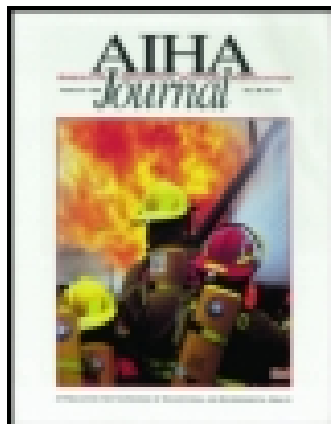


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Chemical and statistical studies of contaminants in urban lungs.

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Lungs taken at autopsy from 100 urban residents were studied to determine concentrations of 21 trace metals, dust, free silica and hydroxyproline. Medical, occupational and environmental histories were collected so that correlation studies might be done. The potential value of this data as a baseline for further studies of occupationally diseased lung tissue was assessed by evaluating the results of an analysis of variance. The personal histories were used as the criteria for classifying the observations (lung concentrations) into groups for the statistical analysis.

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introduction

Beginning over a decade ago, Public Health Service occupational health personnel initiated a study of lung tissue from deceased miners from the West Virginia bituminous coal fields. With the work of several prominent British researchers⁽¹⁻⁴⁾ as a guide, analytical studies were set up, geared to estimate concentration levels for dust, quartz, and certain trace elements in the lungs.

This paper describes a control study performed on a group of 100 lungs from urban residents, and designed to establish baseline concentration levels of the metals and minerals of interest. A contract (no. CPE-R-70-0041) was negotiated with the Pathology Department of the Cincinnati General Hospital for the delivery of 100 whole left lung specimens from routine autopsies at the hospital. They were to be delivered to the NIOSH laboratory within 24 hours of collection, be unembalmed, and be drawn from subjects whose diseases were not related to the inhalation of industrial vapors or dusts. Victims of drowning or acute inflammatory disease of the lungs or patients who had been hospitalized for more than four weeks prior to expiration were excluded from the study. Plans for this study also provided for the collection of an extensive personal history for each subject. This included the information

from the hospital bedchart (on terminal admission), the hospital autopsy report, including microscopic slides of sections of the companion (right) lung, and a completed personal/environmental questionnaire resulting from an interview with next of kin to the deceased subjects.

The organs, after suitable processing, were analyzed quantitatively for dust, free silica, hydroxyproline and selected trace metals. This analytical data and factors from the personal and medical records were subjected to statistical study for the purpose of investigating potential value of urban data as a baseline for futher coal miner study. To evaluate the potential value, a portion of the urban lung data was analyzed using the same type of statistical analysis to be used for the coal miner data after it is obtained. This statistical analysis is available in full from the authors. Briefly, analyses of variance were done to compare average lung concentrations among groups defined by the following factors: sex, race, smoking history, occupational exposure to organic dust, and organ abnormalities at time of death.

Five publications⁽⁵⁻⁹⁾ describe the earlier phases of the broader, continuing study, which at first focused on the coal miners from Raleigh

County, West Virginia. During the course of this work, refinements of both tissue processing and analytical techniques were made. The tissue preparation method has been described in detail.⁽¹⁰⁾ Feasibility studies were conducted to choose a method for trace metals analysis that would offer increased sensitivity and effect the determination of certain elements that had previously been undetected. Neutron activation analysis⁽¹¹⁾ and spark source mass spectrometry⁽¹¹⁾ were evaluated, and the latter technique chosen. These changes were adopted at the beginning of this urban study and will be adhered to throughout all final phases of the overall study.

This urban control study has as its intent two distinct functions. First, it presents an assay of lung tissue drawn from a group of urban residents who have been thoroughly documented as to medical, personal, occupational, and environmental histories. Second, these data will be used as a baseline against which similar analytical data from a group of Pennsylvania coal miners will be compared. With a profile of metal and mineral concentrations found in the lungs of non-miners, already studied as to the influence of smoking, occupational exposures, and certain chronic disease conditions, the effect of coal mine dust can be assessed with more assurance than has previously been possible.

The suitability of using a sample from a single urban population as a baseline for coal miner studies was scrutinized. Ideally, the baseline would be drawn from a sample of a population whose only deviation from the coal miner population would be exposure inside a coal mine; i.e., non-miner residents of the same communities. Unembalmed samples from this source proved unobtainable. Therefore, urban samples were used. Samples from a wide urban base (several communities) would be preferable. However, this could be made available only at a prohibitive cost.

