Scanning Microscopy

Volume 5 | Number 1 Article 9

10-22-1990

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L. E. Stettler
National Institute for Occupational Safety and Health

S. F. Platek
National Institute for Occupational Safety and Health

R. D. Riley
National Institute for Occupational Safety and Health

J. P. Mastin
National Institute for Occupational Safety and Health

S. D. Simon
National Institute for Occupational Safety and Health

Scanning Microscopy, Vol. 5, No. 1, 1991 (Pages 85-94) 0891-7035/91$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

LUNG PARTICULATE BURDENS OF SUBJECTS FROM THE CINCINNATI, OHIO URBAN AREA

L.E. Stettler,* S.F. Platek, R.D. Riley, J.P. Mastin, and S.D. Simon

Division of Biomedical and Behavioral Science
National Institute for Occupational Safety and Health
4676 Columbia Parkway
Cincinnati, Ohio 45226

(Received for publication April 13, 1990, and in revised form October 22, 1990)

Abstract

Because of the relatively small data base existing for lung particulate burdens of subjects with no overt pneumoconioses, the total exogenous lung particulate concentrations of 91 subjects from the Cincinnati, Ohio urban area were determined using an automated scanning electron microscope-energy dispersive x-ray analysis-image analysis system. Four of these subjects were foundry workers and had the highest exogenous particle concentrations seen in the 91 lungs, ranging from 1860 to 2990 x 10^6 particles per gram of dry lung (ppg). The average exogenous particle concentration for the remaining 87 subjects was 476 $\pm$ 380 x 10^6 ppg with a range of 71 to 1860 x 10^6 ppg. The median size of the exogenous particles in the 87 lungs was narrow, ranging from 0.37 to 1.02 μm. The geometric mean particle size over all 87 lungs was 0.60 μm with a geometric standard deviation (σ_g) of 2.35. The total exogenous particle levels were elevated for the male subjects compared to females (p=0.015), and were positively associated with age (p=0.021). However, no correlation was seen between total particle concentration and race or smoking history.

KEYWORDS: human lung particle content, automated particle analysis, non-fibrous mineral particles, electron microscopic particle analysis, quantitative analysis.

*Address for correspondence:
L. E. Stettler, National Institute for Occupational Safety and Health (C-26)
4676 Columbia Parkway
Cincinnati OH 45226 Phone No. (513) 533-8433

Introduction

The investigation of particle-induced lung disease through the analysis of human lung tissue has grown rapidly in the past twenty years. A wide variety of analytical techniques have been used to investigate the particle content of human lungs (Mastin et al., 1988). Electron probe microanalysis, using either a scanning or transmission electron microscope equipped with an energy dispersive x-ray analyzer, has been extensively used to characterize individual fibrous and/or non-fibrous particles found in human lungs (see reviews by Baker et al., 1985 and Shelburne et al., 1989).

Particulate matter has been recognized as a cause of various lung diseases, the pneumoconioses, for many years. Specific pneumoconioses such as coalworker's pneumoconiosis, asbestosis, and silicosis may result from the inhalation of particles at the worksite. Many of the electron probe microanalytical investigations of human lung particulate burdens have been undertaken with the intent of relating the composition and concentration of particles found to any observed lung disease (e.g., Stettler et al., 1977 and 1983; Vallyathan et al., 1980; Gylseth, et al., 1984). However, everyone has a background lung particulate burden resulting from the inhalation of respirable particles present in the ambient environment. Consequently, interpretation of data from analyses of diseased lungs is difficult without a good data base for the particle contents of a control population with no pneumoconioses and/or history of occupational exposure.

While there is a large data base for the fiber content of lungs from the general population (e.g., Tuomi et al., 1989; Roggli, 1989; Churg and Wiggs, 1986; Churg, 1982; Gylseth et al., 1981; Churg and Warnock, 1980), relatively little data exists for non-fibrous particulate burdens of lungs from this group. A small number of subjects from the Vancouver, Canada area have been studied (Churg and Wiggs, 1985 and 1987). In their initial study of subjects who had no history of occupational exposure, Churg and Wiggs (1985) reported the mean particle concentration for 14 males as 261 $\pm$ 175 x 10^6 particles per gram of dry lung (ppg) while the particle concentration for 14 males with lung cancer was 525 ± 369 x 10^6 ppg. Particles from two pieces of upper lobe lung tissue for each subject were analyzed manually by analytical transmission electron microscopy. Approximately 200 particles per sample site were analyzed, but the analysis was limited to particles with at least one dimension 0.5 μm or greater. In a second study (Churg and Wiggs, 1987), the mean particle concentration for 10 male smokers from the Vancouver area who had no history of occupational dust exposure was 465 ± 295 x 10^6 ppg. Four separate pieces of lung tissue from each subject were evaluated. For each piece, 100-200 particles with a dimension greater than 0.1 μm were analyzed manually by analytical transmission electron microscopy.

The lung particulate burdens of 10 subjects (5 males and 5 females) from the Rome, Italy area who had no history of occupational exposure have also been investigated (Paoletti, et al., 1987). The mean particle concentration for these subjects was 180 X 10^6 ppg. Particles greater than 0.1 μm in diameter were analyzed manually by analytical transmission electron microscopy. While tissue from six sites from each subject were analyzed, the total number of particles analyzed was not given.

