HSE
Health and Safety Executive

Machine-made fibres

Airborne number concentration and classification by phase contrast light microscopy

MDHS59/2

Methods for the Determination of Hazardous Substances
Health and Safety Laboratory

Scope

1 The method described here may be used to measure the time-weighted average, personal airborne number concentration or fixed-point concentrations of respirable machine-made mineral fibres (MMFs), defined as fibres longer than 5 µm, narrower than 3 µm, with a length to width ratio greater than 3:1, and visible by the phase contrast technique. All fibres meeting this definition should be counted. Fibres with widths of less than 0.2 µm may not be visible using the techniques described here.

2 If it is necessary to differentiate between fibre types, then the procedures described in *Asbestos: The analysts' guide for sampling, analysis and clearance procedures* (HSG248)[1] may be used.

3 Workplace exposure limits (WEL, 8-hr TWA) exist for MMF[2] either as the respirable airborne number concentration or as inhalable dust determined gravimetrically, using MDHS14.[3] The approach adopted will depend on which of the exposure limit values would be exceeded first (see paragraph 6). It is almost always possible to deduce which will be the appropriate measurement from the type of fibre in use.

Summary

4 Airborne respirable fibres are collected by drawing a measured volume of air through a membrane filter mounted in an open-faced filter holder fitted with an electrically-conducting cowl. For fibres with a refractive index (RI) >1.51 the membrane filter is cleared using the acetone-triacetin technique before counting using phase contrast microscopy (PCM), while those with RI ≤1.51 are plasma etched after acetone clearance to enable satisfactory counting using PCM.

Airborne MMF and its measurement

5 Most MMF production processes give a wide range of particle sizes, with median diameters of a few micrometres in the bulk material. However, in MMF aerosols the median number diameter would be less than 1 micron, because the small diameter fibres stay airborne longer. On average, fibres with greater diameters tend to be longer, but there is a wide range of lengths at each diameter. In some production processes, sub-micrometre fibres can be rare.

6 It is not necessary to monitor routinely using both gravimetric and number concentration, but only by the method best suited to the material in question. The relevant airborne limit is the one which would be exceeded first if exposure were increased, either by raising the mass of dust or the fibre count in the atmosphere.

In most cases it will be the gravimetric limit. It will almost always be possible to deduce which is the appropriate limit from the type of fibre being used:

(a) For mineral wools (and for almost all ceramic fibres), it may be assumed that the gravimetric limit value will be reached before the number concentration value, and only gravimetric measurements will be needed.
(b) For special-purpose or superfine fibres, the airborne number concentration will usually be reached first.
(c) For continuous filament fibres, it is unlikely that the airborne number concentration limit will ever be reached.

7 The sampling flow rate should lie in the range 0.5 to 16.0 $l.min^{-1}$ and its duration will be determined by the purpose of the measurement being undertaken.

8 For testing compliance against the WEL,[2] an 8-hour time-weighted average concentration may be needed (eg 0.5 $l.min^{-1}$ for the whole shift), which may also be derived from two or more consecutive samples. A much shorter sampling duration (eg 8 $l.min^{-1}$ for 15 minutes) may be appropriate for task-specific sampling.

9 The precision of the analysis step depends primarily on the number of fibres counted so that the sample volume should be chosen where possible to keep the fibre density on the filter between 50 and 1000 $fibres.mm^{-2}$. Where low volumes are unavoidable, increasing the area of filter examined may increase the number of fibres counted, but this should not be taken beyond 200 graticule areas because operator fatigue may affect the result.

10 When employing fixed point sampling, the sampling cowls should be positioned at approximately 1–2 m above floor level, away from any walls or large obstructions. The sampling procedures are otherwise the same as for personal sampling.

Prerequisites

11 Users of this method will need to be familiar with the content of the World Health Organisation (WHO) fibre counting method[4] and other relevant documents, eg *Monitoring strategies for toxic substances* (HSG173),[5] *Asbestos: The analysts' guide for sampling, analysis and clearance procedures* (HSG248)[1] and MDHS14/4.[3]

Safety

12 Users of this method should be familiar with normal laboratory practice and carry out a suitable risk assessment. It is the user's responsibility to establish appropriate health and safety practices and to ensure compliance with regulatory requirements.

