Urinary uranium and kidney function parameters in professional assistance workers in the Epidemiological Study Air Disaster in Amsterdam (ESADA)

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Abstract

Background. The Epidemiological Study Air Disaster in Amsterdam (ESADA) aimed to assess long-term health effects in professional assistance workers involved in the 1992 air disaster in Amsterdam. As part of ESADA indications of nephrotoxicity due to exposure to uranium from the balance weights of the crashed aircraft were assessed.

Methods. Data of a historically defined cohort of 2499 (exposed and non-exposed) firefighters, police officers and hangar workers were collected 8.5 years after the disaster. Urinary uranium concentrations were determined by sector field inductively coupled plasma mass spectrometry. Urine albumin–creatinine ratio and fractional excretion of β2-microglobulin were calculated from a single-spot urine specimen and simultaneous blood sample. Exposed assistance workers were compared with their non-exposed colleagues, and associations between uranium and kidney function parameters were explored.

Results. Median uranium concentrations were around 2 ng/g creatinine. Median values of albumin–creatinine ratio and fractional excretion of β2-microglobulin were well below the level for microalbuminuria and for tubular damage, respectively. No statistically significant differences between exposed and non-exposed workers were found in uranium concentrations and kidney function parameters, although exposed hangar workers had lower uranium concentrations. No statistically significant associations were found between uranium concentrations and kidney function parameters in the total cohort.

Conclusions. Occupational exposure to the air disaster in Amsterdam was neither significantly associated with higher uranium concentrations, nor with disturbed kidney function parameters. In this large cohort of professional assistance workers, urinary uranium concentrations were in the low range compared with previously published reference populations. No indications of nephrotoxicity were found at urinary uranium concentrations around 2 ng/g creatinine.

Keywords: albuminuria; β2-microglobulin; ICP-mass spectrometry; nephrotoxic; renal tubular function; uranium

Introduction

Uranium has well been recognized as a nephrotoxic metal [1] and in that respect natural and depleted uranium are indistinguishable [2,3]. The Epidemiological Study Air Disaster in Amsterdam (ESADA) offered the opportunity to study in a large population associations between urinary uranium concentration and kidney function parameters [4].

On the 4th of October 1992, a Boeing 747-F cargo aircraft crashed into two apartment buildings in Bijlmermeer, a densely populated suburb of the Dutch city of Amsterdam. The aircraft, cargo, fuel and remnants of the apartment buildings caught fire and burned for more than 1 h [5]. Firefighters and police officers were called to the scene to extinguish fires, to search and rescue people, to assist in the...
identification of human remains and personal belongings, to secure the surroundings and to clean-up the devastated area. Within a few days, the wreckage of the aircraft was transported to a hangar at Schiphol Airport, where employees (i.e. ‘hangar workers’) sorted and inspected the wreckage. It appeared that 152 kg of (depleted) uranium from the balance weights of the aircraft was not recovered from the rubble [5]. Theoretically, this amount of uranium could have been completely oxidised at high temperatures (in the range 600–1200°C) that occurred during the fire, resulting in the poorly soluble uranium oxides UO₂ and U₃O₈ [2,5]. Respiratory exposure to aerosol-bound uranium oxides could have resulted in deposition of uranium oxides in the respiratory tract and to systemic absorption from the respiratory tract. Due to their pharmacokinetics, poorly soluble uranium oxides are selectively distributed into the body (skeleton, kidney, lungs), resulting in renal excretion during prolonged periods [2,3,6]. Retrospective risk calculations had shown that in the worst-case approach bystanders of the fire had been exposed to airborne uranium concentrations for 1 h that were comparable to the limit for chronic exposure to workers [5]. It was therefore considered improbable that the missing uranium could have led to detectable increases in morbidity [5]. Nevertheless, health concerns arose among professional assistance workers, as in the years following the disaster, individuals expressed health complaints. The aftermath of the disaster was characterized by societal, psychological and finally political consequences (parliamentary inquiry) as described elsewhere [7]. Starting in 2000, a large study was performed on the health effects of the disaster on professional assistance workers: the ESADA [4]. This article focuses on the part of ESADA, aimed at assessing indications of nephrotoxicity of uranium.

