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A normative study of the levels of urinary uranium in the general population was planned to provide a basis for comparison with levels measured in UK military and ex-military personnel who served where armour piercing munitions containing depleted uranium (DU) were used. As preparation, this pilot study trialled the process of collecting 24-hour samples from adult male civilians, and compared the measurements from 24-hour samples with those from spot samples taken over the subsequent 24 hours. Twenty five convalescent hospital in-patients were recruited as participants.

Uranium was detected in all the 24-hour samples, with concentrations ranging from 1 to 10.6 ng.litre^{-1}; the spots ranged from not detectable to 38.1 ng.litre^{-1}. Normalised to creatinine, concentrations in the 24 hour samples ranged from approximately 100 to 800 ng per mol creatinine; those in the spot samples ranged from not detectable to approximately 4000 ng per mol creatinine. The ranges appear similar to those reported for residents of the US.

The distribution of spot sample results indicated that 95% of a participant's creatinine-adjusted concentrations from spot samples would be within the range 40% to 250% of his mean. Adjusting for creatinine almost entirely eliminated a slight indication of diurnal variation in urinary uranium concentration in spot samples.

All the 24-hour samples and 131 out of the 133 spot samples showed ratios of isotopes ^{238}U to ^{235}U consistent with natural uranium (i.e. neither enriched nor depleted). The two spot samples with slightly elevated ratios were not supported by other samples from the same participants and they indicate that slightly elevated ratios may be recorded on very low concentration (<1 ng.litre^{-1}) samples. In the main, quantification of this isotope ratio from spot samples was hardly more variable than from 24-hour samples.

Complete 24-hour urine samples gave better precision than spot samples in estimating uranium concentrations at these low levels, but presented more logistic difficulties in the collection of the samples.

These findings, and the practical lessons learnt from the pilot study, would inform the design of a full normative study.

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SUMMARY

This study piloted procedures that might be used to undertake a normative study to establish the distribution of urinary concentrations of uranium in the general adult population in the UK. One use of such a normative study would be to provide a basis for comparing concentrations currently being measured in military and ex-military personnel.

Generally, urine samples collected over a continuous 24-hour period are considered to be the most representative for bio-monitoring. However, collection of 24-hour samples of urine from the general population is recognised as being logistically difficult. In this pilot study, the process of collecting 24-hour samples from participants was trialled with 25 hospital in-patients. The pilot study also studied the extent to which uranium concentrations in spot samples represented concentrations in 24-hour samples, by comparing the 24-hour samples with sets of spot samples collected over the subsequent 24 hours.

The strategy of using hospital patients as participants enabled two consecutive periods of 24 hours of sampling to be completed. The participants were male adult convalescent cardiac and orthopaedic patients who were fit enough to take part (i.e. ambulatory) but still expected to be in hospital for the requisite 48 hours. In this pilot study, 6 out of the 25 participants completed the sample collection at home.

The samples were analysed by Scientifics Ltd (at Harwell) to determine urinary uranium concentrations as mass per unit volume ($ng.l^{-1}$) and as mass per mol of creatinine. The ratios of the isotopes $^{238}U/^{235}U$ and $^{236}U/^{238}U$ were also measured.

Each sample was analysed using a SF-ICP-MS (Micromass, formerly VG) PlasmaTrace2 operated with a CETAC ultrasonic nebuliser. The instrument is a single-collector system, and uses measurement routines developed to optimise isotope ratio precision (generally counting statistics limited, giving about 3 % measured precision for a typical urine sample). Typical sensitivity (in the selected resolution mode) is 10^{11} counts per ppm ($mg.l^{-1}$) for ^{235}U. Typical limits of detection in urine "as analysed" for ^{235}U and ^{236}U are 0.1 $pg.l^{-1}$. The limit of detection for ^{238}U is typically 5 $pg.l^{-1}$.

In these 25 male participants, the 24-hour sample concentrations of uranium adjusted for creatinine, ranged from approximately 100 to 800 ng per mol creatinine. The concentrations measured in the 139 spot samples (from the same 25 participants) spanned a wider range from not detectable to more than 4000 ng per mol creatinine.

The above ranges of concentration of urinary uranium appear similar to recently published reference range concentrations of uranium in urine samples from a broad spectrum of residents of the United States.

When expressed as ng of uranium /litre of urine, the range of concentrations determined in this pilot study appeared consistent with values previously reported for other similar sized normal groups. The 24-hour samples showed concentrations between 0.9 to 10.6 $ng.l^{-1}$; the spots ranged from not detectable to 38.1 $ng.l^{-1}$.

The uranium concentrations in the urine samples differed significantly between participants, and these differences were more significant after adjustment for creatinine. The residual variation after adjusting the concentration for creatinine was proportionally much smaller than for concentration per unit volume of urine, so adjustment did correct for differences in dilution of the urine.

There was no systematic difference between the concentrations derived from 24 hour or spot sample types. Nor was there any evidence of a participant.type interaction, that is, the differences between participants did not depend on the type of sample.

The statistical analysis showed, through a reduction in the F-ratio for the time-of-day effects, that a small (and not statistically significant) diurnal variation in urine concentration was almost entirely eliminated by expressing uranium concentration normalised to creatinine.

The measurements of the $^{238}U/^{235}U$ ratio showed values that were indicative of natural uranium for all the 24-hour samples and for 137 out of 139 spot samples. The two spot samples with slightly elevated ratios were not supported by previous or subsequent spots for the same individuals nor by their 24-hour samples. This suggests that occasional slightly elevated ratios might be found in spots, which would need to be checked by a repeat testing strategy.

No ^{236}U was detectable in any of the samples. The absence of detectable ^{236}U was also indicative of natural uranium.

The 24-hour samples clearly produce a narrower spread of concentrations than the spot samples. This suggests that a full normative study, if based on 24-hour samples, would describe a narrower distribution of concentrations than a study based on spot samples. This in turn should make it easier to identify individuals who have slightly elevated concentrations of uranium.

On the other hand spot samples are easier to collect, and any unusually high values could be assessed by retesting. It appears that ratios of isotopes can be determined with good precision for spot samples with uranium concentration above 1 $ng.l^{-1}$. Therefore, spot samples may suffice to establish the ratio of isotopes for all samples above that concentration, and – based on the distribution observed in this pilot study - would give a concentration (normalised to creatinine) that would (in 95% cases) be within 40 to 250% of the mean for that individual. Where concentrations are below 1 $ng.l^{-1}$, then a minor elevation of the ratio of isotopes still implies a very low concentration of DU.

1 INTRODUCTION

1.1 BACKGROUND

Concerns have been expressed about the potential health effects of exposure to depleted uranium (DU) following active service in war zones where depleted uranium has been used. It is used by the military as a component of armour piercing ordnance because of its high density, and because it can ignite on impact if the temperature exceeds 600 °C. There are also civil applications where DU's high density is an advantage, including as counterweights in aircraft, radiation shields in medical radiation therapy machines and containers for the transport of radioactive materials.

Depleted uranium is obtained as a by-product from the production of enriched uranium for nuclear power plants. The reactors require uranium with an enriched fraction of the most reactive uranium isotope, ^{235}U. The uranium that remains has about 0.2% ^{235}U compared to 0.72% in natural uranium, and is known as depleted uranium (DU).

A recent review by the Royal Society (2001) concluded that it was unlikely that any excess of fatal cancers from possible radiation effects would be detected in a cohort of 10,000 soldiers followed over 50 years and that very few individuals were at risk of developing kidney disease. The lifetime risk of death from lung cancer in the most exposed individuals, however, might be double that of the general population and they could also be at risk of developing serious kidney disease in later life (a possible chemical effect of uranium). In addition, McDiarmid *et al* (2000) have found evidence of neurocognitive impairment in Gulf War veterans who had retained fragments of DU shrapnel, although these individuals showed little evidence of impaired kidney function. Gulf War veterans known to have been exposed to DU are reported to have had urinary concentrations averaging 80 $ng.l^{-1}$, 7 years post-exposure, with the highest concentrations being about 30 000 $ng.l^{-1}$ (McDiarmid *et al*, 2000).

The Royal Society acknowledged that there was a great deal of uncertainty in their assessment of DU exposure and their recommendations included the need to validate the measurement of urinary DU concentrations as a measure of past exposure.

Since the publication of the Royal Society Report, the MOD's DU Oversight Board has overseen a programme of work (http://www.duob.org.uk/summary.htm). The current programme includes a survey of levels of uranium and depleted uranium in the urine of military and ex-military personnel. However, because the use of DU is not confined to the military, some exposure to DU may have occurred within the UK. Furthermore, uranium is ubiquitous throughout the natural environment, and is found in varying but small amounts in rocks, soils, water, air, plants, animals and in all human beings. Average annual intakes of natural uranium by adults have been estimated to be about 500 µg from ingestion of food and water and about 0.6 µg from breathing air (www.who.int/mediacentre/factsheets/fs257/en). The uranium content of urine in the general population is not well understood but is thought to vary considerably depending on where people live, their diet and their drinking water. Therefore there is a need to be able to compare levels of urinary uranium in personnel with the range of levels to be found in the general population.

The WHO (2001) reviewed data from the early 1990s that suggest that urinary uranium concentrations in the general population range from about 4 to 57 $ng.l^{-1}$. However, measurement methods have improved considerably over the last decade, and these improvements may affect comparisons between new data (for military personnel) and the data from the early 1990s for the general population.

A recent survey of uranium concentrations in the US population found that the median concentration was only 7 $ng.l^{-1}$, but 10% of the measured concentrations exceeded 43 $ng.l^{-1}$ (National Health and Nutrition Examination Survey, 1999). When expressed as concentration normalised to amount of creatinine in the urine samples, the median and upper tenth percentile became, respectively, 5 and 24 ng/g creatinine (or, equivalently, 570 and 2700 ng/mol creatinine). The concentration of uranium per unit volume of urine is usually expected to be more variable than the concentration expressed normalised to the amount of creatinine which is a low molecular weight end-product of muscle metabolism. Measured concentrations did not fit a normal or log-normal distribution but had a marked tail at the higher end of the scale. However, data for levels in the US population were likely to be insufficient as a basis for background levels in the UK general population.

1.2 THIS STUDY

This pilot study was undertaken in parallel with a cross-sectional survey of concentrations of depleted uranium (DU) in urine samples from service personnel.

Given the limited existing information on levels of urinary uranium, it was recognised by the DU Oversight Board that information on the levels in the general population would be needed. A normative study would be needed to survey the level of urinary uranium in the general population.

An important consideration was whether to use spot samples or 24-hour samples. Recently McDiarmid *et al* (1999) described the current view of the discussion in the clinical toxicology literature on the extent to which urine samples that are less than 24-hour collection are representative of the average body burden of the toxicant. Generally, 24-hour samples, taken over a continuous 24-hour period, and with concentration of toxicant corrected as concentration per unit of creatinine, are considered to be the "gold standard" for biomonitoring. However, the logistic difficulties of collecting urine samples over a continuous 24-hour period are well known (e.g. McDiarmid *et al* 1999), and sometimes alternative strategies are used to collect samples over a cumulative period of approximately 24-hours, e.g. over two sequential evening and nights. Alternatively, samples are collected as single spot samples.

Three characteristics of the uranium detected in urine were considered important and were to be measured. The most direct measurement of concentration is mass of uranium per volume of urine. However, concentration of uranium per unit concentration of creatinine corrects for the degree of dilution of urine. Creatinine is a low molecular weight organic molecule released by muscle into the blood, and eventually excreted into urine. The amount of creatinine produced is dependent on the individual's muscle mass. Its concentration in urine largely reflects the degree of concentration of the urine (urine osmolality) which, in turn, depends upon the individual's state of water balance. Standardizing the urinary uranium concentration normalised to creatinine is expected to allow for differences in the degree of dilution of the urine. Therefore concentration was measured in ng of uranium per litre of urine, and as ng of uranium per mol of creatinine. The ratio of the isotopes distinguishes depleted uranium from natural uranium, and therefore the ratios of the isotopes ^{238}U to ^{235}U, and ^{238}U to ^{236}U were to be measured. An elevated ratio for ^{238}U to ^{235}U indicates that the uranium contains less of the most radioactive isotope and therefore contains DU. The detection of ^{236}U is also an indicator of DU.

Therefore, for a survey of urinary levels of uranium in the general population, the plan was to recruit participants who were going to remain in the same place for a period of more than 24 hours by recruiting participants from patients in hospital. However, before undertaking a UK wide survey, it was appropriate to undertake a pilot study to establish efficient procedures and

in particular to determine whether spot samples or 24-hour samples of urine would be needed from a general population survey. When the IOM submitted a proposal to undertake a survey of the levels of urinary uranium in the general population, the DU Oversight Board recommended that the first step should be this pilot study to determine the appropriate methodology.

The participants were recruited at the Royal Infirmary of Edinburgh (RIE), by research Nursing Staff attached to the RIE Clinical Research Facility. The study and protocol were approved by the Local Research Ethical Committee and the hospital management. The Consultants whose patients were recruited were consulted for their consent to, and support for, the study.

2 AIM

The purpose of this pilot study was to test and assess procedures for collecting urine samples if the survey were to be extended to more participants over a wider geographical area. A particular aim was to determine whether spot samples or 24 hour samples would be necessary in a survey of levels of urinary uranium in the general population. Therefore, the pilot study examined the variation in uranium concentrations between spot samples collected individually over the course of 24 hours, and compared those measurements with a total 24-hour sample collected on the preceding day.

The survey of the general population, if undertaken after this pilot study, would provide a normative level against which the results from a survey on urinary levels of uranium in military personnel would be compared. As the military personnel with potential exposure to DU were mainly male, it was appropriate to conduct the pilot study with male, adult participants.

The specific objectives were to examine the concentrations of urinary uranium in terms of four characteristics:

- nanograms per litre of urine,
- nanograms per mol of creatinine,
- ratio of $^{238}U/^{235}U$, and
- ratio of $^{238}U/^{236}U$.

The ratio of $^{238}U/^{235}U$ ratio is a distinctive marker of the proportion of depleted uranium. Detection of ^{236}U is a confirmation of depleted uranium.

3 METHODS

3.1 STRUCTURE OF THE STUDY

3.1.1 Summary

The study comprised:

- production of the pilot study protocol;
- obtaining ethical approval for the study;
- collection of the urine samples, a 24-hour sample and a set of separately bottled spot samples over a further 24 hours from each participant;
- interim storage under suitable conditions, and then transfer of the samples to an analytical laboratory;
- analysis of samples at the separately contracted laboratory (Scientifics Ltd);
- statistical analysis of the uranium measurements.

3.1.2 Design

The study collected urine samples from participants under clinical supervision at the Royal Edinburgh Infirmary. A total 24-hour sample was collected over a 24 hour period, and sequential spot samples were collected over the following 24 hours. Daily supervision was provided by a research nurse (a staff member of the Clinical Research Facility of the Royal Infirmary of Edinburgh, sub-contracted to work on the study).

3.1.3 Participants

The participants were male ambulant participants convalescing from cardiac conditions or orthopaedic procedures especially due to trauma. Participants with known renal disease or undergoing 24-hour urine collection for clinical purposes were excluded.

Participants between the ages of 20 to 59 were sought, and selected to give a broadly balanced spread by age.

The study set out to recruit 25 participants. This number was estimated to be sufficiently large for the purpose of the pilot study. It was also considered sufficient to be able to quantify any large circadian variations. However the data from 25 participants were not expected to be sufficient to characterise other causes of variation, e.g., occupation and location of residence (which could be addressed in a larger general population survey).

3.1.4 Recruitment

The study nurse, based at the hospital, identified eligible participants. When convenient for clinical requirements, and with permission from the medical and nursing staff, she approached eligible participants, explained the purpose and nature of the study, and asked for informed consent after allowing the participants adequate time to consider their decision.

Those agreeing to participate were asked to provide information for a simple questionnaire (see section 3.1.5) and they were given instructions and supplies of containers for collecting the urine samples.

3.1.5 Information about the participants

The information collected about each participant (using a simple questionnaire) comprised:

- name (which was treated as confidential to the hospital and IOM);
- NHS number (similarly treated as confidential);
- gender (confirming, for the study records, male participants only);
- date of birth (to define age at the time of sampling);
- ward (orthopaedic or cardiac);
- consultant in charge;
- medical diagnosis (from the participant, and from the nursing notes);
- dates of admission, and operations;
- location of residence over the past ten years;
- any military service.

If the potential participant had seen any military service, then that was discussed and any subjects with potential military exposure to DU would have been excluded.

3.1.6 Collection of samples

The containers for the samples were prepared at the analytical laboratory Scientifics Ltd, using a thorough cleaning process to remove any contamination. The procedures for storing and transferring the samples had been decided in close collaboration with Scientifics Ltd.

Each participant was given sequentially numbered containers and asked to urinate into a separate container on each occasion for the spot samples. A single container was provided for the total 24 hour sample. Instructions on how to collect the samples without contamination were also given. The study nurse collected samples daily, and recorded any occasions of lost or missed samples.

The participants were asked to complete two record sheets. One showed food and drink consumed. The other sheet recorded the start of the 24-hour sample, and the time and volume of each spot sample.

