# SIMILARITIES IN THE FIBROGENICITY OF ASBESTOS FIBRES AND OTHER MINERAL PARTICLES RETAINED IN HUMAN LUNGS

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## INTRODUCTION

Many new types of fibrous materials are being developed for application in advanced technology and industry. A mathematical model constructed for predicting the fibrosis-inducing potential of airborne particles of such materials has two parts. Data on fibre inhalation required by the first part, which computes from the size distribution of the particles the fraction that would achieve long-term pulmonary retention, were obtained from a uniquely suitable environment in the asbestos mining industry.[1] The asbestos mining industry has also been the source of an index of fibrogenicity for use in the second part of the model which estimates the severity of fibrosis produced by the retained particles.[2]

Asbestosis, the interstitial pulmonary fibrosis induced by inhaled asbestos dust, has long been recognized as a dose-related disease. Epidemiologists and industrial hygienists have mainly used fibre counting methods for estimating exposures. Fibre counting has also been the method used in inhalation studies. In inoculation and 'in vitro' experiments with materials such as the UICC standard reference samples, doses have been measured by gravimetric means. Many authors have suggested that fibrosis is a particle surface effect but no study appears to have been based on the measurement of exposure in terms of particle surface area. Since results of animal experiments are often at variance with epidemiological findings, mainly because the methods for dose evaluation differ, any data that are obtained require epidemiological verification. Three recent studies have therefore been based on human pulmonary material.

## FIRST STUDY

Identification of the fibrosis-related fibre parameter proved particularly elusive until use was made of a South African report[3] that in the period 1959 to 1964 prevalence of 'slight asbestosis' and 'total asbestosis' in asbestos miners had been the same in North Western Cape Province, which produces a small-diameter crocidolite, and in the Transvaal which mines crocidolite and a closely related amosite, both of large diameter. The first part of the mathematical model was applied to data on the size and concentration of airborne fibres in South African asbestos mines[4] in order to determine which concentration parameter of retained fibres (number, surface area or volume) would show equal asbestos dose in the two regions with equality in asbestosis response. The relevant parameter turned out to be the total surface area of retained fibres per unit weight of tissue and fibrogenicity was independent of amphibole type. Analogous evidence on the Finnish anthophyllite mine at Paakkila supported these findings.

## SECOND STUDY

For this study to determine relationships between retained amphibole fibres and fibrosis, tissue specimens were obtained from post-mortem lungs of workers who had been employed at one of four mining locations: Paakkila, NW Cape, Transvaal and the Australian crocidolite mine at Wittenoom. A sample, about 1.5 ml in volume, taken from each lung specimen was sliced into three portions. The middle portion was used to prepare a paraffin section, stained either by haematoxylin and eosin or by a trichrome method. Figure 1 shows the continuous numerical scale of fibrosis[5] one of us (TA) employed in a blind assessment of the severity of interstital fibrosis by scanning the paraffin section in a microscope fitted with a x10 objective. Each successive field was allotted a score between 0 and 8. The mean score for about 50 fields examined was taken as the fibrosis for the sample.

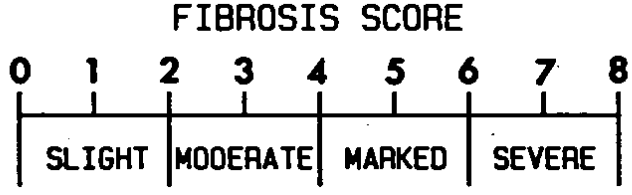


Figure 1. Fibrosis Scale.

The other two portions of the sample were treated together with potassium hydroxide for the extraction of mineral dust. Evaluation of the dust by a method combining magnetic alignment of fibres with subsequent examination by light scattering[6] gave fibre concentration in terms of fibre volume

per microgramme of dry tissue; data on fibre diameters and lengths were used to calculate the concentration in terms of fibre number and fibre surface area.

When surface area was used as the parameter of fibre quantity, the fibre concentrations in specimens showing a given degree of fibrosis were approximately equal:

Wittenoom = NW Cape = Transvaal = Paakkila.

This relationship confirmed the findings of the first study that the severity of fibrosis was related to aggregated fibre surface area and was independent of amphibole type.