The Oak Ridge Laboratory "Standard Man" Study of the 1960's⁽¹³⁻¹⁵⁾ was collected over a wide geographical range both in the United States and abroad. Raw data from five American cities (Baltimore, Dallas, Denver, Miami, and Seattle) were available. A study of the elemental lung concentrations for the eight

elements that overlapped with the NIOSH study (Al, Cr, Cu, Mg, Mn, Pb, Ti, Zn) was made, city by city. Geometric means and standard deviations were calculated and an analysis of variance performed. For four elements, no significant differences were found. For the other four elements, intercity figures differed by a factor of two or less.

This information tends to support the validity of a single urban area as the source of a sample to be used for baseline data in a coal miner study, as long as sufficiently large differences are observed.

the sample group

As mentioned above, the next of kin to each subject in the sampling group was contacted and interviewed to determine personal factors that might be correlated with analytical data. A sociologist performed all the interviews, some in person, some via telephone, obtaining a 100% response. A paper⁽¹⁶⁾ describing the interviewing techniques and quality of response was published.

One hundred deceased urban residents made up the sample group. All were Cincinnati residents, having lived in the community for varying periods of time. They were about evenly divided between men and women, blacks and whites. Ages ranged from 23 to 96 years, with a median age of 66 years. Thirty-six were non-smokers, the balance including light to heavy users of tobacco. Four of these dipped snuff regularly. A broad spectrum of occupations was represented, including foundry workers, machinists, farmers, gardeners, salesmen, domestics, waitresses, clerical workers, and homemakers. Deaths were from normal causes, mostly chronic degenerative conditions of advancing age. There were some acute disease conditions, but no traumatic deaths.

tissue processing

The lung specimens were hand delivered to the NIOSH laboratory within 24 hours of autopsy, in individual plastic bags. Preliminary examination of the specimen was done by a NIOSH pathologist, assisted by a chemist. Precautions against contamination of the tissue were observed. The hilar lymph nodes were

dissected out, trimmed, weighed, sealed in polyethylene bags, and stored in a freezer. Small sections of interest were taken by the pathologist after the lung was weighed. The organ was then sealed in polyethylene and frozen until final processing was begun.

The lung was partially thawed, cut into small pieces and placed in a tared homogenizing flask. After weighing, double deionized water was added in the approximate ratio of two parts water to three parts lung. Exact weights were recorded and the mixture homogenized until a uniform slurry was obtained. All metal parts coming into contact with the tissue were either of tantalum or coated with an epoxy or polyethylene film, so there was no contamination from the metals of interest to the study.

After homogenization, the sample was frozen and lyophilized. Careful records of weights were kept throughout the processing so that ratios between wet, dry, and ashed weights could be calculated for each sample. Ashing was accomplished by a low temperature oxygen plasma system to minimize the losses of volatile metal compounds.

analytical methods

dust determination

Dust concentrations were determined by an enzymatic digestion method.⁽¹⁷⁾ The homogenized, lyophilized tissue was first ground in a liquid nitrogen freezer mill. Particle sizes were thereby reduced to the 5-15 micron range, ensuring high surface area exposure to the enzymatic activity. Ficin, a proteolytic enzyme extracted from the latex of certain tropical trees, digests the organic matrix and leaves the residual dust. The dust was separated from the digested protein by ultra-centrifugation, decanted, redispersed with a stream of deionized water, centrifuged and decanted again. The solid residue was transferred quantitatively into a tared weighing dish, dried overnight at 105°C., and quantitated gravimetrically. This material was designated as total dust. The combustible fraction was determined by difference after low temperature ashing. The remaining solid residue was termed mineral dust, the oxidized fraction, non-mineral dust. (In earlier work,⁽⁵⁻⁹⁾ the residue after ashing was designated non-coal

dust, the oxidized fraction, coal dust. This is obviously a misnomer when dealing with non-minerals.)

free silica determination

Crystalline free silica, or quartz, was determined by a spectrophotometric method.⁽¹⁸⁻²¹⁾ An aliquot of the freeze dried tissue was ashed with redistilled nitric acid to destroy the organic matrix. Crystalline free silica was separated from other silicon-bearing compounds by digestion and quantitated as either the silicomolybdate or the molybdenum blue complex.

hydroxyproline determination

An assay for hydroxyproline concentration was included in the analytical protocol to estimate levels of collagen in the lung tissue. It has been recognized that as progressive massive fibrosis develops in the lungs of coal miners, there is a subsequent rise in the collagen content of the tissue.⁽²²⁾ Hydroxyproline is generally assayed as an index to the amount of collagen. An aliquot of freeze-dried tissue was hydrolyzed in 6 N. HCl, filtered, the pH adjusted to 6, and the volume adjusted to a predetermined level. The hydroxyproline is oxidized with Chloramine T, which then reacts to form a chromagen with Ehrlich's Reagent. An automated analytical system was used for this determination.⁽²³⁾

trace metals determination

As mentioned above, feasibility studies^(11,12) were conducted to select a suitable technique for trace metal analysis. Spark source mass spectrometry was the method of choice and the actual metals analyses were performed under contract no. HSM-99-73-46. Details of the method and the standardization procedures, as well as the raw data, have been published in a NIOSH Technical Report.⁽²⁴⁾