3.1.7 Transfer of samples

On the ward, samples were stored in cool boxes containing ice packs. Samples were transferred from the ward by the study nurse and stored temporarily at 1 to 5 °C at the hospital and then transferred to IOM for storage at 1 to 5 °C. Weekly, the batches of samples were despatched in cool boxes (with ice packs, to keep temperatures in the range 0 to 4 °C) to the analytical laboratory. The cool boxes were packed and prepared for transportation in the hour prior to pickup (between 16:00 and 17:00) by the Courier. The samples were enclosed in tamper-evident sealed bags for transport by the courier. They were transported overnight to Scientifics Ltd at Harwell, and were received by 10:00 the following morning. All despatches were received in good condition with no sample loss and still in the temperature range 0 to 4°C.

The containers were each uniquely numbered, and the sets of containers were accompanied by identically numbered forms.

3.2 ETHICAL APPROVALS

Because the study involved hospital patients, the necessary ethical approval was sought and received from the Local Research Ethical Committee. The protocol approved by this committee is attached in Appendix 1.

For this study, the discussions leading to ethical approval noted that:

- participation was entirely voluntary;
- participants were being given adequate time to consider their decision;
- participants were free to leave the study;
- participation did not involve any invasive procedure; and
- the purpose of the study was worthwhile.

3.3 SAMPLE ANALYSIS

3.3.1 Analytical Procedure for the determination of uranium concentrations and isotope ratios in urine

The same procedure (which has been in use for 7 years) was used for both "spot samples" and for 24 hour collection samples. On receipt in the laboratory, samples were allocated a unique laboratory identification number and stored in a cold room at nominally 4°C until analysed.

Aliquots of the samples were prepared for analysis by a simple procedure designed to lower the Total Dissolved Solids (TDS) of the solution to ca.100 ppm, whilst minimising contamination. The purpose of this procedure was to optimise ICP-MS performance, rather than to pre-concentrate the samples. After addition of a ^{233}U spike, a phosphate precipitation step was carried out, followed by two washing steps. The phosphate precipitate was then dissolved in nitric acid and deionised water and diluted back to the original volume ready for analysis.

High purity semiconductor grade reagents and deionised water were used throughout to minimise the risk of contamination, and all containers used were cleaned with dilute acid and then rinsed repeatedly with deionised water in a clean-room to remove surface contamination.

Each sample was analysed using a Sector Field Inductively Coupled Plasma-Mass Spectrometer (SF-ICP-MS). The SF-ICP-MS used is a Micromass (formerly VG) PlasmaTrace2, operated with a CETAC ultrasonic nebuliser. This instrument is a single-collector system, and uses measurement routines developed to optimise isotope ratio precision (generally counting statistics limited, giving about 3 % measured precision for a typical urine sample). For this type of work, the instrument was operated in low-resolution mode (approximately 300). Typical sensitivity in low-resolution mode is 10^{11} counts per ppm ($mg.l^{-1}$) for ^{235}U. Typical limits of detection in urine "as analysed" for ^{235}U and ^{236}U are 0.1 $pg.l^{-1}$ (i.e. 0.0001 $ng.l^{-1}$). The limit of detection for ^{238}U is typically 5 $pg.l^{-1}$.

The total reagent blank, estimated using a surrogate urine made from synthetic inorganic components, was 0.1 $ng.l^{-1}$ total uranium or less, equivalent to a total value of $\leq$ 5pg in the volume of urine actually used for analysis (50 ml).

All isotope ratio measurements are made relative to a natural uranium reference material (uranium metal EC101 from IRMM, Geel), with a slight (typically 1-2%) mass bias correction to achieve the natural isotope ratio for $^{238}U/^{235}U$ of 137.9. No mass bias correction was applied to $^{236}U/^{238}U$ ratios.

The method used to calculate the uranium concentration in the urine is based on the "isotope dilution principle", using the ^{233}U spike. A range of depleted uranium standards at different concentrations (Glen Spectra, traceable to NIST) have been spiked with ^{233}U and analysed. This has established the range of linearity of the method and has provided an initial estimate of the ^{233}U spike concentration. To measure the actual concentration more precisely, a spiked standard of natural uranium is included in each batch of urine samples and the uranium concentration in the urine established using simple ratio measurements. No depleted uranium standard is analysed at the same time as urine samples to avoid the risk of cross contamination.

The quality control checks included spiking three urine samples. These samples had original (i.e. unspiked) concentrations of 4.2, 7.3 and 10.6 ng U per litre. After addition of depleted uranium with a $^{238}U/^{235}U$ ratio of 286, the concentrations of total uranium increased by approximately 1 $ng.l^{-1}$ (for the two lower concentration samples) and by approximately 2 $ng.l^{-1}$ for the higher concentration sample. These spikes were expected to raise the $^{238}U/^{235}U$ ratio to about 150, and the measurements agreed with the expected to within a difference of 2.1 in the ratios (e.g. 149.2 compared to 151.3).

Normally, uncertainty is estimated from the actual analytical performance obtained for these "in-house" urine materials, based on the performance for different sub-samples, on different days, with different operators and this has been confirmed by analytical performance obtained during the analysis of more than 250 urine samples containing uranium with a natural isotope ratio.

3.3.2 Scientifics Ltd procedure for determination of creatinine in urine

The method used for determination of creatinine in urine was based on the Jaffe reaction, which is a kinetic colour test. Creatinine forms a yellow-orange colour with picric acid in an alkaline medium. The level of creatinine in a sample is proportional to the rate of change in absorbance at 520/800 nm, which was measured with an Olympus AU600 clinical analyser. The analysis was carried out at the Derby laboratory of Scientifics Ltd, and was accredited to UKAS/ISO 17025.

4 DATA PROCESSING AND ANALYSIS

4.1 OUTLINE OF DATA

The data obtained for the study included the outline description of each participant, and the measurements on the samples, i.e. for each sample: volume, total uranium concentration (as mass per unit volume of urine and normalised to creatinine concentration), and ratio of the concentrations of the isotopes ^{238}U to ^{235}U.

4.2 DATA SECURITY

4.2.1 Data protection

All of the study data files were stored on a *Compaq* Server on the IOM's network. The server is located in a secure, climate controlled computer room into which physical access is controlled and limited to IT staff. The IOM's IT Security Policies include issues of data security and the integrity of all computerised data. These procedures included a daily, full-backup procedure, active protection from computer viruses and prevention of unauthorised access to any study data. The study ran in full compliance with the Data Protection Act. (The IOM was the data controller under the Data Protection Act.) No individual participant is identified in this report.

4.2.2 Data Control

The names of participants were held with the IOM's unique number separately from the rest of the study data.

The study data files were subject to standard IOM IT security policies, which include security restrictions that ensure that only project team members had access to these data. These measures ensured that the identities of participants remained confidential, and that the main study data were available only in terms of the anonymous participant identity number.

4.3 DATA PROCESSING

Questionnaire data were entered to computer file. Appropriate procedures were designed and implemented to check the collected data for logical consistency, valid values, valid ranges and cross-record consistency.

The main analysis file was created in MS Excel file. It identified participants only by the IOM's study participant identity number.

The analytical laboratory provided the urine test results electronically in an agreed form (Excel Spreadsheet). Urine test results were checked and validated by Scientifics Ltd before they were sent to the IOM.

The consistent identity numbering of sample results and participants facilitated the matching of sample data with participant questionnaire information.

In any instances where a person was selected and agreed to take part but subsequently did not provide the urine samples, the reasons for non-completion were recorded.

Any issues involving the data collection, data processing and systems design were under the control of the project's systems analyst who also reviewed any other data related issues as appropriate to the requirements of the project.

4.4 STATISTICAL ANALYSIS

Following data validation, the data were tabulated for presentation; total daily volumes for the spot samples were calculated and tabulated.

Formal statistical analyses of the unadjusted and adjusted uranium concentrations, and of the $^{238}U/^{235}U$ isotope ratio, had the following aims:

1. to quantify the variation between individuals;
2. to test for systematic differences between the 24-hour and spot sample collection methods;
3. to test for interactions between participants and methods;
4. to test, in the spot samples, for systematic effects of the time of collection.

Because the design led to an unbalanced data set, the analyses were performed using linear regression techniques, with graphical checks on the distribution of residuals. These checks confirmed that an appropriate scale for analysis of all the variables was the logarithms of the measured concentrations and ratio.

All the analyses were carried out using the statistical package GenStat (GenStat Committee, 2002).

5 RESULTS

5.1 RECRUITMENT AND WITHDRAWAL

The study nurse approached 69 subjects whom she identified as being eligible from their patient records. Eleven refused (or did not feel well enough) to participate. Of the 58 who agreed to participate, 27 were discharged from hospital before the sample collection started. Three were discovered to be ineligible (e.g. due to being catheterised or having a creatinine level that was too high). Of the 28 who did participate, three gave incomplete sets of samples (two of these participants had attempted to complete the sets at home after being discharged early from the hospital, but failed to follow the instructions for spot samples correctly).

The two sets of 24-hours of samples were collected from 25 participants. Six of these completed the sample collection at home after being discharged. They took sample bottles and cool boxes with freezer packs for storage until the samples were picked up by IOM staff soon after the collection period was complete.

Twenty of the 25 participants were recruited on the cardiac Ward (Ward 104) and 5 were from the orthopaedic Ward. Most cardiac patients had been admitted for treatment or investigation of ischaemic heart disease; orthopaedic patients for a range of conditions and some operations, but not major trauma.

The plan was that collection would commence from 12:00 hr, but this was often impractical due to ward administration procedures; the patients were often away from the ward at this time to attend treatments or other procedures. So the time window for starting was widened. Most of the participants (23) started collecting samples between 10:00 and 14:30. One started at 16:30. And in one case (ID=20), the paper record was lost on the ward so the time of starting was not available.

The nurse enquired about any known losses of samples. Participant 21 missed one spot sample out of seven. No other losses were reported by the participants.

As specified in the protocol, the 24-hour sample was collected first. The spot samples were collected in the following 24 hours.

5.2 DATA COLLECTED

5.2.1 Volumes

The volume of each sample was measured by the study nurse, and also by the laboratory at Harwell on receipt of the samples. Table 5.1a shows the Harwell-measured volumes of the individual samples. The nominally 250 ml containers yielded volumes up to 280 ml; a few were as low as 50 ml.

When these volumes were compared with those recorded by the study nurse, there was general agreement and just a few disagreements. Samples where the discrepancy was greater than 25 ml are listed in Table 5.1b. In two samples, i.e. 2/3 and 23/4 there appeared to have been some loss, possibly indicating an incompletely sealed container. Samples 6/4 and 6/5 had apparently been swapped in order either by the nurse or by Scientifics. Sample 14/5 showed an inexplicable increase in volume. Finally, there was a mismatch in the number of samples for participant 29. Harwell received six samples, but the nurse's records show only five, of which the fifth sample matches the sixth Harwell sample, and does not show a sample corresponding to sample 29/5 of Table 5.1.

Table 5.1a Sample volumes measured on receipt at the Scientifics lab.

Participant	24 hours	Spot samples									
		1	2	3	4	5	6	7	8	9	10
1	2050	140	175	250	250	250					
2	1960	260	250	215	250						
3	1980	250	250	250	250	250	210				
4	2175	270	270	255	260	175	270				
6	1980	170	175	230	250	160					
8	2000	200	110	105	210						
9	690	150	225	225	145	75					
10	2200	155	270	255							
11	1080	50	52	150	170	225	150				
12	1420	230	245	75	260	245	125				
14	2000	250	270	270	270	205					
15	2550	265	255	265	255	265	90				
16	2180	265	270	270	270						
17	2150	220	180	275	260	190					
18	990	260	170	240	275	220	120				
19	840	260	270	270	255	240	270				
20	2910	225	225	250	240	225					
21	1900	270	265	250	265	280	275				
22	950	190	250	160	60	50	55	155			
23	1000	145	145	225	60						
25	890	240	255	250	205	220					
26	1825	135	125	115	155	190	210	200	155	140	160
27	1600	265	165	250	125	145	275				
28	2000	225	210	225	180	275	225	255	145		
29	2010	250	250	225	265	150	255				

Table 5.1b Volume discrepancies

Participant	Spot sample	Nurse volume	Scientifics volume
2	3	250	215
6	4	160	250
6	5	250	160
14	5	165	205
23	4	170	60
29	5	250	150

Confusion over the order of a pair of samples has no impact on estimates of within-participant variability, but would affect any attempt to investigate systematic effects associated with time of sample collection. However, there appears to be only one pair of samples affected by some uncertainty over the order of collection.

Table 5.2 shows the total volumes collected for each participant, as 24-hour samples and totalled over the spot samples. Clearly, there was a general trend for the total volume of the spot samples to be lower than the 24-hour collection. This is consistent with the large number of samples measuring around 250 ml. On the occasions when the recorded sample volume indicated a full container (and 61 out of 139 spots samples had volumes of 250 ml or more), we do not know how much urine was lost to collection. However, five participants produced samples that were all below 250 ml, and even in these cases there was less collected in the spots than the 24-hour sample (on average, and for 4 out of these 5 participants).

There were a few cases, e.g. participants 18 and 19, where the 24 hour collection was rather smaller than the spot samples' total; this may be because the total volumes are likely to vary from day to day but it may also be a reminder that 24-hour samples would be deficient if the participant fails to capture individual urination occasions.

5.2.2 Uranium concentration

Table 5.3 shows the individual results for estimated concentration of uranium, in units of $ng.l^{-1}$. In one sample, 27/4, no measurable amount of uranium was recovered. Within a participant, the results showed variation up to tenfold in the range of concentration.

5.2.3 Uranium concentration adjusted for creatinine

Table 5.4 shows the uranium concentrations standardised to the concentration of creatinine. In this measurement, volumes cancel and the units are ng of uranium per mol creatinine.

These results are shown graphically in Figure 5.1. On the y axis, each participant's multiple spot sample results are plotted against his 24-hour sample on the x-axis. Equal logarithmic scales are used, and the line of equality is shown for reference.

The pattern in Figure 5.1 is of general agreement, with a few values unusually far away from the line of equality. These are easily identified in Table 5.2, but in the main are not associated with low sample volumes.

5.2.4 $^{238}U/ ^{235}U$ ratio

Table 5.5 shows the $^{238}U/ ^{235}U$ ratios for each of the samples. In a few cases, recovery was insufficient for a reliable measurement. All of the results from the 24 hour samples were within ± 3 ratio units around the normal value of 137.9. Almost all of the spot samples were also within ± 3 ratio units around the normal value of 137.9.

Scientifics Ltd's guidelines suggest that a sample should be reported as possibly showing depleted uranium with a ratio of between 142 and 145, and as showing depleted uranium if over 145. Here, sample 27/ 5 met the first criterion and 26/3 the second. However, in neither case was the high value confirmed by any of the other spot samples from that participant, suggesting that these high values were results of measurement variation. Furthermore, the uranium concentration in sample 26/3 was below 1 $ng.l^{-1}$ and the laboratory advised that such low concentrations are associated with lower accuracy in the results. For sample 27/5, the chemical recovery was less than optimal, so that again the results are believed to have lower

accuracy than usual. Both samples appear to reflect variation rather real differences from natural proportions of the isotopes.

Table 5.2 Total daily volume

Participant	24 hr sample	Spot samples	
	Vol (ml)	Number of spot samples	Total Vol (ml)
1	2050	5	1065
2	1960	4	975
3	1980	6	1460
4	2175	6	1500
6	1980	5	985
8	2000	4	625
9	690	3	820
10	2200	3	680
11	1080	6	797
12	1420	6	1180
14	2000	5	1265
15	2550	6	1395
16	2180	4	1075
17	2150	5	1125
18	990	6	1285
19	840	6	1565
20	2910	5	1165
21	1900	6	1605
22	950	7	920
23	1000	4	575
25	890	5	1170
26	1825	10	1585
27	1600	6	1225
28	2000	8	1740
29	2010	6	1395
Total	43330	139	29177

Table 5.3 Uranium concentration (ng.l^{-1}) measured in urine samples†

Participant	24 hour	Spot samples									
		1	2	3	4	5	6	7	8	9	10
1	1.1	5.2	2.5	1.5	1.8	3.0					
2	5.7	10.9	3.6	2.0	4.3						
3	1.8	1.9	2.3	1.8	1.7	3.0	18.5				
4	1.7	1.8	1.3	1.3	2.0	3.6	0.8				
6	2.1	5.5	4.4	2.0	2.1	2.4					
8	5.3	6.6	11.2	15.0	13.2						
9	10.6	5.9	6.6	5.1	6.7	6.0					
10	1.9	6.2	5.4	2.5							
11	3.1	7.5	7.7	3.0	5.1	2.7	2.8				
12	1.9	3.8	4.6	6.1	2.5	3.5	6.2				
14	6.9	24.2	11.2	1.1	4.2	7.3					
15	3.3	4.2	2.8	7.8	11.2	7.8	5.5				
16	1.2	0.7	1.5	3.6	2.7						
17	5.9	6.6	5.8	4.2	5.9	11.0					
18	3.0	3.2	1.9	2.1	0.3	3.1	1.6				
19	2.3	0.8	0.6	1.3	0.4	2.0	1.6				
20	2.9	3.8	3.0	2.5	2.1	2.3					
21	1.9	3.8	2.1	0.9	1.1	1.6	2.2				
22	1.8	2.0	0.8	1.1	2.5	4.2	4.2	1.2			
23	4.4	13.3	7.0	6.2	20.9						
25	8.2	5.2	1.9	5.4	7.8	5.2					
26	0.9	0.9	3.2	0.8	1.4	1.9	1.2	2.8	1.3	1.5	1.5
27	9.3	3.3	15.5	10.2	ND‡	2.9	3.1				
28	7.3	5.5	4.1	38.1	5.5	28.3	4.4	4.3	3.2		
29	7.9	4.1	6.7	1.7	7.8	10.9	8.0				

† **Note**: Samples with concentrations less than 1 ng.l^{-1} were subject to larger measurement errors in the concentrations and in the ratios of the isotopes.