The observed nephrotoxic effects of uranium are generally reversible damage to the brush border of the proximal convoluted tubules and to the glomeruli [2,3,8]. The clinical effects are disturbances in reabsorption and thus, excretion of filtered solutes and substances. For example, the appearance of small quantities of albumin in urine below the threshold of microalbuminuria, may result from failure of the proximal tubule to reabsorb normally filtered small quantities of albumin [1,9]. A more sensitive marker of proximal tubular function is fractional excretion of β₂-microglobulin (FE-β₂-microglobulin) [9].

In this part of ESADA, we compared exposed with non-exposed workers per occupational group with respect to (i) urinary uranium concentration and (ii) kidney function parameters i.e. urine albumin-creatinine ratio (UACR), FE-β₂-microglobulin and creatinine clearance (CCr). In addition, we explored associations between urinary uranium concentrations and these kidney function parameters in the total cohort of professional assistance workers to assess possible nephrotoxic effects of uranium.

Subjects and methods

Study population

The ESADA can be characterized as a historical cohort study, with final exposure status to the air disaster based on self-report. The design of the ESADA has been described elsewhere [4]. In short, the study population comprised three occupational cohorts: (i) professional firefighters of the Amsterdam fire department; (ii) police officers of the Amsterdam-Amstelland Regional Police Force; (iii) and hangar workers working at Schiphol Airport. Assistance workers who reported at least one disaster-related task were defined as (occupationally) exposed, and all others as (occupationally) non-exposed. Hangar workers were classified as visitors if they had been present in the hangar while the wreckage was there, but performed no disaster-related tasks. The study was approved by the Medical Ethics Committees of ‘VU University Medical Center’ (VUmc) and the ‘Onze Lieve Vrouwe Gasthuis’ (OLVG) in Amsterdam. All participants signed informed consent and participated voluntarily.

Data collection

Data collection took place at an outpatient clinic in Amsterdam and, for about half the hangar workers, at Schiphol Airport, in the period from 1st January, 2000 to 1st March 2002, i.e. on average 8.5 years after the disaster. The participants were asked to complete questionnaires on occupational disaster exposure and socio-demographic characteristics, and to deliver blood and urine samples. Blood samples and spot urine samples were collected throughout the day (08.30 h to 16.30 h) [4].

Selection of materials for urine samples for determination of uranium

The bowls and vials intended for sample collection and/or for use during the sample preparation preceding analysis of uranium were systematically checked. Bowls and vials manufactured from different materials and obtained from different suppliers were filled with 1.4 M HNO₃. After 24 h, this 1.4 M HNO₃ was analysed for its uranium content. Surprisingly, for some of the bowl and vial types tested, a measurable increase (compared with fresh 1.4 M HNO₃) was indeed observed (uranium levels measured from <LOD up to 1.8 ng/l). On the basis of these tests, polypropylene bowls with a content of 200 ml (PC200 series, Plastiques Gosselin, France) for sample collection and polypropylene vials with a content of 15 ml (T408 series, Simport, Canada) with colourless caps (T401 series, Simport, Canada) for sample preparation were selected. For these bowl and vial types, no leaching of uranium could be demonstrated.

