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Concentrations of Arsenic, Chromium, and Nickel in Toenail Samples From Appalachian Kentucky Residents

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ABSTRACT: Lung cancer rates in Appalachian Kentucky are almost twice national rates; colorectal cancer rates are also elevated. Although smoking prevalence is high, it does not explain all excess risk. The area is characterized by poverty, low educational attainment, and unemployment. Coal production is a major industry. Pyrite contaminants of coal contain established human carcinogens, arsenic (As), chromium (Cr), and nickel (Ni). We compared biological exposure to As, Cr, and Ni for adults living in Appalachian Kentucky with residents of Jefferson, a non-Appalachian, urban county. We further compared lung and colon cancer rates, demographics, and smoking prevalence across the study areas. Toenail clipping analysis measured As, Cr, and Ni for residents of 23 rural Appalachian Kentucky counties and for Jefferson County. Reverse Kaplan-Meier statistical methodology addressed left-censored data. Appalachian residents were exposed to higher concentrations of As, Cr, and Ni than Jefferson County residents. Lung cancer incidence and mortality rates in Appalachia are higher than Jefferson County and elsewhere in the state, as are colorectal mortality rates. Environmental factors may contribute to the increased concentration of trace elements measured in residents of the Appalachian region. Routes of human exposure need to be determined.

KEY WORDS: arsenic, chromium, nickel, Appalachia, toenail, cancer, mining, environmental

I. INTRODUCTION

The state of Kentucky has elevated incidence rates of both lung and colorectal cancers compared to the United States as a whole. Based on recent comparative data across the 50 states for the period 2002–2006,[1] Kentucky ranks highest for lung cancer mortality for men and women (54% and 39% higher than the United States as a whole, respectively), and second and third highest for colorectal cancer, for men and women, respectively (15% and 14% higher than corresponding national rates). In addition, the Appalachian region of eastern Kentucky (Appalachian KY) has high rates of these cancers compared to the non-Appalachian region of the state.[2]

Residents of Appalachian KY, on average, have lower socioeconomic status, higher smoking rates, and higher rates of obesity than the state as a whole. A study in Appalachia found that living in areas of heavy coal mining was associated with increased risk of lung cancer, beyond that explained by smoking or socioeconomic variables.[3] The region has a unique geology that has long supported the country's energy needs with high-quality bituminous coal. Environmental exposures to various trace elements, such arsenic (As),[4] chromium (Cr) VI, and nickel (Ni)[5] have been associated with increased risk of cancers. These trace elements can often be found in pyrite associated with coal from the region, and may contribute, at least in part, to the increased cancer rates found among Appalachian KY residents.[6] Despite the potential for trace metal exposure via ingestion, inhalation, and/or dermal routes in this region, there have been no population-based measurements of biological exposure to trace elements.

Toenails have been shown to be an appropriate biological sample to assess environmental exposures to trace elements or other chemicals.[7,8] Since toenails have a relatively slow growth rate, it has been estimated that toenail measurements usually represent exposures over the last 3–12 months, and are relatively stable over time.[9] In particular, toenail As concentrations have been shown to be a good biomarker of environmental exposure to As.[10–12] The concentration of Cr in toenails has also been used as a biomarker of exposure in epidemiologic studies,[13–15] as well as concentrations of Ni in toenails[16] and fingernails,[17] although to a lesser extent than As or Cr.

For this study, toenail samples that had been collected from a statewide, colon cancer case-control study in Kentucky were made available for analysis. We present the results of an analysis of As, Cr, and Ni concentrations in toenails of residents from selected Appalachian KY counties, compared to residents living in another region of the state distant from the Appalachian region, as a first approach to assess potential environmental exposures to these trace elements. We also present an ecologic analysis of lung and colorectal cancer as well as demographics of the study regions.

