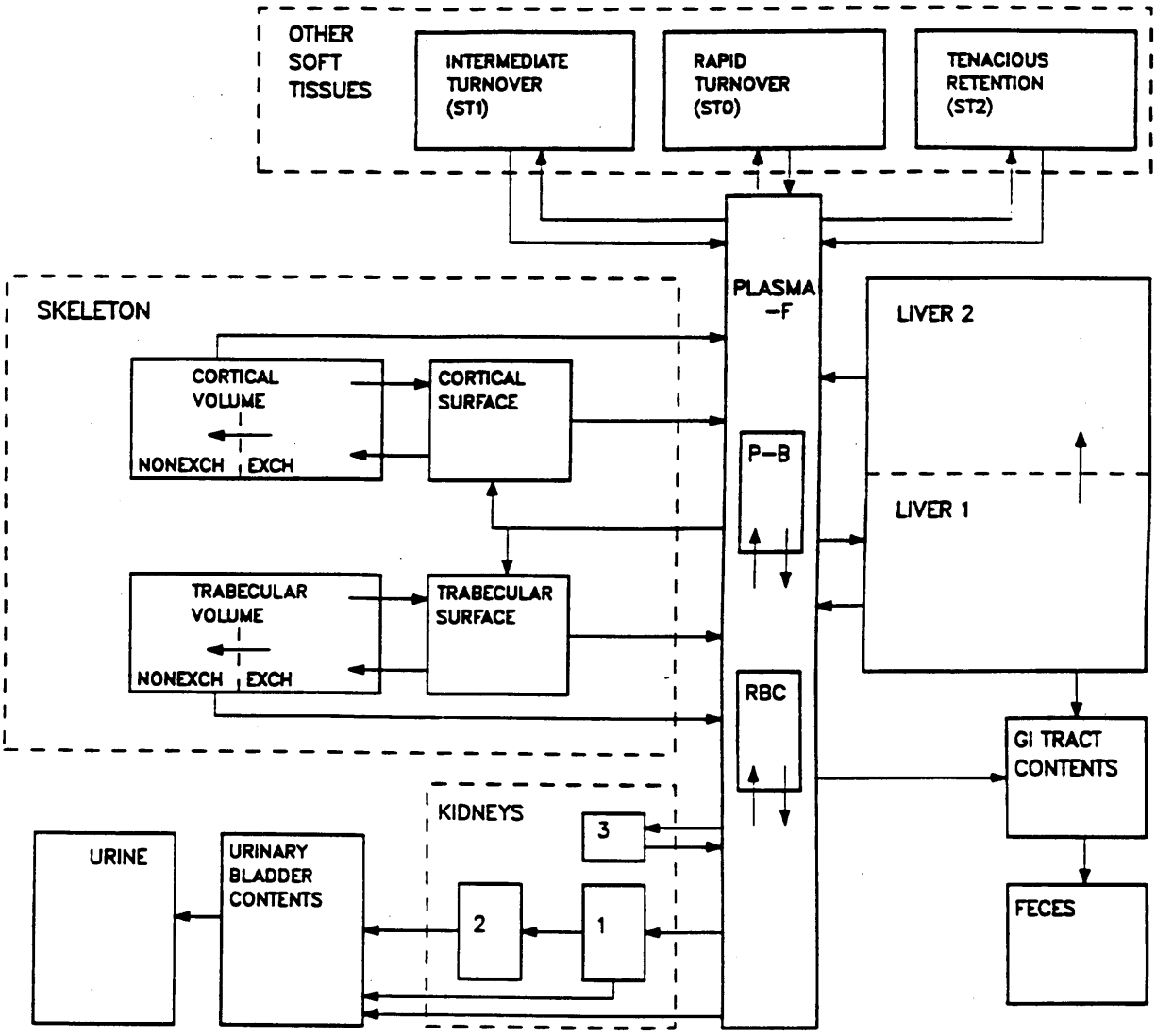

Figure 1. Diagram of the biokinetic model for U (relatively detailed version). PLASMA-F is "filterable" plasma U, P-B is "bound" plasma U, EXCH is exchangeable U in bone volume, and NONEXCH is non-exchangeable U in bone volume.

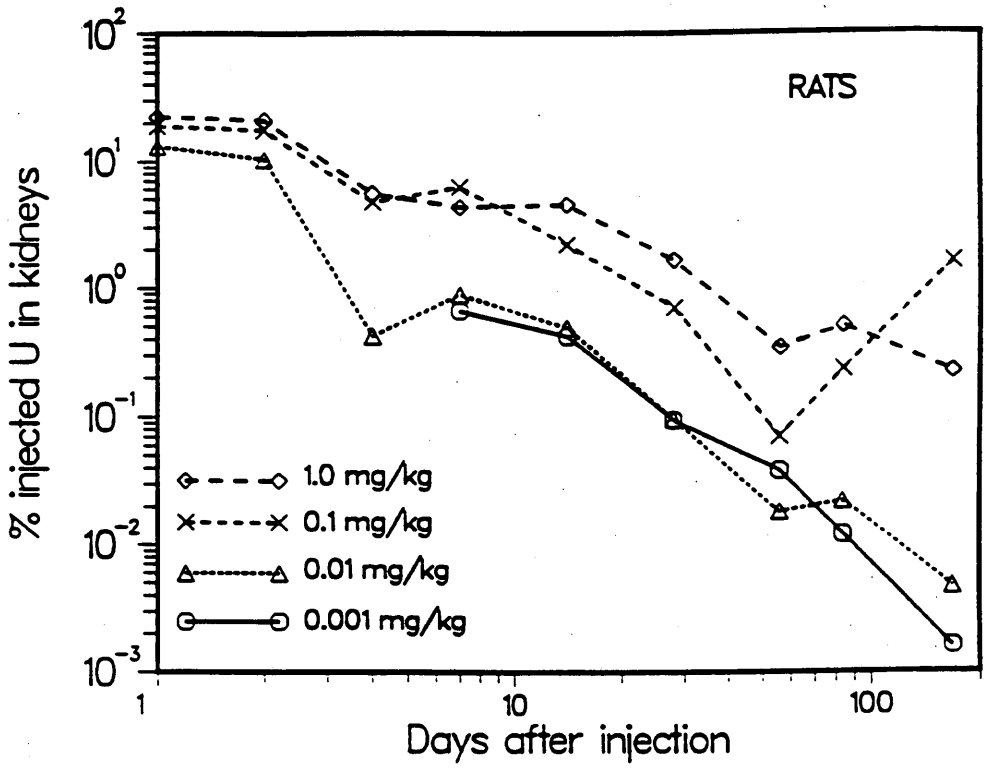

Figure 2. Retention of U in the kidneys of rats as a function of time after injection and administered mass of U (data of Muir et al. 1960, Jones 1966).

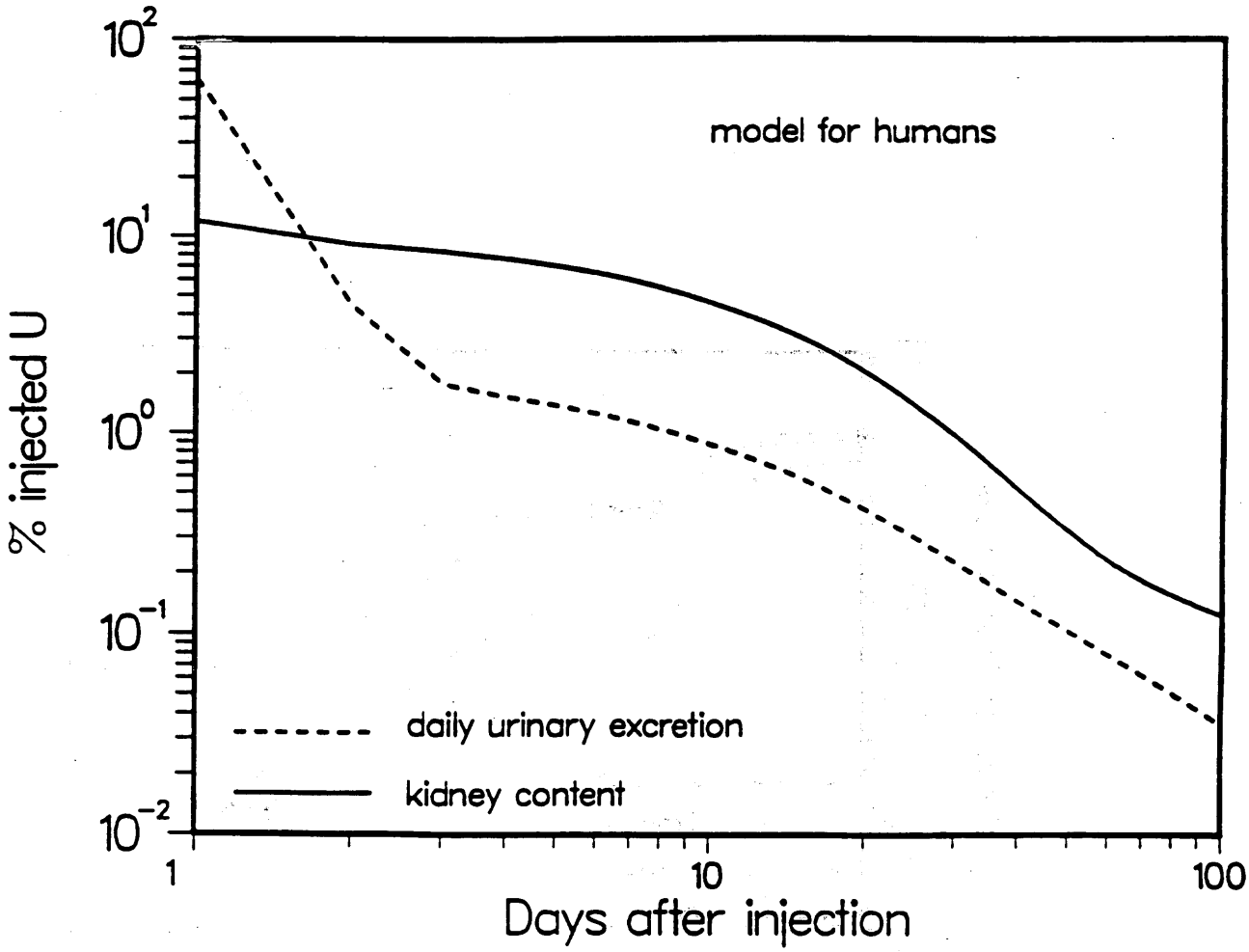

Figure 3. Model predictions of U in the kidneys and urine of a typical adult human as a function of time after intravenous injection of a small mass of soluble U.