Kidney function parameters

Blood samples were collected in blank (β₂-microglobulin) or heparinized tubes (creatinine). Spot urine samples were collected in polypropylene bowls (Plastiques Gosselin, France). After taking urine samples for uranium measurements (see subsequently), urine was transferred into monovettes of 10 ml. The tubes and monovettes were stored at room
temperature and sent to the clinical laboratory within 2 h. Blood samples were tested for creatinine (enzymatically, Roche Modular P800, Roche Diagnostics) and β₂-microglobulin (microparticle enzyme immuno assay, IMx Abbott). Urine samples were investigated for pH (Combur10 test, Miditrion, Roche Diagnostics), creatinine (colorimetrically, Hitachi 747, Roche Diagnostics); microalbumin (immuno-nephelometrically, Beckman Array 360 system) and β₂-microglobulin (microparticle enzyme immuno assay, IMx Abbott). As β₂-microglobulin degrades at urinary pH < 5.5, it was determined only if urinary pH was ≥ 5.5. Alkalization of urine for complete recovery of β₂-microglobulin [9] was not feasible for practical reasons. Calculations were as follows: creatinine clearance (CCr) in ml/min = [[(140-age) × (bodyweight in kg)] / [(R × creatinine in μmol/l in serum)] ^ (-1)], R being 0.86 for males and 1.01 for females (Cockroft-Gault formula); fractional excretion β₂-microglobulin in percentage = 100 × β₂-microglobulin (urine, μg/l) × creatinine (serum, μmol/l) / β₂-microglobulin (serum, mg/l) × creatinine (urine, mmol/l) ^ (-1); albumin/creatinine ratio in mg/mmol = [micro-albumin (urine, mg/l)] / [creatinine (urine, mmol/l)] ^ (-1).

Determination of uranium in urine, sample and sample preparation

Urine was poured into polypropylene vials of 15 ml without acidification, which were closed with caps and frozen at −20°C within 2 h after collection. The samples were stored and transported under refrigeration to the Laboratory of Analytical Chemistry of Ghent University (Belgium). In Ghent, urine samples were handled as follows. After thawing and homogenization of the samples, 1 ml of urine was volumetrically diluted to 10 ml with 0.14 M HNO₃. A quantity of 0.14 M HNO₃ was prepared from 14 M HNO₃ purified by sub-boiling distillation, by dilution with milli-Q water (18 MΩ cm) obtained using a Millipore purification system (Bedford, MA, USA). Rhenium (Re, 1 g/l, Merck, Darmstadt, Germany) was added (final Re concentration: 100 ng/l) to act as an internal standard, correcting for matrix effects (matrix-induced signal suppression or enhancement), instrument instability and/or signal drift.

Determination of uranium in urine, instrumentation, method of measurement

As detection of urinary uranium levels in low concentration ranges (ng/l) should be possible, ICP-mass spectrometry was applied. The analytical requirements for accuracy and precision, as well as the suitability for automation, routine application and low sample consumption are all met in inductively coupled plasma mass spectrometry (ICP-MS) [10]. Sector field ICP-MS was preferred over the more widely distributed quadrupole-based ICP-MS, as it provides superior limits of detection (LODs) [10,11]. The instrument used was a FinniganMat Element (Finnigan, Bremen, Germany). By means of the combination of a concentric pneumatic nebuliser and a water-cooled double-pass Scott-type spray chamber, a representative part of the diluted urine was nebulised into the inductively coupled plasma (ICP). Owing to the high temperature in the ICP, molecules are broken down into atoms that are subsequently ionised efficiently. The ions are extracted via an interface section and introduced into a double-focussing sector field mass spectrometer of reverse Nier-Johnson geometry for mass analysis. Via a very sensitive detection device—an electron multiplier that permits each individual ion that strikes its surface to be detected—the count rate for the nuclides of interest are measured sequentially. The analyzer was operated in low-mass resolution mode (R = 300) and was optimized for maximal sensitivity and minimal effect of the matrix composition on the U/Re signal ratio. Each sample was measured three times (total measurement time < 3 min per sample, total sample consumption < 5 ml of 10-fold diluted urine). Quantification was accomplished via external calibration. The accuracy of the method was secured and demonstrated in various ways—successful analysis of a certified reference material with known U concentration (BCR CRM 668 – Mussel Tissue), quantitative recovery of a 50 ng/l U spike added to a pooled urine sample and a consistent result of about 1.6 ng/l for the aforementioned non-spiked pooled urine sample. With the approach developed, a procedural limit of detection of 0.2 ng of U/l urine was obtained. At a concentration level of 10 ng/l, the precision was typically established to be ~5% RSD.

