Paper

HEALTH SURVEILLANCE OF GULF WAR I VETERANS EXPOSED TO DEPLETED URANIUM: UPDATING THE COHORT

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Abstract—A cohort of seventy-four 1991 Gulf War soldiers with known exposure to depleted uranium (DU) resulting from their involvement in friendly-fire incidents with DU munitions is being followed by the Baltimore Veterans Affairs Medical Center. Biennial medical surveillance visits designed to identify uranium-related changes in health have been conducted since 1993. On-going systemic exposure to DU in veterans with embedded metal fragments is indicated by elevated urine uranium (U) excretion at concentrations up to 1,000-fold higher than that seen in the normal population. Health outcome results from the subcohort of this group of veterans attending the 2005 surveillance visit were examined based on two measures of U exposure. As in previous years, current U exposure is measured by determining urine U concentration at the time of their surveillance visit. A cumulative measure of U exposure was also calculated based on each veteran's past urine U concentrations since first exposure in 1991. Using either exposure metric, results continued to show no evidence of clinically significant DU-related health effects. Urine concentrations of retinol binding protein (RBP), a biomarker of renal proximal tubule function, were not significantly different between the low vs. high U groups based on either the current or cumulative exposure metric. Continued evidence of a weak genotoxic effect from the on-going DU exposure as measured at the HPRT (hypoxanthine-guanine phosphoribosyl transferase) locus and suggested by the fluorescent in-situ hybridization (FISH) results in peripheral blood recommends the need for continued surveillance of this population.
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INTRODUCTION

MILITARY USE of depleted uranium (DU) munitions and DU armored vehicles during and since the 1991 Gulf War has given rise to concerns regarding the long term health effects of DU through exposure pathways unique to combat conditions. Due to the pyrophoric nature of DU, inhalation and ingestion of aerosolized particles of DU, primarily oxides, occur when DU armored tanks or other armored vehicles are hit by DU projectiles (Parkhurst et al. 2005). Soldiers in or on tanks receive inhalation exposures, and may ingest DU particles as they are cleared from the lungs by coughing and swallowing. Wound contamination can also occur under these conditions. In addition to these short term exposures, some soldiers may receive shrapnel wounds in which fragments of DU metal become embedded in muscle tissue. This unique exposure pathway can give rise to chronic systemic exposure to DU as the DU metal fragments oxidize in situ resulting in continued release of DU to the blood (Squibb and McDiarmid 2006).

The chronic health effects of DU in soldiers exposed under combat conditions are not completely understood despite a wealth of information on the toxicity of uranium (U) in other occupational exposure settings. This is due to the unique properties of this radioactive heavy metal and the difficulty in determining initial and long term exposure doses in individual soldiers. Therefore, since the early 1990's, the Department of Veterans Affairs (DVA) and the Department of Defense (DoD) have conducted a medical surveillance follow-up program for veterans and active duty soldiers involved in military DU friendly-fire incidents (Hooper et al. 1999; McDiarmid et al. 2001, 2004, 2006).

DU, a man-made form of uranium, is created during the uranium enrichment process when the ^{235}U and ^{234}U

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isotopes are removed from natural uranium, leaving a uranium metal with a lower $^{235}U/^{238}U$ isotopic ratio and approximately 60% of the radioactivity of natural uranium (Army Environmental Policy Institute 1995). Thus, DU poses a lower radiological hazard than natural uranium, but a similar chemical hazard to human health (The Royal Society 2001, 2002). The tissue distribution and chemical toxicity of U in mammalian systems has been intensively studied and is similar to many other metals (ATSDR 1999; McDiarmid and Squibb 2001; Parkhurst et al. 2005). The kidney is the primary target organ following acute exposures to soluble U compounds (Leggett 1989), while pulmonary damage can occur following inhalation exposures (Leach et al. 1970, 1973; Cross et al. 1981; Mitchel et al. 1999). Uranium has been shown to accumulate in the kidneys with chronic exposures; however, the level of accumulation depends upon whether the rate of exposure exceeds the tissue elimination rate (Squibb et al. 2005).

Based on DU's isotopic composition, its radiation effects are primarily due to alpha particle emissions, which travel short distances in tissue and thus generally have local effects (The Royal Society 2002). A smaller contribution to tissue dose rates is made by beta particles released from the progeny product ^{234m}Pa (protactinium), which is in secular equilibrium with ^{238}U. Beta particles penetrate greater distances in tissue, but beta particle radiation dose rates are one-tenth those from alpha particle emissions (The Royal Society 2002). In addition to the type of radiation emitted, tissue dose over time is also a function of the solubility of the U and the rate of tissue elimination of the metal (Eckerman 1988; Army Environmental Policy Institute 1995; The Royal Society 2002; Parkhurst et al. 2005).

Although in vitro studies provide evidence that natural and DU can be genotoxic (Lin et al. 1993; Miller et al. 1998, 2002, 2003; Stearns et al. 2005; Coryell and Stearns 2006; Knobel et al. 2006), these forms of uranium do not appear to be highly carcinogenic. Some, but not all, in vivo studies have reported induction of tumors in exposed animals (Maynard and Hodge 1949; Tannenbaum 1951; Maynard et al. 1953; Hueper et al. 1952; Leach et al. 1973; Cross et al. 1981; Hahn et al. 2002). In addition, epidemiological findings of occupationally exposed cohorts do not support the extrapolation of the animal and in vitro study results to humans. There is poor evidence for an excess cancer risk specifically of lung, bone or kidney (the most likely targets) in occupational cohorts (ATSDR 1999; Institute of Medicine 2000). The lung cancer excess observed in U miners has been well documented to be attributed to radon present in the mines (Samet et al. 1989; Samet 1989). Radon is a more intensely radioactive constituent than natural U

(Kathren and Moore 1986; Kathren et al. 1989), and this by-product of U decay is not present in DU, since U decay products are removed during the processing of the U ore (Papastefanou 2002).

Based on this background knowledge, uranium's chemical toxicity continues to be the primary focus of the surveillance of the Gulf War veterans, with emphasis on the target organs most likely affected by U and other heavy metals—the kidney, the central nervous system (Pellmar et al. 1999), and the reproductive system. In addition, genotoxic effects continue to be examined as an indicator of potential carcinogenicity of DU in this cohort. To date, six rounds of surveillance (1994, 1997, 1999, 2001, 2003, and 2005) have been conducted on an in-patient basis at the Baltimore VA Medical Center (BVAMC). We report here results of the 2005 clinical assessment of this cohort, an almost 15-y follow-up since exposure first occurred during the Gulf War.

For the first time, we are reporting an assessment of the relationship between health outcomes and cumulative exposure to DU, as well as the traditional current urine U metric used in past surveillance visits. Cumulative exposure is an important consideration when addressing effects from a toxicant which has storage depots with long half lives, as is the case with many heavy metals (Jakubowski et al. 2002; Chia et al. 1997; Bleecker et al. 2003) including U (Pellmar et al. 1999; Squibb et al. 2005).

MATERIALS AND METHODS

A subset of 34 members of a larger dynamic cohort, which currently numbers 74 DU-exposed Gulf War I veterans who were victims of "friendly fire" (Squibb et al. 2005), underwent medical surveillance at the BVAMC between April and June of 2005. Four participants in this group were assessed for the first time; the others have been seen previously.

Uranium exposure assessment

Data analysis for this surveillance visit utilized two types of U exposure metrics. The first is consistent with the current exposure measure used for past surveillance visits (24 h urine U concentration at the time of the visit). The second is a cumulative U exposure metric that takes into account the duration of exposure as well as the intensity of exposure.

Current urine U determination

Twenty-four-hour urine specimens collected during the hospital surveillance visit were sent to the Armed Forces Institute of Pathology's (AFIP) Department of Environmental Toxicologic Pathology (Washington, DC) for quantitative and isotopic composition analysis by an

Inductively Coupled Plasma-Dynamic Reaction Cell-Mass Spectrometer (ICP-DRC-MS) (Ejnik et al. 2000). A detailed methodology was previously reported (McDiarmid et al. 2006). Briefly, urine samples were prepared for quantification by diluting the urine by a factor of four with deionized water. An internal standard at 500 pg ^{233}U mL^{-1} (CRM 111A, New Brunswick Laboratory, Argonne, IL) was used to correct for instrument drift and sample matrix effects. All solutions were prepared in 2% Optima grade nitric acid (Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham, MA 02454). Quantification of U was achieved using an Elan 6100 DRC (Perkin-Elmer, 940 Winter Street, Waltham, MA 02451) ICP-MS. Each sample was measured in triplicate, and each measurement contained five replicates of 49 sweeps. The ratio of ^{235}U ^{238}U^{-1} was calculated from the appropriate measured mass/charge signals. Urine U concentrations were corrected on the basis of urine creatinine concentrations to account for urine dilution to obtain μg U g creatinine^{-1} (Karpas et al. 1998; McDiarmid et al. 2000).

