Could inhalation of depleted uranium have contributed to Gulf War illness? The first direct test

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Abstract

Gulf War Illness is a chronic multi-symptom illness afflicting up to 25 percent of military personnel deployed to the 1991 Persian Gulf War. Of several exposures hypothesized as causes, depleted uranium (DU) has stimulated the most intense international concern. Past research has focused almost entirely on detecting high concentrations of uranium in the urine of veterans harboring DU-containing shrapnel, but with low-precision mass spectrometry, smaller amounts of DU absorbed by inhalation of fumes or dust, the main concern of populations exposed in later wars, have not been evaluated. Also no groups of veterans meeting standard case definitions of Gulf War illness have been studied. We developed high sensitivity mass spectrometry methods capable of detecting as little as 0.07 ng/day of DU in excreted urine, sufficient to detect ≥0.4 *m*g DU inhaled during the 1991 conflict. We applied these to urine samples from 154 individuals of a population-representative sample of U.S. veterans in whom Gulf War illness had been determined by standard case definitions and DU inhalation exposures obtained by medical history. Our analysis of $^{236}U/^{238}U$ and $^{235}U/^{238}U$ isotopic ratios showed no DU signature in any of the veterans. The findings support the conclusion that DU played no role in the development of Gulf War illness.

Main

Gulf War Illness. During or shortly after the 1991 Persian Gulf War, an estimated 25%[1] of the approximately 700,000 deployed U.S. (and additional allied) military personnel developed an unusual chronic multi-symptom illness, referred to as Gulf War Illness (GWI),[2] manifested by fatigue, fever and night sweats, memory and concentration problems, pathogen-free diarrhoea, sexual dysfunction, chronic body pain and other symptoms compatible with autonomic nervous system dysfunction[3] and dysfunction of the brain's cholinergic system.[4] Investigations into the causes have considered potential war theatre exposures including low-level chemical warfare nerve agent(s), pyridostigmine bromide anti-nerve-agent medication, pesticides, multiple immunizations, depleted uranium (DU), and combat stress. The possibility of toxic effects from DU, first raised after the first large scale use of DU munitions in the Gulf War, have stimulated international concern because of potential exposure of civilian populations from the Gulf War and from later conflicts in Kosovo, Bosnia, the Persian Gulf and others. Studies attempting to address this concern have failed to generate a consensus because of limitations in the sensitivity of tests for DU in urine and the lack of any investigation of DU in veterans' meeting accepted case definitions of GWI.

DU and its effects. DU is uranium depleted isotopically in the more fissile ^{235}U isotope which is separated by isotope enrichment methods to produce enriched ^{235}U for use in nuclear reactors and nuclear weapons. Instead of remaining as unused nuclear waste, it has been made into dense armour-piercing munitions used in military conflicts in 1991 and 2003 onwards as well as in tank armour. In a hard target impact a DU-containing projectile efficiently penetrates the target's armour, partially fragments the DU core, and ignites a brief intense fire, combusting

and oxidizing DU into aerosolized oxides. Humans may then internalize DU by inhalation of aerosolized DU oxides, oral ingestion of DU oxide particles that settle in the environment, or retention of DU in metallic form in shrapnel fragments in body wounds.

Adverse effects of significant intake are hypothesized to result from heavy metal toxicity and alpha particle radiation from DU mainly in the lungs, kidneys and bone where it is concentrated. Despite numerous studies demonstrating long-term urinary secretion of DU from industrial exposure and prediction of possible adverse effects on the basis of doses of heavy metal and alpha radiation,[5-8] no actual adverse effects in humans have been described, though Bruess and Snell recently argued that serious effects are being overlooked because of major gaps in research.[9] Nevertheless, DU continues to be considered a plausible cause of GWI,[10] and inhalation of DU in the former Yugoslavia conflicts remains a health concern to both veterans and civilian populations.[11] Recently, courts in the UK and Italy attributed illness and death to inhalation exposure to DU,[12,13] and an intense worldwide political movement works to ban DU munitions.

The 1991 Gulf War presents an opportunity to study the health effects of inhaled DU. Approximately 300 tonnes of DU-munitions were fired by tanks, artillery and aircraft mainly at targets in southern Iraq and especially along the Basra Road where Iraqi tanks were destroyed in large numbers by DU munitions. In addition to combat-related exposures, in July 1991 a post-war explosion and fire at an ammunition storage site within U.S. Army Camp Doha, Kuwait, resulted in a series of explosions involving several tonnes of DU munitions, causing DU-related aerosols to be released into the dense smoke plume from the fire to which many personnel were exposed during containment and cleanup.[14]

U isotopic ratios. Natural uranium (NU) is composed of ^{238}U (99.27%), ^{235}U (0.72%) and ^{234}U (0.0053%), with a $^{238}U/^{235}U$ ratio of 137.80-137.88.[1,15] In the production of nuclear fuel, enriched uranium (EU) high in ^{235}U is extracted from NU leaving the residual DU strongly depleted in ^{235}U and thus with a greatly increased $^{238}U/^{235}U$ ratio of approximately 500.[8,16] Aside from fission of ^{235}U in power generation, nuclear reactions also include neutron capture by ^{235}U generating ^{236}U, which is absent in NU; when re-enriched during nuclear fuel recycling, the resulting DU acquires this rare isotope. While EU has variable and significant amounts of ^{236}U, DU contains ^{236}U in low proportions (~0.003%).[16] The large differences in isotope composition among EU, NU and DU can be exploited by mass spectrometry to quantify even small proportions of DU in humans and the environment.[17,18]

Detection of DU. NU occurs naturally in food, water and soil. A portion (~2%) of ingested or inhaled uranium is absorbed into the bloodstream, concentrated and stored in bone and kidney, and excreted slowly in urine over many years. A large intake of NU, EU or DU can usually be detected by a urine assay for total uranium concentration [U]. Total [U] values above 43 *n*g/g of creatinine—the 95th percentile of the U.S. population[19,20]—indicate an excess body load of uranium, but highly sensitive mass spectrometry is required to determine whether the excess is due to NU, EU, DU or some combination. Two methods of mass spectrometry have been used to detect DU in human urine samples: lower precision sector-field mass spectrometry (SF-ICP-MS) has been used to differentiate DU from NU in Gulf War veterans at $^{238}U/^{235}U$ ratios above 166; whereas, higher precision multi-collector mass spectrometry (MC-ICP-MS) applied to chemically purified U can detect DU at $^{238}U/^{235}U$ ratios as low as 140.

Past studies of DU in Gulf War veterans. A series of studies between 1993 and 2009 was conducted to determine whether an exposure to DU in the 1991 Gulf War resulted in enough

absorption to detect in urine years later.[6] At first, small numbers of U.S. soldiers who had been involved in friendly fire explosions and, later, U.S. veterans who wanted to be tested provided urine samples for assay. All of the studies attempted to identify DU by measuring the total [U]. Some, but not all, veterans with X-ray-documented DU fragments retained in their bodies were found to have extremely high total [U] levels, far above the 95th percentile of the U.S. population—the cut point for distinguishing DU—and no veterans with inhalation exposures but no retained shrapnel did. In the final study,[21] however, measurements with low precision SF-ICP-MS identified 3 veterans out of 1,700 with $^{238}U/^{235}U$ ratio above the cut point of 166 confirming DU in their urine, but 2 of these 3 had total [U] below the cut point for DU and the third was right at the cut point; whereas, 29 veterans with no evidence of DU by SF-ICP-MS were nevertheless above the cut point by total [U], and other veterans who recalled inhalation exposures had far lower total [U] levels. Thus, while these studies demonstrated that a few veterans with retained shrapnel excrete very high levels of DU in their urine, the method was too imprecise to evaluate excretion from inhalation exposures. More importantly, since none of the studies measured the veterans' continuing symptoms or applied the standard GWI case definitions, no study has yet addressed the question of whether the symptoms of GWI could be due to DU exposure.

This study. In this paper we extend prior work to measurement of DU excretion in U.S. veterans with or without GWI who reported information on possible DU exposures. We first calculated the urinary DU concentrations expected as a function of time since exposure to plausible DU oxide levels from aerosol inhalation scenarios in the 1991 Gulf War[7,8,22] based on the Human Respiratory Tract Model relating absorption and excretion.[23] We then evaluated the capability of the following 3 bioassay methods to detect the subtle DU excretion in urine

expected from inhalation absorption: 1) screening for excess total [U]; 2) measurement of $^{238}U/^{235}U$ ratio with high sensitivity multi-collector mass spectrometry (MC-ICP-MS) on chemically purified U; and finally, bivariate analysis of the $^{236}U/^{238}U$ and $^{235}U/^{238}U$ isotopic ratios. We applied these methods to a highly studied population-representative sample of both theatre-deployed and non-deployed Gulf War-era U.S. veterans in whom Gulf War illness (GWI) had been determined by standard case definitions, whose wartime DU exposures had been ascertained by interview, and who provided a 24-hour urine sample 18-20 years after the war. We compared these measured values to those predicted to be found 18-20 years after exposure to medically meaningful DU levels in the war. The goal was to provide the best chance of finding DU if potential disease-causing inhalation exposure had occurred and for the first time provide high confidence in a negative finding.

Results

Predicted current values of DU detection parameters from Gulf War inhalation exposures. Inhaled fine particulates of DU oxide have the potential to lodge deep in the lung, decompose slowly into solution into the blood stream, and become stored in bone, kidney and other organs to be excreted over time with organ and bone re-working, all while undergoing slow radioactive decay. The rate of dissolution is a key parameter that underpins the notion that DU can be detected in urine many years after exposure.

To assess whether DU plays any role in GWI, it was first necessary to predict the concentration of DU expected in a urine sample for a given DU aerosol inhalation exposure as a function of the dose and oxide type of the original DU exposure, the time since exposure, and

likely dietary intakes of NU in the run up to urine collection. Without this prediction, it is impossible to know whether a negative test is due to an inconsequential exposure or an insensitive urine assay method—a valid criticism of past research on this problem.