‡ ND= none detected

Table 5.4 Uranium concentration in ng per mol creatinine

Participant	24 hr	Spot samples									
		1	2	3	4	5	6	7	8	9	10
1	185	229	175	250	305	248					
2	488	908	305	132	263						
3	264	285	300	277	297	323	2467				
4	346	321	331	336	332	311	285				
6	302	345	313	280	277	291					
8	539	543	589	632	503						
9	481	438	557	463	463	507					
10	192	207	278	216							
11	260	270	273	263	295	245	109				
12	158	229	264	251	301	245	247				
14	688	776	1539	665	838	727					
15	436	588	548	592	604	612	587				
16	300	408	498	305	331						
17	568	525	551	584	596	510					
18	256	200	156	172	33	163	68				
19	269	175	180	198	106	175	156				
20	532	564	625	553	483	496					
21	286	390	541	327	295	228	333				
22	240	304	295	327	173	281	271	261			
23	396	538	495	446	609						
25	342	308	305	375	276	210					
26	97	90	366	87	187	188	90	198	85	167	163
27	621	632	697	610	ND‡	637	596				
28	603	468	515	3515	529	4234	454	709	568		
29	789	943	751	125	665	683	706				

‡ ND= None detected

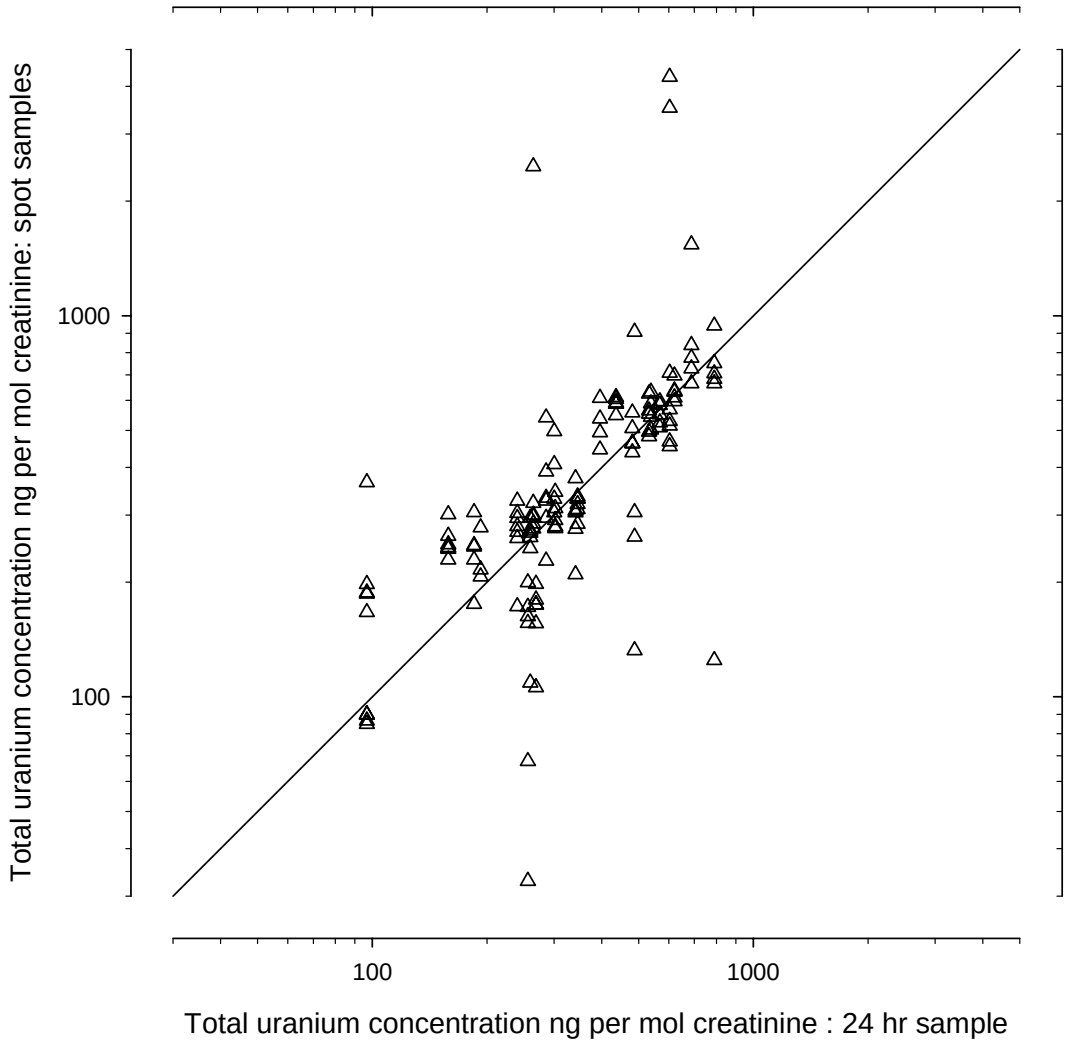

Figure 5.1 Comparison of total uranium concentration per mol creatinine from spot samples and 24 hr collection in the same participants. The diagonal line is the line of equality.

Table 5.5 Ratio of $^{238}U/^{235}U$ concentrations

Participant	24 hr	Spot samples									
		1	2	3	4	5	6	7	8	9	10
1	137.3	139.4	NM‡	136.5	134.7	134.0					
2	134.3	138.2	138.2	135.5	134.1						
3	140.1	138.3	136.3	137.8	135.4	139.4	138.2				
4	135.7	141.4	137.8	134.4	138.2	139.0	137.7				
6	138.5	138.0	136.6	134.6	137.5	137.3					
8	137.2	136.4	136.7	139.1	138.5						
9	138.1	139.4	138.5	137.1	138.0	138.0					
10	138.7	135.9	135.9	134.8							
11	138.9	138.0	137.2	135.3	138.4	137.0	136.4				
12	136.6	138.3	135.7	137.7	136.9	139.0	135.9				
14	137.8	136.6	137.4	140.8	137.3	136.4					
15	137.5	137.7	140.6	137.5	136.7	137.8	137.1				
16	139.0	137.6	137.5	138.4	138.4						
17	137.4	136.9	137.3	137.0	137.0	136.5					
18	135.3	135.6	138.0	137.9	NM‡	137.1	140.1				
19	136.9	136.0	137.8	137.3	NM‡	137.8	140.9				
20	137.8	138.8	134.4	139.2	135.3	136.9					
21	138.0	135.4	136.7	NM‡	137.4	137.1	137.5				
22	136.5	137.3	139.8	138.7	139.3	139.4	138.5	137.1			
23	138.3	137.7	137.7	136.3	139.9						
25	138.4	138.5	139.8	136.1	138.2	140.8					
26	137.9	NM‡	138.1	147.1	137.6	138.2	138.3	135.9	137.4	137.5	139.1
27	138.1	137.5	133.4	136.8	NM‡	143.0	137.7				
28	136.7	138.0	139.5	135.8	139.3	136.4	138.0	138.3	139.6		
29	139.9	134.2	136.1	137.9	136.1	137.9	138.3				

‡ NM= not measurable

Nevertheless, we inspected the dietary records (of these two participants) to see if these samples were associated with intake of material that might contain uranium. Sample 27/5 was collected approximately 2 hours after Participant 27 drank Highland Spring Mineral Water. (The $^{238}U/ ^{235}U$ ratio, in uranium in ground water may be slightly different from the equilibrium value, according to Appendix 2 of the Royal Society report.) However, sample 27/4 was taken approximately 1 hour after drinking the mineral water, and showed a normal ratio. Neither 26/3 nor 27/5 showed elevated uranium levels, compared to other samples from the same participant. Nothing noteworthy in the diet occurred prior to Sample 26/3.

Figure 5.2 shows the agreement between ratios in the individual spot samples, on the y-axis, and the 24-hour samples, on the x-axis, again with equal scales and the line of equality shown. The Harwell lab's limits are shown dotted.

5.2.5 $^{238}U/ ^{236}U$ ratio

This ratio proved impossible to measure in any sample, because ^{236}U concentrations were in all cases below detectable levels. No further analysis was carried out.

5.2.6 Uranium concentration, urine strength and patient factors.

Figure 5.3 shows the distributions of creatinine concentration in urine in the 24 hour samples, separately for the 20 cardiac and 5 orthopaedic patients. The spread of results in the five orthopaedic patients is well within that of the others. The overall impression is of two similar distributions in the two types of patients, plus two cardiac patients with values rather higher than the main range.

Figure 5.4 shows the relationship between the uranium and creatinine concentrations in the 24-hour urine samples. There is a clear trend, and the two highest creatinine concentrations correspond to two of the three highest uranium concentrations, although the points do not all lie on a single line. It is clear that standardisation for creatinine will remove much, but not all, of the variation in uranium concentrations, and that participants' rankings will be altered by standardisation. However, it seems likely from this graph that adjusted concentrations will still contain individual variations.

Figure 5.5 shows the relationships of the urinary creatinine concentrations with age, with 24-hour urine volume, and with time (days) since the participant's admission to hospital. There was no trend with age; the two high creatinine values came from men aged just under 50. There was some evidence of a weak trend with 24-hour urine volume, and the two highest creatinine concentrations were from two of the lowest volumes. In addition, they were from very recent (one day) admissions, but otherwise there was no overall association of concentration with time since admission. Possibly the two men had been mildly dehydrated on admission.

Figure 5.6 shows the relationships of the urinary uranium concentrations in the 24-hour samples with age, urine volume and days since admission. There was no relationship with age, and perhaps a weak trend with urine volume, although the low volumes displayed a wide range of uranium concentrations. A similar pattern was seen with time since admission, where all the highest uranium concentrations were recorded within two days of admission. Again, the recently admitted had the largest range of concentrations, so there was no clear evidence of a trend.

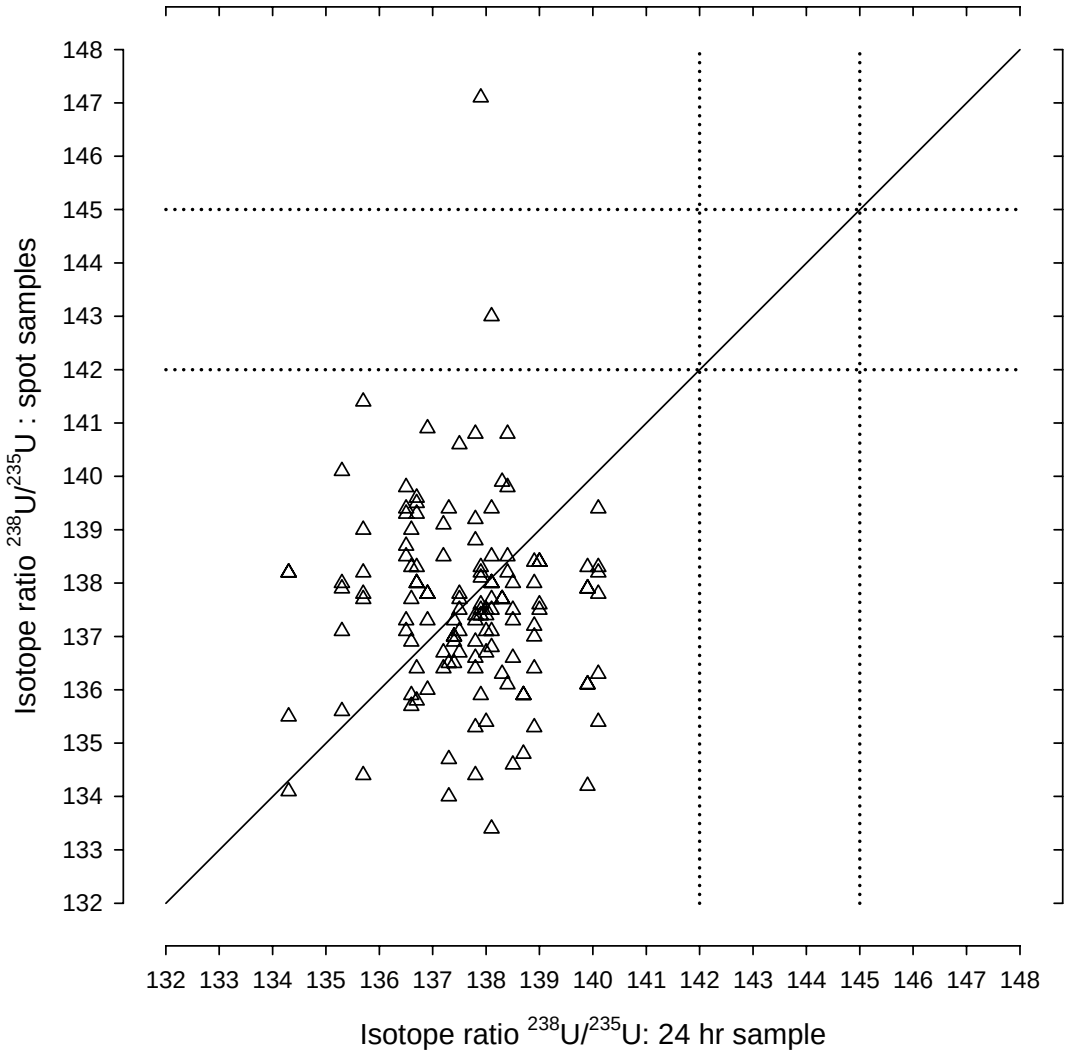

Figure 5.2 Comparison of $^{238}U/^{235}U$ ratios from spot samples and 24 hr collection in the same participants. The diagonal line is the line of equality. The dotted lines correspond to Scientifics Ltd's Guidelines for reporting samples as possibly containing DU (ratios *between 142 to 145*) or containing DU (ratios *exceeding 145 for measurements having good precision*).

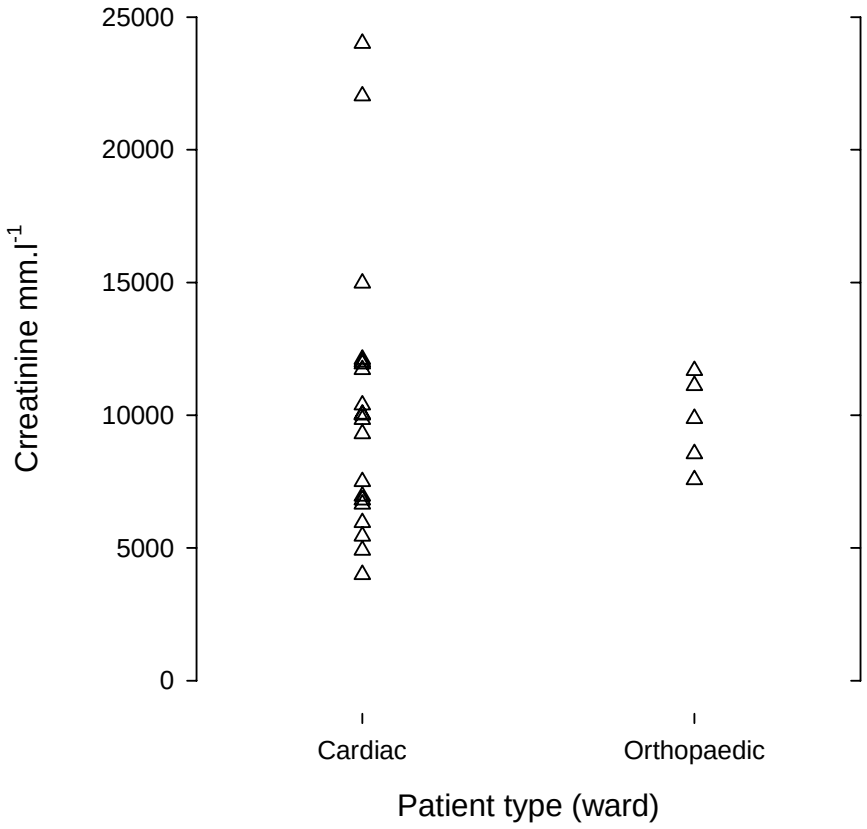

Figure 5.3. Distribution of creatinine concentration in urine by patient type.

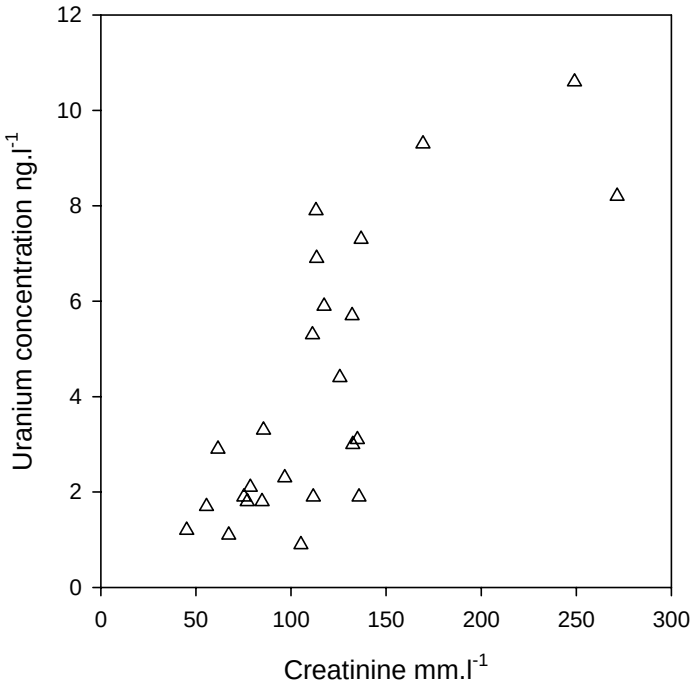

Figure 5.4. Relationship between uranium and creatinine concentrations in urine.

