

SRN 4679

REFERENCE METHODS FOR MEASURING
AIRBORNE MAN-MADE MINERAL FIBRES (MMMM)

WHO/EURO MMMF Reference Scheme

Monitoring Concentration using a
Phase Contrast Optical Microscope

Determining Size using a
Scanning Electron Microscope

prepared by the
WHO/EURO Technical Committee for Monitoring
and Evaluating Airborne MMMF



WORLD HEALTH ORGANIZATION
Regional Office for Europe
COPENHAGEN
1985

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FOREWORD

The increased use of man-made mineral fibres (MMMF) for insulation and as a substitute for asbestos has stimulated national and international epidemiological studies on the biological effects of MMMF. Data from these studies were presented at a WHO/IARC Conference on Biological Effects of Man-made Mineral Fibres in Copenhagen in 1982.

During the course of the epidemiological research in several European countries, it was shown that substantial differences occurred between laboratories when monitoring and evaluating MMMF. Accordingly, a VICC/WHO Consultation on Methods of Monitoring and Evaluating Airborne Man-made Mineral Fibres was held in 1980 to review existing methods and recommend standard procedures to ensure comparability of data for epidemiological research. This meeting recommended a reference method for monitoring the fibre number concentration of airborne MMMF and proposed that the method should be reviewed after three years of experience. It also initiated an international reference scheme under the direction of a WHO/EURO Technical Committee on Monitoring and Evaluating Airborne MMMF consisting of experts from six European countries: Czechoslovakia, Federal Republic of Germany, France, Poland, Sweden and the United Kingdom. The aim of the central reference scheme is to harmonize evaluation, fibre counting and sizing procedures and to assess counting differences between laboratories taking part in the scheme.

The Technical Committee coordinated the work through an appointed central reference laboratory - the Institute of Occupational Medicine, Edinburgh, United Kingdom. National laboratories in the six participating countries took part in technical discussions, experimental work and interlaboratory exchanges of samples for comparative fibre counts and/or fibre size measurements by phase contrast optical microscopy and scanning electron microscopy.

This volume in the Environmental Health series summarizes the progress of the central reference scheme in harmonizing fibre counting levels by the use of standard evaluation procedures and regular interlaboratory sample exchanges. The latter are essential if effective control of subjective counting/sizing differences between observers is to be achieved. The Technical Committee has also agreed on a revised reference method using phase contrast optical microscopy to monitor the fibre number concentrations in MMMF workplaces. This revised method is reported in this volume, together with a reference method using scanning electron microscopy to determine the size of airborne fibres in MMMF workplaces.

The Technical Committee hopes that the methods described will be helpful to those concerned with monitoring MMMF and aid comparability of data for epidemiological research and control of industrial environments. Comments on the recommended methods and their application are welcomed.

The Technical Committee considers that regular interlaboratory exchanges of samples should be continued to maintain good reference standards in fibre counting and size analysis using optical and scanning electron microscopy.

MMMF are now frequently used both in manufacturing and user industries in close proximity to other fibrous materials, including asbestos. There is a need to agree on reference procedures for discriminating between MMMF and other fibrous dusts in these circumstances.

Several countries have adopted gravimetric standards for controlling airborne MMMF, although sampling methods vary considerably. Consideration should be given to the adoption of inspirable dust-sampling methods for this purpose as advocated by the International Standards Organization and the American Conference of Governmental Industrial Hygienists.

J. Dodgson
Chairman, WHO/EURO Technical Committee on
Monitoring and Evaluating Airborne MMMF

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1.1 Introduction

In 1976, the European manufacturing industry initiated an international collaborative retrospective epidemiological study on the possible adverse health effects associated with exposure to airborne MMMFM [1].

The Institute of Occupational Medicine (IOM) in Edinburgh conducted environmental surveys in several European MMMFM factories to determine airborne fibre levels and size distribution [2]. Wherever possible, parallel sampling exercises were carried out by IOM and a prominent occupational hygiene laboratory in the country where the survey took place.

Two major problems concerning measurement were found. One problem was that different methods of measuring airborne MMMFM (both fibre number and mass) were used in different countries. The other problem was that different laboratories did not always produce comparable results. Using the same method, this latter inconsistency was particularly true in fibre counting and sizing, where the subjective nature of the procedure can result in appreciable differences being obtained by different laboratories. This experience parallels that found in asbestos fibre evaluation [3,4].

In 1980, a WHO Consultation considered means of ensuring comparability of environmental measurements in epidemiological studies. Consequently, a scheme was initiated to produce reference methods to sample and evaluate MMMFM and to harmonize inter-laboratory results using these procedures. The aim is to ensure comparability of results produced by different laboratories in epidemiological studies. A more-detailed account of the background and aims of the reference scheme is given in the meeting report [5].

1.2 Organization of the Scheme

The scheme, known as the WHO/EURO MMMFM Reference Scheme, is partially funded by the European manufacturing industry through

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the Joint European Medical Research Board (JEMRB)¹, the Regional Office for Europe of the World Health Organization (WHO/EURO), and participating institutes.

A technical committee of experts was formed to manage the scheme, and the Institute of Occupational Medicine (IOM) was nominated as the Central Reference Laboratory to organize the work. This committee, the WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF, comprises representatives of WHO, JEMRB, IOM, the International Agency for Research on Cancer (IARC), and the following national institutions:

- Health and Safety Executive (HSE), London, United Kingdom;
- Occupational Safety & Health (ASS), Stockholm, Sweden;
- National Institute of Safety Research (INRS), Nancy, France;
- Professional Institute of Occupational Safety (BIA) - St. Augustin, Federal Republic of Germany;
- Institute of Hygiene & Epidemiology, Prague, Czechoslovakia; and
- Institute of Occupational Medicine, Lodz, Poland.

A list of members is given in the Annex.

1.3 Progress

The experimental phase of the WHO/EURO MMMF Reference Scheme, which began in 1981, was designed to minimize interlaboratory differences in fibre evaluation when using a phase contrast optical microscope (PCOM) and scanning electron microscope (SEM). A PCOM reference method (see section 2) was agreed as a consequence of discussions at a WHO Consultation [5], and a SEM reference method (see section 3) was developed during the experimental phase. Several interlaboratory exchanges have been conducted to assess performance with these methods, and special investigations of specific problems were undertaken where necessary. The work, so far confined to laboratories in France, Federal Republic of Germany, Sweden and the United Kingdom has concentrated on fibre number and size measurements.

¹ JEMRB is a nonprofit organization registered with the Charity Commissioners of England & Wales. Through its independent scientific and technical committee (STC) it supports research and studies on the biological effects of MMMF. It is sponsored by the Glass Fibre Producers Group of the Comité international de la Rayonne et des Fibres synthétiques (CIRFS) and the European Insulation Manufacturers Association (EURIMA).

1.4 Outline of Reference Methods

1.4.1 PCOM reference method

The PCOM reference method was developed to measure the personal respirable and nonrespirable fibre concentration. It is based on the membrane filter technique used to determine levels of asbestos fibres in air [6-8]. The sample is collected by using a battery-powered sampling pump to draw a measured quantity of air through a membrane filter. After using the acetone-triacetin technique to make the filter optically transparent [9], a PCOM with a magnification of about 500X is used to count the number of fibres on randomly selected areas.

"Fibres" are conventionally defined as objects with a length greater than 5 μm and aspect ratio (length:diameter) greater than or equal to 3:1. Fibres of diameter less than 3 μm are considered respirable, whereas those of diameter greater than or equal to 3 μm are nonrespirable. The rules used for counting complex fibre/particle groups [5] differ appreciably from the rules commonly used for asbestos evaluations. In particular, fibres in contact with other particles or fibres are counted providing they meet the above criteria. This rule was adopted for the practical reason that, otherwise, few fibres would be counted. It has the added advantage of simplicity. This reference method is referred to in the current draft standard produced by the International Organization for Standardization [10].

1.4.2 SEM reference method

The SEM reference method was primarily developed to assess the size distribution of the airborne MMMF in the workplace, with estimation of fibre number concentration a secondary aim. Samples are collected onto a polycarbonate filter (Nuclepore) or a PVC membrane filter (Gelman DM800) using the same sampling methods as for the PCOM reference method. After preparation, the samples are observed with a SEM at a magnification of 5000X. A series of photomicrographs are recorded from randomly selected fields, and the fibre length and diameter measured from an optically enlarged image of these photomicrographs. Fibres are defined as all objects with an aspect ratio greater than or equal to 3:1, with no maximum or minimum length or diameter specified. The method should be used in conjunction with a SEM fibre visibility test specimen (section 3.5.2) to ensure that fibres with diameter greater than 0.05 μm are visible.

1.5 Results of Experimental Phase

1.5.1 PCOM experimental results

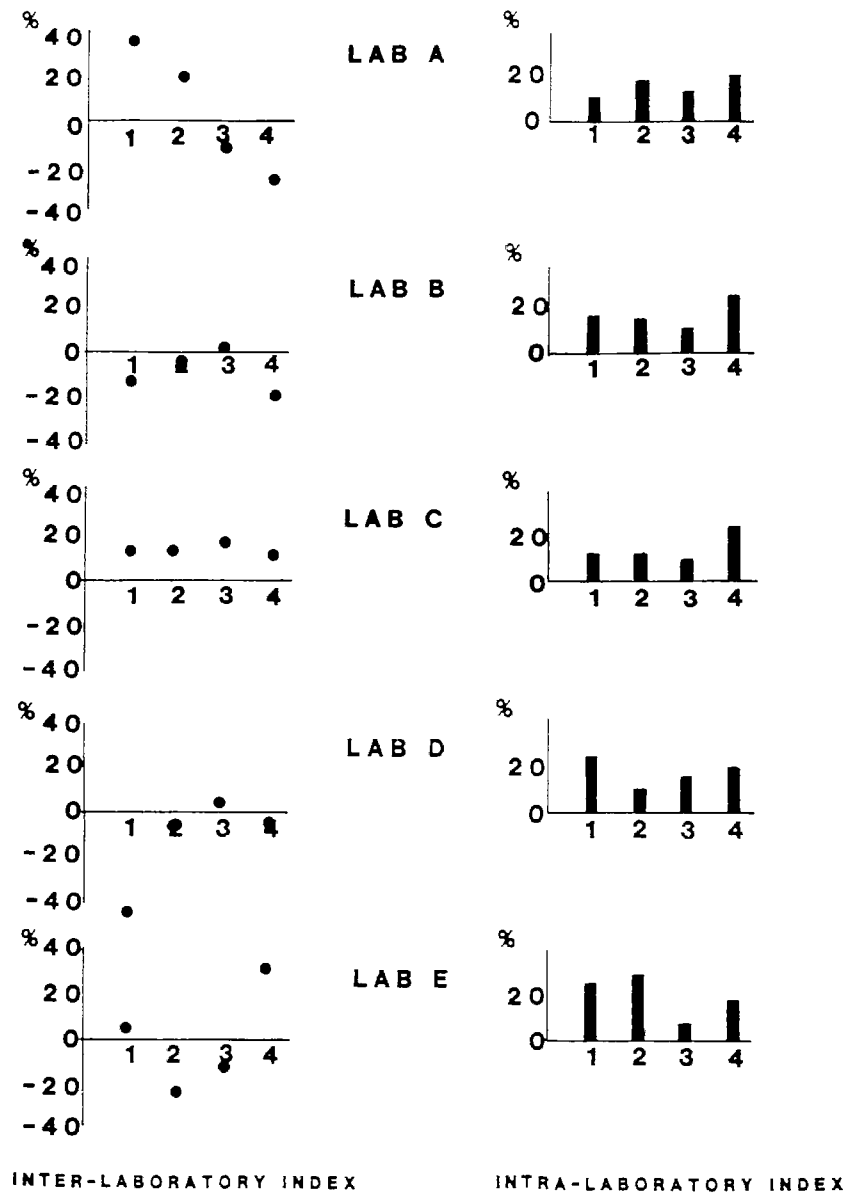
The PCOM reference method has been tested through a series of slide exchanges. Four slide exchanges have been completed: twenty samples were evaluated in the first two exchanges while eight samples were counted in the third and fourth exchanges. All assessments were made on airborne dust samples collected in factories producing rockwool or glasswool. Five laboratories participated in these exchanges, each laboratory using the reference method to make one count on the samples.