The defining studies by the U.S. Department of Defense[7], the World Health Organisation,[22] and particularly the British Royal Society[8] classified battlefield situations of potential DU aerosol exposure into 3 standard exposure levels likely to occur in combat situations: level I-high, level II-medium, and level III-low. Exposure level I includes direct inhalation of an impact aerosol; level II, inhalation of resuspended impact aerosol or oral ingestion within a contaminated vehicle; and level III, inhalation of an aerosol plume at a distance from an impact or fire or resuspension from ground contamination. Doses in milligrams of DU taken into the body in each of these situations were estimated with evidence from other studies, including field studies involving measurements during destruction of vehicles with DU penetrators (**Table 1**).[24]

We used the International Commission on Radiological Protection's (ICRP) Human Respiratory Tract Model[23,25] that considers intake doses representing each of the Royal Society's exposure levels at 18 years after exposure and at 2 likely dietary intake levels of NU in the run up to urine collection to predict current excretion rates of DU oxides (UO_2, U_3O_8, etc.) in a range of excretion levels determined by the level of uncertainty in the ICRP models (**Fig. 1**). The model output[25] allowed calculation of the current expected ranges of the following 3 parameters most used for detecting past DU exposure from urinary assays: total [U], the $^{238}U/^{235}U$ isotopic ratio, and the $^{236}U/^{238}U$ ratio, given in **Table 1**. (See details of the calculation in Online Methods.)

Table 1 | Current ranges of urinary total [U] and U isotopic ratios that identify DU estimated from different levels of DU inhalation exposure during the 1991 Gulf War, time since exposure, and daily dietary intake of natural uranium running up to urine sample collection.

Parameters specified in the prediction model			Ranges of parameters expected 18 years after specified DU inhalation exposure			Can expected levels of DU be detected by criteria used in prior studies?		
Standard DU exposure level	Estimated DU intake (mg) for the exposure level in 1991 Gulf War[a]	Daily dietary U excretion (*n*g) in run-up to testing	Total [U] (*n*g U/g creatinine)	$^{238}U/^{235}U$	$^{236}U/^{238}U$ x10^{-6}	Method 1: total [U] >50 *n*g U/g creatinine[b]	Method 2: $^{238}U/^{235}U$ >166 by SF-ICP-MS[c]	Method 3: $^{238}U/^{235}U$ >140 by MC-ICP-MS[d]
Level I	250	2	14.5-74.5	367-467	25.9-29.2	rarely	yes	yes
		8	20.5-80.5	247-397	18.3-27.0	rarely	yes	yes
Level II	10	2	2.5-4.9	161-241	6.0-17.8	no	yes	yes
		8	8.5-10.9	144-171	1.8-8.0	no	no	yes
Level II	5	2	2.3-3.5	150-198	3.3-12.6	no	no	yes
		8	8.3-9.5	141-155	0.9 -4.6	no	no	yes
Level III	2	2	2.1-2.6	143-165	1.4-6.7	no	no	yes
		8	8.1-8.6	139-145	0.4-2.0	no	no	yes

Numerical values are derived from calculations of[8,23,25] as illustrated in Figure 1, described in more detail in on-line methods.

[a]Typical DU intake doses from Table 1 of the British Royal Society's 2001 report *The Health Hazards of Uranium Munitions*.[8]

[b]In the most recent study of DU in Gulf War veterans, Dorsey et al.[21] screened urine total [U] for DU by this criterion.

[c]Dorsey et al.[21] identified the presence of DU with the $^{238}U/^{235}U$ ratio measured by lower precision sector field–inductively coupled plasma-mass spectrometry (SF-ICP-MS)

[d]The present study identified the presence of DU with the $^{238}U/^{235}U$ ratio measured by the high precision multiple collector–inductively coupled plasma-mass spectrometry (MC-ICP-MS)

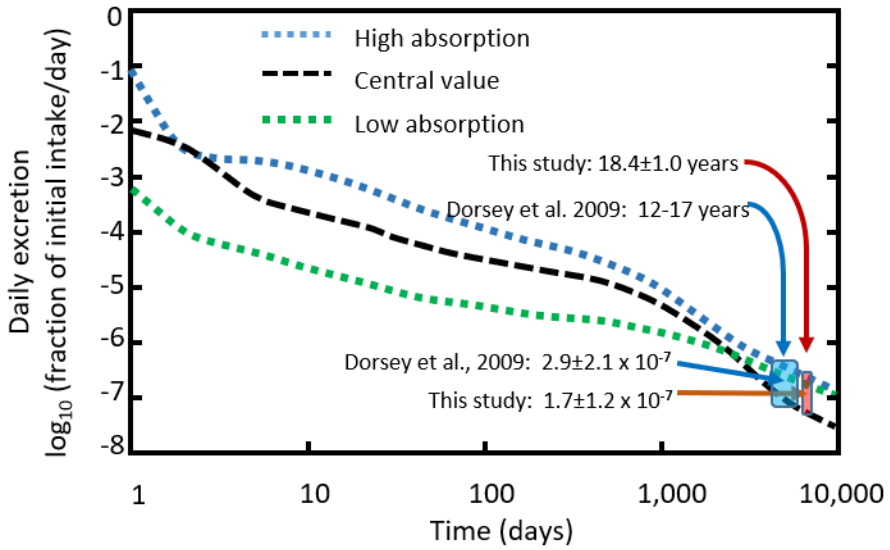

Fig. 1 | Estimation of current urinary DU excretion from DU oxides absorbed 18 years earlier. From the Human Respiratory Track Model (HRTM) for inhalation of uranium oxides we estimated the daily urinary excretion of DU, expressed as a fraction of the original inhaled dose (log scale), and plotted as a function of days since inhalation (log scale). In the sample of veterans studied, the duration between potential inhalation (in the first half of 1991 during the conflict) and the urine collection (November 2008 – June 2010) was 6,720±365 days or 18.4±1.0 years, shown as the width of the small red box. The analogous region from the Dorsey et al.[21] study discussed in the text is shown in the larger blue box. Three curves showing fractional uranium excretion predicted by the combined dissolution-storage-excretion function of the HRTM for uranium oxide particulates are shown; these represent "Low" and "High" absorption curves during dissolution and metabolism of compounds of uranium oxide with contrasting chemical form and solubilities. The figure is modified from Etherington[25] using the HRTM model using aerosol and oxide physicochemical characteristics summarized in Royal Society report,[8] and taking into account the uncertainty in the HRTM model parameters.[23]

Comparison of the ability of three methods to distinguish DU from NU in urine

Total [U]. Applying the criterion of finding >50 *n*g U/g creatinine of total [U] in urine will detect only veterans excreting the very highest total [U] in those with Level I inhalation exposures in the Gulf War, but would be unable to distinguish any with Level II or III exposures from NU excretion (**Table 1**).

$^{238}U/^{235}U$ measured by SF-ICP-MS. The relatively low precision SF-ICP-MS method would be able to differentiate DU from NU in all veterans with Level I exposures in the Gulf War and most in the upper half with Level II exposures but only if they are consuming a diet low in NU (average 2 *n*g NU per day) so that DU is ≥25% of the total U excreted. It would thus be unable to differentiate most with Level II and all with Level III exposures from NU excretion (**Table 1**).

$^{238}U/^{235}U$ measured by MC-ICP-MS after rigorous chemical purification of U. The high precision MC-ICP-MS method, following full chemical separation and purification of U from urine, allows the detection of excreted DU at a rate of >0.068 *n*g/day, our methodological DU detection limit. Our prediction model indicates that it would detect an initial inhalation exposure of as little as 0.40 *m*g of DU from the 1991 Gulf War (see details of the calculation in Online Methods). Thus, it would be able to identify DU in all veterans with Gulf War exposures far less than the lowest Level III exposure (2 *m*g DU inhaled during the Gulf War) even if consuming a diet relatively high in NU before urine collection (**Table 1**). It follows then that only mass spectrometry with high precision MC-ICP-MS is capable of detecting DU from inhalation exposure in the Gulf War or confirming its absence in most Gulf War veterans.

We studied possible DU exposures in a representative sample of GWI cases and controls who experienced a variety of battlefield DU exposure situations. We measured DU in urine samples from a nested case-control sample of Gulf War veterans selected in a 3-stage stratified random sample of the 1991 U.S. military population studied in the U.S. Military Health Survey (USMHS). The methods of sample selection at the 3 stages have been published.[3,26] The first stage involved a computer-assisted computer interview (CATI) survey (N=8,020) which included questions covering battlefield situations likely to involve inhalation of various levels of DU, from which we assigned the standard DU exposure levels. The second stage involved blood collection from all veterans whose symptoms met the 3 widely used case definitions of GWI (cases) and an approximately 10% random sample of those not meeting it (controls), including both deployed and not deployed to the Kuwaiti Theatre of Operations. The third stage constituted a smaller representative sample selected from the larger stage 2 sample, and included 106 who met the 3 standard case definitions of GWI[2,27,28] in which cases comprised of 31 with syndrome variant 1 ("impaired cognition"); 42 with syndrome variant 2 ("confusion-ataxia"); 33 with syndrome variant 3 ("central pain")[2]; and 47 control veterans comprised of 26 deployed to the war theatre and 21 non-deployed not meeting the case definitions. Between November 2008 and June 2010, the 153 veterans in the stage 3 sample were studied extensively in a 7-day clinical research protocol in which each travelled to Dallas, Texas (USA) to be hospitalized in the University of Texas Southwestern Medical Center's Clinical and Translational Research Center. In addition to diverse neuropsychological, autonomic and neuroimaging studies, a 24-hour urine sample was collected in urine containers prewashed with nitric acid to remove any trace uranium; creatinine was measured on an aliquot shortly after collection.