When volume was used as the parameter, the fibre concentration in specimens showing a given degree of fibrosis increased progressively:

Wittenoom NW Cape Transvaal Paakkila.

Table I shows the large differences in fibre size in the four mining areas that account for this relationship. For instance, the average ratio of surface area to volume (which is inversely proportional to fibre diameter) for Wittenoom fibres is about 20 times that for Paakkila fibres; consequently, a given degree of fibrosis was induced by a smaller volume of Wittenoom fibres than Paakkila fibres. When number was used as the parameter, the fibre concentrations in specimens showing a given degree of fibrosis decreased progressively:

Wittenoom NW Cape Transvaal Paakkila.

Differences in fibre size also account for this relationship. The surface area of the average Paakkila fibre is about 25 times that of the average Wittenoom fibre; consequently, a given degree of fibrosis was induced by far fewer Paakkila fibres than Wittenoom fibres.

## THIRD STUDY

The second study gave an intimation that chrysotile and quartz had fibrogenicity similar to that of amphiboles. The third study was designed to pursue this interesting lead and attempt to quantify the fibrogenicity of asbestos and other minerals. As tissue specimens with preponderance of a specified mineral are difficult to find, the study examined the feasibility of a method that would treat specimens as sources of relationships somewhat akin to simultaneous equations and make multiple-mineral specimens an advantage. Far more specimens were required than the number of equations needed in the algebraic analogy, to compensate for the expected wide intra- and inter-subject variations in severity of fibrosis such as had been observed in the second study. Specimens ranging widely in particle concentration and mineral type were obtained from asbestos mines and factories, gold mines, a platinum mine, shipyards and other workplaces. The compositional data presented in Figure 2 show that often the predominant mineral type in a specimen was not the nominal work material.

Dust was extracted from specimens by removal of tissue by either the potassium hydroxide method or low temperature ashing. Scanning transmission electron microscopy was used for identification and size analysis of individual mineral particles. Fibres were modelled as cylinders, the width of the image seen in the electronmicrograph being taken as the fibre diameter. Talc, kaolinite, chlorite, mica, clay and other flaky particles were modelled as elliptical discs lying flat, 0.2 times the length of the minor axis of a disc being recorded as its thickness. Quartz particles were modelled as spheres, the observed projected area diameter of a particle being taken

Table I
Fibre Size Characteristics

| | PAAKKILA anthophyllite | TRANSVAAL amosite crocidolite | NW CAPE crocidolite | WITTENOOM crocidolite |
|---|---|---|---|---|
| MEDIAN DIAMETER (μm) | 0.6 | 0.2 | 0.06 | 0.04 |
| RELATIVE VOLUME | 500 | 50 | 4 | 1 |
| RELATIVE SURFACE AREA | 25 | 10 | 2 | 1 |
| RELATIVE SURFACE AREA / VOLUME | 1 | 4 | 10 | 20 |

as the diameter of the sphere. Because no measurement could be made on the vertical projected area of particles, which for assessing the surface area of quartz particles is as important as the horizontal projected area, the size data for quartz are less accurate than for most other minerals.

The results obtained from the third study provided further confirmation of the findings regarding amphibole fibres, and discussion will therefore be directed to ascertaining what they say about the fibrogenicity of mineral particles in general.

Figure 3a shows the results of plotting the fibrosis score for each tissue sample against the corresponding concentration for all particles expressed in terms of surface area. In this Figure, and more so in Figures 4 and 5, some of the data points lie on or are close to an axis, and in order to avoid

