

## Possible linkages between lignite aquifers, pathogenic microbes, and renal pelvic cancer in northwestern Louisiana, USA

Joseph E. Bunnell · Calin A. Tatu · Rebecca N. Bushon · Donald M. Stoeckel · Amie M. G. Brady · Marisa Beck · Harry E. Lerch · Benton McGee · Bradford C. Hanson · Runhua Shi · William H. Orem

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**Abstract** In May and September, 2002, 14 private residential drinking water wells, one dewatering well at a lignite mine, eight surface water sites, and lignite from an active coal mine were sampled in five Parishes of northwestern Louisiana, USA. Using a geographic information system (GIS), wells were selected that were likely to draw water that had been in contact with lignite; control wells were located in areas devoid of lignite deposits. Well water samples were analyzed for pH, conductivity, organic compounds, and nutrient and anion concentrations. All samples were further tested for presence of fungi (cultures maintained for up to 28 days and colonies counted and identified microscopically) and for metal and trace element concentration by

inductively-coupled plasma mass spectrometry and atomic emission spectrometry. Surface water samples were tested for dissolved oxygen and presence of pathogenic leptospiral bacteria. The Spearman correlation method was used to assess the association between the endpoints for these field/laboratory analyses and incidence of cancer of the renal pelvis (RPC) based on data obtained from the Louisiana Tumor Registry for the five Parishes included in the study. Significant associations were revealed between the cancer rate and the presence in drinking water of organic compounds, the fungi Zygomycetes, the nutrients  $\text{PO}_4$  and  $\text{NH}_3$ , and 13 chemical elements. Presence of human pathogenic leptospires was detected in four out of eight (50%) of the surface water sites sampled. The present study of a stable rural

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J. E. Bunnell · C. A. Tatu · M. Beck ·  
H. E. Lerch · W. H. Orem  
Eastern Energy Resources Team, USGS, US  
Department of the Interior, Reston, VA, USA

C. A. Tatu  
Department of Immunology, University of Medicine  
and Pharmacy, Timisoara, Romania

R. N. Bushon · D. M. Stoeckel · A. M. G. Brady  
Water Resources Discipline, USGS, Ohio District  
Microbiology Laboratory, Columbus, OH, USA

B. McGee  
Water Resources Discipline, USGS, Louisiana  
District, Ruston, LA, USA

B. C. Hanson  
Louisiana Geological Survey, Baton Rouge, LA, USA

R. Shi  
Louisiana State University School of Medicine,  
Shreveport, LA, USA

J. E. Bunnell (✉)  
USGS, 956 National Center, 12201 Sunrise Valley  
Drive, Reston, VA 20192, USA  
e-mail: jbunnell@usgs.gov

population examined possible linkages between aquifers containing chemically reactive lignite deposits, hydrologic conditions favorable to the leaching and transport of toxic organic compounds from the lignite into the groundwater, possible microbial contamination, and RPC risk.

**Keywords** Leptospires · Lignite aquifers · Medical geology · Natural water contamination · Renal pelvic cancer

## Introduction

The state of Louisiana, which is situated between the states of Texas and Mississippi along the shores of the Gulf of Mexico in the southeastern USA, has the sixth highest mortality rate for cancer of the renal pelvis (RPC) in the United States (Ries et al., 2001). Many rural residents in the northwestern part of the state rely on well water for drinking, cooking, and other domestic uses. Northwestern Louisiana contains sizeable lignite (low-rank coal) deposits associated with fresh water aquifers. These deposits naturally contain nephrotoxic compounds that may potentially contaminate ground water used as residential water supplies. The possibility of a link between lignite deposits, drinking water derived from rural wells, and RPC in Louisiana has been proposed based on the results of investigations that have been carried out in Eastern Europe into Balkan endemic nephropathy (BEN) and urothelial cancers and urinary tract cancers such as RPC (Tatu, Orem, Finkelman, & Feder, 1998). BEN occurs in restricted geographic areas characterized by lignite deposits, stable rural populations, and untreated, non-municipally supplied water sources (Tanchev, Evstatiev, Dorossiev, Penchava, & Zvetkov, 1956). The etiology of BEN has not been firmly established, is possibly multifactorial (Toncheva, Dimitrov, & Stojanova, 1998), and may involve toxic organic compounds such as nitrogen-containing aromatic hydrocarbons (Orem et al., 2004). These hydrocarbons have been identified in samples of lignite and well water from endemic regions (Orem & Tatu, 2001; Orem et al., 2002; Tatu et al., 2000). Previous investigations have identified markedly different

