Uranium Risk Statement

The 40 CFR Part 192 rulemaking should be based upon risks since the goal of regulation should be to mitigate risk. The risks posed by natural uranium are not high however, and do not present risks commensurate with this degree of regulation. The 40 CFR Part 192 places an inappropriate level of burden on industry which is not scientifically justified and should be linked to the risk. The discussion below discusses the real as opposed to perceived risks from natural uranium and demonstrates that they indeed low and do not warrant this proposed level of regulation. It also address some of the key elements where the proposed requirements are not in line with best practice or appropriate for the degree of risk.

- Natural uranium consists of three (3) isotopes of uranium all of which are radionuclides, those being:
  - Uranium-238 (99.3% of all naturally occurring uranium with a half life of 4.51 billion years
  - Uranium-235 (0.72% of all naturally occurring uranium with a half life of 703.8 million years and;
  - Uranium-234 (0.0055% of all naturally occurring uranium with a half life of 245,500 years
- The preamble to the proposed rule states:
  - *The proposed rule will reduce the risk of undetected excursions of pollutants into adjacent aquifers. This in turn will reduce the human health risks that could result from exposures to radionuclides in well water used for drinking or agriculture in areas located down-gradient from an ISR. Because radionuclides are human carcinogens, the main health risk averted would be cancer.*
  - The preamble leads the reviewer to conclude that since uranium is a radionuclide it poses a substantial risk that justifies additional stringent regulation.
- Natural uranium (the naturally occurring mixture of the Uranium-238, Uranium-235 and Uranium-234 isotopes) presents a very low risk.
- The U.S. Public Health Service Agency for Toxic Substances and Disease Registry 's Toxicological Profile for Uranium (February 2013) (https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=440&tid=77) clearly states:
  - *Neither the National Toxicology Program (NTP), International Agency for Research on Cancer (IARC), nor the EPA have classified natural uranium or depleted uranium with respect to carcinogenicity.* and;
  - *No health effects, other than kidney damage, have been consistently found in humans after inhaling or ingesting uranium compounds or in soldiers with uranium metal fragments in their bodies.*
  - This document concludes that the only substantial health risk from natural uranium is kidney damage.
  - A hard copy of this document is not attached due to its size however an electronic copy is included in the DVD disk.
- A conclusion that natural uranium presents a substantial risk warranting substantial new and stringent regulation is not supported by the following papers:
  - *Mortality among a cohort of uranium mill workers: an update* - L E Pinkerton, T F Bloom, M J Hein, E M Ward (NIOSH Study) (March 20030
    - This study involved a cohort of 1,484 uranium mill workers who worked in Colorado Plateau uranium mills (a maximally exposed cohort) beginning on January 1, 1940.
    - The study concludes:
      - *Mortality from all causes was less than expected, which is largely accounted for by fewer deaths from heart disease than expected. Mortality from all malignant neoplasms was also less than expected.*
  - *ACUTE CHEMICAL TOXICITY OF URANIUM* - Ronald L. Kathren and Richard K. Burklin (August 2007)
    - This paper examined acute toxicity of uranium and concluded:
      - *Insoluble uranium compounds arc reportedly nontoxic. There has never been a death attributable to uranium poisoning ill humans, and humans seem to be less sensitive to both acute and chronic toxic effects of uranium than other mammalian species studied.*

- *Grand Rounds: Nephrotoxicity in a Young Child Exposed to Uranium from Contaminated Well Water* - H. Sonali Magdo et al (August 2007)
  - This paper discussed a young child exposed to uranium via ingestion of water from a private well in Connecticut. The water according to one analysis contained 1.16 milligrams per liter uranium.
  - The paper concludes:
    - *It* (the exposure incident*) confirms previous epidemiologic studies showing that chronic, low-level exposure to uranium in drinking water may result in mild injury to the proximal renal tubule (Kurttio et al.*
    - *Because of its radioactivity, concern has arisen about the possible carcinogenicity of uranium. However, the levels of uranium that have been observed to induce nephrotoxicity are much lower than those that increase risk of cancer, and uranium intake from contaminated water has not been associated with increased risk of human cancer (Auvinen et al. 2002, 2005; Boice et al. 2003; Kim et al. 2004; Kurttio et al. 2002).*
    - 2002).
  - Three (3) months after consumption of the well water containing uranium ceased, the child's protein excretion rate had fallen to normal levels. The only health risk involved was *"…mild injury to the proximal renal tubule..."* the effects of which disappeared three (3) months after the patient ceased consuming the uranium bearing water.
    - The paper states:
      - *Three months after the family had ceased consuming water from the home well (January 2001), this child's urinary beta-2-microglobulin excretion rate had fallen to 52 µg/mmol creatinine. There was no evidence of other proximal tubule dysfunction, as evidenced (in January 2001) by the absence of glucosuria, phosphate wasting (with normal values for the tubular reabsorption of phosphate), bicarbonate wasting, or metabolic acidosis (Table 3).*
    - There were no lasting effects. This low risk does not justify additional stringent regulation.
- *URANIUM DEPOSITION AND RETENTION IN A USTUR WHOLE BODY CASE* J. J. Russell and R. L. Kathren (October 2003)
  - This paper discusses the autopsy of an eighty-three (83) year old man who died from an acute cerebellar infarct. The paper states, "Over *the course of his employment, USTUR Case 1002 submitted numerous urine samples for uranium, plutonium, and fission product analysis. The highest single uranium value measured during this time period was_30µg/L recorded during the second year of his employment. A urinary bioassay sample taken before termination of employment measured 4.3µg/L. The mean urinary uranium concentration per liter per year calculated from the employee's bioassay records covering the first eleven years of monitoring averaged less than 3 µg/ L*"
    - The paper concludes:
      - *Analysis of all the tissues from a whole body donor with a known occupational history of exposure to uranium showed elevated concentrations of uranium in the respiratory tract and spleen that are consistent with inhalation of natural uranium in a somewhat insoluble form. The low kidney concentrations in this case, other Registry cases, and other cases reported in the literature suggest that the Reference Man data on background quantities of uranium on the kidney are high by about an order of magnitude.* and;
      - *Autopsy results disclosed findings not uncommon in the aged with no indication of pathology possibly attributable solely to exposure to uranium.*
  - Twenty-eight (28) years of exposure to uranium in the course of his work resulted in "*... no indication of pathology possibly attributable solely to exposure to uranium."*
  - This specific case again highlights the low risk posed by uranium and the lack of need for additional stringent regulation.
- *The U.S. Department of Veterans' Affairs depleted uranium exposed cohort at 25 Years: Longitudinal surveillance results* Melissa A. McDiarmid et al (October 2016)

        - This paper discusses a cohort of Gulf War I veterans with fragments of depleted uranium munitions embedded in their bodies that were not surgically removable.
        - Following twenty-five (25) years of observation, the paper concludes:
            - *Now, more than 25 years since first exposure to DU, an aging cohort of military veterans continues to show no U-related health effects in known target organs of U toxicity. The results of an extensive health assessment have also shown no other clinically significant health effects as a function of U burden.*
    - The time period, monitoring requirements and criteria for stabilization of an aquifer is dependent on the site specific factors of the aquifer and the proposed rulemaking should utilize a scientific assessment rather than an arbitrary time period and monitoring approach.
        - The changes proposed by this rulemaking including a thirty (30) year stability monitoring period for restored wellfields (especially after it has been shown at a 95% confidence interval that the levels have stabilized) are inappropriate, not justified by science and not justified by the very small risks posed by natural uranium.
        - Stabilization times for restored wellfields should be based upon the specifics of the site and on the risks posed. The rulemaking proposes arbitrary standards without a scientific risk based justification.
        - The degree of monitoring in the proposed rulemaking is not in line with best practice for assessment of impacts. Specifically the ruling "*sampling no less frequently than quarterly*" during the 30 year long term stability phase monitoring is not appropriate. As the ground water has already been shown to be stable, high frequency monitoring is not necessary as this phase is to detect any longer term trends. This high frequency is a waste of resources with no gain in the ability to detect and quantify any potential impact.
        - In addition, the required restoration efforts and post restoration monitoring should be based upon the initial pre- operational water quality which in the case of water in uranium deposits is often very poor (the proposed rulemaking could lead to a situation where an operation has the impossible task to purify an area to an extent greater than the pre-existing background levels). The Agency admits to this fact in the rulemaking's preamble which states:
            - *We anticipate the objection that the presence of uranium deposits typically results in groundwater of poor quality, and not a pristine source of drinking water. We recognize that this is often the case, and that the volume of water affected by the mineralized zone may be significant*

- Conclusions
    - Natural uranium poses a very low health risk...
    - Natural uranium is a nephrotoxin.
    - *"... humans seem to be less sensitive to both acute and chronic toxic effects of uranium than other mammalian species studied."* - ***ACUTE CHEMICAL TOXICITY OF URANIUM***
    - Individuals with uranium metal fragments embedded in their bodies for twenty-five (25) years show, "*... no U-related health effects in known target organs of U toxicity."* and "*... no other clinically significant health effects as a function of U burden."* - ***The U.S. Department of Veterans' Affairs depleted uranium exposed cohort at 25 Years: Longitudinal surveillance results***
    - A cohort of 1,484 uranium mill workers (a maximally exposed group) show, "*Mortality from all causes was less than expected, which is largely accounted for by fewer deaths from heart disease than expected."* and *"Mortality from all malignant neoplasms was also less than expected."* - ***Mortality among a cohort of uranium mill workers: an update***
    - Symptoms of uranium exposure vanish rapidly upon cessation of exposure since "*Three months after the family had ceased consuming water from the home well (January 2001), this child's urinary beta-2-microglobulin excretion rate had fallen to 52 µg/mmol creatinine."* - ***Nephrotoxicity in a Young Child Exposed to Uranium from Contaminated Well Water***
    - An individual with twenty-eight (28) years of uranium exposure upon autopsy showed, "*... no indication of pathology possibly attributable solely to exposure to uranium."* - ***URANIUM DEPOSITION AND RETENTION IN A USTUR WHOLE BODY CASE***
    - The negligible risks posed by exposure to uranium do not warrant the proposed level of regulation.

- The time period, monitoring requirements and criteria for stabilization of an aquifer is dependent on the site specific factors of the aquifer and the proposed rulemaking should utilize a scientific assessment rather than an arbitrary time period and monitoring approach.
- The proposed rulemaking should consider the site specific aspects of the area and in particular should be based upon the initial pre- operational water quality which in the case of water in uranium deposits is often very poor.

Attachment 1

TOXICOLOGICAL PROFILE FOR URANIUM

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Agency for Toxic Substances and Disease Registry

February 2013

This document at 526 pages is too large to include as a hard copy and is provided on a DVD disk. In addition, it is available on line at:

http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=440&tid=77

Attachment 2

ORIGINAL ARTICLE

Mortality among a cohort of uranium mill workers: an update

L E Pinkerton, T F Bloom, M J Hein, E M Ward

Occup Environ Med 2004;**61**:57–64

See end of article for authors' affiliations

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Aims: To evaluate the mortality experience of 1484 men employed in seven uranium mills in the Colorado Plateau for at least one year on or after 1 January 1940.
Methods: Vital status was updated through 1998, and life table analyses were conducted.
Results: Mortality from all causes and all cancers was less than expected based on US mortality rates. A statistically significant increase in non-malignant respiratory disease mortality and non-significant increases in mortality from lymphatic and haematopoietic malignancies other than leukaemia, lung cancer, and chronic renal disease were observed. The excess in lymphatic and haematopoietic cancer mortality was due to an increase in mortality from lymphosarcoma and reticulosarcoma and Hodgkin's disease. Within the category of non-malignant respiratory disease, mortality from emphysema and pneumoconioses and other respiratory disease was increased. Mortality from lung cancer and emphysema was higher among workers hired prior to 1955 when exposures to uranium, silica, and vanadium were presumably higher. Mortality from these causes of death did not increase with employment duration.
Conclusions: Although the observed excesses were consistent with our a priori hypotheses, positive trends with employment duration were not observed. Limitations included the small cohort size and limited power to detect a moderately increased risk for some outcomes of interest, the inability to estimate individual exposures, and the lack of smoking data. Because of these limitations, firm conclusions about the relation of the observed excesses in mortality and mill exposures are not possible.

In the United States, mining and milling of uranium ores to recover uranium for nuclear weapons began during World War II to support the Manhattan Project. Uranium bearing ores had been mined previously on a small scale, but mainly for the recovery of vanadium. Continued development and expansion of the industry after the war was promoted by a domestic uranium concentrate procurement programme that was established by the Atomic Energy Commission in 1947.[1] As early as 1949, health officials became concerned about the potential health risks associated with uranium mining and milling.[2]

The health risks associated with uranium mining have been extensively studied. Uranium miners have been found to have a substantially increased risk of death from lung cancer, which is associated with cumulative exposure to radon decay products.[3–5] Excess mortality from non-malignant respiratory diseases has also been found.[6] However, existing data concerning the health effects of uranium milling are limited. Waxweiler and colleagues reported a significantly increased risk of ''other non-malignant respiratory disease'' (standardised mortality ratio (SMR) = 2.50; observed (obs) = 39) among 2002 workers at seven uranium mills in the Colorado Plateau.[7] This category included emphysema, fibrosis, silicosis, and chronic obstructive pulmonary disease. Non-significant excesses were observed for lymphatic and haematopoietic malignancies other than leukaemia after 20 years latency (SMR = 2.3; obs = 6) and chronic renal disease (SMR = 1.67; obs = 6). In an earlier overlapping study of 662 uranium mill workers, Archer and colleagues observed an excess risk of mortality from lymphatic and haematopoietic malignancies other than leukaemia (SMR = 3.92; obs = 4).[8] Limited data from morbidity studies suggest that uranium millers may have an increased risk of pulmonary fibrosis[2] and renal tubular injury.[9]

The primary exposures of interest in uranium mills are uranium, silica, and vanadium containing dusts. Inhalation of uranium dust may pose an internal radiation hazard as well as the potential for chemical toxicity. High concentrations of radon and radon decay products, similar to the levels found in underground uranium mines, are not expected in the mills.

Because of continuing concern about the health effects of uranium milling, we extended the follow up of the cohort described by Waxweiler and colleagues.[7] The present report describes the mortality experience of the cohort through 21 additional years of observation. In addition, the risk of end stage renal disease was evaluated among the cohort.

Uranium milling process

The primary function of uranium mills is to extract and concentrate uranium from uranium containing ore to produce a semi-refined product known as yellowcake. Yellowcake is a chemically complex mixture of diuranates, basic uranyl sulphate, and hydrated uranium oxides that contains 80–96% uranium as U_3O_8, UO_3, and/or ammonium diuranate.[10] Yellowcake is used commercially to manufacture nuclear fuel for nuclear power and national defence purposes.

Conventional mills process uranium bearing ores from underground or open-pit mines. Until the mid-1970s, all yellowcake in the United States was produced at conventional uranium mills.[11] The main stages of the process in conventional mills involved: (1) ore handling and preparation; (2) extraction; (3) concentration and purification; and (4) precipitation, drying, and packaging. So-called ''upgrader'' facilities processed virgin ore that was initially too low in uranium content to process economically in a uranium mill. At an upgrader, a series of crushing, grinding, and chemical separation steps were employed to ''upgrade'' the percent

Main messages

- Potential exposures among uranium mill workers that may be associated with adverse health effects include uranium, silica, and vanadium containing dusts.
- We observed a statistically significant increase in mortality from non-malignant respiratory disease and non-significant increases in mortality from lymphatic and haematopoietic malignancies other than leukaemia, lung cancer, and chronic renal disease. These findings were consistent with our a priori hypotheses.
- The SMRs for lung cancer and emphysema among men hired before 1955, when exposures to uranium, silica, and vanadium were presumably higher, were significantly increased and greater than the SMRs observed among men hired in 1955 or later. However, mortality for causes of death observed to be in excess did not increase with employment duration.
- Limitations include a lack of smoking data, small cohort size and limited power to detect a moderately increased risk for some outcomes of interest, and the inability to estimate individual exposures to uranium, silica, and vanadium.

uranium contained in the final product, which was sent to a uranium mill for further processing. Unlike conventional uranium mills, upgrader facilities did not carry out concentration and purification of the uranium, and precipitation, drying, and packaging of yellowcake. In this paper, the term ''mill'' will be used in reference to both conventional uranium mills and upgrader facilities.

METHODS

Cohort description

The cohort was assembled from the personnel records obtained from the companies operating seven uranium mills (five conventional uranium mills and two upgraders). The original cohort described by Waxweiler and colleagues, which is referred to hereafter as the Waxweiler cohort, included 2002 men who had worked for at least one day after 1 January 1940, worked for at least one year in uranium mills, and never worked in underground uranium mines.[7] Because some of the work histories in the Waxweiler cohort were found to be coded inaccurately, we recoded all work histories. We also reviewed documentation from the original study to identify men who met the original cohort criteria, but had been omitted. Personnel records were obtained and work histories updated for cohort members who were still employed in 1971 when the personnel records were originally microfilmed. After re-coding the work histories, we limited the cohort to men who met the original cohort criteria, had never worked in an above-ground or underground uranium mine, and had worked for at least one year in the seven uranium mills before the personnel records were originally microfilmed in 1971 while the mills were operating to recover uranium and/or vanadium concentrates. The final cohort included 1485 men, 1438 (96.8%) of whom were in the Waxweiler cohort. Of the 564 workers not included in the current study, 103 (18.3%) worked in uranium mines, 318 (56.4%) never worked in one of the seven mills comprising the study, 141 (25.0%) worked for less than one year in the seven mills when they were operating, and one (0.2%) was excluded because the work history was incomplete. One woman whose gender was coded incorrectly in the Waxweiler cohort was also excluded.

Follow up

The vital status of all persons in the cohort was determined until 31 December 1998. Follow up included inquiry through the Social Security Administration, Internal Revenue Service, US Postal Service, National Death Index (NDI), and state bureaus of motor vehicles. Death certificates were obtained from state vital records offices for some deceased members of the cohort and coded by a trained nosologist according to the revision of the International Classification of Diseases in effect at the time of death. The causes of death for other deceased members of the cohort were obtained from the NDI.

To identify cohort members with treated end stage renal disease, the cohort was linked with the End Stage Renal Disease (ESRD) Program Management and Medical Information System (PMMIS) by name, social security number, and date of birth. The ESRD PMMIS is maintained by the Health Care Financing Administration (HCFA) and includes all individuals who received Medicare covered renal replacement therapy (dialysis or transplant) in 1977 or later. Approximately 93% of ESRD patients in the United States are included in the ESRD PMMIS.[12]

Analysis

The mortality experience of the cohort was analysed with the use of the National Institute for Occupational Safety and Health (NIOSH) modified life table analysis system (LTAS).[13 14] Each cohort member accumulated person-years at risk (PYAR) for each year of life after 1 January 1940 or completion of the one year eligibility period, whichever was later, until the date of death for deceased cohort members, the date last observed for persons lost to follow up, or the ending date of the study (31 December 1998) for cohort members known to be alive. Cohort members known to be alive after 1 January 1979 (the date that the NDI began) and not identified as deceased were assumed to be alive as of 31 December 1998. The PYAR were stratified into five year intervals by age and calendar time and were then multiplied by the appropriate US gender, race, and cause specific mortality rates to calculate the expected number of deaths for that stratum. The resulting expected numbers were summed across strata to obtain cause specific and total expected number of deaths. The ratio of observed to expected number of deaths was expressed as the standardised mortality ratio (SMR). Ninety five per cent confidence intervals (CI) were computed for the SMRs assuming a Poisson distribution for observed deaths. The mortality analysis was repeated using Colorado, New Mexico, Arizona, and Utah state mortality rates to generate expected numbers of deaths. In addition to analyses of underlying cause of death, all causes listed on the death certificate were analysed using multiple cause mortality methods described by Steenland and colleagues.[15] Multiple cause analyses are particularly important for diseases that may be prevalent at death but that are not the underlying cause of death.[15] In analyses using state or multiple cause mortality rates, person-years at risk started to accumulate on 1 January 1960, when the rates were first available, or completion of the one year eligibility period, whichever was later.

The end stage renal disease experience of the cohort was analysed using methods described by Calvert and colleagues.[16] Briefly, the modified life table analysis system was used to calculate PYAR, expected number of individuals developing ESRD, and standardised incidence ratios (SIRs) for ESRD. Since the ESRD PMMIS is considered incomplete prior to 1977, cohort members who died before this date were excluded from the ESRD analysis. PYAR for cohort members

who were alive on 1 January 1977 began to accumulate on this date. Cohort members accumulated PYAR until the first service date for those with ESRD, the date of death for deceased cohort members, the date last observed for those lost to follow up, or the ending date of the study for those known to be alive. The first service date for ESRD, which generally represents the date on which renal replacement therapy began, was used as a surrogate for the date of onset of ESRD. After the PYAR were stratified into five year intervals by age and calendar time, the PYAR were multiplied by the appropriate US ESRD incidence rates to calculate the expected number of cases for that stratum. The US incidence rates were developed by NIOSH from the HCFA PMMIS data and US census data as described elsewhere.[16] The expected number of treated ESRD cases in all strata were summed to yield the total expected number. The ratio of the observed to expected number of treated ESRD cases was expressed as the standardised incidence ratio (SIR). The SIR for four major categories of ESRD (systemic, non-systemic, other, and unknown) were also calculated.

We stratified SMRs and SIRs by duration of employment (1–2, 3–9, 10+ years), time since first employment (latency) (0–9, 10–19, 20+ years), and year of first employment (<1955, 1955+). In general, the cut points for duration of employment and time since first employment were retained from the original study; however, we lowered the cut point between the lowest and middle duration of employment categories so that the number of deaths in each category would be more similar. The cut point for year first employed was selected a priori based on the assumption that exposures in the earlier years (when there was little emphasis on dust control) would be higher than in later years. Duration of employment was based on employment in the seven cohort mills while they were operating to produce uranium and/or vanadium concentrates and included employment that occurred prior to the start of the follow up period. The analyses were repeated restricting the cohort to those who had worked in a conventional mill and to those who had worked in a conventional mill that produced both vanadium and uranium concentrates. Because of the potential impact of exposures encountered during other employment in the uranium industry, SMRs and SIRs were also conducted restricting the cohort to those without such employment. All analyses were done using the PC version of the LTAS[17] (http://www.cdc.gov/niosh/ltindex.html). Testing for heterogeneity and trend in the SMRs used the methods of Breslow and Day.[18]

Based on previous studies and the known toxic effects of uranium and silica, the a priori outcomes of interest in this study included non-malignant respiratory disease, chronic renal disease, lung cancer, and lymphatic and haematopoietic cancer other than leukaemia. Within the major category of non-malignant respiratory disease, the minor category ''pneumoconiosis and other respiratory diseases'' was of a priori interest.

RESULTS

A total of 1484 men contributing 49 925 person-years were included in the study. Table 1 presents the distribution of the cohort by vital status, plant type (conventional mill, upgrader), duration of employment, time since first employment, and first year of employment. Race was unknown for 642 (43.3%) members of the cohort. Because all workers of known race were white, workers of unknown race were classified as white in the analysis. In the total cohort, 656 (44.2%) men were alive, 810 (54.6%) were deceased, and 18 (1.2%) were lost to follow up. Causes of death were obtained from death certificates or the NDI for 794 (98.0%) of the individuals known to be deceased. Deaths with missing causes of death were included in the other and unknown causes category. The duration of employment of the cohort is relatively short with a median of 3.6 (range 1–36.3) years. Over half of the cohort was first employed prior to 1955. The median time since first employment, based on employment in the seven mills while they were operating, is 37 years.

Table 1 Characteristics of the study population

No. of workers	1485
Excluded from analysis*	1
Person-years at risk	49925
Mill type	
Conventional mill only	1412 (95.1%)
Upgrader only	44 (3.0%)
Both	28 (1.9%)
Vital status as of 31 Dec 1998	
Alive	656 (44.2%)
Dead	810 (54.6%)
Unknown	18 (1.2%)
Year of birth	1921 median
	1872–1951 range
Year of first employment†	
Prior to 1955	799 (53.8%)
1955 or later	685 (46.2%)
Duration of employment†	
1–2 years	634 (42.7%)
3–9 years	547 (36.9%)
10 + years	303 (20.4%)
Time since first employment†	
<10 years	76 (5.1%)
10–19 years	128 (8.6%)
20+ years	1280 (86.3%)

*Missing date of birth.
†Employment in the seven mills while operating to produce uranium and/or vanadium concentrates.