Equipment

Filter holder

13 This should be open-faced, 25 mm in diameter and fitted with an electrically-conducting cylindrical cowl extending between 33 mm and 44 mm in front of the filter, and exposing a circular area of at least 20 mm in diameter. There should also be a cap for the cowl entrance, to protect the filter from contamination when not sampling or during transportation. A suitable design can be seen in Figure 1 but other designs meeting the above requirements are permitted. The filter holder should be cleaned and operated according to the manufacturer's instructions.

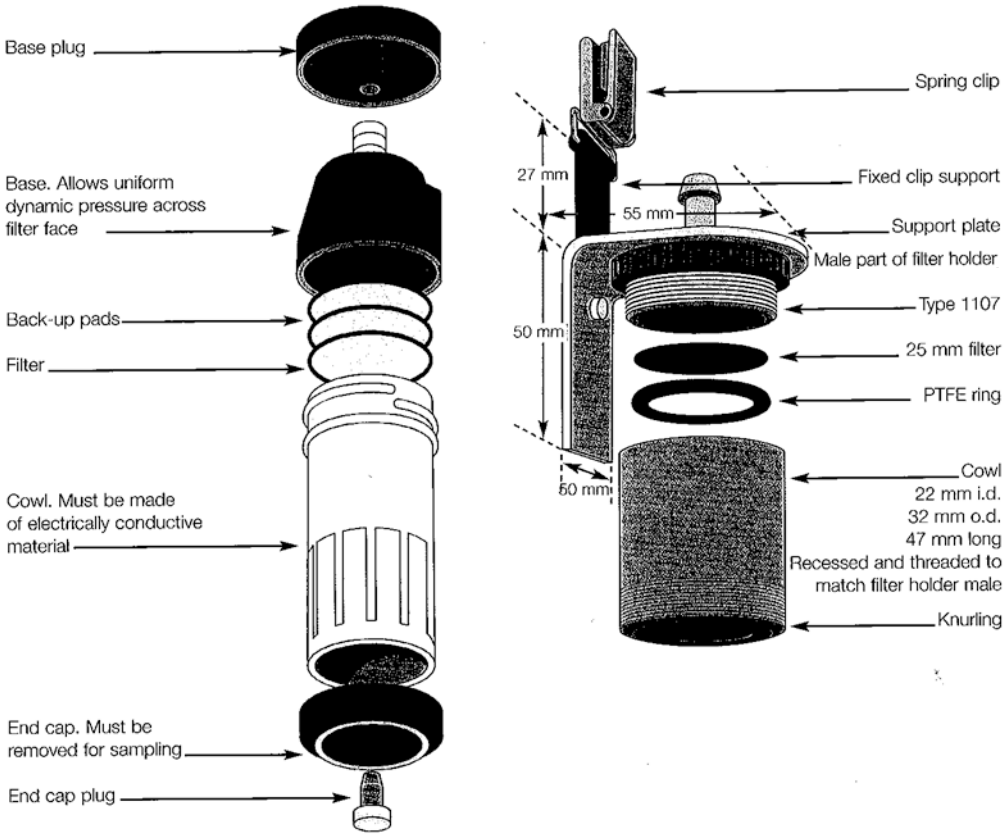

Figure 1 Exploded view of a filter holder with electrically conducting sampling cowl

14 This type of holder is intended to protect the filter, while still permitting a uniform deposit. Even though the sampling head is electrically conducting, the more statically charged the fibre being sampled the more is deposited onto the inner surface of the cowl.[6]

15 In some situations, up to 25% of the airborne respirable fibres can be deposited on the cowl. In these circumstances, the cowls should be capped and retained. After analysis, the cowl from the sample with the largest concentration of fibres should be selected, and the inner surface carefully washed with purified water into a filtration apparatus, with the washings captured onto another filter. This can then be dried, and mounted as described for normal samples. The fibre count on this filter can then be added to the airborne one; if it makes a significant contribution to the total, then consider treating all the cowls in this fashion.

Membrane filter

16 The sampling filter should be a 25 mm diameter, mixed esters of cellulose or cellulose nitrate filter with pore size 0.8–1.2 µm and have a printed grid. The exposed area must be known and should be measured at least every time a cowl type 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.

17 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 (x10) magnification while a diameter of the dark area is traversed by moving the stage. Two diameters should be measured at right angles, and three filters in separate holders should be checked by this method. An uneven appearance of the deposit may show that there is a leak in the sampling head.

Personal sampling pump

18 This must meet the criteria for a Type P pump[7] and be capable of delivering a smooth flow within ±5% of the required flow rate and maintaining this flow rate through the filter to within ±10% (preferably ±5%) during the sampling period.