These data give an indication both of systematic differences between laboratories and of internal consistency within each laboratory. Two performance statistics have been used to describe these data: an interlaboratory index, which gives a measure of the mean position of the laboratory's counts relative to the group; and an intralaboratory index, which measures the variability of this difference. These statistics are calculated as follows. For each sample, the mean count is determined and the ratio of each laboratory's count to this mean is obtained. The arithmetic mean and standard deviation of these normalized results are calculated for each laboratory over all samples in the exchange. The interlaboratory index is obtained by subtracting 1 from the mean of the normalized results and is expressed as a percentage. The intralaboratory index is the coefficient of variation of the normalized results. Values of these indices obtained in each exchange are given in Figure 1.

A marked reduction in the spread of each of the two indices occurred from the first through the third exchange, i.e. from about $\pm 40\%$ of the group mean to $\pm 20\%$ in the case of the interlaboratory indices and from a range of 10-30% to 9-15% for the intralaboratory indices. After this improvement, the reference scheme was extended to include more microscopists, i.e. from one per laboratory to up to three per laboratory. Furthermore, lower-density samples were introduced to provide a more typical representation of fibre loadings encountered in factory situations. Consequently, the performance indices are higher than those observed in the third exchange. However, for fibre densities in the density range generally accepted for optimum precision (i.e. 100-1250 fibres/mm²), the improvement in interlaboratory reproducibility appears to have been maintained (Table 1).

WHO/EURO MMMF REFERENCE SCHEME

Fig. 1. Results from four slide exchanges using the WHO/EURO
MMMF Reference Scheme



WHO/EURO MMMF REFERENCE SCHEME

In achieving improved reproducibility, changes have occurred in the level of count obtained by each laboratory. IOM was responsible for conducting the environmental surveys in the JEMRB retrospective epidemiological study between 1977 and 1980. Five samples counted by IOM and laboratories B & C at this time were included in the second exchange to obtain an indication of long-term changes in level.

IOM counts in this exchange were around three times higher than those made 4 years earlier. The ratio of counts in this exchange was as follows: IOM, 2.8; laboratory B, 4.5; and laboratory C, 1.1. Experience with reference schemes has shown that the mean count of a group rises towards that of the highest counter. This increase has also occurred in this instance; laboratory C has maintained a consistently high level while IOM, freed from the need to keep consistent counting standards for epidemiological research purposes throughout a 4-year survey period, has increased its count level. Laboratory B counts have also increased since 1978. More samples from the survey period are being introduced into the reference scheme to provide a more reliable estimate of changes in level.

1.5.2 SEM experimental results

At the outset of the reference scheme, there was no information about the differences which might be expected between laboratories using SEM to assess fibre size. Four laboratories had experiences with SEM for determining MMMF size, and each had

Table 1. Summary statistics for slide exchanges

Statistics	Exchange			
	1	2	3	4
Number of samples ^a	17	16	8	5
Geometric mean coefficient of variation (%)	30	22	16	19
Maximum interlaboratory difference	2.5	1.6	1.3	1.4

^a Within the density range of 100-1250 fibres/mm².

WHO/EURO MMMF REFERENCE SCHEME

developed its own methods. An interim reference method was agreed upon and tested in two sample exchanges. The time-consuming nature of these evaluations restricted the number of samples to three: two were prepared from liquid suspension of fine MMMF with no organic binder, and one sample was collected in a glasswool factory. Figure 2 shows the results of one of the samples from this exchange.

Interlaboratory differences, both in terms of fibre size and fibre number, were large. For example, in the worst case, the geometric mean length estimates varied from 2.7 μm to 8.4 μm and the corresponding geometric mean diameters from 0.14 to 0.51 μm . In addition to evaluations by the reference method, those laboratories with their own inhouse methods reevaluated some of the samples using them. These data for a second sample from exchange 1 are shown in Figure 3. Clearly, these differences were smaller in magnitude than those between laboratories. It was concluded that the interlaboratory differences were due to variations in instrument performance and subjective judgements by the operators.

To resolve these problems, a special experimental exercise was initiated. One SEM operator visited each laboratory to discuss the reference method and carry out a number of tests to assess resolution, calibration of magnification, and visibility of very fine fibres (diameter about 0.05 μm) using a SEM. At the end of the visit, each laboratory reassessed one of the samples from the first exchange. The results from these evaluations (Fig. 4) show a definite improvement in performance: for example, the geometric mean fibre lengths ranged from 2.3 to 5.8 μm after the visit compared with 2.7 to 8.4 μm before the visit. Similarly, the evaluated fibre density range changed from 4100 to 7700 fibres/ mm^2 to 7900 to 10 600 fibres/ mm^2 . In achieving this improvement, evaluated fibre densities increased while median length and diameter assessments decreased, indicating detection of more short, thin fibres. This change is in fact due to closer specification of various problems in the subjective assessments made for low-aspect ratio fibres.

Immediately after this exercise, a workshop was held at which the SEM operators completed a further series of investigations at one laboratory. These exercises pinpointed areas in the reference method which required closer specification. The following points were incorporated into the reference method. First, the SEM magnification and the magnification of the final image for sizing were specified more precisely. The original definition resulted in a range of final measurement magnifications from 3000X to 50 000X. Second, differences between instruments in image quality were evident (Fig. 5). To overcome this problem, a visibility test specimen was developed against

Fig. 2. Results from one sample in exchange 1 using SEM

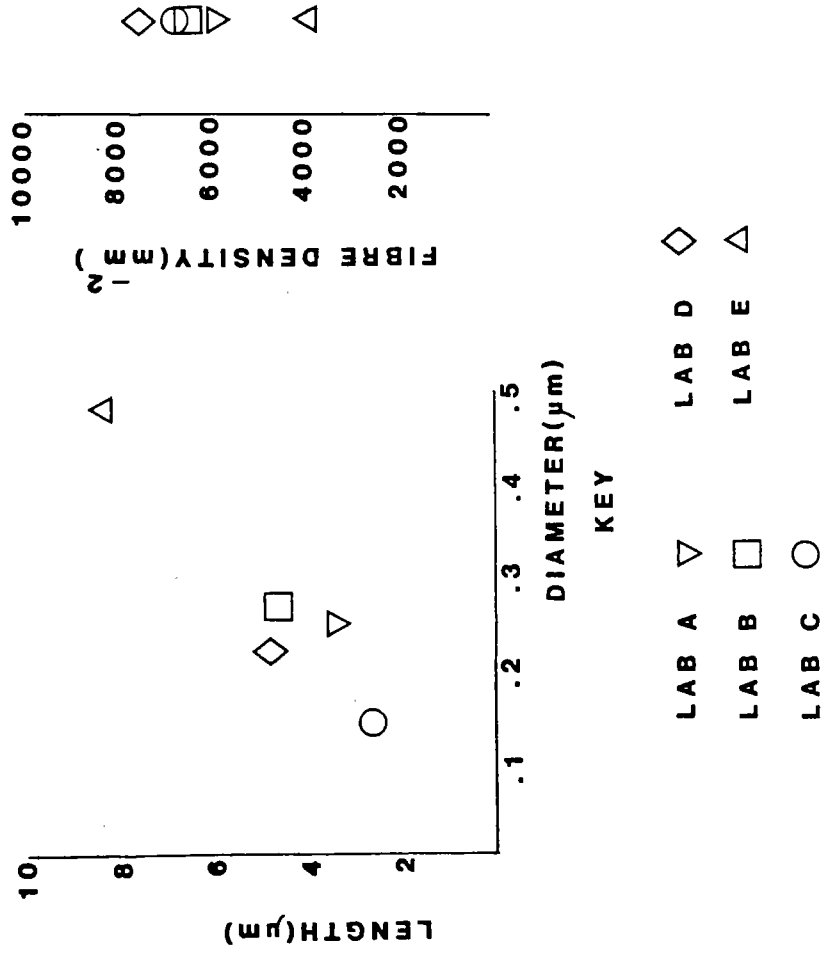
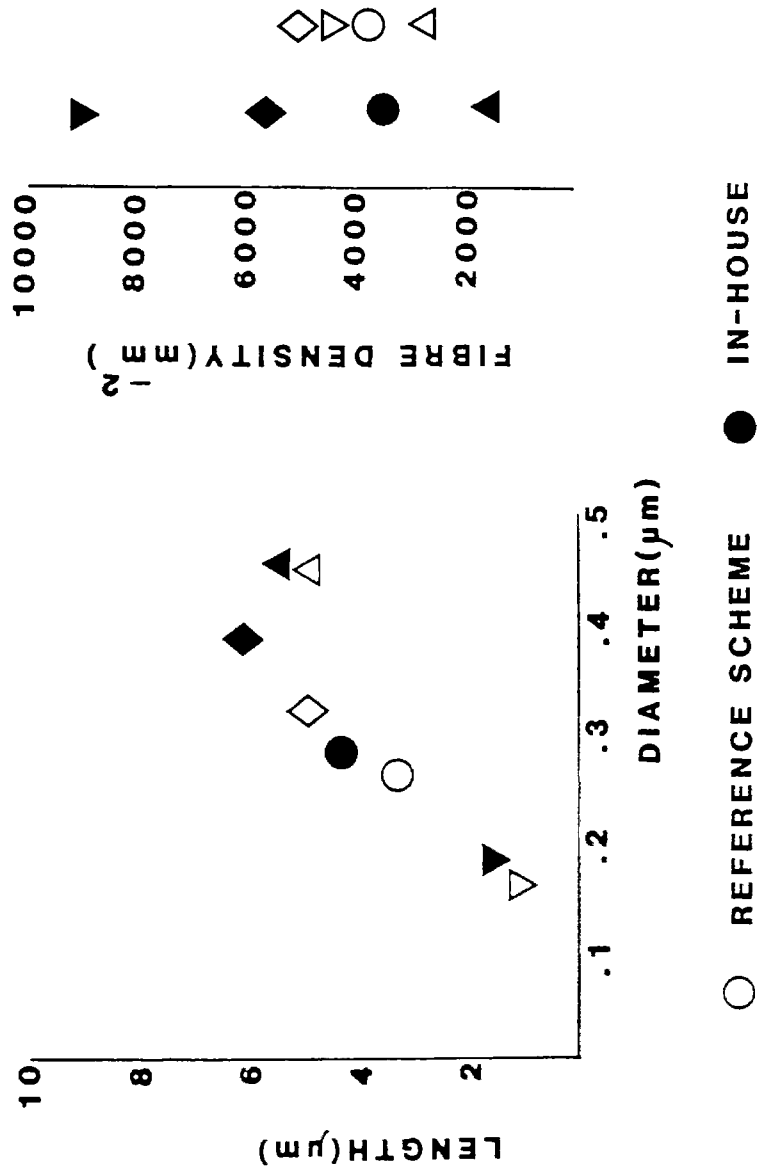


Fig. 3. Comparison of inhouse and reference scheme rules for one sample from exchange 1 using SEM



which SEM operating performance could be judged. These specimens are currently being developed by IOM. Third, for poorer-quality instruments a photomicrograph should be recorded for every field, even if no fibres are visible on the SEM screen. The detection of very fine fibres (diameter about 0.05 μm) on the SEM TV monitor was not always reliable when compared to photomicrographs because of the former's lower signal-to-noise ratio. Finally, subjective errors were greater for short fibres than long ones; one laboratory consistently found more short fibres than the others. To reduce this problem, further work is in progress to assess the reliability of this method for fibre lengths less than about 1 μm .