Almost all of the workers and person-years were from conventional uranium mills. Of the 1440 men who were employed at conventional mills, 1263 (87.7%) were employed at mills that recovered vanadium, 145 (10.1%) were employed at mills that did not recover vanadium, and 32 (2.2%) were employed both at mills that recovered vanadium and mills that did not recover vanadium. Among the entire cohort, 83 (5.6%) men had also been employed in other aspects of the uranium industry according to their employment application or other employment records.

Table 2 shows the results of the analysis for all causes of death. Mortality from all causes was less than expected, which is largely accounted for by fewer deaths from heart disease than expected. Mortality from all malignant neoplasms was also less than expected. Among the outcomes of a priori interest, a statistically significant increase in mortality from non-malignant respiratory disease (SMR = 1.43; 95% CI 1.16 to 1.73; obs = 100) and non-significant increases in mortality from trachea, bronchus, and lung cancer (SMR = 1.13; 95% CI 0.89 to 1.41; obs = 78), lymphatic and haematopoietic malignancies other than leukaemia (SMR = 1.44; 95% CI 0.83 to 2.35; obs = 16), and chronic renal disease (SMR = 1.35; 95% CI 0.58 to 2.67; obs = 8) were observed. The excess in mortality from lymphatic and haematopoietic malignancies was due to an excess in mortality from lymphosarcoma and reticulosarcoma (SMR = 1.74; 95% CI 0.48 to 4.46; obs = 4) and Hodgkin's disease (SMR = 3.30; 95% CI 0.90 to 8.43; obs = 4). Within the major category of non-malignant respiratory disease, mortality from emphysema (SMR = 1.96; 95% CI 1.21 to 2.99; obs = 21) and pneumoconioses and other respiratory disease (SMR = 1.68; 95% CI 1.26 to 2.21; obs = 52) was significantly increased. Among outcomes other than those of a priori interest, non-significant increases in mortality from other and unspecified cancers (SMR = 1.59; 95% CI 0.98 to 2.43; obs = 21) and accidents (SMR = 1.26; 95% CI 0.93 to 1.68;

Table 2 Uranium mill workers' mortality (since 1940, US referent rates): update of cohort to 1998

Underlying cause of death (ICD9 code)*	Obs	Exp	SMR	95% CI
All causes	810	877.66	0.92‡	0.86 to 0.99
All cancers (140–208)	184	204.12	0.90	0.78 to 1.04
Buccal and pharyngeal CA (140–149)	2	5.06	0.40	0.05 to 1.43
All digestive CA (150–159)	33	53.18	0.62§	0.43 to 0.87
Oesophagus (150)	1	5.06	0.20	0.01 to 1.10
Colon (152–153)	12	18.96	0.63	0.33 to 1.11
Rectal (154)	2	4.77	0.42	0.05 to 1.51
Liver and biliary (155–156)	4	5.04	0.79	0.22 to 2.03
Pancreas (157)	6	10.30	0.58	0.21 to 1.27
All respiratory CA (160–165)	78	72.29	1.08	0.85 to 1.35
Trachea, bronchus, and lung (162)	78	68.93	1.13	0.89 to 1.41
Male genital CA (185–187)	15	19.67	0.76	0.43 to 1.26
All urinary CA (188–189)	5	11.03	0.45	0.15 to 1.06
Kidney (189.0–189.2)	4	4.96	0.81	0.22 to 2.06
Leukaemia/aleukaemia (204–208)	5	7.62	0.66	0.21 to 1.53
Lymphatic and haematopoietic CA other than leukaemia (200–203)	16	11.08	1.44	0.83 to 2.35
Lymphosarcoma and reticulosarcoma (200)	4	2.29	1.74	0.48 to 4.46
Hodgkin's disease (201)	4	1.21	3.30	0.90 to 8.43
Other lymphatic and haematopoietic CA (202–203)	8	7.57	1.06	0.46 to 2.08
Other/unspecified CA (194–199)	21	13.20	1.59	0.98 to 2.43
Tuberculosis (001–008)	2	3.88	0.52	0.06 to 1.86
Diabetes mellitus (250)	10	14.60	0.68	0.33 to 1.26
Heart disease (390–398, 402, 404, 410–414, 420–429)	293	349.10	0.84§	0.75 to 0.94
Ischemic heart disease (410–414)	236	280.07	0.84§	0.74 to 0.96
Other circulatory disease (401, 403, 405, 415–417, 430–459)	69	83.06	0.83	0.65 to 1.05
Non-malignant respiratory disease (460–519)	100	70.16	1.43§	1.16 to 1.73
Pneumonia (480–486)	25	23.76	1.05	0.68 to 1.55
Chronic and unspecified bronchitis (490–491)	2	2.20	0.91	0.11 to 3.28
Emphysema (492)	21	10.72	1.96§	1.21 to 2.99
Pneumoconioses and other respiratory disease (470–478, 494–519)	52	30.87	1.68§	1.26 to 2.21
Non-malignant digestive disease (520–579)	23	36.91	0.62‡	0.39 to 0.94
Non-malignant genitourinary disease (580–629)	13	13.03	1.00	0.53 to 1.71
Acute renal disease (580–581, 584)	1	1.16	0.86	0.02 to 4.79
Chronic renal disease (582–583, 585–587)	8	5.91	1.35	0.58 to 2.67
Ill defined conditions (780–796, 798–799)	4	8.01	0.50	0.14 to 1.28
Accidents (E800–E949)	47	37.23	1.26	0.93 to 1.68
Violence (E950–E978)	18	17.73	1.02	0.60 to 1.60
Suicide (E950–E959)	15	14.19	1.06	0.59 to 1.74
Homicide (E960–E978)	3	3.54	0.85	0.18 to 2.48
Other and unknown causes	27†	14.04	1.92§	1.27 to 2.80

*International Classification of Disease codes, 9th revision.
†Includes 16 observed deaths with missing death certificates.
‡95% confidence interval excludes the null value (1.0).
§99% confidence interval excludes the null value (1.0).

obs = 47) were observed. The observed other and unspecified cancers were metastatic cancers of unknown primary site. Mortality from all digestive cancers was significantly less than expected (SMR = 0.62; 95% CI 0.43 to 0.87; obs = 33).

An analysis was also conducted (not shown) using US rate files for 1960 to 1999 which have 99 causes of death instead of 92 because these rate files include more detailed categories of non-malignant respiratory disease and slightly different categories of malignancies of the lymphatic and haematopoietic system. Of the 1484 cohort members, 89 (6.0%) were not included in this analysis because they had either died or were lost to follow up before 1960. Only one death from silicosis (SMR = 5.93; 95% CI 0.15 to 32.94) and two deaths from pneumoconioses other than silicosis and asbestosis (SMR = 2.29; 95% CI 0.28 to 8.25) were observed. The remainder of the excess in non-malignant respiratory disease mortality was due to a significant excess in mortality from emphysema (SMR = 1.83; 95% CI 1.10 to 2.86) and other respiratory diseases (SMR = 1.62; 95% CI 1.19 to 2.15). Most of the observed deaths from other respiratory diseases were due to chronic obstructive lung disease. In the category of malignancies of the lymphatic and haematopoietic system other than leukaemia, mortality was significantly increased for Hodgkin's disease (SMR = 4.01; 95% CI 1.09 to 10.25, obs = 4) and non-significantly increased for non-Hodgkin's lymphoma (SMR = 1.25; 95% CI 0.54 to 2.46; obs = 8).

In order to evaluate whether regional variations in mortality rates could explain the findings, analyses were conducted using state rates as the comparison population (table 3). State rates are not available before 1960 so men who had either died or were lost to follow up before 1960 were also excluded from this analysis. The excess in mortality from cancer of the trachea, bronchus, and lung (SMR = 1.51; 95% CI 1.19 to 1.89) based on state rates was statistically significant and greater than the excess based on US rates since 1960 (SMR = 1.13; 95% CI 0.89 to 1.42). In contrast, the excess in mortality from emphysema (SMR = 1.25; 95% CI 0.75 to 1.95) and other respiratory diseases (SMR = 1.35; 95% CI 0.99 to 1.79) was less than the excess based on US rates. Mortality from chronic renal disease was not increased based on state rates (SMR = 1.02; 95% CI 0.33 to 2.39; obs = 5) and was similar to that based on US rates since 1960 (SMR = 1.00; 95% CI 0.32 to 2.35). This is in contrast to the excess in mortality from chronic renal disease observed based on US rates since 1940.

Tables 4 and 5 show mortality according to duration of employment and time since first employment for selected causes of death based on US rates. Overall mortality was highest among those with the shortest duration of employment and lowest among those with the longest duration of employment. Similar trends with duration of employment were observed for mortality from lung cancer, non-malignant

Table 3 Uranium mill workers' mortality (since 1960) from selected causes of death (state referent rates): update of cohort to 1998

Underlying cause of death (ICD9 code)*	Obs	Exp	SMR	95% CI
All respiratory CA (160–165)	75	51.98	1.44‡	1.13 to 1.81
Trachea, bronchus, and lung (162)	75	49.73	1.51‡	1.19 to 1.89
Leukaemia/aleukaemia (204–208)	5	6.51	0.77	0.25 to 1.80
Lymphatic and haematopoietic CA other than leukaemia (200–203)	15	9.58	1.57	0.88 to 2.58
Non-Hodgkin's lymphoma (200, 202)	8	5.71	1.40	0.60 to 2.76
Hodgkin's disease (201)	4	0.94	4.24†	1.15 to 10.84
Myeloma (203)	3	2.93	1.02	0.21 to 3.00
Other/unspecified CA (187, 194–199)	22	11.93	1.84‡	1.16 to 2.79
Non-malignant respiratory diseases (460–519)	94	79.32	1.19	0.96 to 1.45
Chronic and unspecified bronchitis (490–491)	1	2.74	0.36	0.01 to 2.03
Emphysema (492)	19	15.22	1.25	0.75 to 1.95
Asbestosis (501)	0	0.12	0.00	0.00 to 30.62
Silicosis (502)	1	0.45	2.22	0.06 to 12.36
Other pneumoconioses (500, 503, 505)	2	0.40	5.04	0.61 to 18.19
Other respiratory diseases (470–478, 494–499, 504, 506–519)	47	34.86	1.35	0.99 to 1.79
Non-malignant genitourinary disease (580–629)	10	10.51	0.95	0.46 to 1.75
Acute renal disease (580–581, 584)	1	0.79	1.26	0.03 to 6.99
Chronic renal disease (582–583, 585–587)	5	4.89	1.02	0.33 to 2.39

*International Classification of Disease codes, 9th revision.
†95% confidence interval excludes the null value (1.0).
‡99% confidence interval excludes the null value (1.0).

respiratory disease, and emphysema. A positive trend between mortality and duration of employment was not observed for any of the selected causes of death except other and unspecified cancers. The excess in mortality from Hodgkin's disease was confined to 20 years or more since first employment. Mortality from Hodgkin's disease was significantly increased over sevenfold among this group, but the confidence interval around the point estimate was wide (95% CI 1.96 to 18.40).

Mortality was also examined (not shown) by date of hire (pre-1955 versus 1955 or later). There appeared to be a relation between an earlier date of hire and increased mortality from trachea, bronchus, and lung cancer (prior to 1955: SMR = 1.34, 95% CI 1.02 to 1.74; 1955 or later: SMR = 0.79, 95% CI 0.49 to 1.21). Mortality from emphysema was also higher among men hired prior to 1955 (SMR = 2.22; 95% CI 1.29 to 3.56; obs = 17) than among men hired in 1955 or later (SMR = 1.30; 95% CI 0.36 to 3.33; obs = 4), but mortality from pneumoconiosis and other respiratory disease was similar among men hired prior to 1955 (SMR = 1.69; 95% CI 1.17 to 2.36) and men hired in 1955 or later (SMR = 1.68; 95% CI 0.99 to 2.65).

Analyses of multiple causes of death and end stage renal disease incidence were conducted to further evaluate the risk of renal disease among the cohort. The risk of chronic renal disease mortality was not increased (SMR = 1.05; 95% CI 0.69 to 1.54, obs = 26) in the multiple causes of death analysis. The risk of treated end stage renal disease was less than expected overall (SIR = 0.71; 95% CI 0.26 to 1.55, obs = 6). The risk of treated end stage renal disease of unknown aetiology was increased (SIR = 2.73; 95% CI 0.56 to 7.98, obs = 3). This finding was based on three observed cases and the confidence interval was wide. The primary cause of renal failure was missing in the ESRD PMMIS for two of the three observed cases, raising the possibility that these cases were misclassified. Death certificates were available for these cases; renal disease was mentioned on the death certificate for both, but not a specific type or aetiology of renal disease.

Similar results were obtained when the cohort was restricted to men who were employed in conventional mills and when the cohort was restricted to men who were employed in conventional mills that produced both uranium and vanadium concentrates. Results were also similar when

Table 4 Uranium mill workers' mortality (since 1940) from selected causes of death by duration of employment (US referent rates): update of cohort to 1998

	Duration of employment (years)			
Underlying cause of death	1–2 SMR (obs)	3–9 SMR (obs)	⩾10 SMR (obs)	
All deaths	1.01 (352)	0.91 (295)	0.80 (163)†	‡
All cancers	0.94 (75)	0.91 (68)	0.83 (41)	
Trachea, bronchus, and lung CA	1.35 (36)	1.27 (32)	0.58 (10)	‡
Lymphatic and haematopoietic CA other than leukaemia	1.38 (6)	1.22 (5)	1.90 (5)	
Lymphosarcoma and reticulosarcoma	2.15 (2)	1.15 (1)	2.03 (1)	
Hodgkin's disease	1.91 (1)	4.25 (2)	4.57 (1)	
Other lymphatic and haematopoietic CA	1.03 (3)	0.73 (2)	1.56 (3)	
Other/unspecified CA	1.16 (6)	1.65 (8)	2.19 (7)	
Non-malignant respiratory disease	1.99 (53)†	1.12 (29)	1.02 (18)	‡
Emphysema	2.69 (11)†	1.79 (7)	1.11 (3)	
Pneumoconioses and other respiratory diseases	2.53 (29)†	1.07 (12)	1.35 (11)	
Chronic renal disease	1.27 (3)	1.33 (3)	1.53 (2)	

*95% confidence interval excludes the null value (1.0).
†99% confidence interval excludes the null value (1.0).
‡Test for trend p value <0.05.

Table 5 Uranium mill workers' mortality (since 1940) from selected causes of death by length of time since first employment (US referent rates): update of cohort to 1998

	Time since first employment (years)		
Underlying cause of death	<10 SMR (obs)	10–19 SMR (obs)	⩾20 SMR (obs)
All deaths	0.95 (68)	0.87 (125)	0.93 (617)
All cancers	0.62 (7)	0.88 (25)	0.92 (152)
Trachea, bronchus, and lung CA	0.36 (1)	1.45 (13)	1.12 (64)
Lymphatic and haematopoietic CA other than leukaemia	1.35 (1)	0.00 (0)	1.72 (15)
Lymphosarcoma and reticulosarcoma	3.33 (1)	0.00 (0)	2.24 (3)
Hodgkin's disease	0.00 (0)	0.00 (0)	7.19 (4)**
Other lymphatic and haematopoietic CA	0.00 (0)	0.00 (0)	1.18 (8)
Other/unspecified CA	0.00 (0)	1.21 (2)	1.76 (19)*
Non-malignant respiratory disease	1.32 (4)	1.48 (11)	1.42 (85)**
Emphysema	2.39 (1)	2.21 (4)	1.89 (16)*
Pneumoconioses and other respiratory diseases	3.73 (2)	2.24 (4)	1.61 (46)**
Chronic renal disease	3.95 (3)	1.23 (1)	0.92 (4)

*95% confidence interval excludes the null value (1.0).
**99% confidence interval excludes the null value (1.0).

the cohort was restricted to men without known employment in other aspects of the uranium industry.

DISCUSSION

Uranium exposure presents both chemical and radiological hazard potentials. Both the chemical and radiological toxicity are influenced by the biological solubility of a given uranium compound. Poorly soluble uranium compounds are cleared slowly from the lungs and pose a potential internal radiation hazard. More soluble compounds are absorbed rapidly from the lungs, decreasing the radiation hazard, but increasing the potential for renal toxicity.[19 20] In the ore handling and preparation areas of the mills, the uranium in ore dusts consists mostly of insoluble uranium oxides with a relatively small fraction of the more soluble uranium compounds. The potential for exposure to the long lived alpha emitters (uranium-238, uranium-234, thorium-230, radium-226, and lead-210) is greatest in these areas of the mill. In the yellowcake drying and packaging areas of the mill, the uranium in yellowcake consists of a complex mixture of uranium compounds of varying solubility. The composition and solubility of the yellowcake product depends on the drying temperature employed.[19 21] In mills that dry the product at relatively low temperatures (100–150°C), the yellowcake product is high in ammonium diuranate [$(NH_4)_2U_2O_7$] which is highly soluble in lung fluids; in mills that dry the product at relatively high temperatures (370–538°C), the yellowcake is high in uranium oxide (U_3O_8) which is mostly insoluble in lung fluids.[21 22] Based on available data on drying temperatures and drying equipment, four of the five conventional mills in this study used relatively high drying temperatures. The fifth mill did not prepare a dried yellowcake product; rather, it produced filter press cake or a uranium product liquor, depending on the year of operation. Accordingly, most mill workers in this study worked in mills that probably produced yellowcake of relatively low solubility.

Both human and animal data suggest that insoluble uranium compounds and thorium accumulate in the tracheobronchial lymph nodes.[23–26] Because of this, it has been suggested that studies of early uranium workers evaluate the effects on lymphatic tissues.[25] In the previous study of workers at the mills in this study, a significant increase in mortality from lymphatic and haematopoietic malignancies other than leukaemia was observed after 20 years latency, based on six deaths.[7] We also found an excess in mortality from lymphatic and haematopoietic malignancies other than leukaemia but the magnitude of the excess was less than the excess observed in the previous study. The observed excess was due to an excess in both Hodgkin's disease mortality and lymphosarcoma and reticulosarcoma mortality based on four observed deaths each. The ability to evaluate exposure response relations, using duration of employment as a surrogate of exposure, was limited by the small number of observed deaths from these cancers. Of the eight observed deaths due to Hodgkin's disease, lymphosarcoma, and reticulosarcoma in this study, three were observed in the previous study and one was observed in the study by Archer and colleagues.[8]

Hodgkin's disease and non-Hodgkin's lymphoma, a group of lymphomas which includes lymphosarcoma and reticulosarcoma, have not been clearly linked to radiation.[27 28] Data on the risk of death from Hodgkin's disease and non-Hodgkin's lymphoma among uranium or thorium workers are limited. An increased risk of Hodgkin's disease mortality and lymphosarcoma and reticulosarcoma mortality has been observed among uranium processing workers at the Fernald Feed Materials Production Center near Cincinnati, Ohio (SMR = 2.04, 95% CI 0.74 to 4.43, obs = 6; and SMR = 1.67, 95% CI 0.72 to 3.29, obs = 8, respectively)[29] and thorium processing workers (SMR = 1.64, 95% CI 0.33 to 4.79, obs = 3; and SMR = 1.14, 95% CI 0.23 to 3.34, obs = 3, respectively),[30] but not among uranium processing workers at the Y-12 plant at Oak Ridge, Tennessee[31] and Mallinckrodt Chemical Works in St Louis, Missouri[32] or among a combined cohort of uranium and other miners from 11 studies.[33] Hodgkin's disease mortality and incidence and non-Hodgkin's lymphoma incidence was associated with cumulative external radiation dose among workers at the Springsfield uranium production facility; the effects of internal exposures were not evaluated.[34] In general, these studies, like the current study, are limited by the small number of deaths from Hodgkin's disease and non-Hodgkin's lymphoma among exposed workers.

A new finding in this update not previously reported was a small increase in mortality from cancer of the trachea, bronchus, and lung, particularly relative to state rates. We also observed an increased risk of mortality from non-malignant respiratory disease. Mortality from lung cancer was higher based on state rates than US rates, whereas mortality from non-malignant respiratory disease was lower based on state rates than US rates. This is consistent with the relatively low smoking attributable mortality and relatively high chronic obstructive lung disease mortality in Arizona, Colorado, and New Mexico compared to other states.[35] The reason for the discrepancy in smoking-attributable mortality

and chronic obstructive lung disease mortality in many inland western states is unknown. However, the results suggest that regional differences in mortality may explain, in part, the observed excess in non-malignant respiratory disease mortality based on US rates.

The excess in both lung cancer mortality and emphysema mortality was greater among workers hired prior to 1955, when there was little emphasis on dust control and exposures to uranium and silica containing dusts were presumably higher. However, mortality from lung cancer and non-malignant respiratory disease was inversely related to duration of employment. We found no evidence that workers who were hired prior to 1955 were more likely to be short term workers. The inverse relation between lung cancer and emphysema mortality and duration of employment in this study may be a reflection of the healthy worker survivor effect, in which individuals who remain in the workforce over time tend to be healthier than those who leave.[36] Duration of employment may also be a poor surrogate of exposure in this study since exposures are thought to have varied considerably by mill area and over time.

Some data suggest that uranium workers other than miners may be at increased risk of lung cancer[29 31] and non-malignant respiratory disease.[37] Uranium ore dust has been shown to induce pulmonary lesions in animals[23 38 39] and lung cancer in rats.[40] Silica exposure has been reported to lead to the development of silicosis, emphysema, obstructive airways disease, and lymph node fibrosis.[41] Although the carcinogenicity of silica continues to be debated in the scientific community, several investigators have showed an increased risk of lung cancer among workers exposed to silica.[42–44] Vanadium containing compounds have known acute respiratory effects,[45] but it is less clear whether exposure to vanadium can lead to chronic non-malignant respiratory disease.[45 46] In this study, we only observed three deaths from silicosis and unspecified pneumoconioses. The majority of the excess in non-malignant respiratory disease mortality was due to mortality from emphysema and other respiratory disease.

Other potential explanations also exist for the observed excesses in mortality from lung cancer and non-malignant respiratory disease mortality. Smoking data are not available for this cohort, and differences in smoking habits between the cohort and the general population may partially explain the excesses observed. White men in the Colorado Plateau uranium miners cohort were heavy smokers,[6 47] but it is unknown whether the smoking habits of uranium mill workers who never worked underground in uranium mines would be similar to these miners. Even if the mill workers in this study were more likely to smoke than the general population, other investigators have shown that smoking is unlikely to account for SMRs above 1.3 for lung cancer and other smoking related diseases.[48] Other potential factors that may contribute to these excesses include unknown employment in underground uranium mines and employment in other mines with increased levels of radon and radon decay products. It is unlikely that the cohort included many mill workers who also worked as uranium miners. Mill workers who also worked in uranium mines were identified by reviewing the work history records and by matching the cohort to a NIOSH file of over 18 000 uranium miners. All identified uranium miners were excluded from the final cohort. However, members of the cohort may have been more likely to work in other types of mines than the general population.

We found a small non-significant excess in chronic renal disease when using US rates as a comparison; this excess was not apparent when only deaths between 1960 and 1998 were analysed (both underlying cause and multiple cause). Renal effects have been observed among silica exposed workers. Goldminers and industrial sand workers exposed to silica have been found to be at excess risk of death from renal disease and to have increased renal disease incidence.[16 49 50] Low level β_2 microglobulinuria and aminoaciduria has been observed among uranium mill workers exposed to soluble uranium compounds at a mill not in the current study,[9] but little data on chronic renal disease mortality among uranium workers exist. An increase in mortality from chronic nephritis (SMR = 1.88; 95% CI 0.75 to 3.81) was observed among uranium processing workers at Mallinckrodt, based on six observed deaths.[32] An excess in chronic renal disease mortality has been observed among uranium miners (SMR = 1.6; 95% CI 0.7 to 3.0, obs = 9), but the observed excess was not related to duration of employment.[6]

This study may have underestimated the risk of ESRD and renal disease mortality associated with uranium milling. We observed an excess in chronic renal disease mortality during the follow up period 1940–59, but not during the follow up period 1960–98. This suggests that the exclusion of cohort members who died or were lost to follow up prior to 1960 may have been a significant limitation in our ability to evaluate the risk of ESRD and chronic renal disease mortality using multiple cause of death data. Because the cohort is relatively old, approximately 22% of the cohort was excluded from the analysis of ESRD because they died or were lost to follow up before the ESRD PMMIS is first considered complete, which also reduced the statistical power of the ESRD analysis. In addition, the majority of the mill workers in this study were probably exposed to relatively insoluble forms of uranium. The risk of renal disease may be higher in mills using relatively low drying temperatures where the potential for exposure to soluble forms of uranium is greater. The study evaluated chronic renal disease mortality and ESRD and was not able to evaluate the risk of less severe renal effects.