Flow meter

19 This should be a portable flow meter calibrated against a primary standard at the flow rates of interest, with a measurement uncertainty of less than ±2.5%.

20 Float-type flow meters should be vertical when read. Under normal operating conditions, the measurement of temperature and pressure is not necessary, as it will only make a small difference to the total uncertainty. In the UK, it is therefore not necessary to make corrections to sample volume due to changes in atmospheric temperature and pressure.

21 The length of the flow meter tube, the range of airflow covered and the spacing and number of markings will directly affect the accuracy of reading and the calibration. The airflow and hence the float, must be sufficiently stable in the flow meter tube to enable a precise reading against the tube markings to be taken. From a practical point of view, to set the flow rate to ±10% at 0.5 $l.min^{-1}$ (the minimum recommended value) a minimum tube distance of 10 mm for each 1 $l.min^{-1}$ division is required. This means that the pump flow must be sufficiently stable and adjustable so the float must be able to be positioned and read to within ±0.5 mm of the 0.5 $l.min^{-1}$ flow mark.

22 Longer distances between the markings and the markings at higher flow rates, will give a proportionate increase in the accuracy of reading. A float-type flow meter tube must be marked with an appropriate number and scale of markings to allow the flow rate to be set, within the limits defined in paragraph 18. In general, the primary standard flow meter should have at least twice the tube spacing (eg 0–1 $l.min^{-1}$ >20 mm for flows between 0.5–2 $l.min^{-1}$) as the field flow meters and have sufficient marks on the flow tube to enable sufficiently accurate readings to be made, to calibrate the field flow meter to within ±2.5%.

Other equipment

23 Use a suitable adapter to connect the sampling head to the calibrated flow meter in order to set the flow rate through the sampler accurately.

24 There should be flexible plastic tubing for making a leak-proof connection from the sampler to the sampling pump.

25 A belt or harness should facilitate attachment of sampling pump and sampler to the subjects.

26 Use flat-tipped tweezers for loading and unloading the filters into sampler cassettes.

27 Clean, well-fitting filter tins or cassettes may be used to transport the filters if transport in the capped filter holders is impractical.

Laboratory apparatus and reagents

28 Analytical grade reagents are not essential.

29 Acetone should be free from excessive water.

30 Glycerol triacetate (triacetin) should be free from dust, fibres and moisture (hydrolysis may be indicated by an acetic acid smell). Triacetin is used to provide the interface between the collapsed filter and the cover slip. The mounted slide will keep for years without deterioration, although small-scale movement will occur.

31 Use cargille liquid (RI = 1.51 or immersion oil RI 1.515).

32 Use ultra-pure or double distilled water.

33 Hot block acetone vaporiser should be used (this is commercially available, see Figure 2). It should be operated according to the manufacturer's instructions.