In our laboratory, scanning electron microscopy (SEM)-based automated image analysis has been used routinely to characterize non-fibrous inorganic particles extracted from lung tissue. Since large numbers of individual particles may be qualitatively characterized quickly, and because of the relatively small data base existing for particulate burdens of lungs with no overt pneumoconiosis, a study was initiated to determine the particle contents of 91 lungs from the Cincinnati, Ohio urban area. Earlier, preliminary reports for the analyses of 33, 48, and 75 of these lung specimens have been given elsewhere (Stettler, et al., 1986a, 1986b, and 1989). The particulate data from all 91 lungs will be summarized and discussed in this report.

Materials and Methods

Lung Specimens

During 1970-71, whole left lungs from 100 subjects from the Cincinnati, Ohio urban area were taken at autopsy to serve as a companion, control population for a group of coal miner lungs for which free silica, total dust, and trace element concentrations were being determined. These control subjects had resided in the Cincinnati area for varying periods of time; however, approximately 90% of them had lived in the area for 20 or more years immediately preceding their deaths. The lungs were homogenized, freeze-dried and then extensively analyzed for free silica, total dust, and trace elemental components using bulk analytical techniques (Sweet et al., 1978).

Lung homogenate samples and occupational/social histories for 91 of the original subjects were available for inclusion into the current study. This subject group consisted of 43 males and 48 females. Ages for the males ranged from 24 to 90 years with a mean of 64.9 ± 14.2 years. Ages for the females ranged from 23 to 96 years with a mean of 65.9 ± 16.2 years. A total of 54 of the subjects were smokers, 36 males and 18 females. The male smokers averaged 58 ± 47 pack years, while the females averaged 36 ± 22 pack years. The range of occupations represented in the sample group included domestics, homemakers, clerical workers, farmers, construction workers, foundry workers, machine operators, business executives, and truck drivers. The subjects' deaths were from natural causes, mostly chronic degenerative conditions of advancing age. While there were some acute disease conditions, there were no traumatic deaths. A hospital autopsy report and a personal occupational/social history were available for each subject.

Tissue Processing

Accurately weighed samples (in the range of 0.1 to 0.2 grams) of each freeze-dried lung homogenate were processed separately for microanalysis. Each specimen was ashed for seven hours in a low temperature asher at 90 watts using an oxygen pressure of 2 torr. A suspension of the ash from each sample was made using 50 ml of a 0.05% solution of Aerosol OT® in filtered, de-ionized water. The resulting suspensions were then sonicated in an ultrasonic bath for 15 minutes. The suspensions were then made up to a final volume of 100 ml with filtered, de-ionized water to which 1 ml of glacial acetic acid had been added and allowed to sit overnight. It was found that this acetic acid treatment removed some of the endogenous calcium and phosphorus-containing particles from the ash suspensions. A sample blank was carried through the entire preparation process for each specimen. Aliquots of the suspensions for sample and blank were then filtered onto 25 mm diameter, 0.1 μm pore size Nuclepore® filters.

Particle Analyses

Particles found on the filter preparation for each lung and blank were analyzed using a scanning electron microscope (Hitachi S-570 or ISI Super IIIA) equipped with a Kevex 7000 energy dispersive x-ray analysis system and a LeMont Scientific DA-10 image analysis system using the backscattered electron image. For each lung filter preparation, at least 1000 particles in a minimum of 20 randomly selected fields of view at a magnification of 1000X were analyzed. Particles found in the same number of randomly selected fields of view for the blanks were also analyzed and subtracted from the corresponding lung analysis.

The image analyzer uses a regular gridpoint spacing pattern with a preset point density (off point density) to locate particles. At each off point, the backscattered electron signal is compared to a threshold to determine whether the electron beam is on a feature of interest. For our analyses, this off point density was set at 0.2 μm. Consequently all particles 0.2 μm and larger in any dimension will contain a gridpoint and be counted if the backscattered electron signal from the particle is above the threshold. Features smaller than 0.2 μm can fall between the grid points and be missed. Once a particle was found, a narrower point density (on point density) was used to determine various particle physical parameters. Subsequently, an x-ray analysis for

31 elements using an x-ray spectrum acquire time of 5 seconds was performed. More complete descriptions of automated image analyzers are given elsewhere (Lee and Kelly, 1980; Johnson, 1983).

Analyzed particles were classified using a chemistry definition file which sorts the particles based on their major elemental components and the net fractional x-ray intensities of these components. A listing of the elements analyzed and a listing of the chemistry definition file used in the analyses is given elsewhere (Stettler et al., 1989).

Inorganic particles are sorted into one of the 13 major classes shown in Table 1. Particles classified as silica, alumina-like, iron oxide-like, and rutile-like (TiO_2) contain only one major peak in their x-ray spectrum and are all presumed to be oxides. The aluminum silicate class allows for a broad range of aluminum, silicon, and other element x-ray intensities. The magnesium silicate class contains particles having varying amounts of iron, in addition to magnesium and silicon.

The chemistry file also contains a major category for particles which contain either completely endogenous components or various combinations of endogenous and exogenous components. Generally, the purely endogenous particles in this category are composed primarily of iron and phosphorus. A subclass of the major endogenous category, exogenous-endogenous combination (exog.-endog.comb. in Table 1), contains particles having a net fractional exogenous elemental intensity of at least 0.15 which are counted as exogenous in tabulating the total lung exogenous particulate burden. The particles in this subclass may be either aggregates of exogenous and endogenous particles or exogenous particles which have an endogenous coating. As with the purely endogenous particles, the endogenous portions of these combination particles are also usually composed of iron and phosphorus.