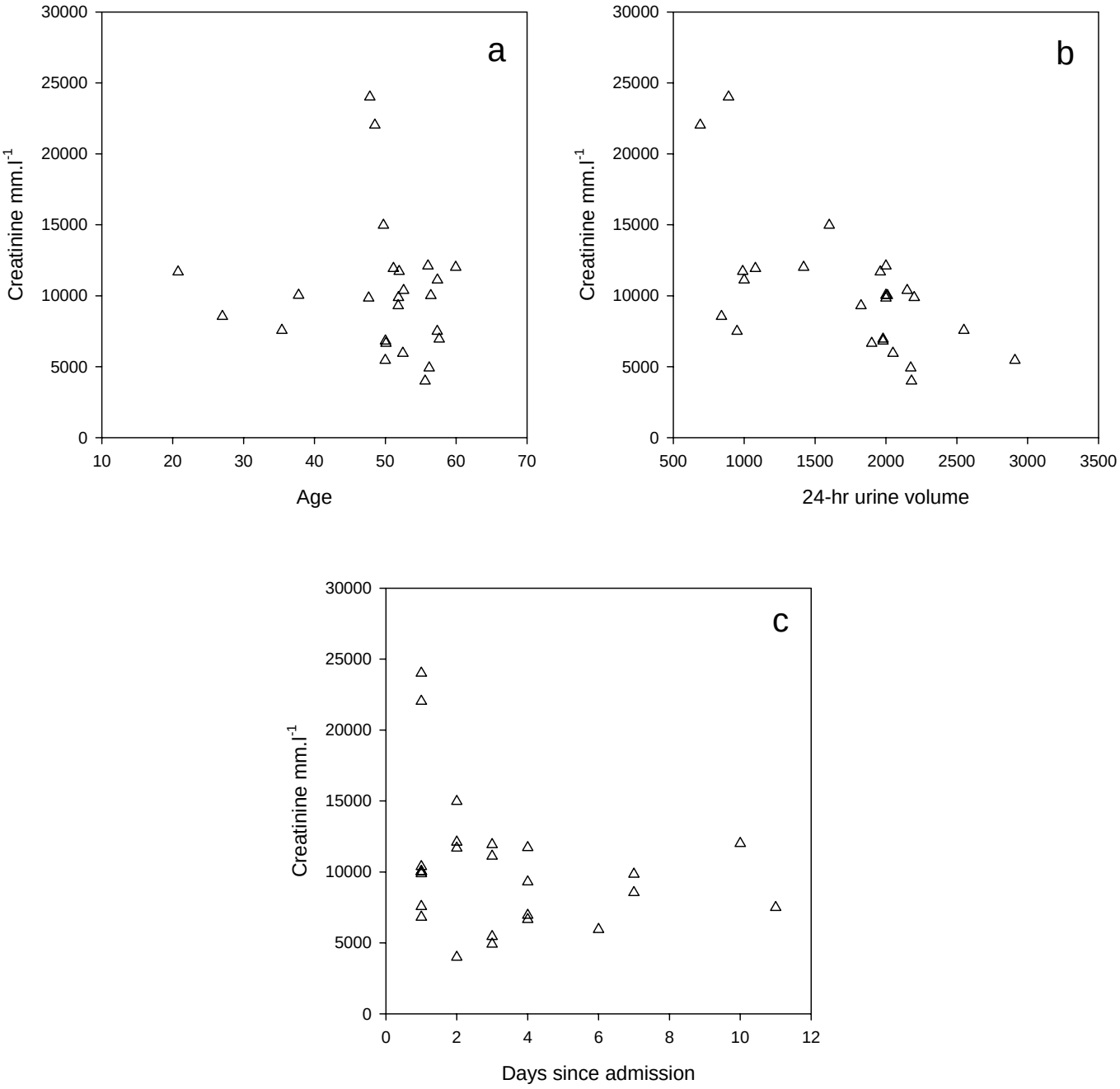

Figure 5.5. Relationship of creatinine concentration in urine with (a) age, (b) urine volume and (c) days since admission to ward.

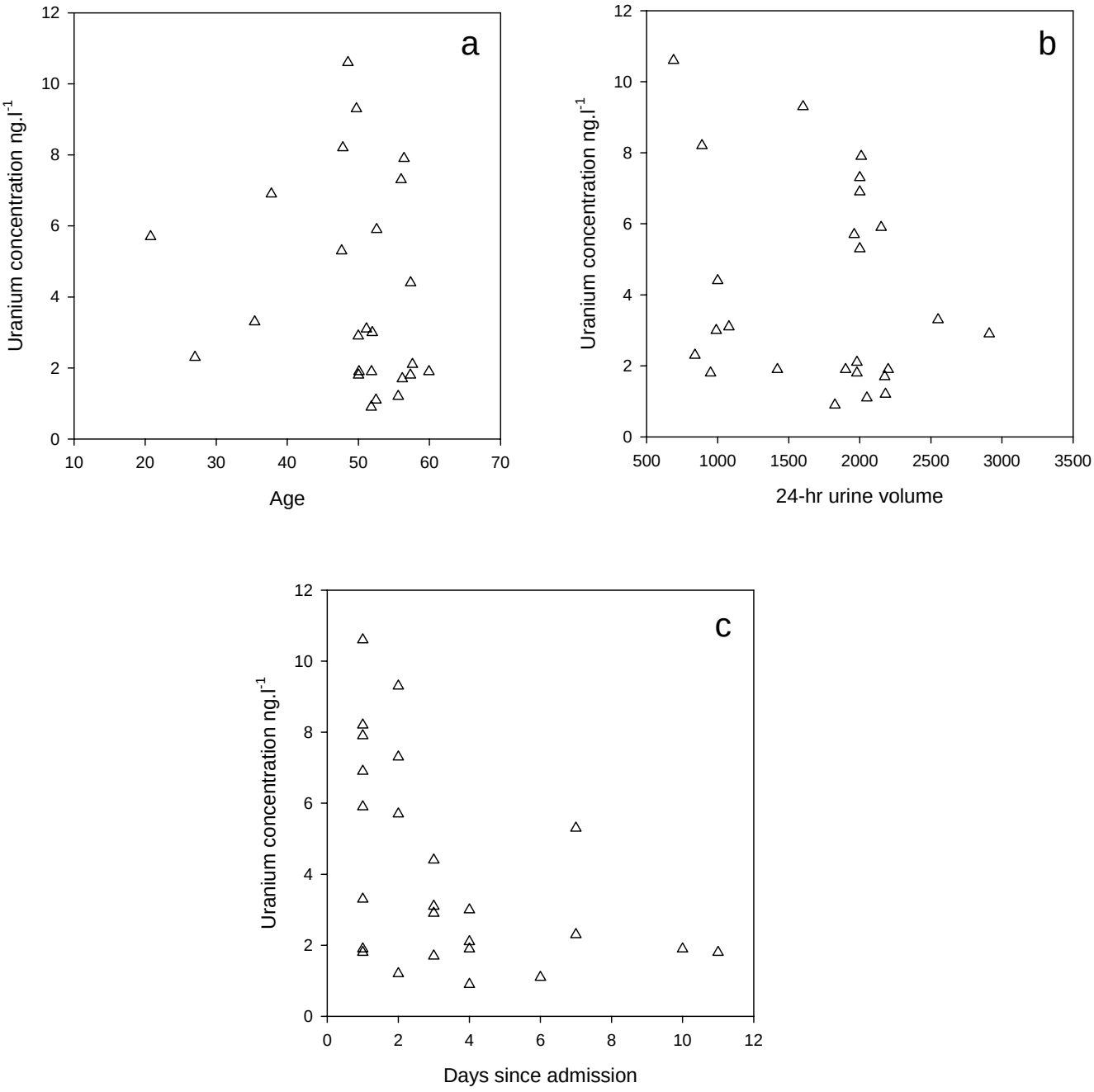

Figure 5.6. Relationship of uranium concentration in urine with (a) age, (b) urine volume and (c) days since admission to ward.

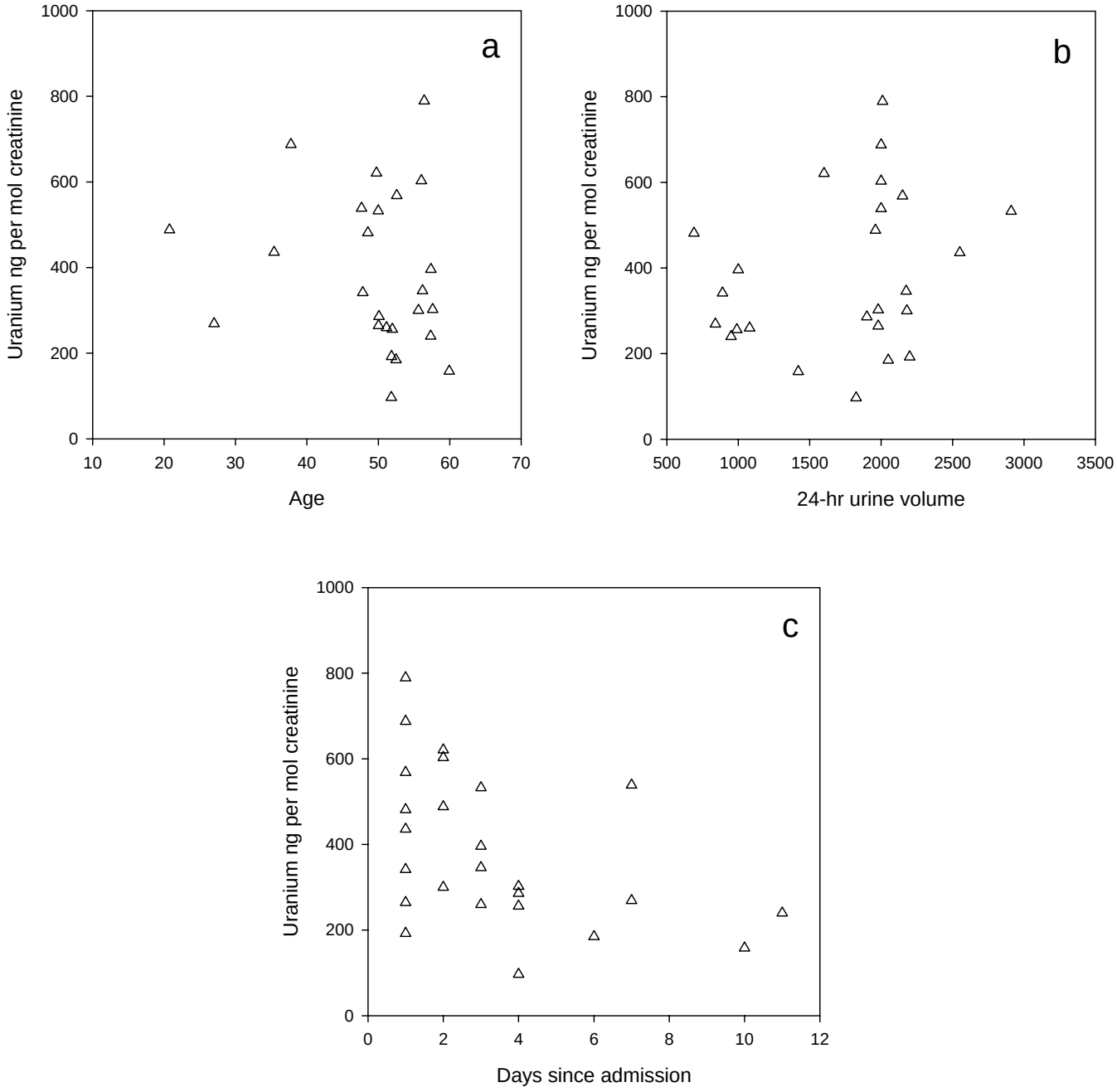

Figure 5.7: Relationship of creatinine-adjusted uranium concentration in urine with (a) age, (b) urine volume and (c) days since admission to ward.

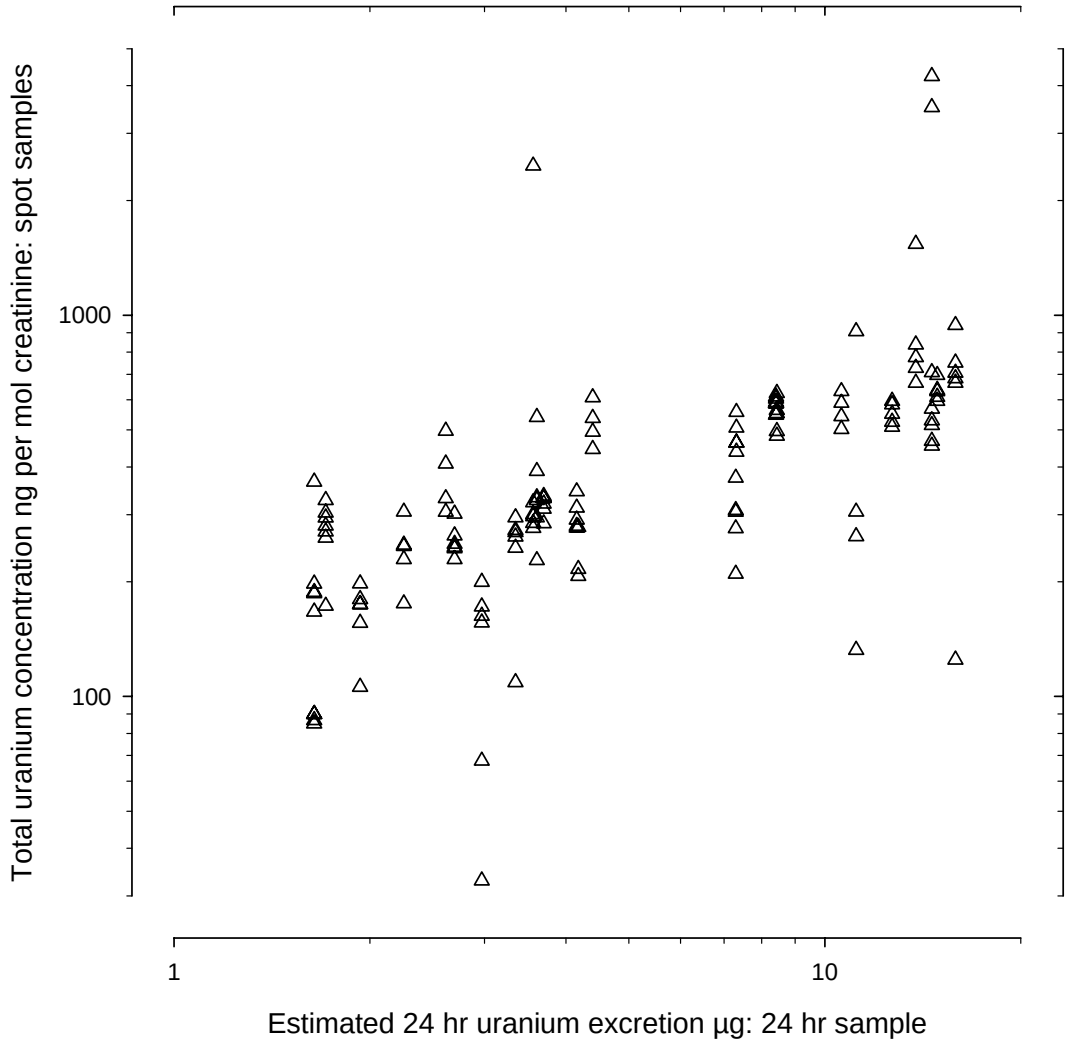

Figure 5.8: Relationship of creatinine-adjusted uranium concentration in spot samples of urine with total estimated 24-hour excretion of uranium (from 24-hour samples).