The results of the determination of uranium are expressed as nanogram per litre. After normalization to the creatinine concentration in urine, to correct for diurnal variation, the results are reported as ng/g creatinine [12]. Measurement of the ⁴⁴⁵⁵⁴⁷⁳⁴⁵⁶⁷⁸⁹⁰⁰⁰ uranium isotope ratio in samples with uranium concentrations higher than 50 ng/l or 50 ng/g, was performed at the Institute for Reference Materials and Measurements (IRMM) in Geel, Belgium, as described elsewhere [13]. For these measurements, the uranium was chromatographically extracted from the selected urine samples (U-TEVA resin, Eichrom Technologies, France) in order to obtain a higher uranium concentration in the solutions submitted to ICP-MS analysis, and thus to obtain a sufficient isotope ratio precision. The ⁴⁴⁴⁵⁴⁷⁳⁴⁵⁶⁷⁸⁹⁰⁰⁰ uranium isotope ratio was determined using an Element2 (ThermoFischer, Bremen, Germany) sector field ICP-MS instrument, operated at low resolution mode (R = 300). In order to increase the analyte introduction efficiency and hence, the signal intensities, ultrasonic nebulization (U-6000AT+, Cetac Technologies, IL, USA) was used as a means of sample introduction instead of the more traditional pneumatic nebulization. Each urine sample to be analysed was first subjected to microwave-assisted acid digestion (with 4 ml HNO₃ + 1 ml H₂O₂), after which uranium was extracted from the digest using a U-TEVA column (Eichrom Technologies, IL, USA). The uranium thus isolated from the sample was subsequently eluted in 0.1 M HNO₃ and the fraction containing the target element analysed.

Statistical analysis

Socio-demographical characteristics of exposed vs non-exposed workers were analysed with t-tests for independent groups (age) and Pearson chi-square tests (all others). In order not to exclude participants whose samples had uranium concentrations or kidney function parameters below the detection level from the statistical analysis, an arbitrary number below the detection level was imputed. This concerned UACR (1.0 for n = 261), urinary β₂-microglobulin (0.99 for n = 13) and urinary uranium concentration (0.2 for n = 100). The statistical analyses comprised adjusted comparisons between exposed and non-exposed workers per occupational group by means of linear regression models. In the case...
of non-normal distribution of outcomes, data were transformed by means of natural logarithm before regression analysis. This concerned uranium, FE β₂-microglobulin and UACR. These statistical analyses were adjusted for the following potential confounders: age, level of education, alcohol consumption, cigarette smoking habits, gender and ethnicity, if applicable [4]. The association between urinary and kidney function parameters was analysed after pooling the data of the three occupational groups, by means of linear regression (with uranium as independent and kidney function parameters as dependent variables) with adjustment for exposure to the disaster, occupational group and the above-mentioned background variables. FE β₂-microglobulin and UACR were again transformed by means of natural logarithm. In these cases, associations are expressed as relative changes in kidney function parameters per 10 units increase in uranium concentration (i.e. the corresponding regression coefficients were multiplied by 10 and back transformed). For CCr, the association is expressed as the mean difference in CCr per unit increase in uranium concentration. The analyses were carried out in SPSS version 10.1, and (two-tailed) P < 0.05 were considered statistically significant.

Results

Participants

A total of 2499 workers were included in the statistical analyses: 528 firefighters, 1468 police officers and 503 hangar workers. The reference group of hangar workers was divided into ‘non-exposed’ and ‘visiting’ workers. Descriptive statistics of socio-demographic characteristics of exposed, non-exposed and visiting workers of each occupational group have been published elsewhere [14]. In general, exposed and non-exposed workers were comparable, with the exception of age of the firefighters. Exposed firefighters were, on average, more than 10 years older than non-exposed firefighters. There were some small statistically significant differences. Exposed police officers were more frequently male than non-exposed police officers (88.5% and 84.9%, respectively). All of the firefighters and the hangar workers included in the analysis were male. Exposed firefighters and hangar workers less frequently had an executive function as compared with their non-exposed colleagues. Exposed firefighters more frequently reported excessive alcohol consumption and exposed firefighters as well as police officers were more often current smokers when compared with their non-exposed colleagues.