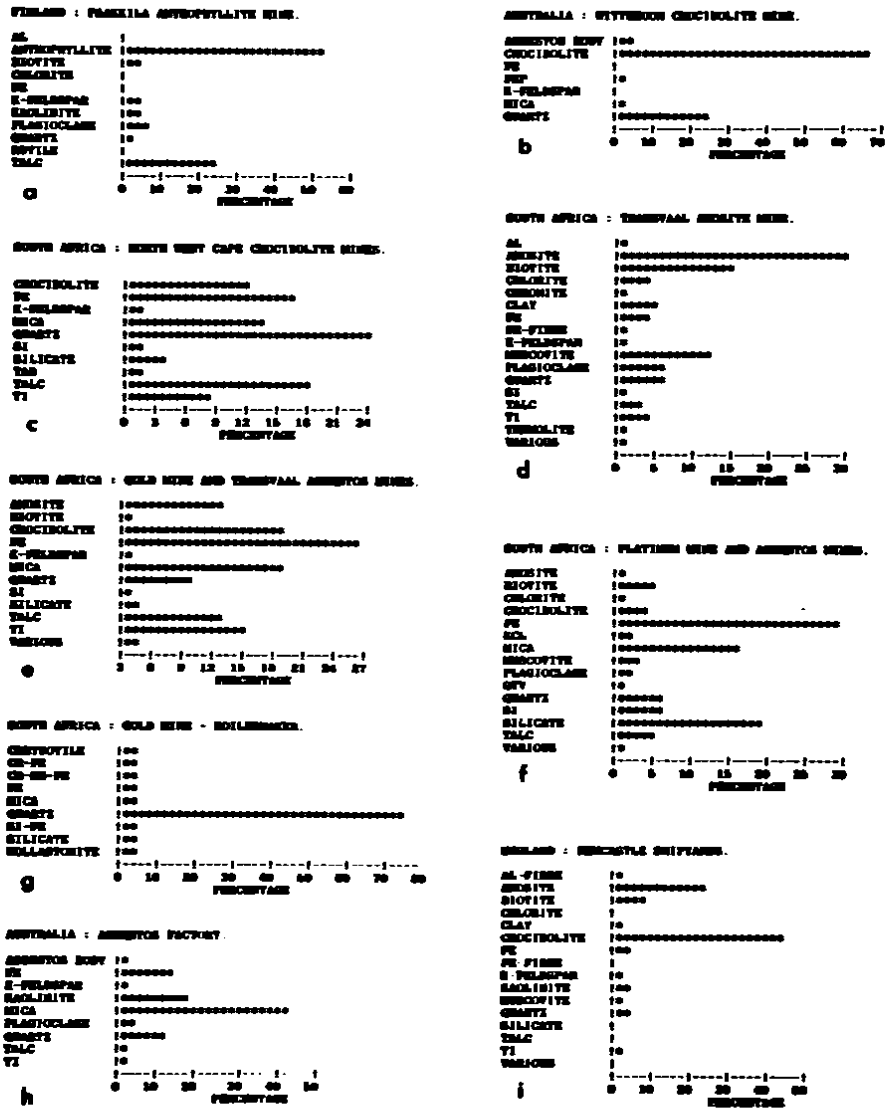

Figure 1. Country of origin of lung tissue specimen; industry; percentage frequency of retained mineral particles.

their obscuration by the scales each scale has been displaced transversely. The regression line is also omitted as it too would otherwise obscure some interesting data points, but may be visualized using the open circles K and E that mark the ends. The point K gives the value of the constant in the regression equation that represents the degree of fibrosis at zero particle concentration. The value of the constant is shown above the graph, together with the correlation and the coefficient (the slope of the regression line) that represents the fibrogenicity for 'all particles.'

Figures 3b-e show the results of plotting the fibrosis score against concentration of various particle fractions ('asbestos + quartz', 'asbestos', 'quartz', 'all-asbestos-quartz'), again with the concentration expressed in terms of aggregated particle surface area. Figures 4 and 5 show data of the type given in Figure 3, and refer to concentrations now expressed in terms of aggregated particle volume and particle number respectively. The statistical data given in the Figures 3-5, are collated in Tables II–IV. Comparison in rows is invalid in Table IV since the fibrogenicity units differ.