At least 50% of the net x-ray intensities for particles in the silicon-, iron-, titanium-, and aluminum-rich categories arise from the element specified in the class title. However, unlike the silica, alumina-like, and rutile-like classes, particles in the element-rich categories also contain at least one other major component. Particles classified as other aluminum silicates have major aluminum and silicon components, however, the net silicon x-ray intensity for these particles is less than the minimum allowed for particles in the initial aluminum silicate class. The other aluminum silicate particles, as well as those placed in the element-rich categories, may be aggregates. All remaining exogenous particles are placed in the miscellaneous class.

Final lung particle concentrations are calculated from the relationship between the weight of dry lung tissue ashed, the aliquot of the lung ash used to prepare the filter, the total filter area and area of the filter analyzed, and the total number of particles analyzed for each filter.

Results

In excess of 145,000 individual particle analyses were performed for the 91 lungs in this study. The overall total exogenous particle concentration distribution for these 91 lungs is shown in Figure 1.

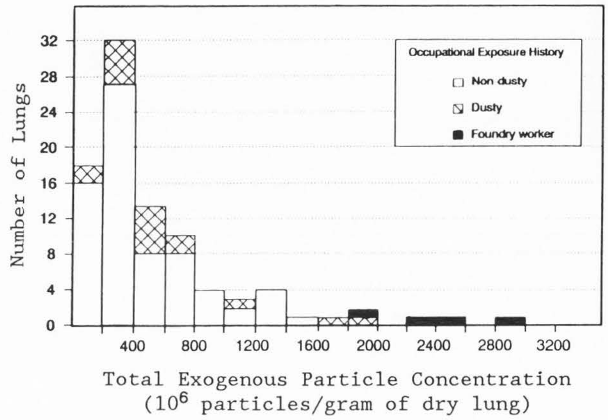

Figure 1. Total exogenous particle concentration distribution for the 91 Cincinnati urban lungs.

The occupational histories for 21 of the 91 subjects indicated some type of dust exposure. The total exogenous particle distribution for these 21 subjects is also shown in Figure 1. Only six of these 21 lungs have total exogenous particle concentrations outside the range seen for the 70 lungs with no history of occupational exposure. Of these, the four subjects having the highest exogenous particle concentrations all had worked in foundries for 8 or more years and also had very high silica levels compared to the other 87 lungs as shown in Table 1. Considering their occupational histories, elevated total exogenous particle concentrations, and elevated silica levels, these four subjects were deemed to have had extensive occupational dust exposures not representative of the general populace. Consequently, these four lungs were separated from the remaining 87 lungs in terms of data analysis.

The results of the individual particle analyses for the remaining 87 lungs are summarized in Table 2 in terms of particle concentration and type and in Table 3 in terms of percentages by particle type. The percentage distributions for selected particle types for the 87 lungs are shown in Figures 2(a) -2(d). Particle concentrations summarized by the subjects' sex, race, and smoking history are shown in Table 4 and Figure 3. In general, the same major particle types were seen in all of the Cincinnati lungs. Percentage quantities of silica, aluminum silicates, and rutile-like particles were seen in all 87 lungs. Magnesium silicates, presumably talc, were present in all 87 lungs, but comprised less than 1% of the exogenous particles in 24 of these lungs. Alumina-like particles were found in 83 of the 87 lungs, but accounted for greater than 1% of the exogenous total in only 13 lungs. Of these 13 lungs, only three had quantities of alumina-like particles greater than 2% (viz., 2.3, 5.0, and 10.1%).

Particles classified as silica, presumably crystalline or free silica, accounted for, on the average, 21.0% of the total exogenous particulate burden for the 87 lungs. Silica concentrations for each lung as determined by the automated SEM analyses were compared to spectrophotometrically-

Table 1. Particle data for four foundry workers' lungs compared to the other 87 lungs.

Particle Type	Particle Concentration (10^6 particles/g of dry lung) Lung 1	Lung 2	Lung 3	Lung 4	Mean of Other 87 Lungs
Total Exog.	2250	2990	2500	1860	476
Al Silicate	655	1250	506	361	179
Silica	740	971	919	973	94
Rutile-like	76	127	19	73	55
Exog.-Endog. Comb.	425	232	375	72	44
Iron Oxide-like[a]	60	134	506	274	35
Iron Rich	40	71	192	64	22
Aluminum Rich	62	58	16	50	22
Miscellaneous	133	47	44	99	15
Titanium Rich	33	94	11	38	15
Silicon Rich	49	74	388	22	12
Mg Silicate	4	27	3	2	9
Other Al Sil.	16	33	5	4	7
Alumina-like	13	9	25	107	3

[a] Iron Oxide-like particles are considered to be endogenous (see text).

Table 2. Summary particle data for 87 urban lungs.

Particle Type	Particle Concentration (10^6 particles/g of dry lung) Mean	Stand. Dev.	Min.	Median	Max.
Total Exog.	476	380	71	374	1860
Al. Silicate	179	144	21	127	762
Silica	94	70	11	72	337
Rutile-like	55	116	2	29	942
Exog.-Endog. Comb.	44	80	1	17	561
Iron Oxide-like[a]	35	33	2	26	157
Iron Rich	22	23	1	15	145
Aluminum Rich	22	22	1	13	115
Miscellaneous.	15	13	1	11	63
Titanium Rich	15	12	1	10	67
Silicon Rich	12	9	2	10	55
Mg Silicate	9	8	<1	6	34
Other Al Sil.	7	7	<1	5	33
Alumina-like	3	6	0	1	53

[a] Iron Oxide-like particles are considered to be endogenous.

