

MDHS

Methods for the Determination of Hazardous Substances

Health and Safety Laboratory



39/4

Asbestos fibres in air

Sampling and evaluation by Phase Contrast
Microscopy (PCM) under the Control of
Asbestos at Work Regulations

November 1995

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INTRODUCTION

Nomenclature, health effects and legislation

1 Asbestos is a term used for the fibrous forms of some naturally occurring silicate minerals which have been exploited commercially for their useful properties of flexibility, high tensile strength, incombustibility, low thermal conductivity and resistance to chemical attack. The term 'fibrous' in this context means asbestiform, consisting of bundles of parallel, very high aspect ratio fibres (generally 20:1 to 1000:1) that split easily, may be curved, or that occur as thin needles or in matted masses. For regulatory purposes in Britain, the Control of Asbestos at Work Regulations (CAWR)^{1,2} define asbestos as any of the following minerals (or any mixture containing them): chrysotile, amosite, crocidolite, fibrous actinolite, fibrous tremolite and fibrous anthophyllite. These fibrous minerals have been associated with the diseases that can result from the inhalation of asbestos, ie asbestosis, lung cancer and mesothelioma. Information on medical effects is given in an HSE Medical Series Guidance Note³ and information on legislation, product types and control measures is given in Approved Codes of Practice^{4,5} and other HSE publications.⁶⁻¹¹ In particular, the use of measurements is detailed in Guidance Note EH10.⁶ Also, the Department of the Environment gives information on the use of asbestos in buildings.¹²

Outline of method and changes from previous MDHS

2 The following method is described for the measurement of airborne asbestos fibre concentrations, and revokes the previously recommended MDHS 39/3. The method involves the collection of air samples and the analysis of those samples using phase contrast microscopy (PCM).

The major changes are as follows:

- The control limits and action levels now apply to two distinct groups of asbestos: (a) all amphibole asbestos minerals; and (b) chrysotile alone;
- The action level for chrysotile is now 96 fibre-hours/ml (f-hr/ml);
- The clearance indicator level is now <0.01 fibres per millilitre of air (f/ml);
- Guidance is given on the situations where discrimination of fibre types is allowed;
- DMF/Euparal for clearing filters is no longer supported; and
- Flow rates up to 16 l/min may be used for static sampling.

Specified concentrations and methods

3 Control limits are specified by CAWR^{1,2} as follows:

- (a) for asbestos consisting of, or containing, one or more of the 5 amphibole asbestos minerals (amosite, crocidolite, fibrous actinolite, fibrous tremolite and fibrous anthophyllite, or any mixture of any of these minerals with chrysotile):
 - (i) 0.2 f/ml averaged over any 4 hours;
 - (ii) 0.6 f/ml averaged over any 10 minutes;
- (b) for chrysotile alone:
 - (i) 0.5 f/ml averaged over any 4 hours;
 - (ii) 1.5 f/ml averaged over any 10 minutes.

The Health and Safety Commission has approved a method for determining airborne concentrations for comparison with the control limits and action levels. The approved method is described in the CAWR regulation 2¹ and is reproduced in Appendix 1. The application of these specified concentrations is discussed in Guidance Note EH10.⁶ This MDHS aims to give further guidance on the method, but departure from it in details not covered by the CAWR Approved Method may be made provided that all changes are validated.

4 Action levels accumulated over any 12 week period are specified by CAWR^{1,2} as follows:

- (a) for asbestos consisting of, or containing, one or more of the 5 amphibole asbestos minerals (amosite, crocidolite, fibrous actinolite, fibrous tremolite and fibrous anthophyllite, or any mixture of any of these minerals with chrysotile): 48 f-hr/ml;
- (b) for chrysotile alone: 96 f-hr/ml;
- (c) where both types of exposure occur separately during the 12 week period: a proportionate number of f-hr/ml may be applied to a composite action level.

Clearance indicator

5 The Approved Code of Practice *Work with asbestos insulation, asbestos coating and asbestos insulating board* (the 'Insulation ACOP')⁵ also refers to an airborne fibre concentration level < 0.01 f/ml. This is fundamentally different from the concentrations specified in CAWR,¹ being "a transient indication of site cleanliness, in conjunction with visual inspections and not an acceptable permanent environmental level".⁵ This level is designated 'clearance indicator' in this MDHS. For measurements related to the clearance indicator the Approved Method does not apply, but the Insulation ACOP⁵ sets a standard of cleanliness of <0.01 f/ml measured by the present MDHS. Any variation from the method described must result in at least as high a standard of cleanliness, and be shown in properly conducted and fully documented tests.

PRINCIPLE

6 A measured volume of air is drawn through a membrane filter, which is subsequently mounted on a microscope slide and rendered transparent. Fibres on a measured area of filter are counted using phase contrast microscopy (PCM), and the number concentration of fibres in the air is calculated.

SAMPLING APPLICATIONS

7 This measurement technique is applicable to different sampling situations as follows:

- (a) **compliance sampling:** this refers to the use of the approved method as detailed in Appendix 1 to assess whether or not the personal exposures of workers are in compliance with the 4h or 10 min control limits and the 12 week action level as defined in CAWR;
- (b) **background sampling:** this is conducted to establish fibre levels prior to any activity which may lead to airborne asbestos contamination;
- (c) **leak (enclosure check) sampling:** this is performed outside the enclosure while asbestos removal work is in progress to check that the environmental control systems are adequate;
- (d) **assessment of the suitability of respirator protection:** this is monitoring inside enclosures while asbestos removal is in progress and is conducted to assess the effectiveness of dust suppression measures and the suitability of respiratory protection;
- (e) **clearance indicator sampling (clearance testing):** this requires air monitoring in a cleaned and visually examined enclosure from which asbestos has been removed or encapsulated;
- (f) **reassurance sampling:** this is monitoring which may be conducted in certain circumstances (such as when an enclosure has been removed) to confirm that the residual asbestos fibre concentrations are <0.01 f/ml.

SCOPE AND LIMITATIONS

8 The method measures the airborne concentration of countable fibres using phase contrast microscopy (PCM). Countable fibres are defined as particles with length >5 µm, width <3 µm and aspect ratio (length : width ratio) >3:1. Fibres having widths <0.2 µm may not be visible using this method,¹³ and the PCM count represents only a proportion of the total number of fibres present. Therefore the count is only an index of the numerical concentration of fibres and not an absolute measure of the number of fibres present. The method does not permit the determination of chemical composition or crystallographic structure of fibres, and therefore cannot be used on its own to distinguish unambiguously between different fibre types. Hence, use of this method requires all fibres meeting the size definition to be counted.

Fibre discrimination

9 It is not permissible to discriminate between asbestos and non-asbestos fibres to determine compliance with the control limit or with the action level. However, it may be possible to discriminate between such fibres for sampling situations other than compliance sampling. Fibre discrimination will be dependent on the range of analytical techniques available and the skills of the microscopist. A hierarchy of methods is available to eliminate non-asbestos fibres such as man-made mineral fibres (MMMMF), vegetable, aramid and other fibre types. Detailed discussion of these techniques is beyond the scope of this MDHS, and other reference documents should be consulted (an MDHS on discrimination strategy is in preparation). The report of the evaluation should include a statement on the type and numbers of interfering fibres which were present and the method by which the number of non-asbestos countable fibres have been eliminated from the original PCM count.

<i>Hierarchy of methods</i>	<i>Application</i>
Phase contrast microscopy (PCM)	Technique for all countable fibres
Polarised light microscopy with dispersion staining (PLM/DS) ¹⁴	Allows subtraction from a count of some sizes and types of non-asbestos fibre
Scanning electron microscopy (SEM) ¹⁵	Allows subtraction from a count of some fibres of regulated sizes: introduce elemental determination to the discrimination
Transmission electron microscopy (TEM) ^{16,17}	Ultimate technique for discrimination; includes quantitative elemental analysis as well as crystal structure determination

Lower concentration limit

10 Errors become very large when small numbers of fibres are counted. Statistical considerations show that, for a mean density of 10 fibres per 100 graticule areas, a count of 5 or fewer fibres per 100 areas will be obtained on about 5% of occasions. This relates to the 'blank count' allowed by paragraph 33, so that it can be argued that 10 fibres per 100 graticule areas should be regarded as the lowest reliably detectable count above background. For a sample volume of 480 litres, this corresponds to a calculated result of about 0.010 f/ml in the air. Moreover, there is some evidence that counters underestimate a blank count if they know it to be so.¹⁸ This MDHS is written so that determination of the specified concentrations in paragraph 3 is never based on counts of fewer than 20 fibres. Bias and inter-laboratory differences will degrade the reliability of low concentration results even further. Therefore, the limit of detection of this method, assuming a 480 litre sample and 200 graticule areas examined, is 0.010 f/ml (see example, Appendix 3).

REAGENTS

11 Acetone and glycerol triacetate ('triacetin') are required for filter clearance. Analytical grade reagents are not essential, although excessive water in the acetone may reduce filter clarity. The triacetin should be clean, free from dust and moisture, and with no evidence of hydrolysis (possibly indicated by a smell of acetic acid) or other contamination.