In conclusion, we observed an excess in mortality from haematopoietic and lymphatic malignancies other than leukaemia, trachea, bronchus, and lung cancer, non-malignant respiratory disease, and chronic renal disease. Some of these excesses were based on a small number of deaths and the confidence intervals around the point estimates were wide. Limitations include the lack of smoking data, small cohort size and limited power to detect a moderately increased risk of some of the a priori outcomes of interest, and the inability to evaluate exposure-response relations using individual estimates of exposure to uranium, silica, and vanadium. Because of these limitations and the lack of a positive trend between the observed excesses and duration of employment, firm conclusions about the relation of the observed excesses and mill exposures are not possible.

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Attachment 3

ACUTE CHEMICAL TOXICITY OF URANIUM

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Abstract—**Although human experience with uranium spans more than 200 years, the LD_{50} for acute intake in humans has not been well established. Large acute doses of uranium can produce death from chemical toxicity in rats, guinea pigs, and other small experimental animals, with variation in sensitivity among species. However, there has never been a death attributable to uranium poisoning in humans, and humans seem to be less sensitive to both acute and chronic toxic effects of uranium than other mammalian species studied. Highly relevant data on uranium toxicity in humans are available from the experience of persons administered large doses of uranium for therapy of diabetes and from acute accidental inhalation intakes. Although the data on which to establish oral and inhalation acute LD_{50} for uranium in humans are sparse, they are adequate to conclude that the LD_{50} for oral intake of soluble uranium compounds exceeds several grams of uranium and is at least 1.0 g for inhalation intakes. For intakes of uranium compounds of lesser solubility, acute LD_{50} values are likely to be significantly greater. It is suggested that 5 g be provisionally considered the acute oral LD_{50} for uranium in humans. For inhalation intakes of soluble compounds of uranium, 1.0 g of uranium is proposed as the provisional acute inhalation LD_{50}.**

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Key words: uranium; ingestion; inhalation; injection

INTRODUCTION

General aspects of acute uranium toxicity

Although more than two centuries have passed since the discovery of uranium in 1789 by German apothecary chemist Martin Heinrich Klaproth, the toxicology of this naturally occurring heavy metal is still incompletely understood. Indeed, despite the long experience with uranium, there is no established or generally accepted level for acute toxicity in humans. The first studies of uranium toxicity were carried out in 1824 by Christian Gottleib Gmelin, some 17 years before the metal was purified and 72 years before uranium was discovered to be radioactive. Death occurred in a rabbit given a rather massive for its size, 2 g dose of uranium chloride by gastric lavage, but no deaths or observable effects were noted in dogs fed up to 0.9 g of various uranium compounds, including the nitrate. Emesis was observed in a dog fed 4 g of uranyl nitrate in 30 cc of water, and death occurred within a minute in dogs intravenously injected with 180 and 600 mg of uranyl nitrate. On the basis of his experimental results, Gmelin concluded that uranium ingested orally was "a feeble poison," but that injection would produce death by blood coagulation and irritability of the heart, a property shared with only two other metals—barium and palladium—whose toxicity he also studied (Hodge 1973).

The initial experiments by Gmelin have been followed by numerous other studies, several hundred alone having been carried out prior to the Manhattan District and an even greater number during and subsequent to the effort to build an atomic bomb. In recent years, interest in uranium toxicity has been heightened by the use of depleted uranium (DU) in munitions used in Iraq and Kosovo. Despite intensive effort and hundreds of scientific papers and reports devoted to the study of uranium in recent years, there is still no definitive or even generally agreed upon acute toxic level in humans. Indeed, what is well known and agreed upon is that uranium is both a chemical and radioactive toxin, with chemical toxicity dominant at enrichments below about 7 to 20% (ICRP 1968; Stannard 1988; Brodsky 1996). As enrichment decreases, so does the radiotoxicity, while chemical toxicity remains constant. Thus, at lower enrichments such as DU (0.2%), the toxic effect is overwhelmingly from chemical and not radiological effects.

Chemical toxicity is independent of enrichment and simply a function the mass of uranium that gets into the blood and other tissues, which in turn is a function of the route of entry and solubility of the specific uranium compound taken into the body. Solubility is thus an important parameter in determining the acute toxic level of uranium. Animal study data indicate a wide variation in toxicity among various uranium compounds. The relatively insoluble compounds of uranium, specifically UO_2, U_3O_8, and UF_4,

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are nontoxic even when given orally in large doses on a daily basis. The more soluble UO_3 and UCl_4 are toxic in large doses, while soluble compounds of uranium such as $Na_2U_2O_7$, $UO_2(NO_3)_2$, and UO_4 are toxic in moderate doses. Because of fluoride content and associated effects, particularly following inhalation, UO_2F_2 and UF_6 are chemically toxic at still lower doses (Tannenbaum and Silverstone 1951).

Radiotoxicity is a function of the specific activity that increases with increasing enrichment; the primary concern with radiotoxicity is stochastic effects—i.e., increased probability of carcinogenesis. Because of the large amount of uranium necessary to exceed the threshold for deterministic effects, these are for all practical purposes not of concern with respect to low enrichment uranium intakes. Although the two types of toxicities produce different biological effects, there may well be overlapping cytogenetic and carcinogenetic effects and it is not possible from the available data to determine what fraction of a particular effect was attributable to the metalotoxicity vis-à-vis the radiotoxicity. Moreover, the increased radiotoxicity of high uranium enrichments may potentiate the heavy metal chemical toxicity. However, irrespective of enrichment, acute effects and certainly acute toxicity will be dominated by the chemical toxicity as the quantity of even highly enriched uranium needed to produce deterministic effects or a radiation dose in the LD_{50} range is likely many fold greater than the mass of uranium that would produce a fatal chemotoxic effect.

Natural uranium has a ^{235}U content of 0.71% by weight, and typical light water reactor fuels have enrichments of 2–5 weight percent. DU is 0.2% by weight ^{235}U. Soluble compounds with enrichments of 5% or less are potentially far more hazardous chemically than radiologically, and it is the potential effects on the kidney produced by chemical toxicity that serves as the basis for the various protective limits and concentrations for natural uranium that have been put forth by national regulatory bodies such as the U.S. Environmental Protection Agency (EPA) and the U.S. Nuclear Regulatory Commission (NRC) and other standards setting bodies such as the International Commission on Radiological Protection (ICRP) and National Council on Radiation Protection and Measurements (NCRP). For high enrichment uranium, the standards are based on possible stochastic effects (i.e., carcinogenesis) from radiation exposure. Because of the paucity of human data, protection standards for occupational exposure and intakes by members of the general public have of necessity been primarily based on animal studies, which may not be fully relevant or applicable to humans. Also, the standards established for protection are by and large concerned with long-term chronic exposures as contrasted with acute intakes and hence not necessarily applicable or relevant to the acute toxicity of uranium in man.

Acute toxicity in animals

There are considerable interspecies differences among animals with respect to uranium toxicity; more than 50 years ago Orcutt (1949) proposed the following order of species sensitivity on a per kilogram of body weight basis to the toxic effects of uranium: rabbit > rat > guinea pig > pig > mouse. Based on comparative evaluation of toxic effects, the dog would seem to fall at the lower end of the level of susceptibility scale; the cat at the higher end. This is consistent with the observations of Tannenbaum and Silverstone (1951) who observed that on a per kilogram of body weight basis, the relative sensitivity of mouse, dog, and rabbit was 40:10:1; in other words, per kilogram of body weight, the mouse was 40 times less sensitive to the toxic effects of uranium as the rabbit. For a given intake of uranium, acute results will not only vary with species but also are dependent upon the specific uranium compound. Not unexpectedly, the more soluble uranium compounds exhibit greater uranium toxicity. Uranium hexafluoride is a special case as the compound rapidly reacts with water to produce hydrofluoric acid, which in itself has highly irritating and toxic properties particularly when inhaled.

Comprehensive reviews of the extensive literature of published animal toxicity studies have been performed by Hodge (1973), Durbin and Wrenn (1975), Yuile (1973), Stannard (1988), ATSDR (1999), and Bailey and Davis (2002) and reveal relatively few studies of acute oral toxicity from a single dose. The earliest studies, carried out in the late nineteenth and early twentieth centuries, clearly demonstrated heavy metal toxicity on the kidneys by whatever route of entry in experimental animals but did not provide adequate or unequivocal data with which to establish an acute LD_{50}. What these studies did establish with respect to acute toxicity was the "marked individual variation in response, especially in rabbits" and the apparent high chemical toxicity of uranium administered parenterally (Hodge 1973). A relatively recent (1987) study by Domingo et al., cited by ATSDR (1999), indicates that the acute oral LD_{50} in rats and mice for a single intake is at least 100 mg kg^{-1}, based on instillation of uranium acetate (soluble) by gastric lavage. If directly and linearly extrapolated to man, this is equivalent to a single oral dose of 7 g or more.

Considerably more data are available for longer term oral intakes. In 30-d oral feeding studies in rats with various uranium compounds carried out during the Manhattan District days, relatively insoluble uranium compounds, specifically UO_2, U_3O_8, and UF_4, were nontoxic even at levels of up to 20% by weight in the diet while six

other water soluble uranium compounds resulted in 100% mortality at levels of 2 to 10% in the diet (Maynard and Hodge 1949). Oral LD_{50} values in rats were estimated to be as small as 540 mg kg^{-1} d^{-1} for uranyl fluoride (soluble) with more typical values for this and other soluble uranium compounds studied in the intake range of 1,000 mg kg^{-1} d^{-1}. By linear extrapolation based on weight, the 540 mg kg^{-1} d^{-1} level corresponds to a daily intake of 37.8 g of the compound for a 70 kg reference man, which equates to a daily intake of 29.2 g of uranium, or 876 g over a 30-d period. Assuming for illustrative purposes the validity of the extrapolation to humans, this corresponds to a peak kidney burden of 794 mg or a peak concentration of 2.6 mg g^{-1} of kidney as calculated using the proprietary Integrated Modules for Bioassay (IMBA-URAN)[†] (James 2002). To achieve this same peak kidney burden would require an acute inhalation intake of 31.6 g of this compound (24.4 g of uranium) in the form of Type F material.

For inhaled uranium, the lethal concentration (LC) is determined by several factors including the specific species, chemical form of the uranium, and length or time of exposure. Such studies as have been done are generally protracted exposures over a period of weeks to years. In short term exposure studies in rats and guinea pigs exposed to UF_6, the LC_{50} for a 2-min exposure was estimated at 35 g of uranium per cubic meter; in rats, the LC_{50} for a 5-min exposure was estimated at 26 g per cubic meter. Longer exposure times resulted in concomitantly lower LC_{50} values, but the relationship of exposure time and LC_{50} was inversely related although not necessarily linear. Although pulmonary effects attributable to HF were observed, death was primarily from kidney damage (ATSDR 1999). What is clear from these animal studies is that inhaled uranium can be fatal, but that massive air concentrations and short term exposures are required to produce death.

The dermal LD_{50} value following application of uranyl nitrate to the skin for 4 h before removal by washing was 4.29 and 1.19 g U per kg body weight, respectively, in mice and guinea pigs (Orcutt 1949). The LD_{50} value could not be established for rats because of insufficient data, but the mortality curve for rats was intermediate between that of the mice and the guinea pigs. When applied to the skin of rabbits in a lanolin vehicle, water soluble uranium compounds exhibited the greatest toxicity and resulted in some deaths from kidney failure; animals similarly treated with insoluble compounds UO_2, U_3O_8, and UF_4 yielded no deaths in the experimental group.

[†] Jointly developed by ACJ and Associates, Inc. and the U.K. National Radiological Protection Board.

Acute human exposure to uranium

It is highly significant to note that there have been no reports of deaths in humans following acute or chronic intakes of uranium by whatever route of entry during mankind's more than two centuries of experience with uranium (ATSDR 1999), and humans as a species seem to have a lower order of sensitivity to the toxic effects of uranium than the other mammalian species that have been studied. Since at least the late nineteenth century until after the discovery of insulin by Canadians Frederick Banting and Charles Best and their coworkers in 1921–1922, uranium was used therapeutically in the treatment of diabetes mellitus. An excellent summary and discussion of uranium administered orally for this purpose has been put forth by Hodge (1973).

Reference to the original literature reveals a total of approximately two dozen cases treated by administration of uranyl nitrate (soluble) by mouth, typically thrice daily in individual doses ranging to a reported 30 grain (2 g) for a total daily intake of 5.8 g, sometimes for extended periods of months or even years (Bond 1898; Bradbury 1896; Duncan 1897; West 1895, 1896; Wilcox 1917). No fatalities were observed among the group so treated, nor was kidney pathology reported. Wilcox (1917) indicated that no untoward symptoms were seen in diabetic patients treated for extended periods of months to years with up to 200 mg of uranium daily. Using the IMBA-URAN software, the kidney burdens from the oral intakes were estimated. Kidney burdens for the patients increased over time, and close to peak burdens may have been sustained for some patients for long periods. Acute inhalation intakes of soluble uranium required to produce the same peak kidney burden were estimated using ICRP 66 defaults for absorption fractions for Type F materials, and ICRP 66 default aerosol characteristics (ICRP 1994a).

Equivalent acute inhalation intakes were calculated for the 11 patients with the greatest uranium intakes and are shown in Table 1. For these 11 cases, the calculated acute inhalation required to produce an equivalent kidney burden ranged from 730 mg to 3.8 g of uranium. In several of these cases, calculated acute inhalation intakes exceeding a gram of soluble uranium were required to produce equivalent peak kidney burdens. Some of the patients may have had higher peak kidney burdens than listed, but the description was inadequate to include the additional exposures. Still other patients receiving high oral doses were not included because of uncertainties of the oral intake quantity. In the most recent of these older studies, Wilcox (1917) stated "In all instances in which I have employed uranium nitrate I have never noted any untoward gastric or intestinal symptoms nor any signs of blood or renal disturbances; careful observation has been

Table 1. Calculated peak kidney burdens and equivalent inhalation intake of Type F material from cases involving large oral intakes of soluble uranium.

Reference	Case number	Estimated oral intake (g U)	Calculated peak kidney burden from ingestion (mg U)	Calculated acute inhalation required to produce equivalent kidney burden (mg U)
Bond (1898)	Case 1	268	25	750
Bond (1898)	Case 9	1,329	120	3,800
Duncan (1897)	Case 1	40	24	740
Duncan (1897)	Case 2	31	33	1,000
Duncan (1897)	Case 3	94	65	2,000
Duncan (1897)	Case 4	111	51	1,600
Duncan (1897)	Case 5	50	32	990
West (1895)	Case 1	101	52	1,600
West (1895)	Case 3	38	39	1,200
West (1896)	Case 3	27	24	730
Bradbury (1896)		178	38	1,200

especially directed toward the early detection of the latter." Wilcox patients were treated for extended periods of months to years with up to 200 mg of uranyl nitrate daily.

For the patient with the highest intake, which included an oral intake in excess of one and a quarter kilograms of soluble uranium in 1 year, the peak kidney burden is estimated to be about 123 mg or about 0.4 mg kg^{-1} of kidney. To achieve a comparable kidney burden by inhalation would require an acute inhalation intake of 3.8 g, which corresponds to 0.4 g U per cubic meter of air for a single 8-h (=1 working day) exposure, some three orders of magnitude greater than the occupational Derived Air Concentration (DAC) assuming 5% enriched soluble uranium and ICRP 66 and 68 parameters (ICRP 1994a and b).

Several planned administrations of uranium have been carried out under controlled conditions. The first of these was a study of uranium metabolism carried out as a part of the Manhattan Project at the University of Rochester in the 1940's (Bassett et al. 1948). Six subjects were injected with doses of 70% enriched uranyl nitrate ranging from 6.3 to 70.9 $\mu g\ kg^{-1}$ of body weight. One subject also received a subsequent dose of 54.5 $\mu g\ kg^{-1}$ of body weight. Ignoring the second dose to this subject, the injections correspond to a dose of 0.44 to 4.96 mg for a 70-kg man. A comparable amount administered by mouth would be about 20 to 250 mg based on a gut absorption fraction of 0.02 (ICRP 1996). There was no mortality and the only effects were noted in the highest exposed individual who exhibited a transient slight rise in urinary catalase and protein, suggestive of minor kidney effects. About 70% of the injected uranium was excreted within 24 h.

A second planned administration—the so-called Boston Injection Cases—was a study to determine the potential value of partially enriched uranium as a treatment for malignancies. If enriched uranium concentrated in tumor tissue, subsequent irradiation of that tissue with a beam of thermal neutrons would induce fissions in the uranium, and in particular in the higher cross section 235 isotope, producing fission fragments which in turn would produce high doses localized in the tumor tissues. The experiment involved injection of eight comatose persons ranging in age from 26 to 63 y, terminally ill with severe central nervous system diseases (Struxness et al. 1956; Leussenhop et al. 1958; Hursh and Spoor 1973). Six of the patients were injected with soluble uranyl nitrate, the doses ranging from 72 to 907 $\mu g\ kg^{-1}$ or 5 to 51.4 mg in total (Hursh and Spoor 1973). The other two patients were injected with 573 and 700 $\mu g\ kg^{-1}$ corresponding to a total of 41.1 and 44.2 mg of the less soluble tetravalent UCl_4. Survival times ranged from 2.5 to 566 d post-injection, but in all cases deaths were attributable to the terminal central nervous system disease and not to the uranium.

An obvious difference between the two compounds injected was the 24-h excretion, which averaged 69% of the injected dose for the six cases injected with uranyl nitrate but only 20 and 16.9% for the two cases injected with the less soluble UCl_4. A similar but less striking effect occurred with respect to total uranium excretion; the six cases injected with the more soluble uranyl nitrate excreted an average of 82% of the dose while the two cases injected with the less soluble uranium tetrachloride excreted 68 and 57%.

There was no correlation between survival time and dose of uranium. The physician who reviewed the extensive clinical test data used to evaluate kidney function concluded that the minimum single dose required to produce caltalasuria and albuminuria was about 0.1 mg kg^{-1}, equivalent to about 7 mg in a 70-kg adult male (Leussenhop et al. 1958). However, at autopsy, none of the injected group showed evidence of any pathology, including kidney damage, attributable to uranium. IMBA-URAN was used to calculate the peak kidney burdens and then to determine the acute intake of

Table 2. Calculated kidney burden from experimental injection of uranium.[a]

Paper	Calculated peak kidney burden from injection (mg U)	Calculated acute inhalation of Type F uranium required to produce equivalent kidney burden (mg U)
Hursh and Spoor—Boston		
Injection Experiment Case 6	3.5	110
Injection Experiment Case 7	2.9	97
Injection Experiment Case 8	3.2	90

[a] Note: For cases 7 and 8, the actual oral intake was Type M material, but the acute inhalation intake was calculated for Type F material. This is because IMBA-URAN calculates the same kidney burden for a given activity in the urine regardless of the type of material.

soluble uranium required to produce equivalent peak kidney burdens. It should be noted that when uranium is injected, the resulting peak kidney burdens are the same regardless of the solubility of the uranium if the urine concentrations are the same. Based on the IMBA calculation (James 2002), the upper dose limit in this series of subjects would produce a maximum kidney burden equivalent to an acute inhalation of 108 mg of soluble uranium salts (Table 2). Using the data from the injection cases and a scaling factor derived from published experimental data in rabbits, Leussenhop et al. (1958) estimated the lethal intravenous injection dose for humans to be 1 mg kg^{-1} or 70 mg for 70-kg reference man. Assuming a gut uptake of 0.02 per ICRP (1996), this corresponds to a single oral intake of 3.5 g, in reasonable agreement although somewhat lower than what was obtained with the human cases.

A third planned administration involved oral administration of 10.8 mg of uranium as uranyl hexahydrate dissolved in Coca-Cola to four hospital patients. With this relatively low dose, the patients reported no subjective symptoms, and no urine abnormalities were noted, indicative of no kidney damage (Hursh and Spoor 1973). Also, in a planned inhalation study, a volunteer inhaled uranium for short periods on 17 different days during a period of 23 d, resulting in an estimated lung deposition of 9.5 mg of uranium without ill effect (Harris 1961).

Butterworth (1955) described a controlled experiment in which a volunteer subject ingested a single intake of 1 g of uranyl nitrate (equivalent to 0.47 g of uranium) in 200 mL of water. Following the intake, the subject experienced violent vomiting, diarrhea, and mild albuminuria. Peak urinary concentrations of uranium, based on two 30 mL specimens, was estimated as 8 mg U per liter. Symptoms cleared within 24 h of the intake. Based on an uptake fraction of 0.02 via the gut, the uptake in this case would have been 9.4 mg. This dose was, of course, not lethal, and the symptoms experienced by the subject may have been attributable to unusual susceptibility on the part of the subject, or to psychological reasons as the subject was aware that he was ingesting a toxic substance (Hursh and Spoor 1973).

In addition to the planned administrations described above is a clinical report from Australia of a deliberate ingestion of 15 g uranium acetate, equivalent to 8.4 g of uranium, along with an unknown quantity of benzodiazipine, by an individual attempting suicide (Pavlakis et al. 1996). Based on 2% uptake via the gut, this would correspond to an uptake of 168 mg; ATSDR (1999) estimated the dose to be 131 mg U kg^{-1} corresponding to an intake of 9.1 g U for a 70-kg reference man. Chelation therapy with both Ca EDTA and Ca DTPA, the former given in conjunction with sodium bicarbonate, failed to significantly increase urinary U excretion. The individual, who survived, suffered from rhabdomyolysis as evidenced by increased serum creatinine kinase, refractory anemia, myocarditis, liver dysfunction with a disproportionate coagulopathy, paralytic ileus, acute renal failure treated by dialysis for two weeks, and glycosuria. Six months after the acute intake, significant renal impairment was present along with a persistent incomplete Fanconi's syndrome. Fanconi's syndrome is an impairment of proximal tubule function characterized by excess amounts of glucose, bicarbonate, phosphates, uric acid, potassium, sodium, and certain amino acids being excreted in the urine. Fanconi's syndrome is relatively rare and may be hereditary and associated with certain genetic defects, or a result of medications or heavy metal poisoning.

Although it is not known whether the individual would have survived without treatment, given his medical history, it is likely that other than the kidney effects and glycosuria, the effects observed in this case were primarily if not wholly attributable to other causes. The observed kidney effects attributed to uranium may have been exacerbated by other medical conditions or potentiated by drugs, as was noted by Pavlakis et al. (1996): "In view of his established history of gastrointestinal ulceration, it is likely that an impaired mucosal barrier aided absorption and significantly increased his toxic insult." His rather extensive medical history included established diagnoses of hyperlipidemia, muscle enzyme deficiency, hypertension, and hypogonadism; he also indicated that suffered from chronic peptic ulcer, asthma, gout, migraine, and more significantly, from renal calculi and urinary tract infections. This patient had been diagnosed with a borderline personality disorder and had undergone psychotherapy for more than 20 y. Perhaps even more notably, he was an admitted self-medicator and abuser of prescription drugs, having regularly taken some 14 drugs orally in the 12 mo preceding his suicide

attempt in addition to using a number of topical agents including antifungals and steroid creams, and topical eye drops. In particular, the Fanconi's syndrome is known to be induced by certain medications (Izzedine et al. 2003). The effect of benzodiazipine, which was taken with the uranium, or any of the other drugs which the individual had taken, on the toxic effects of uranium is unknown, and given his medical and psychological history, even the amount of uranium ingested is open to question as he could have taken doses prior to the reported ingestion. Thus, limited credence should be placed on this case.