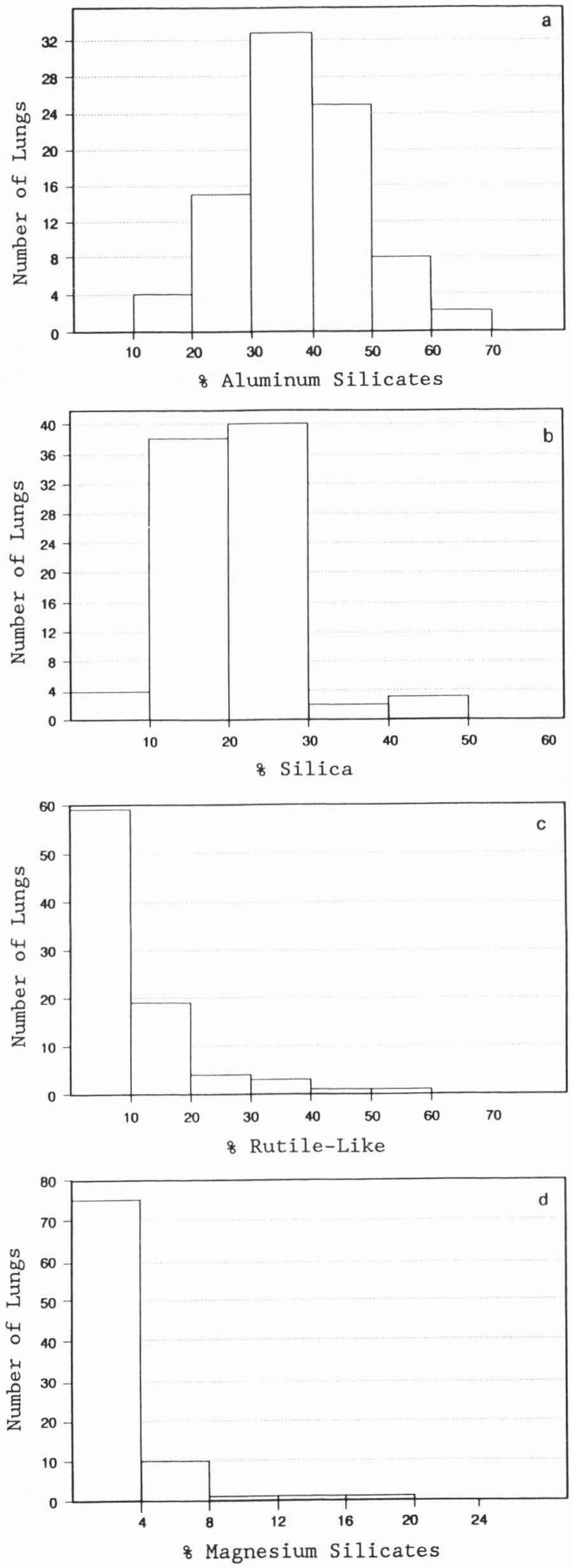

Figure 2. Percentage distribution for selected particle types in the Cincinnati urban lungs. (a) % Aluminum Silicates. (b) % Silica. (c) % Rutile-like. (d) % Magnesium Silicate.

determined free silica levels for this lung set (Sweet, et al., 1978). This data comparison showed that these two techniques correlate reasonably well (Spearman correlation coefficient = 0.50). Consequently, classification of particles having only a major x-ray peak for silicon as silica is appropriate.

As a class, the aluminum silicates were the most prevalent particle type encountered, accounting for, on the average, 38.1% of the total exogenous lung particulate burden. Two major types of aluminum silicates were seen in the lungs; particles whose x-ray spectra were consistent with the mineral kaolinite which contained only peaks for aluminum and silicon, and particles containing varying amounts of potassium and iron in addition to aluminum and silicon which are presumably either mica or a potassium-containing feldspar. Small numbers of particles which had chemistries consistent with the plagioclase feldspars, sodium/calcium aluminum silicates were also seen in most of the lungs.

It should also be noted that approximately 75% of the particles in the exogenous-endogenous combination class appeared to be either silica or aluminum silicates associated with endogenous material. For example, many particles were seen which had x-ray spectra consisting of major peaks for silicon, phosphorus, and iron. These particles could be either silica aggregated or coated with iron and phosphorus-containing endogenous material.

The median circular area equivalent diameters for the exogenous particles found in the 87 lungs ranged from 0.37 to 1.02 μm. Individually, the total exogenous particle size data for each of the 87 lungs were log normally distributed as determined from cumulative log-probability plots of the particle size data for each lung. In addition, the combined particle size data for all 87 lungs for the total exogenous category and the silica, aluminum silicate, rutile, and magnesium silicate classes were also log normally distributed. These data are summarized in Table 5. As can be seen from this data, rutile particles were generally smaller and the magnesium silicate particles larger than either silica or the aluminum silicates.

Table 3. Exogenous particle data for 87 urban lungs - percentage composition.

