DEPARTMENT OF THE AIR FORCE
HEADQUARTERS UNITED STATES AIR FORCE
WASHINGTON DC

5 July 2005

MEMORANDUM FOR NRC HEADQUARTERS
ATTN: PATRICIA HOLAHAN

FROM: AFMSA/SGPR
110 Luke Avenue, Room 405
Bolling AFB, DC 20032-7050

SUBJECT: Receipt of 10 CFR 2.206 Petition (Your Memo, 10 Jun 2005)

The US Air Force (USAF) manages Atomic Energy Act-regulated radioactive material in accordance with its NRC issued Master Material License (MML) # 42-23539-01 AF, docket # 030-28641. Pursuant to that license, the USAF has issued six permits to receive, store and distribute Depleted Uranium (DU) munitions. These permits allow only the handling of intact DU rounds. Most DU round handling is conducted through forklift or Automatic Loading and Unloading Systems, and workers never come into direct contact with DU due to munition cladding and casing (0.8 mm aluminum as a minimum).

In addition to the six permits mentioned above, the USAF has issued two permits under its MML to receive, store, distribute, and test DU munitions. Both permits impose strict operating procedures and radiation safety requirements, including environmental surveillance, intended to prevent human exposure to DU munition residue. Range testing is performed in either geographical remote area or enclosed ranges where no personnel are permitted during testing.

The petitioner expressed specific concerns about the alleged release of uranyl nitrate or UO_3 resulting from the combustion of DU during target impact. The USAF, however, has no data to support the allegation that uranyl nitrate or UO_3 is produced during target interactions. In addition, the U.S. Army has performed extensive research on DU munitions testing and this research does not indicate uranyl nitrate or UO_3 to be a significant combustion product. Environmental sampling generally indicates production of either metallic uranium or oxidized uranium particulates. The attached paper "DU Dust from Fired Munitions" (Health Physics, 87(1): 57-67; 2004) states that the combustion products of DU are U_3O_7 (47%), U_3O_8 (44%) and UO_2 (9%) and makes no mention of uranyl nitrate or UO_3, as claimed by the petitioner.

In summary, management of DU by the Air Force is fully compliant with the Air Force's MML and with all applicable NRC regulations. Air Force use of DU munitions does not pose any significant exposure hazard to either its members or to members of the general public. We strongly disagree with the petitioner's claims that uranyl nitrate is a significant product of munition use or that it poses a serious health risk.

If you have any questions or need further input, please contact Dr. Ram Bhat at 202-767-4306 or e-mail at ramachandra.bhat@pentagon.af.mil. Our telefax is 202-404-8089.

MARK WROBEL Lt Col. USAF, Ph. D., CHP
Chief, Radiation Protection Division
Secretariat, Air Force Radioisotope Committee
Air Force Medical Support Agency
Office of the Surgeon General

Attachment: Health Phys 87(1): 57-67; 2004

CC: NRC Region IV (Mr. Gaines)
NRC Region IV (Ms. Browder)

Paper

DEPLETED URANIUM DUST FROM FIRED MUNITIONS: PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES

R. E. J. Mitchel and S. Sunder*

Abstract—This paper reports physical, chemical and biological analyses of samples of dust resulting from munitions containing depleted uranium (DU) that had been live-fired and had impacted an armored target. Mass spectroscopic analysis indicated that the average atom% of ^{235}U was 0.198 ± 0.10, consistent with depleted uranium. Other major elements present were iron, aluminum, and silicon. About 47% of the total mass was particles with diameters <300 μm, of which about 14% was <10 μm. X-ray diffraction analysis indicated that the uranium was present in the sample as uranium oxides—mainly U_3O_7 (47%), U_3O_8 (44%) and UO_2 (9%). Depleted uranium dust, instilled into the lungs or implanted into the muscle of rats, contained a rapidly soluble uranium component and a more slowly soluble uranium component. The fraction that underwent dissolution in 7 d declined exponentially with increasing initial burden. At the lower lung burdens tested (<15 μg DU dust/lung) about 14% of the uranium appeared in urine within 7 d. At the higher lung burdens tested (~80–200 μg DU dust/lung) about 5% of the DU appeared in urine within 7 d. In both cases about 50% of that total appeared in urine within the first day. DU implanted in muscle similarly showed that about half of the total excreted within 7 d appeared in the first day. At the lower muscle burdens tested (<15 μg DU dust/injection site) about 9% was solubilized within 7 d. At muscle burdens >35 μg DU dust/injection site about 2% appeared in urine within 7 d. Natural uranium (NU) ore dust was instilled into rat lungs for comparison. The fraction dissolving in lung showed a pattern of exponential decline with increasing initial burden similar to DU. However, the decline was less steep, with about 14% appearing in urine for lung burdens up to about 200 μg NU dust/lung and 5% at lung burdens >1,100 μg NU dust/lung. NU also showed both a fast and a more slowly dissolving component. At the higher lung burdens of both DU and NU that showed lowered urine excretion rates, histological evidence of kidney damage was seen. Kidney damage was not seen with the muscle burdens tested. DU dust produced kidney damage at lower lung burdens and lower urine uranium levels than NU dust, suggesting that other toxic metals in DU dust may contribute to the damage.
Health Phys. 87(1):57–67; 2004

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0017-9078/04/0

Key words: uranium, depleted; weapons; health effects; inhalation

INTRODUCTION

Depleted uranium (DU) contains reduced amounts of the isotope ^{235}U and is about 60% less radioactive than natural uranium. While the health effects of inhaled DU are controversial (Abu-Qare and Abou-Donia 2002), many reports (e.g., Bolton and Foster 2002) suggest that the risk to military and civilian personnel is very low. One report (Bleise et al. 2003) suggests that except for crews of military vehicles hit by DU penetrators, observable health effects are not expected and residual cancer risk estimates appear to be a fraction of those expected from natural radiation.