A third sample exchange was subsequently completed, and the results (Fig. 6) confirm the improvement agreement using the revised reference method.

1.6 Discussion

1.6.1 PCOM

A PCOM reference method for estimating airborne MMMF concentrations in the workplace was formulated and tested through a series of interlaboratory slide exchanges. The results show an improvement in interlaboratory agreement for samples in the range of fibre densities recommended for optimal precision (i.e. 100-1250 fibres/ mm^2). The maximum systematic interlaboratory differences being reduced from 2.5 times at the beginning of the MMMF exchanges to 1.4 times in the most recent exchange.

The five laboratories participating in the MMMF exchanges also took part in an international interlaboratory trial involving asbestos fibre counting [11]. The maximum interlaboratory difference was 3.5 times when evaluating chrysotile samples using counting rules published by the Asbestos International Association [8]. These rules have since been incorporated into a European Community directive [7]. The better interlaboratory agreement for MMMF compared with asbestos probably reflects the following factors: (a) the less-ambiguous counting rules inherent in the MMMF reference method; (b) the extent to which these laboratories have collaborated over the years; and (c) the difference in fibre size distribution, MMMF being generally longer and thicker and hence more clearly visible to the microscopist.

This work has particular relevance to the JEMRB epidemiological study [1]. During this study, IOM, which was responsible for making the environmental measurements in the factory, attempted to maintain a consistent counting level. However, in the course of the reference scheme, IOM's level has increased,

Fig. 4. SEM results from one sample evaluated on visits compared with exchange 1

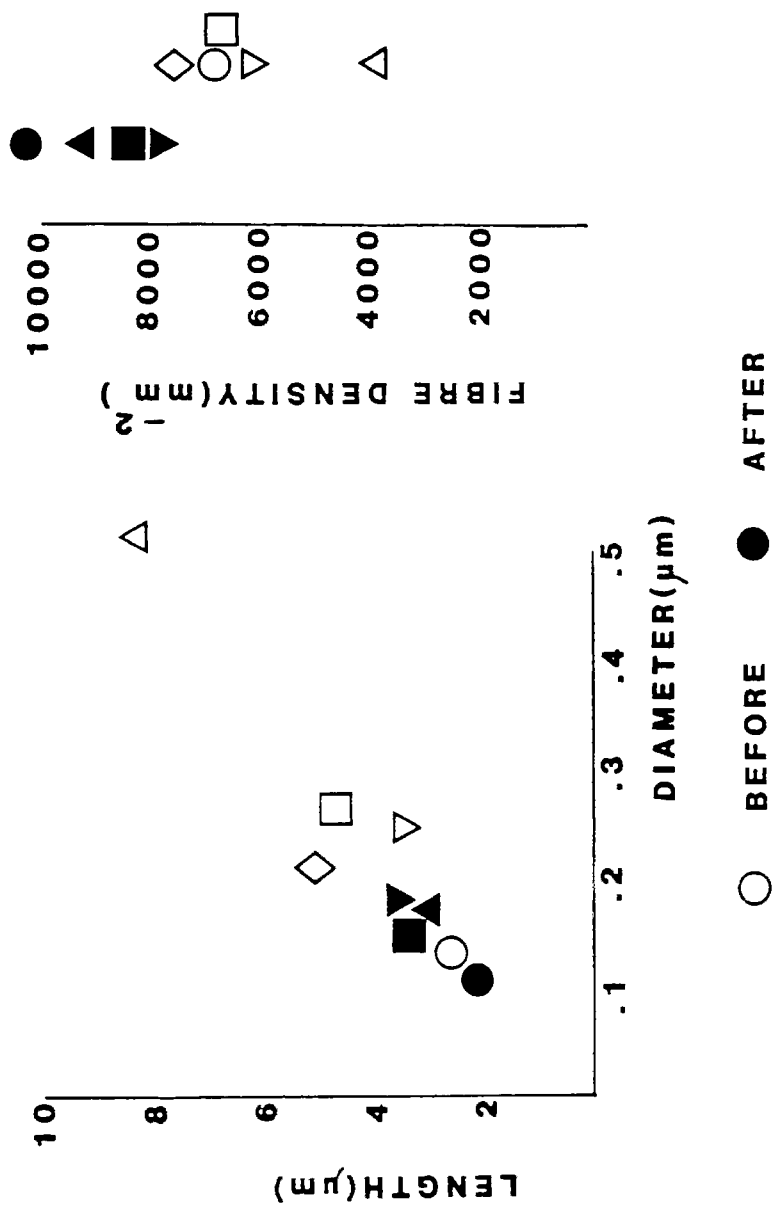
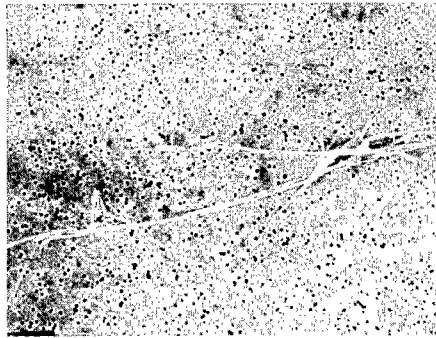
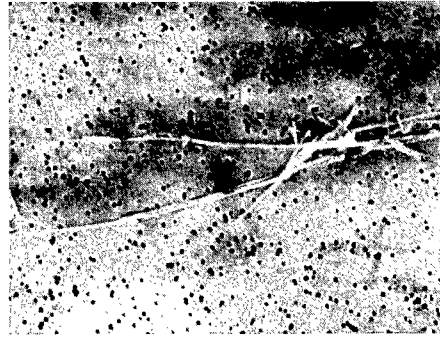


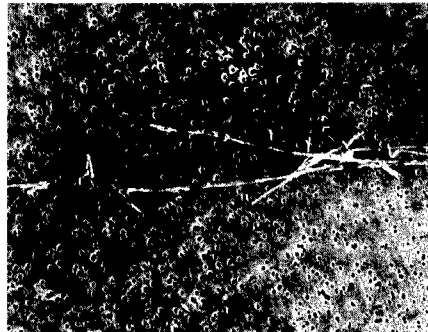
Fig. 5. Test area, containing chrysotile asbestos, as viewed on five SEMs



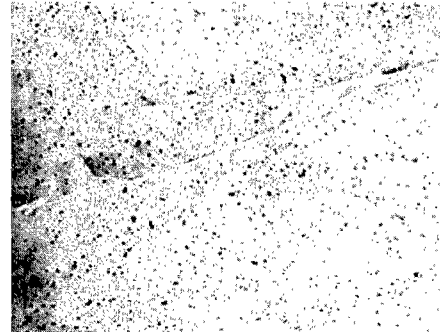
LAB A
4µm



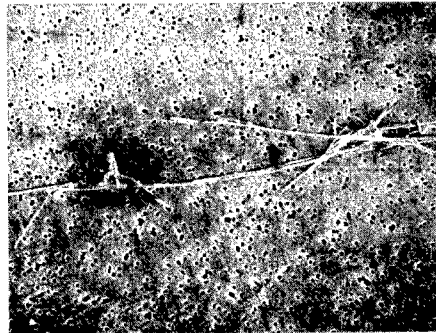
LAB B
4µm



LAB C
4µm

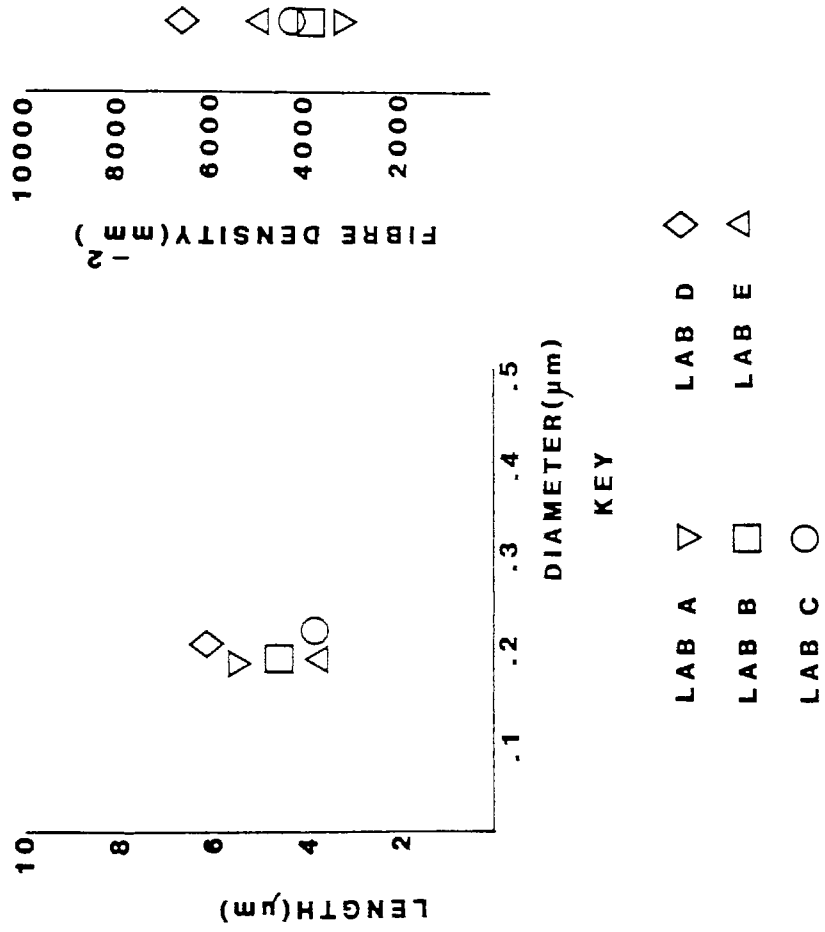


LAB D
4µm



LAB E
4µm

Fig. 6. SEM results from exchange 3



being now approximately two to three times higher than when the factory measurements were made. This represents an under-estimation of exposure compared with the present reference level. Further work is currently being undertaken to quantify better the magnitude of the change in IOM's counting performance.

1.6.2 SEM

A SEM reference method has been developed and has undergone preliminary tests. The extended time required for SEM size analysis has meant that fewer data are available to assess interlaboratory differences than for PCOM. From the limited number of samples exchanged, backed up by the experience of a workshop and laboratory visits, the indications are that some harmonization has taken place between the laboratories. As with PCOM evaluations, the microscopist can make subjective errors in the perception of fibres. This problem has already been shown to be the case for asbestos [12]. These errors can be minimized by using a reference method based on unambiguous counting rules and by continued participation in an interlaboratory quality control scheme. The latter is the most important, for laboratories tend to drift apart if interlaboratory exchanges cease.