Particle Type	Mean (%)	Stand. Dev.	Minimum (%)	Maximum (%)
Al Silicate	38.1	10.1	13.7	61.6
Silica	21.0	6.6	7.6	43.2
Rutile-like	10.2	9.0	2.0	50.6
Exog.-Endog. Comb.	7.9	8.3	0.4	48.4
Iron Rich	4.7	2.3	0.7	13.1
Aluminum Rich	4.4	2.3	0.7	11.0
Miscellaneous	3.3	1.8	0.7	11.3
Silicon Rich	3.1	1.9	0.6	9.0
Titanium Rich	3.0	1.0	1.2	6.1
Mg Silicate	2.5	2.8	0.1	18.1
Other Al Sil.	1.4	0.7	0.2	3.3
Alumina-like	0.7	1.2	0.0	10.0

Table 4. Exogenous particle summary for 87 urban lungs by sex, race and smoking history.

		Total Exogenous Particle Concentration (10^6 particles/g of dry lung)				
Sample Group	N	Mean	Stand. Dev.	Min.	Med.	Max.
All Lungs	87	476	380	71	374	1860
Males	39	582	424	123	478	1860
Females	48	390	320	71	262	1500
Blacks	40	526	432	71	326	1760
Whites	47	434	329	96	376	1860
Black Males	18	656	474	123	562	1760
White Males	21	518	376	141	433	1860
Black Females	22	419	371	71	255	1500
White Females	26	367	275	96	278	1270
Smokers	50	505	434	71	356	1860
Non-Smokers	37	437	293	96	374	1240
Male Smokers	32	594	433	123	502	1860
Male, Non-Smokers	7	527	406	211	380	1210
Female, Smokers	18	438	401	71	212	1500
Female, Non-Smokers	30	416	264	96	368	1240
Black Smokers	21	546	505	71	272	1760
Black, Non-Smokers	19	503	346	173	373	1240
White Smokers	29	476	381	135	376	1860
White, Non-Smokers	18	367	212	96	376	884
Black, Male Smokers	14	651	485	123	566	1760
Black Male, Non-Smokers	4	675	504	244	625	1210
White, Male Smokers	18	550	397	141	466	1860
White Male, Non-Smokers	3	329	103	211	380	397
Black Female, Smokers	7	336	514	71	167	1500
Black Female, Non-Smokers	15	457	297	173	363	1240
White Female, Smokers	11	356	338	135	227	1270
White Female, Non-Smokers	15	375	230	96	374	884

Table 5. Cumulative particle size data for selected particle types for 87 urban lungs.

Particle Type	Geometric Median Diameter (μm)	Geometric Standard Deviation
Total Exogenous	0.60	2.35
Silica	0.66	2.25
Aluminum Silicates	0.74	2.28
Rutile-like	0.34	1.77
Magnesium Silicates	1.05	2.89

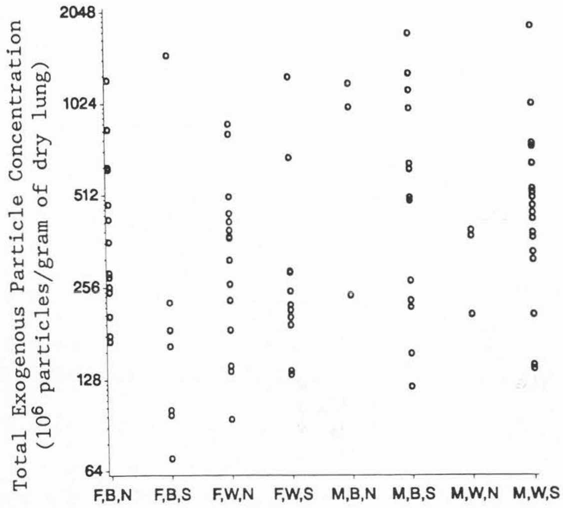

Figure 3. Total exogenous particle concentration distribution by sex (Female or Male), Race (Black or White), and Smoking History (S=Smoker, N=Non-smoker).

Discussion

The first objective of this work was to develop a data base for the exogenous lung particulate burdens for subjects with no overt pneumoconioses. Particles found in the lung which contain only an x-ray peak for iron may be either endogenous or exogenous in nature and, most likely, there are some of each in many of the lungs. While there is no foolproof way to determine the origin of these particles, a decision must be made in reporting the data for this category. In both of the Churg and Wiggs studies (1985, 1987), particles containing only iron were considered endogenous, while these particles were considered exogenous in the Paoletti et al. (1987) study. In our earlier interim reports concerning the analyses of Cincinnati lungs (Stettler et al., 1986a, 1986b, and 1989), iron oxide-like particles were arbitrarily considered to be of exogenous origin if they contained no traces of phosphorus. However, in the final evaluation of the data for all 91 lungs, a correlation between the numbers of particles classified as iron oxide-like and obvious endogenous particles, i.e., particles containing primarily iron and phosphorus, was found (Spearman correlation coefficient = 0.74). In light of this correlation, we feel that a better estimate of total exogenous particulate burden is obtained by considering the iron oxide-like particles to be endogenous. Consequently, these particles have been so classified in this report.

A second consideration in presenting the study data is whether any of the subjects had extensive occupational dust exposures. Inclusion of such subjects in the data base could greatly diminish its value as normative data. However, the determination of whether to remove a subject from the study based on his/her occupational history is not straightforward. Certainly one must be concerned with the accuracy of the work histories obtained. The work histories for the subjects in this study were taken almost twenty years ago. Consequently, we have had to take the histories at face value since follow up would not be possible. Beyond accuracy however, there are issues which are extremely difficult, if not impossible, to resolve concerning whether a subject should be removed. For example, would one remove a subject who has worked for one year in a dusty environment early in his or her career while the remaining work years were spent in an obviously non-dusty environment?