Urinary uranium concentrations

Urinary uranium concentrations in all occupational groups were around 2 ng/g creatinine (Table 1). In all groups, lower concentrations of uranium were found for exposed workers; however it was significant in the hangar workers only. The distribution of the urinary uranium concentrations normalized to creatinine concentration in the total cohort is shown in Figure 1. Five participants (three firefighters and two police officers), of whom four were non-exposed, had urinary uranium concentrations >50 ng/l or 50 ng/g. The ²³⁵U/²³⁸U isotope ratio results of these samples agreed within experimental uncertainty (~2%) with the natural value of 0.00725 [3].

Kidney function parameters

Mean CCr was around 125 ml/min in all occupational groups, except in exposed firefighters, in whom mean CCr was 115 ml/min. Median UACR was around 0.5 in all groups. Median FE β₂-microglobulin was around 0.04% in all groups. Up to 25% of urine samples were discarded for assessment of β₂-microglobulin due to

Table 1. Urinary uranium and kidney function parameters in exposed, non-exposed and visiting professional assistance workers

<table>
<thead>
<tr>
<th></th>
<th>Firefighters</th>
<th></th>
<th>Police officers</th>
<th></th>
<th>Hangar workers</th>
<th></th>
<th>Total cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exposed (n=334)</td>
<td>Non-exposed (n=194)</td>
<td>Exposed (n=834)</td>
<td>Non-exposed (n=634)</td>
<td>Exposed (n=241)</td>
<td>Non-exposed (n=104)</td>
<td>Visitors (n=158)</td>
</tr>
<tr>
<td>Urinary uranium, ng/g creatinine</td>
<td>1.75</td>
<td>1.87</td>
<td>1.94</td>
<td>2.08</td>
<td>1.69*</td>
<td>1.86</td>
<td>1.74</td>
</tr>
<tr>
<td>p25–p75</td>
<td>1.15–2.82</td>
<td>1.25–3.03</td>
<td>1.27–3.04</td>
<td>1.35–3.15</td>
<td>1.06–2.72</td>
<td>1.31–3.0</td>
<td>1.04–2.45</td>
</tr>
<tr>
<td>p95</td>
<td>6.30</td>
<td>6.68</td>
<td>6.65</td>
<td>6.60</td>
<td>5.65</td>
<td>9.36</td>
<td>6.07</td>
</tr>
<tr>
<td>CCR (ml/min) (SD)</td>
<td>115±21</td>
<td>126±23</td>
<td>128±25</td>
<td>124±24</td>
<td>127±27</td>
<td>128±24</td>
<td>126±30</td>
</tr>
<tr>
<td>UACR (mg/mmol)</td>
<td>0.51</td>
<td>0.43</td>
<td>0.46</td>
<td>0.50</td>
<td>0.45</td>
<td>0.48</td>
<td>0.49</td>
</tr>
<tr>
<td>P25–p75</td>
<td>0.35–0.87</td>
<td>0.30–0.65</td>
<td>0.32–0.77</td>
<td>0.34–0.81</td>
<td>0.33–0.68</td>
<td>0.31–0.81</td>
<td>0.35–0.80</td>
</tr>
<tr>
<td>p95</td>
<td>3.48</td>
<td>2.30</td>
<td>2.49</td>
<td>2.67</td>
<td>2.11</td>
<td>4.28</td>
<td>4.31</td>
</tr>
<tr>
<td>FE β₂-microglobulin (%)</td>
<td>0.037</td>
<td>0.038</td>
<td>0.037</td>
<td>0.038</td>
<td>0.036</td>
<td>0.039</td>
<td>0.038</td>
</tr>
<tr>
<td>p25–p75</td>
<td>0.030–0.054</td>
<td>0.030–0.048</td>
<td>0.028–0.053</td>
<td>0.029–0.052</td>
<td>0.028–0.053</td>
<td>0.029–0.056</td>
<td>0.031–0.056</td>
</tr>
<tr>
<td>p95</td>
<td>0.118</td>
<td>0.083</td>
<td>0.115</td>
<td>0.110</td>
<td>0.104</td>
<td>0.141</td>
<td>0.145</td>
</tr>
<tr>
<td>% urinary pH ≥ 5.5</td>
<td>68.9</td>
<td>71.6</td>
<td>75.8</td>
<td>76.0</td>
<td>80.5</td>
<td>71.2</td>
<td>76.6</td>
</tr>
</tbody>
</table>