suites of compounds in water of known BEN areas as compared to non-endemic area control samples (Tatu et al., 2000). Microbial pathogens and their products, such as fungal ochratoxin-A (OTA), have also been implicated in the causation of BEN (Abouzied et al., 2002; Fillastre, 1997; Pfohl-Leszkowicz, Petkova-Bocharova, Chernozemsky, & Castegnaro, 2002; Stoev, 1998; Uzelac-Keserovic et al., 1999). However, studies performed in Romania were unable to find OTA or OTA degradation products in biological samples (urine) collected from BEN patients (C. Tatu, unpublished data).

“Two-hit hypotheses,” such as interactions between toxic organic compounds and microbial pathogens both being necessary co-factors in RPC, are well documented in the cancer literature and have been invoked, for example, in the context of kidney disease (see Knudson, 1996; Pei, 2001). The association between BEN and urinary tract tumors was also recognized early on (Petrsinska-Venkovska, 1960). Thus, BEN is not only a nephrological problem but also an oncological one. Petrsinska-Venkovska (1960) reported that of 33 BEN patients studied, 48.4% developed urinary-tract tumors. Tanchev, Naidenov, Dimitrov, and Karlova (1970) reported a similar finding, with 16% of the BEN patients in their study developing urinary-tract tumors. More recent evidence suggests that up to one-half of all BEN patients suffer from these cancers (Orem & Tatu, 2001; Radonic & Radosevic, 1992). The relative risk of developing urinary-tract tumors among residents of BEN-endemic villages was calculated to be as high as 29 in an epidemiological study in the Vratza district, Bulgaria (Nikolov et al., 1996). Chernozemsky et al. (1977) noted a geographic correlation between BEN and renal tumors in Bulgaria and Serbia. A similar correlation has been found in Romania (Minciu, Botoca, Cumpanas, & Hodor, 2003). The prognosis for RPC is generally poor, and successful treatment rare (Kirkali & Tuzel, 2003).

Low-rank lignite deposits occur in several parishes of northern Louisiana, with lignite actively being mined in DeSoto and Red River Parishes (Chawner, 1936; Meagher & Aycock, 1942; Roland, Jenkins, & Pope, 1976; Snider, 1982). Most of the lignite occurs in Lower Tertiary strata (Mea-

gher & Ayccock, 1942; Roland et al., 1976). Similar lignite deposits extend from Texas, passing through parts of Arkansas, Louisiana, and Mississippi to terminate in Georgia (Tully, 1996); these occur chiefly in the Wilcox Group and, to a lesser extent, in the overlying stratigraphic units of the Claiborne and Jackson Groups (Roland et al., 1976). A complete list of Louisiana lignite localities was compiled in 1921 (Glenk), including every location where lignite was known to outcrop. At present, lignite outcrops have been reported in 13 parishes in northern Louisiana, including DeSoto, Natchitoches, and Red River. However, the issue is not one of mere presence as a surface outcrop, but whether lignite deposits are present in freshwater aquifers currently used by the general population. Of particular interest, Glenk (1921) also compiled a list of localities where both water wells and oil wells occurred in lignite deposits. One of these, the Tertiary-Age freshwater Carizzo-Wilcox Aquifer, serves parts of eight parishes in northern Louisiana at a ground water use rate of about 15 million gallons per day (Sargent, 2002).

In the present study, we examined the hypothesis that rural well water in northwestern Louisiana is contaminated with lignite aquifer-derived toxic, organic compounds. Furthermore, we hypothesized that at low concentrations and when consumed over a long period of time, possibly in combination with microbial pathogens or their products and in association with well or surface water, such organic compounds may cause or contribute to RPC in this region. Study site selection and analysis required geologic, geochemical, microbiological, and epidemiological approaches. This preliminary investigation was designed to assess the feasibility of – and justification for – a more comprehensive analysis using non-aggregated (Parish level) cancer data. Further research is needed to more rigorously test the epidemiological associations and the biological plausibility of the proposed exposure route and resulting disease.