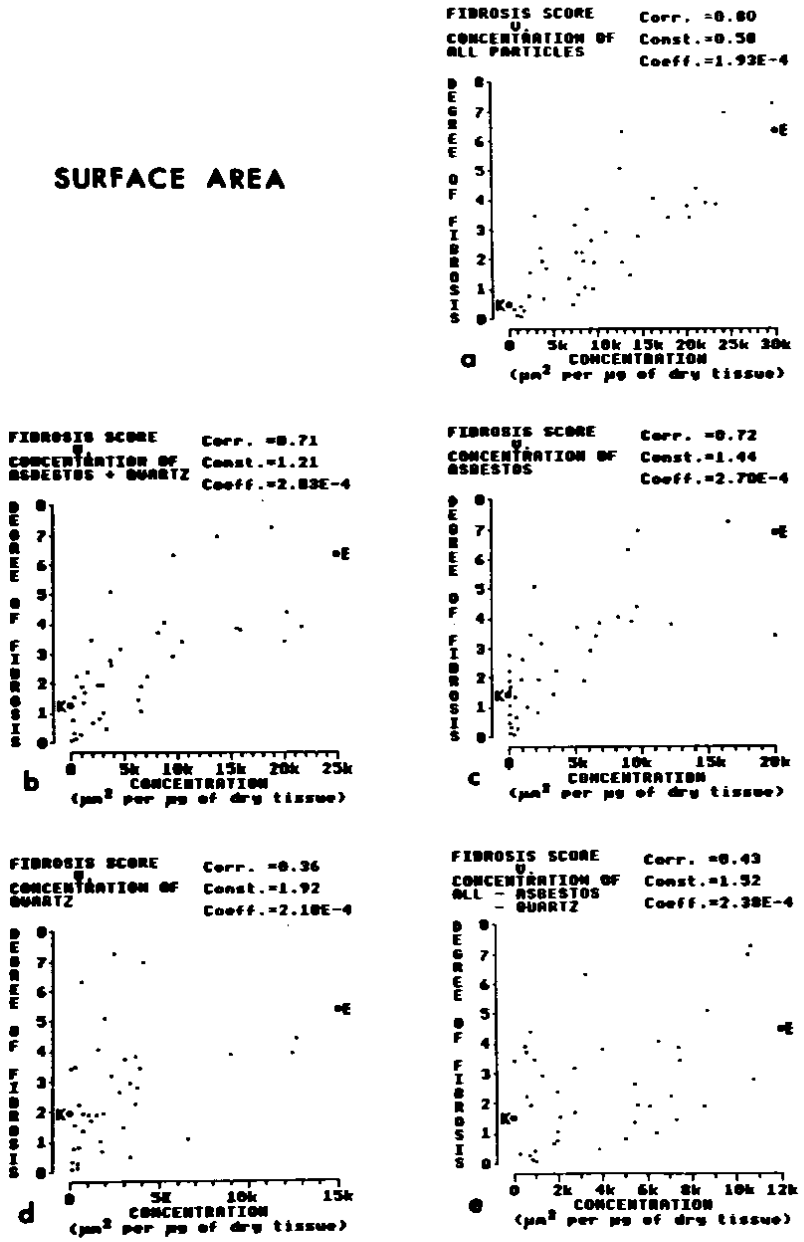


Figure 3. Relationship between severity of fibrosis and particle surface area ($\mu m^2$) per unit weight ($\mu g$) of dry tissue for various fractions of the retained dust.