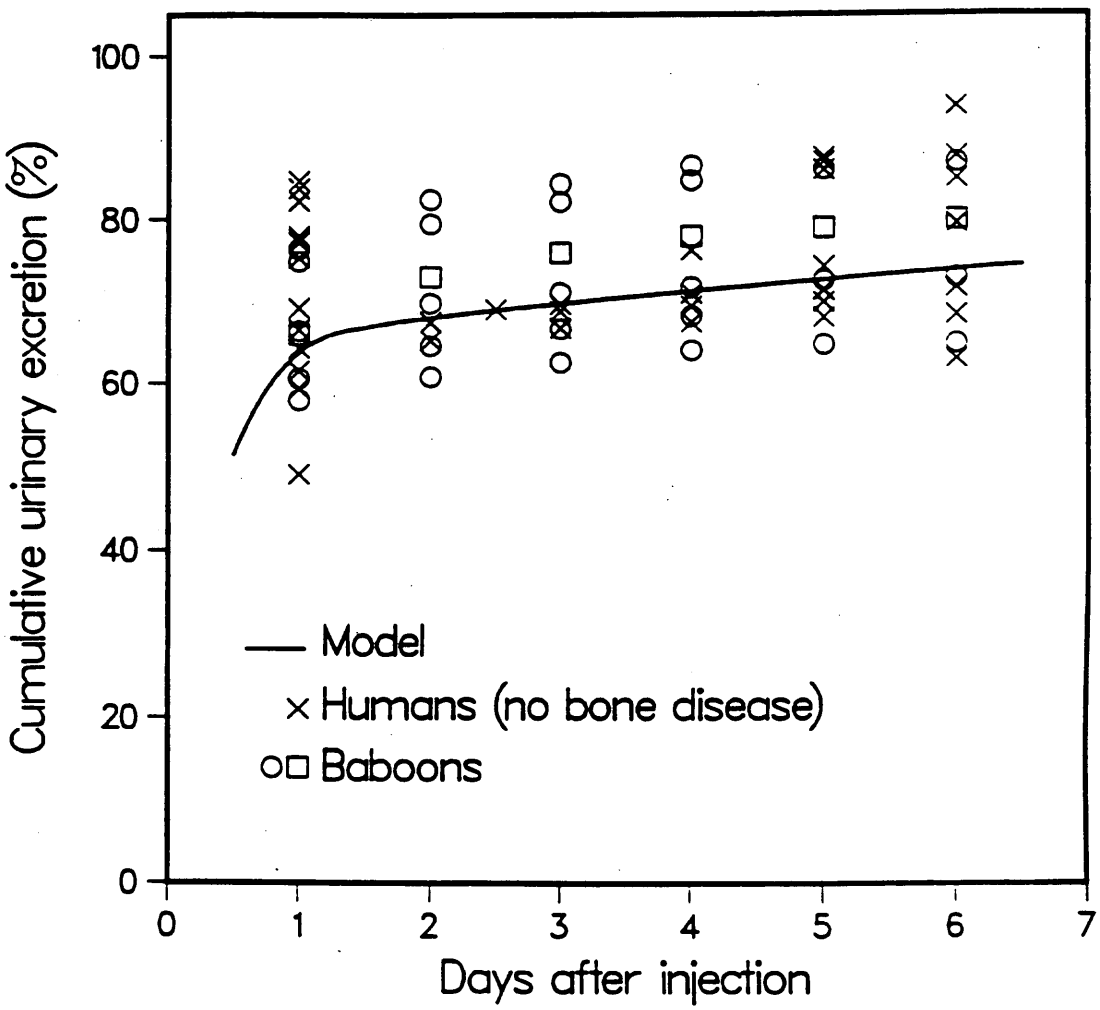

Figure 4. Comparison of early urinary excretion of U by baboons (Lipsztein 1981, circles; Larsen et al. 1984, squares) and humans (Bernard and Struxness 1957, Terepka et al. 1964, and Bassett et al. 1948).

Table 1. Transfer rates for the relatively detailed version of the model (Figure 1). These rates apply to a reference adult.

Path	Transfer rate (d^{-1})
from plasma-F to:	
plasma-B	20.0
ST0	60.0
RBC	1.2
Urinary bladder contents	6.0
Kidney 1	84.0
Kidney 3	0.06
Upper large intestine contents	0.6
Liver 1	1.8
ST1	7.98
ST2	0.36
trabecular bone surfaces	10.0
cortical bone surfaces	8.0
to plasma-F from:	
plasma-B	5.55
ST0	8.32
RBC	0.347
Kidney 3	0.00038
Liver 1	0.092
Liver 2	0.00019
ST1	0.0347
ST2	0.000019
bone surfaces[a]	0.0693
nonexch. trabecular bone volume	0.000493
nonexch. cortical bone volume	0.0000821
from Kidney 1 to:	
Kidney 2	0.594
Urinary bladder contents	3.565
from Kidney 2 to bladder urine	0.099
from Liver 1 to Liver 2	0.00693
from bone surfaces to exchangeable bone volume[a]	0.0693
from exchangeable bone volume to bone surfaces[a]	0.0173
from exchangeable bone volume to nonexchangeable volume[a]	0.00578

[a]Applies both to trabecular and cortical bone compartments.

Richard W. Leggett, Curriculum Vitae

Education:

1967: B.S., Union University, Jackson, Tennessee; major: mathematics
1969: M.S., University of Kentucky, Lexington, Kentucky; major: mathematics
1972: Ph.D., University of Kentucky, Lexington, Kentucky; major: mathematics

Professional experience:

1972-1974: Scientific Coworker, Ruhr University, Bochum, Germany.
1974-1976: Assistant Professor, Dept. of Math, University of Tennessee.
1976-present: Research Scientist, Oak Ridge National Laboratory

Awards:

1986: Martin Marietta Energy Systems (MMES) Significant Achievement Award for development of an improved model of the retention and excretion of plutonium by man.
1989: MMES Publication Award for the paper "A biokinetic model for Rb in humans".
1993: MMES Significant Achievement Award for development of an improved biokinetic model for lead in humans.

Committee memberships:

Member of International Commission on Radiological Protection Task Group on Dose Calculations.
Member of International Commission on Radiological Protection Task Group on Internal Dosimetry.
Corresponding Member of International Commission on Radiological Protection Task Group on Reference Man.
Member of the U.S. National Council on Radiological Protection's Scientific Committee on Dosimetry and Metabolism of Radionuclides.
Member of U. S. National Council on Radiological Protection's Scientific Committee 57-16 on Uncertainties in Applications of Metabolic Models.

Recent Publications

R. W. Leggett, Basis for the biokinetic model for uranium to be used in ICRP Publication 56; to appear in Health Physics.

R. W. Leggett and K. F. Eckerman, Evolution of the ICRP's biokinetic models, to appear in Radiation Protection Dosimetry.

R. W. Leggett, An age-specific kinetic model of Pb metabolism in humans, Environmental Health Perspectives 101:598-616; 1993.

R. W. Leggett, K. F. Eckerman, L. R. Williams, An elementary method for implementing complex biokinetic models, Health Physics 64:260-278; 1993.

R. W. Leggett, A generic age-specific biokinetic model for calcium-like elements, Radiation Protection Dosimetry 41:183-198; 1992.

R. W. Leggett, Fractional absorption of ingested Ba to blood in adult humans, Health Physics 62:556-561; 1992.

R. W. Leggett, A retention-excretion model for americium in humans, Health Physics 62:288-310; 1992.

R. W. Leggett and L. R. Williams, Suggested reference values for regional blood volumes in humans, Health Physics 60, 1991, 139-154.

R. W. Leggett, The behavior and chemical toxicity of uranium in the kidney: a reassessment, Health Physics 57, 1989, 365-383.

L. R. Williams and R. W. Leggett, Reference values for resting blood flow to organs of man, Clin. Phys. Physiol. Meas. 10(2), 1989, 187-217.

R. W. Leggett and L. R. Williams, A biokinetic model for rubidium in humans, Health Physics 55, 1988, 685-702.

ESTIMATED BUDGET

"Dosimetry for uranium-contaminated wound cases"

LABOR	$27.000
TRAVEL	0
SUBCONTRACTS	0
MATERIALS	0
OVERHEAD	12.000
DOE ADDED FACTOR	1.000
TOTAL	$40.000

Department of Energy
Oak Ridge Field Office
P.O. Box 2001
Oak Ridge, Tennessee 37831—

February 14, 1994

Dr. Eric G. Daxon
Defense Nuclear Agency
Armed Forces Radiobiology Research
Institute
8901 Wisconsin Avenue
Bethesda, Maryland 20889-5603

Dear Dr. Daxon:

SPACE AND DEFENSE TECHNOLOGY PROJECT, "DOSIMETRY FOR URANIUM-CONTAMINATED WOUND CASES" (DOE PROPOSAL NO. 0046-H036-A1)

Enclosed for your consideration is one copy of a proposal entitled "Dosimetry for Uranium-Contaminated Wound Cases," prepared by Martin Marietta Energy Systems, Inc. (Energy Systems), a Management and Operating (M&O) contractor for the Department of Energy (DOE). The proposed work covers a performance period through September 30, 1994, with an estimated total cost of $40,000. If approved by your organization, this work would be done as part of the work under the DOE/Energy Systems contract and on the same basis as the DOE work assigned to Energy Systems under the contract. Specific terms and conditions applicable to this project are included with the proposal.

In accordance with DOE orders and procedures, requests for work must be fully funded prior to commencement if it is to be completed within the current fiscal year. For work that transcends the fiscal year, full funding for the current fiscal year, plus three months of the following fiscal year is required. If the Defense Nuclear Agency is unable to provide full funding at this time, an explanation requesting DOE to waive this requirement should be sent to:

Department of Energy
Oak Ridge Operations Office
Attn: Donna J. Phillips
ER-10, Room G-059
P.O. Box 2001
Oak Ridge, TN 37831-8600

To ensure that legal authority exists for DOE to authorize this work, each funding authorization should contain the following statement. If this statement is not provided, DOE will not be able to accept the funding.

This agreement is entered into pursuant to the authority of the Economy Act of 1932, as amended (31 USC 1535), or other statutory authority references and adheres to Federal Acquisition Regulation (FAR) 6.002. To the best of our knowledge, the work requested will not place DOE and its contractor in direct competition with the private sector. This work will be performed in accordance with the DOE/ORO Work for Others Terms and Conditions attached to DOE Proposal No. 0046-H036-A1.

Your funding authorization should be sent to the address shown on the previous page.

Financial/administrative and programmatic points of contact from your organization, along with their telephone numbers, should be included on your funding authorization along with a task description detailing the required work to be performed for the funded amount. If a task description is not provided by you, DOE will provide a task description and obtain your approval before authorizing Energy Systems to perform the work. If there is a need to change the (1) proposal, (2) performance period, and/or (3) funding level, please address all correspondence to the address shown on the previous page.

If you have any questions regarding this proposal, please contact the DOE or Energy Systems personnel listed in Section XIV of the enclosed DOE/ORO Work for Others Terms and Conditions.

Sincerely,

Blox for

Ronald O. Hultgren
Deputy Assistant Manager for Energy
Research and Development

ER-10:Phillips

Enclosure:
Proposal w/Terms & Conditions

cc w/encl:
Jean Marlowe, DOE-OSTI (ORO-UT)
D. C. Cunningham, ER-113, ORO

cc w/o encl:
C. T. Rice, 800TPK, MS-7610
R. B. Honea, ORNL, MS-6230
R. W. Leggett, ORNL, MS-6383

DEPARTMENT OF ENERGY (DOE)
OAK RIDGE OPERATIONS OFFICE (ORO)
WORK FOR OTHERS TERMS AND CONDITIONS

DOE/ORO Proposal No. 0046-H036-A1

I. All work will be performed in accordance with DOE and Martin Marietta Energy Systems, Inc., (Energy Systems) Management and Operating Contract No. DE-AC05-84OR21400.

II. This work will be performed on a best effort basis and neither DOE, Energy Systems nor persons acting on their behalf will be responsible, irrespective of causes, for failure to perform their services or furnish the materials or information hereunder at any particular time or in any specific manner. Furthermore, DOE and Energy Systems hereby specifically disclaim any and all warranties, express or implied, including any warranty of merchantability or fitness for any purpose.

III. This project may be modified by mutual consent of both the Defense Nuclear Agency, Armed Forces Radiobiology Research Institute (AFRRI) and DOE at any time or may be terminated by either agency upon a thirty day (30) advance written notice to the other. In the event of termination by either agency, DOE will be reimbursed by the AFRRI for costs required to terminate and closeout the task activity.

IV. In the event of a dispute between the AFRRI and DOE or Energy Systems, no authorized final decision will be issued without the concurrence of the contracting officers of both the AFRRI and DOE. If the dispute cannot be resolved, the contracting officers of both the AFRRI and DOE will agree upon a third-party forum to settle the dispute.

V. Funds shall be considered obligated upon DOE's acceptance of the funds and issuance of direction by letter to Energy Systems. As DOE is obligating the funds for this project under contract with Energy Systems, it is recommended that no project/fund expiration dates be added to the funding document and that any schedules or delivery dates be expressed in the project task description.

Performance by DOE/Energy Systems shall continue until one or more of the following conditions are met:

a. Completion of the Statement of Work and Task Closeout;

b. Expenditures/commitments equal the amount authorized for this task;

c. Task termination by either party to this project;

d. Work completion date as specified in the funding document. DOE cannot accept work completion dates not mutually agreed to by both parties. (Work completion dates are not to be confused with obligational expiration dates.)

VI. Work performed for other federal agencies shall be fully funded prior to commencement of work if the work is to be completed within the current fiscal year. For work that transcends the fiscal year, full funding for the current fiscal year plus the first three months of the following year shall be required.