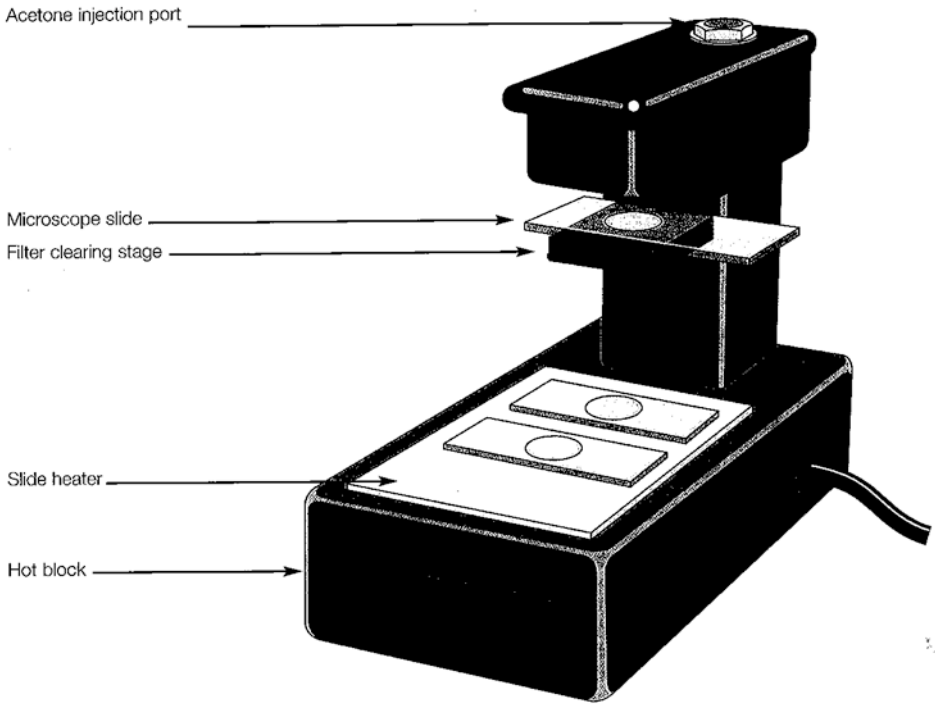

Figure 2 Typical hot block acetone vaporiser for clearing filters

34 Micropipettes or syringe to dispense acetone (0.25 ml) and triacetin (0.1 ml).

35 Plasma etching oven (for etching the filter surface if RI <1.51).

Microscopy

36 The visibility of fine fibres by PCM is dependent on the quality and transparency of the mounted filter, the quality and cleanliness of the microscope's optics, its correct use and maintenance, the operator's eyesight and other factors. Differences between the smallest fibre width observable by phase contrast microscopes will contribute to differences between counters (because fibre diameter distributions can extend below the detection limit for some fibre types). To maintain a uniform level of detection at the limit of visibility, the microscope and accessories should comply with the following specifications:

(a) a binocular stand with Köhler, or Köhler-type, illumination including a field iris (the condenser (sub-stage assembly), objectives and eyepieces specified below must all be compatible with each other and with this stand);
(b) a sub-stage assembly, incorporating an Abbe or an achromatic phase contrast, condenser in a centreable focusing mount with phase annulus centring independent of the condenser centring mechanism;

(c) a built-in mechanical stage with slide clamps and x–y displacement;
(d) a low-powered objective (eg X10 or X4 magnification), which is used for carrying out checks on the evenness of the dust deposit on the filter and locating the stage micrometer and test slide 'tramlines';
(e) a positive phase contrast objective (preferably par focal with the low-powered objective) of magnification X40. 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%;
(f) an optically matched pair of binocular eyepieces, preferably of the wide field, high eye-point type, providing a total magnification of at least X500 (one of the eyepieces must be of the focusing type and must permit insertion of a graticule), some microscope stands may include a tube extension, which increases the total magnification. The total magnification is calculated by multiplying the objective, tube extension and eyepiece magnifications together. This total should not exceed 1000 times the NA;
(g) a Walton-Beckett eyepiece graticule, type G22, with an apparent diameter in the object plane of 100 ±2 µm (when checked against a calibrated stage micrometer) must be used to define the counting area;
(h) various accessories including;
    (i) a phase telescope or Bertrand lens to ensure correct alignment of the phase rings;
    (ii) a green filter (optional) which assists viewing (as the optics are optimised for green light);
    (iii) a calibrated stage micrometer of 2 µm divisions (eg type S12);
    (iv) an HSE test slide (Figure 3, which has been assessed as having band 4 or 5 visible, when the phase contrast microscope is correctly adjusted).

37 The coverslip and slide will also affect the visibility of fine fibres. Both must be of glass and of appropriate thickness. Microscope slides must be of conventional type, eg approximately 76 mm x 25 mm and preferably 0.8 mm to 1.0 mm thick. The coverslip thickness is specified/marked on the objective (eg 0.17) and the appropriate thickness must be used (usually sold as 0.16 to 0.19 mm thick, eg № 1½) and should be about 25 mm in diameter or 25 mm square. The microscope slides and coverslips should be clean and conform to relevant standards.