APPARATUS

Sampling equipment

12 To comply with the standard method, an open-faced filter holder (Fig 1) fitted with an electrically-conducting cylindrical cowl extending between 33 mm and 44 mm in front of the filter, exposing a circular area of filter at least 20 mm in diameter, should be used for sampling. This type of holder is intended to protect the filter, while still permitting a uniform deposit. The cowl will point downwards when sampling. If O-rings are used, they should be made of PTFE or similar material. Flexible tubing is required to connect the filter holder to the pump, and a cap or bung is needed for the cowl entrance to protect the filter from contamination during transport.

13 The exposed area of each filter must be known and should be measured at least every time a type of cowl or O-ring is changed. A suitable method of measuring this is to use the filter holder and cowl to sample from a cloud of dark coloured dust and then to mount the filter on a slide in the usual way. The diameter of the dark deposit can be measured with vernier callipers, or by placing the slide on a microscope stage and observing the filter at low (100x) magnification while a diameter of the dark area is traversed by moving the stage. The distance moved can be obtained from the stage vernier scale. Two diameters should be measured at right angles, and three filters in separate holders should be checked in this way.

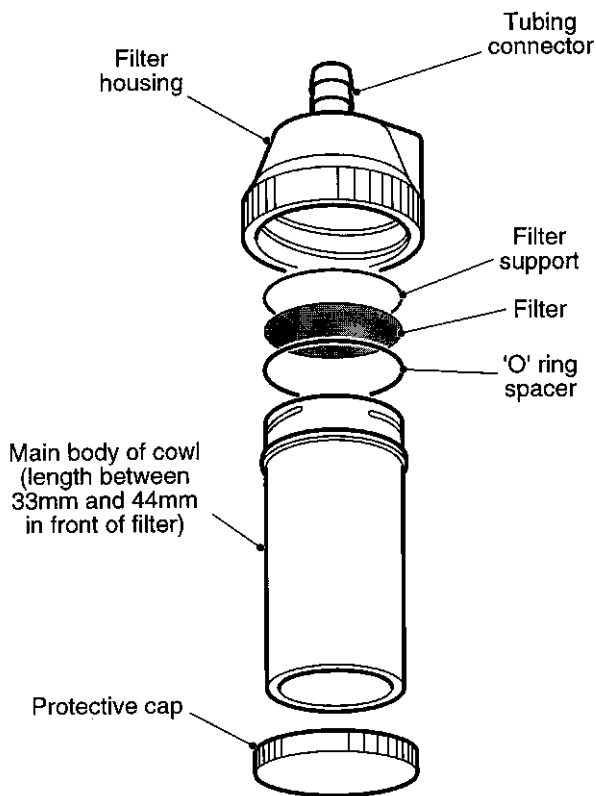


Figure 1 Sample head

(Differences between these six measurements of more than one millimetre may indicate either a poorly fitting filter holder or an unsatisfactory clearing technique.) An uneven appearance of the deposit may show that there is a leak in the sampling head.

14 The membrane filters must be of mixed esters of cellulose or cellulose nitrate, of pore size 0.8 to 1.2 μm (preferably optically clear grade), and 25 mm in diameter with a printed grid. Care must be taken to avoid contamination when handling filters. Printed grids aid both focusing on the plane of fibres and identifying positions: any distortion of grid lines indicates disturbance associated with poor mounting procedure.

15 The pump must give a smooth flow and be capable of having the flow set to within $\pm 10\%$ (and preferably to within $\pm 5\%$) of the required flowrate, and of maintaining this flowrate through the filter to within $\pm 10\%$ during the period of sampling. This variation includes any change of flowrate with pump orientation. For personal sampling, the pump must be light and portable, and fitted to a belt worn by the worker, or carried in a pocket. The pump's battery must have sufficient power to operate within the specified flow limits for the duration of the measurement. When pumps for static samples operated by mains electricity are used, due regard must be given to appropriate safety precautions. Static sampling pumps should have the facility to enable the sampling head to be positioned 1 to 2 m above ground level.

Flow measurement

16 Recommended flowrates for use at various sampling situations are discussed in paragraph 23.

17 The airflow must be measured by a flowmeter, capable of measuring the appropriate flowrate to within $\pm 10\%$ (preferably to within $\pm 5\%$), and which has been calibrated against a primary standard.

- (a) The flowmeter incorporated in the pump may only be used if it has adequate sensitivity, ie that it has been calibrated against a primary standard with a loaded filter in line, that it is read in a vertical orientation if it is the float type, and that it has the facility for the flow to be read with the required accuracy. It is important to ensure that there are no leaks in the sampling train between the sampling head and the flowmeter, since in this event a flowmeter in the pump or elsewhere in line will give an erroneous flowrate.
- (b) The primary standard should preferably be a bubble flowmeter whose accuracy is traceable to national standards and which is used with careful attention to the conditions of the calibration certificate. A bubble flowmeter is an arrangement whereby the pump under test draws a soap film up a calibrated tube. The passage of the film is accurately timed between two marks whose separation defines a known volume. A one-litre burette may be used as a bubble flowmeter for flows up to 4 l/min and film calibrators are available up to 10 l/min. The volume between the marks can be checked by filling the burette with water, allowing temperatures to stabilise, drawing off a known volume and weighing the water, making allowance for the dependence of volume on temperature. A suitable bubble solution can be made by mixing one part of concentrated washing-up liquid, two parts glycerol and four parts water. The burette must be thoroughly wetted with the solution and several attempts at drawing the film up the tube may be necessary before the tube is wet enough for this to be achieved consistently. (Traceability of the calibration will require calibration of the timing device and the use of certificated weights or calibrated balances.)
- (c) If there are large differences in ambient temperature or pressure between the calibration and the sampling site, corrections should be made to the flowrates.¹⁹ This is described in Appendix 4.

Equipment for filter clearance

18 Filter clearing should be accomplished by the acetone/triacetin hot block method (Fig 2). In this, just enough acetone to clear a filter is injected into a block with an integral heater (see paragraph 36). The acetone is vaporised and emerges as a vapour jet from an orifice below which the filter is placed. A washer (or ring of metal or plastic) may be used to form a well, restricting the spread of acetone vapour.

19 Fine-tipped pipettes, or other suitable droppers, are needed to dispense triacetin.

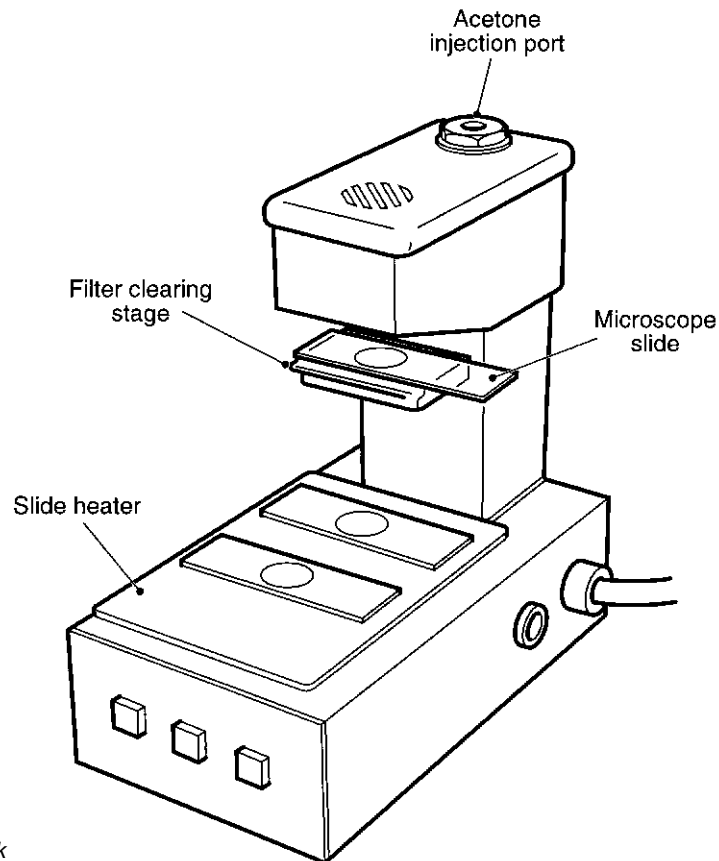


Figure 2 Acetone hot block

Microscopy

20 Differences between the smallest fibre diameters observable by phase contrast microscopes may contribute to inter-laboratory differences between counts (because fibre diameter distributions extend below the detection limit). Thus, it is essential to maintain a uniform level of detection at the limit of visibility; hence the quality of microscope (including adjustment and maintenance) is critically important. The characteristics of a satisfactory microscope system are as follows.

