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THE CLASSIFICATION OF ASBESTOS FIBRES BY SCANNING ELECTRON MICROSCOPY AND COMPUTER-DIGITIZING TABLET

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Abstract—Because of the need to completely and accurately size asbestos bulk samples for toxicity studies, a method was developed to classify asbestos fibres using enlarged micrographs originally produced on the scanning electron microscope (SEM). Individual fibre length and width measurements were performed on a computer-assisted, digitizing tablet. This method, though time consuming, permitted the sizing of all fibres (length and width) and particles (area) in selected fields of view at SEM magnifications of × 100 and × 2500. Final enlargement of the micrographs permitted sizing magnifications of × 10 000. Seven distinct asbestos samples were classified including five chrysotile and two crocidolite samples. Statistical analyses showed good interfilter fibre size correlation for all types of asbestos. In addition, it was determined that a representative sizing of all fibres and non-fibrous particles on a filter preparation could be performed using five sets of micrographs taken at a magnification of × 2500 and enlarged to × 10 000: an inverse square root transformation (−0.5 power) is needed to normalize the distributions of length and width.

INTRODUCTION

MUCH has been written concerning the counting and sizing of fibrous dust samples (SCHNEIDER, 1978; ILES and JOHNSTON, 1983; CARTER *et al.*, 1987; SAMUDRA *et al.*, 1978; ZUMWALDE and DEMENT, 1976; DAVIES *et al.*, 1975). The method of choice may be determined by the type of fibre to be measured, analysis for workplace compliance and the limitation(s) of the analytical method and instrumentation. Such instrumentation includes electrozone sensing (DAVIES, *et al.*, 1975), light-scattering devices and counters (BARTH *et al.*, 1987), optical microscopes (CARTER *et al.*, 1987; MCCRONE and MCCRONE, 1976) and electron microscopes (CARTER *et al.*, 1987; SAMUDRA *et al.*, 1978).

The physical dimensions and aerodynamic properties of fibres make their sizing by sieving, light-scattering, sedimentation, centrifugation, impactors and cyclones impossible or limited in application (BARTH *et al.*, 1987; SILVERMAN *et al.*, 1971). In order effectively to characterize asbestos fibres, from bulk or filter-collected samples, most investigations have used either optical microscopy and/or transmission electron microscopy (TEM) (HWANG, 1983; ROOD and STREETER, 1985; MIDDLETON, 1982). It is well established that optical microscopy (OM) is unsuitable for characterizing small diameter fibres because of the optical limitation of resolving objects smaller than the wavelength of visible light (MCCRONE *et al.*, 1978). The main restriction of using OM is the practical limits of resolution of 0.3 μm (ESMEN *et al.*, 1982). TEM, while capable of

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resolving most asbestos fibres, is extremely expensive in equipment costs, labour intensive in sample preparation and only able to view a small area of particle collection.

Scanning electron microscopy (SEM) has several advantages over TEM. SEM is considerably less expensive than TEM, requires less sample preparation and facilitates a much larger analytical area capability. The use of SEM in asbestos sizing has been reported by several investigators. MIDDLETON (1982) has compared the use of SEM and TEM for asbestos analyses. CARTON and KAUFFER (1980) reported using SEM analyses with counting rules similar to those used in optical microscopy asbestos counting. Advantages of SEM over optical microscopy include better resolution, depth-of-field viewing and the ability to perform X-ray microanalysis for chemical analysis of fibres.

Comparative studies between optical microscopy and SEM asbestos counting have been shown to produce similar results depending upon the type of fibre and the distribution of fibres on a filter (CARTON and KAUFFER, 1980; BECKETT, 1973). Enlarged micrographs or projected images for particle/fibre sizing have been used in both TEM (CARTON and KAUFFER, 1980) and optical microscopy (ANDERSON, 1962).

Fibre counting rules differ from one method to another (SCHNEIDER, 1978; CARTER *et al.*, 1987; SAMUDRA *et al.*, 1978; CARTON and KAUFFER, 1980; LEIDEL *et al.*, 1979). All of these methods have drawbacks such as the overestimation of fibre numbers, over- or underestimation of fibre size, difficulty in counting crossed fibres or fibre bundles, no discrimination of fibre type, filter loading or fibre dispersion problems, fibre/fibril resolution and preparation and/or analysis time.

Fibre counting and sizing methods by optical microscopy are also confounded by the use of light microscope graticules with their inherent error, as well as problems and induced biases with various counting rules (CHERRIE, 1984). Sizing from OM screen projections of particle size distributions also present certain difficulties and problems (CORN, 1965). Micrographs for particle size measurements have been used in other studies and have been reported to induce errors due to non-random sampling of particles from bulk particle lots (ROSS, 1973).