## Materials and methods

In May, 2002, 14 private drinking water wells and four surface water sites (Fig. 1) were sampled. To

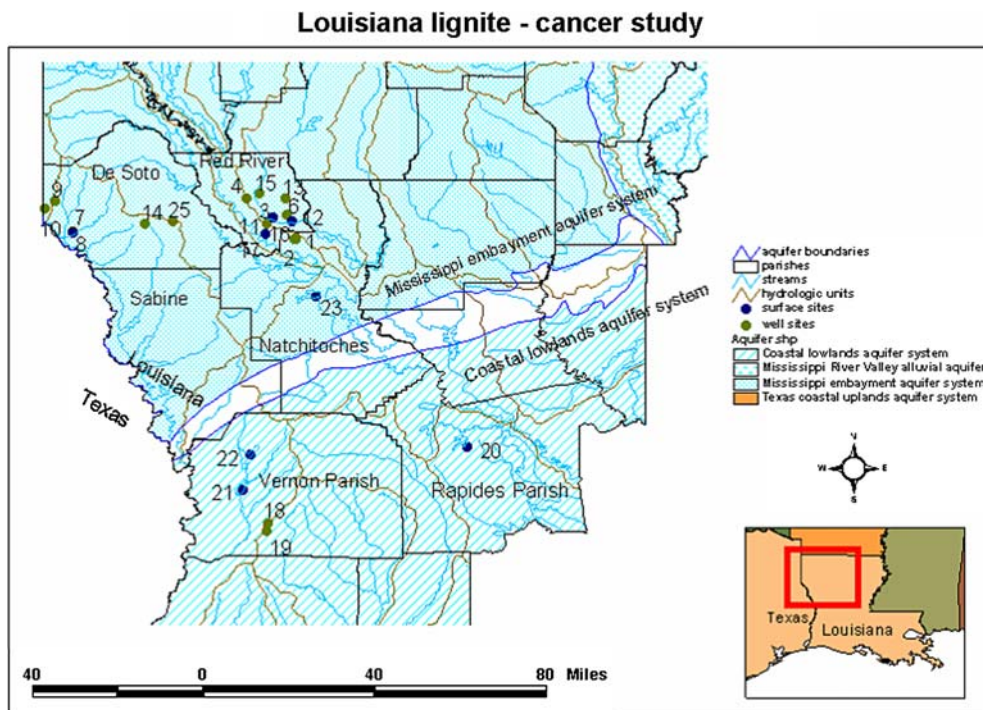
address seasonal variability that might lead to differences in precipitation and ground water activity during different times of the year, we selected a second sampling time point roughly 6 months from the initial sampling event. Thus, in September, 2002, those same wells and sites plus four additional surface water sites were sampled in five parishes (DeSoto, Natchitoches, Rapides, Red River, and Vernon) of northwestern Louisiana. Additionally, dewatering well water and lignite samples were collected from an active coal mine in Red River Parish. A geographic information system (GIS) was used to select 12 residential water wells located in the lignite aquifer area that were likely to be drawing water directly from or just below lignite beds based on the following themes: well depth, lignite bed thickness, amount of overburden, depth of lignite bed below surface, mining lease areas, and geology. Additionally, computer software (STRATIFACT, ver. 4.57; Stratifact Software, Wheat Ridge, Colo.) was used to compare known depths and thicknesses of lignite deposits (based on geophysical well logs) with drinking water well locations and depths (data on file at the Louisiana District Office of USGS, Ruston, LA.). In both May and September, two of the 14 private wells in Vernon Parish served as “control wells,” since they were located in the coastal lowlands aquifer (all of the sample wells were located in the Mississippi embayment aquifer system), and coincided with neither the Wilcox Group nor any known lignite (or coal) beds.

Field and laboratory methods for this type of study have been described in an earlier publication (Bunnell et al., 2003). Briefly, the following methods were employed.

