

Health Consultation

EXPOSURE INVESTIGATION REPORT – III

FOUNTAIN INN/SIMPSONVILLE AREA
(a/k/a FOUNTAIN INN SUBDIVISION)

SIMPSONVILLE, GREENVILLE COUNTY, SOUTH CAROLINA

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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HEALTH CONSULTATION

**Follow-up Exposure Investigation
Retinol Binding Protein Analysis**

**SIMPSONVILLE/FOUNTAIN INN
(a/k/a FOUNTAIN INN SUBDIVISION)**

SIMPSONVILLE, GREENVILLE COUNTY, SOUTH CAROLINA

Prepared by:

**South Carolina Department of Health and Environmental Control
Division of Health Hazard Evaluation
Under a Cooperative Agreement with the
Agency for Toxic Substances and Disease Registry**

Background

Testing conducted by the South Carolina Department of Health and Environmental Control (SCDHEC) in January and February 2001 found elevated levels of uranium in water from some private wells in Simpsonville and Fountain Inn, South Carolina. By the end of April 2001, SCDHEC identified 30-40 wells that produced water with a uranium concentration above the Environmental Protection Agency's (EPA) drinking water Maximum Contaminant Level (MCL) of 30 micrograms per liter ($\mu\text{g/L}$).

On April 25 to 27, 2001, the Agency for Toxic Substances and Disease Registry, in conjunction with SCDHEC, Division of Health Hazard Evaluation (HHE), and the SCDHEC Appalachia II EQC District Office, conducted an Exposure Investigation (EI) in this community. The purpose of this first EI was to assess human exposure to uranium from drinking water. The results of this investigation documented the presence of elevated concentrations of uranium in water samples from many of the private wells that were tested. The findings, conclusions, and recommendations from this EI were presented in a previously released report (ATSDR, 2001).

ATSDR and SCDHEC-HHE conducted a second EI in October 2001. Since 90% of the residents in the first EI had elevated urine uranium levels, the purpose of this follow-up EI (EI-II) was to assess changes in body burdens of uranium and possible kidney effects. In the follow-up EI, a second urine sample was collected from the participants of the original EI and analyzed for uranium and retinol binding protein (RBP), a biomarker of possible damage to the kidney.

The concentrations of uranium in urine samples from the 79 participants in this EI-II ranged from 0.008 micrograms per liter ($\mu\text{g/L}$) to 6.65 $\mu\text{g/L}$. The average urine uranium concentration was 0.376 $\mu\text{g/L}$, and the median concentration was 0.124 $\mu\text{g/L}$. When normalized to creatinine concentration, the urine uranium concentrations in the participants of this EI ranged from 0.009 to 3.144 micrograms of uranium per gram of creatinine ($\mu\text{g/g}$). Details about the urine uranium portion of the Exposure Investigation are presented in the February 2002 ATSDR report (ATSDR, 2002).

Site Description

Simpsonville, South Carolina is located about 12 miles southeast of Greenville, South Carolina. Simpsonville occupies 14,301 square kilometers of land, and its population in 1999 was 11,708. The town of Fountain Inn is about 20 miles southeast of Greenville and about 6 miles from Simpsonville. Public water has been extended to most of the area, but was not completed by the time of the second exposure investigation.

The target population for this EI was the 105 residents who participated in the first EI. A total of 79 residents from the first EI (75 percent) volunteered to participate in this second EI. Details about the study participants can be found in the first Exposure Investigation report (ATSDR, 2001).

Staff from the SCDHEC met with the residents and gave a urine specimen cup to each participant. They were instructed to collect a first-morning void urine sample on the day of the appointment and to store it in a refrigerator until it was collected. Each participant was required to complete a written informed consent/assent form. SCDHEC staff also collected medical and exposure history information, which was used in interpreting the test results.

Biological Sampling and Analysis

On October 29 and 30, 2001, representatives of ATSDR and SCDHEC visited each home to collect urine samples from the participants for this EI. Details about sample collection are included in the February 2002 ATSDR Health Follow-up Consultation (ATSDR, 2002). The urine specimens were stored in a -20 degrees Celsius freezer until they were sent to the Industrial, Toxicology, and Occupational Medicine Unit at Catholic University of Louvain in Brussels Belgium. The samples were shipped on dry ice.