Studies have been performed to investigate fibre toxicity using asbestos from the Union Internationale Contre Le Cancer (UICC), with asbestos size data either supplied by the asbestos manufacturer/processor (STANTON and WRENCH, 1972; BIGNON *et al.*, 1989) or produced by either optical microscopy (WAGNER and BERRY, 1969) or by scanning electron microscopy (e.g. DAVIS *et al.*, 1986; DAVIS and JONES, 1988). Because of a need to size classify several types of asbestos to be used in a chronic toxicity study, a method using scanning electron microscopy, micrograph enlargement and computer-assisted image digitized sizing was developed and evaluated. The resultant method did not permit discrimination of asbestos fibres from other fibrous materials, but it did provide accurate fibre length and width classification for homogenous bulk analysis.

MATERIALS AND METHODS

Preparation of fibre samples

Seven groups of asbestos were classified in this study. Canadian chrysotile was originally size fractionated (to collect respirable fibres) at CERAM-SNA, Inc., Sherbrooke, Quebec, Canada, in an Alpine® classifier. The bulk material was divided

into three equal lots of approximately 500 g each. One sample was retained as the bulk stock of chrysotile. The other two samples were delivered (one each) to Canadian and U.S. manufacturers of modified chrysotile where modification treatments were performed. Previously size-classified chrysotile and crocidolite were obtained from the National Institute for Environmental and Health Sciences, Research Triangle Park, North Carolina, U.S.A. NIOSH size-classified crocidolite as well as UICC chrysotile were obtained from archived samples from NIOSH. Table 1 notes the type of asbestos and abbreviation used in this text.

TABLE 1. TYPES OF CLASSIFIED ASBESTOS

Asbestos	Text abbreviation
Canadian chrysotile (base stock)	CAB
Canadian chrysotile (Canadian modified)	CAM
Canadian chrysotile (U.S. modified)	USM
UICC chrysotile*	UICC
NIEHS chrysotile*	NCRY
NIEHS crocidolite*	NCRO
NIOSH crocidolite*	DCRO

*Notes other classification(s) have been performed.

Four completely independent samples were prepared from each bulk asbestos sample (with the exception of the UICC chrysotile). The preparation required repeated mixing, dividing into quarters, retaining two quarters and repeating until the remaining sample weighed approximately 2–4 g. Filter samples were prepared by suspension and swirling in 100 ml of deionized and filtered water containing one drop of Aerosol OT®. Mild sonication (< 5.0 min) was applied to disaggregate large fibre bundles. The suspension was magnetically stirred and appropriate aliquots of from 0.5 to 2.0 ml were filtered through a 0.1 μm pore diameter Nuclepore® filter.

After filtration, each Nuclepore® filter was mounted on a 25 mm diameter carbon planchet with colloidal graphite. To enhance resolution the filter preparations were sputter-coated with a conductive surface of gold–palladium.

Scanning electron microscopy

The coated filters were examined in the scanning electron microscope at magnifications of from × 60 to × 5000 using the secondary electron image (SEI) at an accelerating voltage of 30 kV. Filters were evaluated for fibre dispersion making sure that fibres were dispersed in a monolayer with a minimum of clumping and overlapping.

Micrograph preparation

A SEM micrograph of a randomly selected field was taken for analysis at × 2500 using the SEI. This micrograph is referred to as the primary micrograph (PM). In taking the PM at × 2500, the Nuclepore® filter was slightly etched by the raster scan, clearly demarking the field of view. The magnification was then reduced, without changing the field co-ordinates, to view the extremities of longer fibres which extended beyond the area of view in the PM and a second, reduced magnification, micrograph (RM), that included the total area of the PM plus the extended borders and ranged in

magnification from × 500 to × 2000, was taken. The etched area was used to determine which fibres extended beyond the field of view in the PM. All PMs (× 2500) were matched with their corresponding RM before proceeding with the actual classification.

Figure 1(a) is a SEM micrograph which shows a PM of Canadian chrysotile originally photographed at × 2500. Figure 1(b) illustrates the RM, originally photographed at × 1000, with the etched area of the PM clearly visible and bounded within the superimposed borders. The on-micrograph sizing and counting rules applied are discussed later.

Sets of micrographs of the PMs at × 2500 and of the RMs at a lower magnification (× 500–× 2000) were composed. Five sets were taken of randomly selected fields on each filter preparation and five additional low magnification micrographs (LM) were taken of each filter at a magnification of × 100. The use of the PM, RM and LM micrographs is discussed in the sizing strategy section.

For each filter preparation a total of 15 micrographs were prepared. Four filter samples were prepared for each type of bulk fibre sample for interfilter comparison, making a total of 60 SEM micrographs (four sets at 15 per set) of each type of asbestos.

Micrograph enlargement

The micrographs were processed in a photographic darkroom to produce prints magnifying the original micrograph by a factor of four, so that the final magnification of the × 100 micrographs was × 400, of the × 2500 micrographs it was × 10 000 and all RMs ranged from × 2000 to × 8000.

MICROGRAPH SIZING METHOD AND COUNTING RULES

Preliminary micrograph evaluation

(1) Particles were considered to be fibres when the length to width (aspect) ratio was ≥ 3:1. These fibres would be sized on the PM only if the *entire* fibre was visible on the PM. All fibres which were not completely visible in the PM (i.e. extended beyond the borders) were identified and marked both on the PM and on the corresponding RM: they would *not* be sized upon the PM but would be evaluated and sized on the RM.