Any health effects will depend critically on the particle size and chemical nature of the inhaled aerosol. During military operations, both military and civilian populations may be exposed to DU dust resulting from exploded munitions, and the concentration, chemical composition, and particle sizes of DU particles in soil from these areas have been investigated (Uyttenhove et al. 2002; Desideri et al. 2002; Salbu et al. 2003; Danesi et al. 2003). As pointed out by Salbu et al. (2003), samples from soil are subject to weathering effects on both particle size and chemical composition. Some analyses of human urine from potentially exposed military personnel have been reported. One study reported that analyses of samples from about half of the people tested indicated a uranium isotopic ratio consistent with DU (Horan et al. 2002), while another report found that the uranium isotopic ratios in all samples were consistent with natural uranium (Ough et al. 2002).

In the current study, we have investigated the physical properties, the chemical composition, and the biological effects of "fired" DU dust, i.e., dust from DU containing munitions that have been fired and have impacted an armored target. Such material may be a more accurate representation of potential military and civilian exposure at times shortly after the firing of such weapons and before any weathering effects can take

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place. The purpose of this study was twofold: to chemically and physically characterize the fired DU and to compare the DU to natural uranium (NU) for toxic properties at similar body levels of dissolved uranium. The physical and chemical analyses describe particle size distribution, elemental composition (by mass spectroscopy), and oxidation state. The chemical state of the uranium in the sample was investigated using x-ray diffraction (XRD). The use of XRD to determine the nature of different uranium oxides has been discussed by several authors (e.g., Choi et al. 1996; Sunder and Miller 1995, 1996; Taylor et al. 1980; and references therein). To develop an in vivo understanding of the properties of the live-fired DU dust in lung, the dust was instilled into the lungs of rats (Henderson et al. 1995; Houpert et al. 1999) whose respiratory system closely mimics humans'. However, since the DU was instilled, not inhaled by the rats, the results are not meant to predict the lung distribution and dissolution behavior of inhaled DU. The behavior of DU in subcutaneous or intramuscular locations was tested by injection into leg muscle to mimic dust impact or contaminated wounds. Such wound contamination may contribute to uranium body burdens particularly where the wound is not severe, does not bleed copiously and may not receive prompt or any medical attention. The effects of wound contamination with dust have not previously been described, unlike the effects of DU metal in shrapnel wounds (McClain et al. 2001, 2002; McDiarmid 2002). The rate of DU dust dissolution was assessed by measuring uranium in urine, and toxicology was assessed by histological examination of various tissues. The rate of dissolution of NU ore dust in lung was also examined for comparison to the previous work on the carcinogenic potential of inhaled natural uranium ore dust (Mitchel et al. 1999) and the transfer of uranium and its decay products from lung to bone (Dewit et al. 2001).

MATERIALS AND METHODS

Fired DU sample

Approximately 100 g of the sample was received as a dry powder from Defence Research and Development Canada. The sample originated from live-fired DU-containing munitions that had impacted an armored target. The dust was therefore expected to contain a mixture of other materials in addition to uranium. Some of the original sample was passed through a 300-μm sieve to divide it into samples >300 μm and <300 μm (Hanson et al. 1974). The <300 μm sample was used for particle size analysis or was further sieved into samples that were particles of <50 μm diameter and 50–300 μm. The samples with diameters <50 μm were used for instillation into the rat lungs and the 50–300 μm fraction for injection into rat muscle.

Natural uranium (NU)

The NU ore sample has been previously described (Mitchel et al. 1999). Briefly, the sample was obtained from the Cluff Lake SK mine. It contained 44% uranium and was milled to a mean mass physical diameter of 1.85 μm (mean mass aerodynamic diameter of 3.15 μm, maximum diameter <15 μm) with about 75% of the mass <5 μm. The carcinogenic properties of this material inhaled in rats (Mitchel et al. 1999), its retention and dissolution in rat lung, and the transfer of uranium and its decay products to bone in rats have been described (Dewit et al. 2001).

Elemental composition analysis of DU

Two sub-samples of ~1 g each were taken from the total sample. A two-step dissolution was performed on each sample: the first step was to heat in concentrated nitric acid and the second step was to fuse the residue followed by more vigorous acid attack. These fractions were analyzed separately by inductively coupled plasma-mass spectroscopy (ICP-MS). Sodium peroxide was used for the fusion. Nitric acid was added, along with a small amount of hydrofluoric acid. The acids were evaporated to near dryness, followed by two more additions of nitric acid with evaporation. A small amount of gel remained undissolved and was not part of the analysis results reported. This residual material is hypothesized to be silica. The addition of HF followed by heating would have caused some loss of silicon and boron as volatile fluorides. In Table 1, this is indicated by reporting the results for these elements as greater than or equal to (≥). A semi-quantitative full-mass scan analysis by ICP-MS was performed, in duplicate, in addition to the quantitative analysis of selected elements. Analytical uncertainties are given.

Particle size analysis of DU

The original sample contained a considerable fraction of material that was too large to measure using a particle size analyzer. The <300 μm material was subjected to particle size analysis using a Horiba LA-900 particle size analyzer (Horiba Ltd., Kyoto, Japan). The sample was dispersed in water with the aid of an ultrasonic bath to break up soft agglomerates in the sample. The analysis was performed while the ultrasonic bath was operating.

X-ray diffraction analysis of DU

The sieved sample with particles of diameter <300 μm was used for XRD analysis. The XRD pattern was

Table 1. Elemental composition of the fired DU dust.

