ASBESTOS

GUIDANCE NOTE ON THE MEMBRANE FILTER METHOD FOR ESTIMATING AIRBORNE ASBESTOS DUST [NOHSC: 3003 (1988)]

CONTENTS

PAG

PREFACE

The National Occupational Health and Safety Commission is a tripartite body established by the Commonwealth Government to develop, facilitate and implement a national health and safety strategy.

This strategy includes standards development, research, training, information collection and dissemination, and the development of common approaches to occupational health and safety legislation.

The National Commission comprises representatives of the peak employee and employer bodies - Australian Council of Trade Unions and Confederation of Australian Industry - as well as the Commonwealth, State and Territory Governments.

In line with the Commission's philosophy of participation, standing committees and working parties are basically tripartite in nature, but also include persons with specialist knowledge and representatives of groups with special needs.

With the establishment of the National Occupational Health and Safety Commission, some functions which were previously the responsibility of the National Health and Medical Research Council (NH&MRC) were transferred to the National Commission. One such function was the continuing development of the Membrane Filter Method for Estimating Airborne Asbestos Dust.

Whereas other Commission Working Parties are of a tripartite nature, the Working Party on the Membrane Filter Method has retained its existing NH&MRC membership of experienced occupational hygienists. Under the direction of the tripartite Standards Development Standing Committee, the Working Party was charged with reviewing the existing NH&MRC Membrane Filter Method and other alternative methods of monitoring asbestos and developing a standard methodology for the determination of airborne asbestos.

Although the Membrane Filter Method for Estimating Airborne Asbestos Dust only addresses the scientific or technical considerations of this technique, this document should be read in conjunction with the National Commission's *Guide to the Control of Asbestos Hazards in Buildings and Structures*,(1) and *Code of Practice for the Safe Removal of Asbestos*.(2) These publications outline the appropriate exposure standards for asbestos and the principles of result reporting and employer-employee consultation which are endorsed by the National Commission.

GLOSSARY OF TERMS

Occupational and Regulatory Sampling	Occupational and regulatory samples are those samples taken within the *Worker Breathing Zone* and give results that are indicative of the worker's exposure under representative working conditions. As they represent actual personal exposure, the results of such sampling can be compared with occupational exposure standards.
Paraoccupational Sampling	Paraoccupational samples are those *static samples* taken as an indicator of the effectiveness of process control techniques, and are not representative of actual occupational exposures. As the results obtained from paraoccupational sampling do not reflect actual worker exposure, they cannot be compared with occupational exposure standards.
Single Sample Duration	Single Sample Duration is the actual time during which a single sample is collected. This duration is usually dependent upon analytical requirements (see section 8.7).
Static Samples	For the purposes of this document static samples are samples taken at fixed locations, usually between one and two metres above floor level.
Total Sample Duration	Total Sample Duration is the sum of the Single Sample Durations taken over the sampling period (see section 8.8.2).
Worker Breathing Zone	The Worker Breathing Zone is described by a hemisphere of 300mm radius, extending in front of the face and measured from a line bisecting the ears. Breathing Zone samples are usually obtained if the filter holder is fastened to the jacket lapel of the worker. **The cowl must always point downwards**. The worker carries the pump on a belt or in a pocket. These samples are known as `Personal Samples'.

INTRODUCTION

While airborne asbestos fibre concentrations from all types of asbestos[3] in the occupational environment are generally determined by the Membrane Filter Method, experience has shown that this method does not always produce comparable results when used by different laboratories and by different workers. Differences can arise due to variations in sampling, preparation of the slide, optical counting, the calculation of the results and other influencing factors. Inter-laboratory comparisons of dust measurements are feasible only if agreement can be reached concerning all details of the method.

This updated version of the original 1976 NH&MRC Membrane Filter Method[4] retains the basic analytical method of phase contrast light microscopy. Any changes should not markedly affect the estimates of airborne fibre concentration but should improve the reliability of the method and produce more reliable results when used by different laboratories. The original NH&MRC method (amongst others) was used extensively in developing the Asbestos International Association (AIA) RTM-1[5] and the present drafts of the International Standard Organisation[6] and the European Reference Method.[7] The National Commission wishes to acknowledge assistance provided by, amongst others, the UK Health and Safety Executive.[8]

This updated version is based largely on the AIA Reference Method, with minor modifications and various additions to adapt it to sampling in paraoccupational situations where the airborne fibre concentration is low relative to that found in the occupational environment where persons are working directly with asbestos-containing materials. Moreover, in these paraoccupational situations (which include sampling positions outside the temporary enclosures erected during the removal of asbestos-based thermal/acoustic insulating materials) asbestos fibres often comprise only a small percentage of the total number of fibres which might be found in a sample of the ambient air. **It must therefore be strongly emphasised that the results obtained by following the paraoccupational method should not be related to exposure standards which apply to occupational situations where the fibres are principally asbestos**.

Most informed authorities consider much of this paraoccupational sampling to be wasteful of resources and money, as the results obtained are often meaningless unless used to confirm the effectiveness of the control measures in use during asbestos removal or asbestos disturbance operations. As these situations now produce the bulk of airborne asbestos sampling, it is necessary to provide a formal method so that results can be more comparable. However, it must be strongly emphasised that the results obtained by following the abovementioned method should not be related to occupational exposure standards because of the different types of fibres found in occupational situations (predominantly asbestos) to those found in paraoccupational sampling (sometimes no asbestos at all).

Persons new to asbestos dust sampling and analysis should not undertake work in this field without making personal contact with an experienced occupational hygienist or scientist to obtain the essential training in the techniques involved.

SCOPE OF THIS METHOD

Part I of this document describes the procedures required to estimate personal exposure and to assist in the control of occupational environments where asbestos processes are in operation and the airborne fibres which are present are known to be predominantly asbestos.

Part II describes the sampling techniques that can be used in the paraoccupational environment where fixed station sampling is used and where airborne fibre levels may be low, or fibres may not necessarily be asbestos.

Part III details laboratory analytical procedures which are common to both the occupational and paraoccupational components of this method.

It should be emphasised that in mixed dust situations the presence of other fibres and fibre-like particles may interfere with the interpretation of any results.

It must also be recognised that the use of this method has limitations when applied to samples containing plate-like or acicular particles (e.g. talc, gypsum and certain other minerals and fibres), and consequently should not be implementated without a full qualitative understanding of the sample. There are analytical methods which can be used to develop a more complete understanding of complex samples. These techniques include polarising light microscopy, electron microscopy, X-ray diffractometry, gravimetric methods etc.

For occupational sampling, in the absence of other technically convincing information, all particles complying with the defined geometric conditions (see section 8.5), are to be considered as asbestos fibres and counted as such, thereby ensuring that under-estimates of asbestos exposure are minimised. This rule should also be applied to paraoccupational sampling but with the knowledge that it frequently over-estimates the asbestos concentration. It is also intended that the procedures described in this document can be used for epidemiology. However, for epidemiological purposes, more complex analysis may be required to achieve a complete understanding of occupational exposure.

Part IV describes the main sources of errors that arise when using the method, and gives several quantitative estimates of the overall `accuracy'.

GENERAL METHOD DESCRIPTION

A sample is collected by drawing a measured quantity of air through a membrane filter by means of a sampling pump. The filter is later transformed from an opaque membrane into a transparent, optically homogeneous specimen. The fibres are then sized and counted, using a phase contrast microscope and eyepiece graticule. The result is expressed as fibres per millilitre of air, calculated from the number of fibres on the filter and the measured volume of air sampled.

PART I

AIR SAMPLING FOR OCCUPATIONAL SITUATIONS (ASBESTOS PROCESSES)

The first part of this method is intended to be used for the sampling of airborne asbestos dust in occupational environments where the airborne fibres are known to be predominantly asbestos. This method shall be used to determine compliance with Exposure Standards[9] or the various regulatory limits throughout Australia which apply to asbestos processes. These include asbestos manufacturing industries, as well as maintenance and construction work directly concerned with *in situ* asbestos-containing products.

Terms such as *Occupational and Regulatory Sampling, Worker Breathing Zone, Single Sample Duration* and *Total Sample Duration* have particular significance to this method and are defined in the Glossary at the beginning of this document.

1. STRATEGY FOR OCCUPATIONAL SAMPLING

1.1 GENERAL PRINCIPLES

Occupational exposure measurements are carried out to achieve one or both of two major objectives:

- to assess exposure relative to an occupational exposure standard and to enable appropriate dust control measures to be implemented
- to provide estimates of exposure for epidemiological investigations of morbidity and mortality

Sampling procedures should be arranged so as to cause minimal interference with the activities of the worker. All sampling must be conducted in the breathing zone of a worker so that the results are indicative of the worker's exposure to asbestos fibres under representative working conditions.

1.2 TOTAL SAMPLE DURATION AND NUMBER OF SAMPLES

Sample duration is influenced primarily by the reason for sampling, the level of fibre concentration to be measured, the concentration of non-fibrous dust and the requirements of the analytical method. This may result in more than one single sample being required. **The Total Sample Duration should never be less than four hours, and preferably over an entire shift**.

Section 8.7 details acceptable minimum and maximum loadings of fibres on the filter, and it is this loading which dictates the range of possible sampling time for different airborne fibre concentrations.

1.3 FLOWRATE

If a 25mm filter is used, the flowrate should be selected in the range 0.4 to 2 litres/min such that a volume of 100 litres $\pm$ 20 per cent (that is 80-120L) is collected over the desired Single Sample Duration. For a 13mm filter the above flowrates and volumes should be divided by five.

As a minimum, the flowrate through the filter holder must be checked both before and after sampling. If the difference is greater than 10 per cent of the initial flowrate, the sample must be rejected.

If an external flowmeter is used to determine the flowrate of the pump, care must be taken to ensure that the flowmeter does not cause unknown changes to the flowrate. Measurement of the Sampling Train flowrate using a soap-film flowmeter, with and without the external flowmeter, is one satisfactory method of determining any change in flowrate.

The flowmeter used must be able to measure flowrate to an accuracy of ±5 per cent of the true flow at the 90 per cent confidence level. (See Appendix B for flowrate calibration.)

1.4 SINGLE SAMPLE DURATION

To assist in the selection of flowrate, the following table gives Single Sample Durations for various flowrates at volumes of 80, 100 and 120 litres.

TABLE 1

SINGLE SAMPLE DURATION FOR VARIOUS FLOWRATES

	Sample Volume (litres)		
Flowrate	80	100	120
L/min		minutes	
0.4	200	250	300
0.5	160	200	240
1.0	80	100	120
2.0	40	50	60

The following Table is based on a 25mm diameter filter, and shows the lowest calculated concentrations that would result from the loadings as detailed in section 8.7. See also section 4 for presentation of results.

TABLE 2

LOWEST CALCULATED CONCENTRATION BASED UPON FILTER LOADINGS

	Sample	Volume	(litres)
	80	100	120
		fibres/mL	
Minimum Filter Loading			
40 fibres/100 graticules	0.24	0.19	0.16
15 fibres/100 graticules	0.09	0.07	0.06
10 fibres/100 graticules	0.06	0.05	0.04
Maximum Filter Loading			
1 fibre/graticule	0.6	0.48	0.4
2 fibres/graticule	1.2	0.97	0.8
10 fibres/graticule	6.1	4.8	4.0

NOTE: that by using these procedures, the concept of `optimum' filter loading[(4)] (usually lying in the range 80 to 300 fibres/100 graticule areas) as required by various other methods is not used. However, unlike these methods, fixing of the sample volume per cm^2 of effective filter area overcomes the now well-known problem where the observed fibre concentration appears to be over-estimated when using the low sample volumes, and under-estimated with high volumes.[(10 - 13)]

The penalty in this approach is that the practical detection limit (approximately 10 fibres/100 graticule areas) resides around 0.05 fibres/mL. Sample volumes and minimum practical detection limits have been designed to allow determination of airborne asbestos dust below the current technical limit for amphibole asbestos. However the reliability of the method decreases below 0.1 fibres/mL for occupational samples. **No attempt should be made to reduce the detection limit in occupational environments by increasing sampling volume**.