(2) Particles were considered non-fibrous when the aspect ratio was < 3:1. They were sized on the PM only if the entire particle was within the PM. Particles which extended beyond the borders of the PM were identified and marked both on the PM and on corresponding RM, and, like the fibres which extended beyond the PM borders, they were sized on the RM only.

Fibre measurements

(3) Using the 'distance' measurement mode of a SigmaScan® computer-driven digitizing tablet and an operator-controlled 'mouse' calibrated for micrograph measurements at a magnification of × 2500 (ALBINGER *et al.*, 1988), each fibre completely within the PM was sized by eye as follows:

(a) the first measurement made was the fibre length. In the case of curved fibres (such as some chrysotile fibres), the measurement was made tracing a 'central line' through the fibre. Straight fibres (such as most crocidolite fibres) were measured simply by measuring the length between the ends of the fibre. Since some fibre bundles have irregularly shaped ends, the longest portion was

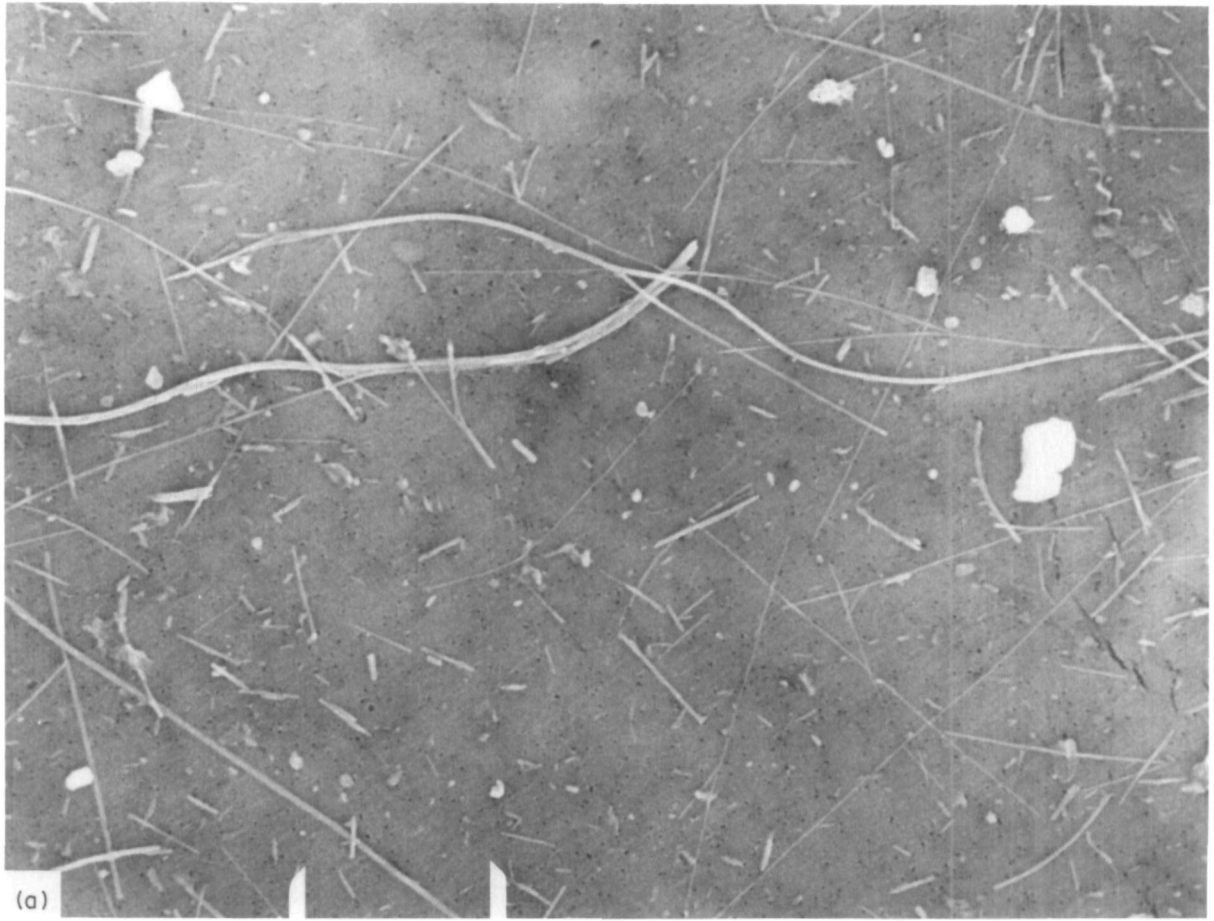

FIG. 1. (a) SEM micrograph of a filter preparation of Canadian chrysotile showing the primary micrograph (PM) taken at ×2500. Marker = 6 μm.