Cumulative uranium exposure

For the cumulative exposure measure, we constructed an integrated metric that is a function of the participant's urine U value at each of the surveillance visits in which they participated and the time interval between each of the urine U concentration measurements. An area under the curve (AUC) calculation was conducted by modifying the method reported by Chia et al. (1997) who utilized a linear formula for determining cumulative lead exposure. Modification of this formula resulted in an analogous integrated metric of U burden, a cumulative U index (CumU), based on urine U concentrations (UUr):

$$\text{CumU} = \sum_{i=0}^{nj-1} [(t_{i+1} - t_i)\,(\text{UUr}_{i+1}) + 0.5(t_{i+1} - t_i)$$

$$(\text{UUr}_i - \text{UUr}_{i+1})],$$

where $\text{UUr}_i - \text{UUr}_{i+1}$ are the ith and $(i + 1)^{th}$ UUr readings and $(t_{i+1} - t_i)$ is the period between readings.

Clinical assessment

Thirty-four veterans participated in a 3-day, in-patient hospital clinical assessment that included a detailed medical history, an extensive exposure history, a thorough physical examination, and laboratory studies. The laboratory battery included hematological and blood clinical chemistry measures, neuroendocrine and genotoxicological parameters, and semen quality measures. Spot and 24-hour urine samples were obtained for measurement of clinical chemistry parameters related to

renal function and for urine U determinations. Participants underwent a battery of neurocognitive tests as well.

Hematological and renal toxicity measures

Hematological parameters, serum and urine creatinine, Ca and PO$_4$, and serum uric acid measures were evaluated by the VA clinical laboratory using standard methodologies. Aliquots of urine were taken and immediately neutralized using 0.5 N NaOH for β_2-microglobulin analysis. They were analyzed by latex-enhanced nephelometry by Quest Diagnostics Incorporated (San Juan Capistrano, CA). Total protein was measured by Baltimore VA Clinical Laboratory using the M-TP microprotein assay from Beckman Coulter (Beckman Coulter, Inc., 4300 N. Harbor Boulevard, P.O. Box 3100, Fullerton, CA 92834-3100) that uses Pyrogallol Red for detection (Watanabe et al. 1986). The 24-h urine samples were collected in multiple containers per study participant and each container was analyzed separately for total protein. Due to fluctuations in protein excretion throughout the day, some of the individual samples contained levels of protein below the limit of detection. In these instances, mean 24-h total protein values were calculated using one-half the detection limit (6 mg dL^{-1}) divided by the sample creatinine concentration for samples with non-detectable protein concentrations. Markers of nephrotoxicity were measured by the Department of Nephrology-Hypertension, University of Antwerp (Edegem-Antwerp, Belgium). These measures included markers of glomerular or tubular dysfunction [urine retinol binding protein (RBP), and microalbumin (mAlb)], and cytotoxicity [urine intestinal alkaline phosphatase (IAP) and N-acetyl-D-glucosaminidase (NAG)]. A detailed description of the methods used to test the markers of nephrotoxicity have been previously reported (McDiarmid et al. 2006).

Neurocognitive/psychiatric assessment

Four neurocognitive and psychiatric impairment indices were constructed from a battery of neurocognitive tests described previously (McDiarmid et al. 2004). Three indices were derived from selected measures of the Automated Neuropsychological Assessment Metrics (ANAM) test system; one index was derived from a battery of six traditional neurocognitive measures. The Accuracy Index (percent correct), Speed Index (median response time for correct responses) and Throughput Index (a computed score combining speed and accuracy) were derived from the ANAM test system. The Neuropsychological Index (NP) was constructed from the six traditional test measures. Norms for the ANAM indices were established from tests given to U.S. Marine Corps recruits, mean age 19 y (range 18–28), mean education 12 y (range 12–17 y) (Reeves et al. 1995). The N varied by test (range 84–196). The impairment index for the

traditional tests was based on published norms (Delis et al. 2000; Heaton et al. 2004; The Psychological Corporation 1997). These indices represent the proportion of scores falling one standard deviation below the mean. Hence, a higher proportional value indicates poorer performance.

In addition to the above index scores used in our previously published work, an additional index based on ANAM test performance was computed. This additional index is called the Index of Cognitive Efficiency (ICE). The ICE was derived by weighting throughput scores from the individual ANAM tests and then combining them into a single score reflecting overall performance on the test battery. The weighting was done so that each score contributed equally to the overall index. For this index, higher scores are indicative of better test performance.

Reproductive health measures

Neuroendocrine parameters. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, thyroid stimulating hormone (TSH), free thyroxine, and total testosterone were analyzed at the Baltimore VA Clinical Laboratory by enzyme immunoassay using a Beckman Coulter Access 2 Analyzer.

Semen characteristics. Semen was collected from study participants who agreed to participate in the semen analysis portion of the 2005 assessment ($n = 25$), including two men not evaluated in prior visits. Data were obtained from only 24 of these men, as one participant was azospermic for reasons unrelated to Gulf War service. Analysis of semen volume, sperm concentration, total sperm count, and functional parameters of sperm motility was conducted as previously described (McDiarmid et al. 2004); however, to accommodate a new semen quality measurement Sperm Chromatin Stability Assay (SCSA), the initial processing of semen was altered for this visit (2005) to limit elevated temperature exposure of the semen until after an aliquot was removed for the SCSA. Semen samples were held at room temperature for the first 45 min after receipt from each participant to allow for spontaneous liquefaction. At the end of this time, aliquots were taken for SCSA. Starting at 1 h after sample receipt, all specimens were incubated for 30 min at 37°C prior to additional analyses. Samples that were not sufficiently liquefied for analysis at the end of the room temperature incubation ($n = 12$) were treated with enzyme followed by bovine serum albumin (BSA) (McDiarmid et al. 2004) during this 37°C incubation. In some cases ($n = 2$), BSA was added after 15 min of enzyme treatment because liquefaction was sufficient. In the remaining cases, BSA was added at the end of the 30 min incubation at 37°C. Only one sample was not liquefied at the end of 30 min of enzyme treatment, for which liquefaction was achieved by limited syringe treatment.

Sperm chromatin stability assay (SCSA). Air displacement pipettors were used to aliquot for SCSA. For semen samples that were not completely liquefied, the pipettor tip was widened to permit aspiration. To determine the appropriate volume and number of aliquots, sperm concentration was estimated by microscopic examination of a cover-slipped wet mount of semen on a conventional slide. Two or three aliquots of semen (200 or 250 mL each), containing a minimum of 100,000 sperm per aliquot, were transferred to cryogenic storage tubes and stored on site in a liquid nitrogen dry shipper. At the end of each week, semen aliquots were sent to SCSA Diagnostics (Brookings, SD) in the same container used for storage. Duplicate analyses were performed on the semen from each participant by previously published methods (Evenson et al. 1980, 2002) and all samples were analyzed in the same assay. SCSA results were reported as DNA Fragmentation Index (DFI) and as the incidence of sperm with High Density Staining (HDS).

Genotoxicity measures

Chromosomal aberration (CA). Peripheral blood lymphocytes were cultured for the examination of background frequencies of CAs using standard methods (Evans and O'Riordan 1975; Swierenga et al. 1991). Briefly, cells were cultured for 48 h. After staining, 50 cells were examined from each sample for CAs.

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutation assay. For HPRT mutation analysis, venous blood samples (30 mL) were obtained in heparinized vacuum tubes in Baltimore and sent at ambient temperature by overnight airmail to the BioMosaics laboratory in Burlington, VT. On receipt, blood samples were centrifuged and the mononuclear cell fractions (containing the lymphocytes) were separated, washed, counted, and cryopreserved in liquid nitrogen. Samples were analyzed as described previously (McDiarmid et al. 2004) within approximately 2 wk. The ratio of cloning efficiency (CE) in the presence of 6-thioguanine to the CE in the absence of 6-thioguanine selection defined the mutation frequency (MF).