1.6.3 Need for fibre identification

With increasing use of MMMF, many situations are developing where workers may be exposed to more than one variety of mineral fibres, either man-made or natural. In such circumstances, it is important to be able to characterize the fibre types present. A reference method for fibre identification based on electron microscopy is therefore being developed.

REFERENCES

1. Cameron, J.D. Man-made mineral fibres: medical research - CIRFS/EURIMA initiative. Annals of occupational hygiene, 20: 149-152 (1977).
2. Ottery, J. et al. A summary report on environmental conditions at 13 European man-made mineral fibre plants. Edinburgh, Institute of Occupational Medicine, 1982 (IOM Report No. TM/82/21).
3. Beckett, S.T. & Attfield, M.D. Interlaboratory comparisons of the counting of asbestos fibres samples on membrane filters. Annals of occupational hygiene, 71: 85-96 (1974).

WHO/EURO MMMF REFERENCE SCHEME

4. Gibbs, G.W. et al. A summary of asbestos fibre counting experience in seven countries. Annals of occupational hygiene, 20: 321-332 (1977).
5. Methods of monitoring and evaluating airborne man-made mineral fibres: report on a VICC/WHO consultation, Copenhagen, WHO Regional Office for Europe, 1981 (EURO Reports and Studies No. 48).
6. The measurement of airborne asbestos by membrane filter method. Rochdale, Asbestos Research Council, 1971 (ARC Technical Note 1).
7. Council directive on the protection of workers from the risks related to exposure to asbestos at work. Official journal of the European Communities, L263: 25-33 (1983).
8. Reference method for the determination of airborne asbestos fibre concentrations at workplaces by light microscopy. London, Asbestos International Association, 1979.
9. Membrane filter method for estimating airborne asbestos dust. Canberra, National Health and Medical Research Council, 1976.
10. Workplace atmospheres - determination of airborne inorganic fibre concentrations by the light microscopy - membrane filter method. Geneva, International Organization for Standardization, 1984 (draft report).
11. Crawford, N.P. & Thorpe, H.L. Effects of counting rule packages on the reproducibility of asbestos fibre counts. In: Proceedings of the Fourth International Colloquium on Dust-Measuring Technique and Strategy. London, Asbestos International Association, 1982.
12. Cherrie, J. An investigation of the reproducibility of counting and sizing of asbestos fibres by SEM. In: Proceedings of the 4th International Colloquium on Dust Measuring Technique and Strategy, Edinburgh. London, Asbestos International Association, 1982.

2. REFERENCE METHOD USING A PHASE CONTRAST OPTICAL MICROSCOPE TO MONITOR NUMBER CONCENTRATION OF AIRBORNE MMMF IN THE WORKPLACE

2.1 Introduction

This method is designed to assess the full-shift dust exposure of workers to MMMF. The method enables the mean fibre number concentration to be measured over an 8-hour period. Full-shift measurements are a good indication of the on-site exposure of personnel and can be used for determining occupational exposure limit values (i.e. the concentration to which a person may be exposed for 40 hours per week) and for epidemiological studies. Other methods may be used, providing they give similar results.

This method, first published in 1981 [1] has undergone some revision in the light of experience gained by the WHO/EURO Technical Committee on Monitoring and Evaluating Airborne MMMF.

2.1.1 Summary of method

Monitoring is carried out with the filter placed in the breathing zone of the subject. The sample is collected by drawing a measured quantity of air through the filter by means of a battery-powered sampling pump. The filter is made optically transparent, and the fibres present within random areas are counted using a transmission phase contrast microscope at a magnification of approximately 500X. The total number of fibres on the filter is estimated, and hence the airborne dust concentration in terms of this estimation. The techniques used are based on those commonly adopted for asbestos monitoring [2,3].

2.1.2 Definition of a fibre

For the purpose of optical counting, a fibre is defined as having a length greater than or equal to 5 μm and an aspect length:diameter ratio greater than or equal to 3:1. Fibres of diameter less than 3 μm are considered to be respirable, whereas those of diameter greater than or equal to 3 μm are nonrespirable. All particles which meet the above fibre definition should be counted.

2.2 Apparatus and Reagents

2.2.1 Sampling equipment

Pumps must be sufficiently light (<1 kg) to be worn for an entire shift without discomfort and be battery-powered and ca-

MONITORING AIRBORNE MMMF NUMBER CONCENTRATION

pable of functioning continuously for at least 8 hours at the selected flowrate without recharging. The flow must also be rendered pulsation free by an external smoothing unit if necessary.

Charging units must be capable of fully recharging the pump battery within 16 hours to allow the use of the pump for one shift every day. It must also be safe to leave the batteries charging for longer than the recharge time without damage to the battery or charging unit. An indicator lamp which registers when the battery is actually charging as opposed to when the charging unit is switched on is also preferable.

Filters must be made of white-gridded cellulose ester membrane with 1.2-um pore size and 25-mm diameter.

Filter holders should have a 25-mm diameter, be light, capable of securely holding the type of filter in use, and unobtrusive. An open-type filter holder must be used which is fitted with a protective metallic cowl (Fig. 7) to help protect the filter from accidental damage.

Tubing should not kink readily and must be capable of maintaining leakproof connections.

Flow meters must be able to measure the flowrate at the filter surface; measurements taken at any other position in the sampling train are subject to errors due to leaks. The flow meter must be able to measure to an accuracy of at least 10% at the selected flowrate. Calibration of flow meters against a suitable primary standard (i.e. wet-gas meter or soap-film meter) should be undertaken annually.

Lapel fixings should be spring clips in preference to, for example, safety-pins, as they do not pierce or damage clothing.

Belts should be supplied to support the pumps when sampling, to improve workers' comfort, and to standardize the position in which the pumps are worn.

Filter holder covers, where not part of the sampling head provided by the manufacturer, must be obtained to protect the filter during transportation.

2.2.2 Optical equipment

The microscope must conform to the following specifications:

Illumination: Koehler illumination, built-in.

Substage assembly: Phase-contrast condenser in centring focusing mount. The phase-annulus centration should be independent of condenser centring mechanism.

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Objective:	40X phase-contrast achromatic objective with numerical aperture at least 0.65. Phase rings should have an absorption coefficient between 70-90%. Positive or negative phase is suitable.
Eyepiece:	Compensating binocular, with total magnification between 500-600X. One eyepiece must permit the insertion of a graticule and focusing.
Graticule:	Walton-Beckett graticule must be used (see section 2.5.2).
Accessories:	Centring telescope or Bertrand lens. Green filter. Stage micrometer, 1 mm long with 2-um divisions. A Health and Safety Executive/National Physical Laboratory (HSE/NPL) Mark I test slide to check the visibility limit of the microscope-observer system.

2.2.3 Filter mounting equipment

Microscope slides should be of best quality, and 25 x 76 mm (0.8-1.0 mm thick).

Cover slips are a necessary part of the slide mount and optical system. They must be of a size marginally greater than that of the filter being mounted and matched in thickness to the calibration of the objective.

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Tweezers must be available in a range of high quality, broad-tipped and pointed-tipped types for filter manipulation.

Lens tissue must be lint free for cleaning cover slips. Industrial paper-cleaning tissue is suitable for slides.

An electrically heated water bath with a double-neck flask and water condenser system must be used for heating acetone (Fig. 8).

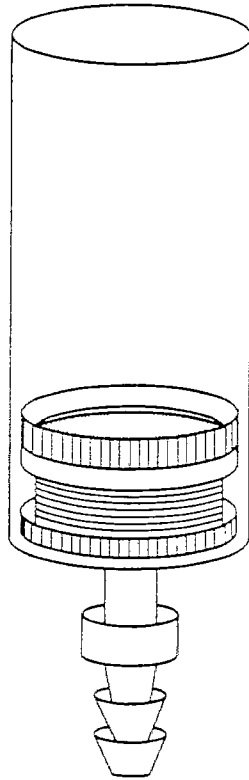
Reagents must be filtered free of fibres.

Acetone of laboratory grade should be used for mounting filters.

Glycerol triacetate (triacetin) of laboratory grade is sufficiently pure, but it should not be used if an odour of acetic acid is present.

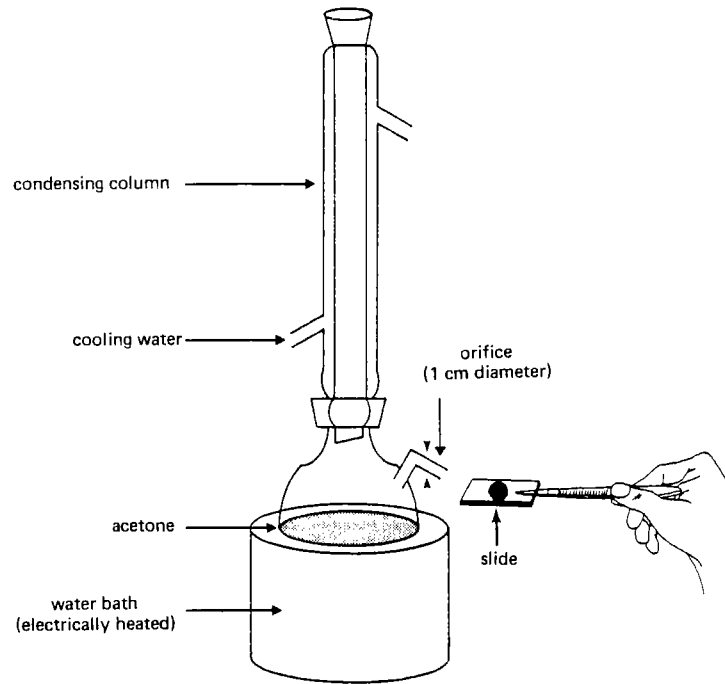
Laboratory-grade ethanol may be used to clean slides, cover glasses, etc.

Fig. 7. Filter holder and protective cowl (From [1])



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Fig. 8. Acetone mounting equipment (From [1])



2.3 Sampling

2.3.1 General information

To estimate the mean shift exposure of an operative, samples must be taken in the breathing zone (i.e. the hemisphere of 300-mm radius extending in front of the face and centred on the nose). The cowed filter holder should be attached to the lapel or shoulder so that the filter surface points downwards.

2.3.2 Flowrate selection

The sampling flowrate must be between 0.5 and 2 l/min. Lower flowrates are to be avoided as particle elutriation takes place and contamination effects are more important.

The sampling time and flowrate should be adjusted to obtain between 50 and 1000 fibres/mm² on the filter to minimize counting errors. In high particle/fibre concentrations, a consecutive series of short-term samples may be necessary rather than one long-term one.

2.3.3 Calibration of sampling pumps

Pumps should be tested before use to ensure that they are operating at the manufacturer's specification. The flow must be preset with the full sampling train (i.e. smoothers, filters) in position. Some types of pumps include an air bleed to enable the flow through the filter to be changed. These bleeds must always be closed and the flow altered by adjusting the piston stroke. If the bleed is open, more air will be drawn through it as the pressure drop across the filter increases due to particulate build-up on its surface. A large decrease in sampling flow is therefore liable to take place.