The samples were analyzed for retinol binding protein using the latex immunoassay methodology (Bernard et al., 1982). To test for urinary dilution, the urine samples were also analyzed for creatinine using an enzymatic assay.

Results

Seventy-nine of the original 105 residents participated in this second EI (EI-RBP). The average RBP concentration in urine was 155 micrograms per liter ($\mu\text{g/l}$). Results ranged from 19 $\mu\text{g/l}$ to 970 $\mu\text{g/l}$. When normalized to creatinine concentration, the average RBP concentration in urine was 119 $\mu\text{g/g}$. Results ranged from 28 $\mu\text{g/l}$ to 431 $\mu\text{g/g}$.

Creatinine is a metabolic product of skeletal muscle, and is excreted by the kidneys at a constant rate regardless of the rate that urine is produced. The results were normalized to creatinine concentration to enhance their accuracy. If the creatinine concentration is outside the normal range of 0.5 $\mu\text{g/l}$ to 3.0 $\mu\text{g/l}$, the urine sample may be either too dilute or too concentrated, respectively. In either case, the RBP concentration reported may not be accurate. Among the samples submitted for analysis, seven samples were too dilute (i.e. had urinary creatinine levels less than 0.5 $\mu\text{g/l}$).

Twenty-five of the 79 participants were children. However, only 24 samples were analyzed for RBP. One sample did not contain enough urine for RBP analysis. The mean RBP level for all children (age ≤ 18) was 123 $\mu\text{g/g}$ creatinine. RBP results for children ranged from 55 to 398 $\mu\text{g/g}$ creatinine. The mean RBP level for adults (age > 18) was 120 $\mu\text{g/g}$ creatinine. Thus, the mean RBP level for children was about the same as the adults.

These data for children were then divided by age group (< 10 years and 10-18 years). The mean RBP levels for children less than 10 years and between 10 - 18 years of age were 170 $\mu\text{g/g}$ creatinine and 89 $\mu\text{g/g}$ creatinine, respectively. The RBP levels ranged from 96 to 398 $\mu\text{g/g}$ creatinine in children younger than 10 years of age. The RBP levels ranged from 55 to 139 $\mu\text{g/g}$

creatinine in children between 10 and 18 years of age. Thus, mean RBP levels were higher for younger children when compared to older children and teens, and when compared to adults.

RBP is one of the biomarkers used as an early indicator of proximal tubular damage in the kidneys. Increased excretion of RBP indicates loss of tubular protein reabsorption capacity (ATSDR, 1998). The test will detect toxic effects to the kidney (nephrotoxicity) in workers with occupational exposures to heavy metals such as cadmium, mercury, and lead. Since uranium affects the same area of the kidney as these other heavy metals, RBP was used to assess kidney changes from uranium exposure.

According to Barton et al., 1997 and ATSDR, 1998, guidelines for interpreting RBP values were developed for workers exposed to cadmium. RBP concentrations in urine $<300 \mu\text{g/g}$ creatinine is considered a normal value. RBP values in workers ranging from $300 \mu\text{g/g}$ to $1000 \mu\text{g/g}$ have been associated with the beginnings of damage to the proximal tubule by cadmium (with the possibility that the effect is reversible if exposure stops) but no change in the rate of glomerular filtration of the kidney. RBP values ranging from $1000 \mu\text{g/g}$ to $10,000 \mu\text{g/g}$ have been associated with irreversible loss of protein through urine which may cause an accelerated loss in kidney filtration with age. RBP values greater than $10,000 \mu\text{g/g}$ have been associated with obvious tubular damage (from cadmium) associated with a decreased kidney filtration rate (Bernard, et al., 1997).

Only three samples exceeded $300 \mu\text{g/g}$; the highest RBP concentration was $431 \mu\text{g/g}$. This means that 96% of the study participants had no evidence of any harmful effects on the kidneys (as reflected by RBP values less than $300 \mu\text{g/g}$).

Discussion

RBP is a low molecular weight protein that is made in the liver to help carry retinol (i.e., vitamin A) to where it is needed in the body. Once it completes this task, it passes to the kidney where it filters through the glomerulus and is reabsorbed by the proximal renal tubule. There, it undergoes decomposition into amino acids and is returned to circulation for use by the body (Lapsley, 1998, Lehnbecher, 1998, Smith et al., 1994). Tubular reabsorption of RBP in a healthy kidney is nearly complete (99.97%) (Smith et al., 1994). Thus, increases in the urinary excretion of RBP is a sensitive indicator of tubular dysfunction, making it useful as a biomarker of harmful effects to the kidney. When a biomarker is continually altered (in this case, elevated) following long-term exposure, it is more likely to indicate a harmful effect (Bernard et al., 1997).