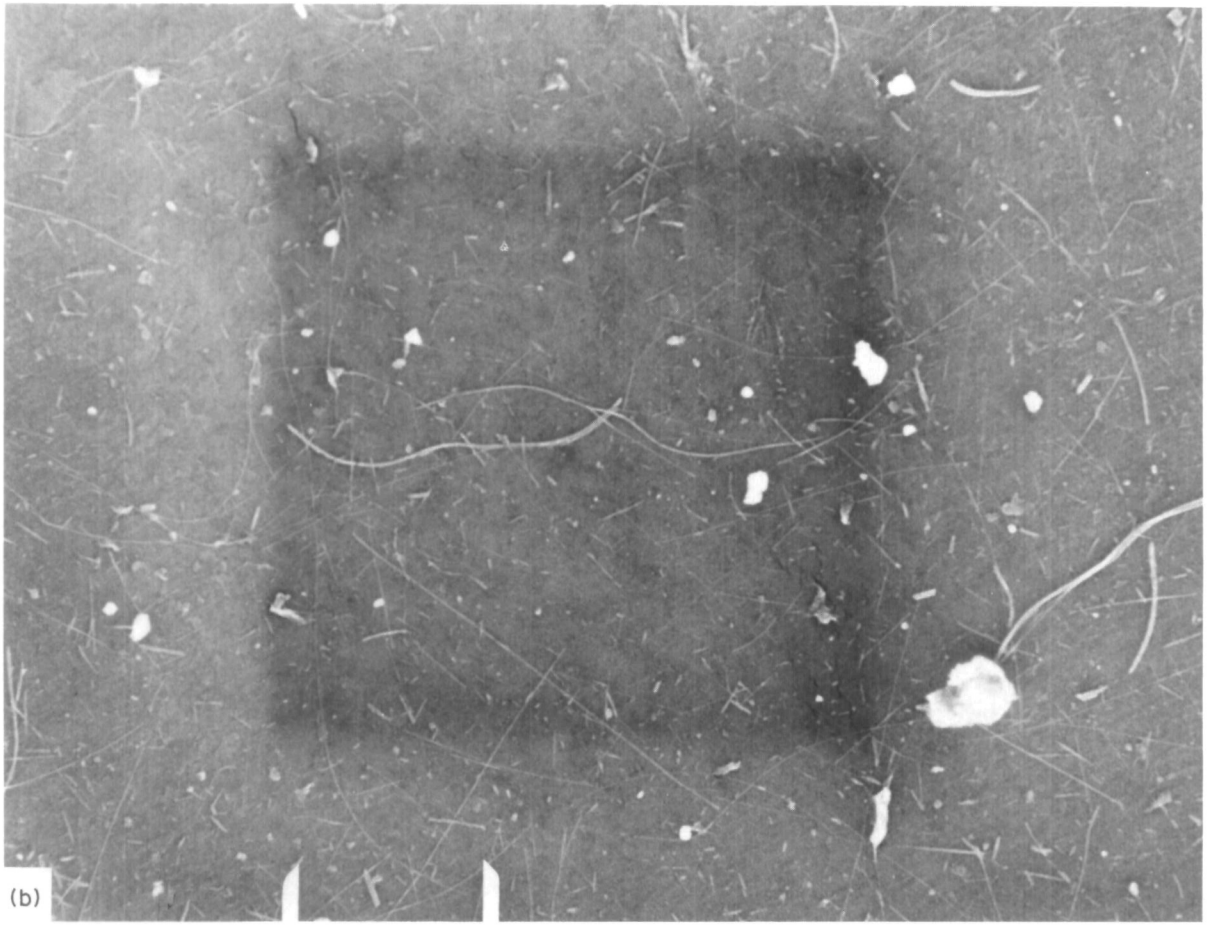

FIG 1. (b) SEM micrograph of the same filter preparation taken at a reduced magnification (RM) of $\times 1000$ with the etched region visible. Marker = 15 μm.

recorded as the length of the fibre. This method permitted the recording of the length of all fibres, straight or curved;

(b) the second measurement was the width of the fibre. This was made at the mid-point of the fibre, measuring edge to edge, perpendicular to the fibre length;

(c) once the fibre's length and width had been recorded by the digitizing computer, it was marked on the micrograph with an indelible pen to prevent it being analysed twice; and

(d) overlapping fibres or loosely-arranged fibre bundles had each length and width of each fibre recorded as individual fibres only if both ends of the fibre were visible. Compact fibre bundles with only single fibre ends visible were counted as particles and are discussed below.

(4) Once all fibres bound within the PM had length and width measurements recorded, the data were stored on a computer disk with a unique file name. The file name was also recorded on the micrograph for future reference.

(5) The SigmaScan® was then calibrated for the magnification of the corresponding RM. All fibres identified as extending beyond the borders of the corresponding PM were sized and recorded *only* if the *centre* of the fibre length was located within the etched region of the PM. Fibres with their centres outside the PM were not sized or counted.

(6) The SigmaScan® was then calibrated for analysis at × 100. All fibres that were completely visible were sized as described above and stored as unique files for future analysis. All low magnification (× 100) micrographs were subsequently sized.