No pump should be operated without a filter on the input; otherwise, damage can occur. All pumps must be allowed to run for a few minutes before setting the flow. Some types are also susceptible to changes in temperature; in these cases, a "warm-up" period of at least half an hour must be allowed before measurement of flowrate.

The mechanical timekeepers built into some types of pump should not be relied upon as they are prone to inaccuracies. Real time must be used to determine the duration of sampling.

2.3.4 Sampling procedure

2.3.4.1 Starting routine

The pump should be attached to the worker by means of a belt or harness, and the filter holder fastened to his or her

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clothing in the breathing zone. The connecting tubing should be secured to clothing by spring clips. At this stage, any covering or cap over the filter must be removed and the filter checked for damage. The switch-on time and any other time or volume reading on the pump is then noted.

2.3.4.2 Progress checks

The flowrate through the filter must be checked and recorded during the sampling period, together with the time. If possible, this procedure should be carried out every 2 hours during long-term sampling. If a change in flowrate has occurred, the pump may be reset to its previous value where practicable. Sampling should be terminated for any sample where the flowrate falls by more than 20%. Damaged filters must be withdrawn and the sample abandoned. Dense samples should be replaced by an unused filter; as a rough guide, a filter should be changed when the printed grid lines are no longer easily visible. Where filters have been changed during a shift, the average concentration should be calculated on a time-weighted basis.

2.3.4.3 Breaks

If an employee takes a lunch or other break away from the work environment, the following procedure should be followed. The sampling equipment should be switched off and temporarily removed if the break is unpaid; if the break is paid, the equipment should be left on and running.

2.3.4.4 Completion of sampling

When the sampling period is completed, the filter holder cap should be replaced and the pump immediately switched off, noting the time. The filter must remain in the filter holder until ready for analysis.

2.3.5 Transporting filters

Filters must be transported in their sampling holders, with a top cap or cover fitted to prevent contamination. After sampling, the filter should be removed from the holder with forceps, care being taken to grasp only the filter's unexposed edge. This operation must be carried out in a dust-free area.

The filter holders or containers should be packed into a rigid case with sufficient foam packing to prevent crushing and to minimize vibrational effects. Care should be taken to avoid undue banging of the case. Fixatives, either cytological or perspex-based, must not be used.

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2.3.6 Blanks

Some control filters (minimum of 2%) must be processed in the same way as for normal samples, but without air being drawn through them or being attached to an employee, to check the level of adventitious contamination. If more than 5 fibres per mm^2 are found on these filters, the entire sampling/analysing procedure must be examined to determine the cause of contamination.

2.4 Filter Mounting

The whole of the 25-mm filter must be mounted on one microscope slide. Dividing the filter into sections increases the chances of sample loss. In addition, large differences may be found between sectors of the same filter [4] which may not be apparent if only one sector is evaluated.

The filter mounting method is based upon the use of acetone vapour¹ [5].

Prior to mounting, all samples should be inspected to ensure that the deposit is not obviously too dense for optical evaluation. Where there is some doubt as to the density of the collected material, further samples should be collected using a restricted sampling time or volume.

The filter is placed, dust side uppermost, on a clean, warm microscope slide, and plunged into hot acetone vapour. The acetone vapour is produced in the double-neck glass flask using an electrically heated water bath until the acetone is gently boiling (Fig. 7). If the acetone is not boiling sufficiently, the filter may twist and buckle before clearing. The condenser outlet operates continuously, but the jet outlet is kept stoppered until the filter is ready for clearing. The filter is placed under the jet. After approximately 2 seconds in the vapour, the filter will clear and should be removed to allow excess acetone to evaporate. The stopper is then replaced and the filter covered with a clean cover slip, using a drop of triacetin (approximately 2 ul/cm^2 of filter) to give a good optical contact between it and the sample.

The use of the acetone-triacetin method is unsuitable for materials with refractive indices between 1.43 and 1.49.

¹ Acetone mounting should be carried out only in a fume cupboard or well-ventilated room, using a water bath for heating. On no account should a naked flame be allowed in the region of acetone vapour.

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2.5. Microscopical and Counting Procedures

The microscopist must have good vision and be trained by someone experienced in fibre counting.

2.5.1 Microscope adjustment

The manufacturer's instructions for setting up the microscope must be followed carefully. The optical alignment of the PCOM should be checked regularly, and always following any lens change or transport of the microscope. An HSE/NPL Mark II test slide should be used to check that the standard of performance of the microscope and microscopist is acceptable. The test slide consists of seven blocks of parallel ridges of varying width, and the microscopist should be able to see the fifth block of ridges.

2.5.2 Eyepiece graticule

The graticule used for MMMF evaluation must satisfy the following criteria:

- (a) markings exactly 5 μm apart (for assessment of fibre length);
- (b) markings exactly 3 μm apart (for assessment of fibre width) (this is particularly important where separate counts are being made of respirable and nonrespirable fibres);
- (c) clearly defined central counting area occupying not more than one-fifth of the full field of view of the microscope.

The Walton-Beckett graticule [6] (Fig. 9) meets these requirements. It was designed specifically for fibre counting but must be manufactured individually for each combination of eyepiece and objective so that the effective diameter is $100 \mu\text{m} \pm 2 \mu\text{m}$.

The graticule must always be in focus, and the eyepiece used should preferably have adjustable focusing. In addition, before use and after any change or adjustment to the microscope lenses the sizes of the markings on the graticule must be calibrated with a stage micrometer. The exact diameter should be measured so that the graticule area may be calculated. (Note: Calibration may change marginally on a binocular microscope if changes in the interocular distance alter the tube length).

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2.5.3 Use of graticule

The central defined area alone is used for counting fibres. The procedure is to count the total number of fibre ends within the graticule area and to divide by two to determine the number of fibres.

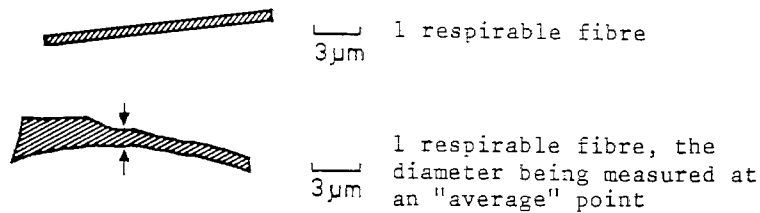
2.5.4 Counting

The aim of the counting procedure is to estimate the total number of fibres on the filter surface so that the original airborne concentration can be calculated from the volume of the air sampled. The number of respirable and nonrespirable fibres is counted in 100 graticule areas, unless more than 100 fibres (either respirable or nonrespirable) are observed. A minimum of 20 graticule areas should be evaluated, even if more than 100 fibres are recorded.

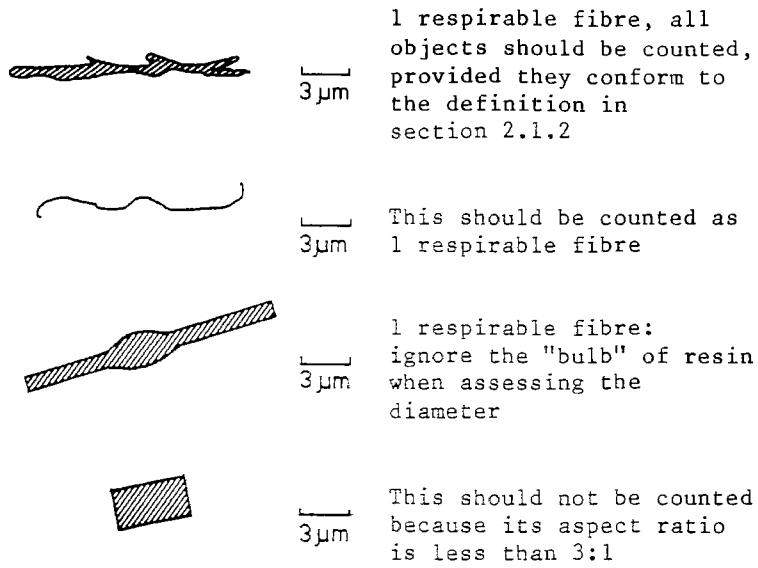
One hundred Walton-Beckett graticule areas constitute only approximately 0.2% of the area of a filter with a 22-mm-exposed diameter: the method assumes that the fibres are homogeneously distributed on the filter. Filters with uneven deposits of sizes of leakage around the filter edge must be rejected. In addition, the randomly selected graticule areas must be representative of the whole area of the mounted sample and not overlap. The method used is to traverse the filter, starting at the top, evaluating randomly selected graticule areas along the traverse. The process is then repeated on randomly selected traverses covering the whole filter area until the requisite number of graticule areas has been evaluated. Once selected, a field should always be evaluated except where over one eighth of the area is obscured by particulate material. Finally, fibres conforming to the size criteria given in section 2.1.2 are counted in each graticule according to the "ends in" rule defined in section 2.5.4.2.

2.5.4.1 Single fibres

Single fibres should be counted according to their geometric dimensions.



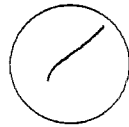
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2.5.4.2 Ends-in rule

A fibre totally within the graticule should be counted as 1 whole respirable fibre.

100-um Walton-Beckett graticule



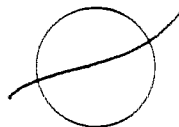
1 fibre

If the fibre crosses the perimeter of the graticule, each end within the area should be counted as 1/2 a fibre.



1/2 fibre

Fibres which pass through the graticule and have no ends within the area should not be counted.

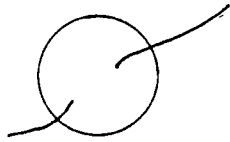


Zero fibres

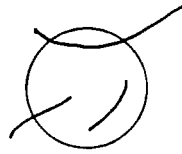
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The following are examples of the ends-in rules.

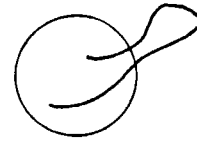
100-um Walton-Beckett graticule



2 fibre ends



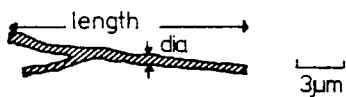
1 fibre plus
1 end



2 fibre ends,
i.e. 1 fibre

2.5.4.3 Split fibres

Split MMMF occur infrequently but should be assessed as if they were single fibres.



1 respirable fibre



2 fibre ends, split counted as 1 end.

Measurement of diameter should be made at an "average" position on the fibre.

Two adjacent fibres which can be clearly resolved as separate fibres should be counted as such.



3µm 2 respirable fibres

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2.5.4.4 Grouped fibres

When several fibres cross or intersect and the individual component fibres can be easily distinguished, each fibre should be counted separately according to its geometric dimensions.

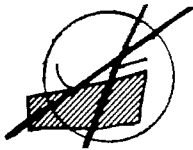


This should be counted as 3 respirable fibres

When crossed fibres form a clump and cannot be easily separated, the whole clump should be ignored.



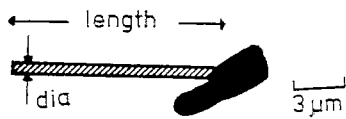
No fibres should be counted



2 respirable fibres

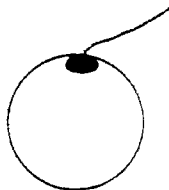
2.5.4.5 Fibres and particles

Fibres in contact with particles should be counted as if the particles were not attached to the fibres.



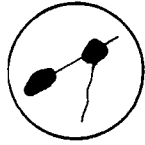
This should be counted as 1 respirable fibre

The following are examples of the use of these rules.