To properly interpret the RBP results, knowledge of the variation in a person's excretion rate of biochemical markers is needed (Bernard et al., 1997). Recent studies suggested a mean RBP biological intraindividual variation of approximately 40% in both normal and proteinuric subjects from spot urine samples over a week (Mason et al., 1998). This wide variation of excreted RBP suggests that care must be taken before concluding that progressive loss in kidney function has been detected. When monitoring individuals at risk, only a persistent change of a biomarker is likely to reflect a clinically significant effect (Bernard et al., 1997).

Only three urine samples had RBP levels above the value considered to be normal (that is, 300 $\mu\text{g/g}$ creatinine). Assuming a 40% intraindividual variation, two of the three RBP levels above 300 $\mu\text{g/g}$ and eight of the RBP levels below 300 were within this percent of variability.

Several situations such as pregnancy, heavy exercise or orthostatic proteinuria, may cause a transient, completely reversible increase in urinary excretion of some proteins or enzymes (Bernard, et al., 1997). The following health conditions/diseases may also cause an elevation of RBP levels: Fanconi syndrome (tubular reabsorption is negligible in this disease), acute tubular necrosis, interstitial nephritis, asymptomatic low molecular weight proteinuria, heavy metal exposures to cadmium, mercury, lead, use of aminoglycoside antibiotics (eg tobramycin, gentamicin, neomycin, streptomycin), use of vancomycin, use of ifosfamide (a chemotherapy agent), kidney diseases involving the glomerulus, urinary tract infections, kidney damage from recurrent urinary reflux, fever, insulin-dependent diabetes mellitus (with accompanying kidney damage) (Tomlinson, 1992). In addition, elevated RBP levels have been associated with Chinese herbs used for weight loss. A secondary contaminant of the herbal preparation was believed to be responsible (Bernard, et al., 1997).

The normal range of RBP in urine (less than 300 $\mu\text{g/g}$) was developed from data on adult workers exposed to cadmium. These data may not be applicable to children. Several investigators have attempted to develop normal ranges of kidney biomarkers, including RBP, for children. RBP ranges reported in the literature, varied considerably (Lapsley et al., 1998; Lehmbecher et al., 1998; Smith et al., 1994; Bangstad et al., 1995; Tomlinson et al., 1990). Four studies had relatively similar upper ranges from 193-262 $\mu\text{g/g}$ creatinine for RBP (Lapsley et al., 1998; Bangstad et al., 1995; Tomlinson et al., 1990). One study with the same analytical methodology for RBP analysis as our investigation, reported 262 $\mu\text{g/g}$ creatinine as a RBP upper limit of normal for children between ages of 12 and 15 years (Bernard et al., 1995). Only one child among the 24 children exceeded these values in our investigation. A repeat of the RBP level and further medical follow-up is warranted for this child.

Other investigators reported quite different ranges for children (Lehmbecher et al., 1998; Smith et al., 1994). For instance, Smith et al., 1994, reported a RBP upper limit of normal for children between the ages of 2-16 years as 440 $\mu\text{g/g}$ creatinine. The RBP upper limit of normal for children between ages 6 months to 2 years was reported as 907 $\mu\text{g/g}$ creatinine. Much of the difference in RBP levels between this study and the others can be explained by the inclusion of much younger children (less than 2 years of age). When age groups were stratified in this study, the RBP upper limit of normal for children aged 5-10 years was reported as 361 $\mu\text{g/g}$ creatinine, and 282 $\mu\text{g/g}$ creatinine for children aged 10-16 years. For comparison, only one child between the ages of 5-10 years exceeded the Smith et al., 1994, RBP upper limit of 361 $\mu\text{g/g}$ creatinine in our investigation. Among the 10-17 year age group, none of the children in our investigation exceeded the Smith et al., 1994, RBP upper limit of normal. Another study reported markedly different normal RBP ranges, with RBP upper limits in the thousands of $\mu\text{g/g}$ (Lehmbecher et al., 1998). Despite the differences in these two studies, both reported that RBP levels declined with age.