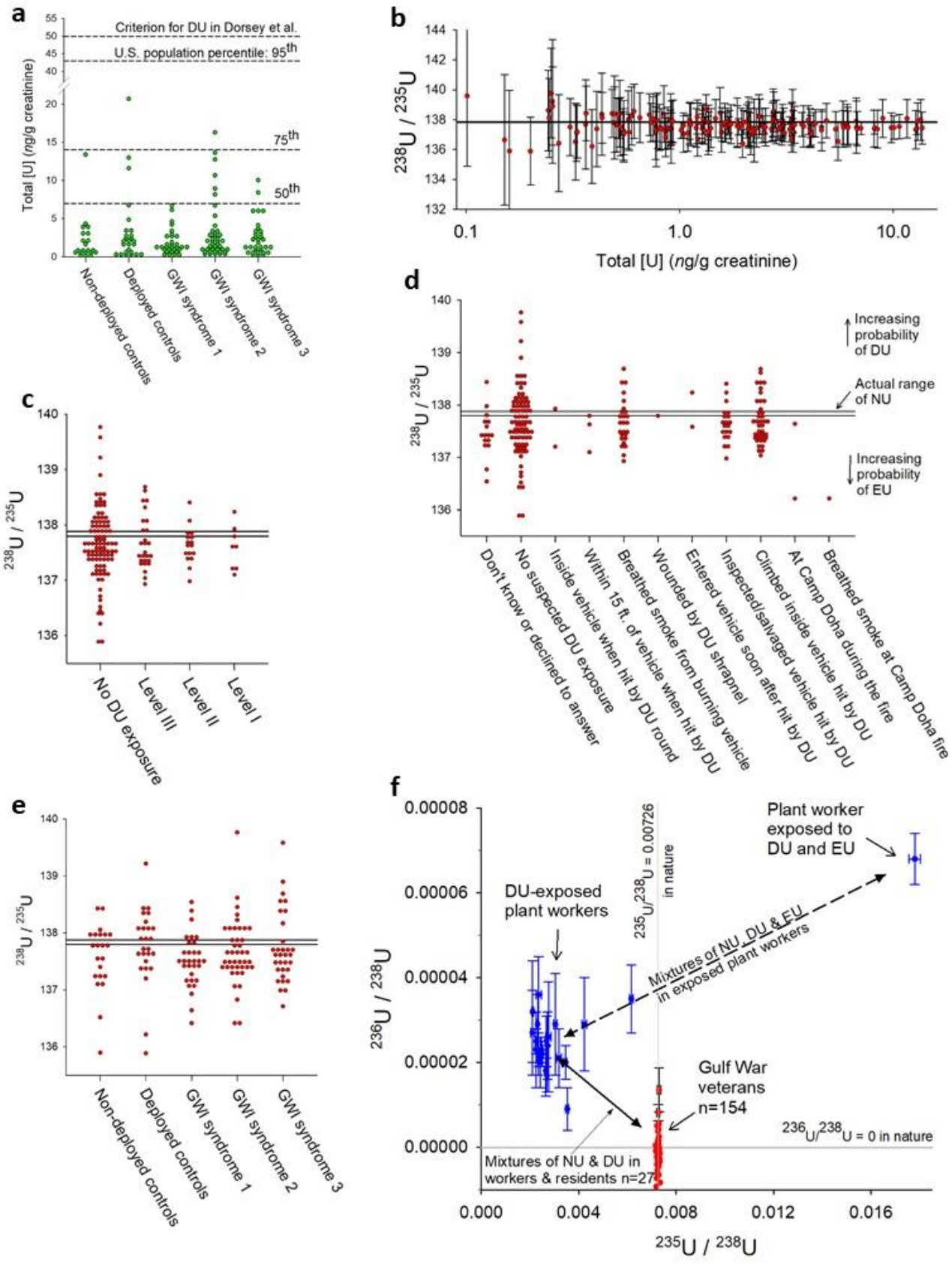
a
Criterion for DU in Dorsey et al.
U.S. population percentile: 95th
75th
50th
Total [U] (ng/g creatinine)
Non-deployed controls
Deployed controls
GWI syndrome 1
GWI syndrome 2
GWI syndrome 3
b
238U / 235U
Total [U] (ng/g creatinine)
c
No DU exposure
Level III
Level II
Level I
d
Increasing probability of DU
Actual range of NU
Increasing probability of EU
Don't know or declined to answer
No suspected DU exposure
Inside vehicle when hit by DU round
Within 15 ft. of vehicle when hit by DU
Breathed smoke from burning vehicle
Wounded by DU shrapnel
Entered vehicle soon after hit by DU
Inspected/salvaged vehicle hit by DU
Climbed inside vehicle hit by DU
At Camp Doha during the fire
Breathed smoke at Camp Doha fire
e
Non-deployed controls
Deployed controls
GWI syndrome 1
GWI syndrome 2
GWI syndrome 3
f
236U / 238U
235U / 238U
Plant worker exposed to DU and EU
DU-exposed plant workers
235U/238U = 0.00726 in nature
Mixtures of NU, DU & EU in exposed plant workers
Gulf War veterans n=154
236U/238U = 0 in nature
Mixtures of NU & DU in workers & residents n=27

Fig. 2 | Total uranium concentration [U] and isotopic ratios in urine from 152 Gulf War-era veterans by reported DU exposure levels and incidents and the Gulf War illness (GWI) clinical classification. a, The creatinine-standardized total [U] (*ng/*g of creatinine) by the veterans' GWI clinical classification; horizontal reference lines indicate the 50^{th}, 75^{th} and 95^{th} percentiles of the distribution of urine U concentrations in the U.S. population[19,20] and the lower limit for detecting DU in Dorsey et al.[21] **b,** The veterans' $^{238}U/^{235}U$ ratios with their individual measurement uncertainty intervals by total [U], demonstrating how the uncertainty intervals and variance of the point estimates increase at lower total [U], producing both high and low outliers. All certainty intervals overlap natural U (horizontal lines). **c–e,** Distributions of the veterans' $^{238}U/^{235}U$ ratios by the standard classification of DU exposure levels[8] (**c**), by specific DU exposure situations (**d**), and by the GWI clinical classification (**e**). The horizontal reference lines indicate the range of the $^{238}U/^{235}U$ ratio of natural U (137.80-137.88), above which values suggest mixtures of natural U and DU and below which, mixtures of natural and enriched U; individual measurement uncertainties of ~1% are omitted for clarity. The standard exposure levels represent aggregations of the specific exposure situations. **f,** Bivariate scatterplot of the $^{236}U/^{238}U$ ratio by the $^{235}U/^{238}U$ ratio for the sample of Gulf War veterans (red symbols), overlaid by those of workers in a DU plant in the town of Colonie, New York, known to have had substantial occupational inhalation DU exposures,[5,29] along with long-term residents that lived near the plant, shown in the white box. The horizontal reference line at $^{236}U/^{238}U = 0$ emphasizes that natural U contains no ^{236}U. The ratios from subjects with various exposure levels of DU would fall along the solid line; whereas, subjects with mixed exposure to DU and enriched U would fall in the upper right where one such DU worker is shown. Six of the Gulf War veterans had elevated $^{236}U/^{238}U$ ratios with certainty intervals that do not overlap zero, but their $^{235}U/^{238}U$ ratios are not reduced, which is incompatible with DU and EU; this is caused by an isobaric interference on mass 236 and is explained in the online methods and the text. The standard DU exposure levels range from level III (lowest) to level I (highest).[8] The GWI clinical classification includes syndrome 1 (impaired cognition), syndrome 2 (confusion-ataxia) and syndrome 3 (central pain).[2]

Table 2. Urinary excretion rate of total U by Gulf War veterans' clinical group

Clinical group	Sample size	Total [U]				Total [U] adjusted for creatinine	
		Mean	95% CI	5^{th} and 95^{th} percentile	range	Median	Mean
Non-deployed controls	21	2.37	-1.04 / +1.86	0.24-11.2	0.2-30.2	1.25	1.24
Deployed controls	27	3.80	-1.60 / +2.78	0.42-50.2	0.32-96.4	2.16	1.87
GWI syndrome 1	31	2.53	-0.78 / +1.12	0.58-10.7	0.22-13.3	1.38	1.41
GWI syndrome 2	42	4.00	-1.25 / +1.81	0.77-21.7	0.70-28.8	2.05	2.15
GWI syndrome 3	33	3.33	-1.05 / +1.54	0.65-12.0	0.44-15.1	1.95	1.74
All controls	49	2.98	-0.98 / +1.45	0.36-25.9	0.17-96.4	1.86	1.57
All GWI	106	3.30	-0.57 / +0.69	0.69-16.2	0.13-23.9	1.84	1.78
All samples	154	3.24	-0.56/ +0.68	0.57-17.6	0.17-96.4	1.84	1.71

Screening with total [U] of urine, the most common previously applied test, fails to rule out potentially important past DU exposure. The first method we used to detect evidence of past DU exposure was to screen the 153 GWI cases and control subjects' urine for an increase in total [U] excretion (**Table 2**). The distribution of total [U] was similar to that of the U.S. population,[19] and no values exceeded its 95th percentile (**Fig. 2a**). The geometric mean of total [U] of the cases meeting the 3 case definitions of GWI was 1.78 *n*g U/g creatinine, statistically indistinguishable from the combined deployed and non-deployed controls (geometric mean 1.57 *n*g U/g creatinine, t-test P=0.18). These values of total [U] are consistent with lack of DU contamination but do not exclude the possibility of the small amounts of DU expected from our prediction model with Level II and III inhalation exposures (**Table 1**). Although continuing dissolution of DU shrapnel in metallic form retained in the body usually increases total [U] beyond the population's 95% percentile, as concluded by Dorsey et al.,[21] our findings further confirm that screening of total [U] is not useful for detecting the far smaller intake doses and the time-limited exposure situations involved in inhalation exposures to DU aerosols (**Table 1**).