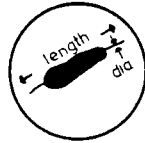


This fibre should not be counted

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This should be counted
as 2 fibres



This should be counted
as 1 fibre

2.6 Precision of Counting

Differences in fibre count may arise from two sources. One is the systematic differences or effects due to differences in techniques (sampling, mounting, counting rules, etc.) or personal factors (subjective errors). The evaluation and discussion of systematic errors constitute an exercise in itself and as such are outside the scope of this document. The second is random errors, i.e. those which are individually unpredictable and variable in magnitude. They arise because the counter examines only a small area of the membrane filter sample, typically less than 0.2%. This implies that repeat counts on the same sample are likely to cover different areas. If a sufficiently large number of measurements are taken in a controlled way, differences arising from random errors may be made unimportant and can, in principle, be made negligible. On the other hand, differences arising from systematic effects cannot be eliminated by increasing the area of the filter examined. Both the magnitude and the source of these systematic differences are difficult to distinguish if the random error is also high.

Tests using man-made vitreous fibre data from environmental surveys have confirmed that the distribution of fibres of membrane filters may be reasonably described by the Poisson distribution. On this basis, when fibres are distributed randomly over a surface, the probability that x fibres are observed in a

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specific field is given by P (x):

$$P(x) = \frac{u^x \exp(-u)}{x!}$$

where u is the mean number of fibres observed in a large number of fields.

Table 2 shows the 95% confidence limits for the Poisson distribution. For example, when the true average fibre density is 4 fibres in a specific area, the observed number in 95 out of 100 such areas will be between 1 and 10 fibres.

Table 2. 95% confidence limits for the Poisson distribution

True number of fibres	Lower limit fibres	Upper limit fibres
1	0	6
2	0	7
3	1	9
4	1	10
5	2	12
10	5	18
20	12	31
30	20	42
40	28	54
50	37	66
100	82	122
200	174	230

Where the evaluation involves samples of similar volume (e.g. approximately 500 l) and all counts refer to the same proportion of the filter area (e.g. approximately 0.2%), the relation between the number of fibres observed on the filter to the density of fibres in the air can be approximated: 50 fibres observed is about 0.05 fibres/ml, and 100 fibres observed is about 0.1 fibres/ml.

Large random variations in the number of fibres observed may occur at the lower densities (Table 2). Nevertheless, the

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variation, expressed in terms of airborne fibre concentration, is relatively unimportant. A detection level of 0.05 fibres/ml can normally be achieved without difficulty. Lower detection levels may be possible in circumstances where contamination from other particles is negligible.

2.7 Calculation

2.7.1 Volume of air sampled

The volume of air sampled is calculated by multiplying the duration of sampling by the measured flowrate. If the flowrate changes during sampling, the air volume should be estimated assuming that a linear change occurred between flowrate measurements.

2.7.2 Airborne fibre concentration

The airborne fibre concentration (C) is determined by dividing the number of fibres estimated to be on the filter (N) by the volume of air samples (V).

$$C = N/V, \text{ and } N = An/ga$$

where A = area of sample deposit (not area of filter) in mm²,
a = area of graticule (mm²) in the object plane,
g = number of graticule areas evaluated, and
n = number of fibres counted in g graticule areas.

Combining these equations gives

$$C = A/ga \quad n/V \text{ fibres/ml where } V \text{ is measured in ml.}$$

REFERENCES

1. Methods of monitoring and evaluating airborne man-made mineral fibres: report on a WHO Consultation. Copenhagen, WHO Regional Office for Europe, 1981 (EURO Reports and Studies, No. 48).
2. The measurement of airborne asbestos by membrane filter method. Rochdale, Asbestosis Research Council, 1971 (ARC Technical Note 1).

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3. Reference method for the determination of airborne asbestos fibre concentrations at workplaces by light microscopy. London, Asbestos International Association, 1979.
4. Bartosiewicz, L. American Industrial Hygiene Association journal, 34(6): 252-259 (1973).
5. Membrane filter method for estimating airborne asbestos dust. Canberra, National Health and Medical Research Council, 1976.
6. Walton, W.H. & Beckett, S.T. A microscope eyepiece graticule for the evaluation of fibrous dusts. Annals of occupational hygiene, 20 (1): 19-23 (1977).

APPENDIX A¹

Pumps

Type T13350	Type C2000
C.F. Casella & Co. Ltd	Rotheroe & Mitchell Ltd
Regent House	14 Aintree Road
Britannia Walk	Perivale
London N1 7ND	Middlesex UB6 7LJ
United Kingdom	United Kingdom

High-flow sampler model P2500 and P4000

E.I. du Pont de Nemours & Co (Inc)
Fabrics and Finishes Department
Applied Technology Division
Brandywine Building 4300
Wilmington, Delaware 19898
USA

¹ The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.

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Filter holder

25-mm-diameter filter holders with cowl

C.F. Casella & Co. Ltd	Millipore SA
Regent House	43 Avenue de l'Europe
Britannia Walk	F-78140 Velizy
London N1 7ND	France
United Kingdom	

Filters

Sartorius GmbH	Millipore SA
Post Office Box 3243	(Same as above)
D-3400 Gottengen	
Federal Republic of Germany	

Eyepiece graticule

Type G22 Walton-Beckett (1977)

Graticules Ltd
Sovereign Way
Botany Trading Estate
Tonbridge, Kent TN9 1RN
United Kingdom

Test slide

HSE/NPL Mark II test slide

Optometrics UK (Ltd)
Unit D9
Cross Green Approach
Leeds LS9 0SG
United Kingdom

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APPENDIX B¹

Ordering a Walton-Beckett graticule:

Graticules Ltd
Sovereign Way
Botany Trading Estate
Tonbridge, Kent TN9 1RN
United Kingdom

When ordering, please specify: Walton-Beckett graticule Type G22 and the diameter of the circle in millimetres which corresponds to 100-um object size. This can easily be determined by taking any available graticule, measuring the actual grid dimensions, (to 3 significant figures) and then measuring the corresponding object length with a stage micrometer. It is then a simple matter to calculate the graticule length corresponding to 100 um in the object plane.

¹ The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.

3. REFERENCE METHOD USING A SCANNING ELECTRON MICROSCOPE TO DETERMINE SIZE OF AIRBORNE MMMF IN THE WORKPLACE

3.1 Introduction

Airborne MMMF size distributions in workplaces are generally measured by sampling onto Nuclepore or membrane filters and manually evaluating the fibre size with a SEM. However, different laboratories using various SEMs do not always produce comparable results, partly due to differences in instrumentation and methodology and partly due to the subjective nature of the manual sizing operation.

The reference method described here was developed to ensure acceptable reproducibility in results in epidemiological studies in the MMMF industry. The method evolved from a collaborative experimental programme undertaken by the WHO/EURO Technical Committee on Monitoring and Evaluating Airborne Man-Made Mineral Fibres. Other methods may be used, providing they give similar results.

The operator dependence of all methods involving manual counting and sizing necessitates that this method be applied with care and used only in conjunction with a quality control scheme such as the one described in Section 1.

For the purposes of estimating the fibre size distribution, a fibre is defined as any object having an aspect ratio (ratio of length:diameter) greater than or equal to 3:1. No maximum or minimum length or diameter is specified.

All fibres satisfying these dimensional criteria are evaluated irrespective of whether or not they touch other fibres or particles. This procedure simplifies the subjective decisions to be made and hence should improve the reproducibility of the method. The method does not permit the determination of chemical composition or crystallographic structure of the fibre and can therefore not be used to distinguish between different fibre types (i.e. MMMF and asbestos). If information of this type is required, an alternative analytical method, such as electron microscopy in conjunction with energy dispersive X-ray analysis, must be used.

3.2 Apparatus and Reagents

Suitable sampling equipment is described in section 2.1.2.

3.2.1 Filters and filter preparation

Filters: These should be 25 mm in diameter, with a nominal pore size of less than or equal to 0.8 μm . Two types are suit-

DETERMINING SIZE OF AIRBORNE MMMF

able: polycarbonate Nuclepore or Gelman DM800 (a polymer of polyvinyl chloride and acrylonitrile). With Nuclepore filters, it is desirable to use as small a pore size as is compatible with the pump being used to ensure the best filtration efficiency. An alpha source or equivalent is required during all filter handling to eliminate electrostatic charge.

Conducting dag: Either aqua dag (colloidal graphite) or silver dag may be used.

Tweezers: A range of high-quality, broad-tipped and pointed tweezers must be available for filter manipulation.

Scalpel: A round-blade scalpel is required for cutting filters.

Low-temperature plasma etching oven (For preparation of DM800 filters only): This should have a forward radio frequency power of 100 W and reflected power of approximately 2 W.

Vacuum coating unit: Either an evaporation unit or a sputter coating unit capable of depositing a thin (c. °250 Å) layer of gold must be used.

Filter clearing solution (DM800 filters only): To provide a smooth surface for SEM evaluation, the porous structure of DM800 filters must be destroyed. A mixture of 33% dioxan and 67% cyclohexanone, filtered free of fibres, should be used for this purpose.

3.2.2 SEM

The techniques described here should provide comparable results on a wide range of SEMs. The minimum requirements of the instrument are accelerating voltage 20-25 keV, magnification of 5000X, capability of recording photomicrographs, and ability to detect fibres of about 0.05 µm diameter. The latter conditions may be assessed using a fibre visibility test specimen (section 3.2.4).

3.2.3 Photomicrograph enlarger

A variety of systems can be used (e.g. photographic enlargers or microfiche readers) provided that a final image enlargement of approximately 20 000X is obtained (i.e. an approximate 12X enlargement of 35-mm photographic film taken at 5000X magnification).

3.2.4 Fibre visibility test specimen

A fibre visibility test specimen has been developed specifically for assessing the performance of the SEM. A test speci-

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men and detailed instructions for its use may be obtained from J.W. Cherrie (see Annex).

3.3 Sampling

General guidance about sampling methodology and strategy can be found in Section 2.

The filters recommended for use with the PCOM (i.e. cellulose ester membrane filters) should not be used for SEM evaluation because they are unstable in the electron beam.

The selection of Gelman DM800 membrane filters may be particularly appropriate when sampling in environments containing organic matter that result from low temperature ashing in the preparation stage.

The sampling flowrate should be 1 l/min. Experience with Nuclepore filters has shown that flowrates in excess of 1 l/min. may produce localized blockages in the filter and consequently an uneven fibre distribution. Since this method aims to evaluate all fibres, not just the respirable fraction, the flow rate must be fixed to avoid size-dependent variations in fibre collection efficiency. The sample duration should wherever possible, be adjusted to give a fibre density in the range 1 to 10 fibres per field at 5000X magnification.

3.4 Sample Preparation

Filters and coated samples should always be handled with tweezers and stored in a dust-free environment.

3.4.1 Initial preparation of Gelman DM800 filters

An initial preparation step is required to render DM800 membrane filters optically transparent. Approximately 70 ul of clearing solution (33% dioxane and 67% cyclohexanone) is placed on a clean microscope slide, the filter placed on top of the solution, and the preparation dried in an oven at 60-75°C for 10 minutes. The cleared filter is then etched in a low-temperature plasma oven for approximately 7 minutes at an oxygen flowrate of 8 cm³/min. The exact low-temperature asher parameters may vary between different units, and optimal settings should be determined for each instrument. Forward and reflected radio frequency power should be 100 W and approximately 2 W, respectively. A detailed description of the technique is given in Le Guen et al. [1].