Element[a]	Mean ($\mu g\ g^{-1}$)	Uncertainty ($\mu g\ g^{-1}$) 95% confidence	Wt%[b]
Ag	105	66	0.02
Al	32,550	1,500	5.06
B	≥135	—	≥0.02
Ba	221	112	0.03
Cd	252	171	0.04
Co	159	87	0.03
Cr	2,415	137	0.38
Cu	1,030	559	0.16
Fe	320,000	37,583	49.79
Mg	9,850	458	1.53
Mn	4,700	482	0.73
Mo	980	498	0.15
Ni	1,435	124	0.22
Pb	188	13	0.03
Si	≥31,285	4,243	≥4.87
Sr	264	151	0.04
Ti	5,900	3,387	0.92
U	227,390	15,887	35.38
W	1,215	70	0.19
Zn	2,125	1,087	0.33
Zr	100	61	0.02

[a] Only elements with Wt% of 0.02% or greater are listed in the Table.
[b] Only elements identified using ICP-MS (see text) are included in the Wt% calculation, e.g., oxygen and hydrogen are not included in this calculation.

recorded using a diffractometer consisting of a Rigaku RU-200BH generator, Rigaku CN2155D5 goniometer with specimen rotation, and a copper anode (Rigaku International Corp., Tokyo, Japan). The powder sample used for XRD analysis was suspended in white petroleum jelly, and the suspension was smeared on a glass slide for recording the XRD. The x-ray pattern was excited using copper Kα1 x rays with $\lambda = 0.154056$ nm. The pattern was recorded for 2-theta values between 10° and 120° using a step scan with a step size of 0.02°.

Crystalline phases present in the samples were identified by comparing the observed pattern with the literature XRD patterns of the crystalline phases using a search and match program (Sunder and Miller 2000). To use the program, information about the elements present in the sample was required and the results given in Table 1 were used. The literature XRD patterns used in the search and match program are from the Powder Diffraction Files (1995). The patterns of the phases selected by the computer were visually compared with the observed pattern to determine the phases present in the sample.

Biological studies

Animals. Pathogen-free male Sprague Dawley rats, obtained from Charles River Canada (St. Constant QC, Canada), were treated at 14–17 weeks of age, with body weights of approximately 361 to 558 g. The rats were divided into 5 treatment groups with 3 or 6 treated rats plus 2 untreated control rats per group. These rats were individually caged and acclimatized over a period of 5 to 7 d to their new surroundings and housing (Thoren ventilated rat shoeboxes). Prior to treatment, each rat was acclimatized in the post treatment metabolic cage, which was fitted with a black "hood" to provide a less open environment. Acclimatization was for 5 to 7 d, or until the original body weight was maintained or increased over three consecutive days. The rats were fed ad libitum. After acclimatization, the rats were treated with either saline solution or a suspension of either depleted uranium or natural uranium in saline solution. All housing and experimental manipulations were approved by the CRL Animal Care Committee prior to beginning the experiment.

Instillation/injection. Different amounts of fired depleted uranium powder with a particulate diameter less than 50 μm or natural uranium ore dust with a particle size less than 15 μm (Mitchel et al. 1999) were suspended in saline solution (100 μL) and instilled into the lungs of male Sprague Dawley rats. Different amounts of depleted uranium with a particulate diameter range from 50 to 300 μm were also suspended in saline solution (100 μL) and injected intramuscularly. The samples of DU or NU were prepared and sonicated the day of the treatment (instillation or injection) as a suspension in sterile isotonic saline solution (100 mg mL^{-1}, pH 6–7). Control rats were instilled or injected intramuscularly with the saline solution. The rats were anaesthetized with isoflurane, injected with butorphanol (2 mg kg^{-1}, approximately 100 μL) in the lumbar muscles and then reanaesthetized with isoflurane. An otoscope and a Tom Cat catheter (Kendall Sovereign Tyco International Co, Mansfield, MA) on a 1 mL syringe were used to deposit the uranium suspension into the lower one third of the trachea. The animal was held in an upright position for 1 min while recovering from the anaesthetic to aid in the progression of the solution further down the airways. The injected rats did not receive an anaesthetic or butorphanol injection but instead were restrained in a rat restrainer for 30–60 s during injection of the 100 μL suspension into the muscle of the hind leg using a 23-gauge needle.

Preliminary experiments were conducted to determine the upper limit of lung DU instillation. Severe toxicity from uranium occurred at a dose double the highest level used here, with the rat being euthanized 6 d post treatment due to loss of 24% body weight.

The animals were not externally contaminated during the instillation or injection procedures. After treatment, the rats were placed in metabolic cages for a period of 7 d prior to euthanization. Lung, kidney, and liver were examined at necropsy and taken for histological

analysis. Urine was collected daily. Lung, muscle, and urine were collected for uranium analysis.

During the preliminary experiments, it was found that maintaining a uniform suspension of either the DU or NU during the time needed for lung instillation or muscle injection was not possible. Because of this difficulty in injecting or instilling reproducible amounts of sample, each rat lung or muscle was analyzed for total uranium after the end of the 7-d experimental period. For the analysis of the rates of dissolution data shown in Figs. 5–7, the amount described as the total amount of uranium instilled or injected was calculated to be the sum of the total amount remaining in the lung or muscle after 7 d plus the total amount excreted in urine over that time. The assumption that this was the total burden was likely to be true for the uranium injected into muscle, but not for the uranium instilled into lungs. Because the lung material was not instilled directly into the deep lung lobes, a substantial but unknown amount would likely be cleared from the upper airways into the gastrointestinal tract where it either would dissolve or be excreted via the feces. The amount in feces was not measured. Solubilization and absorption of this material from the gastrointestinal tract would have contributed to the overall uranium excreted in urine. This situation is likely to mimic a human exposure resulting from inhalation of particulate DU with a wide size range.

