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DEVELOPMENT AND USE OF A PNEUMOCONIOSIS DATABASE OF HUMAN PULMONARY INORGANIC PARTICULATE BURDEN IN OVER 400 LUNGS

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Abstract

Over 400 cases with data from in situ electron microprobe quantitation of non-fibrous inorganic particles (e.g., silica, alumino-silicates, talc, metals) in pulmonary tissue sections, and data from quantitative digestion analyses for fiber content (e.g., asbestos, silica, alumino-silicates, man-made fibers, talc) comprise an extensive microcomputer data set of lung particle burden. When allied with demographic and histopathologic information the result is a comprehensive database of occupational pulmonary pathology. Examples of the kinds of information which can be extracted from the database include: 1) summary information on the types sizes and associations of particles in lungs with a variety of exposures, 2) concentrations of etiologic particle type in cases with recognized pneumoconioses, and 3) correlations between particle type, pathology, occupation and social history. The database provides a powerful tool for assessing such information on statistically meaningful sample sets.

Key Words: Scanning electron microscopy, pneumoconiosis, lung pathology, database, microcomputer, silica, silicates, asbestos, dust analysis, x-ray microanalysis.

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Introduction

The lungs act as a continuous sampler of respirable particulate matter in the environment. Of this material certain particle types are deposited within the lung and of these a proportion are retained. When lung tissue becomes available for examination through either biopsy or autopsy, the analyst can read the record of this pulmonary dust burden. Determining the lung burden of particulate material may provide important clues to occupational and other exposures during the patient's life up to that time. Sometimes it is difficult to obtain a thorough occupational history from patients as they may not be aware of all their exposures, they may be unavailable for questioning (for example at the time of autopsy), or other factors may impede the gathering of accurate and complete information. Although analysis of lung tissue does not provide a complete record of exposures, it does permit a determination of the retained burden in the lung.

Various procedures are available which provide a quantitative measure of lung dust burden. The simplest involves ashing the lungs, weighing the residue and then performing a chemical analysis. While this approach does not allow the individual particles to be studied, by employing an analytical technique such as X-ray diffraction it is possible to determine the amounts of silica present with respect to other dusts. This methodology was used successfully by Nagleschmidt (1960), in studying massive fibrosis (complicated pneumoconiosis) in persons with various occupations. An alternative approach, which can provide similar data but with a greater degree of resolution, is to employ a combination of scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The SEM/EDX procedure by proceeding on a particle-by-particle basis gives detailed information on individual particle size, morphology and individual particle composition.

Over the past ten years we have developed and applied quantitative methods of measurement of pulmonary inorganic dust burden using the SEM/EDX approach. Our analyses have been of two types: inorganic non-fibrous particulates analyzed using an in situ tissue section morphometric method (Abraham and Burnett,

1983; 1989) and a digestion method for the determination of the fibrous particle burden (Abraham et al., 1988). By performing quantitative analyses it is possible to make comparisons between subjects and with general populations that have had or have not had certain exposures. Also, quantitative measures are necessary in epidemiological studies or studies of dose-response and synergy.

Using the tissue section and digestion methods our laboratory has examined many hundreds of lung samples. As a consequence, the amount of data on individual lung dust burdens that has been accumulated has become quite sizeable. Such information provides a valuable record of the types and amounts of particles retained under various exposure conditions. In order to make full use of this resource it is necessary to have the constituent data in a readily accessible form. The solution to this is to bring all the quantitative analysis results (plus other pertinent information) together in a data base format. In this paper we outline the creation of such a database. Potential uses of the database with a few illustrative examples are also described.

Data Base Development: Data Collection

1. Lung Burden Data

a. Non-fibrous Particles: The method for in situ quantitative analysis has been previously reported in detail (Abraham and Burnett 1983; 1989). Briefly, it involves formalin fixation, paraffin embedding, sectioning at 5 micrometers thickness, section mounting on a carbon substrate, deparaffinization and examination in the SEM. Specimen coating is not necessary as the section is conductive, allowing secondary and backscattered electron imaging and EDX analysis. Under standard operating conditions the section is examined using a morphometric point counting approach. This entails the section being scanned at a fixed magnification and the fields of view selected at random. In each field the inorganic particles are identified, sized and analyzed. Elemental data in the form of x-ray counts per second for each major peak are stored. Particle data is subsequently processed on a microcomputer using custom written software. An example of a summary of the results generated by this software is set out in Figure 1. For each case the microanalytic data includes the tissue concentration for total exogenous particles, silica, alumino-silicate, metal and talc particles, as well as other summaries, tabulations and listings of data.

b. Fibers: The concentration of various fiber types (chrysotile, amosite, crocidolite, tremolite, anthophyllite, talc and non-asbestos fibers) is determined using a digestion procedure. In brief, lung parenchymal samples are either wet formalin fixed (alcohol and xylene extracted and rehydrated before digestion) or deparaffinized and rehydrated, weighed, measured, and digested with reagent sodium hypochlorite. All reagents are pre-filtered and appropriate controls are examined. No centrifugation or sonication steps are used. Parallel samples of tissue are weighed wet and dried to determine the wet-to-dry weight ratio. Residues are collected on Nuclepore filters for asbestos body counting by light microscopy or inorganic fiber analysis by SEM/EDX. In the SEM, the uncoated filters are attached to carbon discs with colloidal graphite. The filter surfaces are scanned at 30 kV accelerating voltage, with a random sampling at appropriate magnifications to allow a determination of the fiber burden for that tissue. Fibers

Table 1. Examples of pathology/diagnoses coded in the database.

Asbestosis
Silicosis
Mixed Dust Pneumoconiosis
Silicate Pneumoconiosis
Talcosis
Arc Welder's Pneumoconiosis
Hard Metal Disease (Cobalt)
Emphysema
Diffuse Alveolar Damage (DAD)
Interstitial Pneumonias
Usual (UIP)
Desquamative (DIP)
Giant Cell (GIP)
Fibrosis
Granuloma
Honeycombing
Pulmonary Alveolar Proteinosis (PAP)
Pneumonia
Lung Cancer
Mesothelioma
Normal Lung

Tissue Section Analysis

DATE: 08-17-1987 TIME:14:17:00

Analysis conducted on the Syracuse-Hitachi at 20 kV
Analysis magnification = 3000X
Area of field at 3000X = 2560 μm^2

Sample size (# of fields scanned) = 50
Mean number exogenous particles/field = .86
Standard deviation = 1.95
Standard error = .28

Number of particles analyzed with x-ray = 61
Number exogenous particles counted = 43
Number endogenous particles = 28
which = 39.44 % of total number

Number of fields with particles # > Those x-ray analyzed = 2

Mineral Type	Tot.#	%	#/field	#/cm^3tissue
Total exog. partic.	43	100.00	0.860	1.101×10^8
Silica	8	18.60	0.160	0.205×10^8
Alum-silicate	20	46.51	0.400	0.512×10^8
Misc.silicates	2	4.65	0.040	0.051×10^8
Metals	13	30.23	0.260	0.333×10^8

Figure 1. Illustrative example of the summary results generated from the SEM analysis of a single tissue section.