- (a) A positive phase contrast par focal objective with magnification 40x; the numerical aperture (NA) of this objective (which determines resolving power) must lie between 0.65 and 0.70; the phase ring absorption must lie between 65% and 85%;
- (b) The ridges of block 5 of an HSE/NPL Mark II Test Slide must be visible while only parts of block 6 ridges may be visible and none of block 7 ridges should be seen;
- (c) Koehler, or Koehler type, illumination, with field iris incorporated;
- (d) A substage assembly incorporating an Abbe, or an achromatic phase contrast, condenser in a centrable focusing mount, with phase annulus centring independent of the condenser centring mechanism;
- (e) A mechanical stage with side clamps and x-y displacement;
- (f) A stage micrometer;
- (g) Binocular eyepieces, preferably of the 'wide field' type, with magnification of at least 12.5x to give a total magnification of at least 500x: one of the eyepieces must be of the focusing type and must permit insertion of a graticule;
- (h) A Walton-Beckett graticule,²⁰ with an apparent diameter in the object plane of $100 \pm 2 \mu\text{m}$ (when checked against a stage micrometer) must be used to define the viewing area when using the specified objective and eyepieces;
- (i) The individual components of the microscope should be from the same manufacturer and optically compatible;
- (j) Various accessories include:
 - (i) a phase telescope or Bertrand lens to ensure correct alignment of the phase rings and phase annuli;
 - (ii) a green filter which assists viewing (as the optics are corrected for the wavelength of green light);
 - (iii) a low power objective (par focal with the 40x objective) for locating stage micrometer and test slide grids, and for preliminary checks on evenness of dust deposits;
 - (iv) high eye-point wide field eyepieces for spectacle wearers.

21 Microscope slides must be glass and of conventional type: 76 mm x 25 mm (approximately) and 0.8 mm to 1.0 mm thick. Coverslips must be glass, No 11/2 (0.16 to 0.19 mm) thickness or as specified for the phase contrast objective used, and about 25 mm diameter or about 25 mm square. The microscope slides and cover slips should be clean and manufactured to British Standards.²¹

Ten Minute Sampling Nomogram

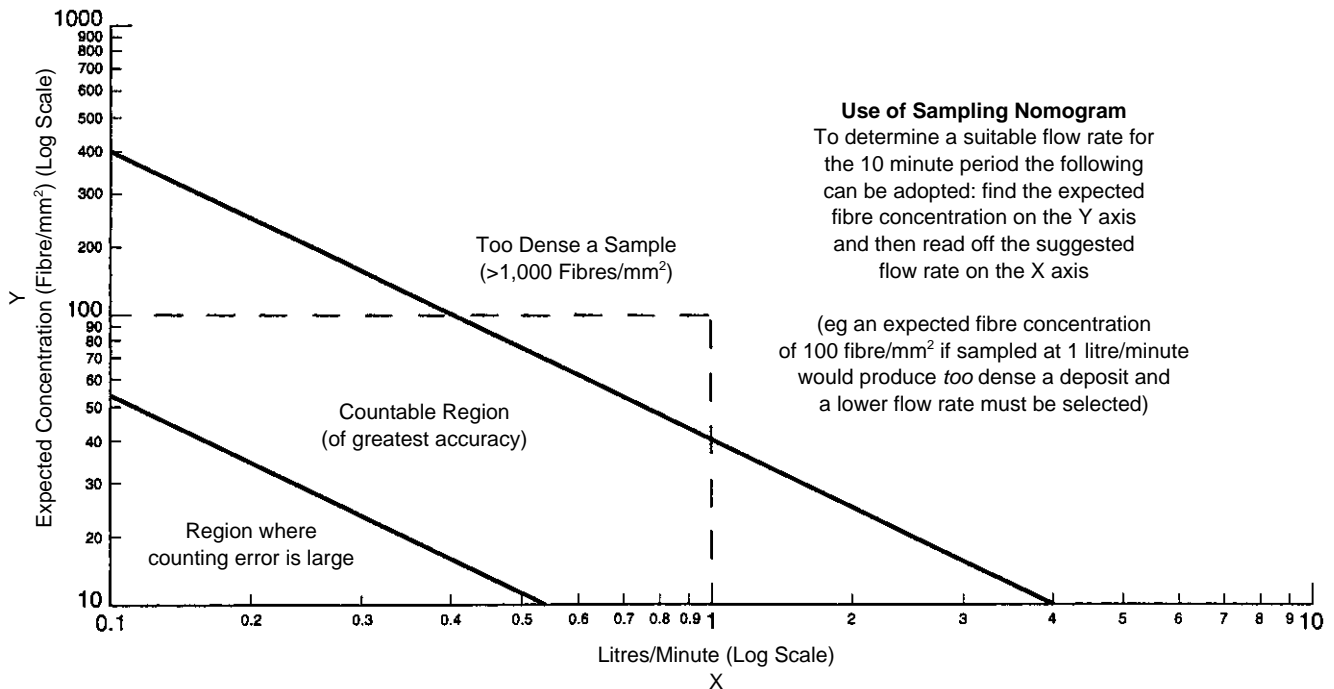


Figure 3 Ten minute sampling nomogram

SAMPLING

Preparation of filters

22 To minimise contamination, the filter holders and cowls must be cleaned before use, and the filters should be loaded, unloaded and analysed in an area as free from fibre contamination as practicable. Care must be taken to handle the filter at all times only with good quality flat tipped tweezers and only gripping the filter at the edge. The entrance to the cowl should be closed with a protective cap or bung when sampling is not in progress. The bung should not be made of plastic or any material which may become electrically charged.

Sampling period, flowrate and volume

23 The following procedures are designed where possible to give sample densities within the range for optimum accuracy and to ensure that at least 20 fibres are counted at important concentrations.

- (a) For personal sampling to determine compliance in relation to 4h control limits, the flowrate must be 1 litre/min and the period of measurement must be representative of worker exposure over a 4h period; exposure at the control limit will give the preferred range of 100 to 400 f/mm² on a 4 hour sample (see Appendix 1).
- (b) For personal sampling in relation to 10 min control limits, a flowrate of 4 litres/min (with a minimum of 1 litre/min) is recommended.

- (c) For personal sampling in relation to action levels, the sampling period should be representative of exposure over a 12 week period. To obtain samples of acceptable fibre densities, it is recommended that individual samples should represent air volumes of about 480 litres (for example, 4 hours at 2 litres/min). These samples can be obtained cumulatively over the 12 week period to obtain a sufficient volume of air through the filter.
- (d) For personal sampling to assess respiratory protection inside enclosures, a suitable strategy should be adopted according to the airborne fibre concentrations expected (especially peak concentrations) and the sampling duration and flowrate varied to produce a suitable fibre density on the filter. A suitable duration can be 10 minutes (but may be shorter if the accuracy of the measurement is not affected seriously). The flowrate should be >0.2 l/min. If the density of fibres collected on the filter exceeds 1000 f/mm², the airborne concentration may be seriously under-estimated. A nomogram is given (Fig 3) for a 10 minute period to assist in the selection of the flowrate.
- (e) For sampling in relation to the clearance indicator, background, leak and reassurance samples, the flowrates should be between 1 and 16 litres/min to generate a total sample volume of 480 litres for each measurement; details are given in paragraphs 29 to 30. Sample volumes greater than 480 litres may reduce the filter area to be counted.

Various strategies for the above are illustrated in Table 1.

Table 1 Examples of fibre counts expected at typical concentrations using the formula given in paragraph 41, assuming an exposed filter diameter of 22 mm and a graticule diameter of 100 µm

Possible application	Volume collected (litres)	Fibres counted	Graticule areas examined	Concentration in air (f/ml)	Legal requirement behind method
4 hr control limit	240	250	100	0.50	European directive/ approved method
10 min control limit	40	124	100	1.5	Approved method
Action level	480	100	100	0.1	Approved method
Assessment of respiratory protection	10	103	100	5	This MDHS
Clearance indicator	480	20	200	0.01	This MDHS

Pump preparation

24 To stabilise flowrate, pumps may need to be run for a few minutes: a separate filter and filter-holder should be dedicated to this, and may be used for several pumps before being discarded. Pumps should be capable of maintaining flow for the intended period as described in paragraph 15. Particular care should be taken with short period samples because flow instability at the start may have a significant effect on the apparent volume collected.

Sampling strategies

Personal sampling for compliance under CAWR

25 The filter holder should point downwards and be fixed to the upper lapel or shoulder of the worker's clothing, as close to the mouth and nose as practicable, and preferably within 200 mm. Due regard must be given to localised concentrations: in such cases, the sampling head should be positioned on the side expected to give the higher result. If a respirator is worn, the sampling head should be positioned away from the clean air exhaust.

Background and reassurance sampling

26 The sampling strategy to be employed in these situations usually will be the same as in clearance indicator sampling. To achieve the limit of detection (0.01 f/ml), each measurement must result from a total of at least 480 litres in volume. Fewer measurements may be generated during background and reassurance sampling than for clearance sampling, but the distribution of samples should cover likely sources of fibre and likely areas of frequent human occupation.