Urine. Urine samples were collected daily from each rat housed separately in a metabolic cage. Each fresh sample was tested using Chemstrips (Roche Diagnostics, Laval QC, Canada) for specific gravity, pH, and protein. To assess kidney damage, microscopic examination of fresh urine for presence of blood and casts was carried out. The average volume of all the daily urine volumes collected was 14.25 mL, with a range of 5–27 mL. Non-uranium analysis required a maximum 0.5 mL sample, which represents 3.5% of the total average urine. The analyses of uranium appearing in urine may therefore underestimate the true average value by about 3.5% (range 1.9–10%).

Biological samples for uranium analysis

The daily urine samples were measured and evaporated to dryness under infrared heat lamps on preweighed Norton Bytac plastic (Labcon, Concord ON, Canada). The plastic and dried samples were placed in 7-mL vials (Becquerel Laboratories Inc., Mississauga ON, Canada) for uranium analysis.

The lung and muscle tissue were also placed in preweighed Becquerel 7-mL vials for analysis of uranium. Lungs from two control rats in each treatment group were subjected to both uranium and histological analysis (approximately 0.2 g, 7% of the total, was removed for histology). The vials containing the wet tissue were prefrozen by flash freezing in liquid nitrogen. The tissues were lyophilized using a Freeze Dry-5 freeze drier (Labconco, Kansas City MO) for at least 24 h or until the vials were at room temperature.

Uranium levels in tissue and urine samples (as ^{238}U) were analyzed by Becquerel Laboratories Inc. (Mississauga ON, Canada) using neutron activation followed by Delayed Neutron Counting (DNC). The detection limit was 0.09 μg. The uncertainties in the measurements are estimated at 5% for uranium content >100 μg, 10% at 10 μg, 20% at 1 μg, and 100% at the detection limit.

Histology. Each rat was necropsied after euthanization using standard gross pathology methods. Gross pathology for each rat was cross-referenced with histopathology to identify any differences in the findings. Histological samples of lung, kidney, and liver were fixed in 10% buffered neutral formalin. Five sections of lung (one from each lobe), one section of liver, and two sections of kidney (one from each kidney) were processed for histopathology interpretation and grading by Dean Percy (pathologist, Ontario Veterinarian College, Guelph ON). Samples of tissues in paraffin-embedded blocks were sectioned and stained by haematoxylin and eosin. Since the whole lung was required for uranium analysis from each test animal that was followed for uranium excretion over 7 d, histological samples of lung from these animals were not available. To estimate the potential for the DU or NU to cause lung lesions, three additional animals were instilled with high but unmeasured amounts in each case. These amounts were chosen to be similar to the three highest measured amounts that were instilled in each case.

Statistics

Differences in histological observations were compared for significance using the non-parametric Mann-Whitney test. Slopes of lines were tested for differences from zero using the *t* test.

RESULTS

Particle size analysis

The original sample was fractionated by passing through a 300-μm sieve. The material <300 μm represented 46.7% of the total mass. Particle size analysis was conducted on this <300 μm fraction. The results, shown in Fig. 1, give the individual particle mass frequency and the cumulative mass frequency. The data indicate that about 14% of the <300 μm fraction was <10 μm.

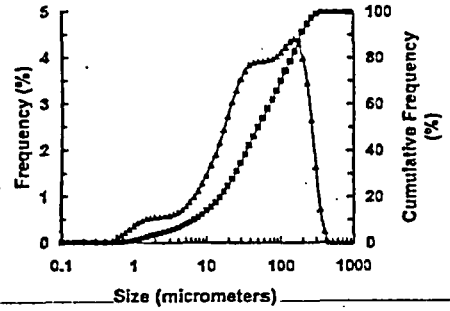

Fig. 1. DU particle size distribution for <300-μm fraction. Mass frequency, ▲; cumulative mass frequency, ■.

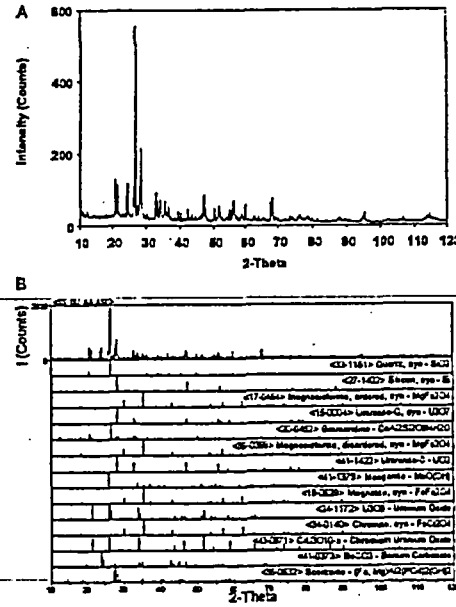

Fig. 2. XRD pattern of a powder sample from the debris created following impact of the DU munitions on an armored target. Panel A. Observed pattern. Panel B. Comparison of the observed pattern (top) with the literature patterns of the possible phases in the sample. The numbers with the literature patterns refer to the file numbers in Powder Diffraction Files (1995).

Elemental analysis

Table 1 gives the elemental composition of the fired DU sample used in the present study. The ICP-MS method was used for all elements except silicon, iron, and manganese, obtained using ICP-AES. The isotopic composition of the uranium in the duplicate samples was closely reproducible, measured as 0.196 ± 0.010 atom% and 0.200 ± 0.010 atom% ^{235}U.

X-ray diffraction analysis of DU

Fig. 2A shows the XRD pattern of the powder sample for 2-theta values between 10° and 120°. Fig. 2B compares the observed XRD pattern with the patterns of the phases selected, using the "search-match" program (Sunder and Miller 2000). This comparison suggests that the sample may contain four uranium compounds (U_3O_7, UO_2, U_3O_8, and CrU_3O_{10-z}) and 10 non-uranium phases. The results suggest that in addition to the uranium phases, quartz (SiO_2), magnetite ($FeFe_2O_4$), and scorzalite [$(Fe, Mg)Al_2(PO_4)_2(OH)_2$] phases make significant contributions to the observed pattern.