A second penalty is that precision suffers at high fibre concentrations (> 5 to 10 fibres/mL), which means that results can only be expressed in broad terms (see section 4). However, this is acceptable because the results are then clearly in excess of the exposure standard, and the reported results reflect the lack of precision by using an appropriate number of significant digits.

1.5 BLANKS

For each batch of filters used in a specific field sampling exercise or for every twenty-five filters in the batch, select one unused filter and subject it to the same treatment of handling and transport as for normal samples, but do not draw any air through it, or attach it to the worker. See section 8.6 for details of blank analysis.

1.6 SAMPLING RECORD

All data necessary for the determination of the fibre concentration must be recorded, along with the sampling details. Furthermore, as much data as available should be recorded for control design and epidemiological studies. Appendix D gives an example of a dust sampling record.

2. LIMITATIONS OF THE METHOD, AND PRESENTATION OF RESULTS

With the parameters specified in this method, that is, a 100 litre ($\pm$ 20 per cent) sample volume and a minimum apparent filter loading of 10 fibres per 100 graticule areas, the practical lower detection limit is approximately 0.05 to 0.1 fibres/mL. This limit arises because it is generally accepted that blank, unused filters can give a reading of several countable fibres per 100 graticule areas. These `fibres' may be unidentified contaminants on the filter, or artifacts from the clearing process which have the appearance of fibres.

It follows that the above detection limit may not be achieved when any of the above factors interfere with the counting process.

It must be recognised that neither counting more fields nor increasing sampling duration overcomes the problem of background dust which has been collected on the filter, especially when asbestos is a minor constituent in the overall dust cloud.

Insufficient information is available to determine at what level the reliability of the method becomes so poor that results have little meaning. It is clear that this level will not be a single value, but will be a range depending at least upon the relative and absolute fibre concentration. There appears to be general agreement amongst those experienced in the field that these limits lie somewhere in the range of 0.05 to 0.5 fibres/mL depending on a variety of conditions. In view of this situation and the inherent variability of the method, all calculated values should be expressed in the manner detailed in Table 3 on the following page.

TABLE 3

REPORTING OF OCCUPATIONAL SAMPLING RESULTS

	Calculated Concentration* (fibres/mL)	Reported Concentration (fibres/mL)
(I)	less than 0.05	< 0.05
(II)	0.05 to less than 0.10	2 decimal places and 1 significant figure
(III)	0.10 to less than 5.00	to 1 decimal place and 2 significant figures
(IV)	5.00 to 10.0	0 decimal place and 1 significant figure
(V)	greater than 10	> 10

Example:

	Calculated Concentration* (fibres/mL)	Reported Concentration (fibres/mL)
(I)	0.049	< 0.05
(II)	0.055	0.06
"	0.084	0.08
(III)	0.65	0.7
"	4.74	4.7
(IV)	6.34	6
(V)	13.42	>10

***NOTE:** If the actual count is less than 10 fibres/100 graticule areas, then the count is not significantly above that of background. The results should be calculated using the minimum practical detection limit of 10 fibres/100 graticule areas and reported as `less than' the calculated value expressed to 1 significant figure and no more than the first decimal place.

Example:

A 100 litre sample with a 25mm diameter filter count of 4 fibres for 100 graticule fields yielded a calculated concentration of 0.0194 fibres/mL. However, because the actual fibre count is below the detection limit of 10 fibres per 100 graticule areas, the concentration when recalculated using this limit is < 0.0484 fibres/mL. When rounded off as required by the above Table, the result shall be quoted as < 0.05 fibres/mL.

PART II

AIR SAMPLING FOR PARAOCCUPATIONAL SITUATIONS

Part II of this method is intended to be used for the field sampling of airborne asbestos dust in paraoccupational situations. These include sampling in the following situations:

- outside asbestos stripping and encapsulating areas
- inside decontamination rooms
- for clearance monitoring after asbestos stripping and encapsulating
- inside buildings or ships which contain asbestos

This type of sampling is often conducted in areas that contain high proportions of non-asbestos fibres or particles which conform with the geometric requirements of a `fibre' as defined by this method. Many experienced occupational hygienists would strongly recommend against this form of sampling as these interferences cause severe problems in interpretation. This is particularly true in situations where the results of the sampling are intended for use in estimating risks to health from suspected environmental contamination by airborne asbestos. **In such situations it is <u>inappropriate</u> to consider that the results from such monitoring have the same significance in terms of health consequences as does occupational monitoring outlined in Part I.** This advice is supported by a number of Australian[14] and international documents.[15,16]

Terms such as *Paraoccupational Sampling, Static Samples, Single Sample Duration* and *Total Sample Duration* have particular significance to this method and are defined in the Glossary at the beginning of this document.

3. STRATEGY FOR PARAOCCUPATIONAL SAMPLING

3.1 GENERAL PRINCIPLES

All sampling must be conducted so that the results are representative of the particular and specified situation being tested. Only static samples are allowed, and these should be taken over a single sample duration of not less than one hour (see section 3.3).

In situations where asbestos is actively being removed or disturbed, dust concentrations may vary widely both within a single day and from day to day, or from place to place. Additionally, variations in work procedures produce concentrations which can vary over one or more orders of magnitude. These factors may influence airborne levels obtained outside the removal area.

Air sampling outside asbestos removal operations is often carried out to ensure that negligible airborne asbestos is present. However, some results so obtained can be misleading due to the non-asbestos fibres which the sampling will detect but not identify. On occasions, air sampling should be used for testing the reliability of enclosures when they are initially installed. Once it has been established that such enclosures are controlling dust emissions, the emphasis should be placed on more efficient methods of testing, such as daily checking of the integrity of the barrier and the work practices that are carried out inside the enclosure. The choice of sampling conditions and interpretation of results should be determined by an experienced occupational hygienist.

Air monitoring in an environment which is representative of normal work activities is acceptable, however the artificial generation of an unrealistic environment is not acceptable.

The use of air monitoring which is associated with the deliberate creation of artificial contamination by sweeping, beating, or the blowing of air on to asbestos-containing or contaminated areas (that is, `aggressive' air monitoring) must not be employed. Data obtained under such conditions do not reflect current or future activities and therefore are of no value in the assessment of risk. Furthermore the practice may result in the transference of contamination from one part of a building to another without significantly affecting the measured airborne asbestos fibre concentration and/or can lead to misleading results due to the disturbance of non-asbestos fibres in the environment.

It is important to understand that air sampling should not be used as a substitute for frequent and thorough inspections by an occupational hygienist experienced in asbestos matters. Careful visual examination will reveal situations that are likely to create future contamination problems. Meticulous cleaning, resulting in the absence of any visible dust, will generally lead to acceptably low level contamination which reduces to less than detectable levels due to the process of normal cleaning.

3.2 TOTAL SAMPLE DURATION AND NUMBER OF SAMPLES

Sample duration is influenced primarily by the reason for sampling, the level of fibre concentration to be measured, the concentration of non-fibrous dust and the requirements of the analytical method. This may result in more than one single sample being required.

The Total Sample Duration should preferably not be less than four hours. In some situations where urgency may dictate, the Total Sample Duration may be reduced to a minimum of one hour. However, it is necessary to ensure that the higher flowrate can be accurately measured and must comply with the total volume range specified in section 3.3 below.

Section 8.7 details acceptable maximum and minimum loadings of fibres on the filter, which therefore dictate the range of possible sampling times for different airborne fibre concentrations.

Single samples of short duration (still within the volume limit of section 3.3) may be necessary if high background levels of particulate matter or fibres are present which would prevent accurate analysis.

3.3 FLOWRATE

The flowrate should be selected in the range 1 to 8 litres/min such that a volume of 500 litres $\pm$ 20 per cent (i.e. 400-600L) is collected over the desired Single Sample Duration. In some situations, ambient levels of airborne dust from other parts of the site may lead to very dense samples which cannot be counted, or the excess dust may obscure some asbestos fibres in the sample.

Where past experience has shown that this is likely to occur, a lower sample volume of 240 litres may be used. Sample volumes of less than 240 litres are not recommended because of the increased loss of precision in the results obtained. Under conditions of very low airborne fibre concentrations or when Single Sample Durations much greater than four hours are desired, it is permissible to increase the sample volume to a maximum of 1000 litres.

The flowrate through the filter holder should be checked at least before and after sampling. If the difference is greater than 10 per cent from the initial flowrate, the sample must be rejected.

If an external flowmeter is used to determine the flowrate of the pump, care must be taken to ensure that the flowmeter does not cause <u>unknown</u> changes to the flowrate. Measurement of the Sampling Train flowrate using a soap-film flowmeter, with and without the external flowmeter, is one satisfactory method of determining any change in flowrate.

The flowmeter used must be able to measure flowrate to an accuracy of $\pm$5 per cent of the true flow at the 90 per cent confidence level. See Appendix B for flowrate calibration.

3.4 SINGLE SAMPLE DURATION

To assist in the selection of flowrates, the following table gives Single Sample Durations for various flowrates at volumes of 400, 500 and 600 litres.

TABLE 4

SINGLE SAMPLE DURATIONS FOR VARIOUS FLOWRATES

	Sample	Volume	(litres)
Flowrate	400	500	600
L/min		minutes	
1.0	400	500	600
2.0	200	250	300
8.0	50	63	75

The following table is based on a 25mm diameter filter, and shows the lowest calculated concentrations that would result from loadings detailed in section 8.7. See section 4 for presentation of results.

TABLE 5

LOWEST CALCULATED CONCENTRATIONS FOR VARIOUS FILTER LOADINGS

	Sample	Volume	(litres)
	400	500	600
		fibres/mL	
Minimum Filter Loading			
40 fibres/100 graticules	0.048	0.039	0.032
15 fibres/100 graticules	0.018	0.015	0.012
10 fibres/100 graticules	0.012	0.010	0.008
Maximum Filter Loading			
1 fibres/graticule	0.12	0.10	0.08
2 fibres/graticule	0.24	0.19	0.16
10 fibres/graticule	1.21	0.97	0.81

Because Part II is concerned with situations generally giving rise to very low airborne asbestos concentrations, the concept of a fixed sample volume/cm^2 of effective filter area as required by Part I is not possible. Note that the use of larger sample volumes than specified in Part I leads to a different estimate of observed fibre concentrations, and should not be compared to present occupational Exposure Standards. At best, these measurements can only assist people in ensuring dust levels are less than certain arbitrary limits.

3.5 BLANKS

For each batch of filters used for sampling, or for every twenty-five filters in the batch, select one unused filter and subject it to the same treatment of handling and transport as for normal samples, but do not draw any air through it, nor attach it to the worker. See section 8.6 for details of blank analysis.

3.6 SAMPLING RECORD

All sampling details and data necessary for the determination of the fibre concentration must be recorded. Furthermore, as much data as available should be recorded for control design and epidemiological studies. Appendix D gives an example of a sampling record.