High precision measurement of the ^{238}U/^{235}U ratio establishes no significant inhalation exposure regardless of exposure situation level or GWI symptoms. The second method we used to detect past DU exposure was analysis of the ^{238}U/^{235}U isotopic ratio measured by the high precision MC-ICP-MS method (**Table 3**). The uncertainty in the ^{238}U/^{235}U ratio, measured by its 95% confidence interval, increases (precision decreases) as the urinary total [U] decreases (**Fig. 2b**), but it was less than ±1% for 95% of the samples (**Fig. 2b**). For urine samples with total [U] above 1 *n*g/g creatinine and a ±1% uncertainty, values of the ^{238}U/^{235}U ratio above 139-140 including their lower uncertainty bound are considered likely to represent the presence of DU

(**Fig. 2b**). This threshold of 139-140 for confirmation of DU is more robust and >10 times more sensitive than the threshold of 166 applied by Dorsey et al. using the lower precision SF-ICP-MS (**Table 1**).[21]

Table 3. Urinary uranium isotope ratios by Gulf War veterans' clinical group

		^{238}U / ^{235}U			^{236}U / ^{238}U	
Clinical group	Sample size	Median	Mean[a]	95% CI[a]	Mean[a,c]	95% CI[a]
Non-deployed controls	21	137.79	137.62	0.26	<LOD	n/a
Deployed controls	27	137.84	137.75	0.25	<LOD	n/a
GWI syndrome 1	31	137.50	137.51	0.16	<LOD	n/a
GWI syndrome 2	42	137.62	137.68	0.16	<LOD	n/a
GWI syndrome 3	33	137.62	137.71	0.19	<LOD	n/a
All controls	49	137.79	137.70	0.18	<LOD	n/a
All GWI	106	137.55	137.64	0.10	<LOD	n/a
All samples	154	137.63	137.66	0.09	<LOD	n/a
In house urine no IRMM184 [d]	4	137.70	137.68	0.27	<LOD	n/a
2 ppb IRMM184+233U [e]	125	137.70	137.71	0.05	1.11E-07	1.3E-08
IRMM184 certified [f]			137.70	0.04	1.25E-07	5.3E-10
Natural uranium			137.82	~0.06	$<10^{-8}$	n/a
DU in munitions			~500		~0.000030	

DU, depleted uranium; IRMM, European Commission's Institute for Reference Materials and Measurements; LOD, limit of detection; n/a, not applicable; U, uranium

[a]Geometric means with 95% CI (confidence intervals calculated using standard deviations of log(values)

[b]Since ^{238}U/ ^{235}U uncertainties are not significantly asymmetrical and are reported as a single value.

[c]The ^{236}U/ ^{238}U ratios were overwhelmingly below the 0.0000015 limit of detection after all corrections and uncertainty propagations were made.

[d]The 4 analyses of in-house urine that had no additional IRMM184 added.

[e]IRMM184 + 233 U values reported are corrected for very minor contributions of other isotopes in the ^{233}U that was added.

[f]IRMM certified value from https://crm.jrc.ec.europa.eu/p/40454/40475/By-application-field/Nuclear/IRMM-184-URANIUM-238-NATURAL-ISOTOPIC-NITRATE-SOLUTION/IRMM-184

Exposure situations. When predictions of DU excretion are applied to groups of veterans with different standard levels of DU inhalation exposures, the $^{238}U/^{235}U$ ratios are below 139 for veterans with all 3 inhalation exposure levels (**Fig. 2c;** Kruskal-Wallis test P=0.74). The 3 veterans with outlying values nearest the threshold between 139 and 140 all had very low values of total [U] and thus very wide uncertainty intervals that generously overlap the $^{238}U/^{235}U$ of NU (~137.8). When the battlefield exposure situations from which the standard exposure levels were generated were broken out, the distributions of the $^{238}U/^{235}U$ ratios showed no pattern suggesting any departure from NU outside of methodological uncertainty' (**Fig. 2d**).

GWI symptoms. Likewise, veterans who continue to have potentially disabling symptoms of GWI had distributions of the $^{238}U/^{235}U$ ratio that did not differ from the deployed and non-deployed control veterans (**Fig. 2e**), and none had values that differed from NU. Moreover, the distribution of the $^{238}U/^{235}U$ ratios in the 3 GWI syndrome variant groups combined was similar to those of the deployed and non-deployed control groups (Kruskal-Wallis test P=0.16).

Bivariate analysis of the $^{236}U/^{238}U$ and $^{235}U/^{238}U$ isotopic ratios further establishes the absence of DU in veterans with GWI. As a further test for the possibility of DU in these veterans, we studied their location on the bivariate distribution of the veterans' ratios of both $^{236}U/^{238}U$ and $^{235}U/^{238}U$ isotopic ratios. This is an important procedure because, since ^{236}U is found only in EU and DU but not in NU, it provides a further direct method of distinguishing DU from NU and from ascertaining whether EU may also have been involved. Since ^{236}U constitutes only ~0.003% of DU used in munitions, it can only be measured by high precision MC-ICP-MS and thus has not previously been measured in research on GWI.

We applied this approach to our representative sample of Gulf War veterans in the context of a prior studies[5,18,29] of groups of U.S. civilians exposed to DU (and EU) aerosol pollution in

New York State from a uranium fabrication plant in the 1960s and 1970s[30] and in part studied with MC-ICP-MS of urine samples using the same methods as in our study.[5,30] These studies demonstrated that significant doses of DU aerosols inhaled by factory workers and residents living nearby can be detected in urine ≥25 years after the plant was closed,[5,29] broadly consistent with predictions of the HRTM model.

In the bivariate plot in **Fig 2f**, an individual excreting pure NU would be located at the point where $^{235}U/^{238}U = 0.00726$ and $^{236}U/^{238}U = 0$, reflecting the usual amount of ^{235}U and the absence of ^{236}U in natural U. A theoretical individual excreting pure DU would be located at a point defined by $^{235}U/^{238}U \approx 0.002$ and $^{236}U/^{238}U \approx 0.00003$, but most DU-exposed workers excrete a combination of DU and NU, which displaces them downward and to the right; whereas, EU added to the mix would displace them upward and to the right. Using published analyses of workers from the plant, mixtures of NU and DU had lower ^{235}U and higher ^{236}U, locating them along the solid line. Mixtures of NU and EU would have $^{235}U/^{238}U$ above that of NU ($^{235}U/^{238}U > 0.00726$), indicating substantially increased ^{235}U, and variably increased ^{236}U. Mixtures of NU, DU and EU would appear along the dashed line in the diagram.

Published studies of the DU plant workers and local town residents with proven non-military DU aerosol exposure[5,29] are shown to illustrate where subjects with proven inhalation exposure to DU typically fall. All but 6 of the Gulf War veterans in our study are located in a narrow zone located exactly at $^{235}U/^{238}U = 0.00726$, the value of natural U, and with $^{236}U/^{238}U$ ratios within the uncertainty zone around $^{236}U/^{238}U = 0$ (**Fig 2f**). The 6 exceptions have slightly elevated $^{236}U/^{238}U$ ratios and values of the $^{235}U/^{238}U$ incompatible with both DU and EU, indicating an artefact of measurement from organic molecule interference in these samples (see explanation in online methods).

One veteran with a Level I battlefield exposure and DU shrapnel retained for 4 months showed no DU in urine. One Gulf War veteran in our study was standing on an Abrams tank when it was hit in a "friendly fire" accident by a DU round which destroyed the tank, threw the individual several meters, peppered him with a mix of sand, pebbles and shrapnel, tattooing his skin and embedding 2 pea-sized pieces of DU shrapnel under his skin. He breathed the hot gases from the explosion for several minutes. Upon return to his base in the U.S. 4 months later, the shrapnel was removed. During his wartime deployment to the war theatre, however, he was also exposed to low-level sarin nerve agent, took pyridostigmine tablets, and has moderately low PON1 type Q isoenzyme level, which are typical risk factors for GWI.[31-33] The veteran had symptoms satisfying the case definitions of GWI, subclassified as variant syndrome 1 ("cognitive impairment"), but his urine showed a total [U] of 1.35 *n*g/g creatinine, a $^{238}U/^{235}U$ ratio of 137.8, and a $^{236}U/^{238}U$ ratio below detection limit (<0.000001)—all typical of natural U with no DU. If residual DU were present it was being excreted at a rate of <0.068*n*g/day, our limit of detection, and our prediction model indicates that he could have absorbed no more than 0.40 *m*g of DU from the Gulf War from both inhalation and DU shrapnel.

Discussion

Our findings, using high precision mass spectrometry MC-ICP-MS on chemically purified U that detects even the lowest level of DU exposure capable of causing illness, demonstrate that a sample of veterans drawn from a large population-representative sample of Gulf War veterans, meeting the case definitions of GWI and reporting a range of inhalation exposures to DU in friendly fire accidents, did not absorb even the smallest amount of DU capable of producing chronic adverse effects on health. The past studies of DU in Gulf War veterans have shown clear

evidence of DU absorption into the body only in individuals with chunks of DU in metallic form as shrapnel retained in tissues from friendly fire wounds. The methods of detecting DU used in those studies—total [U] and limited use of U isotope ratios measured by the less precise SF-ICP-MS mass spectroscopy method—however, had insufficient precision to differentiate the lower levels of DU absorption likely with inhalation exposures from dietary NU absorption. Moreover, no past study has tested the association of urinary DU excretion measurements with GWI defined by the standard case definitions. Consequently, past studies did not address the question more widely concerning to Gulf War veterans and others of whether inhalation of DU in the war caused, or contributed to, the GWI. Our study, however, found no DU excretion in either veterans meeting the case definitions of GWI or control veterans not meeting them. Given the high precision of our methods, our results not only show an absence of evidence for an association but evidence for the absence of that association.

The element of our study design that gives meaning to a negative finding is that we first developed estimates of the amount of DU that would still be excreted in urine 18 years after exposure to amounts of DU found through simulation studies to result from various situations where soldiers inhaled DU oxides from explosions of DU munitions. We then tested urine from a representative sample of ill and well Gulf War veterans with the high precision mass spectrometry method, not used in prior testing of large samples of veterans, that is capable of detecting the levels of DU excretion predicted by the model. We based the predictions on the best estimates of the bodily absorption of DU that results from various exposure events and the modelling on the most widely accepted approach of the International Commission on Radiological Protection's (ICRP) Human Respiratory Tract Model.[23,25] This study design

provided the best chance of finding evidence of DU exposure if it exists but also provides a high degree of confidence in a negative finding as well.