Acute accidental occupational exposures provide additional data relative to acute uranium toxicity. Three men who received a massive airborne exposure to UF_6 in 1944 were evaluated 38 y after their exposure and found to have no apparent long term health effects, including kidney toxicity, attributable to their exposure. The "best" estimate of initial lung deposition of uranium in the three cases was about 40–50 mg, but the range was very broad: 1.21 to 110 mg, 1.3 to 480 mg, and 2.1 to 827 mg in the three cases (Kathren and Moore 1986). The intake, of course, was considerably higher than the initial lung deposition since most of the inhaled material would likely never reach the lungs, being removed in the upper respiratory tract, indicating that an acute inhalation intake of tens to a few hundred milligrams of uranium is well below the LD_{50}. In another acute inhalation exposure also involving UF_6, 31 workers received acute intakes estimated to range from 0.47 to 24 mg with no long-term health effects or significant short-term effects other than possibly mild and reversible effects on the proximal tubules of the kidney (Fisher et al. 1990).

Zhao and Zhao (1990) describe the case of an individual in China who suffered an acute accidental inhalation of natural UF_4 powder. Various renal effects including proteinuria, elevated non-protein nitrogen, and aminoaciduria were observed 1 wk post exposure. Urinary excretion characteristics of this case were quite unusual and not in consonance with recognized biokinetic models for uranium. The case described by Zhao and Zhao (1990) shows a more or less constant initial urinary excretion of about 125 μg for the first 10 d, dropping to 85 μg day 15 and then rapidly increasing to a peak of 3,174 μg at post-exposure day 63. After post-exposure day 63, urinary levels of uranium followed an exponential decrease with a half-life of 11.75 d, declining to normal background level 3 y after exposure.

This unusual urinary excretion pattern is indicative of gradual or slowed clearance from the lung to the blood and it is interesting albeit perhaps a bit speculative to note that the exponentially increasing urinary excretion during the first 2 mo post exposure is reflective of a gradual release of the acutely inhaled uranium from the lungs to the blood, or in other words a holdup in the lungs. Indeed, the excretion curve for this case is rather similar although on a much larger scale to what was observed by Kathren and Moore (1986). In the three of four males acutely exposed to tens of milligrams of UF_6 who exhibited symptoms of pulmonary edema, the initial urinary output dropped to below detection limits on day 5 or 6, followed by a resumption of excretion on post-exposure day 8 in two cases and day 10 in the third. Kathren and Moore (1986) attributed this unusual excretion pattern to a temporary retention of a portion of the U inhaled in a metabolic pool or compartment, likely from the edema resulting from inhalation of corrosive chemicals and physical irritants, specifically including HF, which is produced by the decomposition of UF_6 when combined with water or tissue fluids.

Total urinary excretion for this case is reported as 86.7 mg, which was also considered to be the "estimated inhaled dose." However, to achieve this level of urinary excretion, intake would have to be significantly greater. Using the total urinary excretion of 86.7 over a 1,065-d period, as reported by Zhao and Zhao (1990), and the current ICRP model for U, the intake is calculated as 1.46 g, truly a remarkably large amount. A similar analysis was performed by Bailey and Davis (2002) using the earlier ICRP 30 indicated an inhalation intake of 600 mg.

A second case was originally reported by Jia-Mei and Ji-Xiong in 1982 in the Chinese literature and briefly described by Zhao and Zhao (1990). A worker was burned over 71% of his body with a hot (108°C) mixture of uranyl nitrate and uranium oxide. Urine monitoring from day 1 to 7.5 y post exposure indicated a total excretion of 130 mg of uranium, plus 22 mg excreted in the first 24 h after the accident. This indicates an intake of at least 152 mg, likely mostly through dermal absorption, although inhalation intake could have been significant but is not known. Oliguria began on post-exposure day 1; only 100 mL of urine was excreted on that day, dropping to a nadir of 10 mL on post-accident day 7. Renal function began to improve at post-exposure day 8 as evidenced by increased urinary output, returning to normal 1 mo post exposure and remaining normal throughout the 7.5 y post-accident follow up period. Renal tubule dysfunction was evident in early laboratory studies, which showed grade 3+ proteinuria and elevated non-protein nitrogen and carbon dioxide combining power. It is entirely likely that the oliguria and biomarkers in the urine were mainly, if not entirely, attributable to the severe burns suffered by this individual and the associated loss of water through wound effusion, and not to his intake of uranium.

McGuire (1991) examined the literature and calculated LD_{50} values for uranium derived from reports by Just (1984) and Just and Emler (1984) that he cites. For uranium in a 70-kg person, Table 2 in McGuire (1991) gives 114 mg of uranium in a 70-kg person as the LD_{50} which presumably refers to systemic uranium and thus is in the middle of the range of 70–140 mg for the $LD_{50/30}$ in a reference man put forth a few years earlier in an unpublished work by Lincoln and Voelz (1988; cited in Fisher et al. 1990). McGuire's value is equivalent to an acute oral intake of 5.7–11.4 g based on a gut uptake factor of 0.01–0.02 (Wrenn et al. 1985; Dang et al. 1992; Harduin et al. 1994; Medley et al. 1994; ICRP 1996; Limson Zamora et al. 2002). This is approximately the upper level of therapeutic doses of uranium given daily for treatment of diabetes without apparent ill effect, as discussed above, and thus might be considered a lower limit of the actual acute oral LD_{50}, which is likely several fold greater but indeterminate with the available data.

For inhaled uranium, McGuire uses the 114 mg LD_{50} value for systemic uranium and the now obsolete ICRP 30 lung model to obtain a value of 230 mg for uranium intake by a 70-kg person, defining "intake" as the total amount of material inhaled into the body, and basing his calculation on the following: "For 1-micron (sic) uranium particles in soluble form, about 49% of the intake will be excreted through the kidneys according to ICRP 30 models" (McGuire 1991). However, unlike the ICRP model, McGuire assumes that all the uranium entering the urine over the course of the next 50+ years enters the kidney instantaneously. Thus, relative to the model, NUREG 1391 significantly overestimates the fraction of inhaled soluble particulates that will get into the systemic circulation at any instant in time yielding a conservative underestimate of the LD_{50} based on intake.

This assumption in NUREG 1391 thus ignores the kinetics and assumes that a single inhalation intake that results in a total systemic burden of 114 mg of uranium over the lifetime of the individual is equivalent to the LD_{50}. However, using the current ICRP lung model (ICRP 1994a, 1994b) for a single acute intake of a 5 μm Activity Median Aerodynamic Diameter (AMAD) aerosol, which is the default value, the comparable fraction is 27.6% resulting in a calculated intake 1.77 times greater than that obtained by McGuire (1991) or about 410 mg using his methodology as shown: assuming the 114 mg systemic uranium deposition in a 70-kg person to be in fact the LD_{50}, and accounting for the trivially small additional fraction absorbed from the larger fraction of inhaled material cleared via the gut, suggests that the LD_{50} for an acute inhalation intake of soluble uranium is 410 mg for a 5 μm AMAD aerosol. If inhaled over a single 8-h workday, this corresponds to a mean air concentration of 43 mg m^{-3}. This is, of course, a conservative lower limit on the LD_{50} for inhaled soluble material. The actual LD_{50} for inhaled uranium is likely to be much larger given the actual human data presented in Table 1. Even the example cited above assumes a single intake with essentially immediate and full transfer to the systemic body which of course is not the case. The inhaled uranium will be gradually absorbed into the systemic circulation, and most of it (approximately 70–80%) excreted within 24 h. Thus, the buildup of systemic uranium will be dictated by the kinetics of absorption from the lung and excretion of the absorbed material via the kidneys.

Note that the above assumes that a systemic deposition of 114 mg in a 70-kg person to be the LD_{50} as determined by McGuire (1991) based on the data put forth by Just (1984) and Just and Emler (1984). Reference to the original work by Just is illuminating, and raises the question of whether it provides an adequate basis on which to establish a human LD_{50} for acute intake of uranium. The basis for the LD_{50} proposed by Just (1984) and Just and Emler (1984) was developed as follows:

1. A panel of five scientists—four from the University of Rochester and one from the University of Utah—with expertise in the chemical toxicity of uranium was assembled to develop preliminary guidelines for estimating the toxicity of soluble uranium and HF;
2. This led to a series of toxicity experiments with guinea pigs and rats completed some three years later; and
3. Using primarily data from the animal toxicity experiments, plus limited human data primarily from the injection cases, a panel of four of the original five scientists used the Delphi method to develop, first independently and then by consensus, toxicity levels for soluble uranium and HF.

The expert panel concluded that for soluble uranium absorption 1.63 mg U per kg of body weight was the LD_{50} level. The members of the panel differed on the level, 1.63 mg being the average. The expert panel also put forth an LD_{50} exposure level, defined as the product of air concentration and exposure time of 35,000 mg U per cubic meter-minutes for inhaled uranium based on up to 60 min of exposure and a breathing rate of 7.5 L min^{-1}. This corresponds to a total intake of 15.75 g, a rather significant amount nearly two orders of magnitude greater than that computed by McGuire (1991), and an order of magnitude greater than that calculated using the current ICRP lung model and taking the kinetics into account. Unlike McGuire (1991), who clearly defined the aerosol parameters, the

expert panel report was mute with respect to fractional uptake from inhalation of this quantity of soluble uranium.

The expert panel also examined the chemical toxicity of insoluble uranium compounds, specifically uranium tetrafluoride, but did not come up with a specific LD_{50} value, noting that as insoluble uranium compounds are less toxic than soluble ones, the methodology and values used for soluble uranium compounds could be conservatively applied to insoluble compounds.

Finally, it bears mention that epidemiology studies in human populations, whether occupationally exposed or chronically exposed via high natural uranium levels in drinking water, have revealed no definitive evidence linking uranium exposure to human deaths (ATSDR 1999).

Threshold for chemotoxic effects on the kidney

It has long been generally known and accepted that the kidney is the most sensitive organ for chemotoxic effects of uranium, which manifest as proximal tubule effects with possible glomerular effects at higher exposures. Biomarker indications of kidney damage are many and include glucosuria, albuminuria, elevated beta-2 microglobulin and elevated blood creatinine levels, with specific types of damage indicated by specific biomarkers. The nephrotoxic threshold limit for chronic low-level exposure is typically taken to be 3 μg of uranium per gram of kidney (Alexander 1984; ATSDR 1999; Brodsky 1996; Kathren and Weber 1988; Leggett 1989; Spoor and Hursh 1973; Stannard 1988).

Some have indicated that this level may be too high and recommend additional study if not outright lowering of this nephrotoxic threshold level. Morrow et al. (1982) recommended a five-fold reduction to 0.6 μg U g^{-1} kidney based on animal studies carried out in his laboratory. For prudence, Leggett (1989) proposed a ten-fold reduction to 0.3 μg U g^{-1} kidney "... until more is known about subtle physiological effects of small quantities of U in the kidneys" based on a comprehensive review of the literature. Zhao and Zhao (1990) suggested the permissible kidney burden should not exceed 0.26 μg U g^{-1} kidney, basing this on a single case with an apparently massive acute inhalation intake of UF_4 powder. This case, which was only sketchily reported and was reviewed briefly above, had an atypical urinary excretion pattern and did not exhibit biomarkers of kidney damage until 68 d post exposure, at which time urinary excretion levels were approximately at their peak, some 20-fold greater than on the first day post accident and indicative of a kidney content of several tens of μg U g^{-1} kidney.

More recently, Brodsky (1996) reviewed the literature and concluded that a 3 μg U g^{-1} kidney concentration was "... unlikely to cause kidney damage over a lifetime." Brodsky's conclusion appears to be consistent with recent comprehensive studies and evaluations that have been done, including those by the ATSDR (1999), the U.S. National Academies (Fulco et al. 2000) and British Royal Society (Baily and Davis 2002). Also of interest, a very recent NRC guidance document for personnel responding to radiological emergencies stated "There are no known long term chemical injuries from uranium intakes that are sublethal," which would seem to imply that intakes of uranium no matter how large that did not cause death would not result in permanent kidney damage and further notes that permanent renal damage has never been observed in humans (Athey et al. 2007).

McGuire (1991) also provides an estimate for the threshold intake required for permanent kidney damage. This question was put to the same expert scientific panel described above, but only one member ventured an estimate relative to permanent kidney damage. The estimate of this single scientist was 0.3 mg U kg^{-1} body weight, which corresponds to a body burden of 21 mg for a 70-kg reference man or an inhalation intake of about 40 mg based on ICRP Publication 30 (ICRP 1979) or 75 mg based on the current ICRP model (ICRP 1994a, 1996), and the highly conservative assumption that all the uranium that passes into the urine over the next 50 + years enters into the kidney instantaneously. Assuming the fraction reaching the kidney is 0.12, this corresponds to a peak kidney burden of 8.4 μg U g^{-1} kidney.

Review of the high level acute intake cases described above and especially Table 1 indicates peak kidney burdens of tens of milligrams of uranium, and peak concentrations an order of magnitude or more greater than the 3 μg U g^{-1} kidney considered to be the threshold for permanent kidney damage, apparently incurred without significant or long lasting ill effects. This suggests that the 3 μg U g^{-1} kidney concentration is adequate and quite likely has a safety factor of 10 to 100, as has been suggested by its framers.

CONCLUSION

In summary, large acute doses of soluble uranium can produce death in experimental animals, with variation in sensitivity among species. Insoluble uranium compounds are reportedly nontoxic. There has never been a death attributable to uranium poisoning in humans, and humans seem to be less sensitive to both acute and chronic toxic effects of uranium than other mammalian species studied. Although the data on which to establish oral and inhalation acute LD_{50} for uranium in humans are sparse, they are generally consistent and adequate to conclude that the LD_{50} for oral intake of

soluble uranium compounds exceeds several grams of uranium and is at least 0.41 g, and likely greater, for inhalation intakes of soluble compounds of uranium. It is suggested that 5 g be provisionally considered the acute oral LD_{50} for uranium in humans; for inhalation intakes of soluble compounds of uranium, 1.0 g of uranium is proposed as the provisional acute inhalation LD_{50}.

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Attachment 4

Grand Rounds: Nephrotoxicity in a Young Child Exposed to Uranium from Contaminated Well Water

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Context: Private wells that tap groundwater are largely exempt from federal drinking-water regulations, and in most states well water is not subject to much of the mandatory testing required of public water systems. Families that rely on private wells are thus at risk of exposure to a variety of unmeasured contaminants.

Case presentation: A family of seven—two adults and five children—residing in rural northwestern Connecticut discovered elevated concentrations of uranium in their drinking water, with levels measured at 866 and 1,160 µg/L, values well above the U.S. Environmental Protection Agency maximum contaminant level for uranium in public water supplies of 30 µg/L. The uranium was of natural origin, and the source of exposure was found to be a 500-foot well that tapped groundwater from the Brookfield Gneiss, a geologic formation known to contain uranium. Other nearby wells also had elevated uranium, arsenic, and radon levels, though concentrations varied widely. At least one 24-hr urine uranium level was elevated (> 1 µg/24 hr) in six of seven family members (range, 1.1–2.5 µg/24 hr). To assess possible renal injury, we measured urinary beta-2-microglobulin. Levels were elevated (> 120 µg/L) in five of seven family members, but after correction for creatine excretion, the beta-2-microglobulin excretion rate remained elevated (> 40 µg/mmol creatinine) only in the youngest child, a 3-year-old with a corrected level of 90 µg/mmol creatinine. Three months after cessation of well water consumption, this child's corrected beta-2-microglobulin level had fallen to 52 µg/mmol creatinine.

Significance: This case underscores the hazards of consuming groundwater from private wells. It documents the potential for significant residential exposure to naturally occurring uranium in well water. It highlights the special sensitivity of young children to residential environmental exposures, a reflection of the large amount of time they spend in their homes, the developmental immaturity of their kidneys and other organ systems, and the large volume of water they consume relative to body mass.

Key words: beta-2-microglobulin, drinking water, drinking water standards, groundwater, nephrotoxicity, private wells, uranium. ***Environ Health Perspect*** **115:1237–1241 (2007). doi:10.1289/ehp.9707 available via *http://dx.doi.org/* [Online 22 May 2007]**

Groundwater is the principal source of drinking water for 14–15 million (14%) of the 105.5 million homes in the United States and for approximately 42 million people [Centers for Disease Control and Prevention (CDC) 2003; U.S. Census Bureau 2000; U.S. Environmental Protection Agency (EPA) 2006].

Groundwater is at risk of contamination by a wide variety of industrial pollutants and naturally occurring toxic chemicals. Industrial chemicals that have been identified in groundwater include benzene, methyl *tert*-butyl ether, nickel, perchlorate, perchloroethylene, pesticides, phenol, and trichloroethylene [Agency for Toxic Substances and Disease Registry (ATSDR) 1996, 1997a, 1997b, 1998, 2005b, 2005c; Baker et al. 1978; U.S. EPA 2006]. These contaminants are most commonly found near chemical and pesticide production facilities, hazardous waste sites, roads, and railways. Naturally occurring toxic chemicals that have been documented in groundwater include arsenic, manganese, radon, and uranium (ATSDR 1999, 2005a; U.S. EPA 2006). These materials may be present in especially high concentrations in mining districts, but also occur widely in certain geologic formations, especially in mountainous areas of the United States (U.S. EPA 2006; Walsh 2003).

Private wells that tap groundwater have been associated with episodes of human exposure to toxic chemicals (U.S. EPA 2006). Private wells in the United States are largely exempt from state and federal drinking water regulations, and thus in most states they are not subject to much of the mandatory testing that is required of public water supplies under the provisions of the Safe Drinking Water Act Amendments of 1996 (1996). In most locations, well water is routinely tested only for pH, bacteria, and a small number of chemical contaminants. Uranium is not commonly among the chemicals tested. Because of increasing urban sprawl with continuing movement of populations from urban centers to previously rural areas that lack public water supplies (Frumkin et al. 2004), a growing number of private wells are being drilled in the United States. Unless changes are mandated in current testing requirements, the number of people at risk of exposure to toxic chemicals in groundwater will therefore likely increase.

Exposures to chemical contaminants in groundwater have caused disease and disability in exposed populations. The prevalence and severity of these effects reflect the intensity, the duration, and the developmental timing of exposure. Reported health effects have included diminished intelligence after prenatal exposures to lead and manganese; peripheral vascular disease and skin cancer after childhood exposure to arsenic; and fatal methemoglobinemia after exposure in infancy to nitrates (Ahsan et al. 2006; Campbell 1952; Needleman et al. 1979).

Infants and young children are especially vulnerable to chemical contaminants in drinking water. This heightened vulnerability reflects the disproportionately great water consumption of young children, who drink 7 times as much water per kilogram body weight per day as the average adult (Ershow and Cantor 1989) (Figure 1). It also reflects the inherent biological vulnerability of the young, which is a consequence of their rapid growth and development and their relative inability to detoxify and excrete many exogenous chemicals [National Research Council 1993].

We present a case of a family who was exposed to naturally occurring uranium in groundwater from their private well in Connecticut. Although all family members

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had evidence of exposure, the only family member with evidence of nephrotoxicity was the youngest child.

Case Presentation

In September 2000 a family of seven—two adults and five children 3, 5, 7, 9, and 12 years of age—living in a development in rural northwestern Connecticut discovered highly elevated levels of uranium in the drinking water of the home where they had resided for 5 years. The home water was supplied by a private well that tapped groundwater at a depth of approximately 500 feet. The family had used water from this well for cooking, drinking, and bathing from the time that they had moved into the home until discovery of the contamination.

The family first became aware of the possibility of uranium exposure after a neighbor was found to have markedly elevated levels of uranium in her hair. This neighbor had had her hair tested for a range of metals because she was concerned that she had been exposed to mercury from her dental fillings; mercury levels in the neighbor's hair were not elevated.

After the discovery of elevated levels of uranium in her hair, the neighbor had the water from her well tested for uranium. Her well water was found to contain uranium at a level of 41 pCi/L. Applying the U.S. EPA conversion factor of 0.9 pCi/µg (an estimate based on the ratio of uranium species found typically by the U.S. EPA in well water) this translates to 46 µg/L, a value above the U.S. EPA maximum contaminant level (MCL) for uranium in public water supplies of 30 µg/L (National Primary Drinking Water Regulations 2001a). In response to their neighbor's discovery of uranium in her well water, the index family had their well water tested. Initial testing in a private laboratory (RSA Laboratories, Inc., Hebron, CT) on 28 September 2000 returned a level of 866 µg/L (779 pCi/L), a value also above the U.S. EPA MCL. Repeat measurement by the State of Connecticut Department of Public Health Laboratory (Hartford, CT) on 12 October 2000 returned a level of 1,160 µg/L (1049 pCi/L). Investigation for other contaminants revealed that arsenic was present at a concentration of 0.104 mg/L (above the current U.S. EPA MCL of 0.01 mg/L) (U.S. EPA 2001b) and radium 226 and 228 combined activity was measured at 15.61 pCi/L (above the U.S. EPA MCL for combined activity of 5 pCi/L). The family was advised to immediately stop consuming water from their home well.

Environmental assessment. Environmental investigation was undertaken by the State of Connecticut Department of Public Health (Hartford, CT). Agency staff collected samples from the index family, as described above, and from other nearby homes in the development where the family resided. The State of Connecticut Department of Public Health Laboratory analyzed the water samples for uranium and radon and air samples for radon.

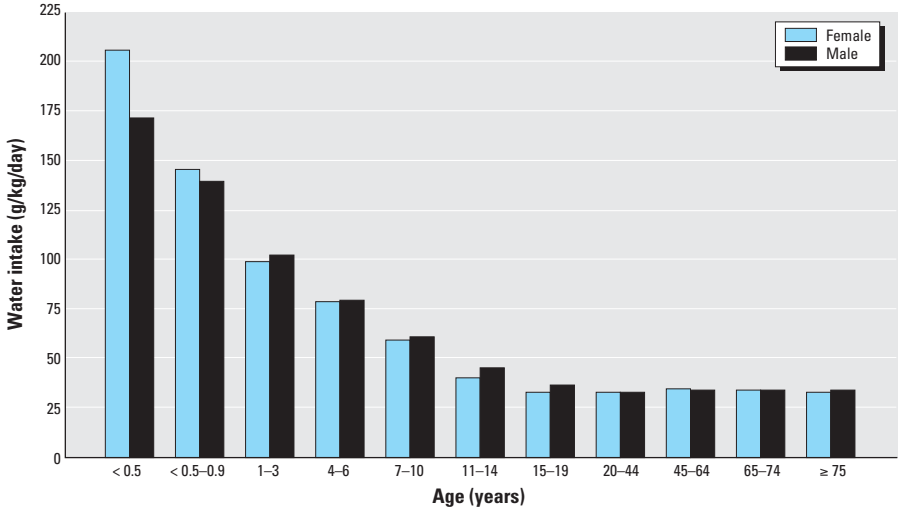

Figure 1. Mean daily intake of total water per unit of body weight by age group and sex. Figure reprinted from Ershow and Cantor (1989), with permission from Life Sciences Research Office.

Environmental assessment revealed that four of 11 homes tested in the development where the index family resided had elevated levels of uranium in their well water. The level of uranium contaminations was quite variable, with very high levels occurring in some homes and almost none in adjoining homes (Table 1).

Geologic assessment by the Connecticut Department of Public Health determined that this housing development had been built in the Appalachian foothills of northern Connecticut and that it sat above the "Brookfield Gneiss," a metamorphic rock formation common throughout the Appalachian ridges of western New England. Geologic formations similar to the Brookfield Gneiss have been shown to contain concentrated pockets of natural uranium (Robinson and Kapo 2003; Walsh 2003). The Connecticut Department of Public Health considered naturally occurring metals in the Brookfield Gneiss to be the most likely source of the uranium and other minerals detected in the index family's well water. Review of town and county records and of business directories revealed no evidence of any current or past metal mining or other industrial source of uranium or other toxic metals in the area.

Clinical assessment. To assess the uranium exposure of family members, a 24-hr measurement of urine uranium was obtained by a pediatric nephrologist in our group (L.S.) from all family members in October 2000, 4 weeks after cessation of well water consumption, an event that had occurred on 28 September 2000 (Table 2). An additional 24-hr collection was obtained from the parents in November 2000, 6 weeks after cessation of well water consumption. At least one urine uranium measurement was found to be elevated in six of the seven family members. Levels ranged from < 1 µg/L to 6.2 µg/L, well above the mean concentration in the U.S. population of 0.009 µg/L (CDC 2005). When adjusted for urinary volume, uranium excretion was 1.1–2.5 µg uranium/24 hr (values above the < 1 µg uranium/24 hr expected in an unexposed population). Although elevated levels of radon, radium, and arsenic were found in the family's water, biological measures were not pursued because these substances are not known to have chemical nephrotoxic effects. The MCLs for these substances are designed to protect against the elevated risk of cancer that exposure confers.