While the preferred experimental design should include a full elemental profile, it would be a monumental and economically impractical task. Choices had to be made. It was decided that approximately 20 elements would be determined. The first three chosen (magnesium, beryllium, vanadium) were the elements whose concentrations had been found in a previous study⁽⁹⁾ to be significantly correlated with

increasing degrees of lung damage from coal workers' pneumoconiosis (CWP). Certain toxic elements were included because of general interest. The major criterion of choice was the data from the spark source feasibility study.⁽¹²⁾ Among samples analyzed in this work were three non-miner lungs and four coal miner lungs. Of the four miner lungs, two were selected because of known advanced fibrotic disease. Interest in examining compositional differences between the characteristic areas of heavy and light pigmentation of pneumoconiotic lungs had been noted⁽²⁵⁾ as a means of gaining maximum insight from a severely limited number of samples. Subsequently, with the guidance and assistance of a pathologist/contractor,⁽²⁵⁾ these two fibrotic lungs were carefully dissected to separate portions of lightly and heavily pigmented tissue. Lymph nodes were removed also. These three samples from each of the two diseased lungs were included in the feasibility study⁽¹²⁾ and analyzed by spark source mass spectrometry. The resultant analytical data was studied for indications of concentration differences between samples. For example, arsenic was not detected in non-miner lungs, but was detected in all samples of miners' tissue. Bismuth was detected only at extremely low levels in non-miner tissue, but was present at quantifiable levels in all miner tissue samples. In addition, bismuth (and several other metals, including magnesium, manganese and germanium) were found in higher concentrations in the heavily pigmented tissue than in the lightly pigmented tissue.

All metals selected for inclusion in this study showed an indication of altered concentration

between miner and non-miner samples and/or between heavily and lightly pigmented tissue. Based on these evaluations of preliminary data, the 20 elements selected for analysis by spark source mass spectrometry were aluminum, arsenic, beryllium, bismuth, cadmium, chromium, cobalt, copper, germanium, iodine, lead, magnesium, manganese, molybdenum, selenium, tellurium, thallium, titanium, vanadium, and zinc. All sample preparation, through low temperature ashing, was performed at NIOSH. National Bureau of Standards SRM 1577, Bovine Liver, was also ashed at NIOSH under identical conditions with the lung and lymph node samples. In addition, mercury was determined, on dry tissue, by the combustion, gold amalgamation, flameless atomic absorption technique. This work is detailed in the NIOSH Technical Report.⁽²⁴⁾

analytical results

dust; total, mineral, and non-mineral

As mentioned above, it was necessary to change the designation for the two dust fractions when dealing with subjects other than coal miners. Therefore, the following terminology will be used. Total dust is the total residue recovered from the lung tissue after enzymatic digestion of the organic matrix. Mineral dust is that fraction of the total dust remaining after ashing. Non-mineral dust is the difference between the total and the mineral fraction, or the portion that is volatilized during the ashing process. This fraction would be analogous to the coal dust of the coal miner studies.

TABLE I
Total Mineral and Non-mineral Dust Concentrations in the Lungs of
Urban Residents and Cases from the West Virginia Miner Study.
Reported as mg/g dry tissue.

	Total Dust		Mineral Dust		Non-mineral Dust	
	Urban	W. Va.*	Urban	W. Va.*	Urban	W. Va.*
Range	19.7 - 263		0.0 - 76.0		18.6 - 231	
Geom. Mean	79.6	72.1	6.3	28.7	68.2	33.1
95% Confidence Limits	70.8 - 89.5		5.1 - 7.7		59.5 - 78.2	
Estimate of Interval Including 68% of Population	43.9 - 144		1.9 - 17.5		34.5 - 136	
Sample Size	98.0	43.0	98.0	43.0	98.0	43.0

*Ranges and standard deviations were not readily available from the W. Va. coal miner data.