Fluorescent in-situ hybridization (FISH) assay. FISH analysis of metaphase cells for the detection of low-level chromosome abnormalities involving targeted chromosomes was conducted by the University of Maryland School of Medicine Cytogenetics Laboratory (Baltimore, MD) using the method described by Zhang et al. (1998, 1999). In this study, metaphase cells from peripheral

blood specimens were prepared using standard cytogenetic procedures. FISH was performed using a D5S721(5p15.2)/ EGR1(5q31) probe set, an ELN(7q11.23)/D7S486(7q31) probe set, an MLL probe set at 11q23, and a D13S319 probe at 13q14 to detect abnormalities involving chromosomes 5, 7, 11, and 13, respectively. These probe sets, which can detect both numerical and structural abnormalities of the targeted chromosomes, were chosen for this assay based on their use for surveillance of other chemically exposed groups, such as for benzene-exposed workers (Zhang et al. 1998) or for the clinical management of newly acquired hematological disorders (Dewald et al. 2005) or second malignancies incurred from cytotoxic therapy (Pedersen-Bjergaard and Rowley 1994). One hundred metaphase cells from each subject were examined with each probe set. These probes were obtained from Vysis (Downers Grove, IL), and were validated in accordance with the recommendations of the American College of Medical Genetics. The hybridization procedures were performed following the manufacturer's instructions. The images were acquired using an Olympus Provis (Olympus America, Inc., Two Corporate Center Drive, Melville, NY 11747) fluorescence microscope and an Applied Imaging system and its software (Applied Imaging Corporation, 120 Baytech Drive, San Jose, CA 95134-2302).

Statistical data analysis

Urine uranium as a binary variable. Two exposure groups, high ($n = 10$) vs. low ($n = 24$) were determined based on each individual participant's current (2005) urine U results or their cumulative urine U exposure metric. As in previous years (McDiarmid 2001, 2004, 2006), high exposure was defined as current urine U concentrations greater than or equal to 0.10 μg U g creatinine^{-1}, a value between 0.034 [the 95th percentile reported for creatinine-adjusted urine U concentration in non-exposed populations in the U.S. (NHANES 2003)] and 0.35 μg U L^{-1} reported as a urine U upper limit that can occur naturally in areas with elevated U in water and food (ICRP 1974). Data were also analyzed using the cumulative U exposure metric using a cut point of 10 μg U g creatinine^{-1} years. This cut point was chosen based on the distribution of the data, which showed a natural break at this cumulative dose. This cut point gave the same "n" value for the high U group as the 0.1 μg U g creatinine^{-1} current U cut point, but the individual participants in each group differed by one participant.

Tests of differences in high vs. low urine U groups. For each outcome, differences in outcome measures of distribution location (e.g., median) between high and low urine U groups were examined using the Mann-Whitney U test (Wilcoxon rank sum test), which assumes equally-shaped distributions (Woolson 1987), although they can differ in their means. SPSS 12.0 (Statistical Products and Service Solutions 2003) was used for these tests. To test the assumption of equally-shaped distributions we used the two-sample version of the Kolmorogov-Smirnov test to compare the shapes of the distributions. In none of the comparisons did we detect significantly unequal distribution shapes. Hence, the Mann-Whitney exact test was used for all comparisons of high vs. low U groups. Mean differences were considered statistically significant when $p < 0.05$. However, attention was paid to differences with p values of 0.2 or less because this is a surveillance program and it is important to look for sentinels of effect and trends in data from year to year.

HPRT MF means were adjusted for cloning efficiency (CE) and age. Correction factors for CE and age were derived by combining the data from all three time points (2001, 2003, 2005) and regressing the natural log of mutation frequency (lnMF) on the age at that time point, CE and time point (a categorical variable). Subject identification (ID) was included as a categorical random effect to take into account the correlation between multiple observations on the same person (some of which is due to exposure).

The coefficients for CE and age were then used to adjust the lnMF values to the average CE (0.28) and age (39). The coefficients were very similar to those previously obtained for healthy individuals (Finette et al. 1994), which is reassuring. The adjusted values were computed as follows:

$$lnMF\_a1 = lnMF - 1.648(0.28 - CE)$$

$$lnMF\_a2 = lnMF - 1.620(0.28 - CE)$$
$$+ 0.0116(39 - age).$$

Test of association between natural logarithm of continuous urine U [ln(urine U)] and health outcome measures. Because of the possible presence of outliers, robust regression, which down-weights outliers, was used to study the association between the neurocognitive indices and the ln(continuous current urinary U). Because any non-linearity of continuous current urinary U in the linear regression model for the outcome measures could produce apparent outliers, we also studied the association between urinary U and FISH and HPRT using fractional polynomial transformations of ln(continuous current urinary U). We tested the relative contributions of covariates to the fit by the process of backward elimination to determine whether any covariate would become significant, or by testing the linear association between each

potential covariate and each outcome. Robust regression and fractional polynomial transformations were done using STATA 2003 (StataCorp 2003).

RESULTS

Thirty-four members of a dynamic cohort of 74 veterans involved in "friendly-fire" incidents during the 1991 Gulf War elected to participate in the health surveillance visit held at the BVAMC in 2005. Demographic characteristics of this sub-cohort, presented in Table 1, are similar to those of the full cohort with respect to age and race.

Biological monitoring for uranium

The distribution of the 24-h total urine U analysis for the 2005 sub-cohort are presented in Fig. 1. Uranium concentrations for this group ranged from 0.002 μg U g creatinine^{-1} to 44.1 μg U g creatinine^{-1}. Values at or above a cut point of 0.1 μg U g creatinine^{-1} were from participants with known retained shrapnel fragments and U isotopic signatures indicative of DU.

A comparison of the current and cumulative U exposure metrics determined for this cohort of Gulf War veterans indicated that they were highly correlated, with an R^2 value of 0.827 ($p = 0.000$). Fig. 2 displays the distribution of the current U exposure metric in μg U g creatinine^{-1} ranked from low to high, paired with the same participant's cumulative U exposure metric in μg U g creatinine^{-1} years. The participants falling into the high U group based on their cumulative U exposure metric being $\geq$10 μg U g creatinine^{-1} years are shown in the top group of circled points. The participants in the high U group based on their current urine U concentration being $\geq$0.1 μg U g creatinine^{-1} are shown in the lower group of circled points. Although the number of participants in each of these groups is the same, the arrows indicate two individuals who changed groups based on these two exposure metrics differing definitions.

Table 1. Demographic characteristics of the 2005 participants[a] compared to all participants.[b]

	2005 cohort n (%)	All participants n (%)
RACE		
African American	9 (26.4)	24 (32.4)
Asian American	1 (2.9)	1 (1.4)
Caucasian	21 (61.8)	40 (54.1)
Hispanic	2 (5.9)	8 (10.1)
Native American	1 (2.9)	1 (1.4)
AGE[c]	40.8 ± 5.7 y	39.2 ± 5.0 y

[a] $n = 34$.
[b] All participants enrolled in DU Follow-up Program ($n = 74$).
[c] Mean age at time of 2005 evaluation (±SE, standard deviation).

Clinical findings

There were no statistically significant differences observed between the high and low urine U groups in the hematology, serum chemistry and neuroendocrine parameters (data not shown).

Renal parameters

Results examining renal function from the 2005 sub-cohort are displayed in Table 2 both as a function of the current U exposure metric (the 2005 urine U value in μg U g creatinine^{-1}) and the cumulative U exposure metric (in μg U g creatinine^{-1} years). There are no statistically significant differences between the low and high U groups for any of the parameters measured regardless of which U metric was used for the comparison, except for serum uric acid which was lower in the high group with a p value of 0.03 for the cumulative U exposure comparisons. The difference between groups is small, is still within the normal range, and is not of clinical concern. In particular, other markers of proximal tubular function, such as concentrations of glucose and the low molecular weight proteins measured in urine (β_2 microglobulin and RBP) were not statistically different between the high vs. low U groups using either U exposure metric.

Neurocognitive evaluation

Responses assessed by the neurocognitive indices shown in Table 3 were within normal ranges regardless of whether respondents were in the high or low U group. There were no significant associations between any of the adjustment variables (age, IQ, and depression) and index outcomes, so these variables were omitted from the robust regressions. Consistent with previous years, there were no statistically significant differences in any of the neurocognitive indices between the high and low U groups, as defined by current U exposure. There were also no differences observed when using the cumulative U exposure metric. However, attention is given to probability levels of 0.2 or less as potential effect indicators, and therefore it is noted that those in the high current U exposure group had a lower mean ICE than the low current U group ($p = 0.158$), indicative of poorer performance. This difference was not seen using the cumulative exposure metric. Fractional polynomial analysis of the NP index, the ANAM indices, and the ICE did not show evidence of a significant relationship between DU exposure and these neurocognitive outcome measures (data not shown).