Leak testing

27 This is testing to support the frequent thorough visual inspections of the enclosure as described in EH51.¹¹ A number of sample positions should be considered: for example, near an air lock, near a bag-lock, and near the exhausts of negative pressure units. For this type of testing it may be possible only to sample for a few minutes, in

which case a high flowrate should be used and the cause of any fibres above background should be investigated. If fewer than 20 fibres are counted, or less than 480 litres of air is sampled, then a calculated result greater than 0.01 f/ml will have a large imprecision and account must be taken of this by proportionally increasing the limit of detection.

Personal sampling to assess respiratory protection

28 This can be achieved by personal sampling with the pump attached to the respirator belt and the filter holder attached to the hood of the wearer's overall. Airborne fibre concentrations may vary from the detection limit of 0.01 f/ml up to a level in excess of 650 f/ml (if dust control is poor).

Clearance indicator sampling (clearance testing)

29 Clearance indicator sampling should take place only when the enclosure is dry and a visual inspection confirms that it is free from dust.⁶ Practical advice on the preparation of the enclosure, and application of clearance tests, is given in Guidance Notes.^{11,22} The filter holders should point downwards, be fixed 1-2 m from the floor and be distributed throughout the enclosure. In tall enclosures (for example, vertical pipework or lift shafts), samplers should be placed at representative exposure heights, especially in areas where residual dust may be difficult to detect. There should always be at least two measurements (unless the volume of the enclosure is less than 10 m³, in which case one measurement is adequate). With that overriding condition, the number of samples should be at least the integer (whole number) next below $(A^{1/3} - 1)$ where A is determined as follows:

- (a) if the enclosure is less than or equal to 3 m in height, or in enclosures which are higher than 3 m but where exposure is likely to be at ground level only, A is the area of the enclosure in square metres;
- (b) in other cases, A is one-third of the enclosure volume in cubic metres; if there are large items of plant (such as boilers) in the enclosure, their volumes may be subtracted from the gross volumes before calculating A.

The formula has no theoretical significance, and merely serves to generate reasonable numbers. It gives the minimum appropriate number of measurements; however, personnel responsible for sampling may judge that more measurements than indicated by this minimum are required. Thus, a larger number of measurements than this minimum may be needed where an enclosure is obviously subdivided, as for example when a whole floor of a building is comprised of many smaller rooms within the enclosure. Table 2 gives examples of the numbers of measurements required.

Table 2 Examples of the numbers of measurements given by the formula ($A^{1/3} - 1$)

Enclosure size		Number of measurements
Area (m ²)	Volume (m ³)	
N/A	<10	1
<50	150	2
200	600	4
500	1500	6
1000	3000	9
5000	15 000	16
10 000	30 000	20

Each measurement should be based on a volume sampled of at least 480 litres. It is permissible to achieve a measurement by pooling two or more simultaneous or consecutive samples having a total volume of at least 480 litres. Samples which are pooled to generate a measurement should be taken within 1 m of each other.

30 Clearance indicator sampling must be accompanied by activities designed to raise dust from surfaces at least to a degree appropriate to possible future activity in the area. Dust disturbance should be conducted in the vicinity of samplers and in areas where visual inspection or the original siting of the asbestos leads to any suspicion of surface contamination. A suitable activity is repeated hitting of accessible surfaces. Other activities may be appropriate: the purpose should be to ensure that workers or members of the public using the area in future are not exposed to asbestos unnecessarily as a result of ineffective cleaning. These dust raising activities should take place for at least 5 minutes near the start of each full hour of sampling, or each time a new filter is used in an area. All equipment used for raising dust should be considered as being contaminated and therefore either cleaned or disposed of as asbestos waste.

Taking the sample

Time and flowrate recording

31 At the start of the sampling period, the protective cap must be removed from the filter holder, the pump started and the time noted. The flowrate should be measured and recorded at the start of sampling and should be checked periodically (for example, hourly) with a calibrated flowmeter during sampling, and should be readjusted to the chosen rate if necessary. At the end of the sampling period, the time should be noted, the pump stopped and

the protective cap replaced on the filter holder. The sampling period must be measured to within $\pm 2\%$.

Filter transportation

32 The preferred procedure is for the filter to be transported in the filter holder, but if for some reason this is not possible, the filter may be removed in a clean area and carefully placed in a clean tin or similar container. Sprays (eg cytology fixative) must not be used to 'fix' the dust to the filter. Adhesive tape can be used to secure the clean unexposed edge of the filter to the tin if one is used, and subsequently can be cut from the filter with a surgical scalpel. Care must be taken neither to contaminate the filter at any stage nor to dislodge any deposit. The filter holder and cowl must be cleaned before re-use.

BLANKS

33 There are three types of blanks:

- (a) Sampling media blanks are generated when filters are extracted from an unused box of filters. They are mounted and counted before sampling to check that the batch of filters is satisfactory; the usual procedure is to select 4 blank filters from each box of 100, prior to the box of filters being used.
- (b) Field blanks are generated when filters are taken from satisfactory batches to the sampling area and subjected to the same treatment as normal samples, but without having air drawn through them and without them being attached to the pump. They are mounted and counted with the main samples; the proportion of field blanks should be about 2% of total samples, unless there are reasons to believe that more may be needed. In the event of contamination, the airborne measurement should be regarded as a rough estimate only.
- (c) Laboratory blanks are generated when filters, extracted from satisfactory filter batches, are mounted and counted to check for laboratory contamination when a field blank has indicated a need for investigation. A laboratory blank may be evaluated with each batch of routine samples, or afterwards if contamination due to laboratory sources is suspected. Laboratories should carefully investigate and monitor the batch-to-batch consistency of membrane filters. Individual blank filter counts should not normally exceed 3 fibres per 100 fields; not more than 1 in 10 of the blank filters tested should have a count of up to 5 fibres per 100 fields, and if laboratory records show that the proportion is higher, the causes (including the source of supply) should be investigated.

Median fibre densities for blank filters included in counting comparisons range from 0.3 f/mm² to 6.7 f/mm². Whenever possible, the identity of blank filters should not be known to the microscopist until all counts have been completed. If elevated counts are obtained, potential internal causes should be investigated (for example, counter error or contamination of the coverslip). If it is

concluded that the problem lies with the filter, the whole box of 100 should be rejected. Blank counts must not be subtracted from sample counts (as may be done in other analytical procedures) because errors at the lower concentration limit are large and of a random nature rather than being systematic.

FILTER CLEARING AND MOUNTING

34 If additional analytical work (eg transmission electron microscopy (TEM)) is required, samples and blank filters may be cut in half with a scalpel using a rolling action, with the filter carefully held at the edge. Half of the filter can then be mounted, and the other half kept for subsequent investigation if necessary. (This may involve preparation and examination by transmission electron microscopy if non-asbestos fibres are suspected, or mounting and counting by PCM to check the result obtained with the first half-filter.)

35 The acetone-triacetin mounting method should be used. The principle is that condensing acetone vapour is used to collapse the filter pores, adhering the filter to the glass slide and turning it into a solid transparent plastic film with any asbestos fibres contained close to the upper surface. Triacetin is used to provide the interface between the collapsed filter and the coverslip. The mounted slide will keep for years without noticeable deterioration, although small-scale fibre movement may occur. Slides should be stored horizontally and not subjected to extremes of temperature. They should be preserved with all relevant records for at least six months so that the result can be checked if necessary.

36 The filter to be mounted is placed centrally on a clean microscope slide, sample side upwards, and with grid lines parallel to the slide edges. It is important that the filter is dry, as water interferes with the clearing process. A ring of metal or plastic forming a 'well' around the filter, but not touching the exposed filter area, helps to localise the spread of acetone and improves the efficiency of clearing, and should mean that 0.25 ml acetone is sufficient to clear the filter. The slide (which must be clean) is placed under the outlet orifice of the hot block (see Fig 2). The acetone is injected slowly into the hot block so that the vapour emerges in a steady stream over the filter. The filter should clear instantly. This small amount of acetone minimises fire and health risks. However, all sources of ignition should be remote, and the acetone storage bottle should be stoppered when acetone is not being extracted. Acetone vapour is highly flammable and slightly toxic, and the appropriate safety precautions should be taken before this procedure is used. (The procedure may be conducted in a fume-cupboard to minimise inhalation of acetone vapour.) The slide may be placed on a hot plate at 50°C for a few minutes to evaporate any excess acetone before applying triacetin and the coverslip. When the acetone has evaporated, a micropipette or other suitable dropper is used to place a drop of triacetin (about 120 µl) on the filter: this must be just enough to cover the filter when the coverslip is in place, without overflow around the edges. The coverslip is lowered gently onto the filter at an angle so that all the air is expelled: it should not be pressed onto

the filter. This procedure should enable counting to take place immediately. If there is no urgency to count, the slide may be kept in a dust-free environment overnight.