Fig. 3 compares, on an expanded scale, the selected sections of the observed XRD pattern with the literature patterns of the uranium-containing phases. The XRD results indicated that the uranium phases present in the sample were U_3O_8, U_3O_7, and UO_2.

The relative fractions of U_3O_8 and UO_2/U_3O_7 in the sample were determined from the relative intensities of their characteristic features (Choi et al. 1996; Sunder and Miller 1995, 1996). Using this procedure, it was estimated that the relative amounts of U_3O_8 and UO_2/U_3O_7 phases in the sample were about 44.2 ± 2.5 and 55.8 ± 3.2, respectively. A procedure based on the work of Taylor et al. (1980) was used to estimate the relative amounts of UO_2 and U_3O_7 in the sample. This analysis indicated that the relative amounts of UO_2 and U_3O_7 were about 16 ± 8.5 and 84 ± 8.4, respectively. Here UO_2 includes U_4O_9 and UO_{2+x} with $x < 0.25$, i.e., phases with cubic lattice and unit-cell size close to UO_2. (It is not possible to distinguish between the UO_2, U_4O_9, and UO_{2+x} phases using the XRD technique used here.) Combining this result with the result for the fraction of U_3O_8 in the sample, the relative amounts of UO_2, U_3O_7, and U_3O_8 phases were about 9 ± 5%, 47 ± 5%, and 44 ± 3%, respectively.

Biological analyses

Fraction of uranium appearing in urine

DU in lung. Depleted uranium dust was instilled into the lungs of 6 individual rats at total sample mass amounts (excluding that amount cleared via the gastrointestinal tract) between about 12 and 200 μg. The percentage of the total that appeared in urine is shown in Fig. 4A. The data show that the fraction of depleted uranium that dissolved and

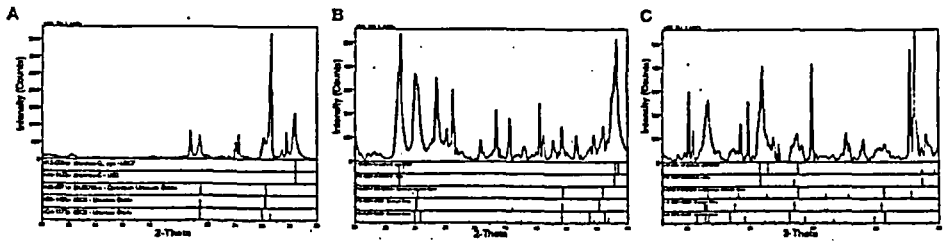

Fig. 3. Comparison of parts of the XRD pattern, on expanded scale, of a powder sample from the debris created following impact of the DU munitions on an armored target (top) with the literature patterns of the possible uranium phases in the sample. Panel A. XRD pattern for 10–30°. Panel B. XRD pattern for 30–48°. Panel C. XRD pattern for 48–70°. In all panels, the numbers with the literature patterns refer to the file numbers in Powder Diffraction Files (1995).

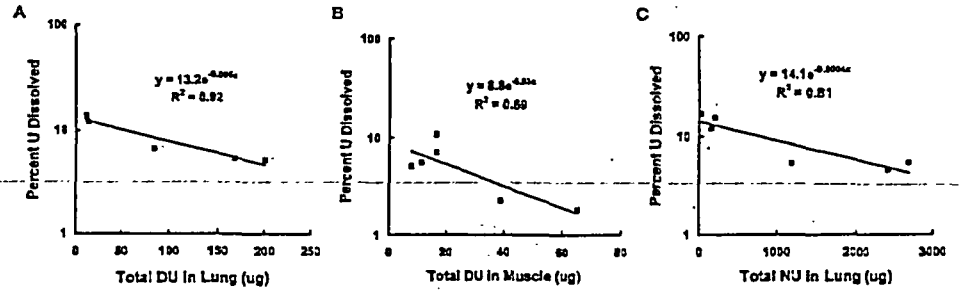

Fig. 4. Relationship between DU dissolution and lung burden. Panel A. Percentage of DU appearing in urine within 7 d of DU lung instillation. Panel B. Percentage of DU appearing in urine within 7 d of DU implantation in muscle. Panel C. Percentage of NU appearing in urine within 7 d of instillation of NU into lungs.

appeared in urine in the first 7 d after exposure declined exponentially with increases in the total amount originally present ($p < 0.05$). Regression analysis indicated that at the lowest lung burdens about 13.2% dissolved in 7 d, declining to about 40% of that amount when the DU lung burden increased to about 200 μg.

DU in muscle. The total mass sample amounts of DU implanted into the muscle of 6 individual rats, and the percentage that appeared in urine within 7 d is plotted in Fig. 4B. As was the case for DU in lung, the total amount of DU that appeared in urine within 7 d was exponentially dependent on the original amount implanted into muscle and decreased with increasing DU burden ($p < 0.05$). At the lowest muscle burdens, regression analysis indicated that the fraction of DU dissolving (8.8%) was less than that for DU in lung (Fig. 4A, 13.2%) and that the fraction of muscle DU dissolving also declined with increasing burden at a much greater rate than for DU in lung.