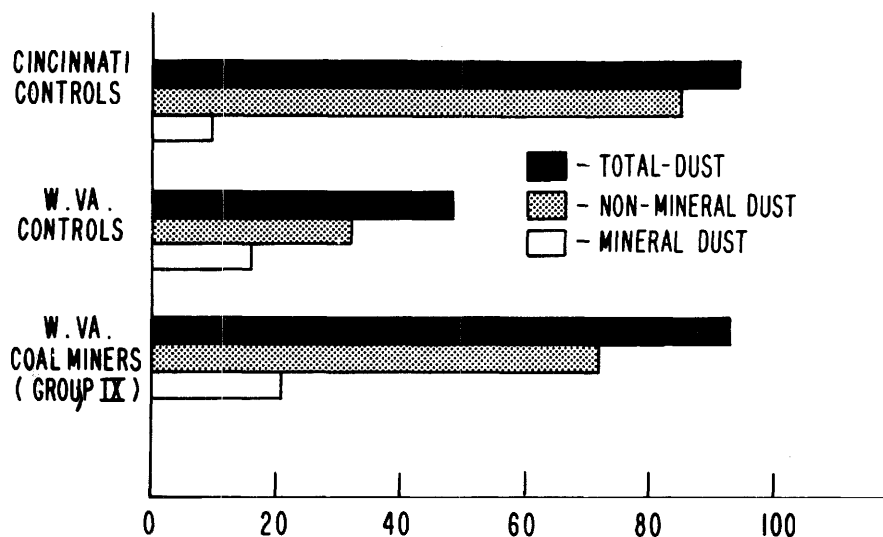


Figure 1 — Total, non-mineral, and mineral dust in lungs. Values shown are the geometric mean concentrations expressed in mg/g dry tissue.

Previous work⁽⁷⁾ has shown that both total dust and coal dust concentrations in miners' lungs show significant correlation with the degree of damage from coal workers' pneumoconiosis. In the lungs of urban residents without mining exposure, much lower concentrations of residual dust levels were expected. Surprisingly, however, both total and non-mineral dust levels were much higher than anticipated. Table I compares the mean dust concentrations of this study with the overall mean of concentrations from the West Virginia miner study. The estimates of the range covered by the central 68% of the population exhibited in Table I (also Tables II and IV) are based on a log-normal distribution model and are comparable to the \pm one standard deviation interval estimate which is often presented as a measure of variability in a normal distribution. The end points of the estimate for a normal distribution are obtained by adding and subtracting the sample estimate of the standard deviation from the estimate of the population arithmetic mean. However, the estimation is not so straight forward for a log-normal distribution because it is not symmetrical. The central 68% range estimate for a log-normal distribution is obtained by dividing and multiplying the geometric mean by the geometric standard deviation (GSD). (The GSD is the antilog of the standard deviation of the logs of the observations).

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Figure I depicts a comparison of geometric means of dust concentrations in the urban controls, West Virginia controls (female residents of the mining community), and one sampling group of West Virginia coal miners.

It is a poor advertisement for modern urban living that the lungs of city residents bear a dust burden comparable to that of underground coal miners. The composition of the dust recovered from the urban lungs is, however, markedly different in appearance from that recovered from miners' lungs.

free silica

The free silica concentrations found in the urban lungs offered no surprises. Increased free silica concentration in the lung develops with prolonged inhalation of the mineral, and is found among miners, sandblasters, quarry workers, and others exposed to respirable quartz (free silica). Table II shows the levels of this mineral found in this study compared with the West Virginia miners.

A review of the personal histories in this study revealed that the single highest free silica level (2.155 mg/g) occurred in a subject who had worked for many years as a construction laborer and for a time in a foundry. This SiO_2 level exceeds the mean concentration from the West Virginia miner study (Table II) and suggests the possibility that there had been a heavy exposure to respirable quartz during his lifetime.

TABLE II
Free Silica Concentrations in Urban Lungs and Miners' Lungs from the West Virginia Study. All concentrations are reported in mg/g dry tissue.

	Urban Lungs	Coal Miners' Lungs*
Range	0.014 - 2.155	
Geom. Mean	0.234	2.08
95% Confidence Limits	0.214 - 0.253	
Estimate of Interval including 68% of the Population	0.098 - 0.576	
Sample Size	98	43

*Ranges and standard deviations were not readily available for the W. Va. coal miner data.

TABLE III
Hydroxyproline Concentrations in Urban Control Lungs, W. Va. Miners Lungs, and W. Va. Control Lungs. Data reported as mg/g dry tissue.

	Urban Controls	W. Va. Miners (Group IX)	W. Va. Controls (Group X)
Range	9.8 - 37.8	17.9 - 29.7	17.3 - 30.0
Arith. Mean	24.5	23.3	23.4
Standard Dev.	5.7	4.0	3.6
Sample Size	98	19	15

TABLE IV
Concentration ranges of trace metals in urban control lungs and hilar lymph nodes, geometric means and the estimate of the interval including 68% of the population. All concentrations expressed in $\mu\text{g/g}$ dry tissue.