EVALUATION

37 The microscope must be adjusted according to the manufacturer's instructions, and its performance must be checked by the analyst at the beginning of each counting session (or more frequently if any adjustments have been made) using an HSE/NPL phase contrast test slide Mark 2. The fine focus and condenser focus may need re-adjustment before a slide is counted. The object plane diameter of the Walton-Beckett graticule should be checked using a stage micrometer with the microscope correctly adjusted for use. With some microscopes, adjustment of the inter-ocular distance changes the magnification, so the microscope in use should be checked for this effect. If it is apparent, the graticule diameter must be measured at the inter-ocular separation used. The diameter should be about 100 µm and must be within the range 98-102 µm; the measured diameter must be used in calculations.

38 The slide with the mounted filter is placed on the microscope stage. The sample may be examined with a low power objective to check uniformity of the deposit and should be discarded if badly non-uniform. Care must be taken if half filters are counted only to evaluate the portion of the slide with the mounted fraction. Fibres on the filter are counted using a 40x objective and at least 12.5x eyepieces (see paragraph 20) according to these rules:

- (a) Graticule areas for counting must be chosen at random to avoid bias and to represent the whole exposed filter area. The fine focus must be adjusted upwards and downwards at each new area to ensure that all fibres are seen. If more than one-eighth of a graticule area is covered by an agglomerate of fibres and/or other particles, that area must be rejected and another selected for counting;
- (b) A countable fibre is defined as any object which is longer than 5 µm, with a width less than 3 µm and having an aspect (length/width) ratio greater than 3:1, which does not touch (or appear to touch) a non-fibrous particle with a maximum dimension greater than 3 µm. A countable fibre with both ends within the graticule area is recorded as one fibre. A countable fibre with only one end in the graticule area is recorded as half a fibre. A countable fibre passing through the graticule area, and having no ends within that area, is not counted;
- (c) A split fibre is taken to be one countable fibre if it meets the definition in (b), otherwise it should be ignored. A split fibre is defined as an agglomerate of fibres which at one or more points on its length appears to be solid and undivided, but at other points appears to divide into separate strands. The width is measured across the undivided part, not across the split part;

- (d) Fibres in a bundle are counted individually if they can be distinguished sufficiently to determine that they meet the definition in (b). If no individual fibres meeting this definition can be distinguished, the bundle is taken to be one countable fibre if the bundle as a whole meets the definition in (b).

Examples depicting countable and non-countable fibres, and which display one or more of the features described in (b) to (d) above, are given elsewhere in a comprehensive review.²³

39 The number of graticule areas counted depends on the sampling situation as follows:

- (a) For evaluations related to personal sampling in connection with compliance sampling or assessment of respirator protection, at least 100 fibres must be counted or 100 graticule areas must be inspected, whichever is reached first; at least 20 graticule areas must be inspected even if these contain more than 100 fibres;
- (b) For evaluations to the clearance indicator, background, reassurance and leak sampling, 200 graticule areas must be inspected on samples of the minimum of 480 litres volume (if the 0.010 f/ml detection limit is to be achieved). If the collected air volume (V) is more than 480 litres, the number (n) of graticule areas inspected may be reduced proportionately according to the formula $n = 96\ 000 / V$. For example, if 960 litres is collected, only one hundred graticule areas need be examined. It may not be necessary to examine n graticule areas if a clear decision can be reached at an earlier stage. For example, if 30 fibres in 200 fields would give a concentration of 0.015 f/ml, it may be possible to report an enclosure as unsatisfactory as soon as a count of 30 fibres is reached (even if only a few graticule areas have been examined). Where two or more samples are being pooled to obtain 480 litres (or a larger volume, as described in paragraph 29), V is the total volume of the pooled samples and n is the same number of graticule areas inspected on each of the pooled filters.

40 Care should be taken to ensure that the working practices and the working environment in a laboratory do not influence the accuracy of counts. Different seating arrangements may influence counts produced by different microscopists. Different practices of recording data also may cause some disagreement between counters due to eye fatigue. Detailed writing of data involves refocusing the eyes after each field, whereas continuous registering with electrical or mechanical counters involves only a single period of continuous concentration. Where possible, the environment should be vibration free and such that the microscopist can sit in a relaxed and comfortable manner. Any peripheral view beyond the microscope should, if possible, be an unobstructed distant view in unchanging subdued light to avoid eye fatigue; alternatively, a matt background shield can be used. Counting should not be performed in bright sunlight (because this may reduce contrast between fibres and background). Limits must be

placed on the amount of fibre counting undertaken by analysts in specified periods because fatigue can adversely affect the quality of counts, and the number of graticule areas examined in any 8-hour period should not normally exceed 2400, the equivalent of 12 samples if 200 graticule areas are examined in each. Counters are recommended to take 10-20 minute breaks from the microscope every one or two hours to limit fatigue. The length and frequency of breaks will depend on the microscopist, samples and laboratory conditions. The number of samples evaluated in a day differs from microscopist to microscopist: typically, counters may take 10-25 min to evaluate a sample with a sparse dust deposit, but longer for more difficult samples.

CALCULATION OF RESULTS

41 The airborne concentration is given by the formula

$$C = 1000 N D^2 / V n d^2 \text{ fibres per millilitre (f/ml)}$$

where: N is the number of fibres counted;
n is the number of graticule areas examined;
D (mm) is the diameter of the exposed filter area;
d (μm) is the diameter of the Walton-Beckett graticule; and
V (litres) is the volume of air sampled.

Where clearance measurement is obtained by pooling two or more samples, V is the total volume sampled, N is the total number of fibres and n is the number of graticule areas examined on each filter (which is the same for each filter and not the total number examined).

Reporting results

42 The following points should be noted in relation to measurements.

- (a) Sampling for comparison with control limits by the above method will give one sample and one result per measurement.
- (b) Sampling for comparison with action levels may give a series of 480 litre samples and a final sample of smaller volume. For each of these, a mean concentration applying to the sampled period in question will have been obtained. Additionally, there may be a number of periods for which the concentration can be estimated with reasonable accuracy, for example from previous measurements. These samples and estimates will be distributed over the 12 weeks to which the action level applies. For each period, sampled or estimated, the concentration (f/ml) is multiplied by the duration of the period (hr) to give a value in fibre-hours per millilitre (f-hr/ml) for the period. The values for all the periods are summed for comparison with the action level. Table 3 gives an example. If exposure is sometimes to amphibole asbestos types and sometimes to chrysotile, this calculation must be applied separately to these classes of asbestos and the results must be combined as explained in EH10.⁶

Table 3 Example of calculation for comparison with action level

Period number	Duration (hours)	Mean concentration (f/ml)	Measured or estimated (M or E)	Number of occurrences in 12 weeks	Duration x concentration x number
1	2	0.22	M	1	0.44
2	2	0.31	M	1	0.62
3-16	5	0.05	E	14	3.5
17	2	0.49	M	1	0.98
18	2	0.10	M	1	0.20
19 - 28	8	0.05	E	10	4.0
29 - 54	4	0.15	E	26	15.6
55	2	0.40	M	1	0.8
56	2	0.15	M	1	0.3

Total fibre hours per millilitre for comparison with action level: 26.44

- (c) Except in very small enclosures, sampling for comparison with the clearance indicator will yield two or more measurements, each based on a sample volume of at least 480 litres. This may be obtained by pooling smaller sample volumes as described in paragraph 26. The 'insulation ACOP'¹⁵ says that in most cases it is reasonably practicable to work in such a way that the concentration after cleaning, as measured by the method described in this MDHS, is less than 0.01 f/ml. The result from each measurement, whether from a single sample or pooled set of samples, should be compared with this value. At least 80% of the results should be less than 0.010 f/ml, and all should be less than 0.015 f/ml. Thus, in small enclosures requiring four or fewer samples, all should be less than 0.010 f/ml, but in larger enclosures one result in five may lie between 0.010 f/ml and 0.015 f/ml, and the ACOP details the action required if <0.010 f/ml is not achieved.

Reports of clearance indicator sampling should include:

- (i) an estimate of the enclosure area or volume;
- (ii) the number of measurements required;
- (iii) the number of samples taken;
- (iv) dust raising activities undertaken;
- (v) time started and finished for each sample;
- (vi) flowrate at the start, finish (and intermediate checks) for each sample;
- (vii) volume of each sample;
- (viii) number of graticule areas examined;
- (ix) measurement of the graticule diameter;
- (x) result from HSE/NPL test slide check;
- (xi) number of fibres counted;
- (xii) concentration result; and
- (xiii) limit of detection for each measurement.

The concentration result must be calculated correct to 3 decimal places to distinguish between 0.009 f/ml (which is acceptable) and 0.010 f/ml (which is unacceptable); the recommended reporting procedure is as follows:

Calculated result	Report as
Result <0.010 f/ml	<0.010 f/ml
0.010 ≤ Result ≤ 0.015	result to 3 decimal places
Result >0.015 f/ml	result to 2 decimal places

The conclusion with respect to the clearance status then should be drawn from the results obtained as indicated above. Examples of measurements for comparison with the clearance indicator are set out in Appendix 3. An example of part of a report for clearance indicator sampling is given (Fig 4), illustrating some of the information required by Guidance Note EH10.⁶

- (d) The laboratory should report the limit of detection of the calculated results.