4. LIMITATIONS OF THE METHOD AND PRESENTATION OF RESULTS

With the parameters specified in section 3, that is, a 500 litres ($\pm$ 20 per cent) sample volume and a minimum fibre loading of 10 fibres/100 graticule areas, the practical lower detection limit is approximately 0.01 fibres/mL.

It is generally accepted that blank, unused filters can frequently give a reading of 1 or 2 countable fibres per 100 graticule areas. The `fibres' may be unidentified contaminants on the filter, or artifacts from the clearing process which have the appearance of fibres. Thus the above detection limit may not be achieved when any of these factors interferes with the counting process.

Because of the inherent variability of the method, especially at the very low concentration levels treated by Part II of this method, all calculated values should be expressed in the manner detailed in Table 6 on the following page. For each sample it is essential to describe conditions existing prior to, and during sampling, as well as the exact position of the static sampler, the area of the location being sampled and any other relevant details.

TABLE 6

REPORTING OF PARAOCCUPATIONAL SAMPLING RESULTS

	Calculated Concentration* (fibres/mL)	Reported Concentration (fibres/mL)
(I)	less than 0.005	< 0.01
(II)	0.005 to less than 0.100	to 2 decimal places and 1 significant figure
(III)	0.10 to 1.00	to 1 decimal place and 1 significant figure
(IV)	greater than 1.00	> 1

Examples:

	Calculated Concentration* (fibres/mL)	Reported Concentration (fibres/mL)
(I)	0.0049	< 0.01
(II)	0.0054	0.01
"	0.063	0.06
(III)	0.356	0.4
(IV)	2.34	> 1

***NOTE:** If the actual count is less than 10 fibres/100 graticule areas, then the count is not significantly above that of background. The results should be calculated using the minimum practical detection limit of 10 fibres/100 graticule areas and reported as `less than' the calculated value expressed to one significant figure and no more than the second decimal place.

Example:

A 400 litre sample with a 25mm diameter filter count of 3 fibres for 100 graticule fields yielded a calculated concentration of 0.0036 fibres/mL. However, because the actual fibre count is below the detection limit of 10 fibres/100 graticule areas, the concentration when recalculated using this limit is < 0.0121 fibres/mL. When rounded off as required in the above Table, the result shall be quoted as < 0.01 fibres/mL.

PART III

LABORATORY TECHNIQUES AND ANALYSIS

The third part of this method details the analytical procedures to be used for both types of sampling as described in Parts I and II. For clarity, aspects common to Parts I and II (such as sampling equipment) will be included in Part III.

5. EQUIPMENT

(See Appendix J for Specifications)

5.1 SAMPLING PUMP

A portable battery powered pump must be used for personal sampling. The capacity of the battery must be sufficient to operate continuously over the chosen sampling time. The pump should be capable of maintaining the required flowrate with a variation within $\pm$10 per cent for the entire sampling duration, allowing for increasing filter loads. Pumps with automatic flow control facilities are recommended. The performance characteristics of field sampling pumps vary considerably and reference should be made to experienced laboratories for selection of pumps for specific sampling applications.

The flow must be sufficiently free from pulsation. As a minimum and tentative criterion there must be no visible vibration of a rotameter float if such a flowmeter is connected to the filter holder with the filter inserted. It may be necessary to install a pulsation damper between the pump and the filter if an internal pulsation damper has not been included or if the pump shows significant pulsation.

Connecting tubing must be constriction-proof and the connections leakproof.

Caution: When sampling in explosive gas or dust atmospheres as defined in AS 2430[(17)], ensure that the sampling pump meets the equirements of AS 2380[(18)].

5.2 FILTERS

Membrane filters (mixed esters of cellulose or cellulose nitrate) of 0.8 micrometre pore size with printed grids must be used, and a diameter of 25mm is preferred. Airborne dust should be collected on the upper (grid) surface of the filter.

It is acceptable to use 13mm filters and filter holders provided that flowrates and sample volumes are adjusted to give identical sampling volumes per square centimetre of effective filter area.

5.3 FILTER HOLDER

It is necessary to use an open faced filter holder fitted with a protective cowl. The cowl helps to protect the filter from accidental contamination. A metallic or conductive coated cowl is preferred because of the possible risk of fibre loss due to electrostatic charge when using plastic cowls. This is especially true when operated under conditions of low relative humidity.

Filter holders and cowls must be meticulously washed with detergent and water and thereafter rinsed thoroughly. After exposure to high asbestos concentrations it may be necessary to dispose of the holders and cowls, or only re-use in environments with high dust concentrations where any potential contamination would not have a significant effect upon results. **Filter holders which have previously been used with fibreglass filters should never be used for asbestos work because of the risk of cross-contamination**.

The design of the filter support utilised in some filter holders requires that a secondary support pad should be used. The purpose of this support pad is to ensure an even distribution of air passing through the primary membrane. These support pads are usually made of pure cellulose and have a much larger pore size than the primary filter upon which the fibres are collected.

5.4 STORAGE AND TRANSPORT

Fixatives must not be used. Experience has shown that fixing fibres to the filter surface with cytological or other types of fixatives is unnecessary and should be avoided.

Filters should be transported in the closed holders in which the samples were collected.

An alternative is to transfer the filter to a petri dish in the following way. In a dust-free area, using forceps, carefully remove each exposed filter from its holder, taking care to grasp only the unexposed filter edge. Place the filter, dust side up, in a plastic petri dish or similar container. Fasten the filter to the bottom of the dish with one or two pieces of adhesive tape attached to the unexposed edge. After transportation, the filter can easily be removed from the dish with a surgical scalpel.

The filter holders or petri dishes should be packed into a rigid container with some soft packing material to prevent both crushing and vibration of the filter. Samples should be clearly and unambiguously labelled, taking care to ensure that filters cannot be accidentally re-used. The filters should not be marked for this purpose because of the risk of damaging the filter.

6. SAMPLE PREPARATION

6.1 CLEANING SLIDES AND EQUIPMENT

Clean conditions should be maintained at all times. A dirty preparation area may result in sample contamination and erroneous results. Particular care should be taken to ensure that the presence of bulk asbestos samples in the laboratory does not cause contamination.

The slides should be cleaned with lens tissue or industrial paper tissue and laid out on a clean surface such as a sheet of lens tissue. Each coverslip should be cleaned with lens tissue immediately before use to ensure that the surfaces are free from contamination.

The scalpel and forceps should be wiped with lens tissue and placed on a clean surface (for example, lens tissue). When mounting a series of filters, the mounting tools must be wiped clean before dealing with each sample.

6.2 FILTER SAMPLE CUTTING

If a 13mm diameter filter is used, the total filter must be mounted. It is preferable to mount only one half of a 25mm diameter filter. All cutting should be done to give a single clean cut, with no disturbance to the filter face. Either a curved bladed scalpel used with a rolling action or a very sharp razor has been found satisfactory.

6.3 MOUNTING THE SAMPLE

For mounting, use the acetone-Triacetin* method only, as described in Appendix A.

* glycerol triacetate

WARNING

ACETONE VAPOUR IS HIGHLY FLAMMABLE, AND MOUNTING SHOULD BE CARRIED OUT WITH ADEQUATE VENTILATION. ON NO OCCASION SHOULD IT BE USED IN THE VICINITY OF AN OPEN FLAME OR OTHER IGNITION SOURCES.

7. OPTICAL REQUIREMENTS

7.1 MICROSCOPE EQUIPMENT

Microscopes with identical `specifications' can give quite different performances; therefore it is necessary that the performance of proposed and existing microscopes be assessed by means of a `Detection Limit Test Slide' (see Appendix F). It is important that experienced practitioners be consulted before selecting microscopes for asbestos dust determination. It is recommended that the following specification be used to select a microscope suitable for asbestos dust counting.

· **Light Source** - Koehler or pseudo-Koehler illumination is preferred. It is preferable for the illuminator to be built-in, but an external lamp with a plane mirror can be satisfactory. A variable light intensity control is necessary for both methods of illumination.

· **Substage Assembly** - An Abbe or achromatic phase-contrast condenser incorporated into a substage unit is required. There must be a means of centering each condenser annulus with respect to the phase plate in the corresponding objective and a means of focusing the condenser.

· **Stage** - A built-in mechanical specimen stage fitted with slide clamps and x-y displacement is required.

· **Objectives** - A rotating nose piece fitted with 10X and 40X parfocal phase-contrast achromatic objectives is required. The 40X objective must have a numerical aperture (NA) of 0.65 to 0.75. It should have a phase ring of not less than 65 per cent and not greater than 85 per cent absorption. Either positive or negative phase-contrast is suitable.

· **Eyepiece** - Binocular eyepieces of the compensating type are recommended. They should be chosen to give a total magnification of between 400X and 650X. At least one eyepiece must permit insertion of a graticule and should be of the focusing type. The use of body magnification changers and wide-field 10X eyepieces is not recommended.

· **Graticule** - The graticule for this method is the Walton-Beckett circular eyepiece graticule (Graticules Limited Type G22). When using the 40X phase objective and an appropriate eyepiece, the image plane diameter of the graticule circle must be 100 micrometres $\pm$ 2 micrometres. See Appendix E for graticule specification, source of supply and ordering information.

7.2 MICROSCOPE ACCESSORIES

Considerations in the selection of microscope accessories:

· A Centering Telescope or Bertrand Lens is essential for checking that the phase rings in the condenser are centred with respect to those in the objective.

· A green or blue filter may reduce eye fatigue.

· The stage micrometer should be from a reputable source, preferably one millimetre in length and must be subdivided into at least 10 micrometre intervals.

· High quality microscope slides should be used.

- The coverslip thickness should be that for which the microscope is designed, usually 0.17mm thickness (that is, No. 1 1/2). Incorrect coverslip thickness will detract from the quality of the final image.

7.3 MICROSCOPE ADJUSTMENT PRINCIPLES

Microscope adjustments and testing with the Detection Limit Test Slide must be part of the daily counting routine. Follow the manufacturer's instruction while observing the following guidelines:

- the object for examination must be in focus
- the illuminator field iris must be in focus, centred on the sample and opened only to the point where the field of view is illuminated
- the image of the light source must be in focus and centred on the condenser iris or annular diaphragm for true Koehler illumination
- the phase rings (annular diaphragm and phase shifting elements) must be concentric
- the eyepiece graticule must be in focus

For more detailed information, see Appendix G.

7.4 EYEPIECE GRATICULE CALIBRATION

Each combination of eyepiece, objective and graticule must be calibrated with a stage micrometer. Should any of the three be changed, the combination must be recalibrated. For some microscopes, calibrations will change for observers with different interoccular distances. (See Appendix E for eyepiece graticule calibration procedures).

7.5 MICROSCOPE/OBSERVER PERFORMANCE ASSESSMENT

Past experience has shown that differences in counts arise from differences in microscope quality, setting up and cleanliness. It is therefore necessary that laboratories following this method should maintain contact with experienced laboratories.

As mentioned in section 7.1, a Detection Limit Test Slide is available which will assist in the regular assessment of microscope and observer performance. If Block 5 on the Detection Limit Test Slide (Mark 2) cannot be distinguished, the microscope (or observer) is not suitable for work with this method and should not be used until this deficiency has been investigated and corrected. Block 7 should not be resolved.