Semen characteristics

No statistically significant differences between urine U groups were noted in the World Health Organization
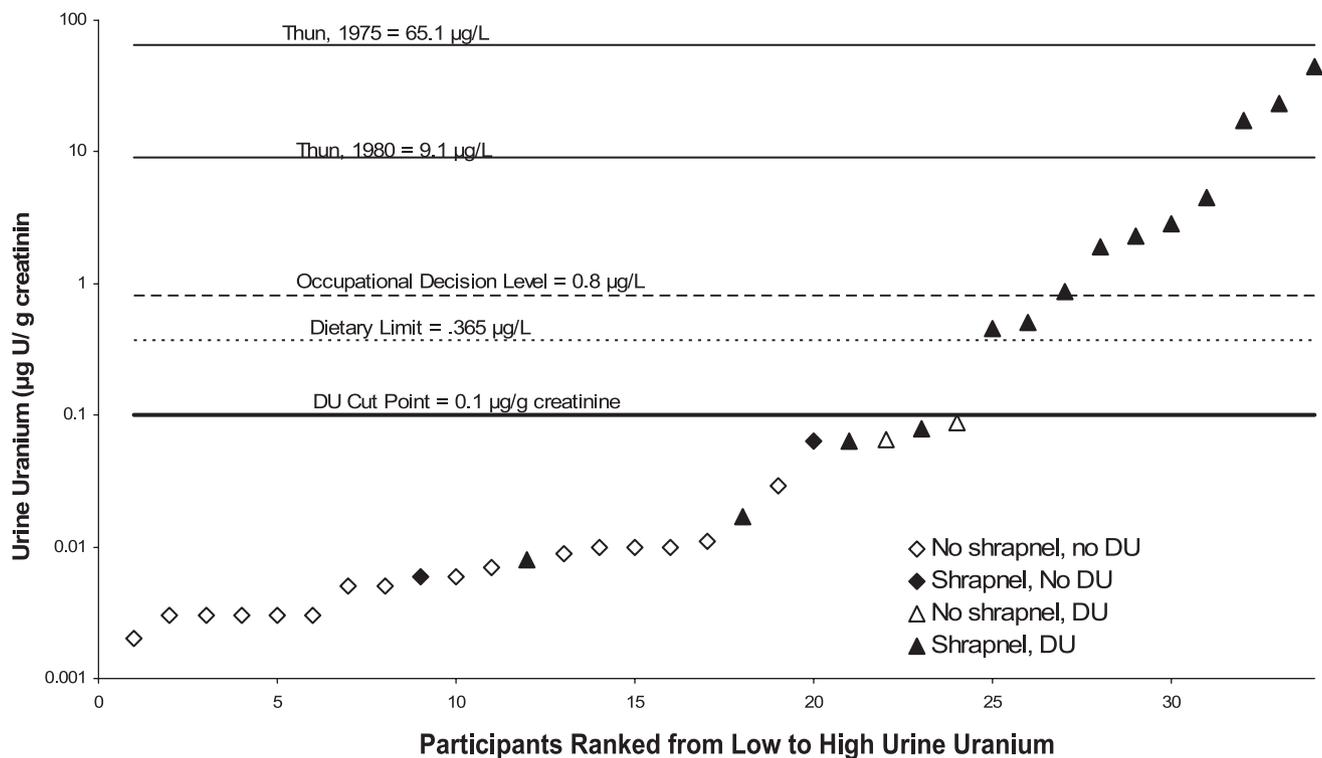
Fig. 1. Distribution of the 24-h total urine uranium analysis for DU-exposed veterans of Gulf War I from the 2005 evaluation. The top two solid lines (65.1 μg L^{-1} and 9.1 μg L^{-1}) represent the mean total urine uranium found in a sub-cohort of uranium fabrication workers in 1975 and 1980, as reported in a study by Thun et al. (1985). The dashed line (0.8 μg L^{-1}) depicts an occupational exposure decision level used at the Department of Energy's Fernald Environmental Management Project (McDiarmid et al. 2000; Fernald Environmental Management Project 1997) as a trigger for investigating work areas for sources of elevated uranium exposure. The dotted line (0.365 μg L^{-1}) is an upper limit for the dietary contribution of uranium in urine for a general population from drinking water (ICRP 1974; McDiarmid et al. 2000). This value was calculated by dividing the upper limit for 24-h uranium excretion for "reference man" by 1.4 L/24 h. It is assumed that corrections per gram creatinine and per liter urine are generally equal for "reference man" and for this group of veterans with normal renal function. The bold solid line (0.1 μg U g creatinine^{-1}) indicates the cut point established by the DU Follow-up Program to identify low vs. high urine uranium concentrations (McDiarmid et al. 2000).

(WHO 1987) criteria semen characteristics using either the current U or cumulative U exposure metrics (Table 4). The mean values for the Percent Progressive Sperm and the Percent Rapid Progressive Sperm, however, were lower in the high U group compared to the low U group using the current exposure metrics ($p = 0.15$ and 0.12, respectively). This was not observed in previous years, and may be due to the changes in sample handling required during this visit for measurement of sperm chromatin stability (see detail in Methods).

Slightly higher mean DFI values were observed in the high U groups established by either of the U exposure metrics (with p values of 0.15 and 0.19 for the current and cumulative U metrics, respectively) (Table 5). The HDS parameter, however, was lower in each of the high U groups compared to their respective low U group means. Since a higher HDS value is indicative of immature chromatin processing the lower mean HDS

value observed in the high U group does not represent an adverse effect.

Genotoxicity

Chromosomal aberrations. No differences between the low and high U groups using either of the U exposure metrics was observed using the Mann-Whitney exact test for baseline chromosomal aberrations in peripheral blood lymphocytes. The same was true when provocative treatment of cells in culture with bleomycin or mitomycin was performed (data not shown).

Hypoxanthine-guanine phosphoribosyl transferase (HPRT) mutation assay. The Mann Whitney Exact Test was used to examine differences in MF by low current urine U levels (<0.1 μg U g creatinine^{-1}) vs. high (≥ 0.1 μg U g creatinine^{-1}) levels, and between low
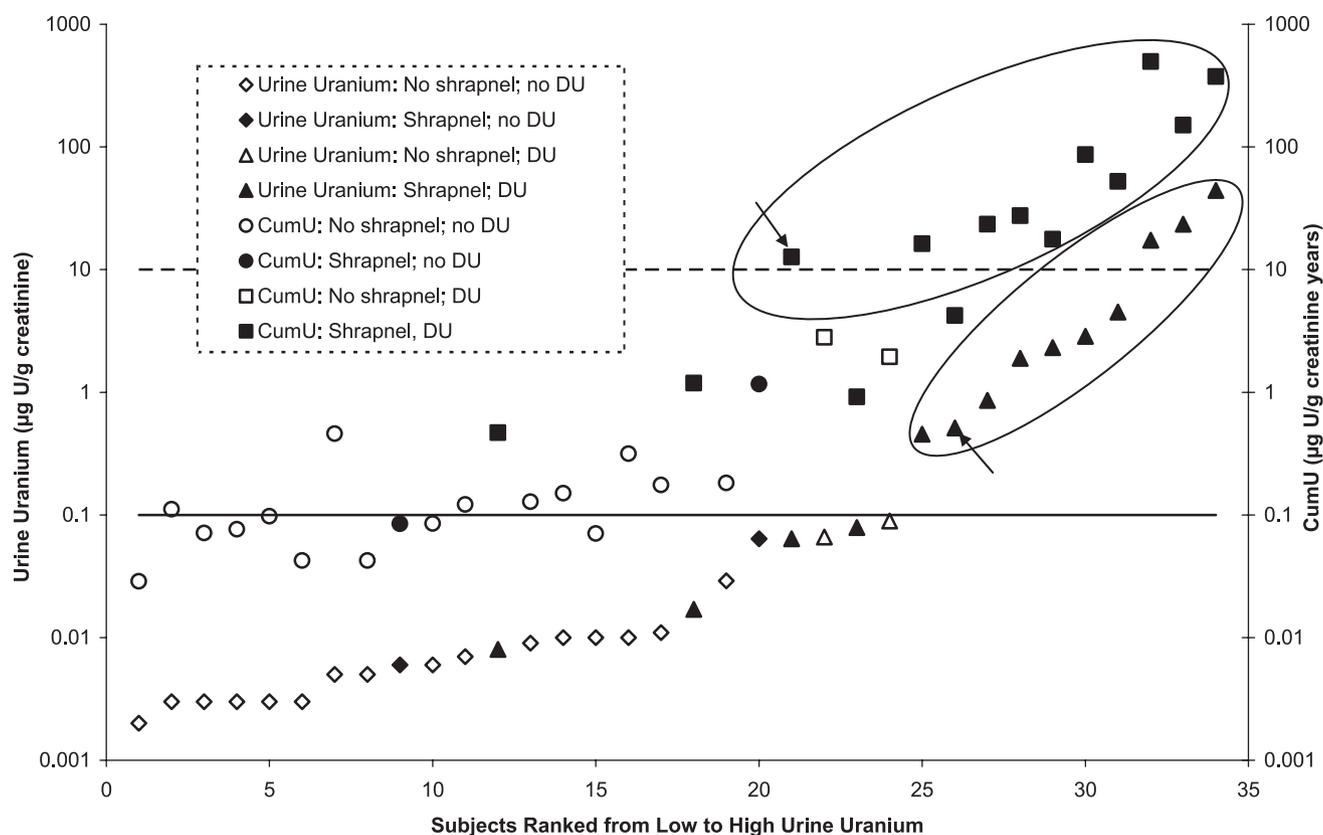
Fig. 2. Distribution of the current and cumulative urine U values for the DU Follow-Up Program 2005 Cohort. The current urine U value for each participant is expressed as μg U g creatinine^{-1} and participants are ranked from lowest to highest along the x-axis based on their current urine U concentration (depicted by diamonds and triangles). The cumulative urine U exposure metric (depicted by circles and squares) expressed as μg U g creatinine^{-1} years was calculated for each participant and is directly above the participant's corresponding current urine U values. The "high" U groups are defined based on either the participant's current urine U (urine U above 0.1 μg U g creatinine^{-1}) or their cumulative urine U exposure (cumulative urine U above 10 μg U g creatinine^{-1} years) and are grouped by the two large circles.