Particles measurements

(7) Returning to the primary micrographs, all particles not classified as fibres (i.e. with aspect ratios < 3) as well as all compact fibre bundles were sized. The SigmaScan® was calibrated in the 'area' mode for the appropriate magnification of the micrograph (ALBINGER *et al.*, 1988). The outer edges of the particles or fibre bundles were traced and the planar area of each particle was calculated and stored in a unique file. Particle size was then reported as the calculated circular area equivalent diameter (CAED) which is defined as the diameter of a circle having the same area as the particle's planar area.

(8) Particles that had regions outside the borders of the PM and completely visible in the RM, were sized on the RM only if the geometric centre of the particle was visible in the PM.

(9) Particles which were completely visible in the low magnification (× 100) micrographs were sized and the data stored in a unique file.

Fibre and particle data were initially saved in American Standard Code for Information Interchange (ASCII) data files by the SigmaScan® software. The files were then imported into Lotus 1-2-3® (LOTUS DEVELOPMENT CORPORATION, 1985) which was then used to sort fibre dimensions and particle area. Files were then translated into Data Interchange Format (DIF) files for further statistical analysis.

RESULTS

Three primary questions became apparent during this attempt to develop a SEM method of classifying asbestos fibres:

(i) is the analysis of four filters per sample preparation adequate and repeatable for fibre size determination?
(ii) are low magnification micrographs necessary for a 'total' view of all fibre sizes present and, if so, how are the data from both low and high magnifications to be combined?
(iii) what contribution do non-fibrous particles make to the total particulate (fibres and non-fibrous particles) burden of the asbestos samples?

To examine filter variability, a nested analysis of variance was applied to individual fibre lengths. Six different fibre types (UICC was omitted since only one filter was used in its classification) represented the main effect. Filters represented a random effect nested within each fibre type. Finally, individual fibre lengths represented an effect nested within filter and fibre types. The fibre lengths required an inverse square root transformation (i.e. -0.5 power) to meet the necessary assumption of normality. Filters and individual fibres represented random effects, leading to computation of the expected mean squares in Table 2.

TABLE 2. ANALYSIS OF FILTER VARIABILITY

Source	Degrees of freedom	Sum of squares	Mean square	Variance component
Fibre type*	5	152.54	30.5072	—
Filters	18	11.69	0.64939	0.0008216
Individual fibres	17017	1283.38	0.07542	0.0754176
Total	17040	1447.61	—	0.0762492

Source	Expected mean square
Filter type*	$\sigma^2_{(\text{indiv. fibres})} + 735.65\ \sigma^2_{(\text{filters})} + Q_{(\text{fibre type})}$†
Filters	$\sigma^2_{(\text{indiv. fibres})} + 698.59\ \sigma^2_{(\text{filters})}$
Individual fibres	$\sigma^2_{(\text{indiv. fibres})}$

Source	*F* ratio	*P* value
Fibre type*	44.87‡	<0.0001
Filters	8.61	<0.0001

*Fibre type is a fixed effect; no variance component exists.
†Notation represents a quadratic function of the fixed effects for 'fibre type'.
‡Approximate *F* ratio using the Satterthwaite approximation with 5 and 17.79 degrees of freedom.

The analysis of variance and expected mean squares in Table 2 were computed by the VARCOMP procedure in SAS. The variance components were also computed by the VARCOMP procedure using the Type 1 method (CARY, 1988). The *F* ratios and *P* values were computed by hand, applying the Satterthwaite approximation (MONTGOMERY, 1984).

The six fibre types show statistically significant differences ($P<0.0001$). This indicates that the four filters from any one type exhibited sufficient uniformity to make it easy to distinguish between the different fibre sample preparations. The filters themselves exhibited significant differences also ($P<0.0001$), but this is due to the extremely large number of fibres counted (over 17000). The variance component for filters, a measure of how much variability to expect from filter to filter, is smaller than

that for individual fibres by almost two orders of magnitude. Thus, the difference detected statistically is too small to be of much practical significance.

Small variability between filters is also shown by the graph in Fig. 2 of median fibre lengths and widths (at × 2500 magnification). This graph shows the four filters for each type of asbestos clustering close together whereas the various asbestos sample preparations are dispersed throughout the graph. Results for fibre widths are similar and are not presented here. The results of this analysis showed there was good filter-to-filter repeatability and supported the use of five sets of micrographs from one filter preparation to obtain a representative analysis.