VII. DOE will account for and control funds by individual funding document as specified by DOE Order 2200.6, Chapter IX, unless specific written instructions to the contrary are received from a certifying officer of the AFRRI.

VIII. Billing will be performed on behalf of DOE by Energy Systems, Central Accounting Department to the billing address indicated on each funding authorization. Billing is performed monthly for the costs recorded during the previous month on an SF 1080 for reimbursement to the DOE appropriation on the basis of actual costs incurred.

IX. The AFRRI cannot withhold or delay reimbursement to DOE. The Economy Act of 1932, as amended, P.L. 98-216, February 14, 1984, paragraph 1535 (b), states, "Payment shall be made promptly by check on the written request of the agency or unit filling the order. Payment may be in advance or on providing the goods and services ordered and shall be for any part of the estimated or actual cost as determined by the agency or unit filling the order. A bill submitted or a request for payment is not subject to audit or certification in advance of payment. Proper adjustment of amounts paid in advance shall be made as agreed to by the heads of the agencies or units on the basis of the actual cost of goods or services provided."

X. Inventions made in performance of this work may fall within the DOE-issued Class Patent Waiver to Energy Systems and Energy Systems may elect to retain title to such inventions subject to retention by the Federal Government of march-in-rights and a non-exclusive, non-transferrable, irrevocable, paid-up license to practice or have practiced for or on behalf of the U.S. the invention throughout the world.

XI. Title of permanent construction will pass to DOE upon completion of construction and acceptance by DOE. If equipment is acquired as part of the project, such equipment will be accounted for and maintained during the term of the project in the same manner as DOE property. When the project terminates, disposition of the equipment will be as previously agreed to or as instructed by the requesting agency. This equipment may be delivered to the requesting agency's location, transferred to DOE or declared as excess.

XII. Security requirements to be followed in performance of the work will be in accordance with applicable DOE Orders. If classified work or access to classified information is required, AFRRI will provide to DOE applicable classification guidance and any new guides/updates as they are developed. Before work begins or classified matter is received or transmitted, a classified mail and/or shipping channel shall be established and approved by the appropriate AFRRI certifying official.

XIII. a. AFRRI recognizes that Energy Systems will perform the work assigned to DOE under this project pursuant to the "Related Services" provision of the DOE/Energy Systems contract. The DOE approved internal procedure governing access to and flow of information between Energy Systems and its affiliate organizations will apply to all work performed under the terms of this project. These procedures are subject to DOE audit at all times. In accordance with the Organization Conflict of Interest terms of said contract, Energy Systems, including any of its officials who may acquire information as part of their management responsibilities, is prohibited from further disseminating any third-party proprietary data or government sensitive data/information, as indicated by restrictive markings identifying the data and information so protected, to its affiliated organizations.

b. In view of the above, the AFRRI hereby agrees that affiliates of Energy Systems shall not be restrained or restricted from competing for any related follow-on contracts or subcontracts to be awarded by the AFRRI which relate to work under this project.

XIV. Department of Energy, Oak Ridge Operations Office, Project Contacts:

David C. Cunningham
Program Manager
Department of Energy
Oak Ridge Operations Office
Post Office Box 2008
Building 4500-N, Mailstop 6269
Oak Ridge, Tennessee 37831-6269
Commercial or FTS (615) 574-9276

Donna J. Phillips
Work for Others Coordinator
Department of Energy
Oak Ridge Operations Office
Post Office Box 2001
Oak Ridge, Tennessee 37831-8600
Commercial or FTS (615) 576-0395

Martin Marietta Energy Systems, Inc., Project Contact:

R. W. Leggett
Martin Marietta Energy Systems, Inc.
Oak Ridge National Laboratory
Health and Safety Research Division
Building 7509, Mailstop 6383
Post Office Box 2008
Oak Ridge, Tennessee 37831-6383
(615) 576-2079

Defense Nuclear Agency Project Contact:

Dr. Eric G. Daxon
Defense Nuclear Agency
Armed Forces Radiobiology Research Institute
8901 Wisconsin Avenue
Bethesda, Maryland 20889-5603
(301) 395-2950

(Revised 05/93)

STATEMENT OF WORK

During the first 6 months of funding a dose-response profile (using the up-down procedure) will be established for determining the acute effects of embedded DU pellets on kidney function in the laboratory rat.

Following the dose ranging study, five groups of rats will be implanted with DU or serve as a control groups. The groups will be: a) low dose DU implant, b) medium dose DU implant, c) high dose DU implant d) tantalum (inert) control implant and e) sham surgical control group. After implantation, rats will be assessed for long-term neurotoxic damage by using a battery of behavioral tests (functional observational battery), peripheral nerve conduction velocity and electrophysiological analysis of brain tissue. Separate groups of implanted rats will be used to evaluate the effects of DU at 1, 6, 12, and 18 months postimplantation.

In addition, all rats will be assessed for signs of nephrotoxicity, capsule formation around the embedded fragments will be characterized and histopathologically analysis of tissues will be ascertained. Finally, in collaboration with Oak Ridge Laboratories we will use the data generated from the rat study to develop a biokinetic model to predict the distribution of DU within the body. The model will also be able to estimate total body burdens of DU based on measurements obtained from excreta or the surface area of a DU fragment.

MILESTONES:

FY95

- Complete dose-ranging study. Estimated time for completion is 6 months.

- Initiate and complete the 1 month time point assessment of the behavioral, neurophysiological, histological, and tissue uranium analyses.

- Initiate 6, 12, and 18 month time points assessments.

- Initiate biokinetic modeling, using early time points.

FY96

- Complete 6 and 12 month time points for the behavioral, neurophysiological, histological, and tissue uranium analyses.

- Continue biokinetic modeling, incorporating 6 and 12 month time points.

- Submit Midterm Report

FY97

- Complete 18 month time point assessments.

- Complete biokinetic modeling.

- Submit Final Report.

TOTAL PROJECT PERIOD COST ESTIMATE

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD

BUDGET CATAGORY TOTALS	INITIAL BUDGET PERIOD 1ST	ADDITIONAL YEARS OF SUPPORT REQUESTED			
		2nd	3rd	4th	5th
DIRECT LABOR COSTS Salary & Fringe Benefits	102,967	108,470	114,211	000000	000000
MAJOR EQUIPMENT	0	0	0	0	0
SUPPLIES	10065	8325	8200	0000	0000
SUBCONTRACTS/SUBGRANTS	178499	0	0	0	0
TRAVEL COSTS	0	0	0	0000	0000
PUBLICATION COSTS	600	600	600	000	000
CONSULTANT COSTS	0	0	0	0	0
OTHER DIRECT COSTS	19617	13091	913	00000	0000
TOTAL DIRECT COSTS*	133,249	130,486	123,924	0	0
INDIRECT COSTS** 45.4	39,975	59,241	56,261	0	0
TOTAL COSTS	351,723	189,727	180,185	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	387,659
TOTAL INDIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	155,477
TOTAL COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	721,635

*This does not include the cost of contracts/subcontracts.

**Indirect rates are 30% for the first year and 45.4% for the remaining.

DETAILED COST ESTIMATE FOR INITIAL BUDGET PERIOD

From 10/1/94 Through 9/30/95

DIRECT LABOR COSTS					DOLLAR AMOUNT REQUESTED			
NAME	ROLE ON PROJECT	TYPE APPT (mo.)	% EFFORT ON PROJECT	BASE ANNUAL SALARY	SALARY REQ.	FRINGE BENEFITS % RATE / $		TOTALS
Pellmar, Terry	Investigator	12	20	79206	15,841	22	3,485	19,326
Landauer, Michael	Investigator	12	15	72797	10,920	22	2,402	13,322
Stiefel, Steven	Pathologist	12	10	00	0	0	0	0
Fergueson, Clifford	Investigator	12	10	00	0	0	0	0
TBA-GS 9	Technician	12	100	28964	28,964	22	6,372	35,336
TBA-GS 5	Technician	12	100	19116	19,116	22	4,206	23,322
TBA-GS 5	Histo Tech	12	50	19116	9,558	22	2,103	11,661
SUBTOTALS					84,399		18,568	102,967

MAJOR EQUIPMENT		0
MATERIALS,SUPPLIES,CONSUMABLES		
Misc supplies - see budget justification	$10,065	10,065
SUBCONTRACTS/SUBGRANTS	$40,000 Biokinetic Model	
DIRECT COSTS	$138,499 Uranium Analysis	
INDIRECT COSTS	0	178,499
TRAVEL COSTS :		0
PUBLICATION AND REPORT COSTS : 1 Report or paper per year @ $600 each		600
CONSULTANT COSTS : Dr. Busch - unpaid consultant		0
OTHER DIRECT COSTS: 373 rats (@$20ea) + per diem (25¢ per day)		19,617
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD*		**133,249**
INDIRECT COSTS FOR INITIAL BUDGET PERIOD (% Rate)	**30**	**39,975**
TOTAL COSTS FOR INITIAL BUDGET PERIOD		**351,723**

*This does not include the cost of contracts.

DETAILED COST ESTIMATE FOR SECOND YEAR BUDGET

From 10/1/95 Through 9/30/96

DIRECT LABOR COSTS					DOLLAR AMOUNT REQUESTED			
NAME	ROLE ON PROJECT	TYPE APPT (mo.)	% EFFORT ON PROJECT	BASE ANNUAL SALARY	SALARY REQ.	FRINGE BENEFITS % RATE / $		TOTALS
Pellmar, Terry	Investigator	12	20	81582	16,316	22	3,590	19,906
Landauer, Michael	Investigator	12	15	74981	11,247	22	2,474	13,721
Stiefel, Steven	Pathologist	12	10	00	0	0	0	0
Fergueson, Clifford	Investigator	12	10	00	0	0	0	0
TBA	Technician	12	100	30828	30,828	22	6,782	37,610
TBA	Technician	12	100	20346	20,346	22	4,476	24,822
TBA	Histo Tech	12	50	20346	10,173	22	2,238	12,411
					0		0	0
SUBTOTALS					88,910		19,560	108,470

MAJOR EQUIPMENT		0
MATERIALS,SUPPLIES,CONSUMABLES		
Misc supplies - see budget justification	8325	8,325
SUBCONTRACTS/SUBGRANTS DIRECT COSTS	0	
INDIRECT COSTS	0	0
TRAVEL COSTS :		0
PUBLICATION AND REPORT COSTS : 1 Report or paper per year @ $600 each		600
CONSULTANT COSTS : Dr. Busch - unpaid consultant		0
OTHER DIRECT COSTS:		13091
TOTAL DIRECT COSTS FOR SECOND BUDGET PERIOD		**130,486**
INDIRECT COSTS FOR SECOND BUDGET PERIOD (% Rate)	**45.4**	**59,241**
TOTAL COSTS FOR SECOND BUDGET PERIOD		**189,727**

DETAILED COST ESTIMATE FOR THIRD YEAR BUDGET

From 10/1/96 Through 9/30/97

DIRECT LABOR COSTS					DOLLAR AMOUNT REQUESTED			
NAME	ROLE ON PROJECT	TYPE APPT (mo.)	% EFFORT ON PROJECT	BASE ANNUAL SALARY	SALARY REQ.	FRINGE BENEFITS % RATE / $		TOTALS
Pellmar, Terry	Investigator	12	20	84029	16,806	22	3,697	20,503
Landauer, Michael	Investigator	12	15	77230	11,585	22	2,549	14,134
Stiefel, Steven	Pathologist	12	10	00	0	0	0	0
Fergueson, Clifford	Investigator	12	10	00	0	0	0	0
TBA	Technician	12	100	32777	32,777	22	7,211	39,988
TBA	Technician	12	100	21631	21,631	22	4,759	26,390
TBA	Histo Tech	12	50	21631	10,816	22	2,380	13,196
					0		0	0
SUBTOTALS					93,615		20,596	114,211

MAJOR EQUIPMENT		0
MATERIALS,SUPPLIES,CONSUMABLES		
Misc supplies - see budget justification	8200	8,200
SUBCONTRACTS/SUBGRANTS	0	
DIRECT COSTS		
INDIRECT COSTS	0	0
TRAVEL COSTS :		0
PUBLICATION AND REPORT COSTS : 1 Report or paper per year @ $600 each		600
CONSULTANT COSTS : Dr. Busch - unpaid consultant		0
OTHER DIRECT COSTS:		913
TOTAL DIRECT COSTS FOR THIRD BUDGET PERIOD		**123,924**
INDIRECT COSTS FOR THIRD BUDGET PERIOD (% Rate)	**45.4**	**56,261**
TOTAL COSTS FOR THIRD BUDGET PERIOD		**180,185**

BUDGET JUSTIFICATION: PERSONNEL

Dr. Terry Pellmar (20%) will be responsible for the coordination of this project. Her expertise is in neurophysiology and she will direct and interpret the data from the experiments on nerve conduction velocity and hippocampal slice electrophy5iology.