cumulative urine U measures ($<$10.0 μg U g creatinine^{-1} years) vs. high ($\geq$10.0 μg U g creatinine^{-1} years) (Table 6). Although mean MF adjusted for age and cloning efficiency was higher in the high current U group (21.64 ± 7.39 vs. 11.94 ± 1.73), the difference between the two groups was not significant ($p = 0.17$). However, that probability is within the level that commands our interest in this surveillance program. The difference in mean MFs for low vs. high cumulative urine U exposure were also approximately 2-fold and not statistically significant ($p = 0.28$).

An analysis of the association between lnMF and ln(urine U) was done using fractional polynomial transformations of the log of the continuous urine U, both current and cumulative. The association with current urine U had a p value of 0.016. The pattern was similar to patterns reported in 2001 and 2003. We did similar analyses in which all covariates (blood cloning efficiency, current smoking, recent x rays, age) were entered

and with backward stepping were removed one at a time for the current U exposure variable. None was significant. No association was seen between lnHPRT MF and ln(cumulative urine U) in the fractional polynomial test.

Fluorescent in-situ hybridization (FISH). Mann-Whitney tests revealed that the total number of chromosomal abnormalities as measured by FISH were higher among those in the high cumulative U exposure group (mean of 0.9) vs. those in the low U group (mean of 0.5) with a p value of 0.08 (Table 7). Although those in the high cumulative exposure group were, on average, 1.7 years older (not a statistically significant difference), this age difference was too small to clinically account for the group difference in total mutations. No significant associations were found between total abnormalities and the natural log of continuously measured U exposure, either the current or cumulative, using the fractional polynomial statistical technique. Of the potential confounders,

Table 2. Renal parameter comparison of low vs. high U groups determined by current and cumulative urine U in the 2005 cohort.

Laboratory test (normal range)	Low uranium group[a] (mean ± SE)	High uranium group[b] (mean ± SE)	Mann-Whitney p	Low cumU group[c] (mean ± SE)	High cumU group[d] (mean ± SE)	Mann-Whitney p
Urine creatinine (1.5–2.6 g/24h)	2.03 ± 0.09	1.91 ± 0.29	0.24	2.06 ± 0.09	1.85 ± 0.29	0.08
Creatinine clearance (97–137 mL/min)	130.21 ± 7.14	135.21 ± 12.13	0.70	130.87 ± 7.26	133.58 ± 11.79	0.90
Urine calcium (100–300 mg/24h)	158.90 ± 21.83	199.04 ± 43.75	0.59	158.33 ± 21.65	200.28 ± 44.07	0.56
Urine PO_4 (0.4–1.3 g/24h)	3.04 ± 2.01	1.11 ± 0.34	0.29	3.06 ± 2.01	1.07 ± 0.33	0.27
Urine glucose (0–0.5 g/24 h)	34.45 ± 33.37	0.26 ± 0.14	0.59	34.45 ± 33.37	0.26 ± 0.14	0.59
Urine β_2 microglobulin (0–160 μg/g creatinine)	63.25 ± 6.75	71.05 ± 11.56	0.22	66.34 ± 6.80	64.25 ± 11.54	0.83
Urine intestinal alkaline phosphatase (IAP) (<2 U/g creatinine)	0.34 ± 0.11	0.46 ± 0.15	0.67	0.33 ± 0.11	0.48 ± 0.14	0.52
Urine N-acetyl-β-glucosaminidase (NAG) (<5 U/g creatinine)	1.51 ± 0.27	1.24 ± 0.15	0.96	1.50 ± 0.27	1.27 ± 0.15	0.90
Urine total protein (1–150 mg/24 h)	122.75 ± 26.80	89.30 ± 17.79	0.50	124.79 ± 26.73	84.40 ± 17.34	0.25
Urine micro-albumin (<25 mg/g cre)	15.17 ± 9.88	3.55 ± 0.62	0.40	15.20 ± 9.88	3.47 ± 0.61	0.32
Urine retinol binding protein (<610μg/g cre)	64.73 ± 6.59	71.45 ± 11.49	0.25	66.46 ± 6.80	67.65 ± 10.98	0.56
Glucose (70–105 mg/dL)	107.00 ± 6.92	109.20 ± 7.41	0.67	107.75 ± 6.89	107.40 ± 7.60	1.00
Serum creatinine (0–1.4 mg/dL)	1.06 ± 0.05	0.99 ± 0.03	0.72	1.06 ± 0.04	0.98 ± 0.04	0.56
Serum calcium (8.4–10.2 mg/dL)	9.32 ± 0.06	9.46 ± 0.09	0.24	9.33 ± 0.06	9.42 ± 0.09	0.56
Serum PO_4 (2.7–4.5 mg/dL)	3.52 ± 0.15	3.47 ± 0.15	0.70	3.53 ± 0.16	3.43 ± 0.12	0.64
Serum uric acid (3.4–7 mg/dL)	6.14 ± 0.27	5.35 ± 0.45	0.10	6.19 ± 0.26	5.22 ± 0.46	0.03

[a] <0.10 μg g creatinine^{-1} ($n = 24$).
[b] ≥0.10 μg g creatinine^{-1} ($n = 10$).
[c] CumU <10.0 μg U g creatinine^{-1} y ($n = 24$).
[d] CumU ≥10.0 μg U g creatinine^{-1} y ($n = 10$).

Table 3. Comparison of measures of neurocognitive function in low vs. high U groups determined by current and cumulative urine U in the 2005 cohort.

Laboratory test	Low uranium group[a] (mean ± SE)	High uranium group[b] (mean ± SE)	Mann-Whitney p	Low CumU group[c] (mean ± SE)	High CumU group[d] (mean ± SE)	Mann-Whitney p
NP index	0.08 ± 0.027*	0.09 ± 0.04	0.89	0.08 ± 0.027*	0.09 ± 0.04	0.89
ANAM accuracy index	0.28 ± 0.05	0.36 ± 0.12	0.84	0.28 ± 0.05	0.36 ± 0.12	0.84
ANAM speed index	0.22 ± 0.05	0.10 ± 0.04	0.24	0.22 ± 0.05	0.10 ± 0.04	0.24
ANAM throughput index	0.24 ± 0.05	0.36 ± 0.12	0.62	0.26 ± 0.05	0.33 ± 0.12	0.90
Index of cognitive efficiency—Revised 2005	366.34 ± 14.272*	316.53 ± 32.61	0.16	359.62 ± 14.739*	331.97 ± 33.47	0.55

[a] <0.10 μg g creatinine^{-1} ($n = 24$ except * $n = 23$).
[b] ≥0.10 μg g creatinine^{-1} ($n = 10$).
[c] CumU <10.0 μg U g creatinine^{-1} y ($n = 24$ except * $n = 23$).
[d] CumU ≥10.0 μg U g creatinine^{-1} y ($n = 10$).

only age had any association with total mutations, so it was retained in the fractional polynomial models. No significant associations were seen in any of the statistical tests between the current measure of U exposure and total chromosomal abnormalities measured by FISH.

It is difficult to interpret a result from so few cases, but this discrepancy between the Mann Whitney and fractional polynomial results for the cumulative exposure and total number of chromosomal abnormalities could be described as an association that is not linear or curvilinear, but rather a general grouping of those with higher numbers of abnormalities (i.e., a threshold) in the high cumulative exposure group. This observation provides suggestive evidence of an association and in this surveillance program is sufficient incentive to continue to monitor the association as the cumulative U dosage increases.

DISCUSSION

The principal finding from this DU Follow-up Program medical surveillance visit continues to be that urine U excretion is significantly higher in veterans with confirmed retention of metal fragments in soft tissue compared to either those DU-exposed veterans without fragments (Hooper et al. 1999; McDiarmid et al. 2000, 2001; Squibb and McDiarmid 2006) or a comparison population of Gulf War deployed, but not DU-exposed veterans (McDiarmid et al. 2000). Multiple smaller fragments remain in some veterans despite surgeries because the fragments are not

Table 4. Comparison of sperm characteristics in low vs. high U groups determined by current and cumulative urine U in the 2005 cohort.

