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ANALYTICAL METHODOLOGY

Uranium quantification in semen by inductively coupled plasma mass spectrometry☆

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Dedicated to the memory of Dr. Larry Anderson.

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ABSTRACT

In this study we report uranium analysis for human semen samples. Uranium quantification was performed by inductively coupled plasma mass spectrometry. No additives, such as chymotrypsin or bovine serum albumin, were used for semen liquefaction, as they showed significant uranium content. For method validation we spiked 2 g aliquots of pooled control semen at three different levels of uranium: low at 5 pg/g, medium at 50 pg/g, and high at 1000 pg/g. The detection limit was determined to be 0.8 pg/g uranium in human semen. The data reproduced within 1.4–7% RSD and spike recoveries were 97–100%. The uranium level of the unspiked, pooled control semen was 2.9 pg/g of semen ($n = 10$). In addition six semen samples from a cohort of Veterans exposed to depleted uranium (DU) in the 1991 Gulf War were analyzed with no knowledge of their exposure history. Uranium levels in the Veterans' semen samples ranged from undetectable (<0.8 pg/g) to 3350 pg/g. This wide concentration range for uranium in semen is consistent with known differences in current DU body burdens in these individuals, some of whom have retained embedded DU fragments.

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Introduction

Uranium is a naturally occurring element with an average abundance in the earth's crust of approximately 2.7 μg/g. Its isotopic composition consists of three naturally occurring radioactive isotopes: ^{234}U (0.0055%), ^{235}U (0.72%), and ^{238}U (99.27%), decaying to ^{208}Pb and ^{206}Pb as final non-radioactive products. The main use of uranium is for nuclear fuel, but because the principal isotope in natural uranium is the isotope with the lowest radiologic activity, ^{238}U, an enrichment of ^{235}U is required to produce a nuclear, fuel-grade product. A by-product of the enrichment process is depleted uranium (DU) which has an approximate isotopic composition of: ^{234}U (0.00061%), ^{235}U (0.2%), and ^{238}U (99.79%) [1]. Additionally, some studies have shown that DU contains small quantities of ^{236}U coming from reprocessed uranium fuel [2]. DU is used commercially in a variety of applications, such as counterweights in aircraft, shielding for radiography cameras and manufactured chemicals containing uranium. DU exposure in civilian populations could occur in occupational settings or in unusual circumstances such as inhalation of burning aircraft materials following a plane crash [3]. DU has also been used by the military. Because of its high density, availability, and low relative cost, it has been incorporated into munitions as high energy kinetic penetrators and into armor plates for military vehicles. Soldiers in battle are therefore at risk of inhaling DU aerosols, ingesting DU particles, and/or experiencing wound contamination by DU particles and/or embedded fragments [4–6].

Because of the toxicological properties of uranium [7–9], the health effects of natural and depleted uranium exposure have long been of concern. Recent exposures to DU in military personnel have led to renewed studies of uranium's chemical and radiologic health effects as an internal toxicant [6,10,11]. While uranium may be stored in the bone, it also accumulates in the kidney which is considered the "critical" organ for uranium toxicity [9,11]. Additionally, as with other metals, there are concerns about potential central nervous system and reproductive health effects [6].

Numerous reports have attempted to evaluate the relationship between essential and non-essential trace element composition of semen and fertility, semen quality, and/or sperm characteristics.

☆ The opinions and assertions expressed herein are those of the authors and are not to be construed as official or as representing the views of the United States Geological Survey, The Joint Pathology Center, the Department of the Army, or the Department of Defense.

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Among the essential elements that are usually and naturally elevated in semen are calcium (Ca) and zinc (Zn), for which concentrations in semen are higher than those in blood [12]. These two metals have important associations with male reproductive functions. Calcium in the semen is essential for sperm motility [13,14]. Zinc in semen may be part of the bacteriostatic action of semen that protects the male reproductive tract [15,16]. Zn may also be one component of the mechanism which maintains the proper balance of oxidants and antioxidants in semen [17,18]. It also has an influence on motility of ejaculated sperm [19].

In addition to the "intrinsic" trace elements in semen discussed above, humans with occupational, as well as certain nonoccupational, exposures have been shown to exhibit elevated semen levels of toxic trace elements. Studies in animals and humans have shown that male reproductive system and semen quality effects are associated with elevated body burdens of such toxic elements as lead (Pb) and cadmium (Cd). Higher semen concentrations of these metals have been reported for certain exposures [20] and levels in semen have been shown to be negatively correlated with sperm concentrations and motility in humans [21].

The effects of uranium at different concentrations in semen have not been reported, but there are recent reports investigating the reproductive effects of depleted uranium using laboratory animals. Arfsten et al. reported no significant changes in sperm motility or sperm count in rats with implanted DU pellets [22,23]. In a follow-up two-generation, reproductive toxicity study in rats, the same authors concluded that DU exposure is not a significant reproductive hazard, with a caution of possible developmental effects (increased mean relative heart weights in rat pups from adult males exposed to DU) [24].