Dr. Michael Landauer (15%) will provide the expertise for the behavioral experiments. He will direct, analyze and interpret the Functional Observation Battery, the locomotor activity tests and the passive avoidance test.

Dr. Clifford Fergueson (10%) will perform the NAG assay and provide expertise on interpretation of the renal biochemistries. Because of his military position, this will be without cost to the project.

Dr. Steven Stiefel (10%) will provide expertise on the histological analysis and on any veterinary concerns in the project. He will direct the histology and necropsy procedures and will interpret the histopathology. Because of his military position, his contribution will be without cost to the project.

All the investigators will contribute to the preparation of results for oral and written presentations.

Dr. David B. Busch will provide consultation without cost to the project. He is a radiation pathologist at the Armed Forces Institute of Pathology and a Diplomat of the American Board of Pathology in combined Anatomic and Clinical Pathology. He currently serves as the Registrar for the Radiation Pathology Registry of the American Registry of Pathology.

GS9 Technician (100%) will be primarily responsible for conducting the electrophysiological studies (both the nerve conduction velocity experiments and the hippocampal slice studies) under the supervision of Dr. Pellmar. These experiments will require a full day for each rat studied. Throughout the project, three rats per week will be evaluated. The remaining two days a week will be used for data analysis and coordinating other components of the project. As a senior technician he/she will help to keep the project organized and the testing on schedule. He/she will ensure that the correct animals are scheduled for behavioral and physiological experiments each week, that the tissue samples are shipped and the biochemical assays performed. He/she will assist with the necropsy, some of the biochemical analyses and sample collections as required.

GS5 Technician (100%) will be responsible for the behavioral testing under the supervision of Dr. Landauer. As with the electrophysiology, 3 rats will be evaluated each week throughout the study. This technician will also be responsible for collecting the urine and plasma samples and provide assistance to the GS9 technician in maintaining coordination of the project.

GS5 Histology Technician (50%) will responsible for preparing the tissue for histological analysis under the supervision of Dr. Stiefel. Tissue will be embedded, sliced, mounted and stained. In addition, he/she will perform the biochemical assays (except for NAG which will be done by Dr. Fergueson).

It is estimated that all personnel will receive yearly a 3% cost of living adjustment of their salary. Technician salaries were estimate at the Step 1 level of their grade (US Government scale for Washington DC). A within grade step increase was added yearly for all technicians but not for the investigators.

REVISED BUDGET

Principal Investigator/Project Director T.C. Pellmar

TOTAL PROJECT PERIOD COST ESTIMATE

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD

BUDGET CATAGORY TOTALS	INITIAL BUDGET PERIOD 1ST	ADDITIONAL YEARS OF SUPPORT REQUESTED			
		2nd	3rd	4th	5th
DIRECT LABOR COSTS Salary & Fringe Benefits	128290	133897	139726	000000	000000
MAJOR EQUIPMENT	0	0	0	0	0
SUPPLIES	10065	8325	8200	0000	0000
SUBCONTRACTS/SUBGRANTS	293499	0	0	0	0
TRAVEL COSTS	0	0	0	0000	0000
PUBLICATION COSTS	600	600	600	000	000
CONSULTANT COSTS	0	0	0	0	0
OTHER DIRECT COSTS	19617	13091	913	00000	0000
TOTAL DIRECT COSTS	158,572	155,913	149,439	0	0
INDIRECT COSTS 30	47,572	46,774	44,832	0	0
TOTAL COSTS	499,643	202,687	194,271	0	0

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	463,924
TOTAL INDIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	139,178
TOTAL COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	896,601

JUSTIFICATION OF REVISED BUDGET

This budget reflects the anticipated cost of doing just Dr. Pellmar's project. The budget for the project has been adjusted to include the cost of the DU pellets ($115,000 contract in year one). In addition, salaries were adjusted to match the personnel that are likely to be assigned to the proposal. Indirect costs have been calculated at 30% for all three years. These changes bring the total expenses up to $899,380 for the three year period.

REVISED BUDGET

Principal Investigator/Project Director T.C. Pellmar

DETAILED COST ESTIMATE FOR INITIAL BUDGET PERIOD

From 10/1/94 Through 9/30/95

DIRECT LABOR COSTS					DOLLAR AMOUNT REQUESTED			
NAME	ROLE ON PROJECT	TYPE APPT (mo.)	% EFFORT ON PROJECT	BASE ANNUAL SALARY	SALARY REQ.	FRINGE BENEFITS % RATE / $		TOTALS
Pellmar, Terry	Investigator	12	20	79206	15,841	22	3,485	19,326
Landauer, Michael	Investigator	12	15	72797	10,920	22	2,402	13,322
Stiefel, Steven	Pathologist	12	10	00	0	0	0	0
Fergueson, Clifford	Investigator	12	10	00	0	0	0	0
TBA GS 12	Res. Assoc.	12	100	42003	42,003	22	9,241	51,244
TBA-GS 7/5	Technician	12	100	26834	26,834	22	5,903	32,737
TBA-GS 5	Histo Tech	12	50	19116	9,558	22	2,103	11,661
					0		0	0
SUBTOTALS					105,156		23,134	128,290

MAJOR EQUIPMENT		0
MATERIALS,SUPPLIES,CONSUMABLES		
Misc supplies - see budget justification	$10,065	10,065
SUBCONTRACTS/SUBGRANTS	$40,000 Biokinetic Model	
DIRECT COSTS	$138,499 Uranium Analysis	
	$115,000 Pellet Fabrication	293,499
TRAVEL COSTS :		0
PUBLICATION AND REPORT COSTS : 1 Report or paper per year @ $600 each		600
CONSULTANT COSTS : Dr. Busch - unpaid consultant		0
OTHER DIRECT COSTS: 373 rats (@$20ea) + per diem (25¢ per day)		19,617
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		**158,572**
INDIRECT COSTS FOR INITIAL BUDGET PERIOD (% Rate)	**30**	**47,572**
TOTAL COSTS FOR INITIAL BUDGET PERIOD		**499,643**

REVISED BUDGET

Principal Investigator/Project Director T.C. Pellmar

DETAILED COST ESTIMATE FOR SECOND YEAR BUDGET

From	Through
10/1/95	9/30/96

DIRECT LABOR COSTS					DOLLAR AMOUNT REQUESTED			
NAME	ROLE ON PROJECT	TYPE APPT (mo.)	% EFFORT ON PROJECT	BASE ANNUAL SALARY	SALARY REQ.	FRINGE BENEFITS % RATE / $		TOTALS
Pellmar, Terry	Investigator	12	20	81582	16,316	22	3,590	19,906
Landauer, Michael	Investigator	12	15	74981	11,247	22	2,474	13,721
Stiefel, Steven	Pathologist	12	10	00	0	0	0	0
Fergueson, Clifford	Investigator	12	10	00	0	0	0	0
TBA GS12	Res. Assoc.	12	100	44704	44,704	22	9,835	54,539
TBA	Technician	12	100	27639	27,639	22	6,081	33,720
TBA	Histo Tech	12	50	19689	9,845	22	2,166	12,011
					0		0	0
SUBTOTALS					109,751		24,146	133,897

MAJOR EQUIPMENT		0
MATERIALS,SUPPLIES,CONSUMABLES		
Misc supplies - see budget justification	8325	8,325
SUBCONTRACTS/SUBGRANTS	0	
DIRECT COSTS	0	0
TRAVEL COSTS :		0
PUBLICATION AND REPORT COSTS : 1 Report or paper per year @ $600 each		600
CONSULTANT COSTS : Dr. Busch - unpaid consultant		0
OTHER DIRECT COSTS:		13091
TOTAL DIRECT COSTS FOR SECOND BUDGET PERIOD		**155,913**
INDIRECT COSTS FOR SECOND BUDGET PERIOD (% Rate)	**30**	**46,774**
TOTAL COSTS FOR SECOND BUDGET PERIOD		**202,687**

REVISED BUDGET

Principal Investigator/Project Director T.C. Pellmar

DETAILED COST ESTIMATE FOR THIRD YEAR BUDGET

From 10/1/96 Through 9/30/97

DIRECT LABOR COSTS					DOLLAR AMOUNT REQUESTED			
NAME	ROLE ON PROJECT	TYPE APPT (mo.)	% EFFORT ON PROJECT	BASE ANNUAL SALARY	SALARY REQ.	FRINGE BENEFITS % RATE / $		TOTALS
Pellmar, Terry	Investigator	12	20	84029	16,806	22	3,697	20,503
Landauer, Michael	Investigator	12	15	77230	11,585	22	2,549	14,134
Stiefel, Steven	Pathologist	12	10	00	0	0	0	0
Fergueson, Clifford	Investigator	12	10	00	0	0	0	0
TBA GS12	Res. Assoc.	12	100	47530	47,530	22	10,457	57,987
TBA	Technician	12	100	28468	28,468	22	6,263	34,731
TBA	Histo Tech	12	50	20280	10,140	22	2,231	12,371
					0		0	0
SUBTOTALS					114,529		25,197	139,726

MAJOR EQUIPMENT		0
MATERIALS,SUPPLIES,CONSUMABLES		
Misc supplies - see budget justification	8200	8,200
SUBCONTRACTS/SUBGRANTS	0	
DIRECT COSTS		
INDIRECT COSTS	0	0
TRAVEL COSTS :		0
PUBLICATION AND REPORT COSTS : 1 Report or paper per year @ $600 each		600
CONSULTANT COSTS : Dr. Busch - unpaid consultant		0
OTHER DIRECT COSTS:		913
TOTAL DIRECT COSTS FOR THIRD BUDGET PERIOD		**149,439**
INDIRECT COSTS FOR THIRD BUDGET PERIOD (% Rate)	**30**	**44,832**
TOTAL COSTS FOR THIRD BUDGET PERIOD		**194,271**

MATERIALS, SUPPLIES AND CONSUMABLES

1. Surgical supplies
. . . . . Surgical implantation in year 1$1865
We estimate that each rat will cost approximately $5 to implant with DU fragments to cover the cost of anesthesia, surgical instruments, towels, blades, etc). In year 1 we will be implanting 373 rats with DU for a cost of $1865. In year 2 we will implant 25 rats for $125. No animals will be implanted in year 3.
In addition surgical supplies (anesthesia, syringes, instruments, towels, blades etc) will be necessary for surgical exposure of the peripheral nerve, removal of the hippocampus and necropsy. Rats will be used at rate of 3 per week throughout the study. . . . . $ 200

2. Electrophysiological supplies $1200
These include: electrodes (stimulating and recording for peripheral nerve and brain slice preparations ($600); small electronic supplies and equipment replacement parts (batteries, cables, connectors, wire, etc.) ($400); Salts for saline and artificial cerebrospinal fluid ($200).

3. Behavioral supplies . . . . . $ 700
Supplies are required for the maintenance of activity monitors and metabolic cages (gloves, towels, disinfectant, small component replacements)

4. Histology supplies $1500
These include: stains, slides, paraffin, cassettes, alcohol, processor upkeep etc.

5. Biochemical assays $2000
Our Institute Pathology Laboratory estimates that each biochemical assay will cost approximately $1 per sample. This cost includes reagents, syringes, plasticware, paper products, equipment upkeep, etc. Eight assays will be performed for each of 373 animal for an average yearly cost of $1000.
Since the assay for NAG is substantially more complex, it is more expensive to run, approximately $10 per sample. We will measure NAG in each of 300 rats over the three years for an average yearly cost of $1000.