II. METHODS

The participants for this study were selected from the controls of a colon cancer case-control study conducted in Kentucky, previously described,[18] following the methodology of other studies drawing from controls as a representative group of healthy individuals to assess environmental exposures using toenail samples.[10,19] Briefly, potential colon cancer cases were identified through the Surveillance, Epidemiology, and End Results (SEER) Kentucky Cancer Registry (KCR), which covers all residents of the state of Kentucky at the time of diagnosis. The KCR was queried every 3 months to identify all incident primary colon cancer cases reported within 6 months of diagnosis. Random-digit dialing was used to recruit a population sample of controls representative of Kentucky. All subjects were recruited between July 2003 and March 2009. Controls were required to be aged ≥30 years, with no personal history of cancer other than skin cancer. For both cases and controls, those with self-reported inflammatory bowel diseases, history of familial adenomatous polyposis, and hereditary non-polyposis colorectal cancer were excluded. The participation rates were 72.2% for the cases and 62.5% for eligible controls. Each participant was mailed a kit for self-collection of toenail specimens, including instructions for clipping them, a sealable plastic bag to store the specimens, and a return envelope for mailing them. Toenail samples were stored at Case Western Reserve University for future analysis.

For the pilot study reported here, a subgroup of controls from the larger case-control study was selected based on geographical location of residence. Specifically, 2 factors were considered for the selection of the potentially exposed subjects: 1) the high rates of lung and colon cancer in the Appalachian region of Kentucky; and 2) the fact that the mountainous, Appalachian region of the state is known to have a unique geology that contains high-quality bituminous coal naturally rich in trace elements.[20] This led to investigating whether residents of the Appalachian region of Kentucky had higher exposures to As, Cr, and Ni as reflected by concentrations in toenail samples, which could in turn support the hypothesis that environmental exposure to these trace elements may partially explain the high lung and colon cancer rates. Therefore, for this analysis we included subjects from 23 contiguous rural counties in the Appalachian region of the state, selected for their high colon and lung cancer rates and their location within the geologically rich bituminous coal region. A comparison group was selected from Jefferson County, the largest, most urban county in Kentucky, which includes the city of Louisville and is located on the Ohio River about 100 miles northwest of the Appalachian region. Another relevant factor that set apart the Appalachian counties from Jefferson County was the high percentage of the population using private versus public water supply.

Toenail samples from 253 controls residing in the target counties were selected for trace element analysis, of which 102 participants were from the Appalachian counties (henceforth referred to Appalachian) and 151 controls were from Jefferson County (henceforth referred to as Jefferson). We excluded two counties that were not contiguous to the core of mountainous Appalachian ones, are more urban, and have an oil refinery in the vicinity (introducing potential exposures from other sources). Therefore, our final study group included 88 Appalachian and 151 Jefferson participants. Basic demographic data for these participants was obtained from the larger case-control study.

The Institutional Review Boards of the University of Kentucky and Case Western Reserve University/University Hospitals of Cleveland approved this study. All participants provided written informed consent.

A. Laboratory Analysis

Elemental analyses of toenails were performed following the methods of Heck et al.[21] External contamination was removed from the toenails first by ultrasonication in acetone, second in 1% citranox (metal free acidic detergent), and finally in 18 MΩ de-ionized water. The samples were then dried to a constant weight at 60°C. A microwave reaction system (MARS Xpress; CEM, Matthews, NC, USA) was used to digest the samples in ultra-pure nitric acid in trace-metal clean polypropylene centrifuge tubes, ramping the temperature to 100°C and holding for 10 minutes. Total elemental analyses were performed after dilution using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500cx; Agilent Technologies, Santa Clara, CA, USA). The ICP-MS is equipped with an octopole collision cell that was pressurized with helium (for Cr and As) to minimize polyatomic interferences. Quality control parameters included analyses of reagent blanks, dilution replicates, samples spiked with a second source standard, and monitoring of isotope ratios where possible. These quality assurance/quality control procedures follow the guidelines set forth by the U.S. Environmental Protection Agency (EPA) method 6020a.[22] The quality control criterion for matrix spike recovery was 80%–110%.

B. Detection Limits

Method detection limits (MDLs) depended on sample mass. Detection limits were calculated using 3 times the standard deviation of the reagent blanks and then were converted to an equivalent dry mass concentration in each toenail based on the actual dry mass of the sample. The dry mass of collected toenail varied widely between study participants; in general, nearly two thirds of the collected toenail samples weighed <20 mg.

C. Statistical Analysis

Standard descriptive statistics were used to summarize distributions for age, race, gender, smoking status, and amount of dry toenail mass between Jefferson and Appalachian samples. Distributions for these variables were compared between the 2 regions using the 2-sample *t*-test for age and toenail mass and Fisher's exact test for gender, race, and smoking status.