Laboratory test (normal range)	Low uranium group[a] (mean ± SE)	High uranium group[b] (mean ± SE)	Mann-Whitney Test (p)	Low cumU group[c] (mean ± SE)	High cumU group[d] (mean ± SE)	Mann-Whitney Test (p)
Days abstinence (2–5)	4.89 ± 0.86	4.25 ± 0.75	0.98	4.74 ± 0.82	4.57 ± 0.78	0.61
Semen volume (2–5 mL)	3.40 ± 0.64	4.08 ± 0.99	0.26	3.38 ± 0.60	4.22 ± 1.14	0.33
Sperm concentration (>20 million/mL)	159.66 ± 39.19	168.75 ± 66.77	0.74	156.64 ± 36.93	177.37 ± 76.45	0.71
Total sperm count (>40 million)	466.96 ± 110.27	535.78 ± 185.40	0.98	459.25 ± 103.87	564.33 ± 211.52	0.95
Percent motile sperm (>50%)	60.38 ± 3.42	49.75 ± 7.44	0.24	59.76 ± 3.27	49.71 ± 8.59	0.32
Total progressive sperm (>20 million)	138.34 ± 44.71	149.70 ± 72.11	0.61	133.17 ± 42.32	163.88 ± 81.64	0.76
Percent progressive sperm (>50%)	28.94 ± 3.12	20.38 ± 4.81	0.15	28.12 ± 3.04	21.14 ± 5.48	0.29
Total rapid progressive sperm (>10 million)	80.70 ± 26.72	95.33 ± 50.50	0.53	77.14 ± 25.35	106.07 ± 56.98	0.66
Percent rapid progressive sperm (>25%)	18.44 ± 2.66	11.63 ± 3.54	0.12	17.71 ± 2.61	12.43 ± 3.98	0.23

[a] <0.10 μg U g creatinine^{-1} ($n = 16$).
[b] ≥0.10 μg U g creatinine^{-1} ($n = 8$).
[c] CumU <10.0 μg U g creatinine^{-1} y ($n = 17$).
[d] CumU ≥10.0 μg U g creatinine^{-1} y ($n = 7$).

Table 5. Comparison of sperm chromatin stability measures in low vs. high U groups determined by current and cumulative urine U in the 2005 cohort.

Semen parameter	Low uranium group[a] (mean ± SE)	High uranium group[b] (mean ± SE)	Mann-Whitney p	Low cumU group[c] (mean ± SE)	High cumU group[d] (mean ± SE)	Mann-Whitney p
DNA fragmentation index	18.28 ± 2.01	18.95 ± 7.66	0.15	18.06 ± 1.90	19.59 ± 8.81	0.19
High density staining	10.34 ± 1.21	7.88 ± 2.32	0.15	10.00 ± 1.19	8.36 ± 2.62	0.38

[a] <0.10 μg U gcreatinine^{-1} ($n = 16$).
[b] ≥0.10 μg U g creatinine^{-1} ($n = 8$).
[c] CumU <10.0 μg U g creatinine^{-1} y ($n = 17$).
[d] CumU ≥10.0 μg U g creatinine^{-1} y ($n = 7$).

Table 6. HPRT mutation frequency comparisons of low vs. high U groups determined by current and cumulative urine U in the 2005 cohort.

	Current uranium exposure 2005			Cumulative uranium exposure through 2005		
	Low uranium group[a] (mean ± SE)	High uranium group[b] (mean ± SE)	Mann-Whitney p	Low cumU group[c] (mean ± SE)	High cumU group[d] (mean ± SE)	Mann-Whitney p
Mutation frequency (MF)	12.12 ± 1.84	22.77 ± 7.52	0.27	12.30 ± 1.84	22.33 ± 7.59	0.40
MF adjusted for cloning efficiency	12.14 ± 1.73	22.32 ± 7.54	0.22	12.23 ± 1.73	22.09 ± 7.57	0.29
MF adjusted for cloning efficiency and age	11.94 ± 1.73	21.64 ± 7.39	0.17	12.09 ± 1.72	21.28 ± 7.44	0.28

[a] <0.10 μg U g creatinine^{-1} ($n = 24$).
[b] ≥0.10 μg U g creatinine^{-1} ($n = 10$).
[c] <10.0 μg U g creatinine^{-1} y ($n = 24$).
[d] ≥10.0 μg U g creatinine^{-1} y ($n = 24$).

easily accessible or due to risk of excessive surgical morbidity associated with their removal.

From the onset of this surveillance program, the U exposure metric employed to assess relationships between DU exposure and health effects has been the veteran's current 24-h urine U concentration measured in μg U g creatinine^{-1}. While we have previously demonstrated that a participant's urine U value is relatively constant from measure to measure over time (McDiarmid et al. 2004), this cross-sectional metric does not take duration of exposure into account, which more accurately reflects a cumulative burden and thus an integrated

Table 7. Comparison of abnormal metaphases detected by FISH analysis of chromosomes 5, 7, 11, and 13 in low vs. high U groups determined by current and cumulative urine U in the 2005 cohort.

Mean abormal metaphases	Current uranium exposure			Cumulative uranium exposure		
Chromosome (100 metaphases counted per subject per chromosome)	Low uranium group[a] (mean ± SE)	High uranium group[b] (mean ± SE)	Mann-Whitney p	Low cumU group[c] (mean ± SE)	High cumU group[d] (mean ± SE)	Mann-Whitney p
Chromosome 5	0.08 ± 0.06	0.10 ± 0.10	0.96	0.04 ± 0.04	0.20 ± 0.13	0.14
Chromosome 7	0.13 ± 0.07	0.30 ± 0.15	0.45	0.13 ± 0.07	0.30 ± 0.15	0.23
Chromosome 11	0.17 ± 0.08	0.10 ± 0.10	0.78	0.17 ± 0.08	0.10 ± 0.10	0.62
Chromosome 13	0.17 ± 0.08	0.30 ± 0.15	0.56	0.17 ± 0.08	0.30 ± 0.15	0.39
Summary findings						
Proportion of subjects with any mutation	0.42 ± 0.10	0.70 ± 0.15	0.21	0.38 ± 0.10	0.80 ± 0.13	0.06
Mean number of total mutations per subject	0.54 ± 0.16	0.80 ± 0.20	0.24	0.50 ± 0.16	0.90 ± 0.18	0.08

[a] <0.10 μg U g creatinine^{-1} ($n = 24$).
[b] ≥ 0.10 μg U g creatinine^{-1} ($n = 10$).
[c] CumU <10.0 μg U g creatinine^{-1} y ($n = 17$).
[d] CumU ≥ 10.0 μg U g creatinine^{-1} y ($n = 7$).

measure of chronic exposure over the years since exposure began. Measuring exposure to heavy metals in terms of cumulative exposure, however, should be considered. This approach has been used to evaluate human health effects such as the neurobehavioral effects of chronic lead exposure (Lucchini et al. 2000). Jakubowski et al. (2002) have also used integrated indices of exposure to evaluate the dose-effect and dose-response relationships between cadmium exposure (Cd blood concentrations in μg L$^{-1} \times$ years of exposure), and RBP excretion in urine (RBP-U) and beta2-microglobulin concentration in serum (beta2M-S). Bleecker et al. (2003) have also noted that a cumulative dose metric is the traditional measure of chronic toxicity for lead (Pb).

In the sub-cohort of our population that carries retained DU metal fragments in their bodies, exposure is ongoing as evidenced both physically by imaging of metal fragments on x ray and by the continuously elevated urine U biomonitoring results. Thus, in this study, we looked for statistical differences between low vs. high U exposed groups determined by both their current measure of U exposure (as in previous years) and their cumulative U exposure metric. We tested the hypothesis that effects on organ systems known to accumulate U, such as kidney, bone and brain (Pellmar et al. 1999; Squibb et al. 2005), would be more highly associated with the cumulative U exposure metric, while changes in other health outcome measures, such as sperm production, would be more influenced by recent exposure intensity. The benefit of our application of the cumulative U metric was to examine and refine the historic method used to stratify our population into low and high U groupings. Examining the urine U distributions from low to high has yielded some obvious cut points which we have typically used (0.1 μg U g creatinine^{-1}) to separate the population. However, there have been some few individuals who have hovered on the border of that cut point and, on occasion, moved from one side to the other. The cumulative U exposure metric was employed to obtain a more integrated accounting of the U burden in hopes of clarifying the true classification of those borderline cohort members. However, there were only two changes in group membership when this was done; one participant moved into and one moved out of the high U group. We therefore see little difference in outcomes when the different exposure metrics are used.