Element	Lungs			Lymph nodes		
	Range	Geom. Mean	Estimate of the interval including 68% of population	Range	Geom. Mean	Estimate of the interval including 68% of population
Aluminum	0.059 - 410	26.5	8.22 - 85.3	0.32 - 1.7×10^4	340	77 - 1460
Cadmium	0.08 - 6.5	0.62	0.21 - 1.94	0.01 - 31	1.31	0.42 - 4.07
Chromium	0.02 - 26.0	0.35	0.11 - 1.13	0.17 - 72	2.96	0.99 - 8.82
Cobalt	0.005 - 4.2	0.053	0.018 - 0.16	0.004 - 8.6	0.05	0.047 - 0.51
Copper	0.83 - 62	8.83	4.19 - 18.6	2.1 - 420	22.4	8.61 - 58.6
Germanium	0.08 - 4.2	0.49	0.24 - 1.00	0.08 - 12	1.98	0.81 - 4.87
Iodine	0.65 - 1.2×10^4	16.5	1.59 - 171	1.0 - 3400	23.7	3.51 - 161
Lead	0.08 - 41	1.72	0.59 - 4.99	0.005 - 56	2.6	0.59 - 11.5
Magnesium	16 - 970	63.1	30.4 - 131	640 - 2.7×10^5	3200	1200 - 8200
Manganese	0.09 - 81	1.20	0.43 - 3.36	0.20 - 300	4.34	1.20 - 15.7
Molybdenum	0.04 - 3.7	0.31	0.15 - 0.62	0.05 - 340	0.77	0.13 - 4.75
Selenium	0.46 - 26	4.10	1.96 - 8.57	0.34 - 29	6.15	2.72 - 13.8
Titanium	3.6 - 1400	34.3	11.0 - 107	9.7 - 2.5×10^4	1700	305 - 9800
Vanadium	0.004 - 1.1	0.083	0.026 - 0.26	0.09 - 55	2.76	0.85 - 8.9
Zinc	22.0 - 780	98.4	41.4 - 206	2.0 - 570	103	45.8 - 232

hydroxyproline

The hydroxyproline data accumulated to date offers no indication of elevated levels in coal miner lungs. However, only a small number of miner cases have been analyzed for this amino acid, and there is no information as to the degree of pneumoconiotic damage to this group of specimens. More data will be required before

any differences may be detected. Table III summarizes the data.

trace metals

Although analyses were run for 21 elements, only 15 yielded definitive data. The results of analyses, by spark source mass spectrometry, are

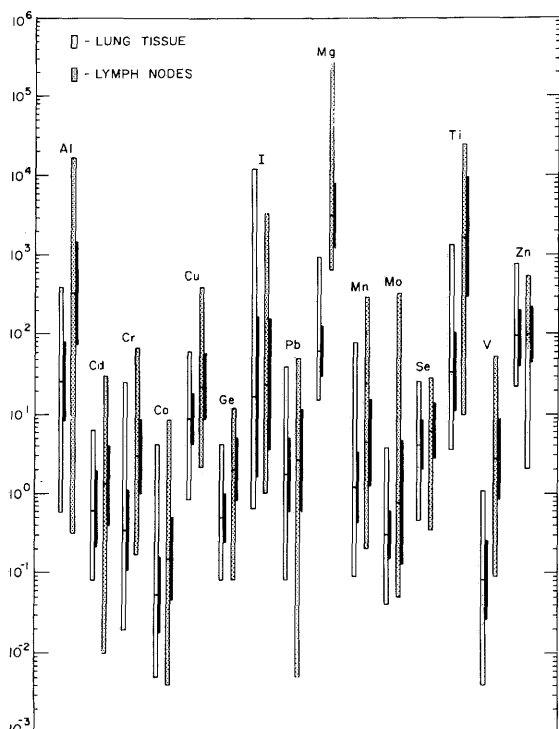


Figure 2 – A comparison of range, geometric means, and estimates of the interval including the central 68% of the population in lung tissue and hilar lymph nodes. Concentrations are expressed in $\mu\text{g/g}$ dry tissue.

summarized in Table IV. Because the data appeared to be log normally distributed, geometric means and standard deviations were used to describe this data. Lung data included duplicates for 30% of the tissue specimens. Where duplicates occurred, the average of each pair of observations was pooled with the other 70%. Values reported in Table IV were calculated from this pooled set. The same information is visualized in Figure 2. Plotted on a logarithmic scale, the length of the bar represents the concentration range. The cross bar represents the geometric mean, and the side bracket the estimate of the interval including the central 68% of the population. The placement of the lymph node data beside the comparable lung data serves to dramatize the difference between metals concentrations in the two kinds of related tissue. Note that the geometric mean values of aluminum, magnesium, titanium, and vanadium levels in the nodes exceed that of the lung tissues by more than an order of magnitude.