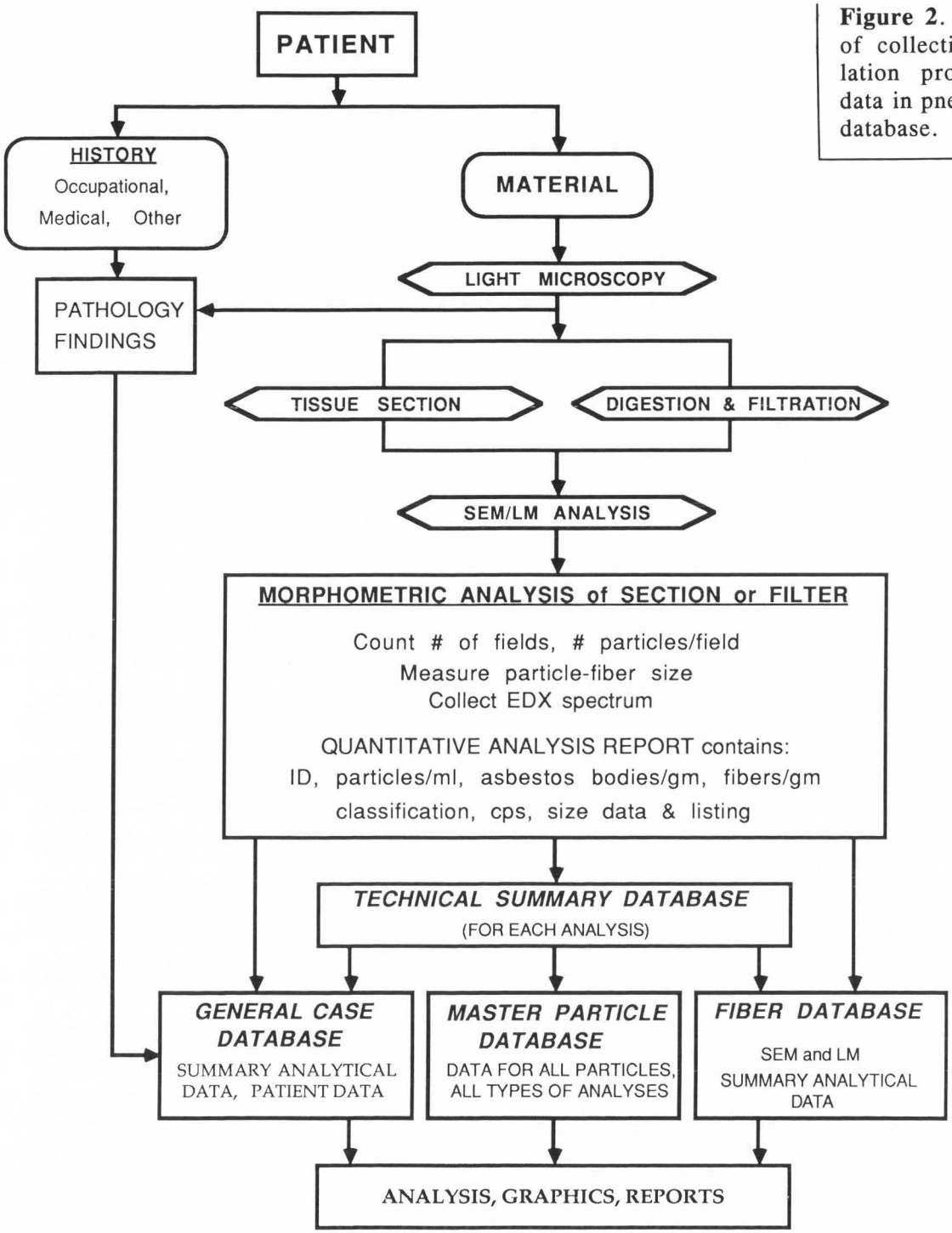

Figure 2. Flow chart of collection and collation procedures for data in pneumoconiosis database.

greater than one micrometer in length are counted, measured and identified by EDX. On occasion, non-fibrous particles may also be analyzed with similar digestion preparation, taking special care to use a fine enough pore size filter (usually 0.1 micrometer) to allow collection of the fine particles. Custom written software produces data for reports and other uses (Abraham et al., 1988, and in preparation).

c. Detection Limits: For both tissue section and tissue digestion type analyses, the detection limit indicates the concentration of particles or fibers represented by the minimum finding of one such particle in the entire number of fields searched. This is influenced by the SEM magnification, the number of fields searched, and the weight of tissue digested and filtered (in the case of digestion type analyses).

d. Comparison with other databases: Our quantitative data, and that from nearly all laboratories pursuing this type of analytical work, has unique features which must be kept in mind when attempting to compare data between laboratories. For example, our tissue section analyses result in concentrations of particles on a volumetric basis, and the digestion analyses result in data on both volumetric and gravimetric bases (although the gravimetric is the most utilized). One can approximate the ratio of volume to tissue weight to convert the volumetric data to gravimetric (the concentration/ml tissue is usually close to the concentration/gram wet weight). Other factors contributing to inter-laboratory variability include: instrumental conditions, magnification, resolution, operator sensitivity, counting acceptance and rejection criteria, digestion methodology, sonication, centrifugation, filtration or film deposition technique. Currently an international fiber counting inter-laboratory study is nearing completion of its initial report (coordinated by the Institute of Occupational Medicine, Edinburgh, UK). Our laboratory's fiber counting data appear to fall near the median values in this international study. We have also nearly completed an inter-laboratory study with the laboratory of Stettler et al. (in preparation). In this study of ten cases representing a wide range of pulmonary inorganic particle concentrations, our results from tissue section analyses rank in order exactly the same as the rankings based on automated particle analyses of ashed samples (expressed in gravimetric basis) (Stettler et al., 1989; 1991). The "offset" constant needed to convert our concentrations to Stettler et al's is approximately 10 (i.e., the volumetric concentrations need to be multiplied by 10 to give the concentrations per gram dry lung), but varies somewhat for different particle types, most likely owing to differences in particle size acceptance criteria, with metal particles often being below the size cutoff routinely used in the Stettler et al.'s automated particle analysis system.

2. Histo-Pathology Data

When sufficient histo-pathologic samples are available then diagnoses, tissue descriptions and pneumoconiosis gradings are recorded (Craighead et al., 1982; 1988). Materials are archived for implementation of other grading systems as desired in the future. Examples of relevant diagnostic and pathologic evaluations are listed in Table 1. These cover normal lungs and a wide range of lung diseases of occupational and non-occupational origin.

3. Occupational and Social History Data

Details of the occupational and life history of a subject, if available, are provided by either the subject or relatives (directly or via medical or medicolegal records). Optimally, the non-occupational record includes such information as age, sex, smoking habits, medical history and documentation of any exposures which are not job related. The occupational history documents the type(s) of exposure, intensity, duration, frequency of occurrence and its timing (during the subject's life).

Table 2. Concentration Data for the Major Particle Types in Tissue Sections from 433 Cases (in millions of particles/ml of tissue).

Particle Type	Median	(range)	Mean	(s.e.m.)[1]
Total Exogenous Particles	71	(1-33,450)	473	(113)
Silica	4	(0-4,038)	49	(11)
Alumino-Silicates	13	(0-29,062)	328	(103)
Metals	23	(0-1,378)	68	(7)
Talc	0	(0-3,468)	14	(8)

[1] standard error for mean

Table 3 Frequency of Various Metal Particles in 498 Tissue Section Analyses from 433 Cases.