We have taken a very conservative approach in attempting to resolve these issues surrounding occupational dust exposures. While subjects were automatically classified as having occupational exposures if there was any mention of dust exposure noted in their work histories, they were not removed from the study unless it was obvious that both their total exogenous particulate burden and their particle concentration profile were consistent with a specific occupational exposure. Thus it was relatively straightforward to remove the four foundry workers from the study because of their elevated total exogenous and silica concentrations. On the other hand, while occupational dust exposures were indicated for two other lungs with high dust levels, no relationship between these subjects' occupational histories and their lung particulate profiles was immediately apparent. Consequently, these lungs were not removed from the data set. The remaining 15 subjects which had occupational exposures noted in their histories also were not removed from the study because there was no apparent relationship between their particle profiles and respective occupations. In addition, the particulate concentrations for these 15 lungs were not elevated; in fact, the particle concentration distribution for these 15 lungs was very similar to that seen for the 70 lungs with no mention of dust exposure in their history as shown in Figure 1. Overall, the inclusion of these 17 lungs with unsubstantiated occupational dust exposures into the 87 lung data base had very little effect. The mean total exogenous particle concentration for the 70 lungs with no occupational dust exposure history was $448 \pm 341 \times 10^6$ ppg, compared to $476 \pm 380 \times 10^6$ ppg for the 87 lungs.

The overall mean particle concentration for the 87 Cincinnati urban lungs, $476 \pm 380 \times 10^6$ ppg, compares quite well with the limited data in the literature for subjects with no pneumoconioses or histories of occupational exposures; i.e., $261 \pm 175 \times 10^6$ ppg for 14 non-cancer subjects and $525 \pm 369 \times 10^6$ ppg for 14 cancer subjects from Vancouver (Churg and Wiggs, 1985), $465 \pm 295 \times 10^6$ for 10 smokers from Vancouver (Churg and Wiggs, 1987), and 180×10^6 for 10 subjects from Rome (Paoletti, et al., 1987. In terms of particle profiles, the data from our study are similar to that found by Churg and Wiggs and by Paoletti, et al. in that the same major particle types were found. Various aluminum silicates were the species encountered most often in all of the studies. Differences were seen in the silica, rutile, and magnesium silicate (talc) concentrations among the studies.

The mean silica levels in the Churg and Wiggs and Paoletti, et al. studies ranged from approximately 10% to 16% and were all somewhat lower than that seen for the Cincinnati lungs (21.0 %). The overall average magnesium silicate (talc) levels for the Churg and Wiggs studies, 7% to 21%, and the Paoletti, et al. study, approximately 16%, were considerably higher than that seen for the Cincinnati lungs (2.5%). On the other hand, the average rutile concentration in the Cincinnati lungs, 10.2%, was much higher than that seen in the other studies which ranged from approximately 2 to 6%. These differences in particle profiles are, most likely, related to differences in the atmospheric environments of the respective cities.

In terms of mean percentage composition, particles classified as silica, aluminum silicates, and magnesium silicates accounted for 61.6% of the exogenous particles in the Cincinnati lungs, whereas these particle types accounted for 75-85% of the total in the initial Churg and Wiggs study (1985), approximately 80% in the second Churg and Wiggs study (1987), and approximately 90% in the Paoletti, et al. study (1987). A more accurate estimation of the total silica, aluminum silicate, and magnesium silicate levels for the Cincinnati lungs would include the large number of aluminum silicate (mean percentage of the exogenous total = 3.9% $\pm$ 4.8) and silica (mean percentage of the exogenous total = 1.9% $\pm$ 2.8) particles associated with endogenous material. These particles were initially counted in the exogenous-endogenous combination class. Including these particles into the total would bring the level of silica, aluminum silicates, and magnesium silicates in the Cincinnati lungs to 67.4%.

A statistical analysis of the Cincinnati lung data was performed to examine the relationships, if any, between subject demographic parameters and exogenous particle concentrations. This analysis used three categorical factors and one continuous covariate to predict exogenous particle concentrations. The three categorical factors were gender (male or female), race (black or white), and smoking history (yes or no). The continuous covariate was age. This analysis incorporated all possible interactions among the categorical factors. Since a preliminary analysis indicated that the dependent variable, total exogenous particle concentration, was skewed, a logarithmic transformation was applied. The gender and age effects were statistically significant (p=0.015 and 0.021, respectively). The mean total exogenous particle concentration for males was 56% larger than the same mean for females. The 95% confidence interval, however, ranged from 10% to 121%, indicating that even with 87 lungs, we do not have a precise estimate of the difference seen between males and females. The effect of age on exogenous particle concentration was positive. Each additional year in age resulted in an increase of 1.3% in average exogenous particle concentration. Again, the 95% confidence interval was very wide (0.22% to 2.5%) indicating that we do not have a precise estimate of the change in exogenous particle concentration due to age. No other effects or interactions were significant at alpha = .05.