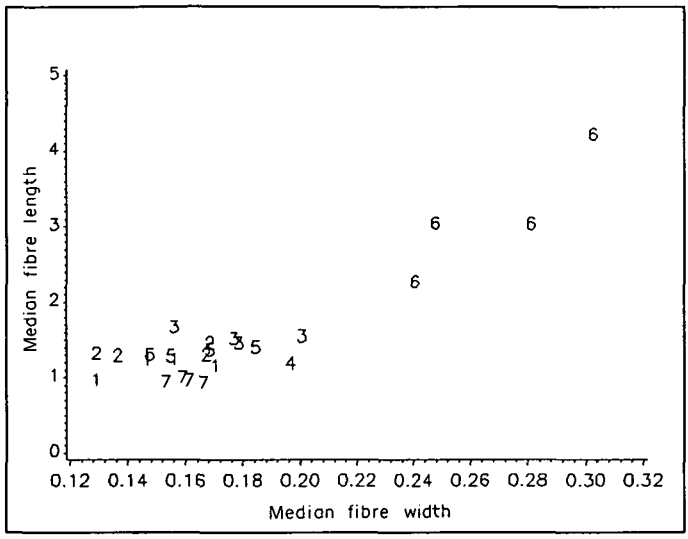

FIG. 2. Median fibre size (in μm) for each fibre type and filter (× 2500). 1 = CAB, 2 = USM, 3 = NCRY, 4 = UICC, 5 = CAM, 6 = NCRO, 7 = DCRO.

The second question, regarding how the data from both low and high magnifications are to be combined and/or weighted, presented the largest obstacle. The protocol for selecting asbestos fibres was designed to avoid bias in the selection of large fibres. To try to establish this lack of bias, micrographs at × 100 magnification were simultaneously processed. While magnification at × 100 did not resolve most of the fibres, it did provide information on the distribution of large fibres.

To resolve this problem, a 'composite' distribution combining those from low and from high magnifications was compared with that from high magnification only. This composite distribution excluded fibres greater than a cut-off size (20 μm in length and 3 μm in width) from the high magnification and replaced them with fibres larger than these cut-offs from the low magnification. The low magnification fibres so included were weighted by a factor of 1/625 to account for the difference in measured filter area between the two magnifications. The composite distribution was very similar to the distribution at high magnification only, indicating that there is little or no bias in the selection of large fibres by this method. The choice of cut-off values was somewhat arbitrary. The values chosen allowed a reasonable number of low magnification fibres

to be used. Other cut-off values were examined, however, and led to the same conclusions.

Table 3 compares percentiles from the composite distribution of lengths with those using high magnification only. The 90th and 99th percentiles are slightly longer for the composite distribution. This indicates that the selection process may not include enough of the large fibres. Nevertheless, the bias appears to be small and of little consequence; thus, for example, the proportion of fibres longer than 5.0 μm is almost identical for both distributions, and the geometric mean and geometric standard deviation change (if at all) only in the third significant digit.

The data in Table 3 are proposed as a standard method for presenting information on the distribution of fibre sizes. Previous methods of reporting fibre sizes relied heavily on percentages falling into somewhat arbitrary size categories, the size categories are inconsistent from one publication to another, and they sometimes include ambiguous categories (e.g. 50 μm and larger) or have gaps between adjacent categories (e.g. 0–0.5, 0.6–1.0). This hinders the graphical display and comparison of different fibre sample preparations. The percentiles, on the other hand, have a simple unambiguous interpretation, and while there is some potential for inconsistency in reporting percentiles, most (e.g. the three quartiles) are fairly commonly reported in many applications. Those listed in Table 3 allow the reader to focus on effects in the middle of the distribution (e.g. between the 25th and 75th percentile) or to focus on the extreme values. Finally, these percentiles can be supplemented by the judicious use of categories. Table 3, for example, notes the percentage of fibres longer than 5.0 μm.

Table 3 also shows the large differences among the seven fibre types. Most striking is the difference between NCRO and the other fibres. The median length is twice that of any fibre type and nearly 30% of the fibres exceed 5.0 μm in length. The other type of crocidolite (DCRO) classified in this study was considerably less in median length with only 2.1% of the fibres exceeding 5.0 μm in length. The NCRY and UICC chrysotile appear similar in the distribution though the NCRY tends to have larger values at the higher percentiles (75% and above).

An alternative method for classifying fibre size distribution is through the five parameters of the bivariate log-normal distribution as described by CHENG (1986). The first four parameters are the geometric mean (GM) and geometric standard deviation (GSD) for both the length and the width of fibres. The fifth parameter represents the correlation between the logarithm of length and the logarithm of width. Table 4 lists estimates for these five parameters for the seven different fibre types (high magnification only).

The bivariate log-normal distribution, however, does not fit our data well. The distribution of lengths and widths are highly skewed, even on a log scale, and an inverse square root transformation (-0.5 power) is needed to achieve normality for these fibre types.

Table 4 is provided to allow comparison with other fibre size distributions that may have been classified by the bivariate log-normal distribution scheme. Since the bivariate log-normal is a poor approximation to these size distributions, the estimated parameters in this table should not be used to derive confidence intervals, percentiles or probability statements. The percentiles shown in Table 3 are actually preferred for characterizing the size distribution of asbestos fibres.