Metal	Number of Particles[1] (Metal = 1st Element)		Number of Cases[2] (Metal Identified)	
Fe	2711	(33.07%)	376	(86.84%)
Ti	1762	(21.49)	347	(80.14)
W	959	(11.70)	79	(18.24)
Al	951	(11.60)	232	(53.58)
Sn	348	(4.24)	115	(26.56)
Pb	289	(3.53)	90	(20.79)
Cr	171	(2.09)	187	(43.19)
Ni	144	(1.76)	125	(28.87)
Ba	129	(1.57)	59	(13.63)
Au	117	(1.43)	30	(6.93)
Zn	104	(1.27)	110	(25.40)
Hg	91	(1.11)	26	(6.00)
Zr	83	(1.01)	34	(7.85)
Cu	78	(0.95)	96	(22.17)
Ce	60	(0.73)	47	(10.85)
Ag	53	(0.65)	34	(7.85)
Ta	22	(0.27)	19	(4.39)
Mo	21	(0.26)	4	(0.92)
Bi	19	(0.23)	16	(3.70)
Co	15	(0.18)	22	(5.08)
V	13	(0.16)	38	(8.78)
Sb	11	(0.13)	19	(4.39)
Os	11	(0.13)	2	(0.46)
Br	6	(0.07)	4	(0.92)
Pd	6	(0.07)	6	(1.39)
Mn	5	(0.06)	71	(16.40)
Cd	5	(0.06)	5	(1.15)
Nb	3	(0.04)	4	(0.92)
Se	3	(0.04)	2	(0.46)
La	2	(0.02)	4	(0.92)
Tc	2	(0.02)	2	(0.46)
As	1	(0.01)	2	(0.46)
Pt	1	(0.01)	1	(0.23)
Ru	1	(0.01)	1	(0.23)
Nd	1	(0.01)	3	(0.69)

[1] n = 8198; [2] n = 433.

Table 4. Comparison of Types of Particles Found in Tissue Section versus Digestion Analyses[1].

Particle Types	Tissue Sections		Digestions for Fibers	
Total Exogenous Particles	20106	(100%)	12765	(100%)
Metals	8013	(40)	885	(7)
Alumino-Silicates	7009	(35)	3297	(26)
Silica	2513	(13)	782	(6)
Miscellaneous Silicates	1289	(6)	676	(5)
Talc	797	(4)	1629	(13)
Gypsum	279	(1)	19	(<1)
Asbestos	206	(1)	5477	(43)

[1] in numbers of individual particles

Data Base Development: Data Base Scope

The way in which these various types of information come together to form the database is illustrated in Figure 2. A major strength of the database is the diversity of exposures which have been investigated. The material submitted for analysis includes normal tissue and tissue which displays conditions including, for example: infectious pneumonia, silicosis, asbestosis, silicate pneumoconiosis, mixed dust pneumoconiosis, talcosis, giant cell interstitial pneumonia (hard metal disease), arc welders pneumoconiosis, desquamative interstitial pneumonia, granulomatous disease and lung cancers (see Table 1).

The database itself consists of three intimately linked sub-databases. The largest and most basic of these (the Master Database), contains information on the size, elemental composition and operational identification of every particle that we have analyzed. Currently, the master database contains results for 32,871 particles from 913 analyses. These are comprised of 20,106 particles from 498 tissue section analyses in 433 cases and 12,765 fibers from 415 digestion analyses in 190 cases. Summaries of this data on a case-by-case basis are contained within the second database (the General Database). Here are recorded the concentrations of the various exogenous particle "types" which have been identified in tissue sections. These types are: alumino-silicate, miscellaneous silicate, silica, talc, gypsum and metal. The latter category may contain any, either singularly or in combination, of 35 metallic elements so far recorded (i.e., Ti, Fe, Al, Cr, Ni, Sn, Zn, Cu, Pb, Mn, W, Ba, Ce, V, Au, Ag, Zr, Hg, Co, Bi, Sb, Ta, Cd, Br, Mo, La, Nb, Se, Os, Pd, Tc, As, Pt, Ru, and Nd). In addition to these particle summaries this database also contains coded information on the histo-pathology, occupational and life history and diagnosis attending each case. The third constituent database (the Fiber Database) mainly contains information on the various types of fiber found in the tissue submitted for digestion analysis. This fiber data consists of a case-by-case record of the total number of fibers and, where present, the concentrations of talc, asbestos fibers (amosite, anthophyllite, crocidolite, chrysotile, actinolite and tremolite), non-asbestos fibers and coated fibers (both asbestos and non-asbestos). This database also contains information on the asbestos body content of tissue submitted for light microscopy analysis.

Data Base Uses

The database which has been described can provide a wide variety of information at several different scales. At the most fundamental level, summary information can be generated which pertains to the different kinds of lung exposure which have been investigated. Cases with recognized pneumoconiosis reveal concentrations of the etiologic particle type greatly elevated (usually orders of magnitude) above that seen in normal lungs or in the lungs of persons with other lung pathology. Similarly, information on the abundances of various particle types can be extracted from the database.

The most commonly occurring particle types in the tissue section analyses are alumino-silicate, silica, metal and talc (Table 2). Frequency distributions for the concentrations of these types, for total exogenous particles and for the detection limits are shown in Figure 3. The concentrations of each typically display a log-normal distribution. While the alumino-silicate, silica and metal particles are present in most of the cases analyzed using this methodology, talc was found in only a relatively small number of cases. In addition to these fairly broad summaries, more detailed information on specific particle types can also be obtained. For instance, set out in Table 3 is a breakdown of the occurrence of various metal particles in 433 cases from the database. These data which are grouped by numbers of particles containing the element as the most abundant ("first") element in any metal particle and by number of cases in which the given element was detected in any metal particle shows in rank order the most commonly occurring metallic elements. These relative frequencies are, of course, influenced by the case mix in the database. For example, the frequency of W is high as a result of the inclusion of the series of hard metal disease cases (see below).

Basic information relating to fibrous particles can also be abstracted from the database. A comparison of the types of fibers found in the digestion analyses with