NU in lung. Natural uranium ore dust was also instilled into the lungs of 6 individual rats at total mass sample amounts between about 27 and 2,700 μg. The percentage of the total that appeared in urine in the first 7 d after exposure is shown in Fig. 4C. The data for NU shows a result qualitatively similar to that observed for DU in lung. The amount appearing in urine also declined exponentially with increases in the total lung burden ($p < 0.05$). At the lowest lung burdens, the fraction of NU dissolving (14.1%) was not different from the fraction of DU dissolving (Fig. 4A, 13.2%). However, compared to equal amounts of DU in lung, the fraction of NU dissolving within 7 d was much greater, remaining at 12–15% with lung burdens up to 200

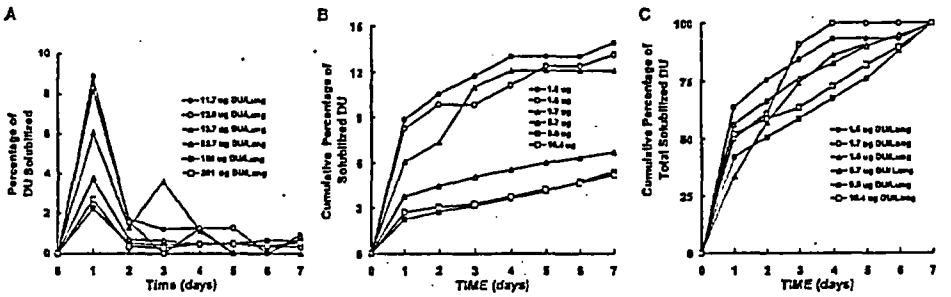

Fig. 5. Time course of DU dissolution in lung. Panel A. The percentage of lung DU solubilized and excreted each day over a seven-day period after instillation. The figure shows the percentage of DU lung burden that appeared in urine as uranium each day. The legend shows the total mass of the DU sample instilled in the lung of each animal. Panel B. Cumulative appearance of U in urine after DU instillation into lung. The figure shows the cumulative daily percentage of uranium appearing in urine, as a fraction of the total mass amount of DU instilled in the lungs. The legend shows the total cumulative mass of U that dissolved and appeared in the urine of each rat over the 7-d period. Panel C. Daily appearance of U in urine after DU instillation into lung. The figure shows the daily cumulative percentage of uranium, as a fraction of the total amount of uranium that appeared over 7 d in the urine of rats with DU instilled in their lungs. The legend shows the cumulative mass of U that dissolved and appeared in the urine of each rat. Each animal is represented by the same symbol in all panels.

μg while the fraction of DU dissolving at similar lung burdens had fallen to about 5%.

Rates of uranium dissolution

DU in lung. The percentage of the total DU sample mass instilled in each lung that appeared as uranium in urine each day for 7 d is shown in Fig. 5A. Fig. 5B shows that percentage on a cumulative basis. Fig. 5C shows the data as a daily fraction relative to the total cumulative amount of DU that appeared in urine over the 7 d. There appear to be two uranium components with different rates of solubilization. The figures show that about half of the total DU that appeared in urine over the 7-d period after lung instillation appeared in the first day. On days 2–7, the amount appearing daily remained relatively constant at the three higher levels of DU instillation but appeared to be completely removed by day 4 in the animals with lower lung burdens.

DU in muscle. The percentage of the total DU sample mass that appeared as uranium in urine each day after injection into rat muscle is shown in Fig. 6A. Fig. 6B shows that percentage on a cumulative basis. Fig. 6C shows the data as a daily fraction relative to the cumulative total of DU that appeared in urine over the 7 d. As in lung, there appear to be two uranium components with different solubilities. The figures show that about half of the total DU that appeared in urine over the 7-d period after muscle injection appeared in the first day. On days 2–7, the amount appearing daily remained relatively constant in the animals with the three higher muscle burdens, but at the lower burdens there may be a relatively faster removal of the slower component, and data from one animal suggests complete removal in 5 d.

NU in lung. The percentage of the total NU sample mass instilled in each lung that appeared as uranium in urine each day is shown in Fig. 7A. Fig. 7B shows that percentage on a cumulative basis. Fig. 7C shows the data as a daily fraction relative to the total cumulative NU that appeared in urine over the 7 d. The solubilization of NU appeared to be more complex than DU. As with DU about half of the NU that appeared in urine within 7 d appeared in the first day. However, NU appearance in urine on days 2–7 appeared not to be constant with time, or was completely removed within 4–5 d as was seen with different burdens of DU. The rates of solubilization appeared to decrease with increasing time, and this was independent of initial burden.

Histology

The effects on rat kidney and liver of the material dissolved from the DU and NU samples, as assessed by histological and pathological observations, are summarized in Table 2.

DU in lung. The two animals exposed to DU lung burdens above about 170 μg/lung (~9–10 μg U excreted/7 d) showed blood in urine and moderate to severe kidney damage, but no visible liver damage. Animals with lung

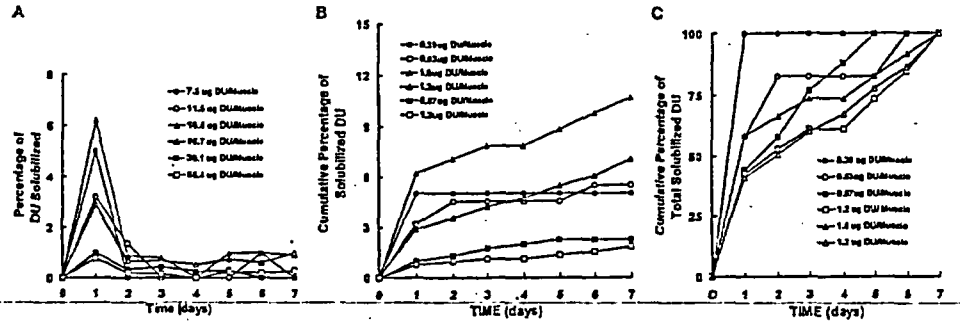

Fig. 6. Time course of DU dissolution in muscle. Panel A. The percentage of muscle DU solubilized and excreted daily over a 7-d period after injection. The figure shows the percentage of DU muscle burden that appeared in urine as uranium each day. The legend shows the total mass of DU sample injected into the muscle of each animal. Panel B. Cumulative appearance of U in urine after DU implantation into muscle. The figure shows the cumulative daily percentage of uranium appearing in urine, as a fraction of the total amount of DU installed in the muscle. The legend shows the total cumulative mass of DU that dissolved in the muscle and appeared in the urine of each rat over the 7-d period. Panel C. Daily appearance of DU in urine after DU instillation into muscle. The figure shows the daily cumulative percentage of uranium as a fraction of the total amount of uranium that appeared over 7 d in the urine of rats with DU injected in their muscle. The legend shows the cumulative mass of U that dissolved in the muscle and appeared in the urine of each rat. Each animal is represented by the same symbol in all panels.