6. Uranium transportation costs $2000
Ten samples from each of 120 rats will be shipped to Battelle Pacific Northwest Laboratories for uranium analysis at a cost of approximately $5 per sample. Shipping costs will average $2000 per year over the three years.

7. Computer supplies . . . . . $ 600
Computers used for data acquisition and analysis and for word processing of reports. We will require floppy disks, tapes for backup, paper, cartridges for printers, etc. at an average of $600 per year.

SUBCONTRACTS

Biokinetic Model (Appendix I-F) $40,000

Drs. Leggett and Eckermann have estimated their costs for development of the biokinetic model at $40000 (see appendix I-F, p. I-70). We plan to contract this work with this group because they are the designers of the code currently used by the Army to assesss the risk from internal emitters. Once this project is completed, they will be able to rapidly add embedded fragments to this code and give the Army a usable product.

Uranium Analysis (Appendix I-E) $138,499

The uranium analysis will be contracted to Battelle, Pacific Northwest Laboratories (PNL). They have agreed to do all of the uranium measurements that we require in this project (all Tasks) for a flat fee of $138,499 (see appendix I-E, p. I-46). This is significantly less than estimates obtained from other sources ($120 for fluids and $195 for tissue samples for a total cost of approximately $180,000 for this Task, from International Technology Corporation (IT), see attached quote). For this Task alone, it is estimated that we will require analysis of 1000 samples (8 tissues and 2 fluids from each of 100 animals). PNL provides the best price for the required services. In addition, the assay performed by PNL is orders of magnitude more sensitive than that of other sources (0.01 g U/l vs 20 g U/l fluids; 10 pg U/g vs 100,000 pg U/g for tissues).

OTHER DIRECT COSTS

Direct costs will vary from year to year with the number of rats purchased and the per diem required to house the rats. The schedule will require the following:

Year 1:	325 + 48 rats purchased @ $20 each	$ 7460
	Per diem for 75 rats for 1 month @ 25¢ per day	$ 563
	Per diem for 48 rats for 2 weeks @ 25¢ per day	$ 188
	Per diem for 250 rats for 6 months @ 25¢ per day	$11406
	Total for year 1	**$19617**
Year 2:	25 rats purchased @ $20 each	$ 500
	Per diem for 25 rats for 1 week @ 25¢ per day	$ 44
	Per diem for 75 rats for 6 months @ 25¢ per day	$ 3422
	Per diem for 100 rats for one year @ 25¢ per day	$ 9125
	Total for year 2	**$13091**
Year 3:	Per diem for 20 rats for 6 months @ 25¢ per day	**$ 913**

Quotation

IT INTERNATIONAL TECHNOLOGY CORPORATION

April 25, 1994

Dr. Kimberly Benson
Armed Forces Radiobiology Research Institute
AFRRI-BHS
8901 Wisconsin Avenue
Bethesda MD 20889-5603

Dear Dr. Benson,

IT Analytical Services-Richland Division is pleased to provide the following quotation for the analysis of biological samples for total uranium.

Sample Type	Detection Limit	Sample Preparation	Analysis	Total
Tissue	0.1 μg/g (wet)	$ 100.00	$ 95.00	$ 195.00
Homogenized Tissue	0.1 μg/g (wet)	$ 50.00	$ 95.00	$ 145.00
Blood	0.02 μg/mL	$ 100.00	$ 95.00	$ 195.00
Blood Serum	0.02 μg/mL	$ 25.00	$ 95.00	$ 120.00

The detection limits quoted above assume a minimum sample size of 1 gram for the tissue samples and 0.5 mL blood or 0.25 mL blood serum. The prices quoted above assumes fewer than ten samples of a particular matrix. If you anticipate a greater number of samples or a continuing effort over an extended period, volume discounts may be available.

If you have additional questions or comments, please call me at (509) 375-3131.

Sincerely,

Lee Scott
Project Manager

xc: Van Petrey, Systems Manager

Regional Office
2800 George Washington Way • Richland, Washington 99352-1613 • 509-375-3131 • FAX: 509-375-5590

ADDENDUM 1: ABBREVIATIONS

AP	--	action potential
ACSF	--	artificial cerebrospinal fluid
C_c	--	creatinine clearance
CNS	--	central nervous system
DU	--	depleted uranium
FE	--	fractional excretion
FOB	--	functional observational battery
Gy	--	Gray (1 Gy = 100 rads)
H&E	--	hematoxylin and eosin stain
HFS	--	high frequency stimulation
Hz	--	Hertz
i.m.	--	intramuscular
I/O	--	input/output
i.p.	--	intraperitoneal
ISI	--	interstimulus interval
LDH	--	lactate dehydrogenase
LTP	--	long-term potentiation
NAG	--	N-acetyl-β-glucosaminidase
P_c	--	creatinine concentration in urine
P_g	--	glucose concentration plasma
pPSP	--	population synaptic potential
PS	--	population spike
Pu	--	plutonium
Ta	--	tantalum
U	--	uranium
U_c	--	creatinine concentration in urine
U_g	--	glucose concentration in urine
V_u	--	rate of urine production (ml/min)
V	--	volts

ADDENDUM 2

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93. Rossi, B., Sartucci, F. and Stefanini, A., Measurement of motor conduction velocity with Hopf's technique in the diagnosis of mild peripheral neuropathies, *J. Neurol. Neurosurg. Psychiatry*, 44 (1981) 168-170.

94. Simpson, J.A., Applied electrophysiology in nerve and muscle disease. Disorders of neuromuscular transmission, *Proc. R. Soc. Med.*, 59 (1966) 993-998.

95. Aminoff, M.J., Layzer, R.B., Satya Murti, S. and Faden, A.I., The declining electrical response of muscle to repetitive nerve stimulation in myotonia, *Neurology*, 27 (1977) 812-816.

96. Brown, J.C., Muscle weakness after rest in myotonic disorders; an electrophysiological study, *J. Neurol. Neurosurg. Psychiatry*, 37 (1974) 1336-1342.

97. Tucker, S.M., Boyd, P.J., Thompson, A.E. and Price, R.G., Automated assay of N-acetyl-beta-glucosaminidase in normal and pathological human urine, *Clin. Chim. Acta*, 62 (1975) 333-339.

98. Luna, G.G., *Manual of histologic staining methods of the Armed Forces Institute of Pathology, American Registry of Pathology*, McGraw Hill Book Co., New York, 1968, pp. 32-49.

99. *Histological Classification and Nomenclature of Veterinary Tumors, Fascicles 1 through 21; Vol. 53*, Bulletin of the World Health Organization, 1976,

100. *Histological Classification and Nomenclature of Veterinary Tumors, Fascicles 1 through 21; Vol. 50*, Bulletin of the World Health Organization, 1974,

ADDENDUM 3
Curriculum Vitae

NAME Terry C. Pellmar ROLE ON PROJECT Principal Investigator

EDUCATION (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training*)

Institution and Location	Degree	Year Conferred	Field of Study
Brown University, Providence RI	ScB	1973	Biology
Duke University Med Ctr, Durham NC	PhD	1977	Physiol & Pharmacol
Neurobiology Dept.,AFRRI, Bethesda MD	Postdoc		Neuroscience
Lab Preclin Studies, NIAAA, Rockville MD	Postdoc		Neuroscience

RESEARCH AND PROFESSIONAL EXPERIENCE: (*Concluding with present position, list in chronological order, previous employment, experience and honors. Identify any Federal Government Service, either current or within the past year, naming the agency and describing the duties.*)

1977-1978 National Research Council Fellowship, Neurobiology Department, AFRRI, Bethesda, MD
1978 Grass Fellow, MBL, Woods Hole, Massachusetts
1978-1981 NIH Postdoctoral Fellowship, Neurobiology Department, AFRRI, Bethesda, MD
1981-1982 National Science Foundation, Postdoctoral Fellowship, National Institute of Alcohol Abuse and Alcoholism, Rockville, MD
1982-1984 Research Physiologist, Physiology Department, AFRRI, Bethesda, MD
1991-1994 Member, Veterans Administration Neurobiology Merit Review Board.
1984-present Project Manager, Neurophysiology Project, Physiology Department, AFRRI, Bethesda, MD

SIGNIFICANT PUBLICATIONS (from a total of 44):

Pellmar T.C. and Somjen G.G. Velocity of supraspinal input and conduction velocity of axons of spinal motoneurons. Brain Res. 120:179-183, 1977.

Pellmar T.C. and Wilson W.A., Unconventional serotonergic excitation in *Aplysia*. Nature 269:76-78, 1977.

Pellmar T.C. and Carpenter D.O., Serotonin induces a voltage-sensitive calcium current in neurons of *Aplysia californica*. J. Neurophysiol. 44:423-439, 1980.

Pellmar T.C., Does cyclic AMP act as second messenger in a voltage-dependent response to serotonin? Brit. J. Pharmacol. 74:747-756, 1981.

Pellmar T.C., Histamine decreases calcium-mediated potassium current in guinea pig hippocampal CA1 pyramidal cells. J. Neurophysiol. 55:727-738, 1986.

Pellmar T.C., Electrophysiological correlates of peroxide damage in guinea pig hippocampus *in vitro*. Brain Res. 364:377-381, 1986.

Pellmar T.C., Single-electrode clamp in mammalian electrophysiology. In: Modern Methods in Pharmacology; Electrophysiological Techniques in Pharmacology (ed. H. M. Geller), Alan R. Liss, Inc., New York, pp. 91-102, 1986.

Tolliver J.M. and Pellmar T.C., Dithiothreitol elicits epileptiform activity in CA1 of the guinea pig hippocampal slice. Brain Res. 404:133-141, 1987.

Pellmar T.C., Peroxide alters neuronal excitability in the CA1 region of guinea pig hippocampus *in vitro*. Neuroscience. 23:447-456, 1987.

SIGNIFICANT PUBLICATIONS: (*continued*)

Tolliver J.M. and Pellmar T.C., Ionizing radiation alters neuronal excitability in hippocampal slices of the guinea pig. Radiation Res. 112:555-563, 1987.

Pellmar T.C., Tolliver J.M. and Neel K.L. Radiation-induced impairment of neuronal excitability. Comments on Toxicology. 2:253-263, 1988.

Tolliver J.M. and Pellmar T.C., Effects of dithiothreitol, a sulfhydryl reducing agent, on CA1 pyramidal cells of the guinea pig hippocampus *in vitro*. Brain Res. 456:49-56, 1988.

Schauer D.A., Zeman G.H. and Pellmar T.C., A low energy X-ray irradiator for electrophysiological studies. Int. J. Radiation Applications and Instrumentation Part A 40:7-17, 1989.

Pellmar T.C. and Neel K.L. Oxidative damage in the guinea pig hippocampal slice. Free Radical Biology and Medicine 6:467-472, 1989.

Pellmar T.C., Neel K.L. and Lee K. Free radicals mediate peroxidative damage in guinea pig hippocampus *in vitro*. J. Neuroscience Res. 24: 437-444, 1989.

Pellmar T.C., Schauer D.A. and Zeman G.H. Time and dose dependent changes in neuronal activity produced by x-radiation in brain slices. Radiation Res. 122: 209-214, 1990.

Pellmar T.C., Hollinden G.E. and Sarvey J.M. Free radicals accelerate the decay of long-term potentiation in field CA1 of guinea pig hippocampus. Neuroscience 44:353-359, 1991.

Pellmar T.C., Fatty acids modulate excitability in guinea pig hippocampal slices. Neuroscience 45:273-280, 1991.

Pellmar T.C. and Lepinski D.L., Electrophysiological consequences of exposure of hippocampal slices to dihydroxyfumarate, a generator of superoxide radicals. Brain Res. 569:189-198, 1992.

Pellmar T.C., Roney D. and Lepinski D.L., Role of glutathione in repair of free radical damage in hippocampus in vitro. Brain Res. 583:194-200, 1992.

Gilman S.C., Bonner M.J. and Pellmar T.C., Peroxide effects on [^{3}H]L-glutamate release by synaptosomes isolated from the cerebral cortex. Neurosci. Letts. 140:157-160, 1992.