8. COUNTING AND SIZING FIBRES

8.1 GENERAL

Airborne asbestos dust collected on membrane filters appears in a wide variety of forms ranging from simple single fibres to very complex configurations of fibres or aggregates. When presented with these, the microscopist may experience difficulty in defining and counting the fibre content in a dust sample. The following notes (and drawings in Appendix H) have been prepared to assist and guide the observer in assessment and interpretation of asbestos dusts collected on membrane filters. It must be recognised that the use of this method has limitations when applied to samples containing plate-like or acicular particles (for example, talc, gypsum and certain other minerals and fibres), and consequently should not be implemented without a full qualitative understanding of the sample.

8.2 LOW POWER SCANNING

With a total magnification of 100X to 150X (that is, 10X objective), scan the entire filter area.

The margin normally covered by the filter holder should be free of dust and fibres. All viewing fields should have similar appearances with respect to total dust loading. If the observed fields show marked differences in loading, or gross aggregation of fibres or dust, the filter must be rejected.

At least half of the mounted filter area must be countable, otherwise it must be rejected.

8.3 GRATICULE FIELD SELECTION

After a satisfactory low power scan, change the microscope objective to 40X phase contrast and focus on the dust plane.

Ensure that the phase rings remain concentric. While most of the fibres and dust will be found on the upper surface of filter, it will be necessary to focus below (up to 10 micrometres) and slightly above the surface.

When counting and sizing, constant use of the fine focus is necessary because of the small depth of field of a 40X objective (that is, 2 to 3 micrometres).

Counting fields should be examined throughout the entire area of the filter or filter segments ensuring that the choice is not biased by the lack or presence of fibres. If the grid of a filter obstructs the view, move the stage to another field. Do not count fields that lie within 3mm of the filter edge and within 2mm of the cutting line.

8.4 LABORATORY WORKING CONDITIONS

The working environment in a laboratory may systematically influence the accuracy of the actual counting. Subjective factors such as lighting, seating and noise should be suitable for the task.

8.5 COUNTING CRITERIA

8.5.1 Accuracy for determining fibre length and diameter is critical, and full use must be made of the eyepiece graticule. Estimate the length of curved fibres along the curve of the fibre (that is, true length).

8.5.2 A *countable fibre* is defined as any object having a maximum width less than 3 micrometres and a length greater than 5 micrometres and a length/width ratio greater than 3:1, and which does not appear to touch any particle with a maximum dimension greater than 3 micrometres. A countable fibre with both ends within the graticule area shall count as one fibre; a countable fibre with only one end within the area shall count as half a fibre; a fibre with both ends outside the area must not be counted.

8.5.3 Graticule areas for counting shall be examined as in section 8.3 above.

8.5.4 An agglomerate of fibres, which at one or more points on its length appears to be solid and undivided but which at other points appears to divide into separate strands, is known as a split fibre. Any other agglomerate in which fibres touch or cross one another is known as a bundle.

8.5.5 A split fibre is regarded as a single countable fibre if it meets the definition in 8.5.2, the width being measured across the undivided part, not the split part.

8.5.6 Fibres in a bundle are counted individually if they can be distinguished sufficiently to determine that they meet the definition in 8.5.2. If no individual fibres meeting the definition can be distinguished, the bundle is a countable fibre if the bundle as a whole meets the definition in 8.5.2.

8.5.7 If more than one-eighth of a graticule area is covered by an agglomerate of fibres and/or particles, the graticule area must be rejected and another counted.

8.5.8 Count as many fields as is necessary to yield a total fibre count of 100 but count a minimum of 20 fields even if more than 100 fibres are counted. Do not count any more than 100 fields if a total of 100 fibres is not reached.

8.5.9 All relevant information must be recorded. It is good practice to record each field and fibre as it is observed. (See Appendix I for an example of a fibre counting form.)

8.6 BLANKS

Parts I and II require that blank filters be used. If any `blank' yields fibre counts greater than 3 fibres/100 graticule areas, the entire sampling and analytical procedure should be examined carefully to find the cause of the contamination. When the blank count exceeds 3 fibres/100 graticule areas, and also exceeds 10 per cent of the actual sample fibre count/100 graticule areas, the samples to which the particular blank is appropriate should be rejected.

It is prudent to check each batch of filters to ensure that blank levels of new filters are not excessive.

For example, if the fibre count of a blank filter was 4 fibres/100 graticule areas (that is, 0.04 fibres/area) while the sample yielded 15 fibres in 100 graticule areas (that is, 0.15 fibres/graticule area).

$$\frac{\text{Blank Count}}{\text{Sample Count}} = \frac{4}{15} \times 100 = 27\%$$

As this ratio exceeds 10 per cent, the sample is rejected. Furthermore, because the blank count exceeded 3 fibres/graticule area the cause of contamination must be found and corrected.

8.7 ACCEPTABLE FIBRE LOADINGS ON FILTERS

8.7.1 **Minimum loading**

For reliable counting, a fibre loading of a filter should exceed 40 fibres/100 Walton-Beckett graticule areas. In special circumstances (for example, when an indication of concentration with low precision is acceptable) it is permissible to lower the acceptable fibre loading to 10 fibres/100 Walton-Beckett graticule areas.

If less than 10 fibres/100 graticule areas is observed, then the figure of 10 fibres/100 graticule areas is the minimum that can be used to calculate airborne fibre concentration.

The lowering of the acceptable fibre loading to 10 fibres/100 graticule areas gives, at best, barely acceptable coefficients of variation. The limitations as described in Part IV should also be considered when measuring very low fibre concentrations.

Note that a sample count of 10 fibres/100 Walton-Beckett graticule areas can just be distinguished from the background `blank' count of 2 fibres/100 graticule areas for typical sampling/analytical coefficients of variation of 0.6 to 0.8. For this reason, it is mandatory to ensure that blank counts are not greater than 2 fibres/100 graticule areas before accepting 10 fibres/100 graticule areas as a minimum loading.

8.7.2 **Maximum loading**

Experience shows that the filter loading should not exceed a maximum of 5 fibres/graticule area (average value for all counted fields) for the majority of sampling situations. This may need to be reduced to an average of about 1 fibre per graticule area when mixed dusts or agglomerates are present, and can sometimes be doubled when only fibres are present. Average filter loadings between 5 and 10 fibres/graticule area tend to result in an under-estimation, and should be treated with caution. Average filter loadings exceeding 10 fibres/graticule area should be rejected.

8.8 CALCULATION OF DUST CONCENTRATION

When the following calculations are applied, the limitation imposed upon the data by the sampling and fibre counting methods must not be disregarded. Results should not be interpreted or reported with false precision.

8.8.1 **Single values**

The fibre concentration for each Single Sample Duration is determined according to the following formula:

$$C = \frac{A}{a} \cdot \frac{N}{n} \cdot \frac{1}{r} \cdot \frac{1}{t} \qquad \text{....... (1)}$$

where:

C =	concentration (fibres/mL)
A =	effective filter area (mm^2)
a =	eyepiece graticule area (mm^2) (see Appendix E)
N =	total number of fibres counted
n =	number of graticule areas observed
r =	flowrate of air through filter (mL/min)
t =	Single Sample Duration (minutes)

Results should be presented in the manner detailed in either sections 2 or 4.

8.8.2 **Time-weighted average values**

When several consecutive samples of different sampling duration are taken, calculate the time-weighted average values from the single values as follows:

$$C_{TW} = \frac{\sum c_i . t_i}{\sum t_i} \quad \frac{c_i t_i + c_2 t_2 + \ldots + c_n t_n}{t_i + t_2 + \ldots + t_n} \qquad \text{....... (2)}$$

where: C_{TW} = time weighted average concentration (fibres/mL)
c_i = single value of concentration (fibres/mL)
t_i = Single Sample Duration (minutes)
t_i = Total Sample Duration (minutes)
n = total number of samples

If the Single Sample Durations (t_i) referred to above are of equal duration, then equation 2 is simplified as follows:

$$C_{TW} = \frac{\Sigma C_1}{n} = \frac{C_1 + C_2 + \ldots + C_n}{n} \qquad \text{...... (3)}$$

Results should be presented in the manner detailed in either sections 2 or 4.

9. QUALITY CONTROL[8]

A good quality control procedure is essential because of the large differences in results obtained both within and between laboratories using all manual fibre-counting methods. Laboratories using the method must participate in systematic checks to assess inter-laboratory variation. It is important to provide a measure of the reproducibility and stability of a laboratory's performance in relation to other laboratories and to an automatic method. These exchanges should be supplemented by checks of internal consistency, which should aim to measure the mean and reproducibility of each counter's difference from the average of the laboratory. It is unsatisfactory for a counter to have an average result equal to the laboratory mean if that counter's average performance conceals considerable variation from sample to sample.

In a large laboratory, a satisfactory procedure is to have all the counters recount a specified fraction of the routine slides. The fraction should be chosen to provide a quality control sample about once a week, and to ensure that these slides are fully representative of the laboratory's routine samples. For example, a laboratory counting 5000 samples a year could select every hundredth filter (whatever its type) for recounting by all of its counters, and could keep a running check of the mean and standard deviation of each individual's difference from the laboratory mean.

A laboratory with only one or two counters would have to maintain a stock of permanently mounted and well-characterised slides for periodic check counting, and could again calculate the mean and standard deviation of the counts in relation to those accepted for the stock slides.

Systematic records of quality control results should be kept and regularly examined to assess individual counter and overall laboratory performance. It should be remembered that, in common with monitoring for other particulates, errors will be introduced in sampling.

PART IV

SAMPLING AND ANALYTICAL ERRORS

The estimation of airborne asbestos dust comprises sampling and analytical errors, each of which has a systematic and random component. These can be minimised by strict adherence to the method and by participating in intra and inter-laboratory Quality Assurance schemes. The following list describes some of the common sources of error.

10. SOURCES OF ERROR

Some common sources of error are listed below:

10.1 SYSTEMATIC ERRORS

10.1.1 **Sampling**

- flowrate
- sampling time
- non-representative or biased sampling
- contamination - deliberate or accidental

10.1.2 **Analytical**

- effective filter area
- counting area
- filter mounting
- microscope
- observer
- contamination

10.2 RANDOM ERRORS

10.2.1 **Sampling**

- flowrate variability
- random fluctuations of the airborne dust cloud

10.2.2 **Analytical**

- fibre distribution on the filter: non-random deposition of dust on the filter leads to gross errors, the magnitude of which cannot be estimated. Twenty or more fields must be counted to ensure that minor divergence from randomness does not bias the result

- Poisson error

As only a small proportion of the fibres deposited on the filter are counted, errors arise in the estimation of the total number of fibres on the entire filter surface. Theoretically, the Poisson Distribution defines the variation in fibre counts resulting from the viewing ofrandomly selected counting fields on the filter. If a minimum of 100 fibres is counted, and if a Poisson Distribution were appropriate to the counting results, the coefficient of variation of the fibre counts would not exceed 10 per cent.

The Poisson error is the minimum inherent error of the Membrane Filter Method and in many other practical situations such as particle `counting', electron microscope methods and blood counts.

11. OVERALL ACCURACY

Because of the nature of the Membrane Filter Method, it is not possible to know the *`true'* airborne fibre concentration of a given dust cloud. For this reason it is not possible to assess the likely accuracy of the method. Even the precision (or repeatability) of the method is difficult to quantify because of systematic errors which tend to arise both within and between laboratories. Taken as a whole, by *`randomly'* selecting observers and laboratories, these systematic errors take on a random nature such that it may be possible in the future to provide estimates of empirical precision (that is the closest approach possible to a statement of accuracy for a method with known *`true'* values).