Of particular interest in these studies is the impact of the accumulation of U on renal function in veterans with ongoing DU exposure (i.e., those with retained DU metal fragments) (Squibb et al. 2005). The kidney is thought to be the most sensitive target organ of U toxicity (Parkhurst et al. 2005; The Royal Society 2002). Results from past surveillance visits (McDiarmid et al. 2004, 2006) have shown increased urinary excretion of the low molecular weight protein, RBP, in the high U exposed group compared to the low U group, which is consistent with predicted effects of U on proximal tubular cell protein reabsorption. Results from this visit showed only a small, non-significant increase in RBP in the high vs. low group based on the current U exposure, however. Also, when RBP urine concentrations were compared based on the veterans' cumulative U exposure burden, there was again no difference between the groups, despite the hypothesis that this exposure metric would be a better measure of renal U concentrations and thus renal effects. Thus, there is little evidence of an impact of DU exposure on renal function in this cohort.

Similarly, there were no significant differences observed in the measures of neurocognitive function when examined using either the current or cumulative U exposure metric, indicating no progression of small differences observed in earlier years and no better relationship apparent when cumulative U exposure was considered.

The weak genotoxicity results obtained from this surveillance visit are consistent with the low risk of increased cancer predicted by The Royal Society (2001, 2002) for Gulf War exposures and with radiation dose estimates measured for Gulf War veterans with shrapnel which were close to the annual occupational exposure limit of 5 rem y^{-1} (McDiarmid et al. 2000). The 2-fold elevation in HPRT mutations in peripheral blood lymphocytes of the most highly DU-exposed Gulf War veterans contributes to the body of evidence documenting the genotoxic nature of DU. We have observed a positive correlation (although not consistently statistically significant) between lnMF and ln(urine U) levels now over three successive biennial assessments in 2001, 2003, and 2005. In the most recent assessment (2005), the effect was dampened as compared to previous years. In this most recent assessment, we also observed a trend toward increased mean abnormal metaphases, as detected by FISH analysis of chromosomes in peripheral blood, in the high vs. the low current U groups. When a cumulative measure of U exposure was used, the mean number of chromosomal abnormalities per subject (summed across four different chromosome markers) was higher in the high U group as compared to the low U group ($p = 0.07$). The increased significance observed with the cumulative U exposure metric is consistent with what would be expected for this measure of stable aberrations.

Despite increasing evidence for the genotoxicity of DU, the mechanism of action by which DU damages DNA remains to be determined. Recent in vitro studies have begun to elucidate the mechanism of genotoxic action of DU as related to its radioactivity and/or chemical toxicity. In a study of the effects of DU on plasmid DNA, Yazzie et al. (2003) observed increased strand breaks in the PBluescript SK+ plasma when in the presence of ascorbic acid, suggestive of a direct chemical mechanism. These results support earlier work by Miller et al. (2002), which demonstrated that DU induced oxidative DNA damage in the absence of significant alpha particle decay. More recently, the formation of U-DNA adducts have been observed in CHO (Chinese Hamster Ovary) cells under conditions that induce HPRT mutations and DNA strand breaks (Stearns et al. 2005), again suggesting a chemical mechanism for the genotoxicity of DU. This was further supported by molecular analysis of HPRT mutations generated by DU (Coryell

and Stearns 2006). Initial results from mutational analysis of the HPRT mutations in the cohort of Gulf War veterans provides weak but suggestive evidence of a radiation effect (McDiarmid et al. 2006). Thus, continued work is needed to fully understand the mechanism of DU's genotoxicity.

Although no clinically significant U-related health effects were observed in DU-exposed Gulf War veterans participating in this surveillance visit, the HPRT and FISH data from this subcohort provide evidence of a weak genotoxic effect of DU derived from their ongoing exposure. These results indicate a need to continue close monitoring of this cohort for evidence of more pronounced adverse effects as their exposure duration increases.

REFERENCES

Agency for Toxic Substances and Disease Registry. Toxicological profile for uranium (update). Washington, DC: U.S. Department of Health and Human Services, Public Health Service, ATSDR; 1999.

Army Environmental Policy Institute. Health and environmental consequences of depleted uranium use in the US army. Arlington, VA: AEPI; 1995.

Bleecker ML, Ford DP, Lindgren KN, Scheetz K, Tiburzi MJ. Association of chronic and current measures of lead exposure with different components of brainstem auditory evoked potentials. Neurotoxicol 24:625–631; 2003.

Chia SE, Chia HP, Ong CN, Jeyaratnam J. Cumulative blood lead levels and neurobehavioral test performance. Neurotoxicol 18:793–803; 1997.

Coryell VH, Stearns DM. Molecular analysis of hprt mutations generated in Chinese Hamster Ovary EM9 cells by uranyl acetate, by hydrogen peroxide and spontaneously. Mol Carcinog 45:60–72; 2006.

Cross FT, Palmer RF, Busch RH, Filipy RE, Stuart BO. Development of lesions in Syrian Golden hamsters following exposure to radon daughters and uranium ore dust. Health Phys 41:135–153; 1981.

Delis DC, Kramer JH, Kaplan E, Ober BA. California verbal learning test. San Antonio, TX: The Psychological Corporation; 2000.

Dewald GW, Therneau T, Larson D, Lee YK, Fink S, Smoley S, Paternoster S, Adeyinka A, Ketterling R, Van Dyke DL, Fonseca R, Kyle R. Relationsip of patient survival and chromosome anomalies detected in metaphase and or interphase cells at diagnosis of myeloma. Blood 106:3553–3558; 2005.

Eckerman K. Limiting values of radionuclide intake and air concentration and dose conversion factors for inhalation submersion and ingestion. Washington, DC: U.S. EPA; Federal Guidance Report No. 11; 1988.

Ejnik EJ, Carmichael AJ, Hamilton M, McDiarmid M, Squibb K, Boyd P, Tardiff W. Determination of the isotopic composition or uranium in urine by inductively coupled plasma mass spectrometry. Health Phys 78:143–146; 2000.

Evans HJ, O'Riordan ML. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests. Mutat Res 31:135–148; 1975.

Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm chromatin heterogeneity to fertility. Science 210:1131–1133; 1980.

Evenson DP, Larson Kl, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 23:25–43; 2002.

Fernald Environmental Management Project. Technical basis for internal dosimetry at the Fernald Environmental Management Project. Fernald, OH: FEMP; FEMP Pub. SD 2008; 1997.

Finette BA, Sullivan LM, Oneill JP, Nicklas JA, Vacek PM, Albertini RJ. Determination of HPRT mutant frequencies in t-lymphocytes from a healthy pediatric population—statistical comparison between newborn, children and adult mutant frequencies, cloning efficiency and age. Mut Res 308:223–231; 1994.

Hahn FF, Guillmette RA, Hoover MD. Implanted depleted uranium fragments cause soft tissue sarcomas in the muscles of rats. Environ Health Perspect 100:51–59; 2002.

Heaton RK, Miller S W, Taylor MJ, Grant I. Revised comprehensive norms for an expanded Halstead-Reitan Battery: demographically adjusted neuropsychological norms for African American and Caucasian adults. Professional Manual. Lutz, FL: Psychological Assessment Resources; 2004.

Hooper FJ, Squibb KS, Siegel EL, McPhaul K, Keogh JP. Elevated urine uranium excretion by soldiers with retained uranium shrapnel. Health Phys 77:512–519; 1999.

Hueper WC, Zuefle JH, Link AM, Johnson MG. Experimental studies in metal cancerigenisis II. Experimental uranium in rats. J Natl Cancer Inst 13:291–305; 1952.

Institute of Medicine. Gulf War and health, vol 1. Fulco CE, Liverman CT, Sox HC, eds. Washington, DC: National Academy Press; 2000.

International Commision on Radiological Protection. Report of the task groups on reference man. Elmsford, NY: Pergamon Press; Vol. 23; 1974.

Jakubowski M, Trzcinka-Ochocka M, Halatek T, Razniewska G, Szymczak W. Integrated indexes of occupational exposure as predictors of kidney dysfunction. Int J Occup Med Environ Health 15:393–399; 2002.

Karpas Z, Kirber A, Eliah E, Marcus P, Roiz Y, Marko R, Kol R, Brikner D, Halicz L. Uranium in urine—normalization to creatinine. Health Phys 74:86–90; 1998.

Kathren RL, Moore RH. Acute accidental inhalation of U: a 38-year follow-up. Health Phys 51:609–619; 1986.

Kathren RL, McInroy JF, Moore RH, Dietert SE. Uranium in the tissues of an occupationally exposed individual. Health Phys 57:17–21; 1989.

Knobel Y, Glei M, Weise A, Osswald K, Schaferhenrich A, Richter KK, Claussen U, Pool-Zobel BL. Uranyl nitrilotriacetate, a stabilized salt of uranium, is genotoxic in non-transformed human colon cells and in the human colon adenoma cell line LT97. Toxicol Sci 93:286–297; 2006.