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3.4.2 Sample preparation of Nuclepore and DM800 filters

It is preferable to mount the whole filter for SEM evaluation. If only a proportion of the filter is to be mounted, this should be cut with a sharp, curved scalpel blade using a rocking motion. Care must be taken not to disturb the dust on the filter surface. The filter or filter section is then attached, dust-side-up, to an SEM stub using conducting dag.

3.4.3 Gold coating

After initial preparation, the sample is placed in a vacuum coating unit and a thin (c. 250 Å) layer of gold applied to the surface. The gold coating is required to suppress electrical charging in the electron beam and to increase the secondary electron emission from the sample surface.

3.5 Measurement of Fibre Size

3.5.1 SEM operating conditions

The operating conditions which should be adopted on any particular SEM are not described here in detail. The general requirement is that fibres with diameters greater than 0.05 µm should be visible and their images sharp. To this end, it is advisable to optimize the various SEM parameters to give best resolution. Detailed advice should be sought from the SEM manufacturer. Various parameters which should be fixed are discussed below. The acceptability of SEM performance may be checked using the fibre visibility test specimen (section 3.2.4).

3.5.1.1 Signal source

For best resolution, the secondary electron image should be used.

3.5.1.2 Magnification

There will be a minimum magnification at which fibres of 0.05 µm diameter are no longer visible. A magnification of 5000X provides comparable results on a wide range of SEMs although a modern research standard instrument with a magnification of approximately 2000X can produce reliable data. Adoption of lower magnifications is not recommended.

Calibration of SEM magnification against a diffraction grating standard, or equivalent, should be carried out at regular intervals. Drift in the internal machine settings may occur

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with time or after maintenance, resulting in errors up to 10% in calibration.

The SEM magnification control switch should not be relied upon to provide reliable information about the true magnification as variations in specimen working distance may result in substantial differences between the nominal indicated magnification and the actual magnification. For the purposes of this method, magnification is defined as the ratio of the apparent distance between two points on the photographic record screen to the actual distance between the points.

3.5.1.3 Accelerating voltage

A voltage of 20-25 keV is recommended. The accelerating voltage affects the contrast between fibres and the filter, with low keVs resulting in less contrast. Lowering the contrast in this way will affect the detection of fine fibres. A reduction in accelerating voltage will also increase the spot size and hence degrade the resolution.

3.5.1.4 Sample geometry

In scanning electron microscopy, the sample is often tilted towards the detector, this configuration ensuring the maximum signal collection. Unfortunately, this geometry distorts the image, resulting in the length of some fibres being foreshortened. Many instruments now have an electronic facility which attempts to correct for any image distortion, but have the consequence of increasing the apparent diameter of some fibres while correcting their lengths. The only way to ensure an undistorted image is to orient the specimen surface perpendicular to the electron beam.

3.5.2 Fibre visibility test specimen

The ability of the SEM to detect very fine mineral fibres may be monitored using a fibre visibility test specimen specifically developed for use with this method. The sample consists of mineral fibres on a small section of gold-coated filter mounted on an aluminium stub. Two types of fibre are present: a MMMF and an UICC¹ chrysotile fibre. The specific area to be used for the test is marked on the filter surface.

¹ Union International Contre le Cancer. Name also used for certain asbestos standards material from the Union of South Africa.

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3.5.3 SEM evaluation

Photomicrographs are recorded for each field of view, and fibre sizes determined by direct examination of the photomicrographs. The specimen is observed using the operating conditions described in section 3.5.1. A field is selected at random and a photomicrograph recorded. If any fibres have only one end within the field of view,¹ a second photomicrograph is recorded at lower magnification, centred on the original field, so that the fibre length can be assessed.

It is important, particularly on older instruments, to record photomicrographs even for fields which are apparently empty. The image on the viewing screen generally contains more noise than found in the final photomicrograph. In these circumstances, very fine fibres visible on the photomicrograph may be invisible on the viewing screen.

The process of recording photomicrographs should be continued until at least 100 fibres have been observed, with a minimum of 50 fibres being longer than 5 μm .

3.5.4 Measurement of fibre lengths and diameters

For the purposes of this method, fibres are defined as all objects with an aspect ratio greater than or equal to 3:1. MMMFs and other mineral fibres cannot be reliably distinguished by this method. If identification of fibre type is required, a different method must be applied. Further advice on fibre identification will be published in due course by the WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF. Scanning electron microscopy cannot reliably assess fibres less than 0.5 μm in length. Where detailed information is required for short fibres, a transmission electron microscope should be used.

Fibre lengths and diameters should be measured according to the rules outlined below. These rules are based on measuring the dimensions of all fibrous objects visible on the photomicrographs irrespective of whether or not they touch other fibres or particles provided that they satisfy the dimensional criteria defined in section 3.1. When a fibre touches, for example, a particle, each is assumed to be separate and the length and diameter of the visible portion of the fibre are measured. This

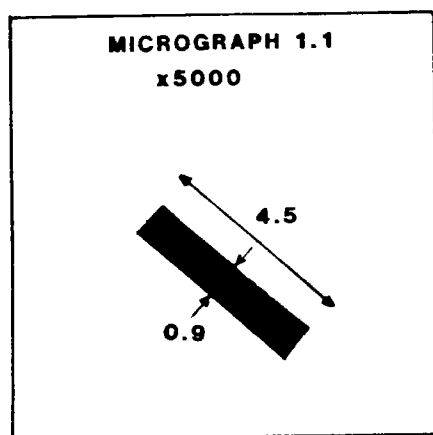
¹ If the field viewed on the SEM monitor screen is different from the area photographed, a graticule should be used to delineate the photographic area.

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eliminates some sources of variation in operator judgment. As a consequence, the rules may result in a small systematic bias in fibre size estimates but they are probably more reproducible.

3.5.4.1 Fibres contained within the field of view¹

1. For each fibre of this type, the length and diameter should be recorded (in μm) along with the photomicrograph number (Photomicrograph 1.1.)

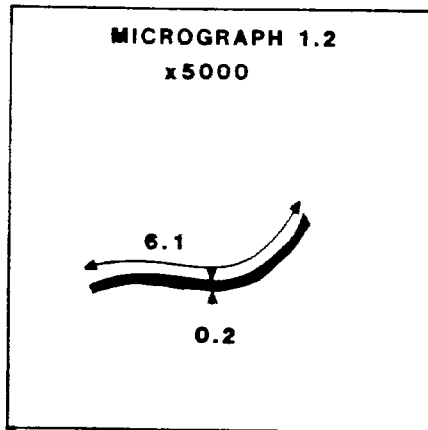


L	D	P
4.5	0.9	1.1

¹ SEM magnification 5000X; viewing magnification 20 000X; and L = length (μm), D = diameter (μm), and P = Photo no.

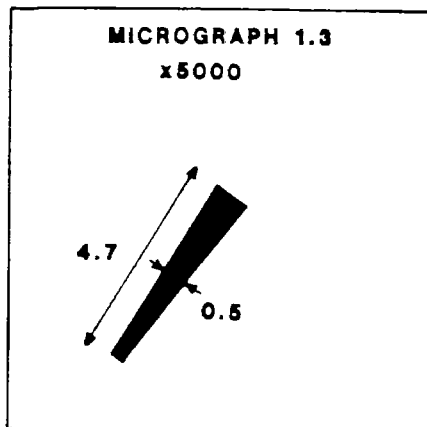
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2. Where fibres are curved, the length should be measured as if the fibre were straightened out. (Photomicrograph 1.2)



L	D	P
6.1	0.2	1.2

3. Where a fibre does not have parallel sides, the diameter should be measured at an "average" point (Photomicrograph 1.3)



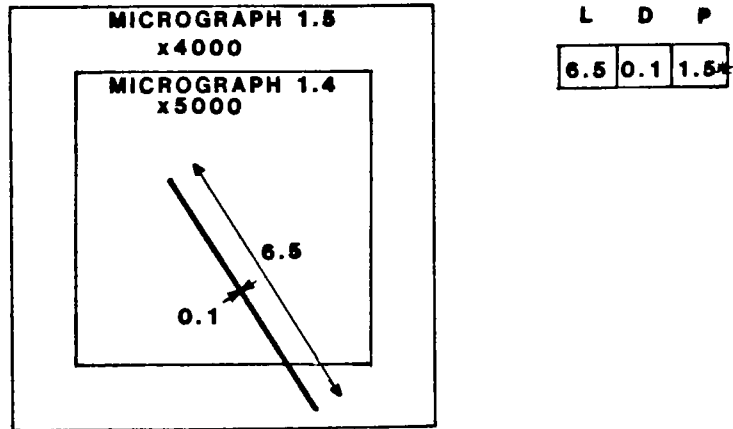
L	D	P
4.7	0.5	1.3

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3.5.4.2 Fibres crossing the boundary of the field of view

A number of rules may be adopted for dealing with fibres which cross the boundary of the field of view. A detailed description of the acceptable variants is given in Schneider [2]. The rule recommended in this method is that all fibres with at least one end in the field should be measured.

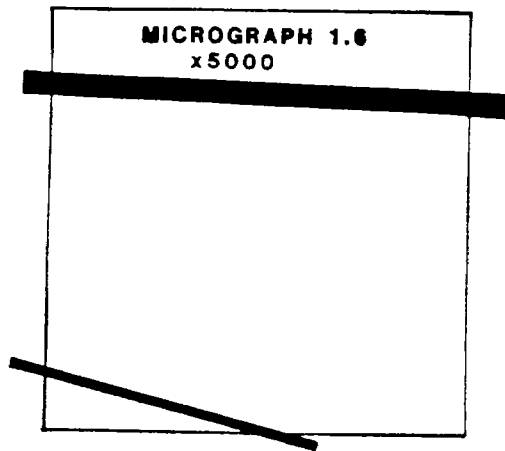
1. One end within the field: Diameter measurements should be made from the 5000X photomicrograph and length from a second photomicrograph at a lower magnification. These measurements have the "weight" of a half fibre.



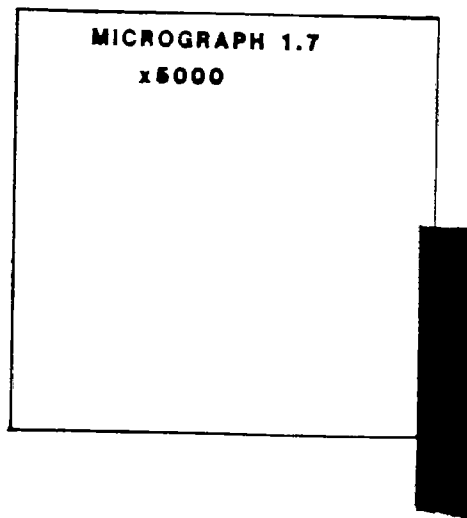
The "*" after the photomicrograph number signifies that these measurements have the "weight" of a half fibre.

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2. Fibres with neither of their ends within the field: These fibres are not measured.