Much work has been done in an attempt to arrive at these estimates, and to date only a partial conclusion has been reached. Examples of confidence intervals calculated from the Poisson Distribution are presented in Table 7 below:

TABLE 7

THEORETICAL CONFIDENCE INTERVAL FOR RESULTS USING POISSON DISTRIBUTION

NUMBER OF FIBRES COUNTED PER 100 GRATICULE AREAS	95% CONFIDENCE INTERVAL FOR RESULT
100	±20% of the calculated result
40	-26% to +36% of the calculated result
10	-50% to +84% of the calculated result (that is, the true result may be in the range of 50-184% of the calculated result)

Confidence limits apply to the measured result and not the final reported result, which is a rounded-off representation of the measured result. Other sources of random and systematic errors add significantly to the uncertainty in estimating the airborne asbestos dust concentration, and these have been known to increase the above confidence intervals by up to a factor of 2 or 3. Tables 8 and 9 present the findings of empirical studies[19] in the United States into the precision of the Membrane Filter Method in estimating airborne asbestos concentrations. There is no reason to assume that this variability would not be reflected in Australia.

TABLE 8

COEFFICIENTS OF VARIATIONS FOR EXPERIENCED LABORATORIES

Total No. of Fibres Counted	Coefficients of Variations* Analytical Only	Sampling & Analytical
10	0.60	0.90
15	0.55	0.80
40	0.45	0.70
100	0.40	0.65

***** The Coefficient of Variation (CV) is calculated by dividing the standard deviation by the arithmetical average of a set of fibre concentrations determined with a number of observers estimating the dust concentration of a specific dust cloud or of a single filter.

TABLE 9

90% CONFIDENCE LIMITS DERIVED FROM EMPIRICAL STUDIES (19)

Total No. of	Analytical		Sampling & Analytical	
Fibres Counted	LCL	UCL	LCL	UCL
10	3	21	2	26
15	6	31	4	37
40	18	74	12	93
100	49	175	31	222

LCL = Lower Confidence Limit

UCL = Upper Confidence Limit

At the lowest count allowed by this method of 10 fibres/100 graticule areas, experience indicates that the inherent variability of the combined sampling/analytical process can yield `real' values as low as 2 and as high as 26 fibres/100 graticule areas. Precision increases as the count becomes higher. However, observing significantly more than 100 graticule areas in an attempt to improve precision often results in no real gain due to operator fatigue and other subjective errors.

APPENDIX A

ACETONE-TRIACETIN MOUNTING PROCEDURE

There are various methods available for generating acetone vapour to `clear' membrane filters. The original NH&MRC Membrane Filter Method[4] employed a flask of acetone heated on a hot plate, water bath or infra-red lamp. An increasing number of Australian laboratories have now adopted the `hot-block' method.[20] This method uses a heated metal block in which a small amount of acetone is rapidly vapourised and directed on to the filter. This has certain advantages in terms of filter preparation and safety. The NH&MRC paper[4] should be referred to for constructional details, bearing in mind that for added safety it is wise to use a low voltage source of power (for example, 12V) and not 240V mains electricity.

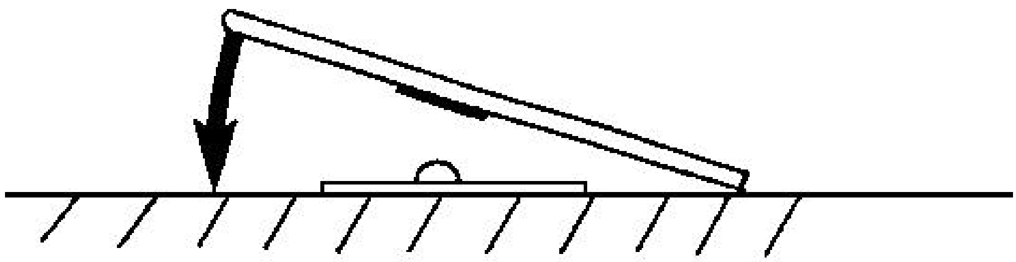
An alternative method recommended by the (UK) Health and Safety Executive[8] consists of a boiling acetone reservoir with a condensing coil to prevent acetone escape. In this method, temperature control is reasonably critical and the larger quantity of acetone poses a potential fire hazard. Other methods are described in Appendix A of the AIA RTM 1 document.[5] However, these are not recommended because they are not as inherently safe as the `hot block' method and generally do not clear filters as effectively.

After acetone clearing, the cleared filter should be left to stabilise for at least five minutes. Thereafter, the slide and its adhering filter is treated, using the following technique:

Lay a freshly cleaned coverslip on a clean horizontal surface and using a variable dispenser capable of consistently delivering a quantity between 5-10uL (for half of a 25mm dia. filter) of glycerol triacetate (Triacetin) on the coverslip. Lower the already (acetone) cleared filter on to the coverslip and allow the weight of the slide to spread the Triacetin evenly. See Figure 1.

Too much Triacetin (as indicated by excess liquid emerging from the edges of the coverslip) can cause the outside edge of the filter to eventually disintegrate to some degree. Insufficient Triacetin will result in uneven clearing of the granularity left from the acetone vapour clearing. Further, the refractive index of the mounted sample will not be suitable for optimum visibility of very fine chrysotile fibres.

FIGURE 1



Heating the cleared filter to approximately 50°C for fifteen minutes accelerates the clearing process and enables analysis to proceed almost immediately thereafter. Otherwise it is necessary to delay counting for up to twenty-four hours until the entire filter has dissolved under the action of the Triacetin. Provided the above procedures are followed, the finished product will be stable, will not disintegrate, nor be subject to significant particle migration.

It is desirable to paint nail polish, or similar lacquer, around the edge of the coverslip if the slide is to be kept indefinitely.

APPENDIX B

FLOWRATE CALIBRATION AND CORRECTIONS

(I) FOR FLOWRATES 0.4 - 2.0 L/min

Internal and external flowmeters must be calibrated with a primary calibration device. One suitable calibration procedure makes use of a soap film flowmeter. The flowmeters described in this section are of the variable area type (that is, `rotameters').

1. Choose an accurate burette (or similar measuring device) of 300-500 mL capacity. Attach a tube to the bottom of the burette, and then clamp it in an inverted vertical position in a stand.

2. Set up the sampling pump, allow flow to stabilise, complete with connecting tube, filter holder and filter as used in the field.

3. Connect the soap film flowmeter. Ensure that the system is leakproof. It is advisable to rinse the burette thoroughly in water immediately prior to the test - this removes accumulated detergent and also assists in wetting the inside of the burette.

4. Switch on the pump, allow the flow to stabilise and adjust the flowrate to the nominal desired figure according to the internal flowmeter (if fitted).

5. Partly fill a beaker or petri dish with water plus the minimum amount of detergent necessary to permit bubbles to be formed.

6. By momentarily placing the beaker against the bottom of the soap film flowmeter, create a bubble such that it will travel the entire length of the burette without bursting.

7. With a stop watch, measure accurately the time that the bubble requires to traverse the tube between the appropriate graduated marks.

8. Repeat steps 6 and 7 at least twice, or more, until good repeatability of the times is achieved.

9. Average the times and calculate the true flow (Q_c) as follows:

NOTE: Theoretically, the water vapour content in the soap film flowmeter air should be taken into consideration in determining the `true' flowrate. However, for practical purposes acceptable accuracy is maintained without this correction.

$$Q_c = \frac{V}{T}$$

where

Q_c = true volumetric flowrate (mL/min) at calibration conditions

V = volume of burette (mL)

T = average time required for bubble to traverse the tube (minutes)

10. If the external or internal rotameter is used under different temperature conditions than those during calibration, it is generally not possible to calculate the different flowrate that will inevitably result.

As all air sampling measurements are concerned only with volumetric flowrate (that is flowrate measured and expressed at the prevailing temperature and pressure) and not mass flowrate (that is flowrate corrected to standard temperature and pressure conditions), recalibration of the pump flowrate is essential if it is operated under conditions substantially different to those of calibration. `Substantial' implies a difference in altitude or temperature by more than 500m or 15^{o}C respectively compared to the calibration conditions.

EXAMPLE:

During the calibration of a pump with an internal flowmeter a soap film flowmeter of 500mL volume gave an average of 31.7 seconds for the bubble to traverse its length.

What is the flowrate under these conditions?

Using the equation in this Appendix:

$$Q_c = \frac{V}{T} = \frac{500}{31.7/60} = 946 \text{ mL/min}$$

The flowrate, under the temperature and pressure conditions as stated above, was 946 mL/min.

(II) FOR FLOWRATES EXCEEDING 2.0 L/min

If an accurate burette of 1,000mL capacity is chosen, then flowrates up to approximately 4 L/min can be calibrated. However, it is not acceptable to manually time the passage of the bubble if the time interval over the measured volume is less than 15 seconds. It is not acceptable to use a soap film flowmeter of capacity exceeding 1 litre. Photocell activated electronic timers can be used if it is proven that no added artifacts are introduced which can cause inaccuracy. At high flowrates, pressure drops across restrictions in the calibration train may cause further inaccuracies, and must be taken into account.

If secondary standard flowmeters (for example, rotameters) are used, it is imperative that they be regularly calibrated against a suitable primary standard, giving due consideration for pressure drop and pulsation problems.[21]

APPENDIX C

MEASUREMENT OF EFFECTIVE FILTER AREA

One convenient way in which to determine the area of the dust deposit (that is, the effective filter area) is as follows:

1. Place a small quantity of dark coloured dust (for example, carbon, cement or road dust) into a 2 to 5-litre container with a lid.
2. Shake the container, remove the lid and draw air through a membrane filter and its holder until the airborne dust in the container forms an obvious visible deposit on the filter.
3. Remove the filter from the holder, and mount on to a microscope slide in the normal manner as described in Appendix A.
4. Measure at least two and preferably four different diameters of the resultant dust spot to within $\pm$ 0.2mm. Among other methods, microprojection measurement, or the use of microscope object stage verniers have been found satisfactory.
5. Provided that the measured diameters of the filter differ by no more than 1mm, a simple arithmetical average is sufficient to provide a good estimate of the effective filter diameter.
6. At least three individual filters must be prepared and measured as described above to give assurance that the final calculated area is sufficiently accurate.
7. Provided that the three individual filter diameters differ by no more than 1mm, an arithmetical average should be taken and the area calculated in the usual manner. This area is then the Effective Filter Area to be used for calculations in this method.
8. If steps 5 or 7 produce differences greater than 1mm, close attention should be paid to the sampling of the dust or to the filter clearing technique.
9. It is necessary to repeat the measurement of the effective filter area if the type of filter or holder, or if any aspect relating to filter clearing, is changed.
10. It is advisable to repeat the entire measurement procedure every twelve months to ensure that the correct effective filter area is known.

APPENDIX D

DUST SAMPLING RECORD
(Example Only)

All data necessary for the determination of the fibre concentration must be recorded in a sampling record. Furthermore, as much data as available should be recorded for control design and epidemiological studies.