Myers L.S., Carmichael A.J. and Pellmar T.C., Radiation chemistry of the hippocampal brain slice. Adv. Space Res. In press.

Gilman S.C., Bonner M.J. and Pellmar T.C., Effect of oxidative stress on excitatory amino acid release by cerebral cortical synaptosomes. Free Radic. Biol. Med. 15: 671-675, 1993.

Pellmar T.C. and Lepinski D.L., Gamma radiation (5-10 Gy) impairs neuronal function in the guinea pig hippocampus. Radiation Res. 136:255-261, 1993.

Gilman, S.C., Bonner, M.J. and Pellmar, T.C., Free radicals enhance basal release of [^{3}H]D-aspartate from cerebral cortical synaptosomes, J. Neurochem. 62:1757-1763, 1994.

Keyser, D.O. and Pellmar, T.C., Glial cells are critical for synaptic transmission. Glia 10:237-243, 1994.

Pellmar, T.C., Reactive oxygen species on neural transmission, Ann. NY Acad. Sci. in press, 1994.

INFORMATION ON OTHER COMMITMENTS OF TIME, SUCH AS SABBATICAL OR ANTICIPATED EXTENDED LEAVE: NONE

PROPORTION OF TIME DEVOTED TO THIS AND TO OTHER RESEARCH: 100%

NUMBER OF GRADUATE STUDENTS FOR WHOM YOU ARE RESPONSIBLE: None

Curriculum Vitae

NAME: Michael R. Landauer ROLE ON PROJECT: Investigator

EDUCATION (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training*)

Institution and Location	Degree	Year Conferred	Field of Study
Rutgers Univ., New Brunswick, NJ	B.S.	1968	Biology
Univ. Illinois, Urbana, IL	M.S.	1970	Psychobiology
Univ. Illinois, Urbana, IL	Ph.D.	1975	Psychobiology
Medical Coll VA, Richmond, VA	Postdoc	1979-1982	Toxicology

RESEARCH AND PROFESSIONAL EXPERIENCE: (*Concluding with present position, list in chronological order, previous employment, experience and honors. Identify any Federal Government Service, either current or within the past year, naming the agency and describing the duties.*)

1976-1979 Visiting Assistant Professor, Department of Biological Sciences, Barnard College of Columbia University, New York, NY.

1979-1982 National Institute of Environmental Health Sciences (NIEHS) Postdoctoral Research Fellow in Behavioral Toxicology and Pharmacology, Medical College of Virginia, Richmond, VA.

1982-1984 Visiting Senior Research Scientist, Chemical Research and Development Center, U.S. Army, Toxicology Branch, Aberdeen Proving Ground, MD.

1984-1989 Research Toxicologist, Principal Investigator, Behavioral Sciences Department, Department of Defense, Armed Forces Radiobiology Research Inst., Bethesda, MD.

1990-present Project Manager, Behavioral Sciences Department, Armed Forces Radiobiology Research Institute, Bethesda, MD.

SIGNIFICANT PUBLICATIONS (from a total of 50):

Landauer, M.R., Attas, A.I., and Liu, S. Effects of prenatal and neonatal androgen on the estrous cyclicity and attractiveness of female hamsters. Physiology and Behavior. 27:419-424, 1981.

Landauer, M.R., Lynch, M.R., Balster, R.L. and Kallman, M.J. Trichloromethane induced taste aversions in mice. Neurobehavioral Toxicology and Teratology. 4:305-309, 1982.

Landauer, M.R. and Balster, R.L. The effects of aggregation on the lethality of phencyclidine in mice. Toxicology Letters. 12:171-176, 1982.

Landauer, M.R., Woolverton, W.L. and Balster, R.L. Effects of chlorpromazine on the lethality of a d-amphetamine and phencyclidine in mice. Research Communications in Substance Abuse. 3:287-295, 1982.

Landauer, M.R. and Balster, R.L. Opiate effects on social investigatory behavior of male mice. Pharmacology, Biochemistry and Behavior. 17:181-1186, 1982.

Landauer, M. R., Tomlinson, T., Balster, R.L. and MacPhail, R. Some effects of chlordimeform (a formamidine pesticide) on the behavior of mice. Neurotoxicology., 5:91-100, 1984.

Landauer, M.R., and Romano, J.A. Acute behavioral toxicity of the organophosphate sarin in rats. Neurobehavioral Toxicology and Teratology. 6:239-243, 1984.

Landauer, M.R. Romano, J.A. and Armstrong, R.D. Neurobehavioral toxicity of sarin in rats. Proceedings of the 1983 Scientific Conference on Chemical Defense Research. 703-711, Special Publication - CRDC-84014, Aberdeen Proving Ground, MD. 1985.

SIGNIFICANT PUBLICATIONS: (*continued*)

Landauer, M.R., Balster, R.L. and Harris L. Attenuation of cyclophosphamide-induced taste aversions in mice by prochlorperazine, delta-9-tetrahydrocannabinol, nabilone and prochlorperazine, delta-9-tetrahydro cannabinol, nabilone and levonantradol. Pharmacology, Biochemistry and Behavior. 23:259-266, 1985.

Landauer, M.R. and Leach, G.J. Comparative behavioral toxicity of physostigmine in ferrets and rats. Journal of the American College of Toxicology. 5:261-266, 1986.

Romano, J.A. and Landauer, M.R. Effects of the organophosphorus compound O-ethyl-N-dimethyl phosphoramidocyanidate (tabun) on flavor aversions, locomotor activity, and rotorod performance in rats. Fundamental and Applied Toxicology. 6:62-68, 1986.

Landauer, M.R., Davis, H.D., Dominitz, J.A., Weiss, J.F. Dose and time relationships of the radioprotector WR-2721 on locomotor activity in mice. Pharmacology, Biochemistry and Behavior. 27:573-576, 1987.

Landauer, M.R., Ledney, G.D. and Davis, H.D. Locomotor behavior in mice following exposure to fission-neutron irradiation and trauma. Aviation, Space and Environmental Medicine. 58:1205-1210, 1987.

Landauer, M.R., Walden, T.L., Davis, H.D., Dominitz, J.A. Alternations in locomotor activity induced by radioprotective doses of 16, 16-dimethyl prostaglandin E2. In: Walden, T.L. and Hughes, H.N., (eds). Prostaglandin and Lipid Metabolism in Radiation Injury, Plenum Press: New York. 245-251, 1987.

Landauer, M.R., Davis, H.D., Dominitz, J.A. and Weiss, J.F. Comparative behavioral toxicity of four sulfhydryl radioprotective compounds in mice: WR-2721, cysteamine, diethyldithiocarbamate and N-acetylcysteine. Pharmacology and Therapeutics. 39:97-100, 1988.

Landauer, M.R., Davis, H.D., Dominitz, J.A., Weiss, J.F. Long-term effects of radioprotector WR-2721 on locomotor activity and body weight of mice following exposure to ionizing radiation. Toxicology. 49: 315-323, 1988.

Landauer, M.R., Walden, T.L., Jr. and Davis, H.D. Behavioral effects of radioprotective agents in mice: Combination of WR-2721 and 16, 16-dimethyl prostaglandin E2. In: Riklis, E. (ed.) Frontiers in Radiation Biology, VCH Publishers: New York. 199-207, 1990.

Landauer, M.R., Davis, H.D. and Walden, T.L., Jr. Behavioral and physiological effects of leukotriene C4. Prostaglandins, leukotrienes and Essential Fatty Acids. 39:247-252, 1990.

Maier, D.M. and Landauer, M.R. Onset of behavioral effects of mice exposed to 10 Gy cobalt-60 radiation. Aviation, Space and Environmental Medicine. 61:893-898, 1990.

Landauer, M.R., Davis, H.D. and Walden, T.L., Jr. Behavioral toxicity of radioprotective bioactive lipids. In: Hohn, K.V., Marnett, L.J., Nigam, S. and Walden, T. (eds). Eicosanoids and Other Bioactive Lipids in Cancer and Radiation Injury, Vol 1, Kluwer Academic Publishers, Boston, MA. 183-188, 1991.

Landauer, M.R., Davis, H.D., Kumar, K.S. and Weiss, J.F. Behavioral toxicity of selected radioprotectors. Advances in Space Research. 12:273-283, 1992.

Landauer, M.R., Weiss, J.F., Gunter-Smith, P.J., Benson, K.A., Blair, M.D., Hogan, J.B. and Hanson, W.R., Behavioral and radioprotective effects of misoprostol in adrenalectomized mice. In: Eicosanoids and Other Bioactive Lipids in Cancer and Radiation Injury, Vol 3, Kluwer Academic Publishers, Boston, MA. in press.

INFORMATION ON OTHER COMMITMENTS OF TIME, SUCH AS SABBATICAL OR ANTICIPATED EXTENDED LEAVE: NONE

PROPORTION OF TIME DEVOTED TO THIS AND TO OTHER RESEARCH: 100%

NUMBER OF GRADUATE STUDENTS FOR WHOM YOU ARE RESPONSIBLE: none

Curriculum Vitae

NAME Steven M. Stiefel ROLE ON PROJECT Investigator

EDUCATION (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training*)

Institution and Location	Degree	Year Conferred	Field of Study
Phillips, University, Enid OK	BS	1972	Biology
College of Veterinary Medicine, Kansas State University, Manhattan, KS	DVM	1979	Vet Med

RESEARCH AND PROFESSIONAL EXPERIENCE: (*Concluding with present position, list in chronological order, previous employment, experience and honors. Identify any Federal Government Service, either current or within the past year, naming the agency and describing the duties.*)

1979-1980 Veterinarian, Small Animal Practice, Wichita, KS

1980-1983 Base Veterinarian, Fitzsimons Army Medical Center, Aurora, CO

1981-1983 Veterinarian, Small Animal Relief Practice, Aurora, CO

1983-1986 Resident, Armed Forces Institute of Pathology, Washington, D.C.

1984-1985 Prosector, Department of Pathology, National Zoological Park, Washington, D.C.

1986-1990 Chief, Comparative Pathology Division, Veterinary Science Department, Armed Forces Radiobiology Research Institute; Instructor: Radiation Pathology, AFRRI MENW Course; Instructor: Tissue and Organ Sensitivity, Medical Officer Course in Nuclear Medicine and Radioisotope Techniques, Bethesda Naval Hospital, Bethesda, MD

1990-1993 Chief, Animal Diagnostics Division, 10th Medical Laboratory, Landstuhl, Germany; Consultant, Veterinary Pathology, Commanding General, 7th Medical Command, Heidelburg, Germany

1993-Present Chief, Comparative Pathology Division, Veterinary Science Department, Armed Forces Radiobiology Research Institute, Washington, D.C.; Instructor: Radiation Pathology, AFRRI MENW Course; Consultant and pathologist, Clinical Pathology Laboratories, Uniformed Services University of Health Sciences, Bethesda, MD

MEMBER: American College of Veterinary Pathologists; American Veterinary Medical Association;
HONORS: National Defense Service Medal with Bronze Medal; Armed Forces Reserve Medal; Overseas Service Ribbon; Army Good Conduct Medal; Army Service Ribbon; Army Achievement Medal; Vietnam Service Medal; Republic of Vietnam Campaign Ribbon; Vietnam Unit Citation of Gallantry with Palm; Army Commendation Medal with Oak Leaf Cluster; Meritorious Service Medal; Defense Meritorious Service Medal

SIGNIFICANT PUBLICATIONS:

Jackson, R.K., Juras, R.A., Stiefel, S.M., and Hall, J.E., Mycobacterium kansasii in a Rhesus monkey, Laboratory Animal Science, 39:425-427, 1989.

Elliot, T.B., Brook, I. and Stiefel, S.M., Quantitative study of wound infection in irradiated mice, International Journal of Radiation Biology, 58:341-350, 1990.

Stewart, D.A., Ledney, G.D., Madonna, G.S., Stiefel, S.M., and Moore, M.M., Synthetic trehalose dicorynomycolate (S-TDCM) increases hematopoietic cell proliferation in fission neutron (N/G=1) irradiated mice, Military Medical Laboratory Science, 19:208-213, 1990.