### Water samples

#### *Inorganic geochemistry*

A Thermo Orion (Beverly, Mass.) semi-micro pH electrode with temperature compensation probe was used to measure pH. Conductivity, salinity, total dissolved solids, and temperature were read simultaneously from the collection bottle using a Thermo Orion conductivity cell. Dissolved oxygen (DO) and temperature readings were



**Fig. 1** Map of study area showing the locations and identification numbers of well and surface water sample collection sites. *Green dots* Wells, *blue dots* surface sites,

hydrologic units, and aquifers. Digital source of data for hydrologic units and aquifer boundaries: the National Atlas of the United States (<http://www.nationalatlas.gov>)

taken at surface water sites only using a YSI (Yellow Springs, Ohio) model 55 handheld DO meter. Fluoride ( $F^-$ ), chloride ( $Cl^-$ ), bromide ( $Br^-$ ), nitrate ( $NO_3^-$ ), and sulfate ( $SO_4^{2-}$ ) concentrations were measured by ion chromatography using a Waters (Milford, Mass) 2795 Separations Module. Anions were analyzed by separating samples with an eluent of 1.7 mM bicarbonate/1.8 mM carbonate (EZ-LUTE; Alltech, Deerfield, Ill.) through an Alltech Allsep Anion Column.  $F^-$ ,  $Cl^-$ , and  $SO_4^{2-}$  were detected using a Waters 432 conductivity detector;  $Br^-$  and  $NO_3^-$  were detected with a Waters 486 Tunable Absorbance Detector at 210 nm. Chromatogram peaks were identified and areas calculated with Waters MILLENNIUM<sup>32</sup> software, ver. 4.00. Phosphate ( $PO_4^{3-}$ ) and ammonium ( $NH_4^+$ ) concentrations were determined colorimetrically at 885 nm and 640 nm, respectively, using a Brinkman PC 900 fiber optic spectrophotometer. Metal and trace element concentrations were measured by inductively-coupled plasma mass spectrometry (ICP-MS) and atomic emission spectrometry (IC-

AES) using an ELAN 6000 (Perkin-Elmer/Sciex, Toronto, Ont., Canada) instrument and a model 3300 DV (Perkin-Elmer), respectively.

#### *Organic geochemistry*

Organic compounds were isolated from the water samples using a liquid/liquid extraction technique in conjunction with pesticide grade dichloromethane. To identify and quantify the extracted organic compounds, samples were analyzed by gas chromatography (GC)/mass spectrometry analysis using a Hewlett Packard (HP) 6890 (Agilent Technologies, Rockville, Md.) and a 5973 mass selective detector (Hewlett Packard/Agilent) set in the SCAN mode for a range of 50–650  $m/z$  (atomic mass to charge ratio). The compounds were separated on an HP-5MS (5% phenyl methyl siloxane) column (30 m × 0.25 mm × 0.25  $\mu$ m). The peak identifications were made using the National Institute of Standards and Technology 2002 and Wiley 7.1 reference libraries.

## Microbiological analyses of water samples

### *Fungi*

Collected samples were aliquotted through pre-sterilized 0.45- $\mu\text{m}$  pore size, 47-mm diameter mixed cellulose ester filters (Advantec MFS, Pleasanton, Calif.). Filter membranes were removed from the funnel and placed on Sabouraud Dextrose Agar (SDA) Emmons Modification, supplemented with chloramphenicol, and incubated for a minimum of 7 days at 22°C. *Aspergillus* and *Penicillium* cultures, obtained from the American Type Culture Collection, were used as positive controls. For each isolation the date of transfer and morphological characteristics, such as color, shape/size, texture, reverse color, and pigmentation in the agar, of the colony on the membrane filter were recorded. Fungi were subcultured onto a potato dextrose agar (Sigma, St. Louis, Mo.) slant for long-term storage at  $-70^{\circ}\text{C}$  (Larone, 2002). Sample fungi were examined microscopically.

### *Leptospira spp.*

Samples were filtered through a series of 0.65- $\mu\text{m}$ , 0.45- $\mu\text{m}$ , and 0.20- $\mu\text{m}$  pore size, 47-mm diameter mixed cellulose ester filters to remove particulates. Cells were harvested from the filtrates by centrifugation at 15,000  $g$  for 15 min, resuspended in 100  $\mu\text{l}$  of phosphate buffered saline, and stored at  $-70^{\circ}\text{C}$ . Following DNA extraction, PCR was performed using pathogenic leptospire primers and conditions described in Gravekamp et al. (1993) and Woo et al. (1997). Purified *Leptospira* DNA obtained from the Centers for Disease Control and Prevention (CDC) reference laboratory was used as the PCR positive control. Gel electrophoresis (2% agarose matrix with  $1 \times \text{TAE}$  buffer) was used to detect leptospiral DNA.