SAMPLING DETAILS

· instrument type and number
· flowrate: (initial, intermediate and final)
· duration
· sampling strategy used
· date, hour
· sampled by

SAMPLING ENVIRONMENT DETAILS

· designation: (job title and work location)
· harmful substances: (for example, types of asbestos.)
· brief description of working process
· variable parameters which can exercise an influence on dust formation
· work practices: (if applicable)
  - working conditions: (normal, abnormal)
  - material: (for example, type, size, condition)
  - airflow: (worker in dust airflow - yes/no
    obvious influence on adjoining working places)
· methods of dust control: (if applicable)
  - exhaust ventilation
  - other methods
  - visual impression
· number of employees for which the measuring value is representative
· personal protection (yes/no) type
· hours per shift
· days per week

APPENDIX E

SPECIFICATIONS FOR EYEPIECE GRATICULE AND CALIBRATION

PART 1: SPECIFICATIONS OF EYEPIECE GRATICULE, ORDERING INFORMATION AND CALIBRATION

The Walton-Beckett graticule described in this method is available from:

GRATICULES LIMITED
SOVEREIGN WAY
BOTANY TRADING ESTATE
TONBRIDGE
KENT
ENGLAND
TN9 1RN.

A technical description of this graticule can be found in a paper in the Annals of Occupational Hygiene.[22]

The desired diameter (d) of the circle to appear as 100 $\pm$ 2 micrometres in the image plane (D) and the overall diameter of the glass disc should both be specified in millimetres when ordering. The graticule can be referred to by the Graticules Ltd Reference No.G22.

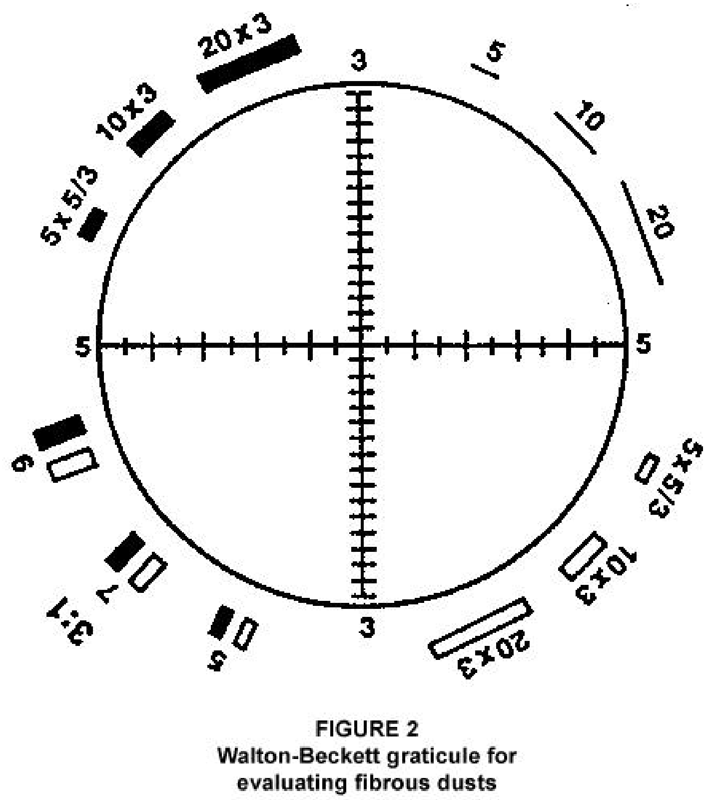


FIGURE 2
Walton-Beckett graticule for evaluating fibrous dusts

The following procedure is one of several methods for determining the diameter (d) of the circular counting area:

1. Insert any available graticule into the eyepiece and focus so that the graticule grid is sharply in focus.
2. Set the appropriate interpupillary distance and, if applicable, reset the binocular head adjustment so that the `tube' length (and thus magnification) remains constant.
3. Ensure that the 40 phase objective is in place, and that the magnification changer position (if used) is known and recorded.
4. Place a stage micrometer on the microscope object stage and focus the microscope on to the graduated lines.
5. Measure the overall object length (l_o) of the graticule grid, using the stage micrometer.
6. Remove the graticule from the microscope and measure its actual overall grid length (l_a). This can be done by using a stage fitted with verniers.
7. Use the following equation:

   diameter to be specified (d) = $(1_a/1_o)$. D

   It is also necessary to specify the overall diameter of the glass disc as detailed by each microscope manufacturer.

   EXAMPLE:

   Step 5 produced an object length of a Porton graticule of 108 micrometres

   Step 6 produced an actual length of 4.50mm

   Step 7: $\frac{4.50}{0.108} \times 0.1 = 4.17$ mm

   For this example, the graticule diameter was found to be 17mm. Thus a 17mm diameter, Type G22 Walton-Beckett graticule of circle diameter 4.17mm, should be specified for the above example.

   To expedite manufacture of `made to order' graticules so as to avoid delay and keep down prices, graticules should be ordered in bulk if at all possible. In most cases individual invoices and deliveries can be arranged on a single bulk order if requested.

PART 2: CALIBRATION OF EYEPIECE GRATICULES

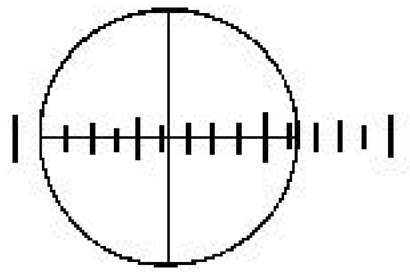
1. Obtain a stage micrometer, preferably with a scale having two or ten micrometre divisions, and place on the object stage of the microscope.
2. Make sure interpupillary distance of eyepieces is set correctly.
3. Note the objective magnification and any intermediate magnification used.
4. Focus the microscope on to the graduated marks of the stage micrometer.
5. Line up the eyepiece graticule with the graduated divisions on the micrometer so that the number of whole micrometer divisions can be counted from one side of the eyepiece graticule graduations to the other.
6. If less than a whole division remains, estimate this fraction to the nearest micrometre and add to the number of whole divisions of the stage micrometer after converting to micrometres.

   This totalled result is the projected or object dimension of the eyepiece graticule.

EXAMPLE:

1. A stage micrometer with ten micrometre divisions was placed on the stage of a microscope.
2. The following diagram depicts the view of the superimposed eyepiece graticule and stage micrometer.

FIGURE 3



   Note that 10 whole divisions span across the graticule (that is 10 x 10 micrometres).

3. The remainder of the 11th division is estimated as being one-third of a whole division (that is, three micrometres).

   Adding these together yields 103 micrometres, which is the object dimension of the eyepiece graticule.

   Note that if the interpupillary distance, objective, intermediate magnification, or even in some microscopes the eyepiece is changed, then this usually changes the object dimension of the eyepiece graticule, thus necessitating recalibration.

APPENDIX F

DETECTION LIMIT TEST SLIDE

The recommended HSE/NPL Detection Limit Test Slide (Mark II) for use in Phase Contrast Microscopy is available from:

PTR OPTICS
UNIT D9
CROSS GREEN APPROACH
CROSS GREEN INDUSTRIAL ESTATE
LEEDS
YORKSHIRE
UNITED KINGDOM

DESCRIPTION

The standard test slides consist of epoxy replicas of a Master Slide produced and certified by the National Physical Laboratory (UK). The replicas are mounted on a 75 x 25mm glass slide which is either 1.2 or 0.8mm thick. The slide is covered by a coverslip 0.17mm thick with a layer of another resin with a different refractive index in betweem.

The test objects consist of a series of seven blocks of grooves of length 8.5mm filled with a resin of refractive index 1.58 in a medium of refractive index 1.485. The grooves have a V-shape profile and have a depth-to-width ratio of about 0.1. The blocks are separated by gaps 20 micrometres wide. A set of four deep marker grooves is placed on either side of the array and a further two sets of two marker grooves spaced at an interval of 120 micrometres intersect the array at right angles. The zone of the test objects to be used is delineated by the rectangle bounded by these marker grooves. This zone can easily be located, as the field of view in which it is found is engraved on the coverslip. This is illustrated in Figure 4.

The widths of the grooves within each block and the calculated phase change (in degrees) associated with the maximum path difference in the light rays passing through the test objects are in Table 10.

METHOD OF USE

Set up the microscope for phase contrast microscopy as recommended for the membrane filter method.

Locate Block 1 (the coarsest set) of the test objects and move the slide to observe adjacent blocks. Determine the block of the finest grooves that can be seen. It is unlikely that all seven blocks of grooves will be detected using optical phase contrast techniques, even on the best research microscope. On the basis of present information, a satisfactory system will detect Block 5.

Full details are supplied with the slide.

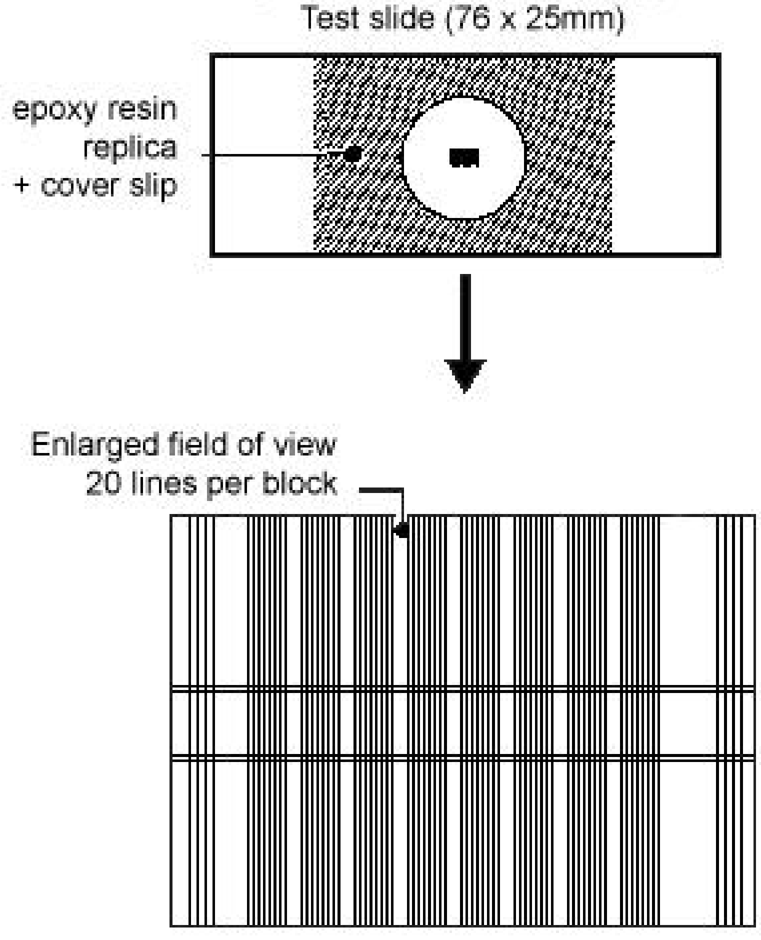
TABLE 10

WIDTHS OF TEST OBJECTS AND CALCULATED MAXIMUM PHASE CHANGE INDUCED IN LIGHT RAYS PASSING THROUGH TEST OBJECTS OF HSE/NPL TEST SLIDE

Block Number	Groove Width (micrometres)	Maximum Calculated Phase Change (in degrees) for light rays*
1	1.08	6.6
2	0.77	4.7
3	0.64	3.9
4	0.53	3.2
5	0.44	2.7
6	0.36	2.2
7	0.25	1.5

*wavelength = 530 nanometres passing through test objects.

FIGURE 4
HSE/NPL Test slide for phase contrast microscopy



APPENDIX G

MICROSCOPE ADJUSTMENT PROCEDURE

Good quality phase contrast microscope equipment should be used as detailed in section 7.2. The equipment should be maintained in first-class condition. Most manufacturers operate a routine maintenance service which includes the stripping down and cleaning of all optical components and the replacement of worn traverse mechanisms. Such services should be used unless skilled maintenance services can be provided by counting-laboratory staff.