The Oak Ridge Laboratory "Standard Man" Study⁽¹³⁻¹⁵⁾ of the 1960's has stood as a major

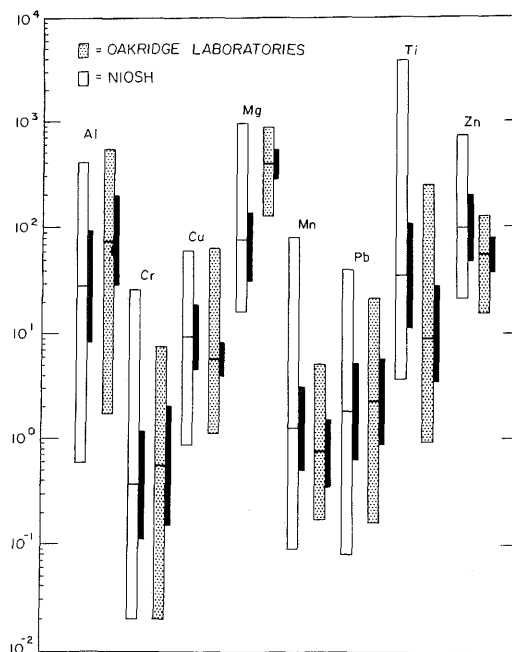


Figure 3 – A comparison of range, geometric means and estimates of the interval including the central 68% of the population for eight trace metals assayed in the Oak Ridge Laboratory "Standard Man" Study and the present study. Concentrations are expressed in the $\mu\text{g/g}$ dry tissue.

reference for trace metals concentrations in human tissues. Interstudy comparisons are difficult to make due to varying of reporting units, differences in tissue processing, and analytical methodology. The "Standard Man" Study used 200 grams of lung tissue, air dried and muffle furnace ashed at 400°C ., and reported the data on the ash weight basis. However, the raw data from Baltimore, Miami, Denver, Dallas, and Seattle was available. This data was converted to the dry weight base used in this study and comparisons made. Aluminum, chromium, copper, magnesium, manganese, lead, titanium, and zinc made up the overlap between the two studies. The "Standard Man" Study also included analysis of lung tissue for cadmium, cobalt, and vanadium. However, there were numerous samples where the concentrations of these three elements were undetermined. This may have been due to the lesser sensitivity of arc emission spectrometry compared to the spark source spectrometric method used in the NIOSH study. These three metals were readily detectable by SSMS (See

Table IV). Figure 3 shows the comparisons of elemental concentration ranges, geometric means, and estimates of the interval including the central interval 68% of the population for eight trace metals. In the cases of chromium, manganese, titanium, and zinc, where the Oak Ridge maximum values are notably lower than the corresponding NIOSH maxima, the more rigorous ashing technique used in the earlier study may have resulted in the loss of volatile metal salts. In general, however, the data from the two studies seem consistent with one another. It should be noted that log transformations were used in reducing the data, since the data from neither study appeared to be normally distributed. Each data set, however, approximates a log normal distribution.

The six elements that were analyzed chemically but not studied statistically were beryllium, bismuth, arsenic, mercury, tellurium, and thallium. The concentrations of these metals were extremely low, and a high proportion of the data fell below or near the detection limit. Therefore, it was not possible even to calculate means for these elements. Only very general observations may be made. In lung tissue, all six elements exhibited concentration levels below detection limit of the method in over 50% of the data. In the hilar lymph nodes, mercury and bismuth exhibited the same phenomenon. Median values for the remaining four metals in nodal tissue are as follows:

Arsenic	0.23	μg/g dry tissue
Beryllium	0.02	μg/g dry tissue
Tellurium	0.006	μg/g dry tissue
Thallium	0.01	μg/g dry tissue

These low values for the six metals are not necessarily negative results when considered in the context of this study. These elements were selected for inclusion in this study because of their presence in coal miners' lungs and low or undetected levels in non-miner tissue tend to support the premise that coal mine dust is the source of these toxic metals.

statistical analysis

A statistical analysis of the data described above was performed, aimed at determining factors from the medical and personal histories of the

subjects which might bear a relationship to altered chemical concentrations in the tissue. In view of the intended use of this data as a baseline for comparison with coal miners' lungs, it is important to determine factors, other than exposure to the coal mine environment, which are correlated with elevations or depressions of metals, dust, silica, and hydroxyproline concentrations. For each substance, the statistical protocol consisted of a preliminary analysis which investigated assumptions for the analysis of variance (ANOVA) and an analysis of variance which investigated possible differences among group means. The groupings for the means were formed from the following characteristics which were selected as being the most promising from the mass of information found in historical records:

1. *Age*
2. *Sex:* Male
Female
3. *Race:* Black
White
4. *Smoking:* Heavy Smoking
Light Smoking
Non-Smoking
5. *Working Conditions:*
Metal Dust (includes welders, foundry workers, machinists, etc.)
Organic Dusts (includes farmers, gardeners, or bakers with exposures to pesticides, fungicides, fertilizers, flour, etc.)
Dust Free (not exposed to metal or organic dust.)
6. *Abnormal physical conditions present at time of death* (as derived from the anatomical diagnoses.)
Cardiovascular (includes myocardial infarction, congestive heart failure, atherosclerotic heart disease, cerebrovascular accident, etc.)
Respiratory (includes severe lung edema, pneumonia, emphysema, etc.)
Kidney (includes acute pyelonephritis, glomerulonephritis, renal failure, etc.)
Liver (includes hepatitis, cirrhosis, etc.)
Cancer (any malignant growth in the body.)