As is often the case with environmental contaminant data, a portion of the values for each trace element in our data set fell below the MDL and are referred to as "non-detects." Such values are characterized in the statistical literature as being left-censored. An MDL value was calculated for each sample independent of whether it was left-censored. The analysis described in this report utilizes the MDL only when there was a non-detect value present.

Due to the relatively high presence of non-detects for As (70.7%), Cr (57%), and Ni (71%), simple methods of substitution (such as using MDL/2) or simple imputation are considered inadequate for statistical analysis.[23,24] A more appropriate method to compare distributional differences is the use of the reverse Kaplan-Meier estimator.[24] This estimator is a left-censored analog to the common Kaplan-Meier estimator. This is a nonparametric method that makes no distributional assumptions regarding the trace elements. It also accounts for the non-detects and has been advocated as a preferred method over other more popular ad hoc methods based on "filling in" a value for the non-detect (e.g., fill in the MDL or ½ × MDL).[24]

The reverse Kaplan-Meier estimator is an appropriate method if there is substantial overlap in the distribution of the actual trace element values and the MDL values for the non-detects. This amount of overlap can be seen by constructing side-by-side box-and-whisker plots. These plots are included in this report (Fig . 1) and show a substantial amount of overlap, which indicates that the reverse Kaplan-Meier method is quite suitable for this application.

Distributions of trace elements were summarized by computing the 10th, 25th, 50th, 75th, and 90th percentiles, and were compared between the 2 groups (Appalachian and Jefferson), as well as by gender and 2 age groups (below and above the median of 61 years). Several of the percentiles ≤25th percentile could not be estimated precisely for any of the trace elements. Where this was the case, we followed the procedure recommended by others,[24] and report an upper bound based on the minimum trace element value observed (e.g., 0.02 was the minimum observed arsenic value in both Appalachian and Jefferson, and thus "<0.02" is the value reported for the 10th percentiles in the results. As is the case with the Kaplan-Meier estimator, the reported *P* values based on reverse Kaplan-Meier estimation reflect overall group differences in trace element distributions between the relevant groups.

D. Ecologic Data Analysis

In addition to the toenail analysis, we also analyzed secondary data sources to characterize the study region with respect to other variables available from the KCR, the Behavioral Risk Factor Surveillance System (BRFSS), the Kentucky Division of Water (DOW), and the 2000 Census. For these comparisons, we grouped counties geographically as follows: Appalachian KY (the 23 counties for which we present toenail trace element analyses), the rest of the Appalachian KY counties, as classified by the Appalachian Regional Commission (31 counties),[25] non-Appalachian KY counties (the remaining 69 counties combined), and Jefferson County. KCR data were used to derive colorectal and lung cancer incidence and mortality rates for 1995–2007. The BRFSS, a national program from the Centers for Disease Control and Prevention, administered by each state, is based on a yearly telephone survey and seeks to obtain information on a number of behavioral risk factors, preventive screening, and health status of the adult population (aged ≥18 years).[26] In Kentucky, it is administered through a contract with the University of Kentucky and the full data set is obtained yearly, which allows for grouping across counties and calendar years, as well as across variables, to meet data analysis requirements. In this case, we used it to calculate prevalence rates of