Neta, R., Stiefel, S.M., Finkelman F., Herrmann, S. and Ali N., Interleukin-12 protects bone marrow from and sensitizes intestinal tract to ionizing radiation, Journal of Experimental Medicine, 1994, submitted for publication.

Gary,J.J., Mitchell, D.L., Stiefel, S.M. and Hale, M.L., Tissue compatibility of non-distilled monomer in heat polymerized methyl methacrylate used in cranial prosthesis, Journal of Prosthetic Dentistry, submitted for publication.

Synnott, S.A., Mitchell, D.L., Stiefel, S.M. and Hale, M.L., Tissue compatibility of heat and autopolymerizing methy methacrylate used in cranial prosthesis for rats, Journal of Prosthetic Dentistry, submitted for publication.

Alderks, C.E., Stiefel, S.M., and Harris, A.H., Radiation-induced lethality and pathology in pigeons (Columba-livia), Laboratory Animal Science, submitted for publication.

INFORMATION ON OTHER COMMITMENTS OF TIME, SUCH AS SABBATICAL OR ANTICIPATED EXTENDED LEAVE: NONE

PROPORTION OF TIME DEVOTED TO THIS AND TO OTHER RESEARCH: 100%

NUMBER OF GRADUATE STUDENTS FOR WHOM YOU ARE RESPONSIBLE: NONE

Curriculum Vitae

NAME Clifford L. Ferguson ROLE IN PROJECT Investigator

EDUCATION (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training*)

Institution and Location	Degree	Year Conferred	Field of Study
Morehouse College, Atlanta, GA	B.S.	1970	Biology
Meharry Medical College, Nashville, TN	M.D.	1976	

RESEARCH AND PROFESSIONAL EXPERIENCE: (*Concluding with present position, list in chronological order, previous employment, experience and honors. Identify any Federal Government Service, either current or within the past year, naming the agency and describing the duties*).

1976-1977	Occupational Medicine Officer, U.S. Army Environmental Hygiene Agency, Aberdeen Proving Grounds, MD.
1977-1978	Flexible Internship, Walter Reed Army Medical Center, Washington, DC
1978-1981	Internal Medicine Residency, Walter Reed Army Medical Center, Washington, DC
1981-1983	Nephrology Fellowship, Walter Reed Army Medical Center, Washington, DC
1981-1983	Teaching Fellow, Uniformed Services University of the Health Sciences, Bethesda, MD.
1983-1989	Clinical Instructor, Department of Medicine, Texas Tech University, Lubbock, TX.
1983-1985	Chief, Internal Medicine Clinic, William Beaumont Army Medical Center, San Antonio, TX.
1985-1986	Staff, Nephrology Service, William Beaumont Army Medical Center, El Paso, TX.
1986-1989	Chief, Nephrology Service, William Beaumont Army Medical Center, El Paso, TX.
1989-1992	Fellow, FDA/ARMY Fellowship, Division of Clinical Pharmacology, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD, Walter Reed Army Institute of Research, Experimental Therapeutics Division, Washington, DC.
1992-present	Staff, Department of Pharmacology, Experimental Therapeutics Division, Walter Reed Army Institute of Research, Washington, DC and Assistant Professor, Division of Clinical Pharmacology, Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD.

HONORS AND AWARDS:
Bronze Star Medal; Vietnam Campaign Medal; Vietnam Service Medal; National Defense Service Medal; Army Service Medal; Army Commendation Medal; Alpha Omega Alpha Medical Honor Society;

LICENSURE: State of Maryland, D23677;
CERTIFICATION: American Board of Internal Medicine

SIGNIFICANT PUBLICATIONS:

Choi Y., Paul J.V. and Ferguson C.L. Parent-child minimal change nephrotic syndrome. American Journal of Diseases of Children. 142:11:114, 1988.

LaGrone R., Jeffrey T.B., and Ferguson C.I. Effects of education and relaxation training with essential hypertension patients. Journal of Clinical Psychology. 44:2:271-276, 1988.

Monahan B.P., Ferguson C.L., Killeavy E.S., Lloyd B.K., Troy J., Catilena, L.R. Torsades de pointes occurring in association with terfenadine use. Journal of the American Medical Association. 264:21:2788-2790, 1990.

Ferguson, C.L. and Cantilena, L.R. Enhanced mercury clearance during hemodialysis with cheating agents. Clinical Pharmacology and Therapeutics. Abstract 49:2:131, 1991.

Ferguson, C.L. and Cantilena, L.R. Mercury Clearance from human plasma during in vitro dialysis: screening systems for cheating agents. Journal of Toxicology - Clinical Toxicology, 30:3:423-441, 1992.

Ferguson, C.L. and Cantilena, L.R. In vitro equilibrium dialysis as a screening procedure for cheating agents effective in enhancing mercury clearance from human plasma. Clinical Pharmacology and Therapeutics. Abstract; 53:2:155, 1993

Ferguson, C.L. and Cantilena, L.R. Changes in tissue distribution of mercury in rats following infusion of N-acetylcysteine. (Manuscript in preparation)

INFORMATION ON OTHER COMMITMENTS OF TIME, SUCH AS SABBATICAL OR ANTICIPATED EXTENDED LEAVE: NONE

PROPORTION OF TIME DEVOTED TO THIS AND TO OTHER RESEARCH: 75%

NUMBER OF GRADUATE STUDENTS FOR WHOM YOU ARE RESPONSIBLE: None

ADDENDUM 4
Existing/Pending Support

Name *Terry C. Pellmar* Active Pending **None**

a. Source
Title
b. Role on Project
c. Dates & costs of entire project
d. Dates & costs of current year
e. Specific aims of project

f. Describe scientific & budgetary overlap

g. If there is overlap, provide justification for USAMRDC interest and support

---

Name *Steven Stiefel* Active Pending **None**

a. Source
Title
b. Role on Project % Effort
c. Dates & costs of entire project
d. Dates & costs of current year
e. Specific aims of project

f. Describe scientific & budgetary overlap

g. If there is overlap, provide justification for USAMRDC interest and support

Existing/Pending Support

Name *Clifford L. Fergueson* **Active** Pending None

a. Source: Uniformed Services University of the Health Sciences

Title: Clearance of mercury during hemodialysis in pigs.
b. Role on Project: Principal Investigator 50% Effort
c. Dates & costs of entire project: 01 Oct 93 - 3- Dec 94/ $18,000
d. Dates & costs of current year: $9,000
e. Specific aims of project
1. Determine if N-acetylcysteine can effect clearance of mercury across hemodialysis membrane.

f. Describe scientific & budgetary overlap: NONE

g. If there is overlap, provide justification for USAMRDC interest and support
NONE

Name: *Michael R. Landauer* **Active** Pending None

a. Source: Veterans Administration/Dept of Defense
Title: Radiation Protection by Prostaglandinds: Mecahnisms & Applications
b. Role on Project: Investigator 10% Effort
c. Dates & costs of entire project: 1 Apr 91 - 1 Mar 95, $354,000
d. Dates & costs of current year: 1 Apr 94 - 1 Mar 95, $ 42,000
e. Specific aims of project:
1. Investigate the influence of prostaglandins on radiation-induced survival and longevity of mice alone and in combination with phosphorothioate radioprotectors.
2. Investigate the influence of prostaglandin-induced protection of villud and/or crpt epithelium.
3. Determine the effect of prostaglandins alone or in combination with phosphorothioates on behavioral toxicity.

f. Describe scientific & budgetary overlap - NONE

g. If there is overlap, provide justification for USAMRDC interest and support

ADDENDUM 5

LETTERS CONFIRMING COLLABORATION

A. See attached letter from Ronald O. Hultgren from the Oak Ridge Laboratories, Department of Energy (DOE) describing contract support of the biokinetic modeling. We are in the process of obtaining from DOE an updated letter extending this proposal to enable the work to continue through FY97 at no increased cost.

B. See attached letter from Dr. Thomas S. Tenforde from Battelle Pacific Northwest Laboratories confirming arrangements for uranium analysis of issues and organs of rats implanted with depleted uranium.

C. See attached letter from Dr. David B. Busch agreeing to provide unpaid consultation on the histopathology.

Department of Energy
Oak Ridge Field Office
P.O. Box 2001
Oak Ridge, Tennessee 37831—

February 14, 1994

Dr. Eric G. Daxon
Defense Nuclear Agency
Armed Forces Radiobiology Research Institute
8901 Wisconsin Avenue
Bethesda, Maryland 20889-5603

Dear Dr. Daxon:

SPACE AND DEFENSE TECHNOLOGY PROJECT, "DOSIMETRY FOR URANIUM-CONTAMINATED WOUND CASES" (DOE PROPOSAL NO. 0046-H036-A1)

Enclosed for your consideration is one copy of a proposal entitled "Dosimetry for Uranium-Contaminated Wound Cases," prepared by Martin Marietta Energy Systems, Inc. (Energy Systems), a Management and Operating (M&O) contractor for the Department of Energy (DOE). The proposed work covers a performance period through September 30, 1994, with an estimated total cost of $40,000. If approved by your organization, this work would be done as part of the work under the DOE/Energy Systems contract and on the same basis as the DOE work assigned to Energy Systems under the contract. Specific terms and conditions applicable to this project are included with the proposal.

In accordance with DOE orders and procedures, requests for work must be fully funded prior to commencement if it is to be completed within the current fiscal year. For work that transcends the fiscal year, full funding for the current fiscal year, plus three months of the following fiscal year is required. If the Defense Nuclear Agency is unable to provide full funding at this time, an explanation requesting DOE to waive this requirement should be sent to:

Department of Energy
Oak Ridge Operations Office
Attn: Donna J. Phillips
ER-10, Room G-059
P.O. Box 2001
Oak Ridge, TN 37831-8600

To ensure that legal authority exists for DOE to authorize this work, each funding authorization should contain the following statement. If this statement is not provided, DOE will not be able to accept the funding.

This agreement is entered into pursuant to the authority of the Economy Act of 1932, as amended (31 USC 1535), or other statutory authority references and adheres to Federal Acquisition Regulation (FAR) 6.002. To the best of our knowledge, the work requested will not place DOE and its contractor in direct competition with the private sector. **This work will be performed in accordance with the DOE/ORO Work for Others Terms and Conditions attached to DOE Proposal No. 0046-H036-A1.**

Your funding authorization should be sent to the address shown on the previous page.

Financial/administrative and programmatic points of contact from your organization, along with their telephone numbers, should be included on your funding authorization along with a task description detailing the required work to be performed for the funded amount. If a task description is not provided by you, DOE will provide a task description and obtain your approval before authorizing Energy Systems to perform the work. If there is a need to change the (1) proposal, (2) performance period, and/or (3) funding level, please address all correspondence to the address shown on the previous page.

If you have any questions regarding this proposal, please contact the DOE or Energy Systems personnel listed in Section XIV of the enclosed DOE/ORO Work for Others Terms and Conditions.

Sincerely,

B Cox for

Ronald O. Hultgren
Deputy Assistant Manager for Energy
Research and Development

ER-10:Phillips

Enclosure:
Proposal w/Terms & Conditions

cc w/encl:
Jean Marlowe, DOE-OSTI (ORO-UT)
D. C. Cunningham, ER-113, ORO

cc w/o encl:
C. T. Rice, 800TPK, MS-7610
R. B. Honea, ORNL, MS-6230
R. W. Leggett, ORNL, MS-6383

Battelle
Pacific Northwest Laboratories
Battelle Boulevard
P.O. Box 999
Richland, Washington 99352
Telephone (509)375-3738

REVISED

May 19, 1994
(94.225)

Lt. Col. E. Daxon
Armed Forces Radiobiology Research Institute
Defense Nuclear Agency
Bethesda, Maryland 20814-5145

Dear Col. Daxon:

The following material is enclosed as the Pacific Northwest Laboratory's contribution to the proposal on toxicity of depleted uranium fragments being prepared by AFRRI:

Item A: "Analysis of Rat Tissues for Uranium After Administration of Depleted Uranium Particles" (Responsible Investigator: A.F. Fuciarelli)

Item B: "Computation of Organ and Localized Dose Rates Around DU Fragments Imbedded in Tissue" (Responsible Investigators: G. Akabani, A.C. James, and M. Parkhurst)

Item C: Appendix to Item B -- "Dose Assessment for an Imbedded DU Fragment"

Item D: Appendix on "Radiological Consequences of Exposures of Military Personnel to Aerosolized DU" (additional project that PNL would like to pursue if adequate funding is obtained)

Items E through H: Brief resumes of G. Akabani, A.F. Fuciarelli, A.C. James, and M. Parkhurst

Item I: Budget sheets for core project proposal (item A)

Item J: Budget sheets for core project proposal (item B)

Item K: Budget sheets for item D on dosimetry of inhaled DU aerosols

This material is being sent to you via Airborne Express, with both hard copies of the various items for the proposal and disks containing the text and budget sheets. The only parts of the proposal that cannot be transmitted to you

Battelle

electronically are the 2 figures in item C (the model dose calculation). Please contact me on May 20 if you have any questions. After that date I will be away on travel until June 2, and I recommend that you contact either Dr. Dennis Mahlum (509/372-1846) or Dr. Al Fuciarelli (509/376-2627) for questions on item A. Dr. Gamal Akabani (509/375-6986), Dr. Tony James (509/375-2035), or Ms. MaryAnn Parkhurst (509/375-6893) should be contacted for questions on items B, C, and D. Ms. Iris Garza (509/376-3119) should be contacted regarding any budget questions.