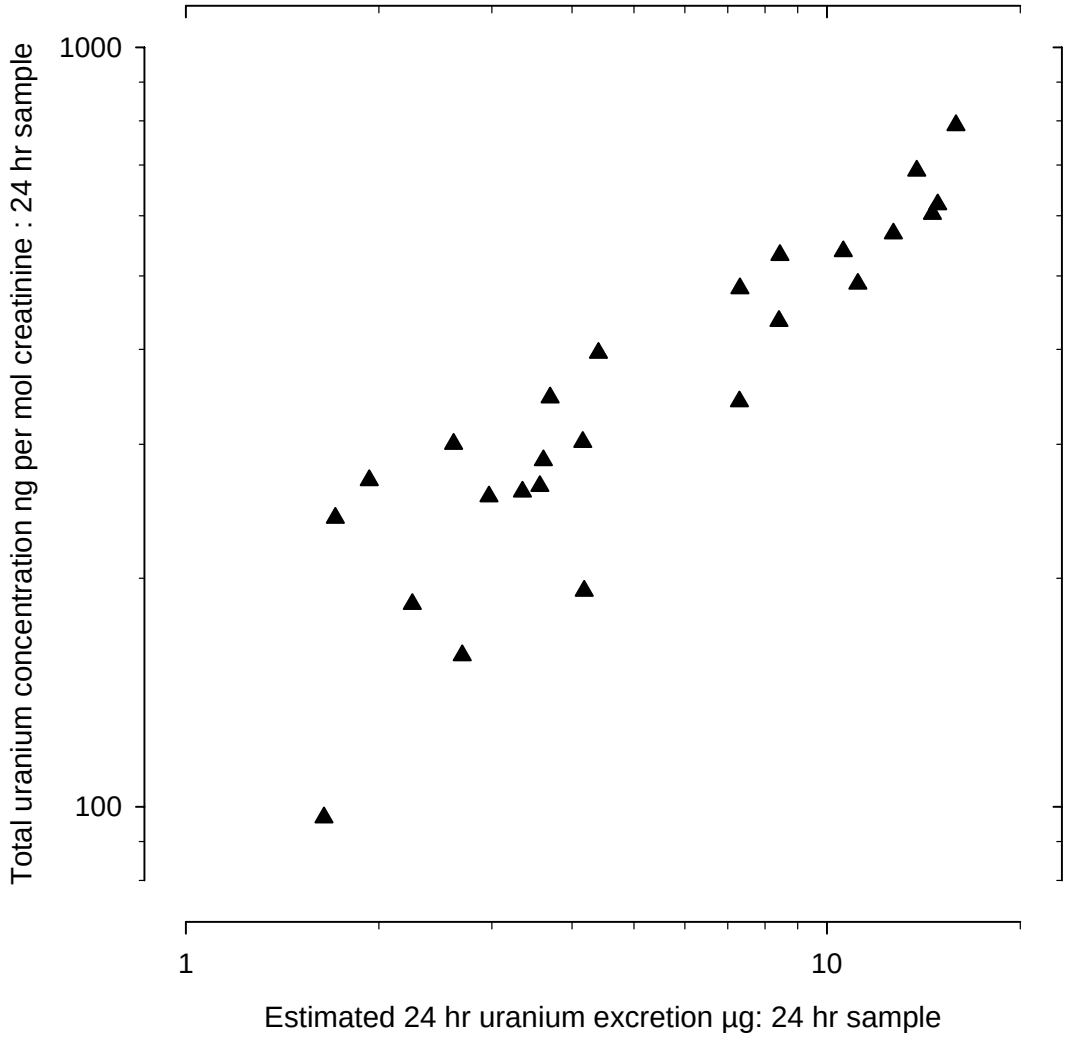

Figure 5.9: Relationship of creatinine-adjusted uranium concentration in urine to total estimated 24-hour excretion of uranium (both from 24-hour samples).

Figure 5.7 shows the relationships of the creatinine-adjusted uranium concentrations with age, urine volume and days since admission. There was no relationship at all with age, nor with urine volume, and the suggestion of a trend with days since admission, already weak in the unadjusted concentrations (Figure 5.5) was reduced in the adjusted values.

We conclude that age has no influence on the raw or unadjusted uranium concentrations; that adjustment for creatinine removes any weak influence of urine volume; and that days since admission may have at best a very weak influence, weaker yet in the adjusted than in the unadjusted concentrations.

5.2.7 Total 24-hour uranium excretion.

The DUOB indicated an interest in the relationship between the creatinine-adjusted uranium concentrations in the spot samples and the estimated total amount of uranium excreted in a 24-hour period. Our data permit this comparison, since we have uranium concentration per ml and total volume in the 24-hour samples. (Attempts to estimate this quantity by totalling the spot samples would not be satisfactory, because we believe that many of the spot samples did not capture the full volume excreted.)

Figure 5.8 shows the creatinine-adjusted concentrations against the estimated 24-hour uranium excretions. The variation between spot samples within individuals is of course the same as in Figure 5.1, but comparison with that figure shows also that that the positions of individuals are very similar in the two graphs.

Figures 5.1 and 5.8 have the same data on the y-axis, and differ only in their x-axes, which are different variables (creatinine-adjusted uranium concentration and total amount of uranium) from the 24-hour samples. Figure 5.9 compares these data. It is seen that these are quite closely related. This explains why Figures 5.1 and 5.8 show very similar relationships of the spot samples with these characteristics of the 24-hour samples.

5.3 SOURCES OF VARIATION

5.3.1 Structure of analysis

The underlying design of the pilot study, in terms of sampling days, is summarised by Table 5.1. Each participant was sampled on two consecutive days by a 24-hour collection and a series of spot samples. This allows estimation of differences between participants in measured variables such as uranium concentration or isotope ratio, plus differences between sample types, and a participant.type interaction (i.e. variation between participant that differs between types, or vice versa). In addition, there is variation associated with differences between spot samples within a participant. Using the numbers (of measurements and participants) in Table 5.1, in the absence of missing data values, the structure of the Analysis of Variance (ANOVA) for this data set is shown by the following breakdown of degrees of freedom (d.f.):

Source of variation	d.f.
Participant	24
Type (spot v 24-hour)	1
Participant.Type interactions	24
Residual (between spot samples)	114
Total	163

Occasional missing values within a particular response variable will reduce the d.f. for Residual.

A subsidiary question was whether the unadjusted or adjusted concentration, or the ratio, was dependent on the time of day at which the sample was taken. This was addressed by regressing terms calculated as the sine and cosine of an angle calculated from the time of day, expressed as a proportion of 24 hours set equivalent to 2π revolutions. Regression on these terms used 2 degrees of freedom from the Residual term (equivalent to estimating amplitude and phase parameters).

5.3.2 Weighted vs. unweighted analysis

One consideration for the formal analyses was whether to give each sample equal weight. Weights are employed in statistical analyses for two (separate, but sometimes confused) purposes. Where a set of data values are from the same distribution but are measured with different precisions, weighting can adjust for measurement precision and give relatively more weight to values with higher precision. Where a set of values represent different strata of a whole, and are sampled with different fractions, it will be necessary to weight the stratum results to reconstruct an average for the whole. These two circumstances correspond to dealing with random imprecision around a single mean, and representativeness in averaging across a set of different means.

In the present circumstance, the second consideration will be important only once differences have been established by an ANOVA, and the crucial question is whether the ANOVA should adjust for different measurement precisions. We might assume *a priori* that measurement precision might be affected by sample volume. This is likely to be an over-simplistic assumption. First, the volume collected is not the volume of the aliquot measured. Second, the 24-hour sample volume represents a level of overkill in material collected. Third, the samples analysed for isotope ratio were different from those analysed for uranium and creatinine concentrations. Nevertheless, we carried out analyses of the uranium concentrations both equally weighted and weighted by Harwell-measured sample volumes, as a test of the sensitivity of the results to weighting assumptions. In the weighted analyses, we have weighted all the spot samples in proportion to their volumes as measured by Harwell. To avoid overweighting, the 24-hr samples have been allotted a weight as if their volume was 500 ml. The weights were normalised so that a 250 ml sample had a weight of 1.0.

5.3.3 Uranium concentration

As is normal for concentrations, the ANOVAs were carried out after transforming the measurements to the logarithmic scale. Residual plots showed that this gave broadly equal variance across the data range.

The results of the equally weighted analysis are shown in Table 5.6. There were significant systematic differences between participants' uranium concentrations, but no difference on average between the 24-hour and spot sampling types, and no participant.type interaction. The harmonic terms showed no significant effect of time of day. Analyses weighted by sample volume gave identical conclusions, and are not shown.

Table 5.6 Summary of Analysis of Variance (ANOVA): uranium concentration in urine (log scale).

Source of variation	d.f.	Mean Square	F-ratio
Participant	23	2.690	6.58
Type (spot vs 24hr)	1	0.256	0.63
Participant.Type interactions	23	0.250	0.61
Residual (between spots)	105	0.414	
Harmonic terms (time of day)	2	0.702	1.71
Residual after harmonics	103	0.409	
Total	152	0.733	

Table 5.7 Summary of Analysis of Variance (ANOVA): uranium concentration adjusted for creatinine (log scale).

Source of variation	d.f.	Mean Square	F-ratio
Participant	23	1.938	9.39
Type (spot vs 24hr)	1	0.034	0.16
Participant .Type interactions	23	0.093	0.45
Residual (between spots)	105	0.207	
Harmonic terms (time of day)	2	0.231	1.11
Residual after harmonics	103	0.206	
Total	152	0.451	

Table 5.8 Summary of Analysis of Variance (ANOVA): Ratio $^{238}U/^{235}U$ (log scale).

Source of variation	d.f.	Mean Square	F-ratio
Participant	23	0.000154	0.92
Type (spot vs 24hr)	1	0.000017	0.10
Participant.Type interactions	23	0.000128	0.76
Residual (between spots)	100	0.000168	
Harmonic terms (time of day)	2	0.000160	0.95
Residual after harmonics	98	0.000168	
Total	147	0.000158	

5.3.4 Uranium concentration adjusted for creatinine

Again, the analysis of variance was carried out on the logarithmic scale, and residual plots confirmed that this was appropriate.

The results of the equally weighted analyses are shown in Table 5.7. Again, there were significant differences between participants, but no difference between 24 hour and spot sample types in their average concentrations, and no significant participant.type interaction. There was no evidence for any systematic dependence on the time at which the sample was collected.

It is notable that, even although the units for the creatinine-adjusted concentrations are two orders of magnitude greater than for the unadjusted, the residual variation is actually smaller for the adjusted, showing that a considerable amount of the variation has been removed by adjustment; on the log scale, the coefficient of residual variation has reduced from 53% to 8%. It is also notable that the F-ratio for the time-of-day effects reduced from 1.71 to 1.11 through adjustment, suggesting that a small diurnal variation in urine concentration is indeed almost entirely eliminated by adjustment for creatinine.

Analysis weighted for sample volume showed almost identical results, which are not shown.

5.3.5 $^{238}U/ ^{235}U$ ratio

The $^{238}U/ ^{235}U$ was analysed on the logarithmic scale, and residual plots confirmed that this was appropriate. The analysis was carried out unweighted.

The ANOVA table for the analysis of the isotope ratio is shown in Table 5.8. There were no significant differences at all between participants or sample type, and no evidence of a time-based trend. The residual plot showed clearly that the two high ratios already identified lay a little outside the range of variation of the other data points, but it was clear that this was not the cause of the lack of detectable systematic effects.

5.4 SUMMARY OF RESULTS

The uranium concentrations in the urine samples differed significantly between participants, and these differences were more significant after adjustment for creatinine. The residual variation after adjustment for creatinine was proportionally much smaller than in the unadjusted values, ratifying the adjustment procedures.

There was no systematic difference between the concentrations derived from 24 hour or spot sample types. Nor was there any evidence of a participant.type interaction, that is, the differences between participants did not depend on the sample type.

There was also no evidence for a systematic effect on spot sample adjusted concentration of the time of day when the sample was taken, particularly after adjustment for creatinine, which would be expected to vary across the day.

The analysis of creatinine-adjusted uranium concentration yielded a residual standard deviation, on the log scale, of $\sqrt{0.207} = 0.455$. This corresponds to a geometric standard deviation of 1.58. Using standard results for the log-normal distribution, we may predict that, for a given mean concentration, 95% of spot samples would lie within a range of 41% to 243% of the mean value, i.e. within a factor of about 6.

Two values of the $^{238}U/^{235}U$ isotope ratio, from spot samples collected from two different participants, lay outside the normal limits, but were not confirmed by other spot samples

within the same 24 hour period. We assume that these results are due to sampling variation in the quantification process, and we note that, even if the anomalous results were used to classify the participants as abnormal, a simple check-test strategy would correct the misclassification.

Overall, the recorded $^{238}U/^{235}U$ ratios provided no evidence for DU contamination in any of the limited number of participants. In contrast to the adjusted concentration results, there was no significant variation in $^{238}U/^{235}U$ ratio between individuals. In addition, no participant had any measurable amount of the ^{236}U isotope, which is also expected to be present in cases of DU contamination. We conclude that any uranium detected was from natural sources.

6 DISCUSSION

6.1 MAIN FINDINGS FROM THIS STUDY

From the results obtained in this pilot study, we assess the implications for a full normative study of the normal levels of urinary concentration of uranium in the general population.

The analysis of the data from this pilot study has shown that the urinary concentrations of uranium did not differ systematically on average between the spots and the 24-hour sample.

The spots were collected over a 24-hour period so each set of spots in total should be equivalent to a 24-hour sample. However, the total volumes were smaller for the spot samples than for the 24-hour samples suggesting that there were some losses. In particular, there probably was some loss as a consequence of overflow of the spot sample containers. There may have been other causes of lower total volumes for the spots. For example, the times recorded for the sample collection suggest that participants did not always void at the end of the second 24-hour period. The difference in volume would mean that while concentrations were not systematically different, the total amount of uranium collected in samples over the second 24-hours would be lower than that from the first 24-hours.

In these 25 participants, the 24-hour sample concentration ranged from approximately 100 to 800 ng per mol creatinine. The concentrations measured in the 139 spot samples (from the same 25 participants) spanned a much wider range, from not detectable to more than 4000 ng per mol creatinine. The wider range for the spots reflects the combination of the systematic difference between participants and the variation within the set of spots for each participant. As described in the previous chapter, 95 % of spots sample results for a participant would be expected to be within a 6-fold range from lowest to highest.

We therefore predict that a wider survey based on 24 hour samples would produce results that have a narrower range for uranium concentrations than a survey based on spot samples. With 24-hour samples, the benefit would be increased precision, but the results obtained from spot samples could well be sufficient for the purpose of providing a normative level against which to distinguish substantially elevated levels in military personnel.

If spot samples are used, a retesting strategy could be used to confirm or refute urinary uranium concentrations that appear to be unusually high.

Where the purpose is to provide a normative level, then the collection of a large enough set of spot samples is likely to characterise the range in the general population.

A limitation that was seen in the results is that the precision of the measurement (of concentration and ratio of isotopes) is lower on samples with concentrations below 1 ng.litre^{-1}, and more spot samples than 24-hour samples were in that low range.

In summary, a set of spots gave on average, the same urinary concentration as each participant's 24-hour sample, but there was substantial (about 6-fold) variation with each participant's set of spots.

6.2 PREVIOUS WORK

McDiarmid *et al* (1999) examined the utility of spot collection for urinary uranium determinations in depleted uranium exposed gulf war veterans. They also found that the standardisation relative to creatinine concentration reduced the variation. However, the ranges of concentration measured in unexposed persons quoted from previous studies, were in ng.l^{-1}. These ranges, shown in Table 6.1, are similar to those measured here.

Table 6.1 also shows (in the lower rows) the uranium concentrations normalised to creatinine as measured by McDiarmid *et al* (1999). Their measured concentrations normalised to creatinine, for non-exposed veterans, span a wider range than that found here for the general population sample. They had single spot samples from 38 non-exposed veterans, and 24-hour samples from 22 of them. The upper end of their ranges were about twice as high (for the spots) and five times as high (for the 24 hour samples) as those from the present study. Some of the exposed veterans produced concentrations that were about 500 times higher.

Table 6.1 Range of concentration of uranium in urine as reported in this and previous studies.

Range of unadjusted uranium concentration measured in urine samples ng.l^{-1}			
Source	**Number of subjects**	**24 hour samples**	**Spot samples**
This study (number of spots =139 from 25 subjects)	25	0.9-10.6	ND-38.1
Medley *et al* 1994 (quoted by McDiarmid *et al* 1999)	6		4-58
Dang *et al* 1992 (quoted by McDiarmid *et al* 1999)	27		3-40
McDiarmid *et al* (1999) For non-exposed veterans	38		<10-10
	23	10-130	
McDiarmid *et al* (1999) For DU-exposed veterans	29	10-50100	10-5220
Range of uranium concentrations adjusted for creatinine in urine samples ng/mol creatinine			
This study (number of spots =139 from 25 subjects)	25	97-790	ND-4200
McDiarmid et al (1999) For non-exposed veterans	38		0-9000
	22	1130-5700	
McDiarmid et al (1999) For DU-exposed veterans	29	1130-3.5×10^6	1130- 3.7×10^6

ND= not detected

The DU-exposed veterans in the study of McDiarmid *et al* (1999) were known to have pieces of DU shrapnel retained within their bodies, as a result of "friendly-fire" incidents. McDiarmid *et al* presented a graph with uranium concentration normalised to creatinine from single spot samples versus values from 24 hour samples. Their graph is similar to that in Figure 5.1 of this report, except that Figure 5.1 has multiple spots corresponding to each participant's 24-hour sample. They reported a high correlation between the creatinine adjusted spot concentrations and the creatinine-adjusted 24-hour sample concentrations. This correlation was higher (with R^2=0.99) for the DU exposed veterans than for the non-DU exposed veterans (R^2=0.44). In their graph, there appeared to be more scatter in the data for the lower end of the range of their measurements, i.e. the range relevant to the non-DU exposed persons. That scatter in the data for non-DU exposed persons is qualitatively consistent with the variation in spot samples seen in the present study.