A limitation of our study is that we included only Gulf War-era veterans who were potentially exposed to inhalation of DU from infrequent, very brief, though often intense, friendly fire accidents and sporadic inhalation of dust potentially contaminated with DU particulates over a 4-5 month period. While study of these Gulf War veterans addresses the question of whether short-term and potentially intense inhalation exposures produce enough systemic absorption to cause chronic illness, it may not adequately address the wider implications of decades of continuous exposure to DU-containing dust faced by the populations in war zones where DU munitions have been deployed. The design of our study applying high precision mass spectroscopy to urine samples could readily be adapted to study residents of former war zones to identify ongoing DU excretion as a quantitative biomarker of true DU absorption. Any effects of this exposure on health could then be addressed with clinical studies comparing sensitive measures of adverse health effects in persons with DU-positive and DU-negative test results.

For now, however, Gulf War veterans need no longer be concerned that a connection between their chronic illnesses and DU exposure in the Gulf War has been inadequately addressed by insensitive methods of measuring DU excretion and studies not relating DU measurements directly to GWI. If DU had been an important cause of GWI, our study, applying the most precise measure of DU to a representative sample of Gulf War veterans meeting the standard case definitions, was sensitive enough to have found it. From our negative results, we conclude that inhalation of DU during the Gulf War was not an important contributor to the GWI. Now scientific attention must be focused even more intensely on the remaining likely

causes, particularly the widespread exposure to cholinesterase-inhibiting toxicants including low-level sarin nerve gas known to have been widely dispersed during destruction of chemical weapons stores by Allied bombing of Iraqi chemical weapons storage depots,[33,34] as well as pesticides and pyridostigmine, for which considerable evidence exists.[32]

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https:// . . .

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Methods

Subjects. The 154 Gulf War-era U.S. military veterans who participated in this study were selected by a 3-stage statistical sampling plan as a representative sample of those who served in the U.S. armed forces during the 1991 Persian Gulf War, the target population. The first stage involved a computer-assisted telephone interview (CATI) survey of a stratified random sample of the target population, known as the U.S. Military Health Survey (USMHS). The sample was selected from the computer personnel file of the Gulf War-era military population between August 2, 1990 and July 1, 1991, obtained from the Defense Manpower Data Center (DMDC, Seaside, CA), stratified by the following design parameters: a flag indicating deployment to the KTO, age (<49 years, ≥49 years), sex, race/ethnicity (non-Hispanic white, other), military rank during the war (officer, enlisted), military component (active duty, Reserve/Guard), military occupation (air flight crew, aircraft maintenance, army special operations, other), location in KTO on 20 January 1991 (deployed only), and 3 special study samples (twin pairs, member of 24th Reserve Naval Mobile Construction Battalion, and parent of a child with Goldenhar complex birth defect). Of the full USMHS sample (n=8020), 6,497 were deployed to the Kuwaiti Theatre of Operations, and 1,523 were non-deployed. With 74.9% of the selected veterans located and contacted and 80.2% of these agreeing to participate, the overall response rate was 60.1%. The methods, extensive pilot testing and initial findings of the USMHS were described in detail elsewhere.[26]

The standardized interview included questions on specific in-theatre scenarios previously defined by the U.S. Army's DU Capstone project for calculating the 4 levels of DU exposure recommended by the U.S. Department of Defense and the British Royal Society for epidemiologic studies.[7,8] It also contained all questions required to define the 3 most often used case definitions of GWI: the Factor,[2] CDC[27] and Modified Kansas[28] definitions. The Factor case definition was developed with principal components analysis of symptom scales to identify groups of veterans with similar patterns related to

deployment and was extensively validated.[26,35] All deployed personnel were present for the 5-week air war and the 5-day ground war in January and early February 1991.

The second stage involved selection of all veterans who met any of the 3 case definitions and an approximately 15% random sample of all who did not. The resulting sample included 2,103 veterans, from whom samples of peripheral blood serum, plasma, DNA and RNA were collected by trained phlebotomists in or near subjects' homes, shipped overnight to the UT Southwestern laboratory, and archived for later studies.

The third stage selected a subset (n=154) of those who participated in the second stage and were randomly selected as a representative sample of Gulf War-era veterans with and without GWI. These were hospitalized between late 2008 and June 2010 in the UT Southwestern Medical Center's Clinical Research Center for a 7-day research protocol involving 25 clinical neurological, neuroimaging, EEG and genetic studies of GWI. This protocol included the collection of a 24-hour timed urine collection supervised and timed by professional research nurses in the hospital's clinical research center. The urine samples were collected in urine collection bottles that had been pre-washed with a HCL solution to remove any trace of uranium. At the completion of collection, the volume of each 24 h urine sample was measured and recorded, and an aliquot was sent to Quest Laboratories for creatinine determination. The urine samples were then stored at 4°C. In late 2017, two 500 ml pre-cleaned HDPE bottles, a 500 ml aliquot was taken from each well shaken urine sample, and sent to the University of Portsmouth in the U.K. for uranium isotope analysis.

Calculation of inhalation exposure using the Human Respiratory Tract Model (HRTM) of the International Commission on Radiological Protection (ICRP)

The predictions of urinary DU concentrations from DU absorbed 18 years earlier, shown in Fig. 1, are based on information in the annexes to the comprehensive study by the Royal Society report on DU,[8] World Health Organisation,[22] and Ethrington.[25] These sources describe the absorption of inhaled material from the respiratory tract and how this can be modelled in terms of accumulation in the kidneys and bone

and excretion in urine over time, dependent upon the type and solubility of uranium oxide particles. These are based upon the Human Respiratory Tract Model (HRTM) of the International Commission on Radiological Protection,[23] Annex A of the Royal Society report[8] presenting available information on absorption characteristics (i.e. lung solubility) of particulate DU from DU penetrator impact and combustion in fires, respectively. Because relatively insoluble UO_2 and U_3O_8 are the predominant oxides from DU combustion,[7,36] they have the slowest dissolution rate constants, approximately 0.0012-0.00035 and 0.0015-0.00049 for U_3O_8 and UO_2, respectively.

The modelling[25] using the Human Respiratory Tract Model[23] is shown in **Fig. 1** of the paper for inhalation of U oxides of various types and illustrates daily excretion as a fraction of the original intake as a function of time. An analogous box is shown for the Dorsey et al. study,[21] Using a range of initial inhalation doses of DU from 250 down to 2 mg, we calculated the DU excretion during the time window of urine collection and added to this two contrasting ranges of daily dietary excretion of natural U of 2 and 8 ng/day of U. For these calculations the $^{238}U/^{235}U$ and $^{236}U/^{238}U$ for DU were 500 and 30 x 10^{-6}, and for natural U 137.82 and 0, respectively. From these excretion rates, we calculated the fractional amount of DU in urine, and the corresponding isotope ratios of $^{238}U/^{235}U$ and $^{236}U/^{238}U$; this allows assessment of measurement capabilities and limits of detection for DU for our method and that of all previous urinary U isotope measurements of US Gulf War veterans,[23] which used a less sensitive SF-ICP-MS methodology.

The predicted isotope ratios $^{238}U/^{235}U$ and $^{236}U/^{238}U$ were calculated from **equations 1 and 2,** using end member DU of $^{238}U/^{235}U$ = 500 and $^{236}U/^{238}U$ = 30 x 10^{-6}, (2) NU $^{238}U/^{235}U$ = 137.82 and $^{236}U/^{238}U$ = 0, and the fraction of excretion that is DU (f_{DU}).

$$^{238}U/^{235}U_{mixture} = [f_{DU} / (^{238}U/^{235}U_{DU}) + 1 - f_{DU} / (^{238}U/^{235}U_{NU})]^{-1} \quad (1)$$

$$^{236}U/^{238}U_{mixture} = f_{DU} * (^{236}U/^{238}U_{DU}) \quad (2)$$

The sensitivity threshold for positive detection of DU using $^{238}U/^{235}U$ measurements was 139-140 for our method (described below) and 166 according to the methods used by Dorset et al.[21] Because we could not

detect DU if its proportion was <2%, corresponding to a $^{238}U/^{235}U$ of 140, we calculate that based on the mean rate of excretion of 3.4 ng/day, the maximum amount of DU excreted would be < 0.068 ng/day. Using the HRTM-based calculation, this leads to a maximum inhalation dose $0.4^{+1.0}_{-0.2}$ mg during the Gulf War.

Chemical procedures

Extraction and purification of uranium. U was extracted from urine samples and purified so that high sensitivity MC-ICP-MC measurements could deliver increased precision of U isotope measurements. Methods of measurement were modified from those standard measures[37,38] by the addition of ^{233}U tracer for quantification, co-precipitation of U from urine with calcium phosphate, and purification of U via ion exchange chromatography using Eichrom© U-TEVA resin, before being re-dissolved in 2% HNO_3 for analysis.

Reagents. Water was from a Milli-Q© system with 18.2Ω resistivity. Acids comprised HNO_3 and HCl and were sPA Romil© reagents, diluted as appropriate. Ammonia and H_2O_2 were also sPA Romil© reagents. Ion exchange resins used were 50-100µm TRU, UTEVA and PFR resins from Eichrom©. Co-precipitation and other reagents comprised $Ca(NO_3)_2$, ammonium phosphate monohydrate ($NH_4H_2PO_4$), and $Al(NO_3)_3$. These latter reagents were not sufficiently clean (i.e. free of uranium) to permit off the shelf use and had to be cleaned by dissolution in 3N HNO3 and either mixed in a slurry with TRU +/- UTEVA resin(s) or repeatedly loaded and rinsed through columns containing TRU and UTEVA to remove U which is retained at this normality of nitric acid. $Ca(NO_3)_2$ and $Al(NO_3)_3$ were easy to clean but with the high phosphate content of ammonium phosphate, the retention of U in UTEVA and TRU resins is degraded, so up to 3 additional steps were necessary and even with this level of cleaning, this reagent contributed a majority of the U introduced in the procedure.

Tracer. A purified ^{233}U tracer from the IRMM (Institute of Reference Materials and Measurements, Geel, Belgium) was used. Its isotope purity is 99.6% ^{233}U; we also measured its full isotope composition for contribution from other U isotopes, including ^{236}U. This solution was prepared to 0.84ng/ml and dissolved in 1N HNO3. Using a pipettor, 0.045ml, or about 38 pg of ^{233}U, was nominally dispensed to samples.