Table 1. Well water uranium in homes, suburban development, northwestern Connecticut, October–November 2000.

Water uranium	Case family (Home 1)	Home 2	Home 3	Home 4	Home 5	Home 6	Home 7	Home 8	Home 9	Home 10	Home 11
pCi/L	1049.00	41.00	0.19	12.50	7.50	12.20	7.50	469.00	14.70	32.00	19.00
µg/L[a]	1165.56	45.56	0.21	13.89	8.33	13.56	8.33	521.11	16.33	35.56	21.11

Testing done by CT DOH Lab.
[a]Mass calculated from activity measurements using EPA conversion factor of 0.9 pCi/µg.

To assess the possible occurrence of renal tubular injury, measurements were made (by L.S.) in family members of urine beta-2-microglobulin levels (Table 2). Beta-2-microglobulin is a low-molecular-weight (11.8 kD) protein that is freely filtered at the glomerulus and avidly taken up and catabolized by the proximal tubule (Brenner 2004). Elevation of urine beta-2-microglobulin is a nonspecific marker of proximal tubule damage. Elevated beta-2-microglobulin levels (normal reference range in adults is < 120 µg/L) were found in five of the seven family members and ranged from 89 to 530 µg/L, thus suggesting the possible presence of proximal tubular injury. To adjust the beta-2-microglobulin for urine volume and body mass in children, a urinary beta-2-microglobulin excretion rate (micrograms beta-2-microglobulin per gram creatinine) is commonly calculated (Tomlinson 1992). The beta-2-microglobulin excretion rate was normal (< 40 µg/mmol creatinine) in all family members except for the youngest child. In this 3-year-old, who unlike other family members had spent virtually all of her life in the house, the urinary beta-2-microglobulin excretion rate was 90 µg/mmol creatinine, a value more than twice the reported upper limit of normal. Three months after the family had ceased consuming water from the home well (January 2001), this child's urinary beta-2-microglobulin excretion rate had fallen to 52 µg/mmol creatinine. There was no evidence of other proximal tubule dysfunction, as evidenced (in January 2001) by the absence of glucosuria, phosphate wasting (with normal values for the tubular reabsorption of phosphate), bicarbonate wasting, or metabolic acidosis (Table 3).

Analytic methods for key environmental and biological tests are reported in Table 4.

Discussion

This case underscores the potential hazards of consuming groundwater from private wells. It emphasizes the need to test drinking water for a wide range of potential contaminants. Specifically, it documents the potential for significant residential exposure to naturally occurring uranium in well water. The case also highlights the special sensitivity of young children to environmental exposures in the home—a reflection of the large amount of time young children spend in their homes, their developmental immaturity, and the large volume of water they consume relative to their body mass.

Uranium is a commonly occurring radioactive mineral. It is found naturally in geologic formations such as the Brookfield Gneiss. In the formation of metamorphic rock, uranium is distributed very unevenly. It typically deposits in areas of low pressure and irregular cracks. Therefore, concentrations can vary significantly within a small area. The level of uranium that appears in drinking water depends on the flow of water through complicated fracture networks within the rock, as well as on the pH, calcium content and other characteristics of groundwater. For these reasons, concentrations of uranium in closely adjoining wells may be quite different, as was seen in this case. This pattern of significant local variability in concentrations of uranium has been observed in various locations across North America (Natural Resources

Table 3. Urine analysis and electrolyte measurements of the five children (1–5).

Characteristic	1	2	3	4	5
Age [years (sex)]	3 (F)	5 (M)	7 (M)	9 (M)	12 (M)
Test date	Jan 2001	Jan 2001	Jan 2001	Jan 2001	Jan 2001
Serum [HCO_3^-] (mmol)	21.9	23.0	24.4	28.1	27.7
TRP (%)[a]	92	92	95	92	91
pH	6	7	6	7	6.5
Specific gravity	1.015	1.025	1.020	1.025	1.020
Glucose dip	Negative	Negative	Negative	Negative	Negative
Protein dip	Negative	Negative	Negative	Negative	Negative

[a]TRP (%) = tubular reabsorption of phosphate = $[1 - (U_p \times S_{Cr})/(S_p \times U_{Cr})] \times 100$, where U_p, S_{Cr}, S_p, and U_{Cr} are urine phosphate, serum creatinine, serum phosphate, and urine creatinine concentrations, respectively. Normal values are > 85%.

Table 4. Uranium and β2-microglobulin analytic methods.

Analysis	Method
Uranium in water (RSA Laboratories, CT)	Ion exchange separation with alpha-spectrometry detection (APHA 1992)
Uranium and radium in water (Connecticut State Department of Public Health Laboratory)	Gas proportional analysis; EPA Method 908-0 (U.S. EPA 1980)
Uranium in urine (quest diagnostics sent out to Medtox Laboratories, St. Paul, MN)	Inductively coupled plasma/mass spectrometry
β2-microglobulin in urine (quest diagnostics, Medtox Laboratories, St. Paul, MN)	Fixed rate time nephelometry
Creatinine in urine (quest diagnostics, Medtox Laboratories, St. Paul, MN)	Spectrophotometry

Table 2. Urine measurements of uranium, β2-microglobulin (β2-M), and creatinine.

Subject	Age [years (sex)]	Test date	Uranium in 24 hr (µg/L)	Urine volume in 24 hr (L)	Uranium excretion (µg/24 hr)[a]	Uranium excretion (ng/mmol creatinine)	β2-M (µg/L)[b]	Creatinine (mmol/L)	β2-M excretion rate (µg β2-M/mmol creatinine)[c]
1	3 (F)	Oct 2000	6.2	0.4	2.5	1,062	532	5.84	90.8
		Jan 2001					267	5.13	52
2	5 (M)	Oct 2000	4.9	0.475	2.3	690	100	7.1	14.1
		Jan 2001					174	9.08	19.2
3	7 (M)	Oct 2000	< 1	0.7	< 1	NA	140	7.4	18.9
		Jan 2001					343	12.24	28
4	9 (M)	Oct 2000	4.1	0.5	2.1	532	90	7.7	11.7
		Jan 2001					89	11.12	8
5	12 (M)	Oct 2000	1.2	1.4	1.7	97	280	12.4	22.6
		Jan 2001					167	9.8	17
6	34 (M)	Oct 2000	< 1	0.85	< 1	NA	100	18.5	5.4
		Nov 2000	1.2	1.1	1.3				
7	37 (F)	Oct 2000	1.3	0.65	0.8	92	130	14.1	9.2
		Nov 2000	1.1	1.025	1.1				

Abbreviations: F, female; M, male; NA, cannot be calculated. Oct and Nov 2000 samples are 24-hr collections; Jan 2001 sample is a random sample. Conversion factor: 1,000 ng = 1 µg. β_2-M excretion rate = urinary β-2-M:creatinine ratio.
[a]Uranium reference level < 1.0 µg/24 hours unexposed population. [b]β_2-M reference level ≤ 120 µg/L (adults 21–57 years of age). [c]β_2-M excretion rate reference level in children: < 40 µg β2-M/g creatinine.

Canada 2005). Given the unpredictability of uranium concentrations in at-risk areas, testing of well water for the presence of uranium at the time of drilling a new well or the sale or transfer of a property with an existing well is a reasonable measure (ATSDR 1999).

Uranium can enter the body via inhalation as well as through consumption of contaminated food or water (Mao et al. 1995). Dermal absorption is seen principally in the instance of military veterans who have been exposed to munitions containing depleted uranium and suffered puncture wounds (Bleise et al. 2003). Ingested uranium is absorbed from the digestive tract and appears initially in the blood, bound to red blood cells. Most is excreted via urine and feces, and experimental studies in humans have shown that about two-thirds of an injected dose of uranium is excreted within the first 24 hr and 75% within 5 days (Taylor and Taylor 1997). Retained uranium accumulates initially in the kidneys and liver and then in the skeleton (Li et al. 2005). Approximately 50–60% of stored uranium in the human body is found in the skeleton (Fisenne and Welford 1986). The biological half-life of uranium in the skeleton is approximately 300 days. The amount of uranium present in skeletal tissue is proportional to cumulative absorption (Hursh and Spoor 1973).

Uranium has the potential to be both chemically and radiologically toxic, but of principal concern in the context of groundwater exposure are the chemical toxic effects of uranium on the kidneys. The most extensive data on the human toxicity of uranium come from studies conducted on workers occupationally exposed in the nuclear industries (Thun et al. 1985); these studies demonstrated increased excretion of beta-2-microglobulin with increasing duration of exposure to uranium. Investigations of Gulf War veterans exposed to depleted uranium did not find clinically significant abnormalities in renal function, but did demonstrate that mean concentrations of microalbumin were significantly elevated in the group exposed to high levels of uranium (Harley et al. 1999; Squibb et al. 2005). There is also evidence that uranium may cause toxic effects in bone (Kurttio et al. 2005).

Within the kidneys, the proximal tubules are the structures principally damaged by uranium (Mao et al. 1995; Zamora et al. 1998). There is no evidence for glomerular injury (Kurttio et al. 2002). Evidence for dose-related proximal tubular injury has been observed after both ingestion and injection of uranium in animal (Diamond et al. 1989; Gilman et al. 1998) as well as in human (Zamora et al. 1998) studies. The histopathologic damage to the proximal tubules is manifest as cytoplasmic vacuolation, interstitial scarring, and destruction of the basal lamina (Gilman et al. 1998).

The pathophysiologic consequences of the proximal tubular injury associated with exposure to uranium include decreased ability to reabsorb water and small molecules, as is evidenced by the presence of elevated levels of the low-molecular-weight protein beta-2-microglobulin in the urine (Kurttio et al. 2002; Mao et al. 1995; Zamora et al. 1998;). Another marker for proximal tubule damage—increased fractional excretion of calcium and phosphate—has been observed to increase in dose-related manner after chronic ingestion of water containing uranium; this change has been observed in the absence of any increase in urinary beta-2-microglobulin to creatinine ratio (Kurttio et al. 2002). There appears to be no clear threshold for these pathophysiologic changes, and they typically become evident before any histopathologic evidence of injury is manifest (Kurttio et al. 2002). The severity of the tubular injury caused by uranium exposure has been shown in rat experiments involving relatively high-dose exposures to range from mild proximal tubular dysfunction to tubular necrosis (Haley 1982).

Although specific studies on the nephrotoxic effects of uranium in children have not been conducted, it is reasonable to assume that children would be at increased risk for adverse effects from exposure compared with adults. Children consume more water and food per kilogram of body weight than do adults (Figure 1) (Ershow and Cantor 1989; National Research Council 1993). Thus children will ingest proportionately greater quantities of any contaminants that are present in the water or food that they consume. For example, the 3-year-old girl in this case series who manifested elevated urinary excretion of beta-2-microglobulin was reported to derive a major portion of her nutritional intake from infant formula that was prepared by mixing powdered formula with contaminated well water.

Terminal differentiation and maturation of the kidneys and other organ systems occur postnatally, and these developing organs are especially vulnerable to the effects of toxic chemical exposures (National Research Council 1993). Recent studies suggest that chronic uranium exposure is associated with increases in blood pressure (Kurttio et al. 2006). The long-term significance of these changes is unclear. However, children's long future life expectancy further places them at increased risk of delayed adverse health effects that may develop years or decades after exposure in early life to uranium or other chemical contaminants in drinking water.

Because of its radioactivity, concern has arisen about the possible carcinogenicity of uranium. However, the levels of uranium that have been observed to induce nephrotoxicity are much lower than those that increase risk of cancer, and uranium intake from contaminated water has not been associated with increased risk of human cancer (Auvinen et al. 2002, 2005; Boice et al. 2003; Kim et al. 2004; Kurttio et al. 2002). A recent study that examined a cluster of childhood leukemia cases in Fallon, Nevada, found that the town had levels of uranium above or greatly above the maximum contaminant level. However, the children in Fallon with leukemia did not have a higher exposure to uranium than children without leukemia (Seiler 2004).

Although levels of arsenic, radium, and radon were elevated in the index family's water supply, none of these substances are known to have nephrotoxic effects.

In summary, this case series demonstrates the potential for significant residential exposure to naturally occurring uranium in groundwater. It underscores the hazards of consuming groundwater from untested private wells (U.S. EPA 2006). It confirms previous epidemiologic studies showing that chronic, low-level exposure to uranium in drinking water may result in mild injury to the proximal renal tubule (Kurttio et al. 2002). It highlights the special sensitivity of young children to environmental exposures (National Research Council 1993). Public health organizations should take the unique exposures and the special vulnerability of children into consideration when setting standards for uranium and other chemical contaminants in drinking water.

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Attachment 5

Paper

URANIUM DEPOSITION AND RETENTION IN A USTUR WHOLE BODY CASE

J. J. Russell* and R. L. Kathren†

Abstract—**This report describes a whole body donation from a person with a documented occupational intake of uranium. USTUR Case 1002 was an adult male who died from an acute cerebellar infarct at the age of 83. He worked as a power operator, utility operator, and metal operator for 28 years in a facility that processed and handled radioactive materials. Although he suffered a number of burns from hot metal and acids, cuts, abrasions, and puncture wounds during his many years of work, there were no corresponding health physics or medical records to indicate that these occurrences needed or required excision or decontamination due to the suspicion of the deposition of radioactive material. Over the course of his employment, USTUR Case 1002 submitted numerous urine samples for uranium, plutonium, and fission product analysis. The highest single uranium value measured during this time period was ~30 μg L^{-1} recorded during the second year of his employment. A urinary bioassay sample taken before termination of employment measured 4.3 μg L^{-1}. The mean urinary uranium concentration per liter per year calculated from the employee's bioassay records covering the first eleven years of monitoring averaged less than 3 μg L^{-1}. The ratio of $^{234/238}$U activity in the lung tissue was about 1, the same as that found in natural uranium. The highest concentration of uranium was found in a tracheobronchial lymph node. The uranium content in the various tissues of the body followed a rank order lung > skeleton > liver > kidney. Concentration of uranium in the kidney tissue was ~1.98 ng g^{-1}, about 3 orders of magnitude less than the generally accepted threshold level for permanent kidney damage of 3 μg U g^{-1} and roughly equal to the 1.4 ng g^{-1} reported for Reference Man. The autopsy disclosed findings not uncommon in the aged: severe atherosclerosis, areas of sclerotic kidney glomeruli with stromal fibrous scarring, and moderate to severe arterionephrosclerosis. Lung sections contained parenchymal areas of acute vascular congestion and a mild degree of anthracosis.**
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Key words: uranium; skeleton; kidneys; Reference Man

INTRODUCTION

In 1789, German chemist Martin Heinrich Klaproth reported the results of his research with pitchblende to the Berlin Academy, identifying a new metallic element, which he named uranium after the planet Uranus, which had only recently been discovered by William Herschel. Toxicity studies of uranium date back to as early as 1824, carried out as part of a larger study of all 18 then-known metals by Christian Gottlieb Gmelin, who observed that uranium was at best a weak poison if taken by mouth, but that injection of the nitrate or chloride into the jugular vein of a dog was sufficient to bring about death within a minute. Gmelin observed that the injected uranium salts produced large blood clots in the heart and great vessels, an effect also produced by the injected salts of palladium and barium (Hodge 1973).

Since the initial studies of Gmelin, there have been numerous experimental studies of the toxicity of uranium whose radioactive properties were discovered in 1896 by Henri Becquerel. These studies generally confirmed the feeble toxicity of ingested or inhaled uranium and indicated that, in dogs at least, damage to the proximal tubules of the kidney could result from uranium intake, and that uranium promoted excretion of sugar via the urine. This latter observation led to its use therapeutically as a treatment for diabetes early in the twentieth century.

Despite this long association and numerous toxicity studies with uranium over the years, there are still many unanswered important questions regarding the biokinetics and toxicology of uranium in humans. To provide answers to these questions, the U.S. Uranium Registry, now a part of the U.S. Transuranium and Uranium Registries (USTUR), was created in 1978. The basic plan of the Registry is to obtain tissue, or in some cases the whole body, at the time of death from volunteer donors with a known exposure to uranium, and to analyze these tissues for their radioactivity content. In this fashion, information could be gained regarding the distribution, dose, translocation, and fate of uranium in the body, which could be combined with autopsy results and personal exposure and medical histories to better understand the possible health implications of uranium in humans and to assure the adequacy of safety standards for workers and the general public. This paper reports on

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the first whole body donation to the USTUR from a person with a known occupational intake of uranium.

MATERIALS AND METHODS

USTUR Case 1002 was a Caucasian male who died at age 83, approximately 20 y after his retirement. Employment records state that he worked as a power operator, utility operator, and metal operator for 28 years in a facility that processed and handled radioactive materials. His duties brought him into areas of the facility where potential existed for exposure to various radioactive materials including thorium, uranium, plutonium, and americium and other transplutonics. Although he suffered a number of burns from hot metal and acids as well as cuts, abrasions, and puncture wounds during the course of his employment, there are no corresponding health physics or medical records to indicate that these occurrences required excision or decontamination from actual or suspected deposition of radioactive material. Moreover, examination of his health physics records did not disclose any acute accidental inhalation intakes of uranium or other radioactive materials.

Over the first 11 y of his employment, a total of 82 urine samples were collected and analyzed for uranium, plutonium, and fission products. Measurable levels of uranium were found in his urine; these data are summarized in Table 1. However, no plutonium or fission products were detected. With the exception of a single sample collected at the time of his retirement, no urine samples were collected or analyzed after his initial 11 y of employment. Present day health physics personnel at the work site state that reassignment of radiation material workers and others to new job duties that might or might not have involved working with uranium or other radioactive materials was common practice at that time. However, mandatory periodic urinary bioassay sampling of all workers handling or working with radioactive materials was required at the time. Thus it is likely that this worker incurred his uranium body burden from chronic low-level exposure to airborne uranium in the workplace during the first decade of his 28 y of employment, or 38–48 y prior to his death. This conclusion is consistent with his film badge results, which indicated a total lifetime whole body exposure of 11.42 rem of non-penetrating radiation and 4.33 rem of penetrating exposure, mostly during his early years of employment. The ratio of non-penetrating to penetrating dose is consistent with work with uranium since the external radiation field associated with uranium metal is primarily beta radiation (Kathren 1975). Case 1002 was removed from bioassay sampling 11 y after starting employment, indicative that his work no longer involved the potential internal exposure after this time.

Table 1. Urinary excretion of uranium in USTUR Case 1002.

Years of employment	Number of samples	Mean conc. (μg L^{-1}) $\pm$ σ[a]	Range (μg L^{-1})	Estimated annual excretion (mg)[b]
1	5	2.0 ± 4.1	0.0–10.2	1.0
2	13	6.9 ± 8.5	0.0–30.1	3.5
3	10	7.6 ± 7.9	0.0–29.5	3.9
4	8	0.7 ± 1.9	0.0–5.7	0.4
5	5	4.5 ± 4.2	0.0–6.3	2.3
6	8	1.0 ± 1.8	0.0–4.2	0.5
7	11	0.9 ± 2.7	0.0–9.5	0.5
8	11	0 ± 0	0	0
9	6	0.7 ± 1.5	0–4.1	0.4
10	3	0 ± 0	0	0
11	2	3.5 ± 1.9	1.6–5.4	1.8
12–24	0	—	—	Not exposed
25	1	4.3	—	—
Total (years 1–11)		2.53 ± 3.14		14.3
All Years excluding zero values and value at termination		3.09 ± 3.83		

[a] Results below detection limit assumed to be zero.
[b] Estimated annual excretion of uranium = mean concentration × volume of urine excreted per day (1.4 L) × 365 d y^{-1}.

After completion of the autopsy, the bones were defleshed in accordance with the established USTUR protocol, and soft tissue and individual bone wet weights were obtained. The tissues were analyzed at Los Alamos National Laboratory for uranium by kinetic phosphorescence analysis (KPA) according to the method of Bushaw (1984). Selected tissue samples were also analyzed for isotopic uranium by alpha spectrometry after ashing and chemical separation of the uranium in accordance with the methods of Gonzales and Willis (1987) and Boyd and Eutsler (1987). Results of the tissue analysis for uranium are summarized in Tables 2 and 3 and given in detail in Appendix Tables A1 and A2 for soft tissue and bone, respectively. In addition, the Appendix tables also contain the wet weight values of the divided bone samples. Uranium content is reported in mass units, and represents the total uranium present in the sample as determined by KPA, which does not provide an isotopic measurement. Results of the isotopic measurements in selected samples are given in Table 4. Table 5 provides a comparison of the uranium content of selected tissues of USTUR Case 1002 with cases previously reported by the USTUR, data on New York City residents (Fisenne and Welford 1986), and data on Reference Man (ICRP 1975).

RESULTS AND DISCUSSION

Tissue content of uranium

Measurable levels of uranium were found in urine and these data are summarized in Table 1. The mean

Table 2. Comparison of calculated organ content of uranium in selected tissues.

	Uranium content, μg					
Organ	USTUR 1002	USTUR 1042	USTUR 0242	USTUR 0213	Reference Man (ICRP 1975)	New York City residents Fisenne and Welford (1986)
Spleen	12.50	—	0.13	0.09	—	—
Liver	2.92	216	0.20	0.20	0.45	0.36 ± 0.56
Lung	249.60	1,550	1.78	1.02	1.00	0.5 ± 0.39
Kidney (2)	0.39	77	0.29	0.63	7.00	0.13 ± 0.08
Skeleton[a]	43.70	4,917	42.60	35.90	59.00	6.6 ± 3.8
TBLN[b]	8.70	—	0.19	0.09	—	—

Case 1002
Total systemic 62.11,[c] μg.
Total in body 364.11[c] μg.
[a] Average concentration in bones assayed $\times 10^4$ g of bone.
[b] Tracheobronchial lymph node.
[c] Includes total fat, skin, and muscle content calculated from Reference Man values.

Table 3. USTUR Case 1002. Systemic soft tissue uranium concentrations.

Tissue/Organ	ng/g
Spleen	102.8
Bladder	23.4
Blood	12.2
Thyroid	9.8
Eyes	7.5
Hair	6.5
Testis, peritesticular	5.4
Liver	2.5
Kidney	2.0
Brain	0.6
Remaining tissue (excluding respiratory tract)	<1.0

annual urinary concentration of uranium during this period was calculated as 2.53 ± 2.60 μg L^{-1}, with the highest single value, ~30 μg L^{-1}, recorded during his second year of work with uranium. Based on the urinary excretion data, the total uranium excreted via the urine during this period is estimated as 14.3 mg, corresponding to a urinary excretion of a few mg annually and an intake of perhaps on the order of a few tens of mg per year based on generally accepted models (ICRP 1994). Analysis of the single urine sample collected at the time of his retirement revealed an elevated uranium concentration of 4.3 μg L^{-1}, which, although appearing to be anomalously high in consideration of his likely exposure history and previous urine results, could be interpreted as suggestive of a long-term deposition of uranium in the body. The result of a single in vivo chest count made at the time of his retirement was less than the lower limit of detectability of <1 mg based on ^{235}U and natural uranium, suggestive, perhaps, that any long term deposition was at least in part not in the respiratory tract, and indicative of an inhalation intake of perhaps a few tens of mg over the 11-y exposure period some four decades or more prior to his death based on various biokinetic models (ICRP 1977, 1979, 1988, 1995a and b; Fisher et al. 1991; Wrenn et al. 1985, 1989).

Not unexpectedly considering the likely mode of exposure, the highest soft tissue concentrations of uranium were found in the respiratory tract, and in particular in the associated lymph nodes (Table 2). The inordinately high value of uranium in the lungs decades after intake is a reflection of a highly insoluble organ burden. Both mechanical and particle dissolution processes influence the clearance rate of uranium from the lung. Likewise, the very high concentration of uranium found in the tracheobronchial lymph nodes (TBLN) reflects the transportable fraction of uranium that was initially either physically entrapped or deposited in the lung parenchyma that subsequently cleared. The amount of uranium found in the respiratory tract is entirely consistent with the failure to detect a lung burden by in vivo counting, being a factor of 4 lower than the lower limit of detectability for the in vivo counting system.