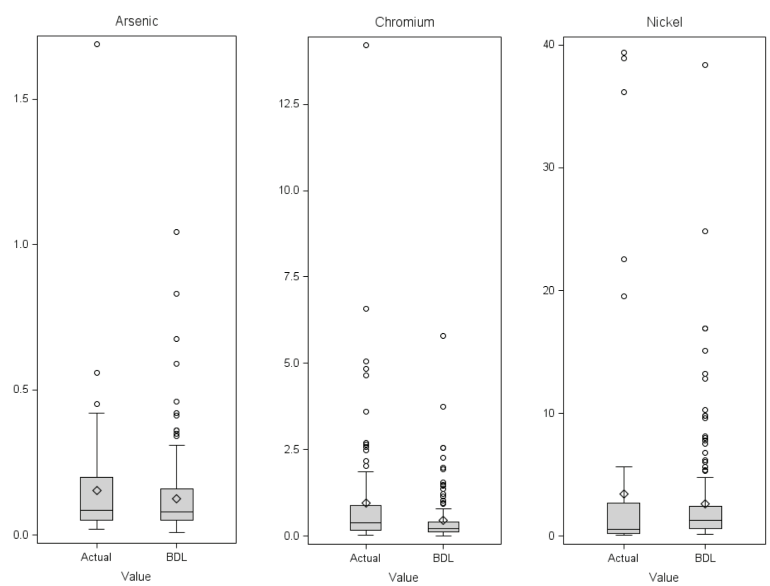

FIGURE 1. Box plots provide side-by-side distributions for actual values (values > LOD) and non-detects (values < LOD) for arsenic, chromium, and nickel. The lower end of the box corresponds to the 25th percentile, the middle line corresponds to the median, and the upper end to the 75th percentile. The diamond provides the estimate of the mean and the whiskers extend to the most extreme value that is within 1.5 times the interquartile range of each end of the box. The left-most vertical axis in each of the 3 panels corresponds to values (in ug/g) of arsenic, chromium, and nickel, respectively.

smoking, being overweight, and consuming ≥3 servings of fruits and vegetables over the 3 most recent years of available data (2007–2009). DOW data were used to estimate private well water usage and Census data were used to characterize the regions by education (percentage of adults without a high school diploma) and poverty levels (percentage below the poverty line).

III. RESULTS

A total of 239 controls from the original case-control study, who had submitted toenail samples and were residents of the 23 selected Appalachian (n=88) and of Jefferson (n=151) counties, were included in this analysis. As shown in Table 1, on average the Appalachian participants were younger than those from Jefferson County (mean 58.2 vs. 61.5, respectively; $P = .016$), had a slightly lower proportion of women (59.8% vs. 64.9%), and a higher representation of whites (94.3 vs. 84.0%, $P = .008$), reflecting the differences in racial distribution between the 2 regions. In terms of smoking rates, there were no significant differences across the 2 groups, although Appalachian subjects were more likely to be current smokers (20.5% vs. 15.9%), also reflecting the smoking patterns across the 2 regions.

The analysis of toenail samples showed that the total self-collected mass was generally small, averaging 18.2 g (slightly higher in Jefferson compared to Appalachian, 19.5 vs. 16.1). This resulted in 70.7% (169/239) of the samples being classified as below the detection limit (DL) for As, 57% (137/239) for Cr, and 71% (170/239) for Ni. Even in the presence of a large portion of below DL samples, the reverse Kaplan-Meier method can offer advantages over many of the ad hoc substitution methods such as substituting with half the detection limit. The utility of the Kaplan-Meier method depends on the overlap between the distribution of the actual trace element values and the distribution of the below DL for the corresponding non-detects. As can be seen in Figure 1, there is substantial overlap in these 2 distributions for all 3 trace elements, indicating that the Kaplan-Meier method of analysis is appropriate for these samples.

The results of the reverse Kaplan-Meier estimations show that, for the 3 trace elements considered, concentrations tended to be higher among the Appalachian than the Jefferson subjects (Table 2). In particular, for As the differences in concen-

TABLE 1. Comparison of Appalachian and Jefferson control study subjects by age, race, and smoking status

Variable	Appalachian	Jefferson	*P* value
Age, y			
n	88	151	
Mean	58.2	61.5	0.016
SD	9.54	10.51	
Min	36	33	
Max	89	86	
Race, %			
White	94.30	84.00	0.082
Black	2	13.00	
Other	3.40	3.00	
Female, %	59.80	64.90	0.094
Smoking status, %			
Current smoker	20.5	15.9	0.55
Former smoker	35.2	41.1	
Never smoked	44.3	43.1	

TABLE 2. Results of toenail Analysis of As, Cr, and Ni, by Appalachian and Jefferson location, by age groups and by gender