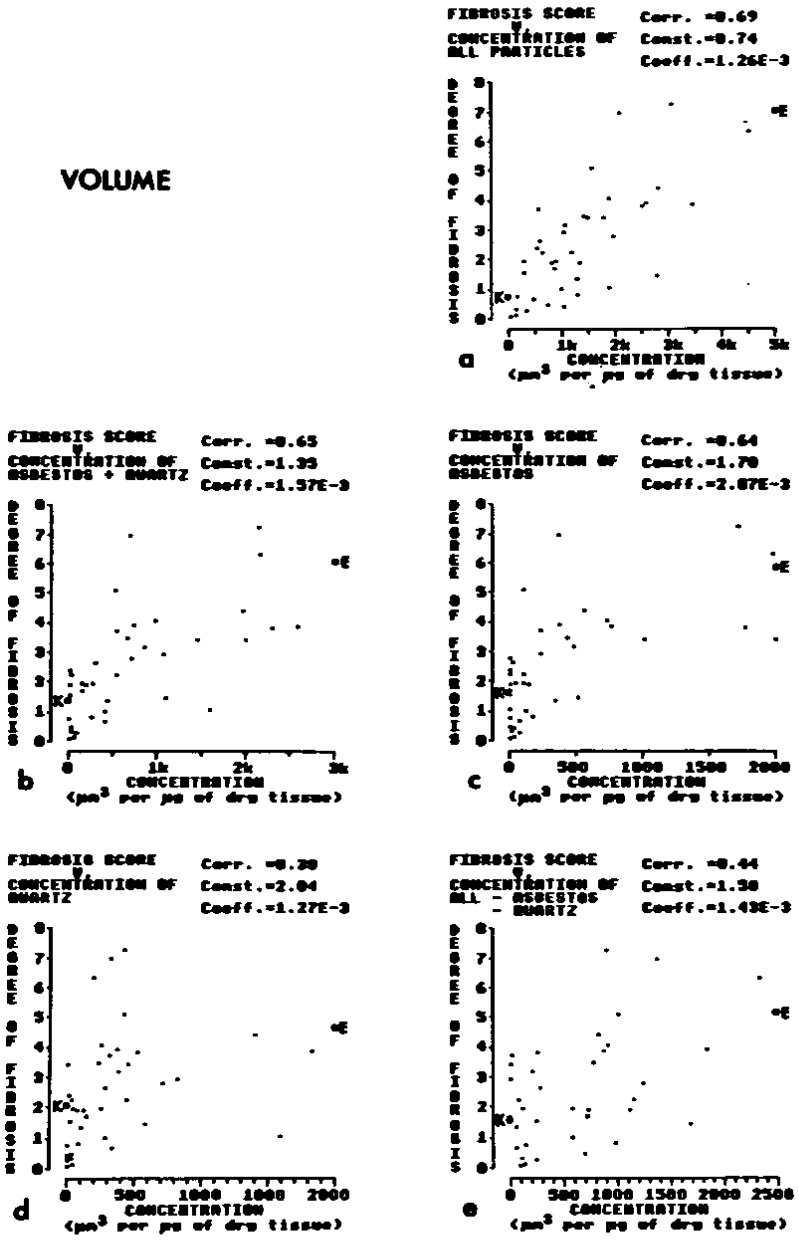


Figure 4. Relationship between severity of fibrosis and particle volume ($\mu m^3$) per unit weight ($\mu g$) of dry tissue for various fractions of the retained dust.

The substantial scatter of the data points in Figure 3a reflects the wide inter- and intra- subject variations in the degree of fibrosis which are associated with a given particle concentration and stem from differences in the cellular composition of samples taken from different parts of the lung. These are random variations however, and the correlation of 0.80 is the highest seen in Figures 3–5. The data points near the origin suggest that the value of 0.5 for the constant overestimates the fibrosis that is associated with zero particle exposure. The fibrogenicity is 1.93E-4 units (significance level 0.0001); or rounding the reciprocal, 5000 million μm of particle surface area per gramme of dry tissue induce one degree of fibrosis.

The greater scatter of the data points in Figure 3b for 'asbestos + quartz' than that in Figure 3a for 'all particles', the decrease of correlation from 0.80 to 0.71, and the increase in the constant from 0.50 to 1.21, all testify to the presence in the tissue specimens of fibrogenic particles that are not asbestos or quartz. The fibrogenicity of 2.38E-4 units shown in Figure 3e for these other particles which constitute the 'all-asbestos-quartz' fraction is, in the present biological context, equal to that for 'all particles'. This fraction, as may be seen in Figure 2, contains a wide assortment of minerals including talc, kaolinite and iron particles. The suggestion that these data indicate similar fibrogenicity of most of the

Table II
Correlation of Fibrosis Score with Particle Concentration

| | SURFACE AREA | VOLUME | NUMBER |
|---|---|---|---|
| ALL PARTICLES | 0.80 | 0.69 | 0.50 |
| ASBESTOS + QUARTZ | 0.71 | 0.65 | 0.49 |
| ASBESTOS | 0.72 | 0.64 | 0.49 |
| QUARTZ | 0.36 | 0.30 | 0.37 |
| ALL - ASBESTOS - QUARTZ | 0.43 | 0.44 | 0.33 |

Table III
Constant in Regression Equation

| | SURFACE AREA | VOLUME | NUMBER |
|---|---|---|---|
| ALL PARTICLES | 0.50 | 0.74 | 1.55 |
| ASBESTOS + QUARTZ | 1.21 | 1.35 | 1.82 |
| ASBESTOS | 1.44 | 1.70 | 1.90 |
| QUARTZ | 1.92 | 2.04 | 1.83 |
| ALL - ASBESTOS - QUARTZ | 1.52 | 1.58 | 1.78 |

of the minerals is more acceptable than that some are not fibrogenic while others are more fibrogenic than asbestos and quartz.