### Coal sample

The lignite sample was collected from the Chemard Lake lignite area of the Naborton Formation, Wilcox Group, Paleocene (channel sample location:  $32.00847^{\circ}\text{N}$ ,  $93.60043^{\circ}\text{W}$ ;

approximately 328 feet a.s.l.). The raw sample was sent to a commercial lab (Geochemical Testing, Somerset, Pa.) for grinding, sieving, and bulk chemical analysis. The sample was extracted for 24 h using 150 ml of pesticide analysis grade dichloromethane (DCM) in a Soxhlet extraction apparatus and analyzed using an Agilent 6890 GC interfaced with an Agilent 5973 Mass Selective Detector (MSD).

### Statistical analysis

Statistical software SAS ver. 8.2 (SAS Institute, Cary, N.C.) was used to analyze the data. Individual measurements of data collected in May and September, 2002, from the same wells at each of the two sample collection timepoints were averaged to correct for possible seasonality confounding and to increase robustness. The incidence of RPC was derived from total number of RPC cases divided by total number of inhabitants in that parish at the same period. The average value of variables was calculated from the several measurements of point and/or time period in that parish. The Spearman correlation method was used to assess the association between the endpoints for these field and laboratory analyses and RPC. Cancer incidence data were obtained from the Louisiana Tumor Registry (<http://publichealth.lsuhs.edu/tumorregistry>).

## Results

The complete set of raw data has been presented in Bunnell et al. (2003; see Table 8). Data from the 12 residential water wells and the two Vernon Parish control wells were included in the statistical analysis. Because the mine dewatering well sample was not collected at the same time, and there were no microbiological, nutrient, or anion data collected from the lignite sample, this sample was evaluated qualitatively. While recognizing certain limitations in the present data set, such as an unequal distribution of sampling points within the four parishes in northwestern Louisiana (e.g., only two well water samples were taken as controls in Vernon Parish), we observed significant associations between the presence of organic

compounds and other water parameters measured between sample and control wells and RPC incidence. There were no characteristics indicative of exposure to lignite leachates observed in the control well water samples analyzed.

The Spearman rank coefficient method was used to test for correlations between RPC incidence and more than 80 measured environmental parameters (including DO, pH, temperature, salinity, conductivity, total dissolved solids, phosphates, ammonium, five anions, 46 inorganic elements and metals, 29 species or classes of fungi, leptospire, total number of organic compounds, and relative concentrations of organics). Significant associations were revealed between the cancer rate and the overall number of organic compounds, the fungi Zygomycetes, the nutrients  $\text{PO}_4$  and  $\text{NH}_3$ , and 13 chemical elements (As, B, Br, Cl, Cr, F, Li, Na, P, Rb, Se, Sr, and W) from the well water (Table 1). Specific organic compounds that have been preliminarily identified include propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester, drometrizole, and a number of nitrogen-containing polycyclic aromatic hydrocarbons.

**Table 1** Spearman correlation coefficients and  $p$  values for parameters significantly associated with incidence rate of cancer of the renal pelvis (RPC) in Red River, Natchitoches, DeSoto, and Vernon Parishes in northwestern Louisiana

Parameter <sup>a</sup>	Coefficient ( $\beta$ )	$p$ value
Element: boron (B)	1.0000	<0.0001
Element: chlorine (Cl)	1.0000	<0.0001
Element: chromium (Cr)	1.0000	<0.0001
Element: fluorine (F)	1.0000	<0.0001
Element: phosphorus (P)	1.0000	<0.0001
Element: rubidium (Rb)	1.0000	<0.0001
Element: sodium (Na)	1.0000	<0.0001
Element: strontium (Sr)	1.0000	<0.0001
Nutrient: ammonium ( $\text{NH}_3$ )	1.0000	<0.0001
Nutrient: phosphate ( $\text{PO}_4$ )	1.0000	<0.0001
Organic compounds: overall number	1.0000	<0.0001
Element: arsenic (As)	0.9487	0.0513
Element: bromine (Br)	0.9487	0.0513
Element: lithium (Li)	0.9487	0.0513
Element: selenium (Se)	0.9487	0.0513
Element: tungsten (W)	0.9487	0.0513
Fungi: Zygomycetes	0.8944	0.1056