The substances (responses) considered were Pb, I, Cd, Mo, Se, Ge, Zn, Cu, Co, Mn, Cr, V, Ti, Al, Mg, total

dust, mineral dust, non-mineral dust, hydroxyproline, and free silica.

The preliminary analysis consisted of an investigation of: (1) the validity of the normality assumptions by inspection of histograms and probability plots, (2) under the normality assumptions (of logs) the presence of homogeneous variances was then tested by using Bartlett's test,⁽²⁶⁾ (3) an effect due to the subjects' ages by using analysis of covariance, and (4) two way interactions by using analyses of variance. The histograms and probability plots were used to check the assumptions of normality before and after the logarithms had been taken. Bartlett's tests were used to check homogeneity of variances before and after the logarithms of the data had been taken. The histograms, probability plots, and Bartlett's tests indicated that taking the logs of the data would better satisfy the assumptions of normality and homogeneous variances than not taking the logs, except in the case of hydroxyproline where no transformation was needed. (In the analysis of mineral dust, there were some zero concentration points, so it was necessary to add unity to the data before the log transformation could be made.) The analyses of covariance yielded no significant effect of age at the .05 probability level. Likewise, the ANOVA's yielded no significant two-way interactions at the .05 probability level. As a result of the preliminary analyses, the effects of age and the interactions were considered as random error in the subsequent ANOVA's. Also, the concentrations were considered as log-normally distributed with the exception of hydroxyproline concentrations which were considered normally distributed.

There were three basic types of ANOVA's for this Cincinnati data: (1) ANOVA's considering all historical characteristics, (2) ANOVA's considering only one historical characteristic and (3) ANOVA's considering more than one but less than all historical characteristics. Each ANOVA's controls are defined by the requirement that they possess a specific set of the following previously defined characteristics:

1. Female
2. Non-smoking
3. Black
4. Dust-free working condition

5. No listed abnormal physical condition present at time of death.

A control from the first must possess all of the characteristics; a control from the second must possess one, and only one, of the characteristics; and a control from the last must possess a specific subset, but not all, of the characteristics. Controls from this third type resulted from the stepwise regression analysis selection procedure which was used as a systematic method to obtain an indication of the most important characteristics to be considered in the ANOVA's.

A summary of the results from the first two types of ANOVA's is presented in Tables V and VI. No summary from the last type of ANOVA is presented in tabular form because it would give little information not already contained in the two tables. Statement 6 below is the only finding presented from the last type of ANOVA. The numbers in the tables may be considered as the probabilities of observing a greater difference between the means of the control group and the group with the indicated characteristic when there is, in fact, no difference between the population means. Actually the ANOVA techniques used to obtain Table VI yielded a t-test (without pairing) which compares the observed mean of the control group and the observed mean of the group possessing the historical characteristic indicated on the left of the table. They yield a somewhat more complicated t-test (without pairing) for Table V involving weighting the observable means of groups which are defined by the historical characteristics considered. Probabilities less than .05 were considered significant, but were reported at the .10 level in summary statements. This was done to compensate for inexactness in the statistical analysis caused by an unavoidably unbalanced statistical design. (An explanation in a more detailed description of the entire statistical analysis is available from the authors.) Note that this change of probability applies to summary statements and should not be confused with the .05 level in preliminary testing nor the presentation of probabilities of .10 or less in the tables. Significant differences in Table V are easy to interpret because there is only one control — subjects who possess none of the characteristics listed in the table and all the control defining

TABLE V
Probabilities from ANOVA'S Considering All the Historical
Characteristics Simultaneously*

Historical Characteristic	SiO ₂	Cd	I	Pb	Se
Sex (male)			.050		
Smoking					
heavy		.000			
light		.000			
Dusts					
metal					
organic					
Abnormal Conditions					
cardiovascular	.049				
respiratory		.058		.076	.004
malignancy					
liver	.031				
kidney					

*Only probabilities less than .10 are presented. Each may be interpreted as the probability of observing a greater difference between the mean of the controls and the mean of the subjects possessing the historical characteristic when in fact, there is no difference between the two groups' population means.

characteristics. Significance in Table VI is harder to interpret because the control is defined by selecting only the control characteristics which are opposite to the corresponding historical characteristics in the left-most column. All other control-defining characteristics may or may not be present in these controls – they are, in effect ignored in these definitions. For this reason, the following summary statements are presented (at the .10 probability level):

1. On the average, males exhibited significantly

greater concentrations of Pb, Cd, V, and Al than did women.