The relatively low correlation of 0.36 shown in Figure 2d for the 'quartz' fraction may be attributed to the inaccuracy, mentioned earlier, in the measurement of the surface area of quartz particles compared with other particles. However, the value of 2.18E-4 units for the fibrogenicity is comparable to those for the other fractions and for 'all particles'.

Examination of Table II shows that evaluating 'all particles' and expressing their concentration in terms of aggregated surface area provides the best correlation between particle concentration and fibrosis. This indicates that an index of fibrogenicity needs to be closely related to surface area, which is dependent on both particle size and shape and may be a major factor in the disease mechanism. The lower correlation values which occur when concentration is expressed in terms of volume instead of surface area are not unexpected; while volume is a function of particle size it is not a function of shape and cannot therefore be a complete substitute for surface area in the quantification of concentration, or in turn, of fibrogenicity. Table IV shows that, for similar reasons, if the index of fibrogenicity is based on particle volume instead of surface area then this changes the ranking of the fractions in order of increasing fibrogenicity. Tables II and IV also show that when concentration is expressed in terms of particle number, the correlation values fall even lower, the fractions differ more in fibrogenicity and the ranking order alters yet again. These marked changes stem from the fact that particle number is not a function of either particle size or shape and consequently, even more than volume, cannot be a complete substitute for surface area. Quartz illustrates the marked influence particle shape and size have on the value obtained for a mineral's fibrogenicity when assessment of concentration is based on particle number. Figure 5d for quartz shows the most complicated of all the relationships represented in Figures 3–5. Notable is the marked difference in Table IV between the fibrogenicity of 6.09E-4 units for quartz and the more equal values for the other fractions. The sources of this difference are the