Table represents median (25th, 75th and 95th percentiles) of urinary uranium, UACR and FE β₂-microglobulin and mean (SD) of CCr for exposed compared with non-exposed workers per occupational group and for the total cohort. CCr, creatinine clearance; UACR, urinary albumin-creatinine ratio; FE, fractional excretion. *P < 0.05; by means of linear regression, adjusted for age, gender, ethnicity, level of education, alcohol consumption and cigarette smoking habits.
low urinary pH, which applied in the same manner to all occupational groups. No statistically significant differences in CCr, UACR, FE-β2-microglobulin and urinary pH were found between exposed and non-exposed workers (Table 1).

**Association between urinary uranium and kidney function parameters**

Associations between urinary uranium and kidney function parameters were explored in the total cohort. All three kidney function parameters, i.e. CCr, UACR and FE-β2-microglobulin were positively associated with urinary uranium concentration normalized to creatinine, yet this was not statistically significant (Table 2).

![Figure 1. In-transformed urinary uranium concentration. Histogram of in-transformed urinary uranium concentrations normalized to creatinine. For uranium concentrations below the detection level (<0.3) 0.2 was imputed (n=100).](https://academic.oup.com/ndt/article-abstract/23/1/249/1922514)

**Discussion**

The present study, as part of ESADA, focuses on indications of nephrotoxicity of uranium from the balance weights of the crashed aircraft among professional assistance workers. It provides data on urinary levels of uranium and kidney function parameters (fractional excretion of β2-microglobulin, urine albumin-creatinine ratio and creatinine clearance) in a large cohort of people. This part of ESADA was primarily set up to assess urinary uranium levels and indications of disturbed kidney function parameters in exposed professional assistance workers in the 1992 air disaster in Amsterdam as compared with non-exposed colleagues. Although retrospective risk calculations had shown that in the worst-case approach of uranium exposure during the disaster, increases in morbidity were improbable [5], and although more than 8 years had passed since possible exposition to uranium, the hypothesis of nephrotoxicity of uranium was included in ESADA. This was done for societal and political reasons [7]. Interestingly, from a nephrological point of view, this study offered the opportunity to study, in nearly 2500 people, urinary uranium concentration and whether it is associated with the aforementioned kidney function parameters.

This study shows that urinary uranium concentrations were not significantly higher among exposed workers than among non-exposed workers. In fact, exposed workers tended to have lower uranium levels than their non-exposed colleagues, which was significant among the hangar workers. However, these differences were small and probably due to chance since the comparison groups were relatively large. In the few workers with uranium concentrations > 50 ng/l or ng/g creatinine (n=5) the 235U/238U isotope ratio did not indicate the presence of depleted uranium. Thus, 8.5 years after the disaster, no evidence was found that among exposed workers uranium had been retained in the skeleton, kidney or lungs and continued to be excreted in urine at the time of the investigation [2,3]. At low urinary uranium levels around 2 ng/g creatinine, FE-β2-microglobulin was well below 0.1%, the cut-off level for tubular damage [9]; UACR was far below the threshold for microalbuminuria and

**Table 2. Associations between urinary uranium and kidney function parameters in the total sample of professional assistance workers**