<sup>a</sup> All other parameters analyzed had  $p$  values equal to or greater than 0.2000

Among the species of fungi from the total of 136 isolates cultured were 12 *Penicillium* spp., at least two *Aspergillus* spp., a number of other genera (*Alternaria* sp., *Eupenicillium lapidosum*, *Cladosporium* sp., *Epicoccum* sp., *Trichoderma* sp., *Paecilomyces* sp., *Chrysosporium* sp., *Chloridium* sp.), and the classes Zygomycetes and Coelmycetes. The two control wells yielded a mean of 6.5 (SD: 3.5) individual isolates, while the mean number of isolates from all other sites was 7.6 (SD: 4.5). Evidence of human pathogenic leptospire was detected in four of eight (50%) surface water samples.

## Discussion

Among the factors that were found to be statistically associated with RPC incidence, there is more supporting evidence in the literature for biological plausibility of the presence and relative concentration of organic compounds present in the water samples than any of the inorganic compounds, and zygomycetes are not known to produce OTA (R. Vilgalys, personal communication). Nitrogen-containing aromatic compounds were found in many of the well water samples; such compounds are similar in structure to acetaminophen, which has been associated with chronic renal failure (Fored et al., 2001). By contrast, none of the inorganic chemical elements found to correlate with RPC incidence in this study are known to be related in a positive dose-response manner to kidney disorders. Exposure to ochratoxins and leptospire, both found in many of the well and surface water samples tested, may contribute to kidney compromise and RPC susceptibility in this region. The lignite sample collected from the mine in the same area as the sample wells generated a total ion current chromatogram that was very similar to that of the water samples in this study, containing many more peaks and higher concentrations of organics than higher rank coal samples.

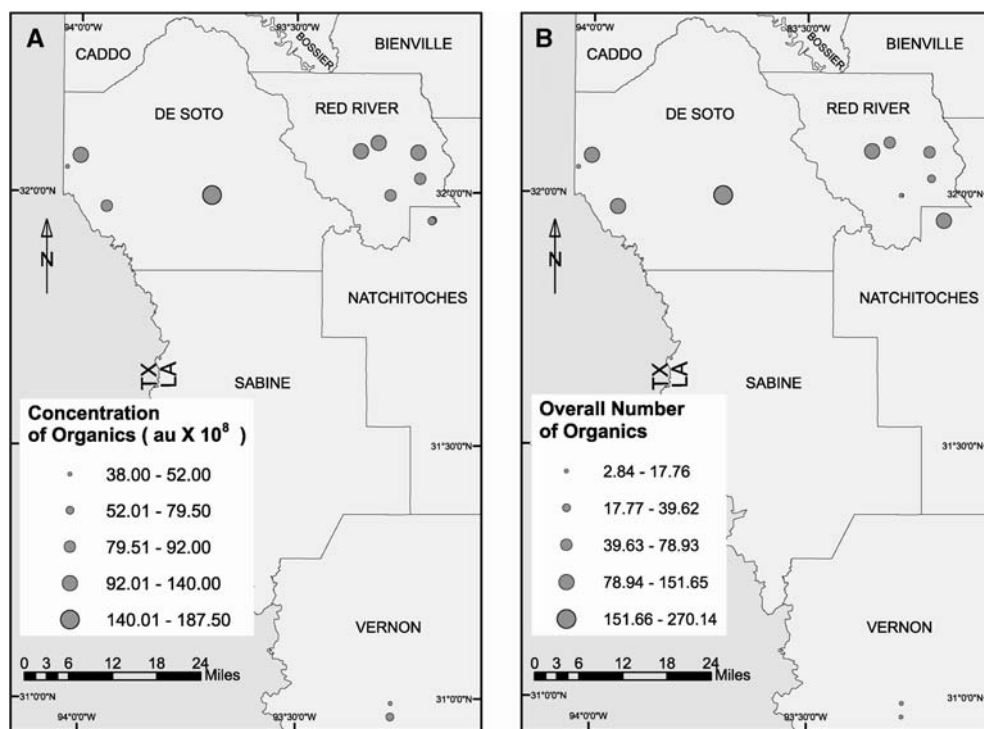
The mean relative (summed) concentration of organic compounds for the sample wells, 8.4 absorbance units (au)  $\times 10^9$ , was greater than that of the control wells, 6.4 au  $\times 10^8$  (Fig. 2a). The overall mean number of organic compounds