Plasticware and environment. Plasticware consisted of Eppendorf© pipettors and pipette tips, PFA Teflon or LDPE bottles for storage, HDPE 250ml bottles, 10ml tapered PE columns from Dowex©, Quartz glass beakers for wet-ashing and evaporation, PFA Savillex© or quartz glass beakers for evaporation, and FEP-enclosed magnet stirring bars. All plastic- and glassware was pre-cleaned with nitric acid and rinsed with purified water. All chemical procedures were done in a class 100 clean lab containing a fume extraction cupboard and a separate HEPA filtered class 100 dedicated clean air hood.

Chemical method of U extraction and purification. The method was modified from the Eichrom© procedure for separating and purifying U from urine but with some steps modified or eliminated. Batches of 24 samples consisted of 20 samples, two aliquots of the in-house large urine sample to which some IRMM184 ('natural' uranium of certified isotope composition) was added, and two blanks with 150ml of MQ H_20 instead of urine. Briefly, samples were weighed (150ml nominal sample), a stirring magnet bar was added and placed onto a stirring hot plate at ~ 75ºC, followed by addition of concentrated nitric acid, 0.045ml of the 0.84ng/g ^{233}U tracer solution, calcium nitrate and ammonium phosphate. The acidity was verified to be > pH 1 using pH strips or pH meter and allowed to heat and stir for about 30 minutes. Following this, ammonia was added to raise the pH to ~9 (checked with strips or pH meter) and further stirred for 30 minutes on the hot plate. The sample was removed from the hotplate and the suspended precipitate was allowed to settle at least overnight. The supernatant was poured off to waste and the precipitate was transferred to a pre-cleaned quartz beaker to which 5ml each of concentrated NHO_3 and H_2O_2 were added. Several cycles of addition of 5ml each of concentrated NHO_3 and H_2O_2 were followed by evaporation to dryness on a 90°C-140°C hotplate to produce a pale or colourless salt, largely free of

organic matter. This was dissolved in 3ml of 1N $AlNO_3$ - 3N HNO_3 and loaded into columns containing 1.4ml UTEVA resin capped by 0.2ml of PFA resin (PFA is a resin that absorbs organic material), both precleaned prior to sample load with H_2O, 0.6N HCl, and preconditioned in 3N HNO_3. Alkali and non-actinide metal salts were removed by rinsing the columns with 3N HNO3 and a modest amount of 6N HCl, and then uranium was removed by eluting with 0.6N HCl. The uranium in 0.6 HCl was evaporated, a small amount of concentrated HNO_3 was added to remove any residual organic matter and to convert the uranium to a nitrate salt, evaporated, and dissolved in 1.2 ml of 2% HNO_3, and transferred to pre-cleaned microcentrifuge tubes, ready for MC-ICP-MS mass spectrometry. Overall, the recovery of U from samples was likely to have averaged about 75% but due to irregularities in the uptake of samples during mass spectrometry, this was difficult to quantify precisely. Samples with very low recovery of <20% were noted as having little if any visible precipitate, but these were still measured; however, for all samples with low recoveries, replicates were processed and a four-fold increase in amounts of co-precipitating reagents were added. This ensured a visible co-precipitate and good recoveries.

For every ~20 samples processed, two blanks and two reference urine samples were processed. The blanks were processed identically to samples save for substituting purified H_2O for urine. From these, the contamination introduced to the procedure (termed blank) was 11-38 *p*g, averaging 8-12 *p*g, but some samples contained even less uranium than blanks and so some adjustments were made to applicable blank amounts for some samples.

A large (several litre) in-house urine sample was prepared, aliquoted, and analysed for reproducibility. Three of every four of these reference samples had ~ 0.4, 0.8, and 1.2 ng of IRMM184 uranium added at the stage in the procedure when addition of acid, tracer and co-precipitating reagents was done. These provided a set of urine samples of natural U composition of varying concentrations to compare to results of samples, at varying but comparable signal levels, and allowed a method to assess the uncertainty and sensitivity in measured parameters from a sample of the same isotope composition. The processing of samples took place over a 4 months period from December 2017 to March 2018. Finally, a ~2*n*g/g solution of 'natural' U isotope standard IRMM184 with sufficient ^{233}U tracer to achieve

a $^{238}U/^{233}U$ ratio of ~100 was prepared in a well-mixed batch and measured 125 times as the IRMM+1%^{233}U reference solution. This was done to assess instrument performance and assess sensitivity and uncertainties of measurement. IRMM184 is a synthetic U certified solution almost identical to natural uranium and so its composition and measurement was analogous to samples in this study.

Mass Spectrometry. A Nu Instruments© MC-ICP-MS instrument at the University of Portsmouth, equipped with multiple faraday and ion counting detectors to measure all U isotopes, was used for isotope analysis (^{233}U, ^{234}U, ^{235}U, ^{236}U, and^{238}U). Sample solution was drawn into a de-solvating nebuliser (DSN) via a Teflon© or quartz nebuliser with a nominal uptake of 0.1ml/minute. At nominal flow rate, the instrument produced ~25M cps/ppb U. Occasionally samples were rerun when adequate sample remained after an analysis, sometimes diluting the sample with 2% HNO3 for additional full repeat measurement if the concentration was sufficient. Parameters of instrumental operation are listed in **Supplementary Table 1**. The IRMM+1%^{233}U reference solution was measured multiple times during every session to ensure quality control. The composition of this solution facilitated ion beam focussing and optimisation of intensity by the adjustment of DSN and mass spectrometer gas flows, torch position, accelerating potential, and focussing of numerous lenses, including quad settings for peak shape and multiple isotope collection peak alignment.

Supplementary Table 1. ICP-MC-MS operating conditions

Category	Condition
ICP-MS Instrument	Nu Instruments© NuPlasma ICP-MC-MS, at University of Portsmouth
Sample introduction	Nu Instruments© DSN aerosol with membrane Ar gas flow 2.8l/m, nebulizer pressure 30
RF power (W)	1300W

Carrier gas (L/min)	Ar = 0.87 L/min
Masses measured	233, 234, 235, 236, 237, 238
Measurement time in each sequence	10s followed by 2s magnet settle after each mass shift to measure the 3 sequences; 5 measurements of this series of sequences for each final analysis
Measurement mode	3 sequences of multicollection, see **Supplementary Table 2**.
Calibration strategy	IRMM184+1%^{233}U solution, natural composition of uranium, for peak centring, peak shape optimization, sensitivity, etc. (IRMM, Institute of Reference Materials and Measurements, Geel, Belgium)
Data processing package used /	Bespoke Excel spreadsheet with corrections for mass bias using IRMM 235/238, gain of ion counters, hydride and mass abundance sensitivity, tailing using 237 mass
Quality control / Validation	125 measurements of IRMM184+1%^{233}U solution, 14 measurements of in-house urine sample to which was added variable amounts of IRMM184+1%^{233}U solution.

During a run, there was initial peak centring, followed by measurement of isotope signals across multiple detectors in three sequences with a shift of one mass unit (1 Dalton) into any given collector. This facilitated measurement in either faraday cups (F) or ion counting electron multipliers (IC) in sequence 1 of ^{238}U(F), ^{235}U(F), ^{233}U(F), ^{236}U(IC), and ^{234}U(IC); in sequence 2 of ^{238}U(F), 237 mass (IC), ^{235}U(IC), ^{233}U (IC), ^{236}U(IC); and finally in sequence 3 of ^{238}U(F), ^{235}U(IC), and ^{233}U(IC). This series of peak shifts in the multiple collector array is shown in **Supplementary Table 2**.

Supplementary Table 2. ICP-MC-MS collector array

Magnet sequence	IC2	L4 far	IC1	L3 far	IC0	L2 far	L1 far	Ax far
Seq 1		**233**	**234**	**235**	**236**		**238**	
Seq 2	**233**		**235**		**237**	**238**		
Seq 3			**233**		**235**			**238**

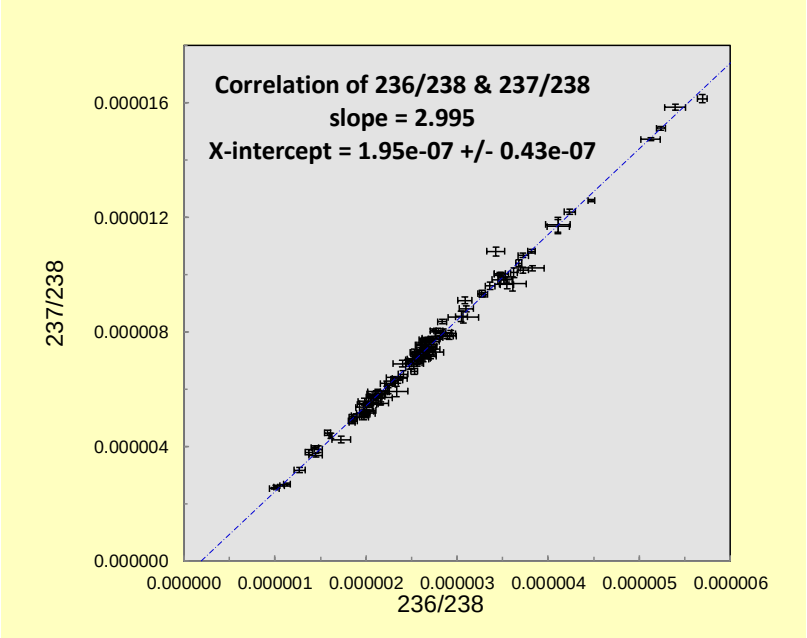
Note: far, faraday cup; IC, ion counter; 238, mass used for peak centering