The $^{238}U/^{235}U$ ratio for natural uranium is 137.88, whereas Horan *et al* (2002) obtained a value for a piece of DU shrapnel as 492.6. The change in the ratio $^{238}U/^{235}U$ can be used to estimate the relative proportions of natural and depleted uranium, if the 492.6 is taken as being representative of the DU.

The ratio of $^{238}U/^{235}U$ in a study with 24-hour samples collected from service personnel, showed 14 results confirming DU, with the $^{238}U/^{235}U$ ratio in the range 143.5 to 426.5, and a mean value of 207 (Horan *et al,* 2002).

Ting *et al* (1999) analysed uranium and thorium in 500 archived urine samples that covered a broad spectrum of the US population. After excluding one outlier specimen from the uranium results (that had 4080 ng.l^{-1}), the distribution of concentrations was found to be as shown in Table 6.2.

Table 6.2 Percentiles of urinary concentrations (ng.l^{-1}) in a US reference population, based on 499 samples, as reported by Ting *et al* (1999)

Percentiles for unadjusted concentration (ng.l^{-1})						**Means**	
5th	25th	50th	75th	90th	95th	Geometric	Arithmetic
1.42	3.84	6.32	11.8	25.6	34.5	6.64	11.0
Percentiles for creatinine normalised concentration (ng per mol creatinine)							
5th	25th	50th	75th	90th	95th	Geometric	Arithmetic
167	375	630	1154	2307	3947	684	1131

The ranges of concentrations obtained in the current study, 1 to 11 ng.l^{-1} in 25 24-hour samples, and not detected to 38 ng.l^{-1} in 139 spots, appear similar to the distribution reported by Ting *et al* (1999). The range of creatinine normalised concentrations found in this study, 100 to 800 ng per mol creatinine for the 24-hour samples and not detected to 4200 ng per mol creatinine for the 139 spots, appear similar to the spread in the results of Ting *et al.*

Gwiazda *et al* (2004) reported on the detection of depleted uranium in urine of veterans from the 1991 Gulf War. They found "*The determination of urine uranium isotopic ratios is necessary for identifying exposure to DU in soldiers with no shrapnel because urine uranium concentration is an ambiguous indicator for the presence of DU in these soldiers."* The concentrations from McDiarmid *et al* (1999), summarised in Table 6.1, distinguished non-exposed veterans without any data on the isotopic ratios in the detected uranium.

Gwiazda *et al* (2004) reported data for soldiers with embedded shrapnel and soldiers without shrapnel. The latter they subdivided into groups with DU detectable by the isotope ratios and those without DU. They found that "*The median creatinine normalised urine uranium concentration of soldiers with urinary DU (3110 ng per mol creatinine) fell between the 90th and 95th percentile for the US population* (See Table 6.2) *and it was six times larger than the median concentration for soldiers with no urinary DU (520 ng per mol creatinine)".* This median concentration of urinary uranium for the soldiers without DU (520 ng per mol creatinine) is in the centre of the range of creatinine normalised concentrations from this study (Table 6.1).

In the present pilot study, all the 24-hour samples had $^{238}U/^{235}U$ ratios that were in the range expected for natural uranium. Two of the spot samples showed slightly elevated ratios, which were not present in previous or subsequent spots (nor the corresponding 24-hour sample). While this suggests that a wider study using a spot sampling strategy would be likely to quantify the ratio adequately in most cases, it would be possible that some spot samples would return slightly elevated ratios, and that retesting would probably be required to address such instances.

6.3 PRACTICAL ISSUES FOR A FULL STUDY

The pilot study tested out the practical procedures involved in setting up and operating a survey with these procedures. The experience gained would be very helpful in designing and conducting a wider normative study.

The 24-hour samples were collected by recruiting hospital patients. The collaboration of a senior hospital consultant was essential in this case, and may well be similarly important if a wider hospital based study were to be undertaken. The administrative procedures in getting approval from hospital authorities and the local ethical committee (as required for any trial involving patients as participants) involved quite a lot of work. Once the administrative hurdles were overcome, the trial went well. However, some practical points are noted below.

The eligibility of patients was restricted by the practical need to choose patients fit enough to participate, but not so well that they were about to be sent home. In this pilot trial, 6 out of 25 participants finished the collection at home after early discharge from hospital.

The time specified for starting collection had to be widened from 12 midday to a window from 10:00 to 14:00 due to patients frequently being taken elsewhere during this part of the day.

The logistics of collecting a single 24-hour sample (in a future wider survey) would be easier than collecting successive 24-hour samples (as in this pilot study). The shorter time period of involvement (for each participant) would make more patients available. So the timescale for collection (of a given number of samples) could be shorter.

Collection of spot samples (with one per participant) would be logistically easier and could be conducted at locations other than a hospital. The amount of laboratory analytical work would presumably be approximately the same regardless of whether the sample is 24-hour or spot.

More work in the collection of 24-hour samples might be more efficient than additional analytical work to characterise a population from more variable spot samples where greater numbers of samples might be needed. However, if the level of variation seen in spot samples in this pilot is acceptable, then spot samples would be the more efficient method.

7 CONCLUSIONS

The pilot study has quantified the amount of variation to be expected in spot samples compared to a 24-hour sample from the same participant.

The participants showed significant differences between each other in the uranium concentrations in their urine samples, and these differences were more significant after adjustment for creatinine. This was largely because the residual variation after expressing the concentration normalised to creatinine was proportionally much smaller than for concentration per unit volume of urine. This finding ratified the use of concentration normalised to creatinine.

There was no systematic difference on average between the concentrations derived from 24 hour or spot sample types. Nor was there any evidence that the difference between participants was affected by the type of sample.

The statistical analysis showed, through a reduction in the F-ratio for the time-of-day effects, that a suggestion of diurnal variation in urine concentration was almost entirely eliminated by expressing uranium concentration normalised to creatinine.

The 24-hour samples clearly produce a narrower spread of concentrations than the spot samples. This suggests that a full normative study, if based on 24-hour samples, would describe a narrower distribution of concentrations than a study based on spot samples. This in turn should make it easier to identify individuals who have slightly elevated concentrations of uranium.

If it is important to know the proportion of individuals with slightly elevated levels, then 24-hour samples would be the best. On the other hand spot samples are easier to collect, and any unusually high values could be assessed by retesting. It appears that ratios of isotopes can be determined with good precision for samples with concentration above 1 $ng.l^{-1}$. Therefore, spot samples may suffice to establish the ratio of isotopes for all samples above that concentration, and – based on the distribution observed in this pilot study - would give a concentration that would (in 95% cases) be within 40 to 250% of the mean for that individual. Where concentrations are below 1 $ng.litre^{-1}$, then a minor elevation of the ratio of isotopes still implies a very low concentration of DU.

When expressed as ng of uranium /litre of urine, the range of concentrations determined in this pilot study appeared consistent with values previously reported for other similar sized groups. The concentrations were in the normal range for non-exposed subjects as described in other studies, and were much lower than the concentrations that have been measured in samples from subjects with DU shrapnel retained in their bodies.

The measurements of the $^{238}U/^{235}U$ ratio showed values were indicative of natural uranium for all the 24-hour samples and for 137 out of 139 spot samples. The two exceptions, with slightly elevated ratios were not supported by previous or subsequent spots for the individuals nor by their 24-hour samples, and appeared to be explained by slightly larger than usual variation in the measurements due to the low concentrations (< 1 $ng.l^{-1}$) of uranium in those two samples. This suggests that occasional slightly elevated ratios might be found in spots, which would need to be checked by a repeat testing strategy. However, this is more likely to occur in samples with very low concentrations (<1 $ng.l^{-1}$) of uranium in urine and the issue might be to improve the guidelines for defining samples as indicating DU present.

There was no ^{236}U detected in any of the samples, and that was also indicative of natural uranium.

The pilot study also demonstrated that 24-hour samples can be collected using hospital patients as participants, and it allowed many of the practical issues in organising and conducting a wider normative study to be identified.

8 ACKNOWLEDGEMENTS

We thank the Ministry of Defence for funding the study, and the Depleted Uranium Oversight Board for advice and encouragement. We thank the volunteers who agreed to participate in the study. We are also grateful to the study research nurse (Narelle Gregor) and her colleagues (notably Fiona McArdle and Sharon Cameron) at the Royal infirmary of Edinburgh for their enthusiastic support. We thank also the Local Research Ethical Committee, the Royal Infirmary of Edinburgh, Dr Douglas Young, and the Senior Consultants (Dr A Flapan, Mr JF Keating), all of whose support was essential to the study. The analysis of the samples was undertaken by staff at Scientifics Ltd.

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APPENDIX : PROTOCOL APPROVED BY LOTHIAN RESEARCH ETHICS COMMITTEE

Institute of Occupational Medicine

General Population Depleted Uranium Normative Value Study Development study

Short title; Methods of testing for uranium levels in the urine of male civilians

IOM Protocol, Version 10
LREC no. 04/S1103/6

03 June 2004
MOD Tender No CBC.MED/0752

1.1	**Project Title**	**General Population Depleted Uranium Normative Value Study: Development study**
1.2	**Chief Investigator**	**Dr Colin Soutar, IOM**
1.2	**Local Senior Investigators**	**Dr Andrew Colvin, IOM** **Dr Simon Walker, RIE**
1.4	**Associated Workers**	**Dr Alan Jones, IOM** **Dr Andrew P Colvin, IOM** **Dr Brian Miller, IOM** **Peter Hutchison, IOM**
1.5	**External Consultants**	**None**
1.6	**Independent Medical Adviser**	**Dr A Flapan**
1.7	**Analytical Laboratory**	**Harwell Scientifics**
1.8	**Customer Name**	**Ministry of Defence**
1.9	**Customer Code No.**	**Contract CBC/MED/0798**
1.10	**Tasking Reference**	**CBC/MED/0798, 12/12/03**
1.11	**Establishments**	**Edinburgh Royal Infirmary, Institute of Occupational Medicine**
1.12	**Location for Study**	**Edinburgh Royal Infirmary , Institute of Occupational Medicine**
1.13	**Experimental Dates**	**April to May 2004**

1 SYNOPSIS

The procedures consist only of a continuous urine collection over one 24-hour period, followed by a series of spot urine samples over a subsequent 24-hour period. A few personal and clinical details will be recorded.

The purpose of the study is to find out whether measurements of uranium on spot samples adequately represent the 24-hour excretion of uranium. This will help to determine the design of a proposed national study of urine uranium concentrations in the general population.

This information will be of value in the interpretation of urine uranium values found in service men and women who have served in the Middle East.

A refinement will be the ability to measure the ratio of Uranium 238 and Uranium 235, since this ratio relates to whether the source of the uranium is natural, from nuclear fuel or from depleted uranium.

The study has been commissioned by the Ministry of Defence.

We will ask to recruit male convalescent, ambulant, patients recovering from orthopaedic procedures or trauma, also convalescent cardiac patients. The total number will be 25 subjects with complete collections.

We will ask to station an authorised research nurse in the hospital (we hope it will be one of the staff of the Clinical Research Centre), who will liaise with ward staff to identify potential recruits, ask for informed consent, and arrange for the urine collections. No work will be required from ward staff, and the procedures will not be allowed to interfere with clinical care.

The data will be kept in confidence, and reports will not enable the identification of individuals.

2 INTRODUCTION

This pilot study will inform the design of an intended national study to determine urine uranium levels in the general population, for the purposes of comparison with the levels in military personnel who served in the Middle East.

The pilot study seeks to determine the extent to which the uranium content of spot urine samples represents 24hr excretion of uranium.

Both uranium 235 and uranium 238 will be measured, since the proportions of these can help to identify the sources. Exposure to depleted uranium in munitions is likely to be associated with lower than normal $^{235}U/^{238}U$ ratios, while exposure to any emissions from nuclear power stations is likely to be associated with higher than normal ratios.

MOD has commissioned the Institute of Occupational Medicine (IOM) to conduct the pilot study.

The MOD's Statement of Requirement is for the following:

- Design of a normative value study in conjunction with the MOD;
- Production of a protocol for the study;
- Obtaining ethical clearance for the study;
- The collection of the urine samples, a combined 24 hour sample and a set of separately bottled spot samples over the following 24 hours from each participant;
- The storage of the urine samples;
- Transfer of samples to a separately contracted laboratory (Harwell Scientifics) for analysis;
- Statistical analysis of the uranium measurements in association with other data, and produce a comprehensive report;
- Publication of the work in a peer review journal.

This research project has been prepared to meet these requirements, and has been produced after discussions with MOD.

Background

Concerns have been expressed by some, about the potential health effects of exposure to depleted uranium following active service in war zones where depleted uranium has been used. A recent review by the Royal Society (2001) concluded that it was unlikely that any excess of fatal cancers from possible radiation effects would be detected in a cohort of 10 000 soldiers followed over 50 years and very few individuals were at risk of developing kidney disease. The lifetime risk of death from lung cancer in the most exposed individuals, however, might be double that of the general population and they could also be at risk of developing serious kidney disease in later life (a possible effect of uranium as a chemical). In addition, McDiarmid *et al* (2000) have found evidence of neurocognitive impairment in Gulf War veterans who had retained fragments of DU shrapnel, although these individuals showed little evidence of impaired kidney function. The Royal Society acknowledged that there was a great deal of uncertainty in their assessment of DU exposure and their recommendations included the need to validate the measurement of urinary DU concentrations as a measure of past exposure. Since the publication of the Royal Society Report, the MOD's DU Oversight Board has overseen a programme of development work.

The uranium content of urine in the general population is not well understood but is thought to vary considerably depending on where people live, their diet and their intake in drinking water. The use of DU is not confined to the military and some exposure to DU is likely to have occurred locally within the UK. The WHO (2001) reviewed data from the early 1990s that suggest that urinary uranium concentrations in the general population range from about 4 to 57 ng/l. Gulf War veterans known to have been exposed to DU are reported to have had urinary concentrations averaging 80 ng/l, 7 years post-exposure, the highest concentrations being about 30 000 ng/l (McDiarmid *et al*, 2000). Measurement methods have improved considerably over the last decade. A recent survey of uranium concentrations in the US population found that the median concentration was only 5 ng/g creatinine (7 ng/1), but 10% of the measured concentrations exceeded 24 ng/g creatinine (43 ng/l) (National Health and Nutrition Examination Survey, 1999). Measured concentrations did not fit a normal or log-normal distribution but had a marked tail at the higher end of the scale.

The Institute of Occupational Medicine was invited to tender for this work, which is funded by the Ministry of Defence (Contract number CBC/MED/0798. Issue Date 12 December 2003, (from Mr Douglas Martin, CB (Com) Med, MoD, 4th Floor, Cerium Building, 55 Douglas Street, Glasgow, G2 7NP). The primary contact and project officer for the MoD is Mr Charles F Williams BSc MPhil (GVIU – Research A) (Floor 7 Zone A, St George's Court, 2-12 Bloomsbury Way, London WC1A 2SH. The Project Officer and associated workers have no conflicts of interest to declare.

An additional summary of this project in lay language is given in the Subject Information Sheet at Annex A and Administrative Officer Information Sheet at Annex D.

This research project is being submitted to the Local Research Ethical Committee (LREC) for ethical approval.

3 AIM

The aim of this pilot study is to establish how much the urine excretion of uranium isotopes varies over the course of a day, and from one day to another; specifically to examine the individual variation in uranium concentrations between single (spot) urine samples within a day, and to compare these with a total 24hr sample on a different day. The study will be conducted in one (hospital) location.

Uranium will be measured (in collaborating analytical laboratories), under a separate contract from the MOD) as nanogrammes per litre, nanogrammes per gramme of creatinine and $^{235}U/^{238}$ ratio, in an adult male study group.

4 METHODS

4.1 Design, location, subjects, exclusions, sample size, recruitment, and collection

4.1.1 DESIGN

The design, as requested by the client, is a clinical study of uranium concentrations in a total 24hr sample on one day and in sequentially collected spot samples on another day. Sequential days are specified by the client. We suggest that if collection is incomplete or not possible on the immediately succeeding day, collection be attempted, if feasible, on the third or fourth day.

4.1.2 LOCATION

The study will be conducted in one large hospital near Edinburgh. The Clinical Director of Laboratories in The Royal Infirmary of Edinburgh (RIE) has kindly expressed interest in helping with the study, and it is thought that a study in the RIE will be possible, subject to approvals. If not, another large hospital will be approached.

4.1.3 SUBJECTS

The subjects will be male ambulant patients convalescing from cardiac conditions, orthopaedic procedures and trauma. We propose a quota-sampling scheme, of eligible patients, with consecutive recruitment up to specified quotas of participants in subgroups defined by age. Patients with known renal disease, or who are undergoing 24-hr urine collections for clinical purposes, will be excluded. Age ranges are within the range 20 to 59 years. About 25 subjects will be recruited. The sample size will be sufficient to quantify between-sample and between day variations. It will also identify any large systematic circadian variations, large effects of urine volume, or large age effects. Other possible causes of variation, such as recent exercise or change in diet or medication, are unlikely to be characterised effectively in this small sample. However, hospital meals and intake of mineral water will be recorded.

The sample will be biased towards those who have accidents or heart attacks. This would be quite a broad selection of the population, and there seems little reason to expect that the selection would induce any bias in respect of uranium fluctuations.