	Percentile	Appalachian Controls	Jefferson Controls	Appalachian Controls, by Age Groups		Jefferson Controls, by Age Groups		Appalachian Controls, by Gender		Jefferson Controls, by Gender	
		All	All	Age <61 y*	Age ≥61 y	Age <61 y	Age 61 y	Women	Men	Women	Men
No. of participants		88	151	54	34	68	83	51	37	104	47
Arsenic	10th	<0.02	<0.02	<0.02	<0.06	<0.02	<0.02	<0.06	<0.04	<0.02	<0.02
	25th	0.02	0.02	0.02	<0.06	<0.02	0.02	<0.06	0.04	<0.02	0.02
	50th	0.06	0.02	0.07	0.06	0.02	0.02	0.06	0.06	0.02	0.02
	75th	0.13	0.03	0.21	0.12	0.03	0.02	0.12	0.16	0.03	0.03
	90th	0.29	0.04	0.34	0.13	0.07	0.04	0.31	0.29	0.04	0.04
		P < .0001		*P* = .231		*P* = .143		*P* = .663		*P* = .986	
Chromium	10th	<0.03	0.02	<0.06	<0.09	0.04	0.02	<0.06	<0.06	0.04	0.02
	25th	0.06	0.02	0.06	0.09	0.04	0.02	0.06	0.06	0.04	0.02
	50th	0.27	0.04	0.16	0.47	0.11	0.02	0.19	0.27	0.08	0.02
	75th	0.65	0.21	0.46	0.82	0.22	0.2	0.49	0.82	0.18	0.35
	90th	1.31	0.65	0.96	1.48	0.89	0.65	0.74	1.73	0.65	1.56
		P = .0001		*P* = .0367		*P* = .266		*P* = .273		*P* = .668	
Nickel	10th	0.08	<0.18	0.1	0.08	<0.27	<0.18	0.07	0.08	<0.41	0.18
	25th	0.12	<0.18	0.12	0.12	<0.27	<0.18	0.11	0.14	<0.41	0.18
	50th	0.28	0.18	0.27	0.33	0.27	0.18	0.16	0.36	<0.41	0.27
	75th	0.59	0.35	0.77	0.59	0.53	0.33	0.31	0.77	0.41	0.33
	90th	3.31	1.41	3.83	1.45	1.73	0.95	2.83	3.47	0.9	3.37
		P = .0591		*P* = .728		*P* = .182		*P* = .117		*P* = .214	

*61 years of age represents the median.

tration were notably higher in Appalachian in the 75th and 90th percentiles. Similar results were observed for Cr and Ni starting at around the 50th percentile.

Interesting patterns were observed when participants were stratified into 2 groups by age (above and below the median age of 61 years) and by gender. Although the overall *P* values for age or gender distributional differences were not significant for As, the younger group had higher toenail As concentrations in both Appalachia and Jefferson, with samples from younger Appalachians having considerably higher concentrations across the 75th and 90th percentiles. No differences were observed with respect to gender. For Cr, higher concentrations were observed for the older Appalachian group (P = .0367), whereas the reverse was true in Jefferson samples. Men were found to have higher Cr levels than women in both regions, although these differences were not statistically significant. Finally, observed Ni levels were higher among the younger group in both Appalachia and Jefferson (the difference being higher in Appalachia) but neither of these differences reached statistical significance.

The results of the ecologic-level analysis by region are presented in Table 3, comparing the following groups of counties: the 23 Appalachian, coal-rich counties for which toenail results were presented; the remaining Kentucky Appalachian counties; all of the non-Appalachian counties in Kentucky combined; and Jefferson County alone. The comparison of age-adjusted cancer rates indicates that colorectal cancer incidence is higher in Appalachia than in Jefferson, and colorectal cancer mortality is higher in the 23-county area than the rest of Appalachia or Jefferson. Lung cancer incidence and mortality rates are higher in the 23 Appalachian counties than in the rest of Appalachia, which in turn is higher than in Jefferson. Smoking rates are also higher in Appalachian counties, but no difference was observed among the 2 Appalachian groups. A similar pattern was observed for overweight rates and consumption of fruits and vegetables. The poverty levels were highest in the 23-county region, followed by the rest of Appalachia, and in stark contrast with Jefferson; a similar pattern was observed for education (although not much difference between the 2 Appalachian

TABLE 3. Age-adjusted incidence and mortality Rates per 100,000 for lung and colorectal cancer, by region (2003–2007) and BRFSS prevalence rates for smoking, overweight, and consumption of ≥3 servings of vegetables per day (2007–2009)