ACCURACY

43 It is not possible to know the 'true' airborne fibre concentration of a given dust cloud and the absolute accuracy of the method cannot be assessed. However, some information is available about relative bias associated with sample evaluation. Microscopists generally undercount dense deposits. When sampling fibres in atmospheres relatively free from interfering particulates, the density range for optimum accuracy should be in the range 100-1000 fibres mm²;¹⁸ for densities above this, the results may be underestimates (but no attempt should be made to correct them). In mixed dust situations, the presence of other fibres and particles may interfere with the accuracy of results. Chance superimposition of non-fibrous particles may cause fibres not to be counted fully, by a proportion which depends on the mean size and concentration of the non-fibrous particles but not on the fibre concentration.²⁴ In practice, the effects of chance superimposition on counts are small compared with subjective effects and will not be important for the counting rules defined in this method. An important factor is that the microscopical counting procedure can result in systematic differences in counts produced by different microscopists within and, more particularly, between laboratories. Such differences must be controlled by proper training and periodic quality checks.

PRECISION

44 Counting precision depends on the number of fibres counted and on the uniformity of the fibre distribution on the filter. The latter may be described reasonably by the

95% Confidence Limits for Asbestos Fibre Counting

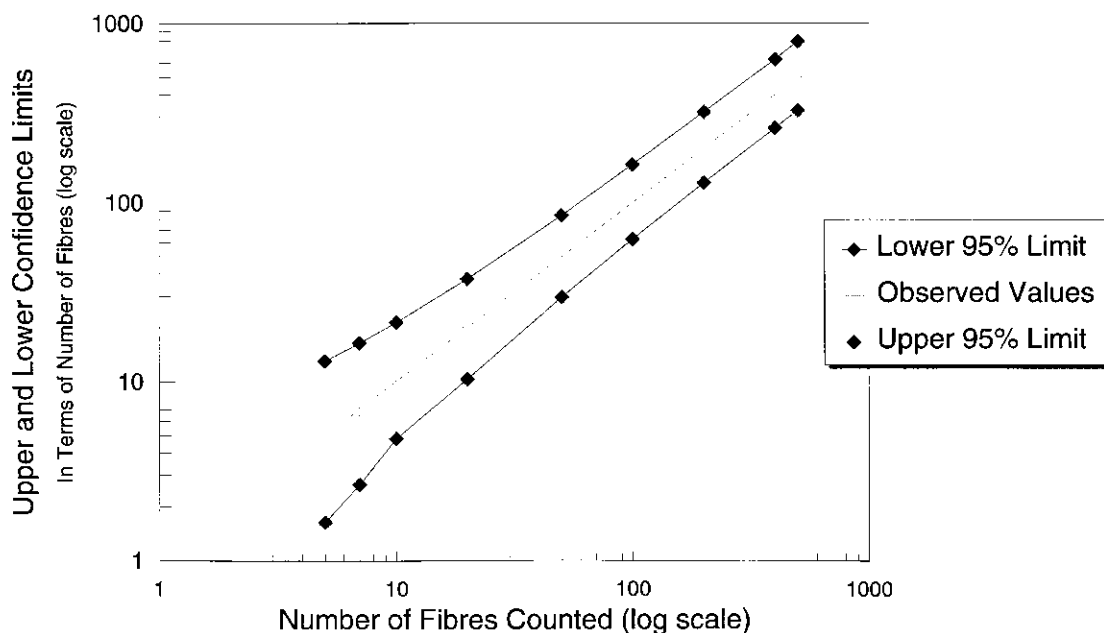


Figure 5 95% confidence limits for asbestos fibre counting

Poisson distribution. Theoretically, the process of counting randomly-distributed (Poisson) fibres gives a coefficient of variation (CV) = $1/\sqrt{N}$, where N is the number of fibres counted. Therefore the CV is 0.1 for 100 fibres and 0.32 for 10 fibres counted. In practice, however, the actual CV is greater than these theoretical numbers due to an additional component associated with subjective differences between repetitive counts by one microscopist and between replicate counts by different microscopists: this CV is given approximately by the formula $(N + 0.04N^2)^{1/2}/N$, where N is the mean number of fibres per evaluation.²⁵ If n fibres are found in a single evaluation, the mean of many repeated determinations on equal areas is expected to lie within the confidence limits $M_{97.5}$ and $M_{2.5}$ on 95% of occasions²⁵ where:

$$0.866 M_{97.5}^2 - (2n + 3.36)M_{97.5} + n^2 = 0,$$

$$0.745 M_{2.5}^2 - (2n + 6.39)M_{2.5} + n^2 = 0.$$

Table 4

N	Expected CV	Expected 95% confidence limits for the mean of repeated determinations	
		Lower	Upper
5	0.49	1.64	13.01
7	0.43	2.66	16.38
10	0.37	4.81	21.32
20	0.3	10.34	37.41
50	0.25	29.66	84.77
100	0.22	62.59	163.16
200	0.21	128.87	319.67

These equations have been used to calculate the upper and lower confidence limits shown in Table 4 and Figure 5. It can be seen from this that counting more than 100 fibres gives only a small increase in precision. Also, the method loses precision as fewer fibres are counted; this loss of precision increases as counts drop below about 10 fibres. Inter-laboratory CVs can be twice the intra-laboratory coefficients, or even greater if quality control is poor.

QUALITY CONTROL

45 Employers are required by the ACOPs^{4,5} to ensure that the laboratories which they use for the sampling and analysis of airborne asbestos meet the necessary standards. Guidance Note EH10⁶ gives details. Employers can satisfy this responsibility by using laboratories who hold NAMAS (National Accreditation of Measurement and Sampling) accreditation for asbestos sampling and asbestos fibre counting. NAMAS publishes documents^{26,27} which discuss accreditation for asbestos sampling and analysis (see also the list of suppliers in Appendix 2).

46 An essential part of quality assurance is participation in internal and external quality control schemes. This is particularly appropriate for this method because of the large differences in results within and between laboratories obtained with all manual fibre counting methods. Laboratories using this MDHS therefore must participate in the Regular Interlaboratory Counting Exchanges (RICE) scheme (see the list of suppliers in Appendix 2). This provides a measure of the laboratory's performance in relation to other counting laboratories.

Participation in RICE must be supplemented by checks on internal consistency which should aim to measure and control the individual counter's performance relative to other counters in the laboratory. The internal quality control scheme should incorporate the use of both reference samples (ie those which have a well defined result established as a mean of a number of determinations) and routine samples (ie those which have been analysed in the course of normal work). Participation and assessment of individual performance should be carried out at least once a month. Systematic records of quality control results must be maintained and the assessment of performance must be to a defined set of criteria.

47 If it is suspected that the HSE/NPL Mark II Test Slide has deteriorated in quality due to damage or wear, it should be re-evaluated. HSL should be contacted for advice (see paragraph 48).

ADVICE

48 Advice on this method may be obtained from the Health and Safety Laboratory (HSL), Broad Lane, Sheffield S3 7HQ (tel: 0114 2892000). Suggestions for improvement should be sent to the same address.

ACKNOWLEDGEMENT

49 Revision of this document was overseen by a Working Group of the Committee on Fibre Measurement, consisting of Mr BE Tylee (HSL), Mr J Michell (HSL), Dr NP Crawford (IOM), Mr T Shenton-Taylor (UKAS), Mr R Webster (ECOS) and Mr B Wilkinson (Wilkinson Environmental Services Ltd).

REFERENCES

1 *The Control of Asbestos at Work Regulations 1987* SI 1987 No 2115 HMSO 1988 ISBN 0 11 078115 5

2 *The Control of Asbestos at Work (Amendment) Regulations 1998* SI 1998 No 3235 Stationery Office 1999 ISBN 0 11 080277 2

3 Health and Safety Executive *Asbestos: Medical Guidance Note MS13* HSE Books 1999 (3rd edition) ISBN 0 7176 2417 X

4 Health and Safety Executive *The control of asbestos at work. The Control of Asbestos at Work Regulations 1987. Approved Code of Practice L27* HSE Books 1999 (3rd ed) ISBN 0 7176 1673 8

5 Health and Safety Executive *Work with asbestos insulation, asbestos coating and asbestos insulating board. The Control of Asbestos at Work Regulations 1987. Approved Code of Practice L28* HSE Books 1999 (3rd ed) ISBN 0 7176 1674 6

6 Health and Safety Executive *Asbestos: exposure limits and measurement of airborne dust concentrations*

Guidance Note EH10 HSE Books 1995 (rev) ISBN 0 7176 0907 3

7 Health and Safety Executive *Working with asbestos cement* HSG189/2 HSE Books 1999 ISBN 0 7176 1667 3

8 Health and Safety Executive *Work with asbestos cement* Guidance Note EH36 HSE Books 1990 (rev) ISBN 0 11 885422 4 (superseded by reference 7 above)