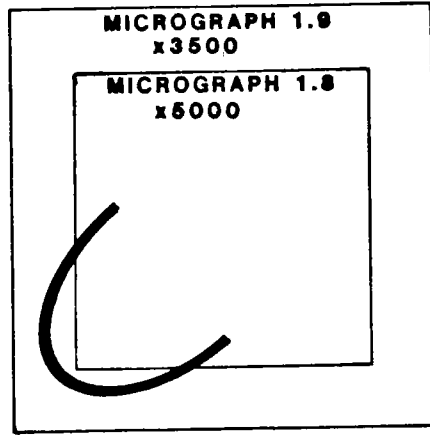


A fibre end is defined as the midpoint of the fibre breadth at the extremity of the fibre. In Photomicrograph 1.7, the fibre end is not within the field and should not be measured.



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3. Fibres which cross out of the field of view but have both ends within: This situation occurs relatively infrequently. Fibres of this type should be measured and be given the weight of 1 fibre.

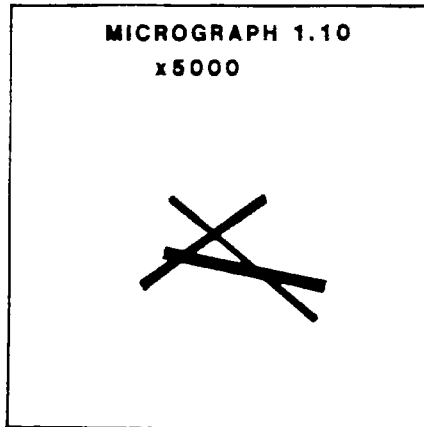


L	D	P
11.	0.2	1.8

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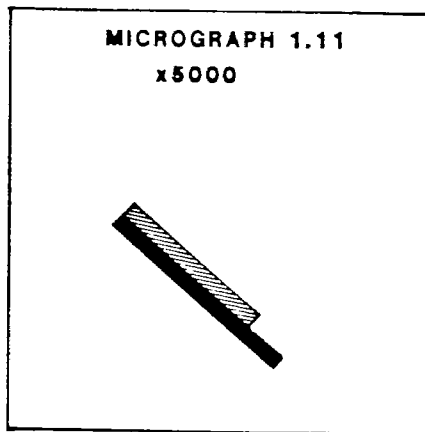
3.5.4.3 Fibre aggregates or groups

1. Where fibres are grouped together, each constituent fibre should be distinguished on the basis of continuity and measured separately (Photomicrograph 1.10).



L	D	P
4.5	0.1	1.10
3.6	0.2	1.10
3.8	0.3	1.10

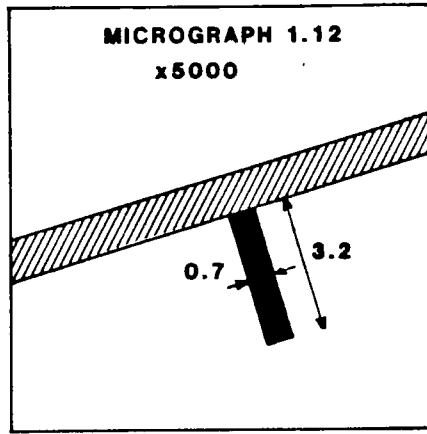
2. In many cases, two or more fibres can be seen to lie parallel to each other. Each fibre should be measured (Photomicrograph 1.11).



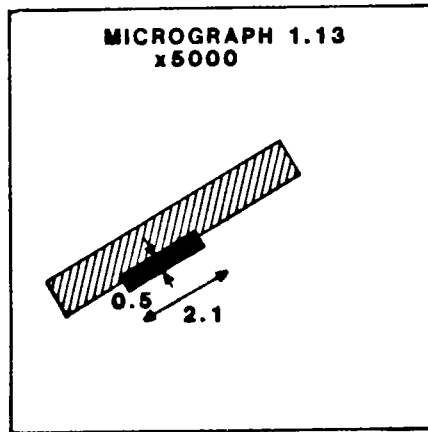
L	D	P
4.0	0.3	1.11
5.1	0.3	1.11

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3. Where two fibres are in contact, one may partially obscure the other. In these circumstances, the observed image should be measured. (Photomicrographs 1.12 and 1.13).



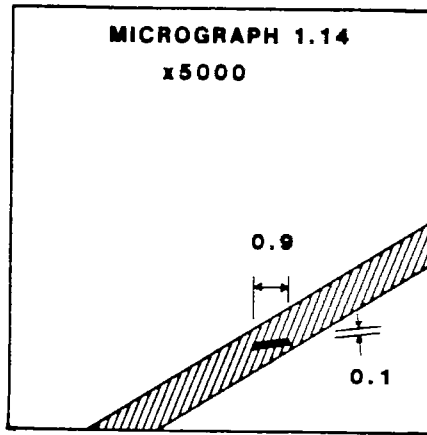
L	D	P
3.2	0.7	1.12



L	D	P
6.5	0.9	1.13
2.1	0.5	1.13

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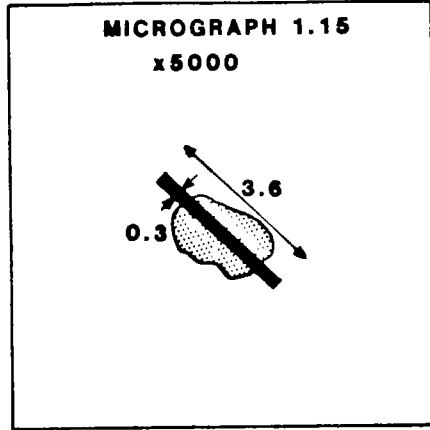
4. In some circumstances, fibres may be observed to lie directly on top of other fibres. Each should be measured as a distinct fibre (Photomicrograph 1.14).



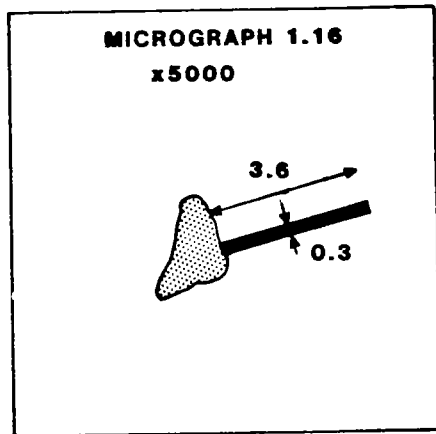
L	D	P
0.9	0.1	1.14

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5. Fibres in contact with particles: Particles in contact with fibres should be ignored and the visible part of the fibre sized according to the rules given in the preceding sections. (Photomicrographs 1.15 and 1.16). Fibres only partially obscured should be measured on the basis of continuity.



L	D	P
3.6	0.3	1.15



L	D	P
3.6	0.3	1.16

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3.6 Calculation of Fibre Concentration and Fibre Size Statistics

These calculations are based on counting and sizing all fibres which cross the periphery of the field and have at least one end within the area, as described in the preceding sections. If alternative rules are used, the algorithms would need to be revised accordingly. Further guidance on acceptable variants on the rules described and calculation of the appropriate statistics may be found in Schneider [2].

3.6.1 Fibre concentration

The airborne fibre concentration determined by SEM (C, fibres/ml) may be determined as follows:

$$C = N/V, \text{ where } N = \frac{A \sum_{i=1}^K W_i}{2ga}$$

where V = volume (ml), N = estimate of total number of fibres on filter, A = exposed filter area (mm²), a = area of one field (mm²), g = number of fields, W_i = fibre weighting, i.e. 2 for fibres with two ends in the field and 1 for fibres with one end in the field, and K = actual number of fibres measured.

3.6.2 Median and quartiles of fibre size distribution

To calculate the size distribution of the fibres, the lengths of all measured fibres should be used, as each fibre makes a valid contribution to the distribution. Because longer fibres have a greater probability of crossing the periphery of the field than shorter ones, allowance has to be made for any bias this may cause in the calculation of the size distribution. This is done by effectively weighting by 1/2 all the fibres with only one end in the area, although to simplify the calculations, in practice, the fibres with both ends within the area are weighted by 2 instead. Therefore, the total number of fibre ends contributing to the size distribution is:

$$N_1 = \sum W_i$$

where W_i = 1 for fibres with one end in the field, and 2 for fibres with two ends in the field.

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To calculate the median and the quartiles, the fibre measurements (either length or diameter) should first be arranged in ascending order of size, with those fibres weighted 2 included twice in the list, giving a total list length of N_1 . The median, m , is defined to be the middle value of the distribution, i.e. the point at which 50% of the distribution is less than m and 50% is greater. For an odd number of values, $m = (N_1 + 1)^{\text{th}}/2$ observation, and for an even number, m is the point calculated by interpolation between the $(N_1)^{\text{th}}/2$ and $(N_1 + 1)^{\text{th}}/2$ observations. In some cases, these 2 observations will refer to the same fibre, where it has been included twice in the list because of its weighting and in this instance m is simply the fibre size (length or diameter). In other cases, these values will refer to 2 fibres of different sizes; if this occurs, an average of the 2 values will have to be used.

The quartiles are calculated similarly to the median, except that for the first quartile q_1 , 25% of the distribution lies below q_1 and 75% above; $q_2 = m$, the median; and q_3 is the point at which 75% of the distribution is less than q_3 and 25% is greater. It follows that 50% of the distribution will fall between q_1 and q_3 .

3.6.3 Geometric mean and standard deviation of fibre size distribution

The geometric mean and standard deviation are best defined as the exponentials of the arithmetic mean and standard deviation of the natural logs of the observations. To calculate these quantities, natural logs (to base e) are taken of the measurements (either length or diameter) of the fibres and the arithmetic mean and standard deviation of the logs calculated. These values are then transformed back using the inverse of natural logs, the exponential function. The potential bias caused by fibres which cross the periphery is again minimized by using a weighting system similar to that described in the previous section.

If S_i is the length or diameter of fibre i , then the arithmetic mean of the logs is defined as:

$$\bar{S} = \frac{1}{N_1} \sum_{i=1}^K W_i Y_i$$

where $W_i = 1$ for fibres with one end in the field and 2 for fibres with two ends in the field, $Y_i = \ln S_i$, $K = \text{actual}$

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number of fibres measured and

$$N_1 = \sum_{i=1}^K W_i$$

Hence, the geometric mean is equal to:

$$\exp \left\{ \frac{1}{N_1} \sum_{i=1}^K W_i \ln S_i \right\} = \left\{ \frac{1}{N_1} \sum_{i=1}^K S_i^{W_i} \right\}^{1/N_1}$$

The arithmetic standard deviation (asd) of the log length is:

$$\text{asd} = \left\{ \frac{1}{N_1 - 1} \left[\sum_{i=1}^K W_i Y_i^2 - \frac{(\sum_{i=1}^K W_i Y_i)^2}{N_1} \right] \right\}^{1/2}$$

(notation as above)

and the geometric standard deviation = exp (asd).

3.6.4 Fibre size matrix

In addition to summary statistics such as median and quartiles or geometric mean size, the composite size distribution, length, and diameter are important to know. These may conveniently be displayed in the form of a matrix showing the percentage number of fibres in various size categories, as shown below.

Fibre length (um)	Fibre diameter (um)						Σ% (length)
	0.2	0.2-0.6	0.6-1	1-3	3-5	5	
<1							
1-5							
5-10							
10-20							
>20							
Σ % (diameter)							

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REFERENCES

Le Guen, J.M. et al., Environmental science and technology,
14 (8): 1008 (1980).

Schneider, T. The influence of counting rules on the number and
on the size distribution of fibres. Annals of occupational hy-
giene, 21 (4): 341-350 (1980).

ANNEX

WHO/EURO Technical Committee on Monitoring and
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