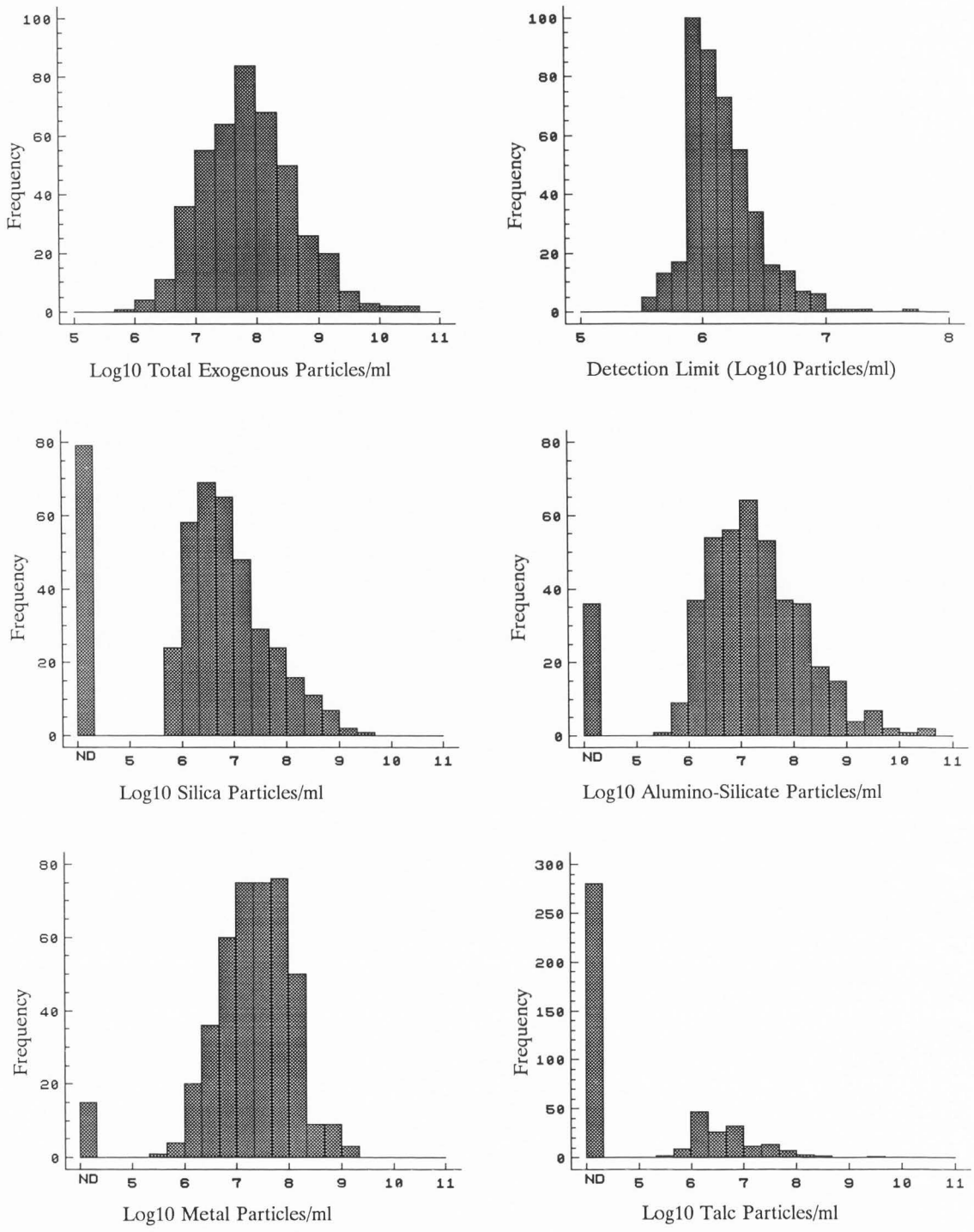

Figure 3. Frequency histograms of the tissue section concentrations of non-fibrous inorganic particles in 433 cases from the database. ND = Not Detected.

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those found in the tissue section analyses is presented in Table 4. Concentration histograms for various fiber types identified during digestion analyses performed at 10,000x magnification (on the viewing CRT of the SEM) are set out in Figure 4. Note that these values are in numbers of fibers per gram wet lung. Of these 172 cases, there was sufficient extra tissue available for determination of dry-to-wet weight ratio in only 113 [mean dry weight as a percentage of wet weight was 19.9 (s.d. = 6.56)]. These data indicate that the different kinds of

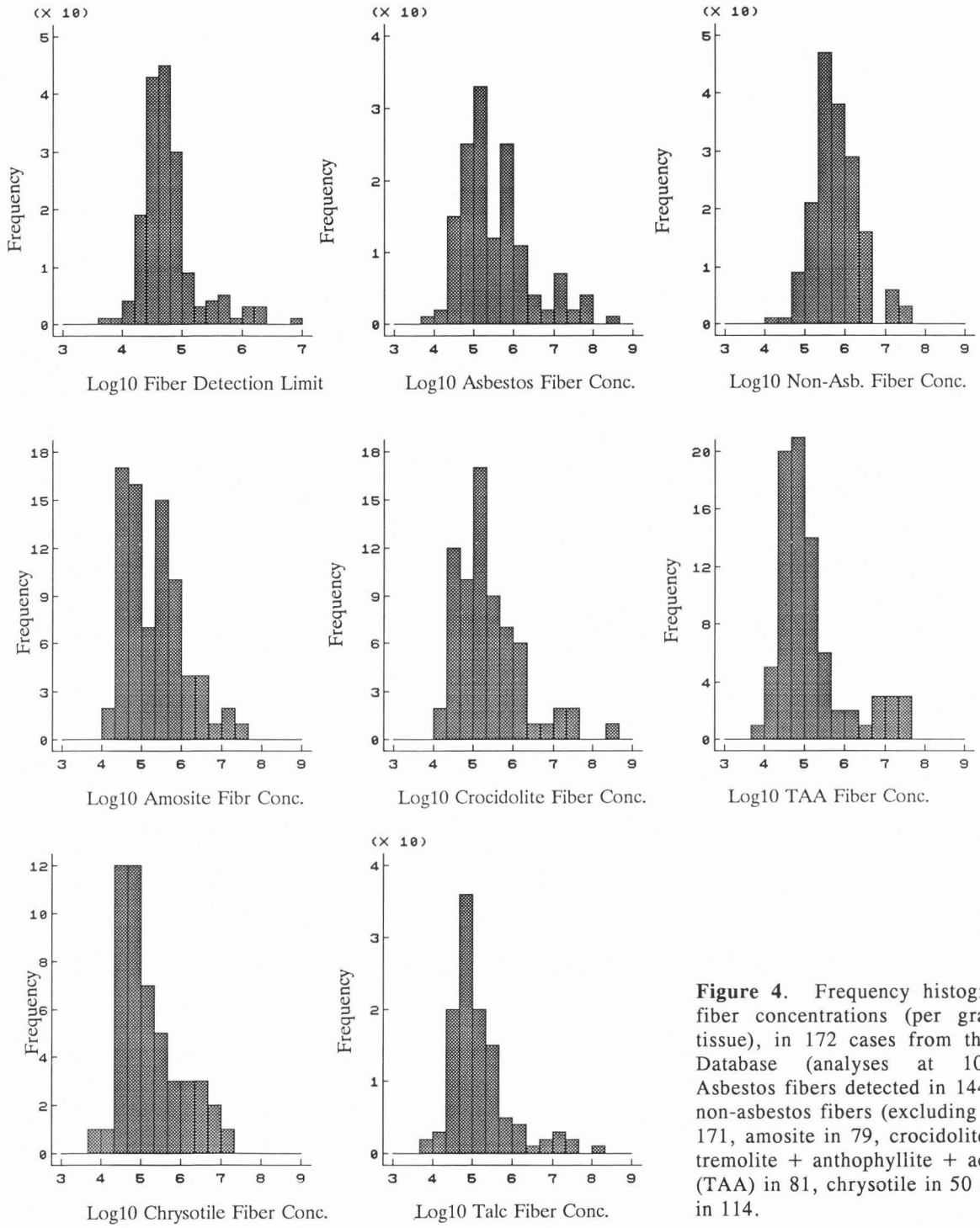

Figure 4. Frequency histograms of fiber concentrations (per gram wet tissue), in 172 cases from the Fiber Database (analyses at 10,000x). Asbestos fibers detected in 144 cases, non-asbestos fibers (excluding talc) in 171, amosite in 79, crocidolite in 70, tremolite + anthophyllite + actinolite (TAA) in 81, chrysotile in 50 and talc in 114.