Inhalation experiments utilizing human subjects exposed to Fe_3O_4 (Cohen, et al., 1979) or polystyrene (Bohning, et al., 1982) have shown that cigarette smoking impairs long-term clearance of particles from the lung. Consequently, one would expect the exogenous lung particulate burden of smokers to be elevated compared to non-smokers. For the small number of lungs analyzed in their studies, the data of Churg and Wiggs (1985 and 1987) and Paoletti, et al. (1987) generally support the association of increased lung particle burdens with smoking. However, no such correlation was seen for the Cincinnati lungs, and clearly, further data needs to be developed on this subject. Interestingly, the data of Churg and Wiggs (1987) indicate that the effect of smoking on lung particle burden may be limited to specific areas of the lung, since a correlation between number of pack years smoked and particle concentration was seen only for the upper lung lobe. We were unable to determine whether smoking had any effect on particle concentration in specific areas of the Cincinnati lungs, since we were dealing with whole lung homogenates.

In summary, the total exogenous particle concentration levels and types of particles found in the 87 Cincinnati lungs with no overt pneumoconioses or documented occupational exposures are very similar to those seen in other studies (Churg and Wiggs, 1985 and 1987; Paoletti, et al. 1987). Considering the differences in analytical techniques used and the vastly different geographic origins of the subjects in these studies, the exogenous particle concentrations determined in each are remarkably similar, with the means ranging from 180 to 476 x 10^6 ppg.

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Discussion with Reviewers

B. Burnett: Inclusion of the 17 or 21 subjects with occupational dust exposures with the rest of the data, especially in the particle type analyses, is simply not valid considering the intent of this study. I suggest that the authors restrict themselves to reporting only the results of subjects without documented occupational dust exposure. If the authors feel that the addition of the occupationally-exposed subjects is necessary, then these data should be included only for comparison and in no way should the two data sets be mixed.

J.L. Abraham: You should show separately the data for 70 lungs with no history of occupational exposure.

Authors: The primary objective of this work was to develop a data base for the exogenous lung particulate burdens for subjects with no overt pneumoconioses. This certainly is the case for all 91 lungs in the study. However, presentation of the data for these lungs in terms of occupational exposures was not straightforward as discussed earlier. From our perspective, we needed a valid reason for excluding cases from the study, since it would be as incorrect to exclude cases with no substantiated occupational exposures as it would be to include cases with substantiated exposures. Consequently, our criteria for exclusion were mention of dust exposure in the subjects' histories and corroboration of this exposure in the lung particulate burden. Having decided on these two criteria, only the four foundry workers were excluded from the 91 lung data set, and summary data for the 87 lungs were presented in Tables 2-4. However, we recognize the reviewers' concern and summary data for the 70 lungs with no mention of occupational dust exposure in their histories has been added in Tables 6 and 7. As can be seen from these data, there is a small decrease in the overall total exogenous means for the 70 lung data set ($448 \pm 341 \times 10^6$ ppg) compared to the 87 lung set ($476 \pm 380 \times 10^6$ ppg), while the particle type profiles for both sets are very similar.

J.R. Millette: Do the authors think that the use of the ultrasonic bath for 15 minutes had any effect on the size distribution of particles? Do larger particles break up into smaller ones in the preparation process?

B. Burnett: It has been shown that the use of ultrasound increases the burden estimates of asbestos from lung tissue. Could ultrasound be affecting the nonfibrous particle estimates in the same way?

Authors: The effect of mild sonication on particle size distribution is certainly of concern. However, we have seen no change in particle size distributions for various minerals (quartz, talc, and feldspars) treated in our ultrasonic bath for periods up to 2 hours. Consequently, we feel that the mild sonication used in this study had no effect on individual lung particles.

Table 6. Summary particle data for the 70 lungs with no history of occupational exposure.

Particle Type	Particle Concentration (10^6 particles/g of dry lung)				
	Mean	Stand. Dev.	Min.	Median	Max.
Tot. Exog.	448	341	71	318	1500
Al Silicate	180	149	21	122	762
Silica	94	74	11	72	337
Rutile-like	41	65	2	25	481
Exog.-Endog. Comb.	36	47	1	18	233
Iron Oxide[a]	35	34	2	24	157
Iron Rich	21	19	1	14	98
Al Rich	21	22	1	12	115
Misc.	13	10	1	10	49
Ti Rich	13	11	1	9	53
Si Rich	12	8	2	10	32
Mg Silicate	9	8	<1	6	34
Other Al Sil.	7	7	<1	5	33
Alumina-like	2	3	0	1	17

[a] Iron Oxide-like particles are considered to be endogenous (see text).

Table 7. Exogenous particle data for the 70 lungs with no history of occupational exposure - percentage composition.

Particle Type	Mean (%)	Stand. Dev.	Minimum (%)	Maximum (%)
Al Silicate	38.9	9.5	18.8	61.6
Silica	21.5	6.6	9.4	43.2
Rutile-like	8.9	6.5	2.0	38.6
Exog.-Endog. Comb.	8.0	8.2	0.4	48.4
Iron Rich	4.7	2.2	1.0	13.1
Al Rich	4.3	2.1	0.7	11.0
Si Rich	3.2	2.0	0.8	9.0
Misc.	3.0	1.4	0.7	8.0
Ti Rich	2.9	0.9	1.2	6.1
Mg Silicate	2.7	3.0	0.1	18.1
Other Al Sil.	1.4	0.7	0.2	3.3
Alumina-like	0.5	0.5	0.0	2.0

B. Burnett: The amount of backscatter for any particle in the SEM varies not only with the elemental composition (i.e., density), but also with its geometry. Thus a light-element composed particle (e.g. SiAlNa) would have less backscatter than a similar geometry particle made up of heavier elements (e.g. Ti). Could the higher percentage of rutile particles in your study compared to TEM studies be the result of missing light-element particles?