In general, the following setting-up procedure should be adopted to obtain Koehler illumination and good phase contrast conditions. The detail may vary according to manufacturer's instructions and the type of equipment.

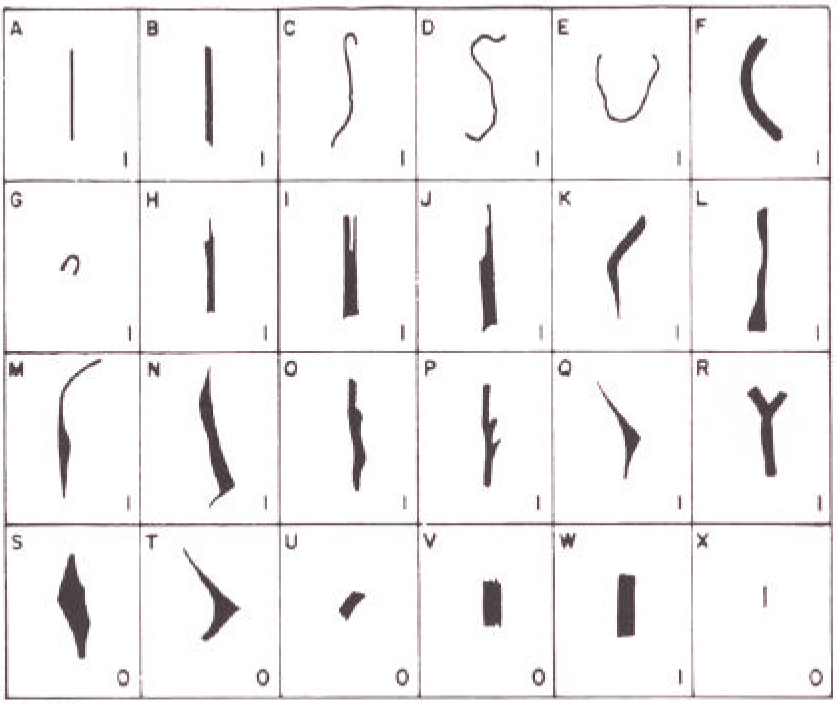
1. Place membrane filter specimen slide on microscope stage.
2. Open both the illuminator diaphragm (often referred to as the field iris) and the substage condenser diaphragm. (Note: at this stage the phase annuli should not be inserted. These are usually based in a rotating drum fitted into the substage condenser unit.)
3. Raise condenser to its upper limit, usually within 1mm of lower face of specimen slide.
4. Using a convenient level of illumination and 10x objective, focus the specimen.
5. Close down the illuminator diaphragm and focus this in the field of view by lowering and raising the condenser. Centre the diaphragm and re-open to fill the field of view.
6. Observe the back focal plane of the objective, using either a Bertrand lens fitted to the body of the microscope or by removing the eyepiece and using an auxiliary telescope.
7. Observe the image of the bulb (removing the diffusing disc if one is fitted) and centre the bulb filament, focusing the bulb if possible with the adjustment provided. The image of the bulb filament should fill the back focal plane of the objective. Re-insert the diffusing disc if appropriate. (Note: if the bulb cannot be focused, adjust to give uniform bright illumination.)
8. Insert the correct phase annulus into the condenser system and centre this, using the appropriate adjusting screws so that the phase plate in the objective and the image of the annulus coincide exactly. *Slightly* adjust the condenser focusing if this is necessary. Ensure that the bright annulus image does not extend beyond the phase ring.
9. Revert to normal viewing and change to 40x objective with no phase annuli in the condenser system. Close down the field diaphragm and re-focus this by appropriate adjustment of the condenser. Re-centre if necessary and re-open to fill field of view.
10. Repeat stages 6 and 8 after inserting the phase annulus appropriate to the 40x objective.
11. Revert to normal viewing.

DRAWINGS OF VARIOUS ASBESTOS FIBRES

<u>NOTE</u>: **All drawings are the same scale (1mm represents one micrometre). The number in the right bottom corner of each drawing indicates the number of fibres (as defined) counted.**

SINGLE FIBRES: These are the simplest of the fibres to identify and count. They are also the most common measurable fibres seen on the membrane filter. Amosite and crocidolite fibres generally assume a straight needle-like form. Chrysotile fibres, while sometimes straight, often assume a curved or curly outline. Fibres which appear irregular and perhaps `unfibre-like' are counted if they conform to the basic requirements of fibre definition.

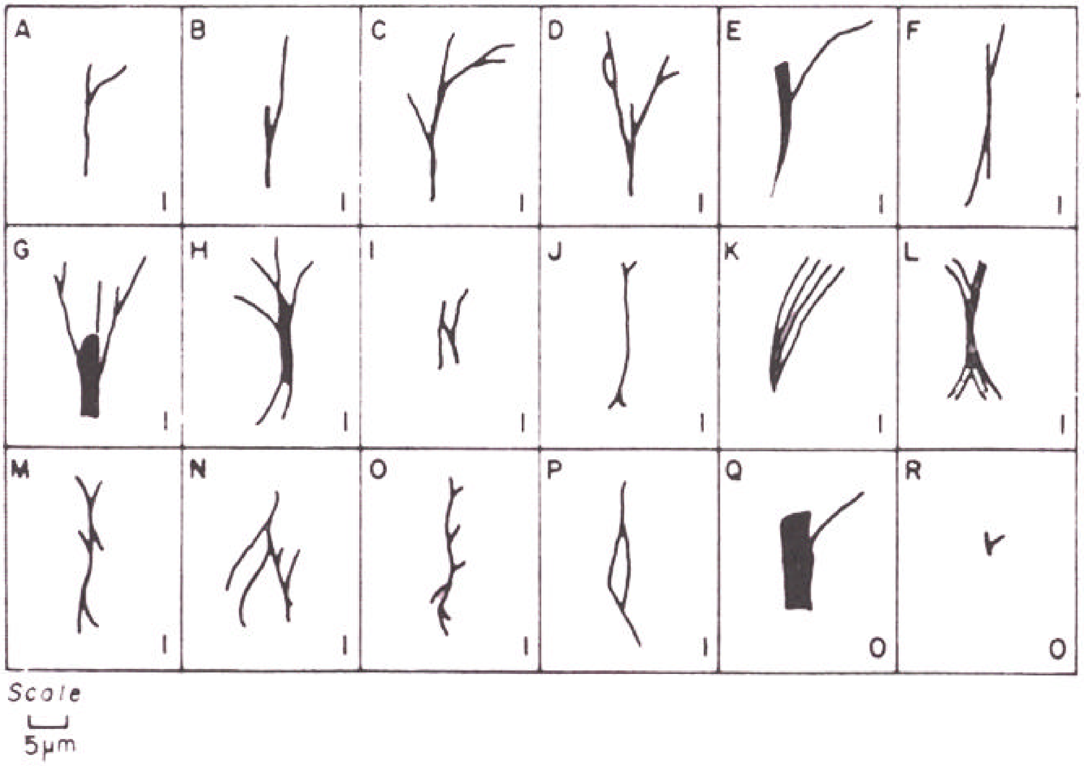
FIGURE 5



Scale:
5μm

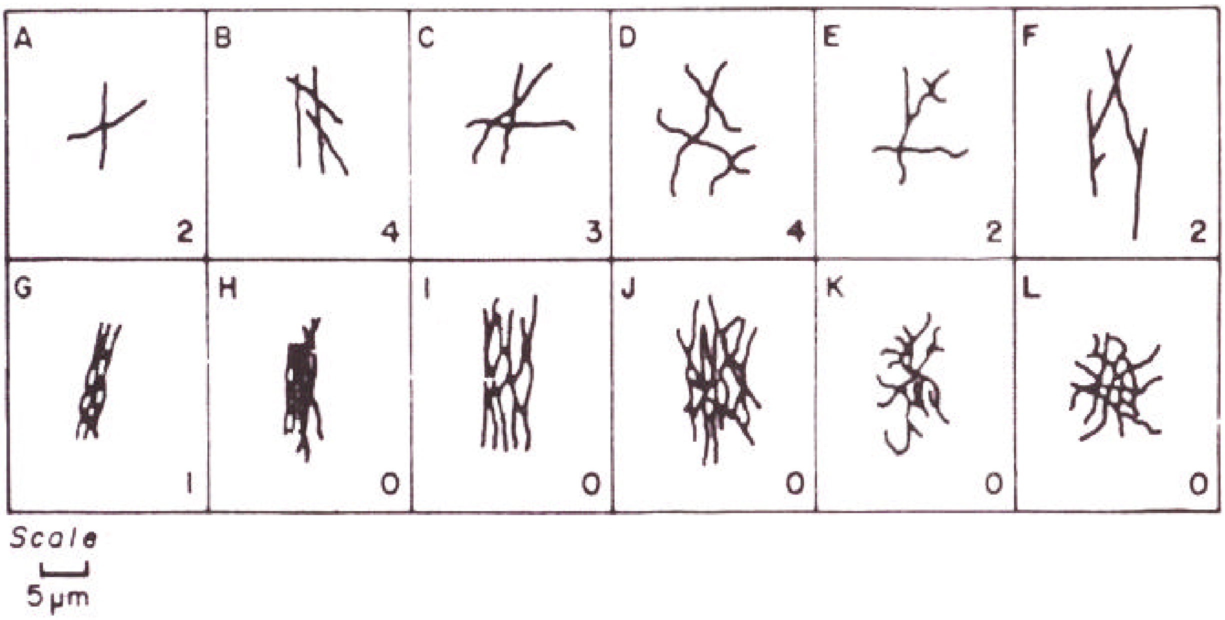
SPLIT FIBRES: These appear generally as a fibre or fibres splitting away from a single stem.