Particle size analysis for all types of asbestos samples is shown in Table 5. The

TABLE 3. DISTRIBUTION OF FIBRE LENGTHS

Asbestos	1%	10%	25%	50%	75%	90%	99%	GM*	GSD†	% > 5.0 μm‡
	% fibres ≤ stated value (length in μm)									
NCRO—composite	0.660	1.01	1.51	2.92	5.67	10.5	53.9	3.16	2.55	29.6
NCRO—high only	0.660	1.01	1.51	2.89	5.66	10.5	49.5	3.16	2.55	29.5
NCRY—composite	0.452	0.726	1.01	1.58	2.65	4.45	15.0	1.73	2.10	8.2
NCRY—high only	0.452	0.726	1.01	1.58	2.64	4.44	14.8	1.72	2.10	8.2
USM—composite	0.518	0.742	0.959	1.38	2.30	4.07	10.7	1.58	1.98	6.8
USM—high only	0.518	0.742	0.959	1.38	2.30	4.07	10.7	1.57	1.97	6.8
CAM—composite	0.408	0.634	0.873	1.36	2.29	4.37	25.9	1.55	2.29	8.2
CAM—high only	0.408	0.634	0.873	1.36	2.29	4.36	25.1	1.55	2.28	8.2
UICC—composite	0.498	0.684	0.880	1.23	1.92	3.10	8.25	1.37	1.84	3.2
UICC—high only	0.498	0.684	0.880	1.23	1.92	3.10	8.25	1.37	1.83	3.2
CAB—composite	0.402	0.618	0.819	1.20	1.94	3.26	13.0	1.34	2.04	4.2
CAB—high only	0.402	0.618	0.819	1.20	1.94	3.26	12.9	1.34	2.04	4.2
DCRO—composite	0.382	0.550	0.715	1.02	1.55	2.40	7.17	1.10	1.86	2.1
DCRO—high only	0.382	0.550	0.715	1.02	1.55	2.39	7.03	1.10	1.86	2.1

*Geometric mean.
†Geometric standard deviation.
‡Percentage of fibres greater than 5.0 μm in length.

TABLE 4. BIVARIATE LOG-NORMAL DISTRIBUTION FOR FIBRES (×2500 MAGNIFICATION ONLY)

Fibre type	Length GM (μm)	Length GSD (μm)	Width GM (μm)	Width GSD (μm)	Log correction
NCRO	3.16	2.55	0.267	1.50	0.63
NCRY	1.73	2.10	0.177	1.52	0.53
USM	1.57	1.97	0.149	1.48	0.25
CAM	1.56	2.28	0.162	1.56	0.49
UICC	1.37	1.83	0.197	1.44	0.54
CAB	1.34	2.04	0.152	1.54	0.49
DCRO	1.10	1.86	0.158	1.53	0.59

median particle area, median circular area equivalent diameter (CAED) and the total number of particles for all particles sized at ×2500 are shown. In all seven types of asbestos the median CAED was between 0.31 and 0.35 μm for particles.

TABLE 5. CLASSIFICATION OF NON-FIBROUS PARTICLES

Asbestos type	Median area (μm^2)	Median CAED (μm)	Total particles
Canadian base chrysotile	0.091	0.341	1464
U.S. modified chrysotile	0.074	0.307	1380
Canadian modified chrysotile	0.081	0.322	904
NIEHS chrysotile	0.078	0.315	403
UICC chrysotile	0.098	0.354	237
NIEHS crocidolite	0.095	0.347	258
DPSE crocidolite	0.086	0.331	1877

Since an important advantage of this method is that it can be used to classify both fibrous and non-fibrous particulates, Table 6 shows their relationship. The actual numbers of fibrous and non-fibrous particulates, and the percentage of fibrous particles by number and by virtue are shown. Certain assumptions must be made in order to calculate the volumes. The fibres classified in this study were regarded as cylindrical, the fibre width being the diameter of the cylinder. The volumes of non-fibrous particles were determined by calculating the volume of a sphere using the particle radius calculated from the planar area. The fibre volume as a percentage of the total volume of all particulates (fibres and non-fibrous particles) ranged from 48% in the USM asbestos to 99% in the NCRO asbestos. This classification method permits the determination of the mass contribution of fibres (or particles) at any specified size range, e.g. the total mass of fibres greater than 5.0 μm in length.

DISCUSSION

One obvious shortcoming of this method is the inability to discriminate between asbestos and other fibrous materials by the use of other analytical techniques (i.e. energy dispersive X-ray analysis and electron diffraction). However, it does provide a means for size classification of samples of homogenous bulk asbestos fibres. With additional work and criteria modification to deal with non-cylindrical fibres and fibres

TABLE 6. COMPARISON OF ALL FIBROUS AND NON-FIBROUS PARTICLES

Asbestos type	No. of fibres	No. of particles	No. of fibres/total burden	Total volume of fibres (μm^3)	Total volume of particles (μm^3)	Volume of fibres/volume of total burden
Canadian base chrysotile	3768	1464	72%	801.7	562.3	59%
U.S. modified chrysotile	2945	1380	68%	360.4	397.9	48%
Canadian modified chrysotile	3174	904	78%	961.3	313.8	75%
NIEHS chrysotile	2745	403	87%	556.1	199.7	74%
UICC chrysotile	2123	237	90%	196.4	32.5	86%
NIEHS crocidolite	1387	258	84%	1971.1	19.5	99%
DPSE crocidolite	3022	1877	62%	478.9	352.4	58%

without parallel sides, it may have wider applications in the classification by size of most types of man-made and naturally occuring fibres. However, such work and modification were beyond the scope of this paper.