Authors: We agree that the strength of the backscattered electron signal is weaker for small, light element particles, as well as for thin, platy particles. Consequently, some of these particles could be missed. However, it is very doubtful that enough particles could be missed to bring our rutile data in line with the TEM studies. We have analyzed some of the lung filter preparations using the secondary electron image and saw no significant changes in the particle type profiles compared to those obtained using the backscattered electron image.

V.L. Roggli: What range of particle numbers were observed on the filter blanks?

J.R. Millette: Please publish a summary of the particle data from the blanks.

Authors: A summary of the particles found in the blank preparations is given below in Table 8. The number of exogenous particles in the blank filter preparations averaged less than 0.5 per field of view. The total number of exogenous particles found in 20 or more fields of view for these preparations ranged from 0 to 54.

Table 8. Particle data for 91 blank samples.

Particle Type	Number Found in Same Number of Fields Used in Corresponding Lung Analysis (20 or More)		
	Mean	Standard Deviation	Maximum
Total Exogenous	9.2	8.9	54
Silica	3.2	4.5	34
Miscellaneous	1.7	3.0	21
Rutile-like	1.4	2.7	17
Iron Oxide-like[a]	1.0	1.5	8
Aluminum Silicate	0.8	1.7	14
Iron Rich	0.6	0.9	4
Silicon Rich	0.4	0.8	3
Titanium Rich	0.3	0.8	6
Exog.-Endog. Comb.	0.2	0.6	3
Aluminum Rich	0.2	0.5	2
Magnesium Silicate	0.2	0.5	3
Alumina-like	0.2	0.6	4
Other Al Silicates	0.1	0.3	2

[a] Considered endogenous in lung analyses

P. Dumortier: Your data were obtained from subjects autopsied 20 years ago. Are those data still reflective of the actual lung particle burdens of today's general population from Cincinnati or would there be differences due to changes in the urban air particulate levels? Do the authors have some data from more recent samples?

Authors: We would expect lung particulate burden to be affected somewhat by changes in the urban air particulate levels. Unfortunately, we do not have any data from more recent samples to compare with the data summarized in this report.

B. Burnett: Has the effect of various degenerative diseases ever been measured on particle burden of the lungs? Would not a better measure of background lung dust burden be from subjects that have had a traumatic death?

Authors: We are not familiar with any definitive studies of the effects of degenerative diseases on lung particle burden. Information on the lung dust burden in trauma cases would be interesting. However, the data from urban lungs, in our opinion, are a better measure of true background lung particle burden since most people, e.g., the "average person", do not die from trauma.

J.L. Abraham: Please describe the lung "homogenate" which, from my observations, is more like a "minced" sample with intact pieces ranging from 0.3 to 1.5 cm in dimension.
Authors: According to Sweet, et al. (1978), whole left lungs were cut into small pieces and then homogenized in a blender "until a uniform slurry was obtained". Then the homogenate was freeze-dried. In point of fact, we agree with the reviewer that some of these "homogenates" contained large pieces of intact lung.

P. Dumortier: What is the capacity of your system to discriminate various types of silicates and alumino-silicates (e.g. feldspars, kaolinite, ...)?
Authors: The system can easily distinguish between various alumino-silicates, through the use of subclasses for the major particle categories in the chemistry definition file. Such data is available for the Cincinnati lungs, but was not presented due to space limitations. However, as described earlier, particles presumed to be kaolinite and either potassium feldspar or mica were the predominant alumino-silicates seen in these lungs.

P.Dumortier: How are carbonaceous compounds (coal and soot) dealt with in your analytical scheme? Aren't they destroyed with the low temperature ashing process?
Authors: Yes, the carbonaceous material is destroyed by low temperature ashing. Consequently, our analytical data represent only the inorganic portion of the lung particulate burden.

J.R. Millette: The size distributions of particles is affected by the size cut off of 0.2 μm for analysis. Is there interest from the health or regulatory communities in looking at smaller particles?
Authors: From our perspective, one should be concerned with potential health effects of any respirable particle, including those less than 0.2 μm in diameter.

J.R. Millette: What was the strength of the glacial acetic acid added at 1 ml to 100 ml of solution?
Authors: The glacial acetic acid used in this study was 99.7% pure. Consequently, our final 100 ml of solution was approximately 1% acetic acid.

B. Burnett: Were farmers in the study considered as having occupational dust exposure?
Authors: Farmers per se were not considered as having occupational dust exposure. As was the case for the other subjects in the study, farmers were classified as having an occupational exposure only if such an exposure was noted in their respective occupational/social histories.

J.L. Abraham: Is it possible to provide your data with classification changes or sorting to allow determination, say, of numbers of particles containing a given element? If so, could you produce such a table to allow comparison with data on frequencies and concentrations of various metal particles as reported by Abraham and Burnett, 1989?
Authors: A table listing the frequency of detection of specific elements is provided by the LeMont system. However, we have not found this data of much value since one can't tell whether the detected element is a major or minor particle component, or with what other elements the metal components are associated. One could reclassify the data using a new chemistry definition file in which specific metal particles were defined, but that would be a major undertaking.