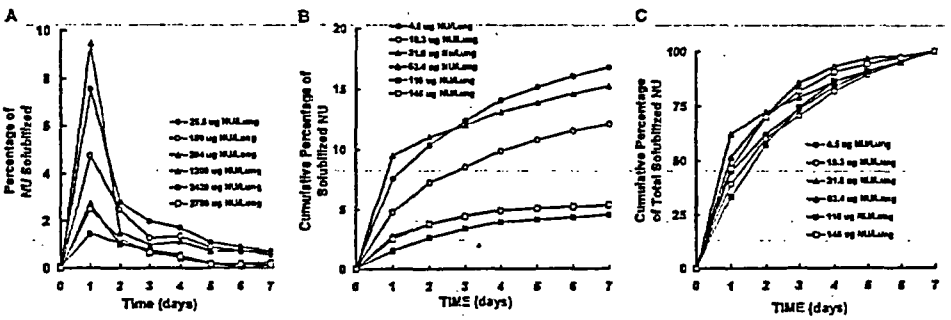

Fig. 7. Time course of NU dissolution in lung. Panel A. The percentage of lung NU solubilized and excreted daily over a 7-d period after instillation. The figure shows the percentage of NU lung burden that appeared in urine each day. The legend shows the total mass of NU instilled into the lung of each animal. Panel B. Cumulative appearance of U in urine after NU instillation into lung. The figure shows the cumulative daily percentage of uranium in urine, as a fraction of the total amount of NU instilled in the lungs. The legend shows the total cumulative mass of U that dissolved and appeared in the urine of each rat over the 7-d period. Panel C. Daily appearance of U in urine after NU instillation into lung. The figure shows the daily cumulative percentage of uranium, as a fraction of the total amount of uranium that appeared over 7 d in the urine of rats with NU instilled in their lungs. The legend shows the cumulative mass of U that dissolved and appeared in the urine of each rat. Each animal is represented by the same symbol in all panels.

burdens less than about 86 μg/lung (<~6 μg U excreted/7 d) showed no visible kidney lesions, but 3 of the 4 animals showed blood in urine, suggesting that a low level of damage may have existed, below the limit of histological detection. In those rats with lungs instilled with DU but not analyzed for uranium, histologically detectable presence of uranium particulates in 3 or more lung lobes was accompanied by histological indications of kidney damage.

DU in muscle. None of the animals (maximum muscle burden about 65 μg/muscle, <~1.8 μg U excreted/7 d) showed histological evidence of kidney or

Table 2. Histological and pathological summary of the toxic effects of DU and NU.

Source burden excreted/7 d	Histological liver damage # Positive / # Animals	Histological kidney damage # Positive / # Animals	Blood in urine # Positive / # Animals
DU in lung <86 μg/lung <~6 μg U excreted	0/4	0/4	3/4
DU in lung >170 μg/lung ~9–10 μg U excreted	0/2	2/2	2/2
DU in muscle <65 μg/muscle <1.8 μg U excreted	0/6	0/6	2/6
NU in lung <200 μg/lung <32 μg U excreted	0/3	0/3	3/3
NU in lung >1200 μg/lung >60 μg U excreted	2/3	3/3	3/3

liver damage. Blood was present in the urine of 2 of the 6 animals tested, and these two animals were among the 3 animals with the highest urine output of uranium, suggesting that these levels may be approaching the point of kidney damage.

NU in lung. All three animals with lung burdens above about 1,200 μg/lung (>60 μg U excreted/7 d) showed blood in urine, severe kidney lesions including casts in the tubules, and two showed liver atrophy. The animals with burdens less than about 200 μg/lung (<32 μg U excreted/7 d) showed no significant kidney or liver damage. The lack of kidney damage in these animals was significantly different ($p < 0.05$) from the result observed in animals exposed to DU lung burdens above about 170 μg/lung (~9–10 μg U excreted/7 d, Table 2). However, positive results for blood in the urine of all three of these latter animals suggests a low level of kidney damage may have existed, below the limit of histological detection.

DISCUSSION

Oxidation state of uranium in DU

Oxidation of uranium metal in air has been investigated by several groups (e.g., Colemenares 1984; Elder and Tinkle 1980; Hayward et al. 1992). The presence of U_3O_8 in the debris suggests that the DU metal in the munitions experienced temperatures higher than 275°C during their firing against armor steel—a very plausible condition during the encounter of armor piercing munitions with a target.

The XRD technique used here probes a sample only to a depth of about 2 μm. Therefore, the results obtained here were heavily biased towards the particles of smaller diameter. Smaller diameter particles are more prone to the aerosolization, and their dissolution is mainly controlled by the chemistry of the outer layers of the particles. Therefore, the results obtained here about the uranium oxidation state are relevant to the investigations on the biological effects of the DU sample.

Solubility and dissolution rate of a uranium oxide increase with an increase in the oxidation state of uranium (Sunder and Shoesmith 1991). In particular, the dissolution rate of a uranium oxide increases significantly if the uranium oxidation goes beyond the $UO_{2.33}$ (U_3O_7) stage (Sunder et al. 1981). The XRD results on the oxidation state of uranium in the DU sample suggest that about 44% of uranium in the DU dust, present as U_3O_8 ($UO_{2.66}$), would dissolve more rapidly in aqueous media than the rest of uranium [present as UO_2 and U_3O_7 ($UO_{2.33}$)].