We hope that AFRRI's proposal will be successful, and look forward to working with you on this project.

Sincerely,

Thomas S. Tenforde

Thomas S. Tenforde, PhD

Chief Scientist, Life Sciences Center
Manager, Health and Environmental
Research Program

Enclosures (11 + 2 disks)

12 May, 1994

Memorandum for: LTC Eric G. Daxon

Subject: my position on depleted uranium (DU) project.

I wish to state in writing that I am aware that you and your associates at AFRRI are interested in my participating in a veterinary research study on tissue effects of depleted uranium (DU), and that I am enthusiastic about participating in such a project, as well as in performing autoradiography studies of verterans with suspected DU injuries that could help determine if their tissues in fact were contaminated with an α emitter.

Sincerely,

David Busch, Ph.D., M.D.
Radiation Pathologist
(202) 576-0222

ADDENDUM 6
Resources and Environment

(x) Laboratory:

Dr. Pellmar has over 600 sq ft containing 3 fully equipped electrophysiology rigs with space dedicated for dissection of the rodents, preparation of hippocampal slices and for making electrodes. A fourth, fully equipped electrophysiology rig and a computer work station for data analysis is available in the laboratory next door (approx. 600 sq ft).

Dr. Landauer has over 650 sq ft of research space. This space used for behavioral testing, including conducting the functional observational battery and the locomotor activity tests.

Dr. Stiefel is director of the histology and clinical pathology laboratories. The histopathology laboratory is 700 sq ft containing all equipment and supplies necessary to process formalin-fixed and frozen tissue sections. Most of the staining procedures are done with H & E stain, but a wide variety of special stains can be performed. The clinical pathology laboratory is 390 sq ft containing all equipment and supplies necessary to perform complete blood and serum/urine chemistries with 18 measures for serum and 8 measures for urine.

Dr. Ferguson has a complete clinical pharmacology laboratory located at the Uniformed Services University of the Health Sciences which consists of 2 areas of approximately 470 sq ft each. The laboratory is staffed by ASCPT certified technicians. The lab provides assay results for both clinical and research specimens and performs most standard and some non-standard procedures and assays on blood, urine and other body fluids.

( ) Clinical:

(x) Animal:

All animal care will be provided by our Institute facility which is AAALAC approved.

(x) Computer:

Personal computers are available for all investigators. Most are connected to the local area network (LAN) to make use of Institute facilities such as Silver Platter-Medline and graphics and word processing software. All electrophysiological and behavioral equipment are interfaced with 486 computers for data acquisition and analysis. Computer work stations, available for use with RS1 or SAS for data analysis, are connected to a high quality laser printers.

(x) Office:

Dr. Pellmar has approx 70 sq ft of office space adjacent to the laboratories. Dr. Landauer has approx 100 sq ft of office space located near the laboratories. Dr. Stiefel has approximately 190 sq ft of office space situated between the histology and clinical pathology laboratories. Dr. Ferguson has approx 100 sq ft of office space adjacent to the clinical pharmacology laboratory.

(x) Other:

MAJOR EQUIPMENT: *List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.*

All behavioral equipment necessary to conduct the functional observational battery (FOB) is currently available and no additional equipment is necessary. Eight computer driven infrared photocell locomotor activity cages (Digiscan Analyzer, Omnitech Electronics) are available for this study. In addition, a digital

strain gauge grip-strength apparatus (San Diego Instruments, San Diego, CA) and four one-way passive avoidance shuttle boxes are available for behavioral testing.

All electrophysiology rigs are equipped with a vibration-free table with Faraday cage, AxoClamp or Dagan single electrode voltage clamp, extracellular amplifiers, three micromanipulators, stimulus isolation unit, calibrator, stimulator and Gateway 486 computer with data acquisition panel and loaded with Axo-Basic and Pclamp software. Three rigs have chart recorders and DeskJet printers. A centrifuge and a spectrophotometer are available for our use. Mettler balance, tissue chopper and electrode pullers are present in the laboratory.

The well equipped AFRRI histology laboratory has the following equipment for use in tissue analysis: Reichert-Jung 2800 Frigocut; (3) Leitz 1512 Microtomes; AO 820 Rotary Microtome; Hacker Instruments 76 Microtome Knife Sharpener; Tissue Tek II Automated Processor; (2) Fisher Slide Warmers; Lab-Line Slide Warmer; (3) Fisher Tissue Mat Water Baths; Bockel Water Bath; Autotechnicon Ultra II Strainer; Shandon Varistain 24-3; Clay Adams Sero-fuge II Centrifuge; Mettler Scale; Olympus AS41 Photomicroscope; Zenith Computer with Epson Printer and Stand; Fisher Isotemp Oven 200 series; Orion Research Digital Ionalyzer 501; (2) Vented hoods; (2) large Vented Hoods; Distilled Water Apparatus; Timer; (2) Refrigerators; Bausch & Lomb Binocular Gross Microscope; Zeiss Binocular Microscope with MC63 Camera Attachment.

The clinical pathology laboratory at AFRRI has the following equipment to be used as necessary for the studies in the proposal. Coulter Counter S Plus II; Kodak Ektachem 700 Analyzer with Printer; Baker 9000 Hematology Counter with printer; Fisher Rotorack mixer; Refrigerator-freezer; (2) AO Binocular Microscopes; Lab Gard Laminar Flow Hood; Imperial II Incubator; IEC Clinical Centrifuge; Progenesis wall-mounted Sterilizer; Ames Hema-Tek II Strainer; IEC Centra-R* Centrifuge; GCA Precision Scientific Incubator; Lab-Line Imperial IV Water Bath; Deluxe Mixer; Timer; Gas/Vacuum/Air Lines. In addition, the use of a HPLC in the Department of Clinical Pharmacology will be available for this project. In addition the laboratory at USUSH is fully equipped (including fluorimeter) for performing the NAG assay.

ADDITIONAL INFORMATION: *Provide any other information describing the environment for the project. Identify support services such as consultant, secretarial, machine shop, and electronics shop, and the extent to which they will be available to the project.*

The Armed Forces Radiobiology Research Institute (AFRRI) is a tri-service research laboratory located on the grounds of the Naval Medical Center in Bethesda, MD. AFRRI was chartered in 1960 as DoD's primary laboratory to conduct biomedical research on the effects of ionizing radiation. A state-of-the art 32,000 sq. ft. animal facility with surgical suites and rooms to house animals implanted with radioactive material such as DU are available at AFRRI. This facility is accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and is staffed by three veterinarians and 20 laboratory animal husbandry personnel. In addition, AFRRI has all the necessary Nuclear Regulatory Commission (NRC) licenses required to work with DU. AFRRI has a central computer facility for both hardware and software support, a design and fabrication shop, electronics shop, graphics department for preparation of posters, illustrations and slides and a centralized radioanalysis laboratory. A specialized radiation library is available within AFRRI and a medical library is located next door at the Uniformed Services University of the Health Sciences (USUHS) on campus. In addition, both the National Library of Medicine and the National Institutes of Health library are located nearby.

CERTIFICATE OF ENVIRONMENTAL AND SAFETY COMPLIANCE

The offeror currently ☒ IS ☐ IS NOT in compliance with applicable national, state, and local environmental and safety laws and regulations.
(If not in compliance, attach details and evidence of approved mitigation measures.)

The offeror has examined the activities encompassed within the proposed action entitled "Health Risk Assessment of Embedded Depleted Uranium: Behavior, Physiology, Histology and Biokinetic Modelling"
(enter title and/or Solicitation number and Principal Investigator's name), for compliance with environmental and safety laws and regulations. The offeror states that the conduct of the proposed action ☐ WILL ☒ WILL NOT violate any applicable national, state, or local environmental or safety law or regulation.
(If a violation will result, attach details describing the nature of the violation and evidence of approved mitigation measures.)

The offeror agrees that if the work required under the proposed action at any time results in a violation of any applicable environmental or safety law or regulation, the offeror will immediately take appropriate action, to include notifying the Contracting Officer, and coordinating with the appropriate regulatory agencies.

CDR DAVID J. SMITH, MC, USN
(Name of Official Responsible for Environmental and Safety Compliance)

Signature

HEAD, SAFETY AND HEALTH
(Title)

27 May 94
Date

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
(Name of Organization)

USAMRDC FORM 65-R
Dec 91

ADDENDUM 8: HUMAN USE – N/A

ADDENDUM 9: ANIMAL USE

A. Assurances:

1. I assure that the animals authorized for use in this protocol will be used only in the activities, the manner, and quantities described herein, unless a deviation is specifically approved by my IACUC and the USAMRDC Animal Use Review Office.

2. I accept full responsibility for the proper care and use of the animals during the conduct of research outlined in the proposal.

3. I verify that I have made a reasonably good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments. (The USAMRDC reserves the right to ask for evidence that a thorough literature search was performed.

4. I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent in those procedures, and have received their training on the use of animals in research, as required by the Animal Welfare Act of 1985.

Terry Pellmar
5/27/94

Terry C. Pellmar, Ph.D.
Principal Investigator

B. Letter of AAALAC Approval for our facility is attached.

C. Justification for animal species use
D. IACUC approval

These forms are in preparation and will be supplied when they are ready.

August 24, 1993

Bill Owen
Armed Forces Radiobiology Rsrch. Inst.
8901 Wisconsin Avenue
Bethesda, MD 20889-5603

Dear Mr. Owen:

The animal care and use program at the Armed Forces Radiobiology Research Institute, Bethesda, Maryland, was granted continued full accreditation by the American Association for Accreditation of Laboratory Animal Care (AAALAC), an independent, nonprofit organization. The animal program has participated in this voluntary accreditation program since 1985.

Achieving and maintaining accreditation is a significant accomplishment, and one in which staff take pride. AAALAC accreditation is widely accepted by the scientific community as assurance that a program exceeds the minimum required by law. We applaud the staff for their commitment to abiding by the essential elements of animal care and use.

We hope you will consider doing a story on the program's accreditation and its value. You may want to use the enclosed sample release for a short story. When printing a story about animal research or testing, you are encouraged to mention that the animal program is accredited by AAALAC. You may wish to speak with the AAALAC contact, Dr. Albert H. McCullen, about the importance of accreditation.

The enclosed brochure provides a brief description of our organization. This brochure is available, free of charge, to use with local media or to distribute to staff, students and the community at a general information desk or an upcoming event. Please contact AAALAC if you would like to order copies or need additional information about the accreditation program.

Sincerely,

Holly M. Sears

Holly M. Sears
Communications Coordinator

/hms:613

cc: Albert H. McCullen, D.V.M., Head, Veterinary Sciences Department

Enclosures

ADDENDUM 10

HAZARDOUS MATERIALS USE

These forms are in preparation and will be supplied when they are ready.

ADDENDUM 11

MEMORANDUM OF ENVIRONMENTAL AND SAFETY ANALYSIS

These forms are in preparation and will be supplied when they are ready.