<table>
<thead>
<tr>
<th></th>
<th>CCr</th>
<th>UACR</th>
<th>FE-β2-microglobulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>2462</td>
<td>2490</td>
<td>1873</td>
</tr>
<tr>
<td>Mean difference per unit increase in [uranium]</td>
<td>0.15</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Relative difference in geometric mean per increase of 10 units in [uranium]</td>
<td>NA</td>
<td>1.05</td>
<td>1.04</td>
</tr>
<tr>
<td>95% CI</td>
<td>−0.07−0.37</td>
<td>0.98−1.12</td>
<td>0.98−1.10</td>
</tr>
<tr>
<td>P-value</td>
<td>0.19</td>
<td>0.13</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Analyses adjusted for age, gender, ethnicity, level of education, alcohol consumption, cigarette smoking habits, exposure to the disaster and occupational group. CCr, creatinine clearance; UACR, urine albumin-creatinine ratio; FE, fractional excretion; n, number; [uranium], urinary uranium concentration; NA, not applicable; CI, confidence interval; A, linear regression after transformation by means of natural logarithm of kidney function parameter.
creatinine clearance was normal. No statistically significant differences between exposed and non-exposed workers were found in kidney function parameters, in particular in FE-β₂-microglobulin as a marker of proximal tubular function. Thus, no indications were found for nephrotoxic effects in exposed professional assistance workers as compared with their non-exposed colleagues. In addition, at urinary uranium concentrations of 2 ng/g creatinine no statistically significant associations between uranium, FE-β₂-microglobulin, UACR and creatinine clearance were found in the total cohort.

The median urinary uranium concentration in our study was just below 2 ng/g creatinine in the total cohort. This concentration is in the low range of continuous excretion of uranium resulting from normal exposure, as levels of urinary uranium in reference populations are reported to be around 10 ng/g creatinine [15]. Compared with these literature values, the urinary uranium levels in the present study seem to indicate low natural uranium abundance and uptake from food and drinking water in the area around Amsterdam.

Renal function parameters have been assessed in chronically exposed uranium mill workers [16]. Compared with a matched control group of local cement plant workers, fractional excretion of β₂-microglobulin was higher in the uranium mill workers and showed a dose-effect relation to the length of time that the workers had been exposed to uranium. Also, the renal effects of uranium have been studied in people chronically and continuously exposed to drinking water from wells with high natural uranium concentrations [17–20]. Urinary uranium concentrations were either reported [19,20] or could be estimated [17,18], using uranium exposure data and a fractional absorption of 2% for uranium in the gastrointestinal tract [2,3]. In contrast to the present study, in which a very small fraction of subjects had uranium concentrations exceeding 10 ng/g creatinine, chronic ingestion of uranium in drinking water in those studies yielded a wide variability over orders of magnitude of urinary uranium concentrations, from <10 ng/g creatinine in the control or low exposure groups up to 10³–10⁴ ng/g creatinine in the high exposure groups. In those studies renal effects of uranium exposure such as microalbuminuria [17], excretion of alkaline phosphatase and β₂-microglobulin [18], excretion of glucose [18,20] and excretion of calcium and phosphate [19] were reported. Possibly, the fact that all urinary uranium concentrations in the present study were in the lowest range as compared with the wide range of urinary uranium concentrations in those studies [17–20], accounts for the absence of renal effects of uranium in the present study.

In addition to the aforementioned chronic exposure studies among workers and residents, renal function parameters have been studied in a follow-up study of Gulf War veterans involved in friendly fire incidents in 1991 with depleted uranium weaponry, including veterans with and without embedded ammunition fragments of depleted uranium [12,21,22]. Based on urinary uranium concentration, the veterans were defined in the low or high uranium group (cut-off point 100 ng uranium/g creatinine). In the high uranium group (n = 14) urinary uranium concentrations up to 10³–10⁴ ng/g creatinine were found. In the 1997 cohort, no statistically significant differences in renal function parameters were found between the low and high uranium group [12]. In the next surveillance in 2001 a statistically significant higher serum creatinine and higher urinary total protein were reported [21]. In addition, urinary retinal binding protein (RBP) tended to be increased in the high uranium group, yet this did not reach statistical significance. However, from a clinical perspective, the higher values of urinary total protein and urinary RBP were within the normal range. In the 2003 cohort, no statistically significant differences were found, although urinary RBP again tended to be increased in the high uranium group compared with the low uranium group, but not statistically significant [22]. Thus, in the veterans no consistent effects on renal function were found when comparing low vs high uranium groups. Should the cut-off point for low and high urinary uranium groups have been defined at a lower level, a more consistent effect of uranium on renal function parameters might have been found in the veterans.