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fiber vary markedly in their frequency of occurrence. Fibrous particles were identified in 172 cases, whereas asbestos fibers were only identified in 144 of the cases. Also, from this set of frequency distributions it is clear that the concentration values for each fiber type are log-normally distributed.

Data of this type can easily be linked to other information in the fiber database. The importance of detection limits is underscored by the following example. In Figure 5 the ratio of the amphibole fiber to asbestos body concentration is plotted against the asbestos body concentration for 87 database cases. For these

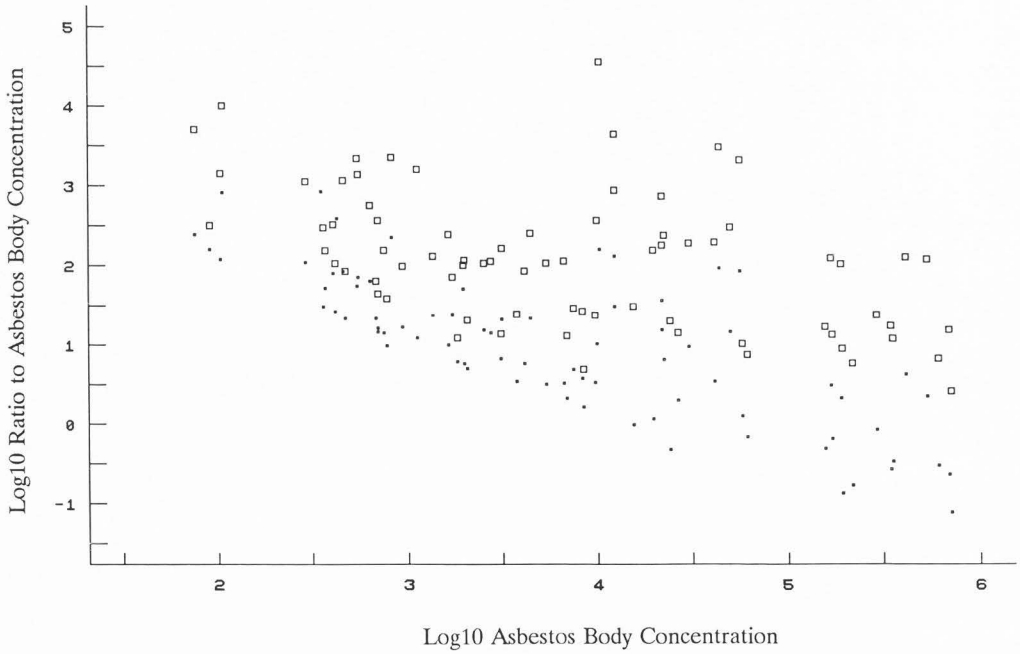

Figure 5. Scatterplot of the ratio of asbestos fiber concentration to asbestos body concentration (□) and the ratio of fiber detection limit to asbestos body concentration (•) (both per gram wet tissue) against asbestos body concentration.

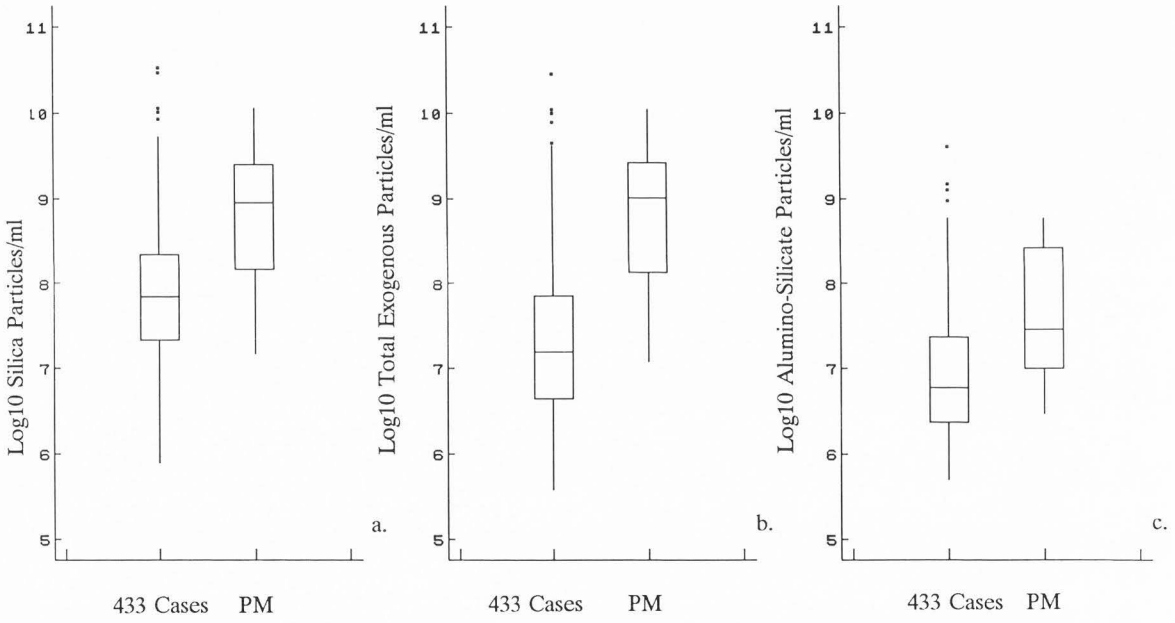

Figure 6. Box plots comparing the concentrations of total exogenous (**a**), alumino-silicate (**b**) and silica (**c**) particles in 433 cases in the database with the concentrations in 17 sections of Pre-Columbian mummy (PM) lung tissue originating in Chile (the central line defines the median, the box length the inter-quartile range, the whiskers the range and the points any value greater than 1.5 times the inter-quartile range).

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cases there is an apparent decrease in the ratio as the asbestos body concentration increases. Also plotted in this figure is the ratio of the SEM fiber detection limit to the asbestos body concentration. This ratio forms an envelope of values below which fibers will not be detectable and explains the apparent decrease in ratio with increasing asbestos body concentration. This is an important consideration for such comparisons, as certain fibers may be present at higher than normal concentrations, but will not be detected if the detection limit is raised as a result of a very high burden of other fiber type(s).

At another level the information stored in the database can act as a reference for other lung dust burden analyses. This applies in two senses. Firstly, it is