Biological studies with DU

Live fired depleted uranium dust showed a fast dissolving component that appeared in urine within the first day after instillation and constituted about 50% of the sample that was solubilized within 7 d. At the lower lung burdens, the total maximum amount dissolved was about 14%. This indicated, therefore, that the DU (< 50 μm size class) instilled into lungs contained a maximum of 6–7% uranium that was rapidly soluble (within 1 d) and about another 6–7% that was soluble within an additional 6 d. Since 44% of the uranium was present as the more soluble U_3O_8, it is likely that this oxide formed the major component of the fast dissolving component. These amounts do not reflect any DU cleared from the lung and solubilized or excreted via the gastrointestinal (GI) tract. Dissolution and absorption from the GI tract would have contributed to the total excreted uranium. Other mechanical clearance routes were assumed to be constant.

As the initial DU lung burden increased, the fractional amount of uranium solubilized and appearing in urine within 7 d declined exponentially. At about 170–200 μg DU/lung, about 5–7% of the total lung sample mass appeared in urine within 7 d. The slow component showed no signs of source depletion since the rate of excretion remained about constant to 7 d. The fast component remained at about half of the total amount excreted in these high lung burden animals. These higher total amounts of uranium excretion (~9–10 μg) for both the fast and slow component were accompanied by histological evidence of kidney damage, but not liver damage. Below these higher lung burden levels, some pathological observations suggested mild kidney damage

(blood in urine), but this was not evident on the histological sections. These observations suggest that histologically detectable impaired kidney function occurs when weekly excretion levels of uranium from the fired DU sample in lungs exceed about 6 μg. The presence or absence of kidney damage should therefore be assessed on the uranium level apparent in urine and not on the assumed lung total, which did not include the material cleared via the GI tract.

When depleted uranium (50–300 μm size class) was injected into muscle, the total fraction solubilized within 7 d was somewhat lower than in lung. This lower solubility could reflect a uranium compound content slightly different from the <50 μm size class instilled into lung (see above comments on the XRD composition studies), or it could reflect a lower rate of solubilization in muscle compared to lung. Most likely, however, the difference in solubility may reflect the smaller surface area of the larger particles.

At the highest muscle burdens, where the maximum 7-d excretion was <2 μg, the kidney showed no histologically apparent lesions. However, even at this low level, there were indications of kidney damage (blood in urine) in two out of six of the animals. This can be compared to the results obtained with DU in lung, where, after 7-d excretion levels of about 6 μg, 3 out of 4 animals showed this level of kidney damage. These results suggest that this type of damage can occur at uranium weekly excretion amounts <6 μg, but becomes less probable as the level decreases.

Natural uranium ore dust instilled into rat lung behaved in a manner qualitatively similar to DU in lung, declining exponentially with increasing burden. Lower lung burdens resulted in about 14% of the NU appearing in urine within 7 d, and at higher burdens about 5%, a result similar to DU. However, this rate of decline occurred more slowly with NU than it did with DU. At NU lung burdens up to about 200 μg, about 14% of the material dissolved in 7 d, while the rate of dissolution of DU had fallen to about 5% at these lung burdens. The exponential decline of dissolution, seen in all samples, may suggest that as burden increases, increasing amounts become locally unavailable for dissolution.

Like DU, NU also showed a fast uranium component that appeared within 1 d and represented about half the amount excreted in 7 d for all lung burdens. However, excretion of other uranium components in the NU sample appeared more complex, and the appearance in urine was curvilinear with time, suggesting more than one component. Histological evidence for kidney damage was evident at NU lung burdens over about 1,200 μg/lung, but not at burdens up to about 200 μg/lung.

Lung levels of DU that resulted in 3.8–5.4 μg of uranium excretion on day one, and thereafter about 0.8–1.0 μg d^{-1} resulted in histologically apparent kidney damage. However, lung levels of NU that produced about 8.6–19.3 μg of uranium excretion on the first day (about 2–3 fold higher than DU) and subsequently about 1–2 μg d^{-1} (about 2 fold higher than DU) produced no histologically apparent kidney damage. This result suggests that kidney damage in the DU exposed animals was not due solely to blood uranium levels. Chemical analysis of DU debris indicated the presence of other metals in the sample (Table 1), which may have contributed to the higher toxicity. We do not know the particle size distribution or solubility of these compounds relative to DU, and these factors will influence the observed differences in toxicity. At very high levels of uranium excretion (~30–60 μg uranium on day 1) from high NU lung burdens, both kidney and liver damage were histologically apparent. Since the radioactivity of the DU per unit mass was substantially less than the NU, none of the increased biological effect of DU seen here could be attributed to radiation effects, supporting the idea that any effects of dust from exploded DU munitions seen in humans could only be attributed to chemical toxicity. Based on these rat studies, such effects in humans would likely be seen only at very high lung burdens.

CONCLUSION

We have examined the physical, chemical, and biological properties of samples of dust resulting from fired munitions containing depleted uranium (0.198 ± 0.10 atom% ^{235}U). The uranium oxides were present as U_3O_7 (47%), U_3O_8 (44%), and UO_2 (9%). DU dust, instilled into the lungs or implanted into the muscle of rats, contained a rapidly soluble uranium component and a more slowly soluble uranium component. NU in lungs also showed rapid and slowly dissolving uranium components. The fraction of DU dissolving in lung showed a pattern of exponential decline with increasing initial burden similar to NU. At high lung burdens, both DU and NU showed histological evidence of kidney damage. Chemical analysis of the DU dust indicated the presence of other metals. DU dust produced kidney damage at lower lung burdens and lower urine uranium levels than NU dust, suggesting that other toxic metals in DU dust may contribute to the damage.

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