Strengths of the present study are the availability of data in a large cohort and the power to detect differences between exposed and non-exposed. Historical records of employment were available to define the occupational cohort. Since 99% of this cohort could be traced, and 70% of those participated, the sample size was considerable. Even if we consider the smallest group (i.e. non-exposed hangar workers), the minimal detectable difference between exposed and non-exposed workers was 0.01, 0.03, 0.78 and 9.49 for urinary uranium concentration, FE-β₂-microglobulin, UACR and Cr, respectively. Some limitations of the study should also be addressed. As no reliable exposure data were available, we used a detailed questionnaire to assess occupational exposure to the disaster. Although this is based on retrospective self-report, it seems reasonable that the workers were able to recollect whether or not they had performed any disaster-related tasks, which was used to define exposure status. Therefore, misclassification with respect to being exposed or not, is unlikely. A second limitation is that the data collection was performed long after exposure. Should the data collection have been performed shortly after exposition, we cannot exclude the possibility that clinically relevant differences between exposed and non-exposed workers in urinary uranium concentrations and kidney function parameters had been demonstrated. However, even in the worst-case approach of uranium exposure during the disaster [5], we did not expect to find increased urinary uranium concentrations or disturbed kidney function parameters in the exposed workers as compared with the non-exposed workers. A third
limitation is that exposed firefighters were on average 10 years older than their non-exposed colleagues. However, we statistically adjusted for this systematic age difference. For example, the logistic regression analysis of the association between exposure status and creatinine clearance among firefighters showed that higher age was associated with both lower creatinine clearance (mean difference of 1.034 per year) and exposure status (exposed being older than non-exposed firefighters). Hence, age confounded the association between exposure and creatinine clearance. After adjustment for age, no significant difference in creatinine clearance between the exposed and non-exposed fire fighters was found. A fourth limitation is that up to 25% of urine samples were discarded for assessment of β2-microglobulin due to low urinary pH [9]. However, selection bias seems unlikely, because the percentage of workers with such samples was comparable between exposed and non-exposed groups. Finally, up to 10% of urine samples were below the detection levels for albumin, β2-microglobulin or uranium. In order not to exclude these data from the analyses, arbitrary values based on the distribution, were imputed. This concerned UACR (1.0 for n = 261), urinary β2-microglobulin (0.99 for n = 13) and urinary uranium concentration (0.2 for n = 100). For UACR, a sensitivity analysis was performed with a lower (0.5) and a higher (1.99) imputed value, which yielded essentially no different results.

In conclusion, occupational exposure to the air disaster in Amsterdam was neither significantly associated with higher uranium concentrations, nor with disturbances in kidney function parameters. In addition, at low urinary uranium levels of 2 ng/g creatinine, we found no statistically significant associations between urinary uranium concentrations and tubular or glomerular kidney function parameters. No indications of nephrotoxicity were found at urinary uranium concentrations around 2 ng/g creatinine.

Acknowledgements. The study was funded by the Dutch Ministry of Health, Welfare and Sports, the City of Amsterdam, the Amsterdam-Amstelland regional police force, and KLM Royal Dutch Airlines. The funding sources had no role in the collection, analysis, or interpretation of the data, nor in the decision to submit a manuscript for publication.

Conflict of interest statement. None declared.

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Received for publication: 30.9.06
Accepted in revised form: 13.6.07