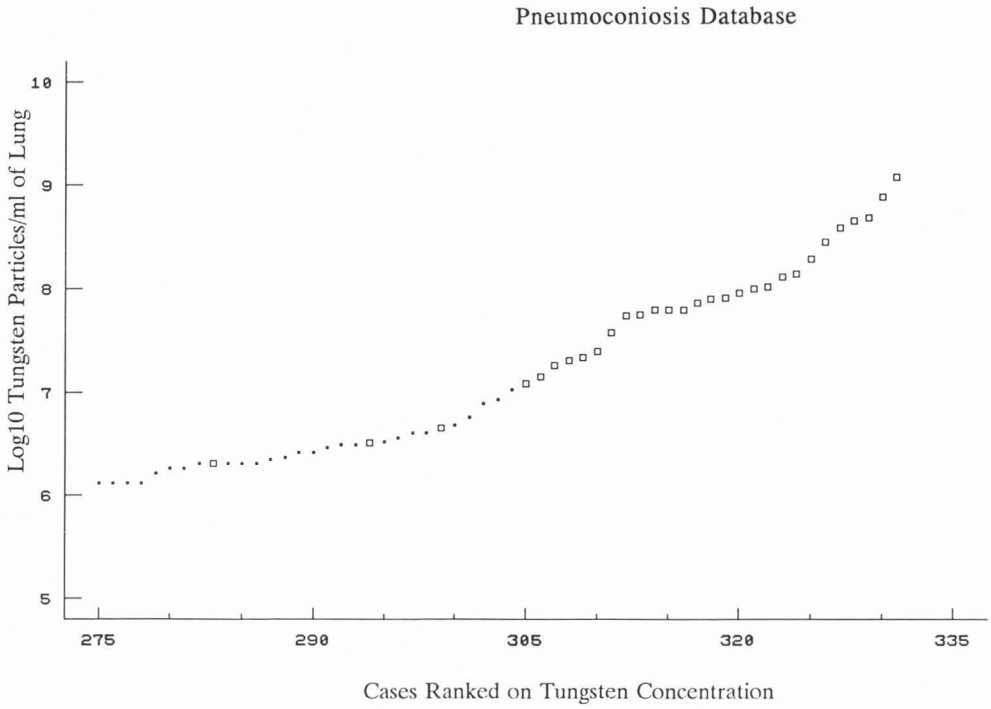

Figure 7. Tungsten particle concentration plotted against database case number (ranked on tungsten concentration) for 331 database cases with GIP cases highlighted (after Abraham, 1990). (□) GIP Cases; (•) Other Cases.

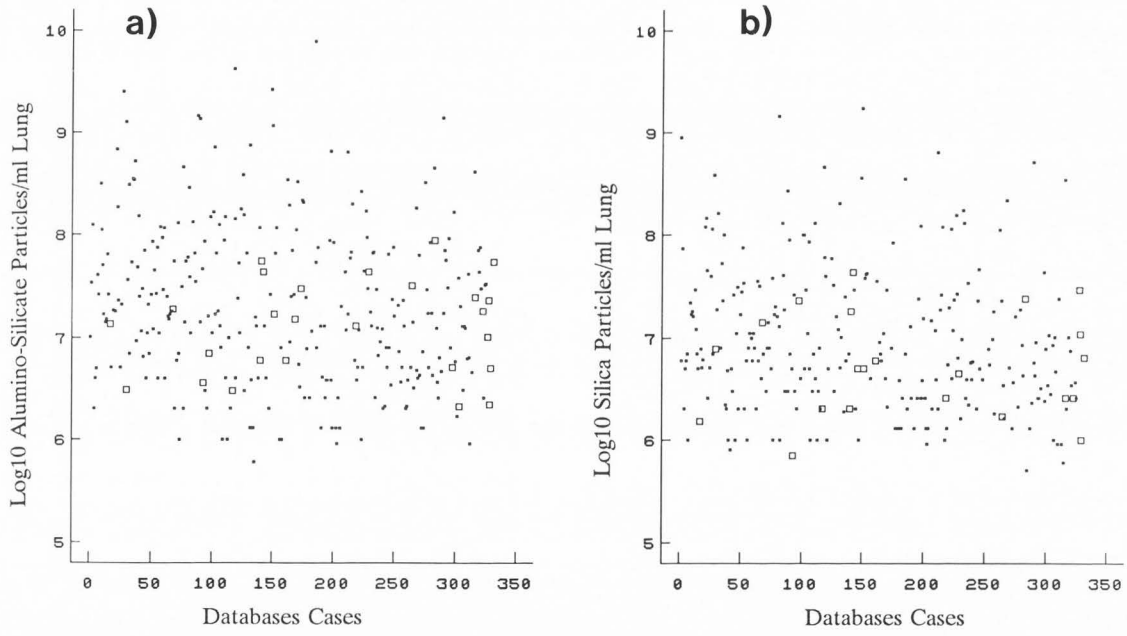

Figure 8. Scatter-plots of alumino-silicate particle concentration (a) and silica particle concentration (b) against case number for 331 database cases with GIP cases highlighted (after Abraham, 1990). (□) GIP Cases; (•) Other Cases.

possible to use certain sub-sets of the data as a "normal population" reference data set. For instance, while silica concentrations in the silicosis cases (with relatively pure silica exposure) will be elevated, the metal concentrations should be representative of those levels in the general population. Similarly, certain sub-sets of the data can be employed in the reverse sense to stand as an "exposure population" database. For example, in a recent study Abraham et al. (1990) found that the lung dust burden in a population of pre-columbian Chilean mummies was markedly elevated. Box-and-whisker plots for silica, alumino-silicate, and total particle concentrations for both the Chilean mummy population and for cases in the pneumoconiosis database are set out

in Figure 6. These results show that the dust burden in the mummy lungs is comparable with that of contemporary pneumoconiotic lungs (Sherwin et al., 1979), which are at the upper range seen in our database.

At a more sophisticated level the pneumoconiosis database can provide information on the relationships between the different types of constituent data items. This can be illustrated by those cases in the database which presented the rare histologic pattern of Giant cell Interstitial Pneumonia (GIP). This finding is characteristic of "hard metal disease" (Abraham 1990). This is the interstitial lung disease resulting from the inhalation of dusts generated in the cemented tungsten carbide (WC) industry which has largely been attributed to the presence of admixed cobalt (Co). Of the 31 GIP cases in the database 30 were found to be amongst the 50 cases with the highest (W) concentrations. In fact the top 27 all displayed GIP (Figure 7). In view of the fact that the concentrations in the GIP cases of silica and aluminosilicate particles are not markedly elevated (Figure 8) then this is a highly significant result. This is particularly so given that of the 30 GIP cases with high W 27 were known to have been employed in the WC industry but of these only 6 had detectable Co levels in the lung.

Conclusion

Creating a database which contains information on an individual's social and occupational history, lung particle burden with accompanying pathology and diagnosis has several advantages. From the viewpoint of data handling, having such a large amount of diverse information in a structured form facilitates record management. By employing a database format specific sub-sets of data which are of interest are easily accessed. This means that almost any combination of the data items can be extracted for the purpose of statistical analysis.

In terms of information content, assembling a database that contains such varied information fulfills a number of purposes. The most obvious of these are:

1. It provides basic data, across a range of exposures, on lung particle burden for various types of pneumoconiosis.

2. It exists as a source of information on both "normal" and "exposed" populations.

3. It enables connections to be made between exposure, pathology and the patients history for reasonable population samples.

Furthermore, it is unlikely that relationships between patient lung burden and other factors will be readily identified unless such information is linked in a form which can be searched for various clusters and types of cases.

Over time, such functions will be continually enhanced. This is because the database is continuously updated as further analyses are performed.

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Discussion with Reviewers

V.L. Roggli: Normal ranges for comparison are very important for this type of database. How many cases have the authors studied in the "Normal Lung" category, and what criteria were used to classify cases in this category as normal (i.e., macroscopically normal? Non-smokers? No history of a dusty occupation?)?
Authors: Criteria for normal in our database include pathologic criteria only, as this category is under the field of "Pathology". Thus we would need to see lungs which are normal both grossly and by light microscopy. This could theoretically include cases with a dusty occupation but which had no recognizable pathologic changes (this would include the absence of recognizable increase in dust by bright field and polarized light microscopy). At this time, we cannot answer the first part, since all the links in the database are not yet complete.