Uranium is typical of many heavy metals in that it exhibits a strong affinity for biological molecules such as those containing phosphate groups, i.e., glucose phosphate and phospholipids; sulfhydryl groups, i.e., glutathione; and proteins and anions containing oxygen, i.e., carbonate and bicarbonate. Thus, free ions of heavy metals do not exist in the blood stream as such except in some transient sense, but they do exist as complexes with such biological molecules as those described. Inhaled particulate material makes first contact with lung epithelial lining fluid, which contains a variety of proteins, antioxidants, and surfactant lipids, which in the case of uranium forms a variety of complexes with the uranium particles, some of which are subsequently phagocytized. Although uranium does not form colloids in blood, aggregations of two or more uranium particles have been seen in macrophages following inhalation intake. The exact nature of how particle laden alveolar macrophages

Table 4. Summary of uranium content and concentration in bone. USTUR Case 1002.

	Weight (g) wet	Weight (g) ash	ng U	ng U/g wet	ng U/g ash
Skull	540	280	3,667	6.79	13
Vertebrae	846	186	2,743	3.24	15
Pelvis	528	128	917	1.74	7
Ribs	433	135	1,438	3.32	11
Sternum	78	11	85	1.09	8
Humerus	272	97	987	3.63	10
Radius	88	32	783	8.87	25
Ulna	103	43	613	5.95	14
Hand	140	37	2,167	15.48	59
Clavicle	56	18	273	4.89	15
Scapula	157	48	371	2.36	8
Femur	808	260	2,773	3.43	11
Patella	31	9	127	4.07	14
Fibula	110	40	582	5.29	15
Tibia	524	156	1,991	3.8	13
Foot	361	84	3,178	8.79	38
Sacrum + coccyx	243	43	544	2.24	13
Costal cartilage	24	0.4	127	5.3	318
Total for skeletal samples analyzed	5,342	1,607	23,366		

Table 5. Isotopic concentration of uranium in selected tissues of USTUR Case 1002.

Sample ID	Wet wt. LANL	ng U sample	± SD	^{238}U mBq per Sample	± SD	^{235}U mBq per sample	± SD	^{234}U mBq per sample	± SD
Liver	1,178	2,924	340	4.9	0.001	1.80	0.001	7.8	0.002
R-lung	548	132,745	10,735	1,207	0.068	40	0.008	1,202	0.068
Kid-R	91	240	19.8	3.1	0.001	0.15	0.015	3.5	0.001
Femur MS	147	783	54.8	10.5	0.002	1.30	0.001	15.7	0.002

migrate to the pulmonary and tracheobronchial lymph nodes via the lymphatics or migrate to the bronchioles to be transported by mucociliary action to the gastrointestinal tract is not completely understood.

Table 2 also provides a comparison of the uranium content of selected tissues of USTUR Case 1002 with those from USTUR Case 1042, another occupationally exposed case (Kathren et al. 1989); two whole body background cases previously reported by the USTUR (Kathren 1997); data on New York City residents reported by Fisenne and Welford (1986), and Reference Man (ICRP 1975). The relative content of uranium in the kidneys in the USTUR and NYC cases was low, on the order of 1–2% of the amount in the skeleton, as compared with 12% for Reference Man. Moreover, the kidney content of uranium in the two USTUR background cases and NYC residents was in all cases less than 1 μg, averaging 0.13 ± 0.08 in the latter and 0.46 ± 0.24 in the former, about an order of magnitude or more lower than the 7 μg reported for Reference Man (Table 2). Clearly, the Reference Man value for uranium in kidney is too high and should be reduced by at least a factor of 10.

Most systemic (i.e., rest of the body) soft tissues had mean uranium concentrations of <1 ng g^{-1} wet weight (Table 3), approximately the same level seen in two background or unexposed whole body cases previously reported by the USTUR (Kathren 1997). A number of soft tissues showed clearly elevated concentrations, most notably the spleen, which, with a concentration of 102.8 ng g^{-1} was two orders of magnitude greater than most of the soft tissues. Other tissues with significantly (i.e., order of magnitude) higher than average concentrations of uranium were the urinary bladder (23.4 ng g^{-1} wet weight), blood (12.2 ng g^{-1} wet weight), and thyroid (9.8 ng g^{-1} wet weight), followed by eyes (7.5) and testis and peritesticular tissue (4.5). The concentration of uranium in hair was 6.5 ng g^{-1}, at the upper end of the range of concentration values for the general public reported in the literature, i.e., a few tenths of ng to several ng g^{-1} (Byrne and Benedik 1991). Concentration in kidney and liver were lower still, but still about two to three fold greater than the average systemic soft tissue concentration, while concentration in fat was an order of magnitude lower than that in most of the tissues.

The elevated concentration of uranium in the spleen is most likely due to uranium bound to red blood cell membranes. One function of splenic macrophages is to remove fragments of abnormal red blood cells (RBC's)

and whole damaged RBC's from the circulation and store the iron as ferritin; some iron is attached to the protein transferrin and released back into the bloodstream. Thus, any uranium bound to such RBC's would be internalized (phagocytized) along with the damaged red blood cells/fragments and retained in situ. This observation is consistent with that of Hedaya et al. (1997) who observed high concentrations of uranium in the spleen of rats following intraperitoneal injection. In addition, uranium dissolved in blood circulates bound not only to erythrocytes but also to transferrin, plasma proteins, and a variety of diffusible ligands (Voegtlin and Hodge 1949).

The reason for the apparently elevated thyroid concentration of uranium in USTUR 1002 is unknown but is consistent with what was observed in one of the two USTUR background cases, and the modest concentration in the hair might be explained by the fact that uranium is a heavy metal, and heavy metals are normally excreted in the hair and nails, although the concentration in the nails in this case were not significantly higher than the soft tissue average. However, this value may be suspect as hair samples are easily contaminated by shampoos and hair dyes, many of which contain trace metals, which in turn can cause analytical interferences.

The uranium content of the skeleton is summarized in Table 4. The results of each specific bone sample analysis are given in Appendix A2. Following established USTUR procedures (McInroy et al. 1985), approximately half the skeleton was analyzed for uranium content in order to calculate the total skeleton burden outlined in Table 4. All vertebrae except C-1 were separated into the vertebral body, which is mostly cancellous bone with a large surface to volume ratio and the arch, which is mostly compact bone. The total wet weight (including red and yellow bone marrow) of skeletal samples analyzed for uranium was 5,342.14 g, slightly more than half of Reference Man skeleton weight of 10,000 g, and contained 23,366 ng U. This corresponds to an average uranium concentration of approximately 4.4 ng U g^{-1} of bone (wet weight) or 14.5 ng U g^{-1} ash.

The highest uranium concentration in a single skeletal sample was found in the costal cartilage (318 ng U g^{-1} ash); the lowest concentration was in the third thoracic vertebrae body and the third lumbar vertebrae body (4.7 ng U g^{-1} ash). Elevated concentrations of uranium were also found in the bones of the hand and foot (59.30 and 37.94 ng U g^{-1} ash, respectively) and in some vertebral arches and bodies. Among the bones of the hand and feet, the higher concentrations of uranium were found in some of the smaller generally odd shaped bones primarily from the phalanges with low ash weights. Elevated concentrations of uranium were also seen in the phalanges of USTUR 0213 and 0242, so-called "background" cases (Kathren 1997). This same observation has also been reported before for americium retention in at least one other USTUR whole body case, USTUR 0102 (McInroy et al. 1985), and possibly in other cases for plutonium and americium.

In the long bones, the highest concentration of uranium was found in the radius (24.50 ng U g^{-1} ash); the lowest concentration of uranium in the humerus (10.20 ng U g^{-1} ash). With few exceptions, the concentration of uranium, a volume seeker, in the more highly trabecularized ends of the various long bones was not greatly different from that of the shaft or compact bone areas. This contrasts somewhat with the long bone retention pattern generally seen with plutonium, a surface seeker, in which the long bone ends that have more trabecular bone (more surface area) also have greater cellular activity, i.e., bone remodeling and bone turnover rate retains a greater concentration of plutonium than compact bone. The concentration of uranium in the ends of long bones ranged from 7.6 to 113.9 ng U g^{-1} ash in the proximal end of the femur and the proximal end of the radius, respectively. By comparison, the concentration of uranium in the shafts of long bones ranged from 7.7 to 18.3 ng U g^{-1} ash in the distal shaft of the humerus and the proximal shaft of the radius, respectively.

The concentration of uranium activity in the 11 vertebrae that were divided and analyzed for uranium was inconsistent, ranging over an order of magnitude and with no apparent or obvious pattern. The uranium concentrations in the vertebral arches ranged from 4.7 to 58.4 ng U g^{-1} ash. Intravertebral concentrations were likewise inconsistent; the highest concentration in the arches was observed in C5, and significantly elevated concentrations were also noted in the arches of T1 and T5. Elevated concentrations of uranium in vertebral bodies were observed in T5 and T7, which had the greatest concentrations, and to a much lesser extent in T9 and L1.

Table 5 shows the results of isotopic measurements in selected tissues. The ratio of $^{234/238}$U in the lung tissue is about 1, same as that found in natural uranium. Although the $^{234/238}$U ratios for the liver and bone are somewhat elevated (i.e., greater than unity), the difference is not significantly different at the 95% confidence level, indicating that the chronic exposure suffered by USTUR 1002 during his early years of employment was likely to natural uranium.

Lack of knowledge of the exposure characteristics specific to this case limits application of the data from USTUR 1002 to biokinetic modeling. Additionally, the fact that the postmortem tissue concentration data for this

case are temporally many years after the intake(s) occurred obviates evaluation of the biokinetics at relatively short times after intake. It is of interest, however, to note that the long term systemic distribution of uranium in the soft tissues of this single occupationally exposed individual differs from the initial or short term pattern of tissue deposition observed in the so-called Boston injection cases who died relatively soon after their intake of uranium (Bernard and Struxness 1957; Luessenhop et al. 1958; Struxness et al. 1956), agreeing somewhat better but still not completely with the pattern of deposition observed in these cases after time.

In consonance with various systemic models for uranium (ICRP 1977, 1979, 1988, 1995a and b; Fisher et al. 1991; Wrenn et al. 1985, 1989; Durbin 1984), the skeleton contained the largest amount of uranium, about three-fourths of the systemic content, an observation in good agreement with post mortem tissue measurements (Fisenne and Welford 1986; Gonzales and McInroy 1991; Kathren 1997; Kathren et al. 1989) and with what would be predicted by the current ICRP model for a person of this age many years after intake (Leggett 1994). The ratio of uranium content in the skeleton to that in liver was approximately 15, albeit a factor of two smaller than the ratio of 30 that would be predicted by the ICRP Publication 69 model (ICRP 1995a and b) but clearly within the expected range of variability for a single case. However, contrary to most human models, an appreciable amount of uranium was also found in the spleen, suggestive as discussed above of RBC-membrane bound uranium clearance by the reticuloendothelial system with deposition or storage in the spleen. Even more striking than the total splenic content of uranium is the concentration (Table 3), which, at 102.8 ng g^{-1} of tissue was about an order of magnitude greater than the concentration in blood, and certainly consistent with preferential uranium clearance, retention, and/or deposition in the spleen. In any case, in consideration of refinements to the widely accepted and applied ICRP model, it would seem important to consider the spleen as a potential depot for inhaled uranium in insoluble form. Elevated levels of uranium were also found in the thyroid and urinary bladder of USTUR 1002. USTUR Case 0242, one of the two whole bodies whose tissues were analyzed for uranium by the USTUR, was an individual not known to have incurred an occupational or other exposure to uranium, and also exhibited an elevated concentration of uranium in the thyroid (Kathren 1997). In the second case assayed, USTUR 0213, the uranium concentration in the thyroid was typical of soft tissue concentrations generally. There is no obvious cause or explanation for the observed elevated thyroid concentrations in USTUR Cases 0242 and 1002, and the significance is unclear from a toxicological standpoint. However, the observed elevated thyroid concentrations relative to other soft tissue concentrations in two of the three USTUR whole body cases bears additional investigation with an eye towards factoring this into future biokinetic models.

Both kidney content and concentration were small, and the ratio of skeletal content to kidney content is in excellent agreement with the age dependent model put forth by the ICRP (ICRP 1995a; Leggett 1994) for an individual 25 y or so after uranium intake as a young adult. This observation also argues against the existence of a long term kidney compartment, although it is not inconsistent with a small kidney compartment with what might be termed an intermediate residence half time of 1,000 to 1,500 d as put forth in some models (Fisher et al. 1991; ICRP 1979; Wrenn et al. 1985, 1989) as well as in agreement with Durbin (1984) and other models that do not postulate a long term kidney retention compartment (ICRP 1995a; Leggett 1994; Leggett and Harrison 1995). The apparent equivocal long term retention of uranium in the kidney is indicative of both the complexity of uranium biokinetics in humans and of the dangers of extrapolation from a single case, and has been succinctly characterized by Leggett (1989) who wrote "Retention of uranium in the kidneys cannot be accurately characterized without consideration of the continual but diminishing inflow of uranium released from the bone and other tissues."

The relatively large retention in the lung is consistent with various models (ICRP 1995b) and recent work by Bertelli et al. (1998a and b), but inasmuch as the characteristics of exposing aerosol are unknown (e.g., particle size distribution, chemical form, time of exposure), it does not seem productive to speculate about respiratory tract biokinetics. The presence of uranium in the respiratory tract this long after exposure is clearly indicative that some unknown fraction of the inhalation intake was to highly insoluble material.

Taken as a whole, the systemic distribution of uranium in the soft tissues of USTUR 1002 is in good agreement with current models, as exemplified by the widely accepted and utilized model put forth in ICRP Publication 69 (ICRP 1995a; Leggett 1994). However, the observations of elevated concentrations in the thyroid and spleen pose interesting questions and hint at potential refinements to this model, which is primarily concerned with deposition in bone, liver and kidney, and are suggestive of a need to consider potential long term toxicological ramifications and perhaps include more specificity with respect to these tissues in future models.

The autopsy disclosed severe atherosclerosis involving the coronary vessels, the aorta, and the vessels at the base of the brain (Circle of Willis). An acute cerebellar infarct was present and was the immediate cause of death. Microscopic examination of H and E stained paraffin sections disclosed specific calcific atherosclerosis with high-grade stenosis of the coronary artery. In addition, sections of the myocardium showed small areas of fibrous scar tissue with moderate intimal thickening. The kidneys displayed areas of sclerotic glomeruli, with parenchymal vessels showing intimal thickening. The kidney also displayed areas of stromal fibrous scarring with some lymphocytic infiltration with moderate to severe arterionephrosclerosis. Lung sections with parenchymal areas of acute vascular congestion and a mild degree of anthracosis were also noted. Sections of the basal ganglia demonstrated slight gliotic scarification, consistent with old areas of cerebral infarction. Sections of occipital cortex with an area of cyst formation, gliosis, and deposition of pigment-laden histocytes consistent with an old cerebral cortical infarct were also observed.

CONCLUSION

Analysis of all the tissues from a whole body donor with a known occupational history of exposure to uranium showed elevated concentrations of uranium in the respiratory tract and spleen that are consistent with inhalation of natural uranium in a somewhat insoluble form. The low kidney concentrations in this case, other Registry cases, and other cases reported in the literature suggest that the Reference Man data on background quantities of uranium on the kidney are high by about an order of magnitude. The relative amount of uranium in the various organs of this case were lung $>$ skeleton $>$ spleen $>$ liver $>$ kidney, which is in agreement with other reported observations from the literature but not with Reference Man, which indicates that the amount of uranium in kidney is greater than that in liver. Autopsy results disclosed findings not uncommon in the aged with no indication of pathology possibly attributable solely to exposure to uranium.

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APPENDIX

Table A1. Uranium content in soft tissue of USTUR Case 1002.

Sample ID	Wet weight (g)	ng U	± SD	Concentration (ng U/g wet weight)
Liver	1,178	2,924	340.0	2.48
Lung-R	548	132,745	10,735.0	242.24
Lung-L	436	116,840	9,440.0	267.98
Larynx	126	232	20.0	1.84
Trachea	60	22,600	1,850.0	375.48
LN (Aortic)	5	4,438	358.0	840.53
TBLN	8	8,684	700.0	1,133.83
Kid-R	91	240	19.8	2.64
Kid-L	107	152	12.8	1.42
Adrenal	77	50	5.0	0.65
Heart	415	265	22.0	0.64
Pericardium	352	908	75.0	2.58
Periaortic scrap	108	1,552	124.0	14.33
Aortic Arch	124	503	42.0	4.04
Des-aorta(part)	56	77	6.9	1.38
Blood	9	109	9.3	12.20
Pancreas	121	74	6.5	0.61
Spleen	121	12,480	1,020.0	102.80
Esophagus	45	32	2.9	0.71
Diaphragm	66	161	13.0	2.48
Stomach	161	81	8.1	0.50
Small intestine	729	1,088	89.0	1.49
Large intestine	1,221	878	72.0	0.72
Omentum	463	279	24.0	0.60
Mesentary	589	188	16.0	0.32
Fat—abdominal	180	11	1.1	0.06
Epidura	71	39	3.9	0.55
Bladder	220	5,141	417.0	23.37
Prostate	37	58	5.0	1.57
Testis-R	6	37	3.5	5.92
Testis-L	12	42	3.7	3.43
Peritesticular tissue	5	28	2.7	5.37
Penis	67	25	2.5	0.37
Scrotum	102	32	3.2	0.31
Cerebrum	1,156	647	58.0	0.56
Cerebellum	78	79	8.8	1.01
Eyes	5	40	3.6	7.50
Thyroid	7	68	6.8	9.81
Hair—head	9	57	5.1	6.48
Skin				
Head	626	361	36.1	0.58
Up R arm	740	69	6.9	0.09
R forearm	290	143	14.3	0.49
R hand	176	59	5.3	0.34
R-foot	249	11	1.1	0.04
Salivary gland	13	7	0.7	0.52
Ear-R	18	9	0.9	0.49
RF-1	286	42	4.6	0.15
RF-2	398	13	1.6	0.23
RF-3	727	22	2.2	0.31
RF-4	324	4	1.2	0.01
RB-1	351	29	3.2	0.08
RB-2	315	16	1.8	0.05
RB-3	455	22	2.2	0.31
RB-4	546	31	3.1	0.06
Thigh-R1	648	84	8.4	0.13
Thigh-R2	928	806	80.6	0.87
Calf-R1	274	44	4.8	0.16
Calf-R2	233	31	3.5	0.13
Muscle				
Head	696	108	9.2	0.16
Tongue	68	10	1.0	0.15
R up arm	526	65	5.8	0.12
R forearm	1,098	115	11.5	0.10
R hand	143	100	8.6	0.70
Abdominal	1,909	1,640	132.0	0.86
R foot	292	25	2.3	0.09
R psoas	333	36	3.2	0.11
RF-1	740	210	21.0	0.28
RF-2	321	41	4.1	0.13
RF-3	675	36	3.6	0.05
RF-4	458	38	3.9	0.08
RB-1	826	37	3.7	0.04
RB-2	471	2,976	208.3	6.32
RB-3	688	26	2.6	0.04
RB-4	1,416	330	33.0	0.23
Thigh-R1	1,826	108	10.8	0.06
Thigh-R2	1,285	36	3.6	0.03
Calf-R1	592	30	3.3	0.05

Table A2. Uranium content in skeleton of USTUR Case 1002.[a]

Sample	Wet weight (g)	Ash weight (g)	ng U	± SD	ng U/g wet	ng U/g ash
Skull						
Frontal-1	33	19.7	282	24.1	8.5	14.3
Frontal-2	15	8.9	113	9.7	7.5	12.6
Frontal-3	15	8.6	113	9.6	7.4	13.2
Parietal-1	79	46.4	513	43.8	6.5	11.0
Parietal-2	92	53.6	615	52.4	6.7	11.5
Occipital	84	46.6	648	55.3	7.7	13.9
Temporal-1	23	13.8	155	13.2	6.7	11.2
Temporal-2	67	31.6	183	15.7	2.7	5.8
Temporal-3	6	2.3	110	9.4	20.0	47.8
Maxilla	79	24.9	398	34.0	5.0	16.0
Mandible	47	23.3	537	45.8	11.5	23.0
Vertebrae						
C-1	30	9.7	72	7.9	2.4	7.4
C-3a	24	5.4	30	3.4	1.2	5.6
C-3b	34	9.2	50	5.5	1.5	5.4
C-5a	16	3.4	252	38.0	16.0	74.1
C-5b	24	6.1	30	3.3	1.2	4.9
T-1a	22	6.3	128	11.2	5.8	20.3
T-1b	18	3.0	23	2.5	1.3	7.7
T-3a	21	6.1	70	6.3	3.3	11.5
T-3b	21	3.0	14	2.9	0.7	4.7
T-5a	22	6.7	220	44.0	9.9	32.8
T-5b	25	3.9	178	28.4	7.0	45.6
T-7a	25	7.8	90	10.0	3.6	11.5
T-7b	36	5.5	324	29.2	8.9	58.9
T-9a	32	9.3	92	10.1	2.9	9.9
T-9b	42	6.5	124	19.4	2.9	19.1
T-11a	33	10.1	95	10.5	2.9	9.4
T-11b	61	8.4	112	10.0	1.8	13.3
L-1a	44	12.6	182	15.6	4.2	14.4
L-1b	71	8.7	178	17.4	2.5	20.5
L-3a	46	13.9	181	16.3	3.9	13.0
L-3b	80	13.8	65	7.2	0.8	4.7
L-5a	49	13.5	132	11.9	2.7	9.8
L-5b	69	13.1	99	10.9	1.4	7.6
Pelvis						
Ilium crest	81	19.4	140	12.6	1.7	7.2
Ilium body	192	42.9	318	28.6	1.7	7.4
Ischium	254	65.8	459	36.8	1.8	7.0
Sacrum	241	42.9	515	41.2	2.1	12.0
Coccyx	2	0.2	29	3.2	15.3	145.0
Spinal cord	19	0.2	4	1.2	0.2	20.0
Ribs						
Rib ends	18	20.4	52	5.2	2.9	2.5
Rib#1	29	7.0	360	32.4	12.5	51.4
Rib#2	34	7.4	64	6.4	1.9	8.6
Rib#3	39	9.5	95	8.5	2.4	10.0
Rib#4	35	10.6	95	8.6	2.7	9.0
Rib#5	42	12.8	135	12.1	3.2	10.5
Rib#6	47	14.4	124	11.8	2.6	8.6
Rib#7	56	14.8	105	9.5	1.9	7.1
Rib#8	39	11.7	114	10.3	2.9	9.7
Rib#9	41	11.1	102	9.2	2.5	9.2
Rib#10	29	8.6	82	8.2	2.8	9.5
Rib#11	19	5.8	84	7.6	4.3	14.5
Rib#12	4	1.1	26	2.6	5.8	23.6
Costal cartilage-R	24	0.4	127	12.7	5.3	317.5
Sternum	78	11.4	85	8.5	1.1	7.5
Arm Bones						
Humerus PE	100	17.0	171	14.5	1.7	10.1
Humerus PS	82	27.9	243	21.9	3.0	8.7
Humerus DS	33	34.9	270	24.3	8.3	7.7
Humerus DE	57	17.0	303	24.9	5.3	17.8
Radius PE	9	2.0	228	19.8	26.5	114.0
Radius PS	34	14.1	258	24.0	7.7	18.3
Radius DS	27	11.9	170	14.8	6.2	14.3

Table A2. Continued.