detected in the sample wells was 104.9, while that of the control wells was only 60.0 (Fig. 2b). When those organic data were statistically correlated with RPC incidence, a strong association between the overall number of organic compounds extracted from the water ( $\beta=1.0000$ ,  $p<0.0001$ ) was observed, as was a slightly weaker association between RPC incidence and overall relative concentration ( $\beta=0.8000$ ,  $p=0.2000$ ). This finding is noteworthy because many of the compounds accounting for the organic compound concentration in the control (as well as the test well) water samples were not lignite aquifer-derived; rather, they were identified as anthropogenic phthalates and phthalic derivatives, ubiquitous compounds (plasticizers) commonly found in relatively high concentrations in well water. These compounds are thus more likely over-represented with respect to their relative concentration values, but the overall number of compounds is not.

The fact that the number of individual compounds (or classes of compounds) in the

samples versus controls were significantly correlated to RPC incidence, with the relative concentrations less strongly associated, suggests that only a small subset of the organic compounds present in the well water actually contributes to cancer risk. The fact that many of the phthalate peaks, while few in number, are relatively high (corresponding to their generally high concentrations) in the control samples suggests that the lignite-derived organic compounds, and not the more uniformly distributed naturally-occurring or anthropogenic organics (phthalates), are associated with RPC incidence. If this were not the case, one would likely not observe a statistically significant difference between the sample and control groups for either organic measure (number or concentration of compounds).

The phthalates that were identified in the water samples do not represent post-collection contamination, but they may be considered to be confounders. Our intention was to analyze the water that residents were actually consuming



**Fig. 2** Relative concentration (in absorbance units, au) (a) and overall number (b) of organic compounds found in well water samples. Note that by both measures, well

water from the northern samples is appreciably richer in organic compounds than the Vernon Parish samples which are not in the lignite area

(while being as unobtrusive as possible; thus water was generally sampled from outdoor spigots rather than from kitchen sinks), which in most cases meant that the water contacted plastic at some point(s) between extraction from the lignite aquifer and ingestion. Such potential contacts included PVC well casings and any plastic parts in the pump or delivery system (e.g., spigots, tubing, hoses, etc.). Subtracting out the phthalate peaks from the total ion chromatograms would run the risk of inadvertently deleting smaller peaks masked by the phthalates – peaks that could represent lignite-derived hydrocarbons with molecular mass to charge ratios similar to those of the phthalates.

Some of the other factors, such as ochratoxin-producing fungi and inorganic chemical elements, may contribute to kidney disease, but these do not account for the apparent geographic association of RPC incidence to lignite deposits. At a cellular level, necrosis of kidney tissue may in fact be a more likely outcome of OTA exposure rather than tumorigenesis, as it induced apoptosis in an *in vitro* experimental study (Schwerdt, Freudinger, Mildenerger, Silbernagl, & Gekle, 1999). However, OTA was found to have possible carcinogenic potential to renal tubular cells in a rat model (Boorman, 1989).

In none of the well water samples tested was the concentration of any trace metal found to be at a level higher than the US Environmental Protection Agency's (EPA) maximum contaminant level. The EPA does not promulgate criteria for private residential wells as they do for municipal utilities, but EPA utility criteria are mentioned for comparison. Of the thirteen chemical elements found to be significantly linked to RPC incidence in our analysis, two (chromium and strontium) were measured in the remains of BEN fatalities in a previous investigation in the Balkans (Makarov, Topakbashyan, & Dinev, 1967). In that study, Cr was detected at elevated levels in only three of 16 samples (Makarov et al., 1967). An environmental source of Cr in hemodialysis patients cannot be assumed, as needles used in that treatment procedure release substantial amounts of Cr to the body (Gallièni, Brancaccio, Cozzolino, & Sabbioni, 1996). Additionally, trivalent Cr is not nephrotoxic (Baruthio,

1992). The other elements found to be present at high levels (Al, Zn, Cd, Cu, Ni, Pb, and Mn) in that earlier study (Makarov et al., 1967) were not significantly associated with RPC incidence in our analysis. Even the detection of elevated levels of nephrotoxic trace elements in chronic renal failure patients, however, is not indicative of causality, as such elements tend to accumulate in individuals with declining kidney function (Vanholder, Cornelis, Dhondt, & Lameire, 2002).