9 Health and Safety Executive *Work with asbestos insulating board* Guidance Note EH37 HSE Books 1989 (rev) ISBN 0 11 885423 2 (superseded by reference 7 above)

10 Health and Safety Executive *The provision, use and maintenance of hygiene facilities for work with asbestos insulation and coatings* Guidance Note EH47 HSE Books 1990 (rev) ISBN 0 11 885567 0

11 Health and Safety Executive *Enclosures provided for work with asbestos insulation, coatings and insulating board* Guidance Note EH51(rev) HSE Books 1999 ISBN 0 7176 1700 9

12 Department of the Environment *Asbestos and man-made mineral fibres: Practical guidance* Thomas Telford 1999 ISBN 0 7277 2835 0

13 Rooker SJ, Vaughan, NP and Le Guen, JMM The visibility of fibres by phase contrast microscopy *Am Ind Hyg Assoc J* **43** 1982 505-515

14 Vaughan, NP, Rooker, SJ and LeGuen, JMM 'In situ identification of asbestos fibres collected on membrane filters for counting' *Ann Occup Hyg* 1981 **24** (3) 281-290

15 LeGuen, JMM, Rooker, SJ and Vaughan, NP 'A new technique for scanning electron microscopy of particles collected on membrane filters' *Environ Sci Technol* 1980 **14** (8) 1008-1011

16 Burdett, JG and Rood, AP 'Membrane filter, direct transfer techniques for the analysis of asbestos fibres or other inorganic particles by transmission electron microscopy' *Environ Sci Technol* 1983 **17** (11) 643-648

17 ISO/TC 146/SC 3 *Ambient air: determination of asbestos fibres: direct transfer transmission electron microscopy procedure*

18 Cherrie, JW, Jones, AD and Johnston, AM The influence of fibre density on the assessment of fibre concentration using the membrane filter method *Am Ind Hyg Assoc J* **47** (8) 1986 465-474

19 World Health Organisation *Determination of airborne fibre number concentrations: A recommended method by phase contrast optical microscopy (membrane filter method)* WHO 1997 ISBN 924 1544 961

20 Walton, WH and Beckett, ST A microscope eyepiece graticule for the evaluation of fibrous dusts *Ann Occup Hyg* **20** 1977 19-24

21 British Standards Institution BS7011 *Consumable accessories for light microscopes Part 2 Slides and Part 3 Cover glasses: specifications for dimensions and optical properties* BSI 1989 ISBN 0 580 16816 6 and 0 580 16823 9 respectively

22 Institute of Occupational Hygienists *Operating code of practice for asbestos clearance certification* Report Number 10 A May 1988 (rev)

23 Walton, WH 'The nature, hazards and assessment of occupational exposure to airborne asbestos dust: a review' *Ann Occup Hyg* 1982 **25** (2) 117-247

24 Iles, PJ and Johnston AM 'Problems of asbestos fibre counting in the presence of fibre-fibre and particle-fibre overlap' *Ann Occup Hyg* 1984 **27** (4) 389-403

25 Ogden, TL *The reproducibility of asbestos counts* HSE Research Report No 18 HMSO 1982 ISBN 0 11885716 9

26 National Accreditation of Measurement and Sampling *Asbestos analysis: accreditation for fibre counting and identification by the use of light optical microscopy* NIS 11 NAMAS Executive 1997 (2nd ed)

27 National Accreditation of Measurement and Sampling *Accreditation for asbestos sampling* NIS 24 NAMAS Executive 1992

HSE priced and free publications are available by mail order from:

HSE Books
PO Box 1999
Sudbury
Suffolk CO10 2WA
Tel: 01787 881165
Fax: 01787 313995

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APPENDIX 1 The Health and Safety at Work etc. Act 1974; Control of Asbestos at Work Regulations 1987

Notice of Approval

The Health and Safety Commission has on 15 December 1987 approved the methods of measurement and calculation set out in the Schedule to this notice for the purpose of determining whether the concentrations and cumulative exposures of asbestos in air respectively exceed the control limits and action levels specified in Regulation 2(1) of the Control of Asbestos at Work Regulations 1987.

Signed AJ Lord
Secretary to the Health and Safety Commission
17 December 1987

Schedule

Sampling method

1 Samples for assessment shall be taken by drawing a known volume of air through a 25 mm cellulose ester membrane filter with a printed grid and of pore size 0.8 to 1.2 μm using a battery operated pump. The pump shall give a smooth flow and the flowrate shall be maintained within $\pm 10\%$ of the initial rate during the sampling period. The sampling time shall be measured to within 2%.

2 An open-faced filter holder fitted with an electrically conducting cylindrical cowl shall be used. The cowl shall extend between 33 mm and 44 mm in front of the filter and expose a circular area of the filter at least 20 mm in diameter. In use the cowl shall point downwards.

3 Filters shall be loaded into holders, unloaded and analysed in an area free from asbestos contamination. During transport through contaminated areas the entrance to a loaded holder and cowl shall be sealed.

4 The pump shall be attached to a loaded filter holder using flexible tubing and allowed to run at approximately 1 litre/min until the flowrate is stable. This step may be omitted if the pump is such that the flowrate is stable when the pump is switched on. Either contamination of the filter during warm-up shall be prevented or the filter used during warm-up shall be discarded.

5 The filter holder shall be fixed to the worker's clothing as close to the mouth and nose as possible, but in any case within 200 mm of them. The pump shall be carried by the worker on a belt or in a pocket.

6 At the end of the sampling period, the filter shall be placed in a clean container for transport, or transported in the filter holder which shall be sealed to prevent contamination. Fixatives shall not be used on the filter.

Filter clearing and mounting

7 The filter shall be placed on a microscope slide and cleared by immersion in acetone vapour. The sample

shall be treated with triacetin and covered with a glass cover slip, using sufficient triacetin to cover the whole filter when the cover slip is in place.

Microscope specification

8 A binocular phase contrast microscope shall be used for counting and shall have the following features:

- (a) Koehler or Koehler-type illumination;
- (b) a substage assembly incorporating an Abbe or achromatic phase contrast condenser in a centring focusing mount, with a phase contrast centring adjustment independent of the condenser centring mechanism;
- (c) a 40x positive phase contrast achromatic objective with a numerical aperture of 0.65 to 0.70 and phase ring absorption within the range 65 to 80%;
- (d) eyepieces of the wide field type with a magnification of at least 12.5x; at least one shall be of the focusing type and permit the insertion of a graticule;
- (e) a Walton-Beckett circular eyepiece graticule with an apparent diameter in the object plane of 100 µm ± 2 µm when using the specified objective and eyepiece, checked against a stage micrometer.

Evaluation of samples

9 At the beginning of each day the microscope shall be set up according to the manufacturer's instructions and the detection limit checked using an HSE/NPL phase contrast test slide Mark 2. The microscopist shall be able to see group 5 on the slide when the microscope is used as specified by the manufacturer. The object-plane diameter of the Walton-Beckett graticule shall be checked at suitable intervals using a stage micrometer, and the measured diameter shall be used in calculations.

10 Samples shall be counted in accordance with the following rules:

- (a) a countable fibre is any object which is longer than 5 µm with a width of less than 3 µm and a length:width ratio greater than 3:1 which does not touch or appear to touch a particle with a maximum dimension greater than 3 µm;
- (b) a countable fibre with both ends within the graticule area shall be counted as one fibre; a countable fibre with only one end within the area shall be counted as half a fibre;
- (c) graticule areas for counting shall be chosen at random within the exposed area on the filter;
- (d) an agglomerate of fibres which at one or more points on its length appears solid and undivided but at other points is divided into separate strands (a split fibre) is counted as a single fibre if it conforms to the

definition in 10(a), the diameter measured being that of the undivided part, not that of the split part;

- (e) in any other agglomerate of fibres in which individual fibres touch or cross each other (a bundle), the fibres shall be counted individually if they can be distinguished sufficiently to determine that they conform to the definition in 10(a); if no individual fibre meeting the definition can be distinguished, the bundle shall be considered to be a countable fibre if, taken as a whole, it conforms to the definition;
- (f) if more than one eighth of a graticule area is covered by an agglomerate of fibres and/or particles, the graticule area shall be rejected and another counted;
- (g) at least 100 fibres shall be counted or 100 graticule areas examined, but in any case at least 20 graticule areas shall be examined.

11 The effect on the count of marks on the filter and contamination shall be kept below 3 fibres/100 graticule areas and shall be assessed using blank filters.

12 The airborne concentration is given by:

$$(1000ND^2)/(Vnd^2) \text{ fibres/ml}$$

where N = number of fibres counted;
n = number of graticule areas examined;
D (mm) = diameter of exposed area of filter;
d (µm) = diameter of Walton-Beckett graticule as measured with a stage micrometer;
V (litres) = volume of air sampled.