A good comparison of our classification of the four types of fibres (UICC, NCRY, NCRO, DCRO) with other characterizations performed by other investigators was not readily available, and in some cases comparison was quite difficult for a number of reasons. When sizing was performed exclusively by optical microscopy, the fibre length analysis was biased for the longer and larger diameter fibres since the shorter and thinner fibres are not resolved. Fibre diameter measurements are rarely performed by optical microscopy. Transmission electron microscopy is capable of resolving the smallest asbestos fibre but may well be limited in its ability to size the extremely large fibres and matted fibre bundles.

Additionally, the method of reporting fibre lengths differed between most of the type of previously size-classified control fibres. Without having the actual fibre data (length and width) for each fibre, a direct comparison of our classification with others was nearly impossible or required making unjustifiable size range separations. If it has achieved nothing else, our method has shown that there is a need for a standardized fibre data reporting scheme. A few specific comments about each type of control fibre are noted below.

UICC chrysotile

TIMBRELL (1970) performed some of the original characterization of the UICC chrysotile both by optical microscopy and by transmission electron microscopy. Optical microscopy did not size fibres less than 4.0 μm long. Non-fibrous particles were not sized or noted.

NIEHS chrysotile and crocidolite

CAMPBELL *et al.* (1980) used both OM and SEM to classify the length and width both of the chrysotile and of the crocidolite asbestos samples. All measurements were made directly on the display screen of the SEM by comparison with latex sphere generated scales. The non-fibrous particles were not counted or considered in this document. The methodology for selecting and sizing fibres is not detailed nor is the criterion for inclusion/exclusion of the larger fibres mentioned. There does not appear to be a fibre length weighting factor used when considering the range of magnifications which they reported. Without knowing the specific fibre counting rules and how fibre counts at various magnifications were treated the fibre distributions appear to be biased in favour of the larger fibres. Our counting rules detail when and how to size fibres and non-fibrous particles when they extend beyond the edges of the prescribed field of view: in CAMPBELL *et al.*'s (1980) paper such information on these analyses was not clear or available.

NIOSH crocidolite

The contractor-prepared sample sized only 584 fibres, by optical microscopy with no fibre below 7.0 μm in length being counted. The non-fibrous particles, which were considered to be agglomerates of short, fine fibres resulting from a knife-milling process, were observed but not counted (GRAF *et al.*, 1979). This problem of producing asbestos 'balls' by milling asbestos has been reported previously (PLATEK *et al.*, 1985).

The analysis of the fibre length data reported in Table 3 also demonstrated several differences in asbestos groups. The difference in fibre length between the two types of crocidolite may be due to the method of fibre size reduction by knife milling performed on DCRO and air jet milling performed on NCRO. The USM and CAM, both prepared from the same base Canadian chrysotile, are very similar in their size distribution with the exception of the 99th percentile. Both appear to be somewhat larger than the base chrysotile throughout the entire range. This may be due to the manufacturing steps including washing processes, used in the production both of USM and of CAM, which may remove the smaller fibres.

The method we have detailed using scanning electron microscopy classification of asbestos fibres provides a relatively accurate method of measuring a large number of asbestos fibres and non-fibrous particulates. It also provides a permanent record of the classification. It is felt that the resolution of the modern SEM (with micrograph enlargements to $\times 10\,000$) is capable of detecting most asbestos fibres. The minimum resolution of the SigmaScan® on a $\times 400$ and $\times 10\,000$ PM micrograph was determined to be ~ 0.35 and ~ 0.015 μm, respectively, using the smallest detected grid location difference on the appropriately calibrated, digitizing tablet. Though time consuming, the micrograph enlargement process affords a more accurate and complete classification than do those by optical microscopy and most TEM methods. Additionally, both fibre length and width are measured as well as the area of all non-fibrous particulates.

While a number of computer-based image analysis vendors have endeavoured for years to develop a program to classify fibres accurately, hitherto a number of problems (i.e. crossed fibres, fibre bundles and curved fibres, etc.) have delayed the successful development of such a system. The micrograph processing and enlargement steps of our method may be eliminated by the use of digital image storage and on-screen measurements of computer-stored images. Our future efforts will investigate this possibility.

With the $\times 2500$ magnification, we found good correlation of fibre length distribution between one filter preparation and another of the same bulk asbestos sample using this classification method. The use of the $\times 100$ magnification LM micrographs did not resolve enough short fibres or enough long, thin fibres to allow much overlap between the low and high magnification micrographs. This makes identification and quantification of any selection bias difficult. However, we have shown that any bias in the selection of large fibres using the $\times 2500$ magnification would probably be small. The method described has shown that the use of enlargements of scanning electron micrographs of filter preparations containing uniformly dispersed asbestos fibres from homogenous bulk samples provided a reproducible and fairly accurate total particulate size classification.

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