Among those 13 elements found to be positively correlated with RPC in our study, only (excess) lithium is known to contribute to kidney disease. While we did find selenium (Se) in the water samples to be positively correlated with RPC incidence, there was no difference in Se levels in the serum of dialyzed patients versus nondialyzed controls in an earlier study (Zima, Tesar, Mestek, & Nemecek, 1999). Moreover, Se in the water samples tested was positively correlated to RPC, but Se *deficiency* has been suggestively linked to kidney cancer and BEN (Lindeman, 1989; Maksimovic & Djujic, 1997). Thus, the evidence for trace elements as a major etiologic factor for BEN and/or RPC seems less clear than that for lignite-derived polycyclic organic compounds.

The EPA also issues secondary standards for contaminants that are not considered to be health threats, but rather have nuisance characteristics, such as unpleasant odor, color, or taste. Only one well, in both May and in September, 2002, exceeded EPA secondary standards for nitrates.

The risk of developing kidney cancer or other chronic conditions leading to end-stage renal failure due to long-term exposure to low levels of naturally occurring nephrotoxic organic xenobiotics could be elevated in individuals having been infected at an early age by a nephrotropic microbial pathogen, such as *Leptospira* spp., the bacterial etiologic agent of leptospirosis, or Weil's disease. Conversely, a history of long-term exposure to low levels of nephrotoxic organic compounds could predispose a leptospirosis patient to a more severe, fulminant disease course upon infection. Interestingly, four of the eight (50%) surface water sites sampled in our study area were found to have DNA evidence of the presence of pathogenic leptospires. Thus, the possibility for



exposure to this bacterium exists in areas where lignite deposits interact with groundwater.

It has been suggested that there may be a genetic component to RPC susceptibility (Blot & Fraumeni, 1979). While the present investigation did not attempt to address this question, a given sample of residents in a relatively sparsely populated rural setting like northwestern Louisiana may possibly be characterized by less genetic heterogeneity in general than a similar sample size of randomly selected residents in larger cities because of the former populations' relative long-term stability and lower ethnic diversity. Genetic susceptibility in relation to metabolism of the xenobiotic compounds could also be a controlling factor in the induction of renal and urothelial cancer in certain groups of residents from Louisiana.

This preliminary investigation into environmental factors that might cause or contribute to the development of RPC identified several variables that should be tested in future studies for biological plausibility. One limitation of the present analysis is that tumor registry data were available only at the Parish level of geographic scale. Future studies are planned that will take advantage of the fine geospatial referencing of the environmental database by incorporating cancer data at a finer scale of resolution (e.g., aggregated at the census block level) that is expected to be made available. Future epidemiologic studies involving human subjects will need to control for cigarette smoking, as this risk factor is a likely cause of RPC (Armstrong, Garrod, & Doll, 1976; McLaughlin, Blot, Devesa, & Fraumeni, 1996), as well as for analgesic abuse, another known cause for RPC, upper urinary tract cancer, and chronic renal failure (Kirkali & Tuzel, 2003).

## Conclusion

Overall, the presence of hydrocarbons and microbes with known or suspected links to kidney disease, including cancer of the renal pelvis (RPC) appears to be greater in ground water sources derived from lignite aquifers of the Wilcox group in northwestern Louisiana than in water from nearby sources not coincident with known lignite or coal deposits. Some of the

organic compounds found in the water samples are structurally similar to those found in lignite collected from a commercial lignite mine in the area, suggesting that lignite beds may in fact be the source of some of the water contaminants identified in residents' wells. The infection of humans by pathogenic leptospires that were found in 50% of the surface water sites in the studied region provides an additional potential aggravating factor contributing to high rates of kidney diseases, including cancer. Preliminary epidemiological evidence suggests that there are significant associations between organic compounds in well water and several other variables measured and kidney cancer rates. Future work is planned to conduct additional water sampling and to investigate biological and toxicological mechanisms to test for causation in the observed statistical correlation.

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