Several analytical methods have been described for measuring uranium in environmental, geological, and biological samples, including thermal ionization mass spectrometry (TIMS), instrumental neutron activation analysis (INAA), delayed neutron counting (DNC), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES), α-spectroscopy, spectrophotometry, fluorometry, and kinetic phosphorescence analysis (KPA) [25]. However, this metal has not been measured in semen to our knowledge. In this study we focused on ICP-MS as a technique of choice, as it provides low detection limits suitable for situations with low metal concentration and where limited sample is available, short sample preparation and instrument analysis time, and high sample throughput. Although uranium levels have been quantified in various types of biological samples including tissues and biological fluids, a number of investigations have focused mainly on urine U and to a smaller extent on blood U quantification [25–28]. The sample preparation for urine uranium analysis (ranging from digestion and wet or dry ashing to no pretreatment and simple dilution) for ICP-MS varies greatly between the different laboratories, based on the detection limits desired and the sample introduction system used for the analysis [29–34].

The primary objective of this study was to develop an assay that accurately measures the quantity of U in semen specimens. A simple robust method is presented herein using acid digestion followed by ICP-MS analysis. This method provides low detection limits and high accuracy and precision for determination of uranium in human semen at low levels.

Materials and methods

Semen sample collection and processing

Collection of samples for a pool of semen used in the development of this assay was performed at the University of Maryland Baltimore (UMB) School of Medicine. Collection of samples from Veterans of Gulf War I was performed at the University of Maryland Medical Center under the auspices of the Depleted Uranium Surveillance Program of the Baltimore Veterans Affairs Medical Center. All study participants gave informed consent and the protocol was approved by both the VA and University Institutional Review Board (IRB). All semen processing occurred at the UMB School of Medicine.

All men participating in the collection of semen were requested to abstain from ejaculation for two days prior to sample production. Semen was produced by masturbation and collected in a sterile 118 mL polystyrene specimen container. Samples for pooled control semen were collected from two non-Veterans over a period of 7 months with reported abstinence of 1–5 days. Volumes ranged from 0.5 to 3.5 mL for each sample. Samples were obtained from Gulf War I Veterans during their 2009 biennial surveillance visit [10]. For these samples, abstinence ranged from 2 to 30 days (6.3 ± 7.7 days, mean ± s.d.) and semen mass ranged from 0.46 to 7.998 g (approximately 0.45–7.95 mL). After transport to the laboratory, all samples were incubated for 3 h at 37 °C to encourage liquefaction before subsequent processing. Additives which can be employed to increase liquefaction of the samples were not added to the semen during sample processing.

After incubation at 37 °C, samples for pooled semen were transferred to 50 mL polystyrene centrifuge tubes to a maximum of approximately 40 mL (semen from the two donors was kept separate) which were stored at −20 °C. To produce the semen pool, all aliquots were thawed at 4 °C for 5 h. Aliquots were vortexed and poured into acid-washed teflon beakers, first combined by donor and then combined into a common pool. For sampling, the pooled semen was initially mixed in the covered teflon beakers with an acid-washed teflon-coated stir bar, and stirring continued during sampling (speed decreased as volume remaining decreased). Aliquots of 2 mL from the final pool were prepared in the vials used for uranium determination (screw-capped, teflon, 15 mL, numbered precleaned vials; Savillex, Eden Prairie, MN) with an air displacement, polystyrene-tipped pipettor. Aliquots were stored at −20 °C.

For Veterans, each ejaculate was transferred in its entirety after incubation at 37 °C with a graduated glass pipet directly into a teflon vial for uranium determination. These samples were stored at −20 °C and all Veteran and pooled semen samples were transferred frozen (on ice) to the Division of Biophysical Toxicology – Depleted Uranium and Embedded Fragment Laboratory (DBT-DU/EMF) for uranium analysis. All specimens, including pooled semen at the time of aliquotting and Veteran samples processed immediately after collection, were transferred into teflon vials of known individual weights and the weight of vial plus semen was recorded. Semen weight was determined as the difference between weight of vial plus semen and the weight of the empty vial. All subsequent quantification calculations for uranium in semen were based on semen weight rather than volume as the volume measured was approximate (particularly in samples with incomplete liquefaction or high viscosity). At DBT-DU/EMF laboratory, the samples were stored at −70 °C until analysis.

Preparation of spiked samples

Two milliliter (2 mL) aliquots (weight 2.081 ± 0.252 g mean ± s.d., $n = 97$) from the pooled control semen were spiked at the following three levels for method validation and generation of quality control samples: (1) low level spike at 5 pg/g (added as 0.02 mL at 500 pg/mL to give a nominal concentration of 5.0 pg/g), (2) medium level spike 50 pg/g U (added as 0.02 mL at 5 ng/mL to achieve a concentration of 50 pg/g) and (3) high level spike at 1000 pg/g U (added as 0.02 mL at 100 ng/mL for a nominal

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