A significant fall in RBP seems to occur within the first few months to first year of life (Lehrnbecher et al., 1998; Smith et al., 1994). It is believed that the decline in RBP excretion with age may reflect the normal development of the proximal tubule and the increased reabsorption of the protein (Smith et al., 1994). In general, many of the studies report that children have higher RBP levels than adults. Our results also support the findings in the literature. Among the 24 children in this investigation (EI-RBP), the RBP levels did show an age-dependent decline.

In light of this information, RBP excretion rates in children should be interpreted with the age of the child in mind, as well as any other factors that may contribute to rate of excretion of the RBP. Even if RBP levels greater than 300 $\mu\text{g/g}$ creatinine truly reflect a harmful effect to the kidney from uranium exposure, the changes or effects may be transient and reversible after exposure stops (Bernard et al., 1997). It is not known how long it takes for the changes in the proximal tubule to return to normal.

The health impact, if any, of long-term exposure to uranium is not known. Studies of workers with occupational exposure to uranium have not demonstrated convincing epidemiological evidence of serious renal disease or other health effects (ATSDR, 2002). However, these studies had limited statistical power to detect an increased rate of disease, if it had been present (ATSDR, 2001). Also, the healthy worker effect may prevent full disease expression.

Other than for cadmium, the usefulness of tests, such as the RBP, to detect early harmful effects to the kidney that lead to kidney failure from other toxic substances are not known. Prospective, longitudinal studies are needed to assess the ability of these tests to detect harm from exposure to other environmental agents, like uranium. It is important to note that an elevated kidney biomarker is not always associated with an increased risk of progression to kidney failure. A one-time elevation in a biomarker might merely represent a transient and reversible effect, and not necessarily be an indicator of future, irreversible kidney damage. However, persistent elevations in these biomarkers should be interpreted as changes in normal kidney function that might be considered early indicators of abnormal function leading to increased risk of disease. Such indicators might help identify groups or individuals who required additional evaluation and follow-up (ATSDR, 1998).

Reporting Results

SCDHEC provided the participants with their individual test results and an explanation of their meaning. A toll-free telephone number was also provided so participants and their health care providers could contact SCDHEC to further discuss their test results.

Conclusions

The majority (96%) of people whose urine was analyzed for RBP had normal levels in their urine indicating that people exposed to uranium had either no adverse effects on the kidneys or the effects were reversible. However, three of the samples were above the normal range and this EI

represents only a one time spot sample and further follow-up is warranted. The majority of people who participated in both of the ATSDR/SCDHEC Exposure Investigations did have urine uranium levels above the 90th percentile of the background United States population. Additional follow-up is warranted to determine the long-term effects, if any, that could result from this exposure.

The participants in the ATSDR/SCDHEC Exposure Investigations were exposed to uranium in their drinking water at concentrations that substantially exceeded the EPA's Maximum Contaminant Level. Therefore, past exposures to uranium posed a public health hazard. At this time, most of the study participants are either using bottled water, have an in-home treatment device, or have been supplied with public water. It cannot be determined whether past exposures to uranium pose any current public health hazard.

Recommendations

1. Residents with elevated levels of RBP should consult with their own physician for follow-up and possible referral to a nephrologist.
2. SCDHEC-HHE will follow-up with the people who had elevated levels of RBP.
3. Residents with elevated levels of RBP will have their wells tested for other heavy metals (i.e. cadmium, mercury, lead) by SCDHEC.
4. Residents with uranium-contaminated wells should continue to use alternate sources of water for potable use until public water is available or an appropriate water treatment system has been installed.

Public Health Action Plan

Planned

1. SCDHEC will submit a proposal to obtain funding from ATSDR to conduct a community Health Investigation. This study will assess the health impact of exposure to uranium from drinking water on the residents of Simpsonville/Fountain Inn.
2. ATSDR and SCDHEC will develop educational materials on the health effects of exposure to uranium and medical testing for uranium-related disease. Relevant information will be developed in printed and electronic formats for the general public and health professionals.

Completed

1. After completion of the first EI, SCDHEC held a public availability session to discuss (1) groundwater contamination with uranium in the Simpsonville area, (2) options for home treatment to remove uranium from groundwater, (3) installation of public water lines, and (4) to respond to questions from the EI participants about their individual test results.
2. A physician from SCDHEC-HHE met with physicians in Greenville County to discuss the health effects of exposure to uranium and the medical evaluation of uranium-exposed residents.

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