Sample	Wet weight (g)	Ash weight (g)	ng U	± SD	ng U/g wet	ng U/g ash
Radius DE	19	3.9	127	11.0	6.8	32.6
Ulna PE	37	12.9	231	20.8	6.2	17.9
Ulna PS	32	15.8	216	21.6	6.8	13.7
Ulna DS	27	12.5	140	14.0	5.1	11.2
Ulna DE	6	1.4	26	2.6	4.0	18.6
Hand Bones						
Scaphoid	6	1.6	45	3.6	7.3	28.1
Lunate	5	1.2	312	74.0	63.7	260.0
Triangilar	3	0.9	72	7.4	21.2	80.0
Pisiform	2	0.5	55	6.2	23.9	110.0
Hamate	6	1.3	95	20.4	15.1	73.1
Capitate	7	1.6	50	12.6	7.1	31.2
Trapezoide	3	0.8	70	7.0	22.6	87.5
Trapezium	5	0.9	42	3.0	9.1	46.7
Metacarp-1	11	2.7	28	3.1	2.6	10.4
Metacarp-2	14	3.9	112	10.2	8.2	28.7
Metacarp-3	13	3.8	122	10.4	9.5	32.1
Metacarp-4	8	2.1	121	11.7	15.9	57.6
Metacarp-5	7	1.8	35	0.0	5.1	19.4
P-1	8	1.8	160	0.1	21.3	88.9
P-2	6	1.9	18	2.7	2.8	9.5
P-3	8	2.3	76	6.8	9.9	33.0
P-4	6	1.7	173	16.2	30.3	101.8
P-5	4	1.1	85	7.8	23.6	77.3
M-2	3	0.8	21	3.0	7.8	26.2
M-3	4	1.1	97	9.4	24.8	88.2
M-4	3	0.9	108	10.8	36.0	120.0
M-5	1	0.4	106	10.4	75.7	265.0
D-1	2	0.5	12	1.2	5.2	24.0
D-2	1	0.2	79	8.8	60.8	395.0
D-3	1	0.3	0	0.0	0.0	0.0
D-4	1	0.3	30	3.3	25.0	100.0
D-5	1	0.2	38	3.0	54.3	190.0
Fingernail	1	0.02	5	1.3	3.6	250.0
Clavicle, sternal	14	3.0	61	6.1	4.3	20.3
Clavical shaft	23	9.6	159	16.0	7.0	16.6
Clavical acromion	19	5.1	53	5.3	2.8	10.4
Scapula, proximal	35	9.0	107	9.6	3.0	11.9
Scapula, spine	50	14.9	112	10.5	2.3	7.5
Scapula, distal end	73	23.9	152	15.2	2.1	6.4
Leg Bones						
Femur PE	220	55.2	419	33.5	1.9	7.6
Femur PS	101	50.5	487	48.7	4.8	9.6
Femur MS	147	71.7	783	54.8	5.3	10.9
Femur DS	104	35.1	345	34.5	3.3	9.8
Femur DE	235	47.8	739	51.7	3.1	15.5
Patella-R	31	8.8	127	11.4	4.1	14.4
Patella-L	30	8.3	108	9.8	3.6	13.0
Fibula PE	16	2.7	118	11.2	7.5	43.7
Fibula PS	41	17.3	273	23.2	6.7	15.8
Fibula DS	37	15.6	147	13.2	4.0	9.4
Fibula DE	17	3.8	44	4.4	2.6	11.6
Tibia PE	162	25.9	289	26.0	1.8	11.2
Tibia PS	186	68.4	830	70.0	4.4	12.1
Tibia DS	109	46.6	544	48.0	5.0	11.7
Tibia DE	66	14.6	328	28.8	5.0	22.5
Foot Bones						
Talus	69	17.0	272	24.0	3.9	16.0
Calcaneus	106	22.7	445	37.9	4.2	19.6
Cuboid	21	4.1	173	14.7	8.0	42.2
Navicular	22	5.6	236	34.0	10.6	42.1
M cuneiform	17	3.9	72	7.2	4.2	18.5
I cuneiform	8	2.1	97	8.6	12.1	46.2
L cuneiform	10	2.3	46	4.0	4.6	20.0
Metatarsal 1	24	5.9	210	18.2	8.6	35.6
Metatarsal 2	15	4.1	117	18.0	8.0	28.5
Metatarsal 3	12	3.3	99	9.0	8.1	30.0
Metatarsal 4	12	3.4	194	19.2	15.5	57.1

Table A2. Continued.

Sample	Wet weight (g)	Ash weight (g)	ng U	± SD	ng U/g wet	ng U/g ash
Metatarsal 5	12	3.7	131	12.0	10.9	35.4
P-1	9	2.0	86	8.0	10.0	43.0
P-2	3	0.7	114	9.0	34.5	162.9
P-3	3	0.6	136	11.6	50.4	226.7
P-4	2	0.4	70	7.8	35.0	175.0
P-5	2	0.3	87	8.6	51.2	290.0
M-2	1	0.2	129	12.2	107.5	645.0
M-3	1	0.2	109	18.9	108.7	545.0
M-4	1	0.1	89	8.0	111.2	890.0
M-5	1	0.1	73	12.0	146.0	730.0
D-1	5	0.6	63	6.0	12.6	105.0
D-2	1	0.1	81	6.8	115.7	810.0
D-3	1	0.1	—	—	—	—
D-4	1	0.1	41	3.2	82.0	410.0
D-5	1	0.1	4	1.2	8.0	40.0
Toenails	2	0.03	4	1.3	2.2	133.3

[a] Notes: P = proximal; M = medial; and D = distal.

Attachment 6

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The U.S. Department of Veterans' Affairs depleted uranium exposed cohort at 25 Years: Longitudinal surveillance results

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ABSTRACT

Background: A small group of Gulf War I veterans wounded in depleted uranium (DU) friendly-fire incidents have been monitored for health changes in a clinical surveillance program at the Veterans Affairs Medical Center, Baltimore since 1994.

Methods: During the spring of 2015, an in-patient clinical surveillance protocol was performed on 36 members of the cohort, including exposure monitoring for total and isotopic uranium concentrations in urine and a comprehensive assessment of health outcomes.

Results: On-going mobilization of U from embedded fragments is evidenced by elevated urine U concentrations. The DU isotopic signature is observed principally in participants possessing embedded fragments. Those with only an inhalation exposure have lower urine U concentration and a natural isotopic signature.

Conclusions: At 25 years since first exposure to DU, an aging cohort of military veterans continues to show no U-related health effects in known target organs of U toxicity. As U body burden continues to accrue from in-situ mobilization from metal fragment depots, and increases with exposure duration, critical tissue-specific U concentration thresholds may be reached, thus recommending on-going surveillance of this veteran cohort.

1. Introduction

In a series of desert combat events in February 1991, U.S. armored tanks and fighting vehicles were mistakenly fired upon by other U.S. forces using depleted uranium (DU) penetrators (Office of the Special Assistant for Gulf War Illnesses (OSAGWI), 2000). As a result of this traumatic event, exposure to DU likely occurred via three routes: (1) DU fragments embedded in tissues, (2) particulate aerosols inhaled and deposited in the lung and (3) (superficial) particulate contamination of the skin. In the twenty-five years since that time, longitudinal surveillance has been performed by the U.S. Department of Veterans Affairs for the group of veteran service members who were victims of these “friendly fire” events.

A finding observed during the first surveillance assessment in 1993, was the apparent systemic absorption of metal ions from soft tissue-embedded DU fragments that act as a metal ‘depot’. This novel exposure ‘mode’ has been observed to this day, as documented by on-going excretion of high concentrations of urinary uranium (uU) in the surviving members of the cohort who continue to retain such metal fragments (McDiarmid et al., 2000, 2013; Squibb and McDiarmid, 2006). With surgical morbidity precluding the complete removal of all embedded fragments in those wounded, exposure has been on-going since its initial occurrence, now almost twenty-five years ago.

Thus, assessing health effects over time in a longitudinal surveillance program was instituted. Such an approach has proven helpful in cases such as these, when long-latency effects are unknown due to incomplete exposure assessment, novel exposure routes or when the exposure is on-going, allowing a toxicologic burden to accumulate (McDiarmid et al., 2009; Squibb et al., 2012).

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2. Uranium toxicity

DU is about 99.8% U^{238} by weight and is a man-made by-product of the U-enrichment process. It is derived when U^{234} and U^{235} are removed from natural U obtained from mined ore, to create a product 'enriched' in specific activity and thus suitable for reactor fuel (Army Environmental Policy Institute (AEPI), 1995). With the removal of most of these two isotopes, the remaining by-product is now 'depleted' of approximately 40% of its specific radioactivity. However, it retains its chemical toxicity due to its 'heavy metal' properties which are exploited for armor-piercing capabilities in military applications (Squibb et. al., 2005; AEPI, 1995; Agency for Toxic Substances and Disease Registry (ATSDR), (2013)).

While DU was only first widely deployed in the 1991 Gulf War, animal and cell culture studies had previously characterized the toxic effects of acute U exposure demonstrating that soluble U primarily targeted the kidney and insoluble forms, from inhalation exposures, targeted the lung (Voegtlin and Hodge, 1949; Morrow et al., 1972; The Royal Society, 2001, 2002; ATSDR, 2013). Epidemiology studies of exposed U workers employed in the defense industry also demonstrated renal effects, although of lesser severity considering the exposure concentrations (Thun et. al., 1985; National Research Council (NRC), 2008; The Institute of Medicine (IOM), 2008; ATSDR, 2013).

Other health risks relate to local tissue reactions and foreign body effects from prolonged contact with metal fragments (Hahn et al., 2002). These fragments have also been seen to oxidize in situ, thus enabling metal ion mobilization to the systemic circulation (as described above) and permitting metal accumulation in tissues remote from the metal depot location (Hooper et al., 1999; Pellmar et al., 1999; Squibb et al., 2005).

3. Surveillance for other metals in fragment alloys

Importantly, the metal fragments retained in soft tissue by our cohort members, are not exclusively DU, but contain other alloy constituents including titanium (Parkhurst et al., 2005) and may include material carried into the patient's wound from a secondary impaction of other material in the trajectory of the projectile.

Evidence from other patient cohorts of metal absorption suggest that other metals in embedded materials may mobilize from in situ tissue depots. These include reports of elevated cobalt and chromium levels in the circulation of hip implant patients (Machle, 1940; Dillman et al., 1979; Sunderman et al., 1989; International Agency for Research on Cancer (IARC), 1999; Jacobs et al., 1998; Keegan et al., 2007).

The chemical analysis of other surgically removed, combat-related fragments such as those from improvised explosive devices (IED) used during the recent Iraq and Afghanistan conflicts has further informed our exposure assessment protocol. These analyses have yielded a suite of 14 metals identified as the most commonly observed metal components of surgically removed fragments from IED injured service members (Centeno et al., 2014). Due to concern about retained fragments from other metal sources, the bio-monitoring battery of the DU cohort has been enlarged to include this broader suite of metals encountered in IEDs, to assess the potential presence and toxicity of non-DU retained metals.

To characterize the long-term health consequences of exposure to DU and possibly other metals and to inform medical management of this cohort, biennial surveillance has been conducted for these veterans since 1993. We report here clinical outcomes of interest from the 2015 surveillance assessment and describe the health implications for and medical management challenges of these patients.

4. Materials and methods

Between April and June of 2015, 36 members of a larger dynamic cohort, currently numbering 80 Gulf War I veterans who were victims of 'friendly fire' involving DU munitions and DU-armored tanks, participated in medical surveillance at the Baltimore, Maryland Veterans Affairs Medical Center (VAMC). Although all members of this cohort were invited to participate and travel and per diem costs were supplied, on-going military deployments and personal obligations typically limit participation to about half of the total cohort. All participants have been seen on previous biennial surveillance visits, with the number of visits per participant averaging more than seven. The surveillance protocol used in this study was approved by the University of Maryland School of Medicine's and the Baltimore VAMC's IRB programs. All participants signed a written informed consent document.

4.1. Exposure assessment for uranium and other metals of concern

Total U concentration and the $^{235}U/\ ^{238}U$ isotopic ratio of 24-hr urine samples collected during the 2015 surveillance visit were measured by the Joint Pathology Center (JPC) Biophysical Toxicology Laboratory (Silver Spring, MD) (previously the Armed Forces Institute of Pathology's Department of Environmental and Toxicologic Pathology [Washington, DC]). Prior to the metal analysis, urine aliquots were slightly acidified with 1% nitric acid (highly-pure Ultrex tarce metal-free HNO3) in acid-washed polypropylene 15 ml plastic tubes. Acidified uranium aliquots were analyzed for total U using a quadrupole-based Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (ELAN 6100; Perkin-Elmer Corporation, Connecticut, USA) (Ejnik et al., 2005; Gray et al., 2012), while uranium isotopic ratios ($^{235}U/\ ^{238}U$) were determined employing a high-resolution magnetic sector ICP-MS (HR-ICP-MS) (Thermo Finnigan Element-2; ThermoFisher Scientific, Connecticut, USA) (Gray et al., 2012). Urine U concentrations were standardized on the basis of urine creatinine concentrations to account for urine dilution to obtain μg U/g creatinine (McDiarmid et al., 2000; Karpas et al., 1998).

The 24 h urine specimens were also analyzed for 13 additional metals including: aluminum, arsenic, cadmium, cobalt, chromium, copper, iron, lead, manganese, molybdenum, nickel, tungsten and zinc. These metals were chosen based on their presence in analyzed embedded fragments (Centeno et al., 2014) and their potential toxicity and carcinogenicity (Gaitens et al., 2010). This panel of 13 additional metals was simultaneously analyzed employing HR-ICP-MS. Preparation of urine samples and metal analysis by sector field high resolution ICP-MS were conducted as described by Gray et al. (2012). Detection limits (DL) for most of the metals ranged from of 0.1–1 ppb (=ng/ml).

Urine arsenic species, including arsenobetaine (AsB), arsenocholine (AsCh), trimethylarsine Oxide (TMO), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenous (III) acid, arsenic (V) acid, were measured using high performance liquid chromatography (HPLC) are inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS). The sum of AsIII, AsV, DMA and MMA was used to determine inorganic arsenic exposure (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (USDHHS CDC), 2014).

4.2. Clinical assessment

A three-day, in-patient clinical assessment has been performed since the mid-1990s and consists of a detailed medical history, an extensive exposure history, a thorough physical examination, and laboratory studies. The laboratory battery includes hematological blood clinical chemistry and bone metabolism measures. Hematological and neuroendocrine parameters, serum and urine creatinine, calcium and phosphate, serum uric acid measures and urine glucose and total protein were evaluated by the Baltimore, MD VAMC clinical laboratory using standard methodologies. Spot and 24-hr urine samples were

obtained for measurement of clinical chemistry parameters related to renal function and bone metabolism as described below. Neurocognitive performance was again assessed as a measure of central nervous system (CNS) insult as per our previous protocols. Specifically, participants completed the Automated Neuropsychological Assessment Metrics, a computerized neurocognitive battery originally developed by the Department of Defense. Four neurocognitive indices of accuracy, speed, throughput (i.e., speed/accuracy) and cognitive efficiency were computed (McDiarmid et al., 2004, 2007, 2009).

4.3. Renal assessment

Markers of nephrotoxicity in urine [retinol binding protein (RBP), microalbumin (mAlb), intestinal alkaline phosphatase (IAP), N-acetyl-D-glucosaminidase (NAG), Kidney Injury Marker -1 (KIM-1), Neutro Gelatinase-Associated Lipocalin (NGAL) and IL-18 and β_2 microglobulin] were measured and analyzed as previously described (McDiarmid et al., 2013).

4.4. Pulmonary functional assessment

Spirometry was performed using a Sensormedics Carefusion Vmax system (Yorba Linda, CA) according to American Thoracic Society guidelines by Registered Respiratory Therapists certified by the National Board for Respiratory Care (Miller et al., 2005). Pulmonary function testing was performed on a flow-sensor spirometer. Predicted normal values for spirometry were obtained from Morris et al. (1971). Spirometry values included forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), and the ratio of FEV1 to FVC.

5. Statistical analysis

5.1. Urine uranium as a binary variable

As in previous years, the cohort was sorted into two (categorical) exposure groups (low-high) based on each individual participant's current (2015) uU result (low n=26; high n=10). As in previous years (McDiarmid et al., 2000, 2001, 2004, 2006, 2007, 2009, 2011, 2013, 2015), the low uU group is defined as those having uU < 0.1 μg U/g creatinine. High exposure was defined as current uU concentrations ≥0.1 μg U/g creatinine, a value between 0.034 μg/g (the 95% percentile reported for creatinine-adjusted uU concentrations in non-exposed populations in the U.S. (USDHHS CDC, 2003)) and 0.35 μg/g U/L which is reported as a uU upper limit that occurs naturally in areas with elevated U in water and food (International Commission on Radiological Protection (ICRP), 2002).

5.2. Tests of differences between low versus high urine U (uU) exposure groups

We present here each outcome by U exposure category (low vs high) in tables using mean values and standard errors. We tested for significance of differences between U groups using the Mann-Whitney *U* test (Wilcoxon Rank sum test). Hence, the Mann-Whitney test was used for all comparisons of the low versus high U groups. Differences were considered statistically significant when $p < 0.05$.

5.3. Analyses of other metal concentrations

For each of the 13 additional metals examined, we determined if a metal concentration was "elevated" by comparing the result to a reference value for unexposed populations. As described in Gaitens et al. (2010), when available, the 2003–2004 National Health and Nutrition Examination Survey (NHANES) 95th percentile creatinine adjusted value was used as the reference value; otherwise, other reported reference values, such as the upper values reported by clinical laboratories, were used. We used the Fisher's Exact test to determine if there was an association between elevated metal concentrations and high exposure to U as measured by uU concentrations.

Table 1
Population characteristics of the 2015 surveillance participants.

	2015 cohort (N=36)		All Gulf War I participants (N=80)	
	N	(%)[a]	N	(%)[a]
Race/Ethnicity				
African American	13	(36)	24	(30)
Asian American	1	(2)	1	(1)
Caucasian	17	(47)	46	(56)
Hispanic	5	(14)	8	(10)
Native American	0	(0)	1	(1)
Age[b]				
Mean (S.D.[c])	50.94 (5.31)		49 (4.76)	
Body mass index (BMI) by U group[d,e]				
Mean (S.E.[f]) Low- 30.83 (1.02) High- 30.37 (3.24)				
Current smoker				
Number (%) Low- 3 (11) High- 1 (10)				

[a] May not add to 100% due to rounding.
[b] Age in 2015.
[c] S.D.=Standard deviation.
[d] Low group- urine uranium (uU) < 0.10 μg/g creatinine (n=24); High group- uU≥0.10 μg/g creatinine (n=10).
[e] BMI and "current smoker" status information not available for the full cohort of 80.
[f] S.E.=Standard error.

6. Results

Between April and June of 2015, 36 members of a larger dynamic cohort, currently numbering 80 Gulf War I veterans, who were victims of 'friendly fire' involving DU munitions and DU-armored tanks, participated in medical surveillance at the Baltimore Veterans Affairs Medical Center (VAMC). Table 1 displays the demographic characteristics of the subgroup of veterans participating in the 2015 surveillance visit and depicts a similar race and age structure to that of the larger complete cohort. All have participated in multiple previous assessments, with the average number of assessments attended by participants being almost eight. Importantly, those with a high uU, who are the most at-risk of U-related health effects, tend to more regularly attend these surveillance visits and thus it is unlikely that we are missing an important health signal contributed from the non-participants.

Urine U concentrations have been used as a marker of systemic exposure to U for the affected cohort since the inception of the surveillance program. Fig. 1 displays the uU distribution from low to high values of the 2015 cohort of participants. In addition to total uU, the figure, using various data point symbols, depicts isotopic U signature and patient fragment status. Because the isotopic signature determination requires a total uU of > 10 μg/g creatinine (which is a limit of detection of 10 μg/g creatinine), the isotopic signature for the lower uU values cannot be determined. The figure includes various uU comparison values reported in other populations which are indicated by horizontal lines on the graph. The top line (65.1 μg/L) represents the mean total urine uranium found in a sub-cohort of uranium fabrication workers in 1975, as reported in a study by (Thun et al., 1985). The U.S. Dietary Limit (0.320 μg/L) is an upper limit for the dietary contribution of uranium in urine for a U.S. general population from drinking water (ICRP, 2002). This value was calculated by dividing the upper limit for 24-h uranium excretion for "reference man" by 1.6L 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 (Ting et al., 1999). The next line (0.1 μg/g creatinine) indicates the cut point established by the

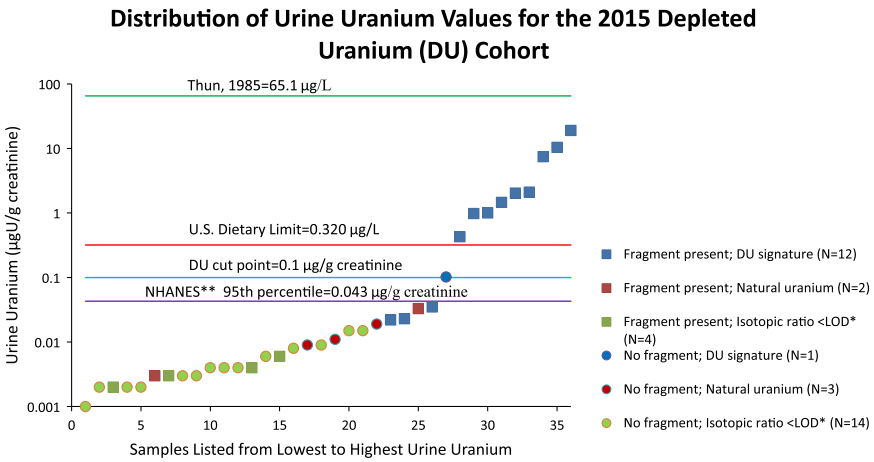

Fig. 1. Displays the urine uranium (uU) distribution from low to high values of the 2015 cohort of participants with comparison populations. The symbols used represent: total uU, isotopic signature and fragment status of individual. The top line (65.1 µg/L) represents the mean total uU found in a sub-cohort of uranium fabrication workers in 1980 (Thun et al., 1985). The next line (0.320 µg/L) is an upper limit for the dietary contribution of uranium in urine for a general population from drinking water (ICRP, 2002; McDiarmid et al., 2000). The next line (0.1 µg/L) indicates the cut point established by the DU Follow-up Program to define low vs. high uU exposure groups (McDiarmid et al., 2000). The next line (0.043 µg/g creatinine) represents the 95th percentile for uU concentration for adults from the 2001–2002 National Health and Nutrition Examination Survey (NHANES) (USDHHS CDC, 2012). * Limit of detection.

DU Follow-up Program to define low vs. high urine uranium exposure groups (McDiarmid et al., 2000). The bottom line (0.043 µg/g creatinine) represents the 95th percentile for urine uranium concentration for adults from the 2001–2002 US. National Health and Nutrition Examination Survey (NHANES) (USDHHS CDC, 2012). The NHANES survey assesses the health and nutritional status of adults and children in the United States using interviews, physical examinations and laboratory assessment.

For the DU cohort results reported on here, the concentrations of uU ranged from a low of 0.001 µg U/gram creatinine, to a high of 19.044 µg U/gram creatinine. Eighteen of the 36 participants, (50%) of the group, had retained metal fragments as determined previously by plain film skeletal X-ray (Hooper et al., 1999; Squibb and McDiarmid, 2006). Those participants possessing metal fragments and a DU isotopic signature are seen at the high end of the distribution, suggesting again that the fragment is a depot of on-going U metal mobilization into the systemic circulation. One participant with an isotopic signature had previously had a fragment removed. We note also that many of the values below the cut point of 0. 1 µg U per gram creatinine in cohort members with only an inhalation exposure are at or below the NHANES U 95th percentile value for the population, and that many of these lower values approach the laboratory limit of quantification to assess the isotopic signature for the sample (~10 ng/L). Participants with fragments, but without a DU isotopic signature, are thought to possess non-DU metal fragments from other sources, such as from non-DU tank armor and/or from secondary impaction of other material in the trajectory of the projectile and carried into a wound. These observations of fragment status (yes/no) generally tracking with elevated uU have been consistent throughout the almost 25 years of follow-up.

Table 2 displays the urinary concentrations of 13 other metals which were also assessed, as they have been shown to be present in fragments analyzed from other veterans of the Iraq/Afghanistan conflict (Squibb et al., 2012; Centeno et al., 2014). It is hypothesized that a participant with a retained DU fragment would be more likely to have other urinary metal elevations resulting from other metals mobilizing from either the same alloyed-DU fragment or from other fragments embedded at the time of injury. Only aluminum concentrations were seen to be statistically different between the two uU exposure groups, with measures higher in the high U group. One may posit that aluminum may also be present in the retained fragments and is thus available for mobilization in the urine, as is U. However, other dietary and environmental sources of aluminum must also be considered as an explanation for this elevation. Importantly, however, the mean concentration of aluminum was found to be within normal ranges. We note also that the arsenic concentrations, while not statistically different, are just outside the normal range for the low uU group. Arsenic speciation revealed that all results were from organic (dietary) sources, so this observation is not a threat to health.