4.1.4. RECRUITMENT

A study nurse will be based at the hospital, and will identify eligible subjects. When convenient for clinical requirements, and with the permission of the responsible medical and nursing staff, he/she will explain to eligible subjects the purpose and nature of the study, and ask for informed consent. Subjects agreeing to participate will be asked a brief questionnaire, and given instructions and supplies for collecting the urine samples. The nurse will return the next day(s), record any lost samples, deliver the samples for storage, and arrange for them to be picked up. The subject will be asked to collect over a second 24hr period, either the next day or after a brief interval. Since decisions on discharge from hospital can be made at short notice, it is recognised that either 24hr collection may be interrupted, and subjects with two 24hr collections may be less numerous than subjects with one. Within the constraint of the recruitment period, additional subject will be sought to replace those with incomplete urine collections.

Assuming eligible subjects agreeing to participate become available at a rate of about five to ten per week, a recruitment period of about five weeks will be required. We plan to recruit as many as possible during this period, then stop and review with MOD the numbers achieved. A further period of recruitment could be arranged if needed.

4.1.5 QUESTIONNAIRE

A simple questionnaire will focus on the requirements of the development study. The questionnaire will record name, NHS number, sex, date of birth, ward, consultant in charge, medical diagnosis from the nursing notes and patient, and dates of admission and operations. A simple dietary history for the period immediately preceding and during the collection will be obtained, for subsequent reference should any unusual results require explanation.

4.1.6 COLLECTION OF SAMPLES

Subjects agreeing to take part will be given a large (5000ml) wide, necked container for the first 24-hr pooled urine sample, and sequentially numbered 200ml wide-necked containers for the second 24 hours, and instructions. He will be asked to pass all urine in the first 24 hours into the large container, and on the next day to urinate on each naturally occurring occasion (from 10 pm to 10 pm on the next day) into a different container, making a note of the time. Instructions will be given on how to collect samples without contamination. The nurse will collect the samples each day, and will record any occasions of losses to collection.

4.2 Ethical approvals, and consideration time for volunteers' consent

Ethical approval will be sought from the Local Area Medical Research Ethics Committee. The study protocol conforms to the "Draft Code of Ethics on Human Experimentation" presented to the World Medical Association at Helsinki in 1961 and as amended thereafter.

The relevant clinical staff supervising the patients will be asked for their permission to approach the patients.

Each volunteer will be invited to sign a consent form having been given adequate time to consider their decision to volunteer. Individuals will be provided with information by a printed information sheet and given the opportunity to ask questions. A copy of the subject information sheet and consent form are attached.

4.4 Procedures

4.4.1 Outline

In order to minimise the risks of sample degradation through poor or inappropriate treatment, a protocol has been developed in close collaboration with the laboratory where the analyses are to be undertaken. This includes the following:

1. IOM stores, for no longer than week, the urine samples in tamper evident sealed bags at 1° to 5° C and processes the questionnaire data.
2. IOM sends the urine samples on to the Harwell Scientifics laboratory in secured coolbox containers. The carrier is TNT, who are accustomed to transporting clinical samples. Transport will be timed to avoid the weekend.

3. Harwell Scientifics analyses the urine uranium content, isotope ratio, uranium content relative to creatinine content, and passes the results back to IOM. We understand that some samples will be divided and sent to other laboratories for comparative measurements, but IOM is not involved in this.
4. IOM analyses the results, consulting with analytical laboratory, and drafts the report.

4.4.2 Detailed description

Sample collection

The study nurse will;

- Liaise with ward staff to identify eligible subjects, and on a convenient time to speak to the subject
- Brief the eligible study subjects on the purpose and nature of the study, and request signed consent
- In the event of consent, administer the personal data questionnaire, give instructions for collection of the samples, and provide labelled containers and a marker pen
- Return the next day, enquire about the success of the sampling, record any losses or other difficulties, record diet, and give instructions for the next day's sampling
- Check the labelling of the samples, put into labelled sealed bags, and store at 1-5°C (either in the hospital laboratory or in the IOM laboratory).
- Return the next day, repeat the retrieval and recording procedures
- Thank the patient for assistance, and answer any queries.

Sample containers

The sample bottles and containers used for storage and transport of the samples will be supplied by the analytical laboratory. The sample bottles are to be composed of low density polyethylene. Tests on a representative number of sample bottles have found no evidence that the bottles either leach uranium into or remove uranium out from the sample. The sample bottles will be labelled with a unique number and provided with an identically numbered form.

The sample bottles will be labelled and put in an individual sealed 'tamper evident' plastic bag following collection. They will then be secured in a locked container during transport.

The containers are likely to be suitable for use as supplied by the manufacturer, and will be provided by the Harwell Scientifics laboratory before sample collection is undertaken. The sample bottles will be cold packed using cool packs to maintain a temperature of 1° to 5° C during transport.

Sample storage

During the collection phase the samples will be placed in a fridge at 1° to 5° C before being transferred to the analytical lab in a sealed coolbox.

Harwell Scientifics will store the samples until analysis is complete and up to two years after collection. After two years, or earlier by agreement with MoD, the samples will be disposed of as clinical waste. It is unlikely that long-term storage of the samples will affect the creatinine content of samples. Urine samples are routinely stored for periods of weeks to months without serious degradation, however, it is not possible to be certain that no degradation will occur over two years.

Staffing

Dr Andrew Colvin is the local IOM senior investigator. Dr Simon Walker, Clinical Director of Laboratories, Royal Infirmary of Edinburgh, has kindly agreed to be a Senior Investigator, and senior member of the scientific team, Dr Alan Jones will direct the operation of the project, and prepare the reports. A nurse will be hired to supervise sample collection and the transport of samples back to IOM headquarters. The nurse may be a member of the hospital's own research unit, or an agency nurse, and in any case will be appointed an honorary member of the hospital staff, to authorise working in the hospital. Dr Brian Miller (Senior Statistician at IOM and Director of Research Operations) has advised on the study design and will provide the statistical analysis. Dr Colin Soutar, Chief Executive and medical epidemiologist will provide supervision and support.

Foreign research workers

No foreign research workers will be employed on this contract.

Quality assurance

IOM is committed to providing high quality services. Research studies are conducted by project leaders with multidisciplinary teams. Detailed project plans are completed and approved before the project starts, and the project is conducted with frequent project meetings in which each member of the project team contributes expertise. Regular project reviews are conducted by senior staff. Draft reports and final reports are reviewed in house by senior staff and revised accordingly. Clients are kept informed of the progress of the project, and may be closely involved in the overview of the progress of the work if desired. Papers describing the work are subsequently prepared for the peer review literature, demonstrating that the work is conducted to a high standard. Draft reports are submitted to clients for comment before finalising. On the Consultancies side we have UKAS accreditation for a wide range of laboratory analysis, occupational hygiene sampling and asbestos work. Our staff are accustomed to working within quality systems. For all work, whether formally accredited by UKAS or not, we ensure that staff are properly trained for the tasks to be undertaken, that there are written instructions and that the staff involved have read and understood instructions and that proper records are kept. We have an internal audit system to ensure that the highest standards of quality are met.

Timescale

Subject to ethical approval, and on obtaining informed consent, and on the basis of collection from a total of 25 volunteers, it is intended that samples will be collected as soon as possible following contract placement (beginning of January 2004). This is likely to be in May and early June, or earlier.

Data processing will proceed once the samples have been analysed by the contracted laboratory. It is anticipated that the statistical analysis, and reporting (in draft) can be completed two months after the last urine sample results have been supplied by the laboratory.

4.5 Measurements

Personal data needed for each individual by questionnaire includes:

- full name;
- gender;
- date of birth;
- home address and telephone number
- reason for being in hospital
- date of admission and/or operation
- dates and times of sample collection;
- ward, supervising consultant
- from hospital notes, result and date of a recent serum creatinine estimation, if available
- dietary record

Laboratory analysis will be undertaken for total urinary uranium contents and for the isotopic ratio of the uranium present and creatinine. Results will be reported in terms of total uranium, scaled relative to creatinine, and isotopic ratio of ^{235}U and ^{238}U.

4.6 Data analysis

Data protection and control

IOM will be Data Controller under the Data Protection Act, and has many years of experience in handling confidential research data. No individual study participants will be identified in the report.

Data processing

Questionnaire data will be entered to computer file. Data will be double entered with all discrepancies checked and verified. Appropriate procedures will be designed and implemented to check the collected data for logical consistency, valid values, valid ranges and cross record consistency.

The study subject's names will be held separately along with the IOM's study subject identity number in an Access database that will use the standard security model and include data encryption.

The main analysis file will also be created in Access database format and will only identify subjects using the IOM's study subject identity number. This database will be subject to standard IOM IT security policies, which include security restrictions that ensure that only project team members have access to these data. These measures ensure acceptable levels of data anonymisation.

The analytical laboratory will provide the urine test results electronically in a form to be agreed during the study. Care will be taken to ensure consistency in labelling the sample results so that they can be matched to the questionnaire data using a unique subject identifier. Urine test results will be checked and validated by Harwell Scientifics before they are sent to the IOM.

On receipt of the data, IOM will construct a computer file system using Microsoft Access which links the personal data, questionnaire data and urine test results for each participant in the survey. For non-participants who were selected and agreed to take part, but who subsequently were unable to attend at survey or provide the urine sample reasons for non-completion will be recorded. Similarly exclusions will also be recorded. All data in the database will be checked for completeness, consistency and validity before a fully anonymised file is produced for use in the statistical analysis.

All of the study data files will be stored on a *Compaq* Server on the IOM's network. The server is located in a secure, climate controlled computer room into which physical access is controlled and limited to IT administration staff. The IOM's IT Security Policies include issues of data security and the integrity of all computerised data. These procedures include a daily, full backup procedure, active protection from the threat of computer virus and prevention of unauthorised access to any study data. The study will run in full compliance with the Data Protection Act.

Any issues involving the data collection, data processing and systems design will be under the control of the project's systems analyst who will also review any other data related issues as appropriate to the requirements of the project.

Statistical analyses

The principal data to be analysed will be concentrations of uranium in urine (both as measured, and standardised for creatinine concentration) and ratios of uranium isotopes, particularly the $^{235}U/^{238}U$ ratio. If convenient or necessary to meet assumptions regarding statistical distributions, any of these variables may be transformed to a suitable scale, e.g. logarithmic, prior to formal analysis. Knowledge of the technical aspects of the measurements will be essential to give scientific context to the measurements, the analyses and their interpretation, and both the design and the results of the statistical analyses will be discussed with appropriate personnel from the analytical laboratories.

For each study participant, uranium concentrations will be available separately for each urine sample collected during the second 24-hour period, and as totals for the combined 24-hour samples. 24-hour variation in uranium concentrations for individuals will be described using tabular and graphical methods as appropriate. Comparisons of differences in uranium concentrations between and within individuals; between samples and between days, will be carried out using standard Analysis of Variance (ANOVA) techniques for nested random effects models. Uranium concentrations calculated from the total 24-hour urine sample will be compared with 'spot' urine samples taken at different times of day (e.g. first thing in the morning, midday, late evening) to determine whether there is a significant temporal variation. Analyses of the uranium concentration data will include investigation of any differences in the results between participants of different ages.

Conclusions will be drawn on the value of spot samples in estimating total 24hr uranium excretion.

The results will be anonymous, and no individuals will be identified.

Personnel at Harwell Scientifics will be invited to comment on the design and results of the statistical analyses as they may beneficially be influenced by knowledge of the technical aspects of the measurements and the scientific context.

4.7 Reporting

The report, in the form of an IOM Research Report, will include a brief executive summary, a scientific summary, introduction, methods, results and discussion. Personnel in Harwell Scientific will be invited to contribute to the authorship of the report. A draft will be submitted to MOD for comment before finalising. IOM will take these into account, but will retain final editorial control. To demonstrate openly the independence of the study, the contract stipulates IOM freedom to publish. IOM Research Reports are publications with a small circulation, and are available on request. In addition, the work will be prepared for submission in due course to a peer-review scientific journal.

A copy of the principal report(s) covering this work will be submitted to the Secretary of MoD(N) PREC on publication.

It is not planned routinely to release individual results, but it is a requirement under the Data Protection Act to release individual information if requested in writing by individual study subjects. This will be

complied with provided the true cost of supplying the information to the study subject by IOM is met prior to disclosure.

5. MEDICAL AND ETHICAL CONSIDERATIONS

IOM will obtain ethical clearance for the study by submitting a study protocol to the Lothian Research Ethics Committee for scrutiny and ethical approval. The study will observe and comply with all the Guidance in notes provided by the Lothian Research Ethics Committee.

Each volunteer will be invited to sign a consent form having been given adequate time to consider their decision to volunteer. Individuals will be provided with information by a printed information sheet and given the opportunity to ask questions of a member of the study team (either in person or by telephone). A copy of the subject information sheet and consent form will be included with the protocol submitted for ethical approval.

5.1 Medical cover

IOM staff will supervise the collection and transport of urine samples. The deputy project leader, Dr Alan Jones, will be on call by telephone as a source of advice or guidance to volunteer subjects, and study staff during the study. Dr A Flapan, the independent advisor, will be on call for advice and guidance to study participants if required by telephone before, during and after the study sample collection phase.

5.2 Withdrawal criteria

Withdrawal criteria will be failure to collect adequate samples, or a request by the subject to withdraw, or major interruptions to the collections due to illness, clinical procedures or discharge from hospital.

5.3 Compliance

This study complies, and at all times will comply, with the Declaration of Helsinki, as adopted at the 52nd WMA General Assembly, Edinburgh, October 2000, and with the Draft Additional Protocol to the Council of Europe Convention on Human Rights and Biomedicine on Biomedical Research (CGBI/INF (2001) 5 dated 18 July 2001).

5.4 Compensation

Not applicable.

5.5 Abnormal findings

If clearly abnormal results are found then the study participant, the supervising consultant and the independent medical officer will be informed in writing. Note that there is at present very limited information on what uranium levels constitute abnormality. No results will be passed to any other parties without specific written consent from study participants.

6. HAZARDS TO SUBJECT SAFETY

6.1 Electrical safety

No monitoring or other electrical equipment is connected to subjects.

6.2 Adverse effects

No adverse affects on health and safety risks are anticipated from this research project.

6.3 Risk assessment

Assessment of the risks for this study lead to the conclusion that the risks are minimal given the innocuous nature of the project and the fact that only anonymised or group results will be routinely released.

If it becomes apparent during the course of the study that this assessment of risk changes in any way, the clinicians responsible for the patients and those participating will be informed fully and without delay.

6.4 Pregnancy and lactation

Women are not included in the study.

7. SUBJECT PAYMENTS

In view of the innocuous nature of the trial, no subject payments are proposed.

About the IOM

The IOM employs over 120 staff, most of whom are employed in our Edinburgh office. IOM was founded as a charity in 1969 by the UK coal industry in conjunction with the University of Edinburgh and our first major research programme on coalminers' lung diseases soon became a benchmark for studies in occupational epidemiology and hygiene. Over the next 20 years the IOM's applied research work spanned other industries (asbestos, chemicals, steel, textiles, construction) and other conditions (back pain, ULD, hearing loss) as well as extending into many areas of basic research, and in 1990 the Institute became a fully independent charitable organisation with its own Board of Governors. We have continued to develop our activities, focusing always on the expanding UK and international needs for independent high quality research in occupational and environmental health, hygiene and safety. In addition to our links with Edinburgh University, we have thriving links with the Universities of Aberdeen, Heriot-Watt and Napier. Since independence we have developed a rapidly expanding consultancy business (IOM Consulting) that provides a wide range of services in occupational health and hygiene, environmental consultancy, ergonomics, occupational psychology, and chemical analysis.

The Health and Safety Executive is our biggest single client and we have also undertaken a number of projects for the Department of Health, the Scottish Executive and the Department for International Development and its predecessor, the Overseas Development Agency. IOM scientists are members of the EPAQS, the Department of Health's Committee on the Medical Effects of Air Pollutants (COMEAP), the HSE Advisory Committee on Toxic Substances (ACTS), the HSE Advisory Committee on Pesticides (ACP) and the HSE Pesticides Incidents Appraisal Panel. IOM staff are actively involved in the HSE's "securing health together" programme.

Harwell Scientifics: Analytical Procedure for the Determination of Uranium Concentrations and Isotope Ratios in Urine

The same procedure which has been in use for 7 years is used for both "spot samples" and for 24 hour collection samples. On receipt in the laboratory, samples are allocated a unique laboratory identification number and are stored in a cold room at nominally 4°C until analysed.

Aliquots of the samples are prepared for analysis by a simple procedure designed to lower the Total Dissolved Solids (TDS) of the solution to ca.100 ppm, whilst minimising contamination. The purpose of

this procedure is to optimise ICP-MS performance, rather than to pre-concentrate the samples. After addition of a ^{233}U spike, a phosphate precipitation step is carried out, followed by two washing steps. The phosphate precipitate is then dissolved in nitric acid and deionised water and diluted back to the original volume ready for analysis. High purity semiconductor grade reagents and deionised water are used throughout to minimise the risk of contamination, and all containers used are cleaned with dilute acid and then rinsed repeatedly with deionised water in a cleanroom to remove surface contamination.

Each sample is analysed using a SF-ICP-MS (Micromass, formerly VG) PlasmaTrace2 operated with a CETAC ultrasonic nebuliser. The instrument is a single-collector system, and uses measurement routines developed to optimise isotope ratio precision (generally counting statistics limited, giving about 1 % measured precision for a typical urine sample). For the purpose of this work the instrument is operated in low-resolution mode (approximately 300). Typical sensitivity in low-resolution mode is 10^{11} counts per ppm (mg/l) for ^{235}U. Typical limits of detection in urine "as analysed" for ^{235}U and ^{236}U are 0.1 pg/l. The limit of detection for ^{238}U is typically 5 pg/l.