Region	Colorectal cancer incidence	Colorectal cancer mortality	Lung cancer incidence	Lung cancer mortality	Smoking rates, %	Overweight rates, %	≥3 fruits and vegetables/d, %	Less than high school education, %	Below poverty (mean), %	Public water (mean), %
23 Appalachian counties	59.5	22.6	116.3	91.1	30.77	70.39	54.03	20.80	30.20	46.64
Rest of Appalachian counties	59.5	20.4	101.8	78.8	30.07	70.32	53.83	19.57	22.32	65.62
Non-Appalachian counties	56	20.3	97.1	72.3	24.66	66.63	58.76	10.22	13.59	76.74
Jefferson County	57	21.6	97	72.4	24.14	64.09	61.38	8.70	12.36	98.51

groups). In terms of water supply, clear contrasts were observed, with <50% of the population of the 23-county region as a whole receiving public water supply, versus 66% in the rest of Appalachia and 98% in Jefferson.

IV. DISCUSSION

The results of this pilot study show that residents from the selected region of Appalachian KY tend to have higher toenail As, Ni, and Cr concentrations than residents from Jefferson County. The interpretation of these results is complex, and involves consideration of potential exposure pathways as well as comparison of the concentrations with other studies. For these reasons, we will first give a general characterization of the 2 regions and then address each of the 3 trace elements separately.

Exposure to these trace elements has been associated with increased risks of various cancers across a spectrum of exposure sources globally, and the evidence in the area suggests that the high lung cancer risks (almost twice than those in the United States as a whole) can only be partially attributed to the higher smoking rates in Appalachian KY.[27] In addition, colorectal mortality rates are elevated compared to other parts of the state, although not as much as lung cancer.

Among the specific trace elements analyzed in this study, As is likely the most clearly established as both an occupational and environmental carcinogen. Data from copper smelter workers several decades ago demonstrated increased lung cancer associated with arsenic inhalation, and similar findings have been reported among other occupations, including mining.[4,28] Environmental exposure to As, mainly from ingestion of naturally contaminated drinking water, has been found to increase the rates of several cancers, particularly lung and bladder neoplasms.[29,30]

Arsenic is metabolized through a methylation process and excreted primarily through the urine, while also accumulating in keratin-rich tissues such as hair and nails, due to its affinity for sulphydryl groups.[4] Several studies have shown that toenail arsenic concentrations are a useful indicator of As exposure over several months.[12] A New England study established a correlation of 0.65 ($P < .001$) between As concentrations in well water and toenails of residents consuming water above 1 μg/L.[10] Their regression line shows that at a water concentration of 10 μg/L, the EPA's maximum contaminant level for As,[31] the predicted toenail value would be, on average, around 0.2 μg/g. In our pilot study, the 90th percentile toenail concentration of 0.29 μg/g interpolated from the same regression line[10] would be equivalent to the exposure expected from drinking water containing around 30 μg/L of As, which is 3 times higher than the EPA standard.

It is important to clarify that we do not have any environmental measurements related to the participants who provided the toenail samples, and there are limited quantified data on direct human exposures to As in the region. Therefore, we cannot ascertain if the As exposures that resulted in the higher toenail As concentrations in the Appalachian participants are from ingestion of contaminated drinking water, occupational or environmental dust inhalation or dermal exposures, food grown or hunted locally, or a combination of various sources. Since over half of the population of the region uses privately owned well water, which is not subject to As testing and regulation, there is scant information on drinking water chemical contaminants. A small study in the region,[32] including several of the Appalachian counties from our study, reported that some wells had arsenic water concentrations between 0.5–10 μg/L, with only 2 higher than 10 μg/L. It is therefore quite possible that other routes of exposure are contributing to the body burden of As reflected in the toenail samples. Inhalation may be one of those routes, given that the geometric mean of As in coal from this region is almost twice that of the coal in the United States as a whole (11 vs. 6.1 ppm),[6] and that surface mining, increasingly common in the area, liberates large amounts of dust into the environment,[33,34] in which pH and other factors may increase the availability of bioaccessible arsenic.[35]