FIGURE 6



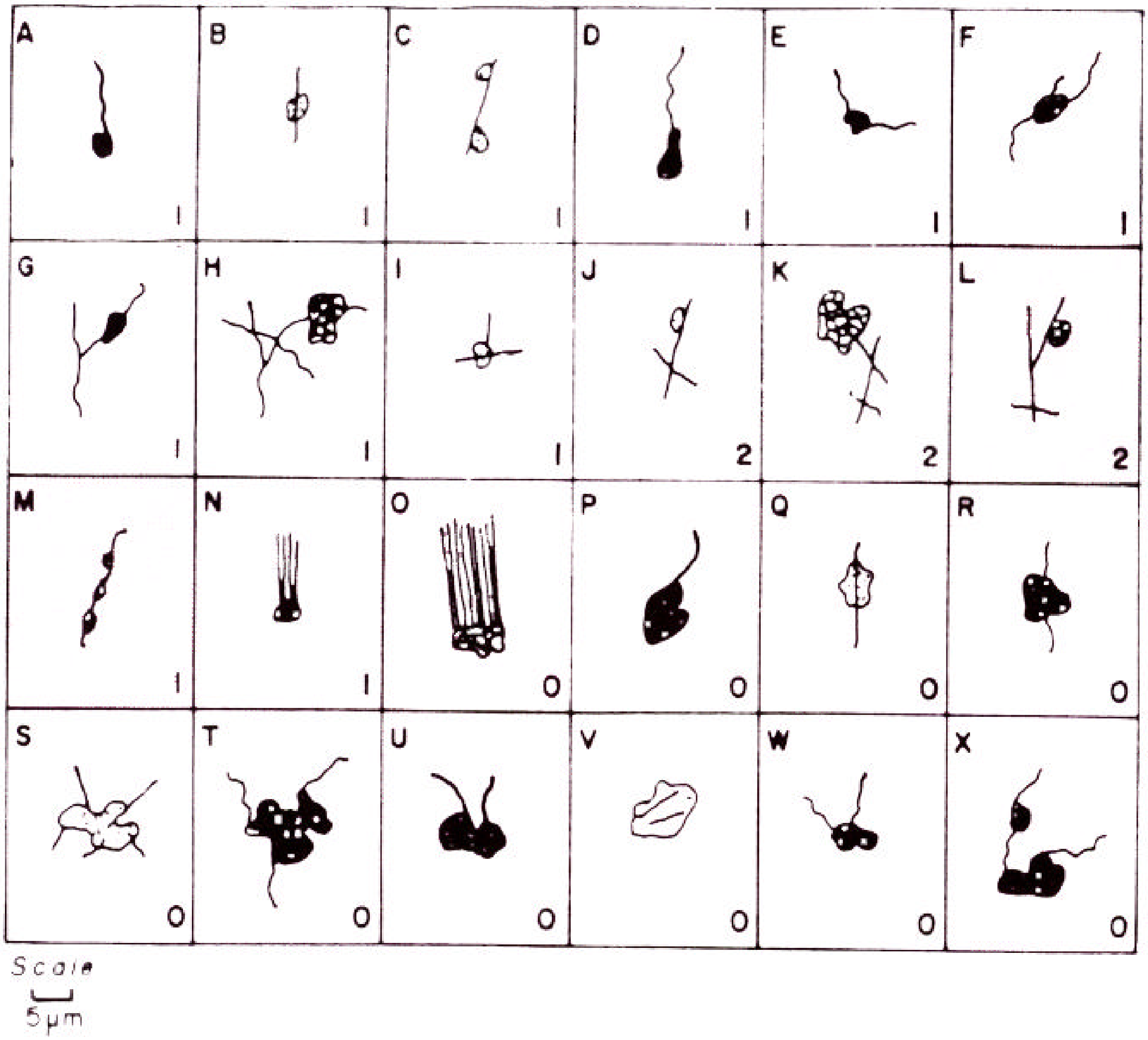
GROUPED FIBRES: These are formed when fibres overlap, intertwine or pack together. The simplest form is when two fibres overlap and cross each other. In this case, each fibre in the group appears as a discrete entity. In more complex form, fibres lie nearly parallel and appear to originate from the same bundle.

FIGURE 7



FIBRES WITH OTHER PARTICLES: This group consists of fibres attached to, or embedded in, particulate matter. For example, this latter material could be parent asbestos rock, or resins, cement, silicates used in manufactured products. Under the microscope some fibres, especially chrysotile, appear to project from the particulate matter with only part of the fibre seen. Other fibres (often amosite) are seen as embedded in the particulate matter.

FIGURE 8



APPENDIX I

EXAMPLE OF FIBRE COUNTING RECORD

Name of job ______________________________	Date
	Pump No.
Location of Sample ______________________________	Filter No.
	Flow
Additional Remarks ______________________________	Time off
	Time on
	Total time
	Sample taken by

LAB USE ONLY

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	TOTAL
Fibres Per Field																					
Accum. Total																					

	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Fibres Per Field																					
Accum. Total																					

	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
Fibres Per Field																					
Accum. Total																					

	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	
Fibres Per Field																					
Accum. Total																					

	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	
Fibres Per Field																					
Accum. Total																					

Scanner's remarks ______________________________

A simplified formula for calculating dust concentration is:

$$\text{Dust concentration } C = 1000 \times \frac{\text{No. of fibres}}{\text{No. of fields}} \times \frac{\text{effective filter diameter(mm) squared}}{\text{graticule diameter}(\mu\text{m}) \text{ squared}} \times \frac{1}{\text{volume(L)}}$$

$$C = 1000 \times \frac{\boxed{}}{\boxed{}} \times \frac{\boxed{}}{\boxed{}} \times \frac{1}{\boxed{}}$$

$C = \boxed{}$ fibres/mL

Sample analysed by	Date / /	Checked

APPENDIX J

SPECIFICATIONS FOR FILTERS, FILTER HOLDERS AND PUMPS

(1) FILTERS

Membrane filter (mixed ester cellulose or cellulose nitrate), 13 or 25mm diameter, pore size 0.8um, with grid. Some manufacturers include:

Gelman Sciences Inc., Type GN4 (gridded)
Nuclepore cellulosic white gridded
Millipore Corp., Type MF (gridded)
Sartorius, mixed cellulose esters

(2) SUPPORT PADS

Millipore Corp
Nuclepore Corp

(3) FILTER HOLDER AND COWL

Some cowls are supplied with an end piece which has a small opening of approximately 3-4mm in diameter. These end pieces must be removed during sampling such that a large opening approximately the size of the effective filter area is used.

(i) **Filter Holder, 25mm diameter**.

Supplier:
Gelman Sciences Inc., Product No. 1107

A cowl must be used with the Gelman Filter Holder. If cowls are not available directly from Gelman, figures 9 and 10 give details of alternative cowls that can be made to order.

FIGURE 9

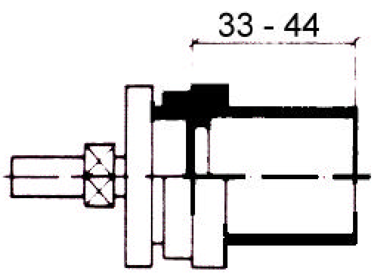
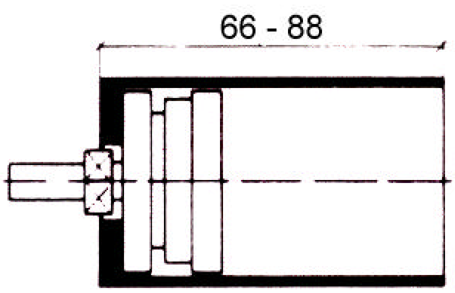


FIGURE 10



(ii) **Filter Holder (monitor), 25mm diameter**.

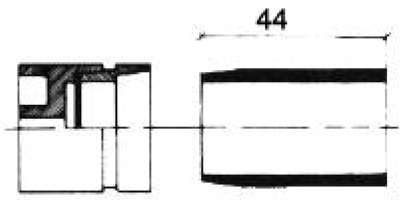
Supplier: Nuclepore, Stock No. 300015 (3-piece cassette including 50 mm cowl) Refer figure 11.

Nuclepone, Stock No. 300075 (3-piece cassette including 50 mm cowl - manufactured with electrically conducting [anti-static] material).

Supplier: Millipore Corp, Order No. M000025AO (3-piece cassette). An anti-static cowl (order no. M000025RO) should be used with the cassette. Refer to figure 12.

FIGURE 11

FIGURE 12



(4) PUMPS

Sampling pumps used for estimating airborne asbestos dust should be capable of maintaining the appropriately chosen flow rate within ± 10% for the entire sampling duration. Pumps with automatic flow control facilities are recommended. Because performance characteristics of field sampling pumps can vary considerably, reference should be made to experienced laboratories for selection of pumps for specific sampling applications.

When sampling in explosive gas or dust atmospheres as defined in AS 2430[17], ensure that the sampling pump meets the requirements of AS 2380[18].

APPENDIX K

REFERENCES

1. National Occupational Health and Safety Commission (NOHSC), *Guide to the Control of Asbestos Hazards in Buildings and Structures*, NOHSC, Sydney, 1988, attached.

2. National Occupational Health and Safety Commission (NOHSC), *Code of Practice for the Safe Removal of Asbestos*, NOHSC, Sydney, 1988, attached.

3. United States Department of the Interior, *Selected Silicate Minerals and their Asbestiform Varieties - Mineralogical Definitions and Identification Characterisations*, Bureau of Mines Information Circular 1977, IC 8751.

4. National Health and Medical Research Council, *Membrane Filter Method for Estimating Airborne Asbestos Dust*, NH&MRC, Canberra, 1976.

5. Asbestos International Association, *Reference Method for the Determination of Airborne Asbestos Fibre Concentrations at Work Places by Light Microscopy (Membrane Filter Method*), Recommended Technical Method No.1 (RTM 1), London, 1982.

6. International Standard Organisation, *Determination of the Number Concentration of Airborne Inorganic Fibres by Phase Contrast Optical Microscopy - Membrane Filter Method*, 3rd Revision, ISO/TC, 146/SC2 WG5, 1986.

7. Official Journal of the European Communities, *Council Directive*, 19 September 1983, (83/477/EEC), Annex 1.

8. Health and Safety Executive, *Asbestos Fibres in Air*, MDHS 39, HSE Occupational Medicine and Hygiene Laboratory, HMSO, London, 1984.

9. National Occupational Health and Safety Commission, *Exposure Standards for Atmospheric Contaminants in the Occupational Environment*, NOHSC Sydney, (in press).

10. Beckett, S.T., `The Effects of Sampling Practice on the Measured Concentration of Airborne Asbestos,' Ann. Occup. Hyg., 23, pp.259-272 (1980).

11. Peck, A.S. et al, `Airborne Asbestos Measurement: Preliminary Findings Identify a New Source of Variability in Membrane Filter Method,' Am. Ind. Hyg. Assoc. J., 46, B14-16, (March 1985).

12. Peck, A.S. et al, `Sample Density and the Quantitative Capabilities of PCM Analysis for the Measurement of Airborne Asbestos.' Am. Ind. Hyg. Assoc. J., 47, A230-234, (April 1986).

13. Cherrie, J. et al, `The Influence of Fibre Density on the Assessment of Fibre Density Concentration Using the Membrane Filter Method,' Am. Ind. Hyg. Assoc. J., 47(8), 465-474, (1986).

14. National Health and Medical Research Council, *Report on the Health Hazards of Asbestos*, NH&MRC, Canberra, 1982.

15. Her Majesty's Stationery Office, Asbestos, *Final Report of the (UK) Advisory Committee on Asbestos*, Volumes 1 & 2, HMSO, 1979.

16. Pinchin, D.J., in Royal Commission on Matters of Health and Safety Arising from the Use of Asbestos in Ontario, *Asbestos in Buildings*, Study No.8, Ministry of the Attorney-General, Toronto, 1984.

17. Standards Association of Australia, AS 2430-Part 1 (1987) Explosive Gas Atmospheres, and AS 2430-Part 2 (1986) Combustible Dusts, SAA Sydney.

18. Standards Association of Australia, AS 2380 - Part 7 (1987), *Electrical Equipment for Explosive Atmospheres - Explosion Protection Techniques: Intrinsic Safety i*, SAA Sydney.

19. Asbestos Information Association (North America), *A Study of the Empirical Precision of Airborne Asbestos Concentration Measurements in the Workplace by the Membrane Filter Method*, AIA, Virginia, USA, 1983.

20. Baron, P.A. and Pickford, G.C., `An Asbestos Sample Filter Clearing Procedure', Appl. Ind. Hyg., 1(4), 169-171, 1986.

21. Nelson, G.O., *Controlled Test Atmospheres*, Ann Arbor Science Publishers, Michigan, 1971.

22. Walton W.H. and Beckett S.T., `A Microscope Eyepiece Graticule for the Evaluation of Fibrous Dusts,' Ann. Occ. Hyg., 20, 19-23, 1977.