The total reagent blank, estimated using a surrogate urine made from synthetic inorganic components, is 0.1 ng/l total uranium or less, equivalent to a total value of <= 5pg in the volume of urine actually used for analysis (50 ml).

All isotope ratio measurements are made relative to a natural uranium reference material (uranium metal EC101 from IRMM, Geel), with a slight (typically 1-2%) mass bias correction to achieve the natural isotope ratio for $^{238}U/^{235}U$ of 137.9. No mass bias correction is applied to $^{236}U/^{238}U$ ratios.

The method used to calculate the uranium concentration in the urine is based on the "isotope dilution principle", using the ^{233}U spike. A range of depleted uranium standards at different concentrations (Glen Spectra, traceable to NIST) has been spiked with ^{233}U and analysed. This has established the range of linearity of the method and has provided an initial estimate of the ^{233}U spike concentration. To measure the actual concentration more precisely, a spiked standard of natural uranium is included in each batch of urine samples and the uranium concentration in the urine established using simple ratio measurements. No depleted uranium standard is analysed at the same time as urine samples to avoid the risk of cross contamination.

The accuracy of the method for isotope ratio determinations has been assessed by the analysis of a range of isotopic reference materials for uranium (NBL). Two "in-house" urine materials have also been run at regular intervals to establish the analytical performance including uncertainty for both the measurement of total concentration of uranium and the $^{238}U/^{235}U$ isotope ratio. These urine samples contain natural, and slightly depleted uranium respectively, with concentrations of 1.5 and 2.5 ng/l of total uranium.

Normally, uncertainty is estimated from the actual analytical performance obtained for these "in-house" urine materials, based on the performance for different sub-samples, on different days, with different operators and this has been confirmed by analytical performance obtained during the analysis of more than 250 urine samples containing uranium with a natural isotope ratio.

The method used for determination of creatinine in urine is based on the Jaffe reaction, which is a kinetic colour test. Creatinine forms a yellow-orange colour with picric acid in an alkaline medium. The level of creatinine is a sample is proportional to the rate of change in absorbance at 520/800 nm, which is measured with an Olympus AU600 clinical analyser. The analysis is carried out at Harwell Scientific's Derby laboratory and is accredited to UKAS/ISO 17025.

References

Lothian Research Ethics Committee. General Guidance for researchers. (April 2001).

Ministry of Defence (Navy), Personnel Research Ethics Committee. Administrative Guidelines for Ethical Approval and Conduct of Non-Clinical Research Conducted on Human Volunteers (The Schedule of Approved Procedures). June 2002.

McDiarmid MA, Keogh JP, Hooper FJ, McPhaul K, Squibb K, Kane R, DiPino R, Kabat M, Kaup B, Anderson L, Hoover D, Brown L, Hamilton M, Jacobson-Kraum D, Burrows B, Walsh M (2000) Health Effects of Depleted Uranium on Exposed Gulf War Veterans. Environmental Research 82, 168-180.

National Health and Nutrition Examination Survey (1999) US National Centre for Environmental Health. www.cdc.gov/nceh/dls/report/results/uranium

The Royal Society (2001) The health effects of depleted uranium munitions, Parts I and II. The Royal Society London (available from their website)

World Health Organisation, Department of Protection of the Human Environment, "Depleted Uranium: Sources, Exposure and Health Effects," Geneva, Switzerland, April 2000

Colin Soutar
15 April 2004

ANNEX A:

Research Study Letter and Information Sheet

Methods of testing for uranium levels in the urine of male civilians

Dear ……..
I would be most grateful if you would agree to take part in a research study to investigate the amount of uranium in the urine of normal people.

The study
You have probably heard of concerns about the health of soldiers who have fought in the Middle East. One possibility is that uranium from shells might be affecting their health, so measurements of uranium in some soldiers are planned.

All of us eat or drink extremely small traces of uranium in our diet, and information is needed on the amounts and types of uranium in the urine of normal people. The present study is designed to find out whether it is better to make the measurements in small samples of urine or in a 24 hour combined sample.

I would be most grateful if you would agree to donate some urine samples while you are in hospital. You will be asked to collect all your urine in one container for 24 hours, and in a series of smaller containers on a second day. We will also ask about your age and address, the illness for which you were admitted to hospital, and ask for our nurse to look up the result, in your hospital notes, of a routine test of kidney function (serum creatinine).

We will send your urine samples, identified only by a number, not your name, to a research laboratory that will measure the uranium in your urine, and some other normal components of urine for comparison (urine creatinine). Your results will be treated in strict confidence, and will not be released without your permission. You can ask to view them if you wish. It is the intention that the results of the study be published, but the results will be anonymous, and no-one will be identifiable in the report.

Participation in the study is entirely voluntary. It will not interfere with your normal treatment or the length of your stay in hospital. You can withdraw at any time, and we will destroy your research records.

If you wish to ask for independent advice on whether to participate, you can ask the independent advisor, Dr A Flapan. The research nurse will explain how you can contact him.

The study has been commissioned by the Ministry of Defence, and is overseen by the Depleted Uranium Oversight Board, which includes Veterans' representatives as well as experts. The Institute of Occupational Medicine, an independent research charity, is conducting the study.

You will be given time to consider. If you agree to participate, you will be asked to sign the attached consent form, and will be instructed how to collect the urine samples. You will also be given a copy of this information sheet and your consent form. If you require further information please contact Alan Jones, telephone 0870 850 5131.

You are asked to read this form carefully. If you consent to take part in this study, you should sign the consent form. If you have any query, or are unsure about anything, you should not sign until your problem has been resolved and you are willing to volunteer. Please feel free to contact either Alan Jones, or myself or the Independent Medical Adviser, Dr A Flapan.

I do hope that you will agree to help with this simple but important study.

Yours faithfully

Dr Andrew Colvin, Director of Clinical Services, Institute of Occupational Medicine, Edinburgh.
Telephone 0870 850 5131.

Additional details

You have a right to obtain copies of all papers, reports, transcripts, summaries and other material so published or presented, on request to Dr Colvin. All information will be subject to the conditions of the Data Protection Act 1984 and subsequent statutory instruments.

Experimental records, including paper records and computer files, will be held for a minimum of 30 years, in conditions appropriate for the storage of personal information. You have right of access to your records at any time.

The study has been approved by the Lothian Research Ethics Committee. This protocol complies with all current legislation, including the Draft Additional Protocol to the Council of Europe Convention on Human Rights and Biomedicine Research (CDBI/INF (2001) 5 dated 18 July 2001). Further details of the approval will be provided if you wish, and you have a right to have a copy of the full protocol to retain, if you so request, of the IOM nurse or the IOM project leader.

Project Leader:	Dr Andrew P Colvin Institute of Occupational Medicine Research Park North Riccarton Edinburgh EH14 4AP Telephone 0870 850 5131 Mobile 07811 165513
Independent Medical Officer:	Dr A Flapan, Edinburgh Royal Infirmary, telephone 0131 536 1000

ANNEX B: IOM Subject Consent Form

Methods of testing for uranium levels in the urine of male civilians

1. I have read the information sheet, which provides an outline of this study, and have had the opportunity to raise and discuss any questions with the IOM nurse and the Independent Medical Advisor, with regard to the general nature, object, potential risks and duration of the study, and understand what is expected of me.
2. I understand that the aim of this study is to determine whether the measured uranium contents of spot urine samples represent total 24hr excretion of uranium.
3. I agree to volunteer as a subject for the study described in the information sheet. I give my full consent to my participation in this study. I understand that my urine sample will be tested for total uranium, isotopic ratio of uranium and creatinine.
4. I understand that if abnormal results are found, then I and my hospital doctor will be informed in writing. No results will be passed to any other parties without my specific written consent.
5. This consent is specific to the particular test described in the information sheet attached, and shall not be taken to imply my consent to participate in any subsequent experiment or deviation from that detailed here.
6. I agree that I may be contacted directly by a member of the IOM research team if any further information is required or to request further urine samples. This does not imply any agreement to any further study participation.
7. I reserve the right to withdraw from this study at any time, and to have my urine samples disposed of.

Signed _____

Name _____ Date _____

Witnessed _____

Name _____ Date _____

Project Leader: Dr Andrew P Colvin
Institute of Occupational Medicine
Research Park North
Riccarton
Edinburgh
EH14 4AP
Telephone 0870 850 5131
Mobile 07811 165513

Independent Medical Advisor: Dr Andrew Flapan

IOM Study ID number	

ANNEX C: SUBJECT QUESTIONNAIRE

Methods of testing for uranium levels in the urine of male civilians

INTRODUCTION

The IOM is an independent centre of research. We have been asked by the Ministry of Defence to look at Depleted Uranium (D.U.) levels in the general population for comparison with those in personnel in the military services in the UK.

Any information we collect will be strictly confidential and no names or other identifying information will be published or released to the M.O.D.

INSTRUCTIONS

This questionnaire will be administered by the IOM nurse or technician. Please discuss with them any queries you might have about the information you are being asked to supply.

1. Some of the questions have a list of possible answers with a box printed beside each one.

   Please choose your answer and put a tick in the box beside it, for example:

Male	✓
Female	

2. Some of the questions have boxes for you to write the response in.

Hospital: Royal Infirmary Edinburgh	**Ward:**
Today's Date: / / 2004	

Please complete the following information:

Q1. Surname

Q2. Forename *(include up to two forenames)*

Q3. Date of birth (DD/MM/YYYY) / /

Q4. Gender *(Please tick appropriate box)* Male ☐ Female ☐

Q5. Current home address

Home address:		
Postal Town:		
Region:		**Postcode:**
Telephone Number:		

Q6. Please tick the box corresponding to the condition that caused you to be in hospital

Orthopaedic ☐

Cardiac ☐

Accident /trauma ☐

Other (please specify)

Q7. Date of hospital admission (DD/MM/YYYY) / / 2004

Q8. Please give very brief details of any surgical procedures undergone since your hospital admission.

Date:	**Operation:**

ANNEX C2

Patient Name:
IOM Study ID number:

Day 1 Pooled Sample	**Start Date (dd/mm/yyyy)**	**Start Time (hh:mm)**	**End Date (dd/mm/yyyy)**	**End Time (hh:mm)**	**Notes**

Day 2 Spot Samples	**Sample Number**	**Date (dd/mm/yyyy)**	**Time (hh:mm)**	**Volume (mls)**	**Tick if missed (✓)**	**Tick if spilled (✓)**	**Additional Notes**
	01						
	02						
	03						
	04						
	05						
	06						
	07						
	08						
	09						
	10						
	11						
	12						
	13						
	14						
	15						

Checked by:
Date (dd/mm/yyyy) & Time (hh:mm):

Dietary History

Date (dd/mm/yyyy)	Time (hh:mm)	Meal	Description (list of dishes, snacks, beverages - for mineral water include brand information)

ANNEX D: Instructions for the Nurse, recruiting patients and collecting samples

INTRODUCTION

The study is described in the protocol (attached). These instructions include specific details.

BACKGROUND

The back ground to the study is explained in the protocol.

An additional summary of this project in lay language is given in the Subject Information Sheet at Annex A.

AIM

The pilot study seeks to determine the extent to which the uranium contents of spot urine samples represent 24hr excretion of uranium.

This will inform the design of an intended national study of urine uranium levels in the general population, for comparison with levels in military personnel who have been deployed in the Middle East.

METHODS

By the time recruitment starts, approvals will have been obtained from the ethics committee, hospital administration, and the consultants whose patients will be invited to participate. You will be informed of the patients you may approach, and will be introduced to the ward nursing and administrative staff.

Subjects
Potential subjects will be male, of the correct age, ambulant, and likely to be available to collect urine samples for the next two days (plus time for the patient to reflect on consent). By liaison with the ward staff you will identify possible subjects, and will approach them at a time convenient for ward procedures.

Study Subjects will be representative of ages ranging between 20 and 59. You will be given instructions for recruiting quotas of subjects in age groups. The aim is to achieve at least 25 subjects with complete sets of samples and questionnaires. The maximum length of the recruitment period is five calendar weeks.

Recruitment
Explain to each potential subject the nature and purpose of the study, and invite him to participate. Give him the information sheet and consent form, and allow a day before returning to ask for his decision. Written informed consent will be obtained from each subject. Pass these to Dr Jones or Peter Hutchison, IOM, who will arrange with you for a copy of the consent form to be returned to the patient. The Subject Information Sheet is attached at Annex A and the Consent Form at Annex B. Please keep a record of the number of refusals as well as successful recruitments. Also keep a record of subsequent dropouts, and the reasons.

The questionnaire and recording forms
Also attached is the Subject Questionnaire at Annex C, which you should complete (you may ask the subject to make a start on this, but check the completeness and accuracy yourself. The form also provides for you to record the urine samples collected, and dietary record. You can also record any difficulties with the study, or with the questionnaire. Completed questionnaires should be passed to Dr Jones, and IOM will examine them for any difficulties. Make a note of any difficulties with the questionnaire yourself.

Urine collection
Subjects will be asked to discharge the bladder immediately before 12 midday and then collect all further urine over the next 24 hours, including a final sample immediately before 12 midday on the following day. For the first 24 hours, urine should be collected as a pooled sample in the large container (a second large container is available if needed). Collection for the second 24 hours can start immediately after completion of the first, though it can start a day or two later if necessary. In this 24 hour period samples should be collected as individual samples, each one in a separate 200 ml container. These should be numbered sequentially. Patients should be asked to note if any loss occurs through accident or omission. Record this on the questionnaire. The labels are pre-printed with the same study number as on the questionnaire. Make sure the containers are numbered according to their natural sequence. Seal them with the plastic tamper-evident seal provided, and place each one in its own sealable plastic bag (provided). Each day store the completed samples in the agreed refrigerator, and arrange for them to be transported to the IOM. At least once a week place the samples in a sealed cool box (supplied), label, and call the carrier to transport them to Harwell Scientific Laboratory (address provided). Do not send on a Friday or over the weekend.

Other records
Each day of the collection make a simple written record of food and drink consumed (not amount) during the period of collection, starting from 4pm on the day the first collection starts. Information on any consumption of mineral water should be recorded (and the make). Use the hospital daily menus as a prompter.

Please obtain from the clinical notes the most recent measurement of serum creatinine (a measure of renal function), and enter into the subject questionnaire (if none available, write “none”).

EXCLUSION CRITERIA, AND WITHDRAWALS

The patient should be ambulant. Any patient with an obvious renal abnormality should be excluded, or any one already with a catheter or already collecting urine for clinical purposes.

Patients can withdraw from the study at any time, or can be withdrawn by the supervising doctor. Some subjects may be discharged before the collection is complete, or collection may be interrupted by a clinical procedure or illness.

STAFF

Project organisation will be run from IOM by Dr Alan Jones, and is your main contact. The Project leader is the IOM’s Director of Medical Services, Dr Andrew Colvin. Data handling will be supervised by Mr Peter Hutchison. Statistical analysis will be provided by Dr Brian Miller (Head of Research Operations at IOM, and Senior statistician).

Institute of Occupational Medicine

WORLD HEALTH ORGANISATION
COLLABORATING CENTRE
FOR OCCUPATIONAL HEALTH

Applying science for a better working environment

The Institute of Occupational Medicine

The IOM is a major independent centre of scientific excellence in the fields of occupational and environmental health, hygiene and safety. We aim to provide quality research, consultancy and training to help to ensure that people's health is not damaged by conditions at work or in the environment. Our principal research disciplines are exposure assessment, epidemiology, toxicology, ergonomics and behavioural and social sciences, with a strong focus on multi-disciplinary approaches to problem solving.

Our beginnings

Our first major research programme began in the 1950s, on respiratory health problems in the coal mining industry. Major themes were quantification of airborne dust concentrations in different jobs, characterisation of types and constituents of the dusts, measurement of health effects, relationships between exposure and disease, and proposals for prevention. This research became an international benchmark for epidemiological studies of occupational health, and was the primary influence on dust standards in mines in the UK, US and other countries.

Current themes

Our current work spans many other industries including asbestos, MMMF, pesticides, chemicals, energy, telecoms, metals, textiles, construction, agriculture as well as the environment. While diseases of the respiratory tract remain a major interest, our scope now extends to many other health outcomes such as mortality, cardiovascular effects, cancer, back pain, upper-limb disorders, hearing loss, skin diseases, thermal stress and psychological stress. Related work includes the development and application of measurement and control systems, mathematical models and survey methods.

Who we work for

Our work in these areas is conducted for a wide range of organisations in the UK, the EU, and the US, including Government departments, international agencies, industry associations, local authorities, charitable organisations, and industrial and commercial companies. The IOM is a World Heath Organisation (WHO) collaborating centre and is an approved institute of the Universities of Edinburgh and Aberdeen, enjoying collaborative research links with NIOSH, IARC, and many other institutes throughout the world.

Publication

We believe that our research findings should be publicly available and subject to the scrutiny of the international scientific community. We publish our findings in the peer reviewed scientific literature and through our own series of Research Reports.

Contact

For further information about the IOM's research capabilities:

Dr Robert Aitken
Director of Research Development
Rob.aitken@iomhq.org.uk