Sampling period and flowrate

13 When a concentration is to be compared with a control limit specified as an average over a 4 hour period, the pump shall be capable of being set to 1 litre/min ±5% through the membrane filter used, and shall be set at this flowrate at the beginning of the sampling period. The sampling period shall be either:

- (a) a continuous period of 4 hours ; or
- (b) a shorter period which is nevertheless:
 - (i) representative of the exposure during the 4 hour period for which an average is to be calculated; and
 - (ii) sufficient for the expected fibre density on the filter to lie within or as close as possible to the range 100 to 400 fibres/mm².

14 When a concentration is to be compared with a control limit specified as an average over a 10 minute period, the sampling time shall be 10 minutes. A flowrate of up to 8 litres/min shall be used such that the expected fibre density on the filter lies within or as close as possible to the range 100-400 fibres/mm².

15 When a measured concentration is to form part of a calculation of cumulative exposure for comparison with the action level, a flowrate of 1 to 8 litres/min and an appropriate sampling time shall be used such that the

expected fibre density on the filter lies within or as close as possible to the range 100 to 400 fibres/mm², but in any case the sampling period need not be longer than 4 hours.

Action level for mixed exposure

16 Where a cumulative exposure to be compared with the action level is made up partly of exposure to amphibole asbestos or mixtures containing amphibole asbestos and partly of exposures to chrysotile only, the action level shall be either:

- (a) the action level for amphiboles asbestos minerals; or
- (b) deemed to be exceeded if

$$\frac{\text{Exposure 1}}{\text{AL1}} + \frac{\text{Exposure 2}}{\text{AL2}} > 1$$

Exposure 1 = the cumulative exposure to amphibole asbestos minerals and mixtures of them with chrysotile

Exposure 2 = the cumulative exposure to chrysotile alone

AL1 = the action level for amphibole asbestos minerals and mixtures of them with chrysotile

AL2 = the action level for chrysotile alone

APPENDIX 2 Suppliers of equipment and services

Acetone 'hotblock' vaporiser

Casella London Ltd
Regent House
Wolseley Road
Kempston
Bedford MK42 7JY

J S Holdings
Unit 5
Willows Link
Stevenage
Hertfordshire SG2 8AB

Filters

Millipore (UK) Ltd
The Boulevard
Blackmore Lane
Watford
Hertfordshire WD1 8YW

Filter tins

Barnsley Canister Co Ltd
Sackville
Barnsley
South Yorkshire S70 2DF

HSE/NPL Test Slide Mark II

Health and Safety Laboratory
Broad Lane
Sheffield S3 7HQ

Optometrics (UK) Ltd
Unit C6
Cross Green Garth
Leeds LS9 0SF

NAMAS accreditation

United Kingdom Accreditation Service
Queens Road
Teddington
Middlesex TW11 0NA

RICE inter-laboratory QC scheme

Institute of Occupational Medicine
8 Roxburgh Place
Edinburgh ES8 9SU

Walton-Beckett graticules and stage micrometers

Graticules Ltd
Morley Road
Tonbridge
Kent TN9 1RN

APPENDIX 3 Examples of measurement related to the clearance indicator

Example 1

Enclosure area 20 m², height 2.5 m.

Enter A = 20 in the formula ($A^{1/3} - 1$) (see paragraph 29).

The whole number next below ($A^{1/3} - 1$) is one, but this is overridden by the minimum requirement to generate two measurements, each based on a total volume sampled of at least 480 litres, unless the enclosure volume <10m³.

Take two samples at 8 l/min for 60 min (480 litres/sample). Diameter of exposed area of filters D = 22 mm; diameter of Walton-Beckett graticule = 99 µm. Referring to paragraph 39(b), 200 graticule areas must be examined in each case.

Sample A. 17 fibres found. Referring to paragraph 41, N = 17, D = 22mm, V = 480 litres, n = 200 and d = 99 µm. Use the formula in paragraph 41 to calculate the concentration.
Concentration = $(1000 \times 17 \times 22^2) / (480 \times 200 \times 99^2) = 0.009$ fibres/ml.

Sample B. 24 fibres found. As sample A, but N = 24.
Concentration = $(1000 \times 24 \times 22^2) / (480 \times 200 \times 99^2) = 0.012$ fibres/ml.

Because only two measurements are required and one of these results exceeds 0.010 fibres/ml, the laboratory cannot recommend that clearance is satisfactory.

Example 2

Enclosure area 30 m².

Enter A = 30 in ($A^{1/3} - 1$) (see paragraph 27). ($A^{1/3} - 1$) = 2.1 in this case. Therefore, two measurements are required, each based on a total volume sampled of at least 480 litres. It is decided to use small pumps and to generate each of these measurements by pooling two samples using the procedure in paragraph 29. Thus, measurement A results from two filters, each exposed within 1 m of each other at 4 l/min for 60 min, and measurement B results from two filters, each exposed within 1 m of each other at 4 l/min for 60 min.

For each measurement, V (total volume) = 480 litres. From paragraph 39(b) it is necessary to analyse 200 graticule areas on each filter (n = 200). In each case, exposed filter diameter D = 23 mm, Walton-Beckett graticule diameter d = 101 µm.

Measurement A. 8 fibres counted on one filter, and 4 on the other.
N = 8 + 4 = 12. Use the formula in paragraph 41.
Concentration = $(1000 \times 12 \times 23^2) / (480 \times 200 \times 101^2) = 0.006$ fibres/ml.

Measurement B. 7 fibres counted on one filter and 9 on the other.

N = 7 + 9 = 16. Use the formula in paragraph 41.
Concentration = $(1000 \times 16 \times 23^2) / (480 \times 200 \times 101^2) = 0.009$ fibres/ml.

Both of these results are less than 0.010 fibres/ml, so the laboratory can report that the airborne concentrations do not exceed the clearance indicator.

Example 3

Enclosure is more than 3 m high. Volume is 190 m³. Enter A = 190/3 = 63.3 in ($A^{1/3} - 1$) = 2.99. Taking the whole number next below 2.99 means that two measurements, each based on a total volume sampled of at least 480 litres, are required. After consideration of pump and counter availability, it is decided to generate one measurement at a flowrate of 6 l/min and the other at 8 l/min, each for 100 min.

Measurement A. V = 6 x 100 litres. From paragraph 39, n = 160 graticule areas must be examined. N = 22 mm, Walton-Beckett graticule diameter = 100 µm. Use the formula in paragraph 40.
Concentration = $(1000 \times 29 \times 22^2) / (600 \times 160 \times 100^2) = 0.015$ fibres/ml.

Because this exceeds 0.010 fibres/ml, clearance cannot be given and it is unnecessary to examine the other sample. Had it been necessary, the volume V = 8 x 100 = 800 litres, so from paragraph 39 it would only have been necessary to examine n = 120 graticule areas on this filter.

Example 4

Enclosure is more than 4 m high. Volume is 7500m³. Enter A = 7500/3 = 2500 in ($A^{1/3} - 1$). ($A^{1/3} - 1$) = 12.6. Twelve measurements are required. Evaluating as in previous samples, the results obtained are: 0.008, 0.008, 0.004, 0.014, 0.003, 0.010, 0.002, 0.009, 0.008, 0.007, 0.004, 0.003 fibres/ml.

At least 80% of these results are less than 0.010 fibres/ml, and all are less than 0.015 fibres/ml, so under the terms of paragraph 42(c) the air in this enclosure is acceptably clean.

Example 5

One sample is taken to detect a suspected leak from an enclosure. Only 240 litres are taken and only 100 fields are examined; 10 fibres are counted. Applying the formula in paragraph 41:
Concentration = $(1000 \times 10 \times 22^2) / (240 \times 100 \times 99^2) = 0.021$ fibres/ml

However, because the recommended volume and fields were not taken, the limit of detection = $(96\ 000 / V n) \times 0.01 = (96\ 000 / 240 \times 100) \times 0.01 = 0.04$ fibres/ml, and not 0.010 fibre/ml.

APPENDIX 4 Correction of flowrate for pressure and temperature differences

If differences in ambient temperature and/or pressure between the calibration and sampling sites are greater than 5%, a correction should be made to the flowrate.

Working flowmeters are used in widely varying environmental conditions. All air sampling measurements are concerned with volumetric flow (ie flowrate measured and expressed at the prevailing temperature and pressure) and not mass flowrate (ie flowrate corrected to standard temperature and pressure). Re-calibration or correction of flowrate is therefore essential if the pump is operated under conditions substantially different from those of calibration (eg differences in altitude). If possible, calibration should be conducted at the sampling site. If this is not possible, a correction may have to be made if the pump is affected by temperature and pressure changes. The actual flowrate will be given by:

$$Q_A = Q_C \sqrt{\frac{P_c T_a}{P_a T_c}}$$

where Q_A = actual flowrate

Q_C = calibrated low rate (the rotameter value)

P_c = air pressure at site of calibration

P_a = air pressure at sampling site

T_a = air temperature at sampling site ($^{\circ}K$)

T_c = air temperature at site of calibration ($^{\circ}K$)

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