7. Clinical findings

A clinical battery of hematology, chemistry, bone metabolism, neuroendocrine and thyroid parameters have been performed in this DU-exposed cohort since inception of the surveillance protocol with the results revealing no consistent U-related differences observed between the low and high U groups over time. For the present assessment, no statistically significant differences between U exposure groups were observed for hematologic endpoints and all values were within normal limits. A complete chemistry panel reflecting electrolytes, lipids and liver function were likewise not different between U exposure groups and mean group results were also within normal limits with the exception of LDL cholesterol, mildly outside the normal range, and higher in the low U group (data not shown).

Four neurocognitive indices of accuracy, speed, throughput and cognitive efficiency were computed. Mann-Whitney *U* tests were conducted to evaluate neurocognitive performance between the low versus high U groups. No significant differences were observed between the two U exposure groups as displayed in Table 3.

Measures of bone metabolism outcomes have also been of interest as bone is a long-term storage depot of U. Only the blood estradiol level was found to be different in the battery of bone metabolism measures, Table 4, with the results in the high U group (35.9 pg/ml) being greater than in the low group (27.9 pg/ml), but still below the upper limit of normal (39 pg/ml). The smoking status of the participants was not observed to affect this outcome. This statistical difference in estradiol was not observed previously, and the many outcomes measured imply that a significant difference will be observed on occasion, due to chance alone. We will watch for this effect during future health appraisals to assess its persistence, which would imply a potentially important clinical observation.

7.1. Biomarkers of renal effects

Surveillance for kidney effects has been a focus of this health assessment since its inception as the kidney is the known 'critical'

Table 2
Urinary metals concentrations.

Laboratory test (reference range[a])	Low uU group[b] Means+S.E.[d] (µg/g creatinine)(N=26)			High uU group[c] Means+SE (µg/g creatinine) (N=10)			Mann-Whitney *p*
Aluminum (< 30 µg/g cre[e])	3.79	±	(0.53)	8.20	±	(0.98)	0.00
Arsenic (< 60 µg/g cre)	55.04	±	(22.01)	14.00	±	(5.50)	0.26
Cadmium(< 1.0 µg/g cre)	0.24	±	(0.02)	0.20	±	(0.02)	0.61
Chromium (< 2.0 µg/g cre[e])	0.26	±	(0.06)	0.39	±	(0.09)	0.18
Cobalt (< 1.0 µg/g cre)	0.21	±	(0.03)	0.21	±	(0.04)	0.99
Copper (< 40 µg/g cre[f])	5.21	±	(0.80)	4.63	±	(0.49)	0.64
Iron (< 250 µg/g cre[f])	6.73	±	(1.31)	5.77	±	(0.92)	0.96
Lead (< 2.0 µg/g cre)	0.39	±	(0.08)	0.26	±	(0.04)	0.77
Manganese (< 2.0 µg/g cre[f])	0.24	±	(0.15)	0.13	±	(0.05)	0.41
Molybdenum (< 122 µg/g cre)	34.72	±	(6.07)	32.04	±	(4.85)	0.57
Nickel (< 8 µg/g cre[f])	0.23	±	(0.05)	0.29	±	(0.18)	0.26
Tungsten (< 0.4 µg/g cre)	0.04	±	(0.01)	0.06	±	(0.02)	0.27
Zinc (< 1100 µg/g cre[f])	591.02	±	(60.44)	686.12	±	(111.04)	0.39

[a] All reference values are rounded from calculated and available data (U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2012, Lauwerys and Hoet, 2001, Burtis and Ashwood, 2001, University of Iowa, Department of Pathology, 2013, Cleveland Clinic Laboratories, 2015).
[b] Low urine uranium (uU) group < 0.10 µg/g creatinine (n=24).
[c] High uU group≥0.10 µg/g creatinine (n=10).
[d] S.E.=Standard error.
[e] Source reference value converted from µg/L to µg/g creatinine (cre) assuming urine output of 1.2 L/24 h and 1.2 g cre/24 h (American Conference of Governmental Industrial Hygienists (ACGIH, 1991)).
[f] Source reference value converted from µg/24 h to µg/g creatinine assuming urine output of 1.2 g cre/24 h (American Conference of Governmental Industrial Hygienists (ACGIH, 1991).

organ, that is, the organ first perturbed, for soluble U toxicity (Parkhurst et al., 2005). Table 5 displays the common, clinical renal function measures obtained, which are within normal limits both for endpoints measured in blood and urine matrices. There are no statistical differences between mean results when comparing the low versus high uU groupings for any measure in Table 5, implying the absence of a U-driven effect.

7.2. Urinary low molecular weight proteins: biomarkers of renal proximal tubule effects

It is specific segments of the kidney's proximal tubule that are the target of soluble U and therefore, we also obtained spot urine samples to measure low molecular weight, filtered proteins [microalbumin, $\beta_2$microglobulin, $\acute{\alpha}_1$microglobulin and retinol binding protein (RBP)], which are normally reabsorbed in the proximal tubules, to assess renal tubular function. We also obtained measures of renal tubular cytotoxicity (IAP and NAG) which may be found in the urine as a consequence of the sloughing of cells into the tubular lumen.

Similar to results obtained during recent past surveillance visits, there were no statistically significant differences (at the $p < 0.05$ value) between the mean values for proteins in the low vs high uU groups (Table 6). This observation remained unchanged when the cohort members diagnosed with diabetes were removed from the analysis.

This lack of differences between U groupings extends to the three acute kidney injury markers we also assessed (IL-18, KIM-1 and NGAL). Borrowed from the pharmaceutical industry, these markers are used as more sensitive indicators for detecting acute kidney injury (AKI) in drug induced renal toxicity (Coca et al., 2008). These biomarkers have also recently been used to detect adverse effects of chronic exposures to renal environmental toxicants such as metals and have allowed early detection of injury (Prozialeck et al., 2009; Zhou et al., 2008; Zhang et al., 2014). Although not reaching statistical significance, results presented in Table 6 for KIM-1 and NGAL demonstrate a 40–100% higher mean value in the high uU group compared to the low uU group potentially suggesting signs of a U-effect. The lack of statistical significance may be due to the high variability associated with the data, to the broad range of normal values and the standard error observed. Of interest, however, this trend of higher mean results in these kidney injury markers have been observed previously in the high uU group (McDiarmid et al., 2015) and may suggest detection of an early U-related renal effect.

7.3. Pulmonary effects

Pulmonary function measurements obtained by spirometry provide a non-invasive test to monitor for physiologic changes to the lungs, a known target of insoluble uranium particulate exposure. Spirometry results for the 2015 cohort are shown in Table 7 and demonstrate values that fall within the normal clinical range for the group overall.

As well, for all parameters displayed (FVC% predicted, FEV1%

Table 3
Neurocognitive measures.

Automated Neuropsychological Assessment Metrics (ANAM)	Low uU group[a] Means+S.E.[c] (N=20)			High uU group[b] Means+S.E. (N=9)[d]			Mann-Whitney *p*
Accuracy index	0.16	±	(0.11)	0.19	±	(0.14)	0.53
Speed index	0.14	±	(0.12)	0.15	±	(0.19)	0.83
Throughput index	0.16	±	(0.20)	0.24	±	(0.29)	0.63
Index of cognitive efficiency	771.11	±	(124.13)	727.80	±	(187.00)	0.42

[a] Low urine uranium (uU) group < 0.10 µg/g creatinine.
[b] High uU≥0.10 µg/g creatinine.
[c] S.E.=Standard error.
[d] The N does not add up to total number of participants for 2015 due to the fact that some were unable to complete neurocognitive testing.

Table 4
Markers of bone metabolism.

Laboratory test (normal range)	Low uU group[a] Means+S.E.[c] (N=26)			High uU group[b] Means+S.E. (N=10)			Mann-Whitney *p*
Serum/Blood							
Estradiol (0–39 pg/ml)	27.86	±	(1.66)	35.90	±	(2.63)	0.01
Parathyroid hormone (intact) (10–65 pg/ml)	48.04	±	(2.92)	46.40	±	(6.88)	0.57
Bone specific alkaline phosphatase (7.6–14.9 µg/L)	12.38	±	(0.69)	10.91	±	(1.16)	0.45
Vitamin D 1,25 (18–72 pg/ml)	49.42	±	(3.33)	53.50	±	(6.41)	0.57
Vitamin D 25–0H (30–100 ng/ml)	34.31	±	(2.27)	37.59	±	(5.70)	0.82
Calcium (8.4–10.2 mg/dL)	9.08	±	(0.06)	9.26	±	(0.11)	0.10
Phosphate (2.7–4.5 mg/dL)	3.57	±	(0.12)	3.65	±	(0.19)	0.82
Urine							
Sodium (40–220 mEq/24 h)	173.31	±	(14.92)	172.90	±	(23.85)	0.88
Calcium (100–300 mg/24 h)	153.79	±	(19.88)	177.49	±	(32.26)	0.44
PO_4 (0.4–1.3 g/24 h)	0.90	±	(0.05)	0.85	±	(0.08)	0.59
N-Telopeptide (9–60 nMol BCE/mMol creatinine)	20.38	±	(1.35)	17.20	±	(2.11)	0.34

[a] Low urine uranium (uU) group < 0.10 µg/g creatinine.
[b] High U≥0.10 µg/g creatinine.
[c] S.E.=Standard error.

Table 5
Renal parameters.

Laboratory test (normal range)	Low uU group[a] Means+S.E.[c] (N=26)			High uU group[b] Means+S.E. (N=10)			Mann-Whitney *p*
Urine							
Creatinine g/24 h (0.6–2.5)	1.94	±	(0.09)	1.87	±	(0.14)	0.76
Glucose g/24 h (0–0.5)	1.24	±	(0.96)	18.47	±	(18.38)	0.52
Ca mg/24 h (100–300)	153.79	±	(19.88)	177.49	±	(32.26)	0.44
PO_4 g/24 h (0.4–1.3)	0.9	±	(0.05)	0.85	±	(0.08)	0.59
Magnesium mEq/24 h (1.4–14.0)	7.84	±	(0.84)	7.9	±	(1.27)	0.32
Total protein conc mg/dL (0–12)	13.02	±	(4.79)	4.05	±	(1.12)	0.16
Uric acid g/24 h (0.25–0.75)	0.67	±	(0.05)	0.65	±	(0.07)	0.14
Potassium mEq/24 h (25–125)	65.38	±	(4.27)	65.5	±	(4.45)	0.74
Serum/Blood							
Glucose mg/dL (70–105)	106.5	±	(6.53)	111.6	±	(14.52)	0.54
C-Reactive Protein (0–10 mg/L)	10.07	±	(4.30)	6.32	±	(2.25)	0.85
Creatinine mg/dL (0.9–1.3)	1	±	(0.04)	0.97	±	(0.05)	0.69
Calcium mg/dL (8.4–10.2)	9.08	±	(0.06)	9.26	±	(0.11)	0.50
Phosphate mg/dL (2.7–4.5)	3.57	±	(0.12)	3.65	±	(0.19)	0.09
Uric acid mg/dL (3.4–7)	6.59	±	(0.27)	5.79	±	(0.39)	0.82
Sodium mEq/L (133–145)	138.54	±	(0.28)	137.8	±	(0.73)	0.37
Magnesium mg/dL (1.8–2.5)	2.04	±	(0.03)	2.12	±	(0.06)	0.56
Calculated Glomerular Filtration Rate ml/min/SA[d] (90–120 ml/min/SA 1.73 m^2)[e]	88.27	±	(3.59)	94.7	±	(8.67)	0.61
Beta 2 Microglobulin µg/L (0–2510)	1937.37	±	(113.43)	1902	±	(109.25)	0.61

[a] Low urine uranium (uU) group < 0.10 µg/g creatinine.
[b] High uU ≥0.10 µg/g creatinine.
[c] S.E.=Standard error.
[d] Surface area.
[e] National kidney foundation.

predicted, and FEV1/FVC ratio) mean values are within normal clinical limits for both the low and high uU groups, and there is no statistical difference in outcomes between U exposure categories. This is consistent with observations made during the 2011 and 2013 surveillance visits (McDiarmid et al., 2013, 2015). Ever smokers tended to have lower FEV1/FVC ratios which could represent a smoking effect. A greater proportion of smokers were found in the low uU group. When adjusted for smoking, there still remained no differences in any spirometry measure in low compared to high uU groups.

8. Discussion

The epidemiology of U fabrication workers and animal evidence from the DU implantation experiments, have informed the content and conduct of the Department of Veteran's Affairs longitudinal surveillance program initiated in the early 1990s. The dual toxicities of U derived from both its chemical, heavy metal properties as well as from its radioactivity have influenced the protocol as have likely exposure routes. Both inhalation and ingestion exposure routes could be anticipated for respirable aerosols encountered in confined spaces, such as inside a tank hatch, likely permitting acute exposure at the time of DU penetrator impact. In addition, chronic absorption of metal ions from retained fragments of shrapnel which were either embedded in tissue or which contaminated superficial wounds posed an added health risk to those affected and argued for on-going surveillance.

Two primary target organs of the DU oxide exposures sustained during these friendly fire events were thought to be the kidney and the pulmonary system, for the soluble and the insoluble DU particles respectively (The Royal Society, 2001, 2002; Parkhurst et al., 2005). For this reason, we have continued to focus on these organ systems. While kidney U concentration in animal experiments have been shown to reach peak levels at six months and plateau thereafter (Pellmar et al.,

Table 6
Biomarkers of renal proximal tubular effects.

Laboratory test[d] (normal range)	Low uU group[a] Means±S.E.[c] (N=26)			High uU group[b] Means±S.E. (N=10)			Mann-Whitney *p*
IAP U/g creatinine (< 2 U/g)	0.44	±	0.11	0.41	±	0.12	0.79
NAG U/g creatinine h (< 5U/g)	1.00	±	0.18	0.85	±	0.12	0.90
Micro Albumin mg/g creatinine (< 17 mg/g)	46.29	±	33.38	10.84	±	3.02	0.39
beta2 microglobulin mg/g creatinine (< 0.20 mg/g)	0.07	±	0.01	0.08	±	0.01	0.37
alpha1 microglobulin mg/g creatinine (< 8 mg/g)	2.79	±	0.49	2.48	±	0.65	1.00
RBP mcg/g creatinine (< 300 mcg/g)	35.33	±	3.71	47.83	±	17.62	0.90
IL-18 ng/g creatinine (< 20 ng/g)	10.11	±	1.47	12.94	±	3.14	0.54
KIM-1 ng/g creatinine (< 560 ng/g)	273.75	±	40.11	366.70	±	91.25	0.43
NGAL ng/g creatinine(< 730 ng/g)	492.08	±	41.75	965.79	±	339.15	0.61

[a] Low urine uranium (uU) group < 0.10 μg/g creatinine.
[b] High uU ≥0.10 μg/g creatinine.
[c] S.E.=Standard error.
[d] Lab test abbreviations defined:IAP (intestinal alkaline phosphatase);NAG ((N-acetyl-β-D Glucosaminidase);RBP (retinol-binding protein);IL-18 (Interleukin–18);KIM-1 (kidney injury molecule-1);NGAL (neutrophil gelatinase-associated lipocalin).

1999), uranium concentrations in bone and other organ systems showed accumulation of U over a long period of time. Therefore, in light of the potential for build-up of U concentrations exceeding tissue-specific threshold concentrations over time, we obtain a full clinical laboratory battery at every biennial visit. The protocol thus continues to examine the impact of uU exposure on other organ systems, such as hematological, immune, CNS, bone metabolism, and neuroendocrine systems.

With the exception of the on-going mobilization of U from fragment depots in soft tissue, manifesting as elevated uU concentrations, the comprehensive surveillance assessment reported here demonstrates no clinically significant uranium-related effects, now twenty-five years since initial exposure. Fig. 1 may help explain this observation as it includes uU results for occupationally- exposed U fabrication workers' (Thun et. al., 1985) (upper line of figure) for comparison with the DU cohort. As shown, only the highest DU cohort members' uU results approach those of the mean uU values recorded in the U fabrication workers in 1975. When the Thun cohort of 36 U mill workers was compared to cement worker referents, the authors showed renal tubular abnormalities including elevated urine B2-microglobulin as a function of exposure duration and mild aminoaciduria in five of 23 amino acids assessed. Although uU results for the entire group were not available, the authors reported historical mean uU values for a sub-group of the population at 65 μg U/L in 1975 as depicted here in Fig. 1. After significant hygiene improvements, the mean uU reported in 1980 was 9 μg U/L. Importantly, the uU biologic action limit was evaluated as acceptable at 15 mcg/L as recently as 2014 (U.S. Nuclear Regulatory Commission (U.S. NRC), 2014).

Also, consistent with the lack of renal findings in the DU cohort are the reports on populations exposed to U via drinking water. In a Canadian cohort whose drinking water source contained uranium at concentrations exceeding 1 ppb and exceeding 100 μg/L for 50% of the group, a positive correlation between proximal tubular markers including urinary glucose, alkaline phosphatase and B2-microglobulin was reported with U intake (Zamora et al., 1998). Urine U concentrations in this cohort ranged from 0.1 to 1.7 μg/L (Zamora et al., 2002).

Kurttio et al. (2006), likewise reported only subtle proximal tubule function effects in a Finnish cohort also exposed via high background U in drinking water. Here, an association between cumulative uranium intake and urinary glucose excretion was reported in a cohort for whom 68% of their current uU samples exceeded 0.03 μg/L, the 95th percentile reference level for uU in a U.S. population sampled between 1988 and 1994 (Ting et. al., 1999). This value is close to the 95th percentile reference level of 0.043 μg/g creatinine reported for a more recently sampled U.S. population (see Fig. 1).

Another North American cohort environmentally exposed to U via drinking water had proximal tubule function assessed using RBP levels in urine with only four of 156 participants having a value above the normal range when the geometric mean for their uU values was 0.100 μg/g creatinine and their arithmetic mean, 0.300 μg/g creatinine (Wyatt et al., 2008). Returning to Fig. 1 in this paper, these uU values reported by Wyatt would lie between the 'DU cut point' used to stratify the DU population into a low or high uranium category, and a reported uU dietary upper limit for other U.S. cohorts exposed via diet or water source, reported by (ICRP, 2002). Such comparisons provide context and orient the DU cohort uU distribution among other industrial and environmentally U-exposed populations, which sustained little to no kidney injury from exposure at these concentrations, similar to our findings here.

Kidney injury thresholds, based on both animal and human

Table 7
Pulmonary function test (PFT) outcomes.

PFT parameter	Reference values	Overall (n=36)	Urine U burden sub-group		Ever smoker	
			Low uU group [a] (n=26)	High uU group [b] (n=10)	No (n=20)	Yes (n=16)
Spirometry[c]	Normal	Mean (S.D.[d])	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
FVC % predicted	≥80	88.56 (13.79)	88.19 (14.0)	89.5 (14.0)	87.05 (14.49)	90.44 (13.09)
FEV1% predicted	≥80	93.81 (14.83)	93.54 (15.5)	94.5 (13.7)	93.5 (14.05)	94.19 (16.2)
FEV1/FVC	≥70	77.69 (6.17)	0.78 (0.07)	0.77 (0.04)	79.4 (4.67)	75.56 (7.24)

[a] Low urine uranium (uU) group < 0.10 μg/g creatinine.
[b] High uU ≥0.10 μg/g creatinine.
[c] Spirometry measuress defined.FVC (forced vital capacity).FEV1 (forced expiratory volume in 1 s).
[d] S.D.- Standard deviation.

evidence, have been set historically to limit harm to Uranium workers, with the traditional threshold of 3 μg U/g kidney set by the ICRP in 1960 (ICRP, 1960). Informing the biokinetic modelling in humans, Pellmar et al. (1999) had previously shown that the biokinetics of DU migrating from implanted pellets in animal experiments had similar biokinetics to forms of U which had been injected or absorbed. This work provided the support for use of this model in assessing mobilization from U-contaminated wounds (Leggett and Pellmar, 2003).

Using the ICRP 69 biokinetic model for forms of U which had been injected or absorbed, Squibb et al. (2005) showed that only the highest U-burdened members of the DU cohort are now approaching that threshold, after 20 years of exposure.

Controversy exists as to the relative importance of the kidney as a long term storage site for U (discussed in Russell and Kathren (2004)). A new report of U tissue content in three males not thought to have occupational exposure to U, revealed measured renal concentrations an order of magnitude lower than the estimate for Reference Man, thus challenging the notion of a long term storage compartment of U in the kidney (Kathren and Tolmachev, 2015). In the veteran cases presented here, the on-going mobilization of U from fixed metal depots in soft tissue and its excretion from the systemic circulation chronically 'presents' U to the kidney. In addition, uranium's deposition in bone (Priest et al., 1982) and potential mobilization from bone due to age and other factors, further adds to continued 'exposure' of the kidney to U. Leggett thus, underscores the need to consider the "continual but diminished inflow of uranium released from the bone and other tissues" when assessing the kidney's retention of uranium.

This line of reasoning suggests that a focus on kidney surveillance remains appropriate for the cohort described here. Also recommending the continued assessment of kidney insult is the debate surrounding the evidence used to set the traditional "safe" threshold value of 3 μg U/g kidney. Questions regarding laboratory methods of decades ago determining urine U on the one hand and measures of renal injury, on the other have led some to suggest that the injury threshold value should be lowered, perhaps by an order of magnitude (Leggett, 1989).

Also a target of soluble U exposure, the CNS has been assessed using markers of neurocognitive function. Likewise here, consistent with results from previous assessment visits, no significant differences in neurocognitive function emerged between the two U exposure groups. The lack of a cross-sectional association between neurocognitive function and uranium burden does not preclude the possibility of a prospective relationship. That is, the impact of accumulated U, especially in light of its ability to penetrate the blood-brain barrier in animals (Pellmar et al., 1999) and given that the nervous system is a principal target of other heavy metals (Clarkson, 1987), may have a latent impact on brain-behavior relationships. It is possible that over time, as the U concentration continues to increase, a critical threshold may be exceeded, leading to a discernible, deleterious impact on neurocognitive function. Therefore, continued surveillance of neurocognitive function is planned and neurocognitive performance over time is currently being assessed longitudinally.

We note also that the present DU surveillance protocol is quite broad, permitting the identification of perturbations in most any organ systems, not only those in expected target organs. Hence, regarding the provocative observation made by Kathren and Tolmachev (2015) suggesting the thyroid as a potential long term storage site for U, we are able to report no apparent differences in thyroid function parameters (data not shown), but will remain vigilant to this possibility, as these endpoints will be retained in our surveillance battery.

As described above, the consequences of insoluble U oxide exposure would manifest in the pulmonary system. Veterans with high U body burdens continue to have no significant differences in lung function compared to those with low U body burdens. Mean FEV1 and FVC % predicted values obtained in 2015 were similar to values obtained in 2011 (93.9% and 90.9% predicted, respectively). While mean percent predicted values for each year's surveillance results appear clinically normal, an assessment of mean rate of decline in FEV1 and FVC over time will provide a better understanding of longitudinal change in lung function in this population. Furthermore, evaluation of the small airways using Impulse Oscillometry may provide additional understanding of distal lung function in DU-exposed Service members. This technique may allow us to recognize whether there are abnormalities that would otherwise be unrecognized using conventional measures of pulmonary function (Oppenheimer et al., 2007).

9. Conclusion

Now, more than 25 years since first exposure to DU, an aging cohort of military veterans continues to show no U-related health effects in known target organs of U toxicity. The results of an extensive health assessment have also shown no other clinically significant health effects as a function of U burden. However, with the accrual of new data challenging historical assumptions regarding target organs and storage depots, a broader net may need to be cast in surveillance efforts for exposed populations, as has been done for this cohort, so as not to miss an unexpected sentinel result. Also, as tissue concentrations of metals from retained fragments continue to accrue with exposure duration, critical tissue-specific U concentration thresholds may be reached, thus recommending continued surveillance for populations such as the one described here (NRC, 2008).

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The work described in this article has been carried out in accordance with *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans*.

The opinions and/or assertions expressed herein are the private views of the authors, and shall not be construed as official or as reflecting the views of the Department of Veterans Affairs, the U.S. Department of Health and Human Services, the U.S. Food and Drug Administration or the U.S. Federal Government.

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