V.L. Roggli: The authors state that while silica concentrations in silicosis cases will be elevated, metal concentrations should be representative of those levels in the general population. This is a dangerous assumption, since individuals employed in dusty occupations may be exposed to relative excesses of a variety of dusts other than the ones associated with their specific pathologic process. Furthermore, excess exposure to toxic dusts such as silica may interfere with clearance of other inhaled particulates. Please comment.
P. Dumortier: It is speculative to say that in a silica-exposed subject the number of metallic particles must be representative of those levels in the general population. Effectively the presence of pathological and functional changes in the lungs of these subjects modify the pattern of particles deposition and clearance and thus the number of metallic particles with respect to the one encountered in the general population. The best choice for controls always remains subjects from the general population not occupationally exposed to particles.
Authors: The reviewers are fundamentally correct. We do not mean to imply that elevated concentrations of metals, for example, in cases of silicosis, are representative of "background". What we mean is that in those with pure or relatively pure exposures to one material, the data obtained regarding levels of other materials in the lungs may be normal or insignificantly elevated. This is examined by looking at the particle concentration distributions, for example in the hard metal disease cases.

V.L. Roggli: The authors note that among 31 cases of giant cell interstitial pneumonitis (GIP) studied, 30 were found to have among the highest tungsten concentrations that were detected, whereas only six had detectable cobalt levels. Do the authors mean to imply that tungsten rather than cobalt is the causative agent in GIP?
Authors: No. Most of the available evidence suggests that cobalt is the main etiologic agent. The relative contribution of the tungsten-containing compounds in the mixed exposure is not known, but there is some evidence that the tungsten exposure is not inert, in that some studies suggest that the dose of cobalt required to produce toxicity is reduced when mixed with tungsten compounds, and also there is some evidence for toxicity of tungsten in the absence of cobalt (See NIOSH Criteria Document on Cemented Tungsten Carbides, 1977 for a review to that time). Since the major exposure to cobalt which has been recognized to produce hard metal lung disease has been with cemented tungsten carbide exposure, the tungsten-containing particles serve as a marker for the more difficult to detect cobalt in the vast majority of cases.

V.L. Roggli: I find Table 4 to be confusing with respect to fibrous and non-fibrous particulates. How do the authors define a fiber? Do the total exogenous particles in the 3rd column include fibrous as well as non-fibrous particles? What proportion of the 1629 talc particles identified by digestion were classified as fibrous? Why do talc particles account for only 4% of total exogenous particles by in situ analysis but 13% of the total by digestion?
Authors: We define fibers as particles with at least 3:1 aspect ratio and parallel sides. Total exogenous particles in tissue section in situ analyses refers to non-fibrous particles (since fibrous particles are much less prevalent than non-fibrous particles in lungs) and in digestion analyses refers to fibrous particles (since we are reporting only fibrous particles in the digestion analyses here, although on occasion we do analyze non-fibrous particles in digestion samples). Therefore, all the 1629 talc fibers in the digestion analysis are fibers. We cannot answer precisely "why" the percentages of talc differ between in situ and digestion analyses, but the most likely explanation relates to the greater concentration of non-fibrous than fibrous particles, and the difference in detection limits between the in situ and digestion analyses.

V.L. Roggli: With respect to Figure 5, the authors imply that the decrease in ratio of amphibole fibers to asbestos body concentrations with increasing asbestos body concentration is apparent rather than real because as asbestos body concentrations decrease, amphibole fiber levels drop below the limits of detectability by SEM. This effect would only explain the observed data if cases with low asbestos body counts by light microscopy and undetectable amphibole fibers by SEM (lower left quadrant of graph) were omitted from the analysis. Were such cases encountered, and if so, how many? I doubt that this is the real explanation for this phenomenon, as we have observed a similar decrease in the uncoated fiber to coated fiber ratio by SEM with increasing uncoated fiber concentration.
Authors: We are pleased to know Dr. Roggli's group has observed similar apparent decrease in this ratio by SEM with increasing uncoated fiber concentration. This issue of the effect of detection limits on interpretation of

such results needs more data, and we would like to see a similar graphing of Dr. Roggli's data. Dr. Roggli did not offer an alternative explanation for this observation. We suppose an alternative explanation could be that as fiber burden increases, the capacity for forming asbestos bodies reaches saturation, and thus the ratio of bodies to fibers could decrease as lung fiber burden increases. This mechanism might be expected to show a change over time: with longer time intervals of fibers remaining in the lung, the proportion which could be coated would increase. This could be examined further in our database and Dr. Roggli's, and would be of interest to pursue. To be included in the analysis, cases had to have both asbestos bodies detected by light microscopy and amphibole fibers detected by SEM analysis. The ratio cannot be determined without values greater than zero, of course.

L.E. Stettler: Have you looked at duplicate sections of the same lung? If so, what types of variations in total exogenous particle concentrations and particle type profiles do you see?
Authors: We have looked at replicate analyses, and this has been published in the cited references by Abraham and Burnett.

L.E. Stettler: I assume that you consider iron- and phosphorus-containing particles as endogenous. Do you classify particles containing only iron as endogenous or exogenous?
Authors: We have classified particles containing only iron as exogenous.

L.E. Stettler: What magnification is typically used in your in situ procedure?
Authors: Our standard magnification is nominally 6000X as viewed on the viewing screen of the SEM. The important corollary to this is what is the minimum size particle detected for analysis? This information should be available for comparison between different laboratories by looking at the size distributions for specific types of particles (e.g., silica, silicates, talc, iron, titanium, etc.).

L.E. Stettler: Fiber identifications made by SEM/EDX may, at times, be quite tenuous. For instance, how do you distinguish between an asbestiform tremolite fiber and a non-asbestiform tremolite cleavage fragment of similar morphology? What criteria do you use to distinguish between anthophyllite and talc fibers?
Authors: We have defined fiber criteria, as mentioned in reply to Dr. Roggli. With regard to asbestiform tremolite versus cleavage fragments we cannot always make this distinction from a single fiber. We use the criteria of asbestiform fibers showing parallel bundles with frayed ends or splitting longitudinally (this is fine only when such fibers are found). Alternatively, we use the methodology suggested by Wylie et al (Characterizing and discriminating airborne amphibole cleavage fragments and amosite fibers: implications for the NIOSH method Am. Ind. Hyg. Assoc. J. 46:197-201, 1985.) in which a population of fibers is analyzed and the regression of the log of the aspect ratio on the log of the length and of the log of the diameter on the log of the length are examined. This method allows separation of true asbestiform fibers from cleavage fragments, or shows commonly that preparations of material samples and/or fibers isolated from lungs are in fact mixtures of cleavage fragments and asbestiform fiber populations (see Abraham, JL. Non-commercial amphibole asbestos fibers and cleavage fragments in lung tissues of New York state talc miners with asbestosis and talcosis. Amer. Rev. Resp. Dis. 141: A244, 1990 (abstract)). Anthophyllite usually has a higher Fe/Si ratio than that shown by talc (Russ JC, NBS special publication 533, pp 13-19, 1980). Our reference standards support this relationship and we distinguish anthophyllite from talc by Fe composition of the fibers.