2. On the average, heavy smokers exhibited a greater concentration of Pb and Cd than light or non-smokers.
3. On the average, subjects who worked in a metal dust environment exhibited greater concentrations of Pb, Cd, Mn, and Al than workers not exposed to metal dusts.
4. Those subjects with an abnormal respiratory condition present at the time of death exhibited a smaller concentration of Pb, Cd, Se, Zn, and Cu.

TABLE VI
Probabilities from ANOVA's Considering Only One Historical Characteristic at a Time.*

Historical Characteristic	Total Dust	Min. Dust	Non- Min.	Free SiO ₂	Al	Cd	Co	Cr	Cu	Ge	I	Mg	Mn	Mo	Pb
Sex (male)						.001		.069					.086		.006
Race (white)											.076				
Smoking						.000									.067
light						.000									
heavy						.000							.080		.043
Dusts					.056	.002		.092					.015		.010
metal dust					.027	.017		.068					.004		.026
organic dust															
Abnormal Condition															
cardiovascular			.084												
respiratory						.011			.018						.042
malignancy						.077	.060								
liver	.047	.004				.058									
kidney										.045	.088	.059		.014	

*Only probabilities less than .10 are presented. Each may be interpreted as the probability of observing a greater difference between the mean of the controls and the mean of the subjects possessing the historical characteristics when in fact there is no difference between the population mean of the two groups.

5. The subjects with an abnormal kidney condition present exhibited significantly greater concentrations of Mo, V, and Ge.
6. Subjects who were white or who had an abnormal kidney condition exhibited a greater concentration of Mg than subjects who were black or had no abnormal kidney condition, respectively.
7. On the average, white subjects exhibited a significantly lower concentration of iodine than black subjects.
8. On the average, higher concentrations of total dust and mineral dust existed when an abnormal liver condition was present at the time of death.

These statements may not be taken out of context. In any statistical analysis limitations are imposed by the basic assumptions made, the definition of the controls, and by the sample size. In this study, the variables considered in the statistical analysis are interrelated and the statistical significances mentioned above appear, except in a few instances, only when all but one of the variables are ignored. Thus, these effects could be construed to be significant only in groups of workers whose historical characteristics were interrelated and distributed in a manner similar to these urban subjects. Cause and effect relationships, of course, cannot be validly inferred. It should also be kept in mind that larger sample sizes might reveal additional significant relationships.

summary

A control sample of lungs from 100 urban

Se	Ti	V	Zn
		.015	
		.058	
		.068	
	.066	.054	
.005			.010
		.028	.058

residents was analyzed for 21 trace metals, free silica, dust, and hydroxyproline. Hilar lymph nodes were also assayed for trace metals. Analytical data for the three latter substances were compared to earlier coal miner studies. Dust levels were comparable to miners' dust levels, but the dust itself was of a different composition from one group to the other. Free silica mean concentrations were lower by a factor of 10 in the urban group. No differences were detected in hydroxyproline levels. Six of the 21 trace metals concentrations were very low in both lungs and nodes. For the 15 metals with a full range of definite values, a graphic comparison was made between lung and nodal tissue. Data from this study was also compared with the Oak Ridge "Standard Man" Study covering the elements Al, Cr, Cu, Mg, Mn, Pb, Ti, and Zn. The differences in concentrations found between these two studies of "normal" tissue appear to be within a range which may be attributed to differing methodology and relative sensitivities of the analytical systems. The more sensitive spark source mass spectrometric method of analysis has extended the number of trace metals that may be detected and quantitated in human lung tissue.

The magnitude of the significant differences among group means found indicate the potential value of this urban data as a baseline in an analysis of coal miner data. If near optimal sample sizes are considered (~100 coal miners are needed) and true differences in population means are near the magnitude of the significant differences found in this study, then a statistical analysis is likely to yield significant differences between coal miners and urban controls. (Prior data indicate that differences of this magnitude appear among means of groups of coal miners classified by degree of severity of coal workers' pneumoconiosis.⁽⁷⁾)

It would be desirable, in future analyses of data for coal miners, to be able to make comparisons with controls without consideration of historical factors. This could be done if: (1) the analysis in this report has shown no effects of the historical factor to be excluded, and (2) no significant differences were found between group means of coal miners (grouped by historical factors). The interpretation and possible follow-up investigation of results from

this type of simplified statistical analysis will be facilitated without the presence of historical factors.

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