This protocol of measurement allowed all peaks to be measured in an IC simultaneously with ^{238}U in a faraday detector (F); this allowed for the gain of each of the three multipliers to be measured using the IRMM+^{233}U reference solution and compared to $^{233}U(F)/^{238}U(F)$ or $^{235}U(F)/^{238}U(F)$. The measurement of the 237 mass on an IC was designed to monitor the 'tail' arising from ion scattering from the large ^{238}U peak as a function of pressure within the mass spectrometer. This 'tailing' arises from imperfect ion beam focussing and is a function of mass spectrometer design, compounded by collision of ions with occasional gas molecules/atoms, and causes broader peak shape, a narrower flat top of any given peak, and a tailing of lower energy U ions down-mass. The MC-ICP-MS design should result in a 'tailing' or mass abundance sensitivity of as little as 1-2 ppm of the ^{238}U signal detected at mass 237, without any additional lenses to supress low energy ions. Given that the University of Portsmouth instrument experienced pressure variations during measurement sessions, the signal on mass 237 varied from 2-16 x 10^{-6} of that of 238, which required a correction procedure to take account of this temporal variation. This is an important effect because the study aimed to measure $^{236}U/^{238}U$ to a detection limit of ~$1x10^{-6}$ to help confirm or refute the presence of non-natural uranium in conjunction with the $^{235}U/^{238}U$ ratio much like was done in.[5,37]

Each sample measurement consisted of 5 cycles, each containing the 3 sequences, and took a total of about 8 minutes, consuming between 40-80% of the sample, depending upon the DSN uptake rate. The IRMM184+1%^{233}U standard solution was measured approximately every 5 unknowns and is sufficiently concentrated to allow good precision measurements of all ratios while ensuring that no signals on any IC exceeded 10^6 cps. The correction procedures for isotope ratio measurements are described below. The dead time of multipliers applied to corrections was 20ns. Output from mass spectrometry measurements consisted of arithmetic means and their standard errors (standard deviation / $\sqrt{n}$) of intensities of the peaks on all masses and relevant isotope ratios using mass 238 as the usual denominator for isotope ratios.

Corrections to raw isotope data. Both samples and the IRMM184+1%^{233}U standard solution were measured with the same mass spectrometric protocol. ^{235}U(F)/^{238}U(F) was measured and normalised to the IRMM184 certified ratio (0.0072623±0.0000033) after subtracting a negligible contribution from the ^{233}U tracer (whose ^{238}U/^{233}U value was 0.00563±0.00006) to determine the mass bias, which was 0.726%/unit mass over the course of the study, with heavy masses being preferentially measured in the MC-ICP-MS instrument. Deadtime corrections of 20*n*s were then applied to all IC measurements. Next, the gains of each IC were determined by comparing a F/F ratio with its IC/F pair, for example ^{235}U(F)/^{238}U(F) of sequence 1 divided into the ^{235}(IC)/^{238}U(IC) of sequence 3. A time sequence of gains for each detector was determined for each session with samples using interpolated values. Where a value is the result of division or multiplication of other ratios, the uncertainties were propagated in quadrature, neglecting any small co-variance terms.

Next, the contribution to ^{236}U from the 'tailing' effect of ion scattering of ^{238}U was done. This was facilitated by noting that a comparison of mass bias- and gain-corrected ratios of $^{236}U(IC)/^{238}U(F)$ of sequence 1 with $237(IC)/^{238}U(F)$ of sequence 2 of the IRMM184+1%^{233}U solution yields a highly linear array. The dispersion in $237(IC)/^{238}U(F)$ in sequence 2, using the same IC for these two minor isotopes (and therefore reducing any uncertainty in IC gain) is a result of changes of pressure within the flight tube of the mass spectrometer, which varied during the course of the study and on a daily basis from 2 x 10^{-8} to 5 x 10^{-9} torr. The slope of this array was determined to be 2.995±0.050 (<2%) at the 95% confidence level, with an X-intercept of 1.95 x 10^{-7} ± 0.43 x 10^{-7}, which is very close to the certified $^{236}U/^{238}U$ value of the IRMM184 standard (1.244 x 10^{-7}), and closer still once the very small ^{236}U contribution from the ^{233}U tracer is taken into account. This plot is shown in **Supplementary Fig. 2.**

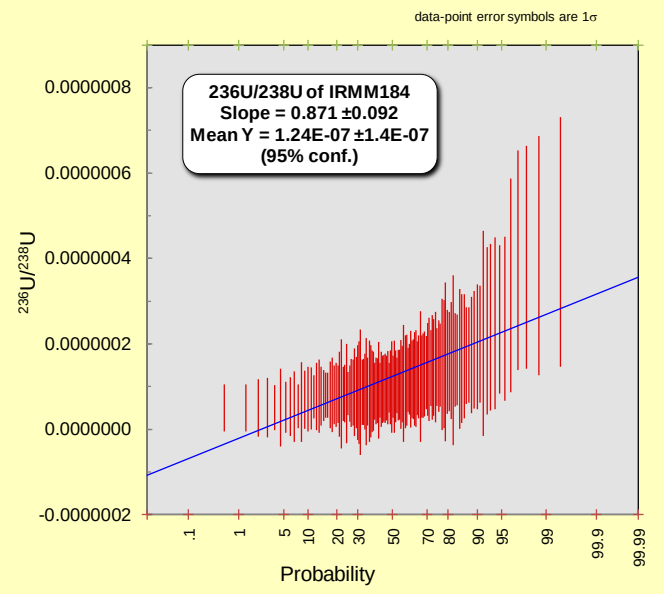


Supplementary Figure 2. Plot of signal ratios of 236/238 v. 237/238 in the IRMM184+1%^{233}U measurements illustrating the strong correlation between 237 and 236, a result of tailing from the much larger ^{238}U isotope.

The correction for tailing from ^{238}U on mass 236 can be done two ways. Because all standards have a $237(IC)/^{238}U(F)$ measurement, and following corrections for mass bias and gain, the 236 counts attributed to tailing are calculated by dividing the 237 counts by 2.995 and subtracting these from the total. When applied to the IRMM184+1%^{233}U measurements, this allows a precision of ~1.5 x 10^{-7} on the final $^{236}U/^{238}U$ ratio for the IRMM184+1%^{233}U measurement. After subtraction for the ^{233}U tracer contribution, the mean $^{236}U/^{238}U$ was slightly different than the certified value though nearly overlapping within

uncertainty. In addition, upon measurement of 2% HNO3 solutions and in conjunction with this slight difference (~0.2 x 10^{-7}) from the $^{236}U/^{238}U$ certified value (1.22 x 10^{-7}) we noted excess counts on mass 236; we had measured 1-5 cps on mass 236 when measuring 2% HNO_3 solution and so a 2.5cps subtraction on the ^{236}U measurement for all samples and standards was done; this brought the mean measured value of $^{236}U/^{238}U$ into coincidence with the certified value, the former being 1.24 ± 1.4 x 10^{-7}. The 'noise' or limit of detection of this correction is approximately the dispersion in $^{236}U/^{238}U$ at any given $237/^{238}U$ value, or about ±1.5 x 10^{-7}, but it is also affected by the ~2% uncertainty in the slope of the line. The dispersion in $^{236}U/^{238}U$ corresponds to approximately 2-13 cps, depending upon pressure and therefore magnitude of 237/238 measurement. This additional uncertainty has been taken into account for all analyses by applying a ± 5% uncertainty to the slope of the correction and in addition adding ±2 cps uncertainty to the $^{236}U/^{238}U$ ratio after this correction has been applied.

The final data for samples and in-house urine samples, and from the IRMM184+1%^{233}U solution are shown in **Supplementary tables 3 and 4**, respectively. Once all of these corrections are made, the analyses of the IRMM184 reference solution, yielded $^{234}U/^{238}U$, $^{235}U/^{238}U$ and $^{236}U/^{238}U$ values of 5.344 x 10^{-5} ± 2.0 x 10^{-6}, 0.0072614 ± 0.0000025 and 1.1 x 10^{-7} ±0.1 x 10^{-7}, respectively, within the certified value of the IRMM184.

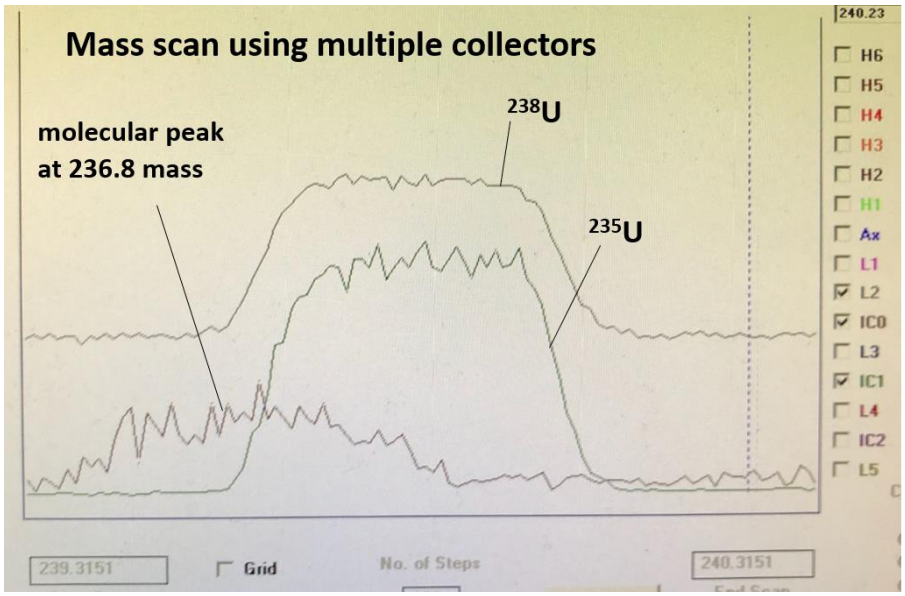


Supplementary Figure 3. Linearized probability plot of final $^{236}U/^{238}U$ measurements on the IRMM184+1%^{233}U solution showing predominantly Gaussian behaviour of measurements aside from higher values beyond the 95th percentile that have excess 236 counts.

Supplementary figure 3 shows a linearized probability plot of $^{236}U/^{238}U$ in the IRMM184+1%^{233}U solution and indicates that although generally there is excellent coherence of the measurements, there is a tendency of excess counts on mass 236 to be present in a small minority of the IRMM184+1%^{233}U

measurements, which raises the $^{236}U/^{238}U$ isotope value in some measurements. This is likely to be the same type of interference as is described below for samples, though very much less prevalent, with a magnitude of excess counts of up to 3cps. The above method of tailing correction is reliable for uranium reference solutions but is not reliable for urine samples.