L.E. Stettler: The number of fibers (43%) compared to the number of non-fibers (57%) in the digestion analyses for the lungs reported in Table 4 seem quite high. Are these data from subjects with known fiber exposures?
Authors: These percentages do not reflect fibers versus non-fibers. As noted in the column heading in Table 4 the values represent the results of Digestions for Fibers. Thus 43% of fibers were classified as asbestos and 57% were classified as other types of fibers. The data are from subjects with known as well as unknown fiber exposure. (Also see answer to Dr. Roggli's question related to Table 4).

L.E. Stettler: We have seen silica and aluminum silicates in every lung analyzed in our laboratory. According to your data in Figure 3, silica was not detected in approximately 20% of the lungs and aluminum silicates were not detected in nearly 10% of the lungs. To what do you attribute this absence of silica and aluminum silicates?
Authors: The fact that we did not detect silica or aluminum silicate particles in an analysis does not mean these mineral particles were not present. It just means that if present, these particles were at concentrations below our detection limits. The digestion method used in your laboratory has a lower detection limit than the in situ method.

P. Dumortier: The use of backscattered electron signal would probably favor the detection of small metallic particles and thus enhance their concentration with respect to silicates or carbonaceous compounds. Do you have any idea of the magnitude of this effect?
Authors: For particles of equal size, you are correct, and it is very likely that the use of backscattered electron imaging (BEI) favors the detection of the very small metallic particles over silicates (we are not able to analyze carbonaceous particles by EDX). The measurement of the magnitude of this effect would have to be

studied for each density and size particle, which we have not done.

P. Dumortier: How do you handle minerals like chromite ($FeCr_2O_4$) or illmenite ($FeTiO_3$) in your classification scheme of metallic particles?
Authors: At present, we have not attempted to specifically sort out all mineral types, but the data on relative x-ray intensity for each particle is contained in the master file and could be the subject of future analysis.

D.L. Luchtel: There is an apparent error in Figure 1 as the total number of particles analyzed should be 71, not 61.
Authors: Dr. Luchtel astutely points out that in Figure 1 the number of particles analyzed was 61, whereas the number of exogenous particles was 43 and the number of endogenous particles was 28 (which total 71). The Figure is correct. Note the line which states that 2 fields had particle number greater than the number analyzed by x-ray. This reflects methodological details not repeated in this paper (see Abraham and Burnett). If fields of view are encountered with a large number of particles, a sub-sample of these is analyzed and the total number is counted, with the results for those analyzed by x-ray being multiplied by this ratio of counted versus analyzed. This extrapolation procedure is a measure designed to reduce the dependence of the results on the findings in a small number of fields in cases with higher particle concentrations.

D.L. Luchtel: What distinguishes exogenous from endogenous particles? Presumably, endogenous implies that the particle was not inhaled. What are some examples of such endogenous particles? What are some examples of exogenous particles in addition to those most commonly found (alumino-silicate, silica, metal and talc); that is, beyond these fibrous particles listed in table 1 (talc, gypsum, asbestos)?
Authors: This is discussed in earlier publications (Abraham and Burnett) and by Stettler et al. (see text references). Most particles found in the lungs fall into the major classifications you mentioned. The only other categories we have are miscellaneous silicates (which contain Si and other elements but not Al and are not talc or asbestos).

D.L. Luchtel: Concerning the digestion method used for the determination of the fibrous particles burden, have any experiments been done to determine if equivalent amounts of lung tissue do in fact give equivalent weights after digestion when previously formalin fixed only versus tissue that has been embedded and deparaffinized. The latter type of tissue sample might weigh significantly less given tissue extraction that could take place during paraffin embedding and subsequent deparaffinization.
Authors: We have done measurements of tissue weights before and after lipid extraction which would be a normal part of paraffin embedding and also deparaffinization. Our results show approximately 20% loss of weight with the extraction.

D.L. Luchtel: In Fig. 5, what is the rationale for plotting, on the vertical axis, log of the ratio of amphibole fiber to asbestos body concentration rather than log of amphibole fiber concentration only?
Authors: The rationale of plotting the ratio of asbestos fibers to asbestos bodies and of detection limit for asbestos fibers to asbestos bodies instead of the concentration is to examine the relationship of the ratio to the detection limit and to the asbestos body concentration.

D.L. Luchtel: In an example of a comparison with other databases, it is stated that an "offset" constant of 10 is needed to convert to the database of Stettler and colleagues since their data is standardized to 'per gram dry lung' (while the volumetric basis of the database in this paper is in 'per gram wet weight'). However, it is mentioned later (in the Database Uses section) that the mean dry weight of lung as a percentage of wet weight is 19.9. It seems that this would imply that the above offset constant would be 5, would it not?
Authors: The observed "offset" constant of 10 brings into near congruity the distributions for total particles. This does not match the "theoretical" value of approximately 5, as you mentioned. Some of the reasons for this discrepancy are suggested in the same sentence of text you cited.

D.L. Luchtel: Is this database available on a disc or tape that can be obtained by other researchers?
L.E. Stettler: The size of your database is quite impressive and the data contained therein are most important. Since particle data for "normal lungs" are very limited, I hope you have plans to split out and publish these data in the near future. It also would be informative if you could discuss in the paper whether and/or how others can access your database.
Authors: At present, our database is not "user-friendly" enough to distribute, but we would welcome any inquiries for uses with which we could assist, and would be glad to have visiting scientists spend time in our laboratory working with us on development and usage of the database.

F.H.Y. Green: Have you used the database to generate information on particle concentrations and types according to demographic stratification? Specifically, I would be interested to know if you have found differences by sex, by age, by rural versus urban residents, by smoking status.
Authors: We do not yet have all the data coded and linked to permit other than manual analysis of many of these parameters. As mentioned, completion of coding and linkages between files is a major task in making this type of database fully operational.

F.H.Y. Green: How do you reconcile your random sampling technique for X-ray analysis in the SEM with the non-random distribution of tissue and airspace in the lung and the non-random distribution of particles within the parenchyma.

Authors: If one wants to determine the concentration of particulates in a three dimensional structure a volumetric sampling as we have done seems logical. If one wants to investigate the sub-distributions of particles (e.g., peribronchial, in alveolar macrophages, etc.) then one needs a further classification and sampling. This was described as a possible extra portion of data available in our original description of the method (Abraham and Burnett, 1983).

F.H.Y. Green: Numerous individual particles are often contained within one macrophage, and even within the same lysosome. How do you cope with the problem of overlapping elemental spectra?

Authors: If particles cannot be spatially resolved, then they are of course not recognized as separate particles, and are counted as single particles. When adjacent particles can be resolved spatially (which usually is limited at 0.05 to 0.1 micrometers) our approach to overlapping spectra is to collect a spectrum or spectra adjacent to the particle of interest and use that as the 'background' which is subtracted instead of the standard use of the spectrum from the carbon disc as background.

F.H.Y. Green: Notably absent from Table 1 is any mention of large and/or small airways disease. The latter is known to be associated with dust exposure, and there is evidence that larger, inhalable particles cause industrial bronchitis. Have you examined for relationships between particle types, sizes and/or shape and the presence/absence of airways disease?

Authors: The listing in Table 1 is not meant to be exhaustive, and there are certainly cases of large and small airways disease (such as early, Stage 1, asbestosis) in our database. Such studies as Dr. Green proposes are one of the reasons for developing this database, and when information on small airways disease is quantifiable and entered into the database such analyses will be performed. For large airways, one should probably have a database of large airway analyses rather than, or in addition to, data on lung parenchymal analyses.