Leach LJ, Maynard EA, Hodge HC, Scott JK, Yuile CL, Sylvester GE, Wilson HB. A five-year inhalation study with natural uranium dioxide (UO2) dust—I. Retention and biologic effects in monkey, dog and rat. Health Phys 18:599–612; 1970.

Leach LJ, Yuile CL, Hodge GC, Sylvester GE, Wilson HB. A five-year inhalation study with natural uranium dioxide (UO2) dust—II. Postexposure retention and biologic effect in the monkey, dog and rat. Health Phys 25:239–258; 1973.

Leggett RW. The behavior and chemical toxicity of U in the kidney: a reassessment. Health Phys 57:365–383; 1989.

Lin RH, Wu LJ, Lee CH, Lin-Shiau SY. Cytogenetic toxicity of uranyl nitrate in Chinese hamster ovary cells. Mutat Res 319:197–203; 1993.

Lucchini R, Albini E, Cortesi I, Placidi D, Bergamaschi E, Traversa F, Alessio L. Assessment of neurobehavioral performance as a function of current and cumulative occupational lead exposure. Neurotoxicol 21:805–811; 2000.

Maynard EA, Hodge HC. Studies of the toxicity of various uranium compounds when fed to experimental animals. In: Voegtlin IC, Hodge HC, eds. Pharmacology and toxicology of uranium compounds, National Nuclear Energy Series (VI). New York: McGraw-Hill; 1949: 309–376.

Maynard EA, Down WL, Hodge HC. Oral toxicity of uranium compounds. In: Voegtlin C, Hodge HC, eds. Pharmacology and toxicology of uranium compounds. New York: McGraw-Hill; 1953.

McDiarmid M, Squibb KS. Uranium and thorium. In: Bingham E, Cohrssen B, Powell C, eds. Patty's industrial hygiene and toxicology, Vol III. New York: John Wiley & Sons; 2001: 381–422.

McDiarmid MA, Keogh JP, Hooper FJ, McPhaul K, Squibb KS, Kane R, DiPino R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Hamilton M, Jacobson-Kram D, Burrows B, Walsh M. Health effects of depleted uranium on exposed Gulf War veterans. Environ Res 82:168–180; 2000.

McDiarmid MA, Squibb K, Engelhardt S, Oliver M, Gucer P, Wilson PD, Kane R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Jacobson-Kram D. Surveillance of depleted uranium exposed Gulf War veterans: health effects observed in an enlarged "friendly fire" cohort. J Occup Environ Med 43:991–1000; 2001.

McDiarmid MA, Engelhardt SM, Oliver M, Gucer P, Wilson PD, Kane R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Handwerger B, Albertini RJ, Jacobson-Kram D, Thorne CD, Squibb KS. Health effects of depleted uranium on exposed Gulf War veterans: a 10-year follow-up. J Toxicol Environ Health Part A 67:277–296; 2004.

McDiarmid MA, Squibb KS, Gucer P, Engelhardt SM, Ardell SK, Albertini RJ. Genotoxic effect at the HPRT locus and in metaphase chromosomes analyzed by FISH in depleted uranium (DU) exposed Gulf War veterans. Environ Mol Mutagen 47:54; 2006.

Miller AC, Blakely WF, Livengood D, Whittaker T, Xu J, Ejnik JW, Hamilton MM, Parlette E, John TS, Gerstenberg HM, Hsu H. Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. Environ Health Perspect 106:465–471; 1998.

Miller AC, Stewart M, Brooks K, Shi L, Page N. Depleted uranium-catalyzed oxidative DNA damage: absence of significant alpha decay. J Inorg Biochem 91:246–252; 2002.

Miller AC, Brooks K, Stewart M, Anderson B, Shi L, McClain D, Page N. Genomic instability in human osteoblast cells after exposure to depleted uranium: delayed lethality and micronuclei formation. J Environ Radioact 64:247–259; 2003.

Mitchel REJ, Jackson JS, Heinmeiller B. Inhaled uranium ore dust and lung cancer risk in rats. Health Phys 76:145–155; 1999.

National Health and Nutrition Examination Survey. Second national report on human exposure to environmental chemicals. Atlanta, GA: Centers for Disease Control and Prevention; NCEH Publication No. 02-0716; 2003.

Papastefanou C. Depleted uranium in military conflicts and the impact on the environment. Health Phys 83:280–282; 2002.

Parkhurst MA, Daxon EG, Lodde GM, Szrom F, Guilmette RA, Roszell LE, Falo GA, McKee CB. Depleted uranium aerosol doses and risks: summary of U.S. assessments. Columbus, OH: Battelle Press; 2005.

Pedersen-Bjergaard J, Rowley JD. The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation. Blood 83:2780–2786; 1994.

Pellmar TC, Fuciarelli AF, Ejnik JW, Emond C, Mottaz HM, Landauer MR. Distribution of uranium in rats implanted with depleted uranium pellets. Toxicol Sci 49:29–39; 1999.

Reeves DL, Levinson D, Batsinger K, Winger B, Gastaldo E. ANAM-USMC normative data. San Diego: National Cognitive Recovery Foundation; Report No. NCRF-SR-95-05; 1995.

Samet JM. Radon and lung cancer. J Natl Cancer Inst 81:745–757; 1989.

Samet JM, Pathak DR, Morgan MV, Marbury MC, Key CR, Valdivia AA. Radon progeny exposure and lung cancer risk in New Mexico U miners: a case-control study. Health Phys 56:415–421; 1989.

Squibb KS, McDiarmid MA. Depleted uranium exposure and health effects in Gulf War veterans. Phil Trans R Soc B 361:639–648; 2006.

Squibb KS, Leggett RW, McDiarmid MA. Prediction of renal concentrations of depleted uranium and radiation dose in Gulf War veterans with embedded shrapnel. Health Phys 89:267–273; 2005.

StataCorp. Stata statistical software. College Station, TX: Stata; 2003.

Statistical Products and Service Solutions. SPSS statistical software. Chicago: SPSS; 2003.

Stearns DM, Yazzie M, Bradley AS, Coryell VH, Shelley JT, Ashby A, Asplund CS, Lantz RC. Uranyl acetate induces hprt mutations and uranium-DNA adducts in Chinese hamster ovary EM9 cells. Mutagenesis 20:417–423; 2005.

Swierenga SH, Heddle JA, Sigal EA, Gilman JP, Brillinger RL, Douglas GR, Nestmann ER. Recommended protocols based on a survey of current practice in genotoxicity testing laboratories, IV. Chromosome aberration and sister-chromatid exchange in Chinese hamster ovary, V79 Chinese hamster lung and human lymphocyte cultures. Mutat Res 246:301–322; 1991.

Tannenbaum A. Toxicology of uranium. New York: McGraw-Hill, Inc.; 1951.

The Psychological Corporation. Wechsler adult intelligence scale. San Antonio, TX: The Psychological Corporation; 1997.

The Royal Society. The health hazards of depleted uranium munitions part I. Policy document 6/01. London, UK: The Royal Society; 2001.

The Royal Society. The health effects of depleted uranium munitions. Summary. Policy document 6/02. London, UK: The Royal Society; 2002.

Thun MJ, Baker DB, Steenland K, Smith AB, Halperin W, Berl T. Renal toxicity in uranium mill workers. Scand J Work Environ Health 11:83–90; 1985.

Watanabe N, Kamel S, Ohkubo A, Yamanaka N, Ojsawa S, Makino K, Tokudo K. Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 Automated Analyzer. Clin Chem 38:642–647; 1986.

Woolson RF. Statistical methods for the analysis of biomedical data. New York: John Wiley & Sons, Inc.; 1987.

World Health Organization. WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. Cambridge, UK: Cambridge University Press; 1987.

Yazzie M, Gamble SL, Civetello ER, Sterns DM. Uranyl acetate causes DNA single strand breaks in vitro in the presence of ascorbate (vitamin C). Chem Res Toxicol 16:524–530; 2003.

Zhang L, Rothman N, Wang Y, Hayes RB, Li G, Dosemeci M, Yin S, Kolachana P, Titenko-Holland N, Smith MT. Increased aneusomy and long arm deletion of chromosomes 5 and 7 in the lymphocytes of Chinese workers exposed to benzene. Carcinogenesis 9:1955–1961; 1998.

Zhang L, Rothman N, Wang Y, Hayes RB, Yin S, Titenko-Holland N, Dosemeci M, Wang YZ, Kolachana P, Lu W, Xi L, Li GL, Smith MT. Benzene increases aneuploidy in the lymphocytes of exposed workers: a comparison of data obtained by fluorescence in situ hybridization in interphase and metaphase cells. Environ Mol Mutagen 34:260–268; 1999.

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