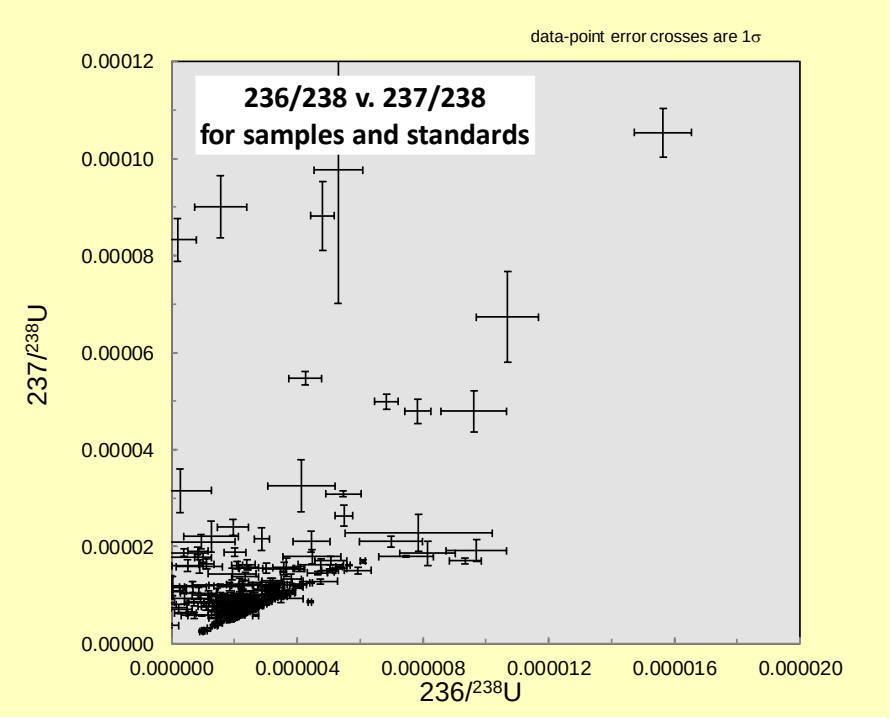
A second correction method for tailing from ^{238}U on mass 236, applicable to samples, is to derive a correction from ^{238}U on the ^{236}U by using the value of correction to $^{236}U/^{238}U$ of IRMM184+1%^{233}U solution that were measured alongside groups of samples; this allows one to take into account any time-dependent variations in tailing during the daily measurement session. This is essential for urine samples because of the variable presence of stray excess counts on the 237 mass, a real effect that is probably produced by a charged organic molecule with a mass of ~ 236.8. This anomaly has been observed in mass scans with the offending excess 237 peak slightly offset from the nominal 237 mass, as shown in **Supplementary figure 4**. The molecule(s) has not been identified, but it appears to be present in measurable quantities only in samples and is likely related to incomplete removal of all traces of organic matter not generally present in standard analyses.



Supplementary Figure 4. Screen capture mass scan of ^{238}U (faraday detector, top scan), ^{235}U (ion counting detector, middle scan), and 237 mass (ion counting, lower scan offset to lower mass at about 236.8.

Supplementary figure 5 shows mass bias- and gain-corrected measurements of $^{236}U(IC)/^{238}U(F)$ v. $237(IC)/^{238}U(F)$ of urine samples and IRMM184 reference solutions. If compared to **Supplementary figure 2**, one can easily observe a great deal more scatter, mainly in excess 237 counts, but a potential lesser magnitude additional contribution from excess 236 counts. The magnitude of excess counts in part

is far higher than the counts arising from the tailing effect. The highly correlated array for standards is also included in **Supplementary figure 5** as the linear lower right bound of the cluster of data) defines the lower limit of sample analyses, with only a few measurements falling below this bounding array of reference solution data. It is not inconceivable that such interferences could also be present at other masses (i.e. 234, 235) but be masked by much larger signals and therefore very difficult to detect, though they are likely to have been negligible if present.



Supplementary Figure 5. Plot of 237/238 v. 236/238 to illustrate the scatter towards excess 236 and 237 counts above the diagonally bounding linear array of constant 236/237 slope from that of IRMM184 standards (see **Supplementary Fig 2**).

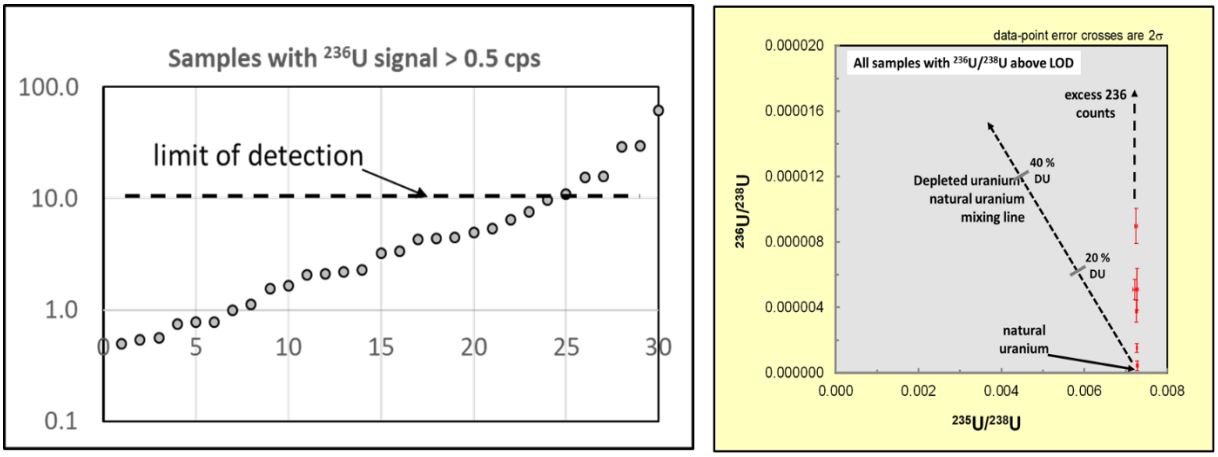
The excess scatter is a result of the presumed organic ion at mass 236.8 adding stray counts to the measured 237 signal. The actual magnitude of the excess 237 varied from 0-200 cps, and for 236 0-~20 cps. For urine samples containing no ^{236}U, 3 standard deviations of the 236 mass was 11 cps; as a result, no analyses can be considered reliably measured that have <11 cps on 236 mass. Samples with either the 95% confidence measurement uncertainty exceeding the $^{236}U/^{238}U$ value or being <11 cps are regarded as below the limit of detection (LOD) as shown in **Supplementary table 3**. Only 6 measurements of the dataset had measurements above the LOD and these had a far higher measured $^{236}U/^{238}U$ than should be expected given the maximum 95% confidence value of $^{238}U/^{235}U$ value, and as such, are likely caused by higher excess 236 counts than the cut-off for LOD. In effect the

higher $^{236}U/^{238}U$ are an artefact of organic molecule interference, and have nothing to do with presence of DU.

Analytical discussion of results

The $^{236}U/^{238}U$ measurements of 148 urine samples fall below the LOD, limited to about 11 cps on the 236 mass **(Supplementary Table 3)**. For the 6 samples above LOD, $^{236}U/^{238}U$ is elevated, but their $^{235}U/^{238}U$ ratios are natural. **Supplementary figure 6** shows the trajectory of samples towards excess 236 counts, the value of natural uranium with no ^{236}U, and the trajectory/mixing line between DU and natural uranium, with percentages along the DU-NU mixing line. If the higher values of $^{236}U/^{238}U$ were to be attributed to DU, then the $^{235}U/^{238}U$ (or $^{238}U/^{235}U$) values should follow the mixing array, which they clearly do not. Although not plotted, the replicate analyses of the in-house urine (with additions of IRMM184+^{233}U) also showed one analysis with ~10 excess 236 counts per second, just below the LOD.

Statistical assessment of data.



Supplementary Figure 6. Plot of rank order of samples with ^{236}U signal > 0.5cps and $^{236}U/^{238}U$ v. $^{235}U/^{238}U$ for the 6 samples with $^{236}U/^{238}U$ above LOD of 11 cps. Lines of mixing of DU and NU, and of the trajectory of excess 236 counts are shown.

The statistical parameters of importance cited in the paper are means and 95% confidence uncertainties of various U isotope ratios and concentrations for subgroups of the study, and probability or *P*-values of the *t*-test that compare the similarity of separate datasets with similar variances and two tailed distributions. All means derived from this study, whether of a sub-group or the whole group of samples, were derived using geometric means, appropriate for skewed distributions. The standard deviations, standard errors of the mean, and 95% confidence intervals of a group or sub-group population were calculated using the uncertainty bounds added or subtracted from the mean while values are in logarithmic notation, and finally taking the exponent of the uncertainty bounds either side of the mean; this produces asymmetric uncertainties, and these are quoted in tables where relevant. P-values of the *t*-test were calculated in the standard way using Excel functions using measured values for sub-groups, assuming a two-tailed distribution with similar variances. *P*-values <0.05 demonstrate lack of similarity between two groups of values and falsify the null hypothesis that the two groups are consistent with sub-sampling from sub-groups sharing the same mean composition. Where a Kruskal-Wallis test is cited, this has been calculated using an Excel addin from the Real Statistics Resource Pack software (Release 6.8), copyright (2013 – 2020) of Charles Zaiontz (www.real-statistics.com).

Data availability

The authors declare that all data supporting the findings of this study are available within the text, figures and Supplementary Information.

References for methods

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Author Contributions

R.R.P. developed the analytical procedures, refined methods of mass spectrometry for these samples, conducted all of the chemical extractions and mass spectrometric measurements. R.W.H. designed the GWI study and Gulf War veteran sample selections, and obtained and provided aliquots of urine samples. R.R.P. and R.W.H. co-wrote the manuscript.

Competing financial interests

The authors confirm declare no competing interests.

Additional information

Extended data are available for this paper at https://doi. . . .

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