Chromium can occur in several valences, but only the trivalent Cr III and Cr VI have environmental stability.[36] Human exposures to these 2 forms seem to have very different effects. Cr VI is an established human carcinogen, based on a number of inhalation studies in occupational settings[22]; Cr VI by ingestion is recently being recognized as a possible carcinogen,[36] although a meta-analysis of studies of occupational exposure to Cr VI showed no association with cancers of the gastrointestinal tract.[37] Recent experimental evidence suggests a role for Cr VI in gastrointestinal tumors.[38] On the other hand, Cr III is considered to be an essential element. Several studies have found low biological concentrations of Cr to be related to different adverse health outcomes. Lower toenail Cr concentrations have been associated with increased risk of diabetes[14] and myocardial infarction,[39] whereas a recent study showed that higher blood concentrations of Cr associated with increased risk of oral cancer.[40] Other studies have shown no association between toenail Cr and melanoma[15] or breast cancer.[13] An important limitation of finding associations between total Cr measurements and specific health effects is separating the beneficial, protective effects of Cr III and the adverse effects of Cr VI. Moreover, in some cases, such as drinking water, Cr III can oxidize to Cr VI under certain conditions, thus rendering the final human exposure product a higher toxicity.[36]

The results of our pilot study showed that residents in Appalachian KY had higher toenail Cr concentrations than those in Jefferson, suggesting the possibility that environmental exposures could account for some of the observed differences. However, even the median concentration for Appalachia, of 0.27 μg/g, was lower than that found in other studies with values around 1.2[39] and 1.40.[15] In a sample of the large Health Professionals Follow-Up Study of men aged 45–75 years, the toenail median for Cr was 0.50.[14] Since the data do not allow us to elucidate if the source of the toenail Cr is Cr III or Cr VI, we are not able to draw conclusions regarding either lower dietary Cr III intake in general and/or higher environmental Cr VI exposures in Appalachia.

Inhalation is the main route of elevated exposures to Ni, and is most often associated with occupational exposures to dust or airborne particles.[41] Based on a number of epidemiologic studies, inhalation of Ni was established as a lung carcinogen in the 1990s.[5] A recent study also showed higher concentrations of blood Ni, along with Cr, associated with oral cancer.[42]

Although there are no published studies showing toenail Ni concentrations in relation to health outcomes, there are a few publications with nail and toenail measurements for different groups. A Danish occupational study found the relative Ni fingernail concentrations to be higher among heavily exposed workers (median 29.9 μg/g) than among moderately exposed workers (median 13.8 μg/g),[17] and in turn were much lower among a control group (median 0.49 μg/g). On the other hand, a reference study in Detroit, Michigan, showed the median concentration of Ni in non-exposed subjects' toenails to be 37.4 μg/g. It is difficult to interpret our findings within the context of these few published results and the lack of apparent validation of toenail Ni as an adequate biomarker of exposure. However, it is worth noting that the levels were higher among Appalachian participants.

One of the main limitations of this study was the collection and analysis of the toenail samples, particularly the large percentage of non-detects for all 3 trace elements. In general, with accurate measurement techniques, such as the one used in our laboratory analysis, non-detects can be considered almost synonymous to low concentrations. However, this is dependent on having sufficient sample mass. The presence of a non-detectable value was dependent, in part, on the amount of mass (toenail weight) available, and this varied from sample to sample. Non-detects were also present in many samples that had sufficient mass indicating low exposure. The reverse Kaplan-Meier statistical approach allows for use of this "partial" information to the extent that one knows that the trace element level was at least no greater than the detectable limit value itself.

Since the study subjects were a subgroup of those from a colorectal cancer case-control study, some selection bias could have occurred in the inclusion of study participants. However, since we only included controls, identified through random-digit dialing, the two main sources of bias would be having a land-line telephone and agreeing to participate. Since the study covered an area of 23 counties in Appalachia, there is no reason to believe that either of these conditions would differentially select individuals who are more, or less, likely to be environmentally exposed to the trace elements investigated. The same is true for Jefferson County participants.

Another limitation of this pilot study is the limited information we had on the study subjects that did not allow us to explore if the differences in Cr toenail concentrations, for example, were associated with differences in dietary practices, or if differences across the 3 trace elements could be at least partially explained by differences in occupational activities.

The results of the ecologic data analysis showing higher lung cancer risks in the coal-rich Appalachian region, despite having the same smoking rates as the rest of Appalachian, Kentucky, support a possible role of arsenic as a contributor, based on the established evidence of arsenic as a lung carcinogen.[4] The findings are also consistent with a recent study in the West Virginia coal-producing region.[43]

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