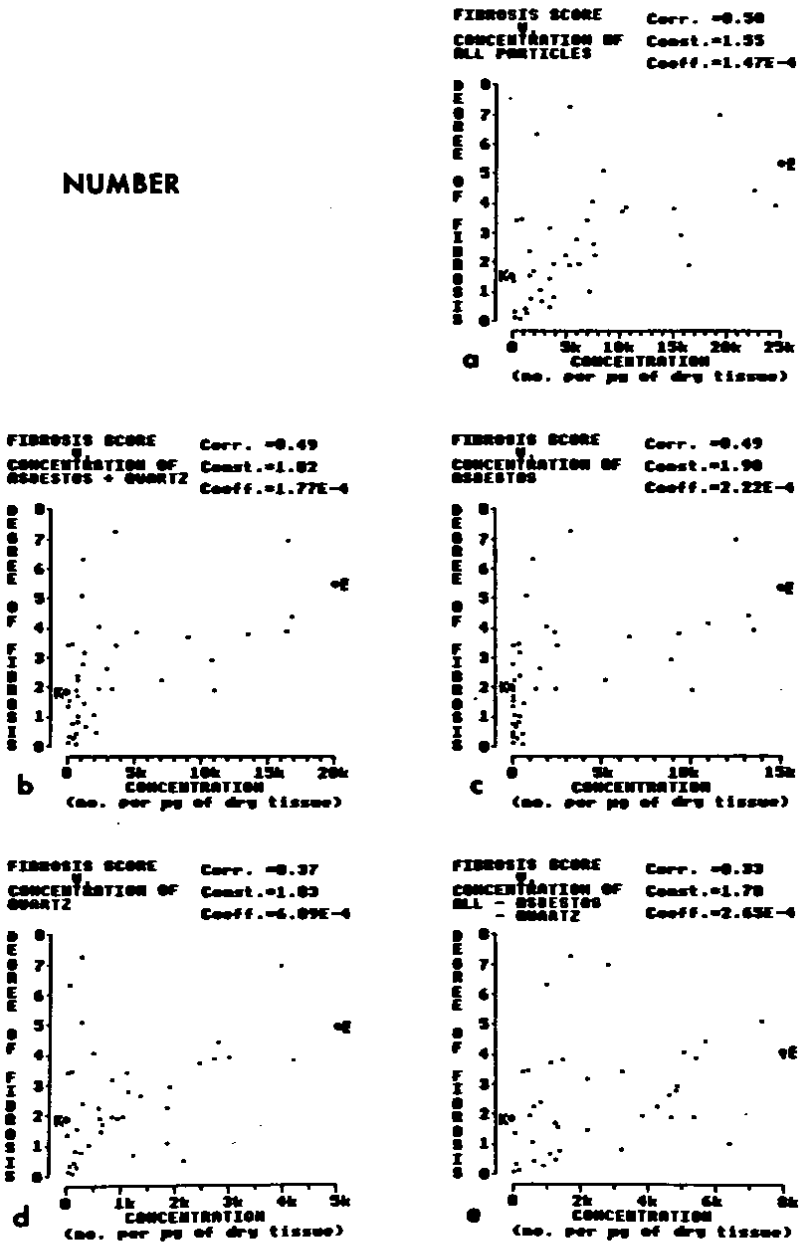


Figure 5. Relationship between severity of fibrosis and particle number per unit weight (µg) of dry tissue for various fractions of the retained dust.

dissimilarities between quartz and the majority of the other minerals in particle shape and size, factors that particle number cannot represent.

Thus the third study showed that evaluating the aggregated surface area of a retained dust provided the best index of its fibrogenicity. Evaluation of aggregated particle volume provided a reasonable index. The index based on particle number was unrealistic, especially when evaluation did not include all particles.

## IMPLICATIONS

Many tissue specimens used in the third study showed mineral contents markedly different from those implied by the type of industry from which they came. Figure 2h shows the recorded contents, which include an asbestos body but no asbestos particle, of a specimen from the lungs of a man who had worked in an asbestos factory. The specimen gave a fibrosis score of 6.96, the penultimate score obtained in the second and third studies. Results of the third study indicate that, at a concentration of 5000 particles per micro-

Table IV
Fibrogenicity by Particle Surface Area, Volume or Number

| | SURFACE AREA (degree of fibrosis /$\mu m^2$ /µg dry tissue) | VOLUME (degree of fibrosis /$\mu m^3$ /µg dry tissue) | NUMBER (degree of fibrosis /no. /µg dry tissue) |
|---|---|---|---|
| ALL PARTICLES | 1.93E–4 | 1.26E–3 | 1.47E–4 |
| ASBESTOS + QUARTZ | 2.03E–4 | 1.57E–3 | 1.77E–4 |
| ASBESTOS | 2.70E–4 | 2.07E–3 | 2.22E–4 |
| QUARTZ | 2.18E–4 | 1.27E–3 | 6.09E–4 |
| ALL – ASBESTOS – QUARTZ | 2.38E–4 | 1.43E–3 | 2.65E–4 |

gramme of dry tissue, the contents, which are typical of asbestos-associated minerals, could have made a substantial contribution to the fibrosis observed. This specimen, together with others which show similar features, suggests that even in the asbestos industry evaluation of air samples should include all mineral types and, preferably, should assess the aggregated surface area of the particles which would achieve long-term pulmonary retention.

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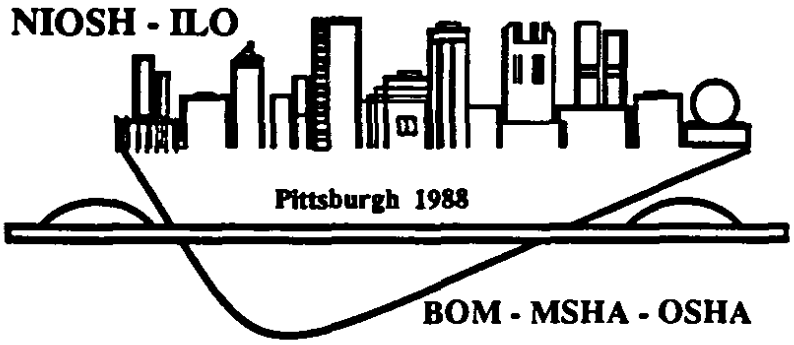


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