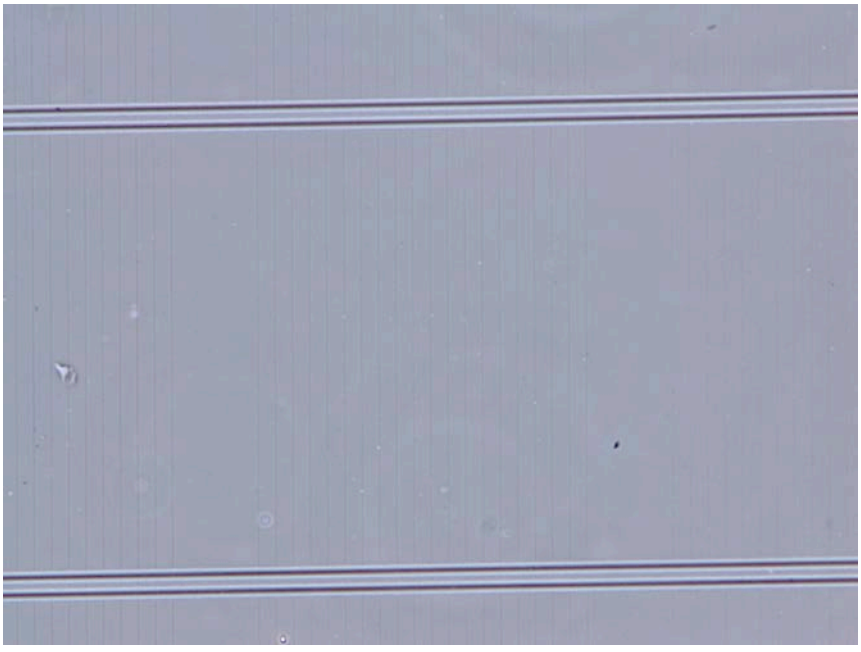

Figure 3 View under phase contrast microscopy of visible blocks on a HSE mark II/ mark III test slide

Preparation and sampling

Blanks

Sampling media blanks

38 For each new batch of filters, before sampling, select at least four blank filters (or a minimum of 1% from larger batches) for quality and background fibrous contamination checks. Individual blank filter counts should not normally exceed 3 fibres per mm^2 (2½ fibres per 100 fields) and should be investigated and rejected if found to be so.

39 In addition, when carrying out a sampling exercise, for every 25 filters (or part of 25) used at least one filter must be reserved to act as a counting blank.

Field blanks

40 Field blanks from a satisfactory batch of filters are generated when subjected to the same treatment as filters used for sampling apart from the actual period of sampling. The designated field blank (in capped, cowled filter holder) should be taken to the sampling area and the cap removed and replaced after a few seconds.

41 A field blank should normally be nominated for each job or for longer jobs, each day of sampling activity is undertaken. These must be mounted and retained alongside the actual samples.

42 Field blanks need only be counted if all of the actual samples have more than 20 fibres counted. If counts on field blanks are high (ie more than 20 fibres counted) all samples should be rejected and resampling carried out.

Laboratory blank

43 A laboratory blank from a satisfactory batch may be evaluated with each batch of routine samples, or afterwards, if contamination due to laboratory sources is suspected.

Use of blanks

44 The type and number of blanks that are available for analysis, and are analysed, will depend on a number of factors.

45 The sampling organisation is responsible for initiating field blanks and these should be labelled accordingly to ensure they can be identified. The on-site analyst should always ensure that at least one field or one laboratory blank is prepared for each batch or group of samples, so that, if necessary, the source of any fibre contamination can be checked to determine whether it was due to the filter preparation.

46 Normally, if low counts are obtained from some of the field samples it will not be necessary to analyse the field or laboratory blanks. If elevated counts are obtained on all the field samples, at least one field blank (or if not available, one laboratory blank) must be counted for each batch to exclude the possibility of contamination. The source of any blank contamination should be investigated and the batch-to-batch consistency of membrane filters monitored.

47 Blank counts must not be subtracted from sample counts. The WHO method[4] calls for subtraction of the blank count but this guidance has not been adopted in

this procedure except as an additional stage, if contamination on the blanks has been found. Evidence shows that the blank count should normally be low and will make little difference to compliance measurements.

Sampler preparation

48 Handle the filters by the edges at all times using tweezers.

49 Load, unloaded and analyse the filters in an area free from fibre contamination.

50 Collect the fibres on the gridded side.

51 Set the required flow rate using the calibrated flow meter and adaptor. Perform a leak test by covering the sampler inlet or 'kinking' its tube. If the pump does not stall this indicates a leak and this should be rectified. Switch off the pump and recap the sampler if satisfactory.

Sample volume

52 The sampling flow rate should be in the range 0.5 to 16.0 $l.min^{-1}$ and the sample duration should have regard to the purpose of the measurement. For example, in testing compliance to the WEL, an 8-hour time-weighted average concentration may be needed (eg 0.5 $l.min^{-1}$ for the whole shift), but this may be derived from two or more consecutive samples. A much shorter sample may be appropriate if the purpose is, for example, to investigate dust production at one particular point in the process.

53 The precision of the evaluation step depends primarily on the number of fibres counted so the sample volume (flow rate x time) should be chosen where possible to keep the fibre density on the filter between 50 and 1000 fibres/mm^2.

54 Where low volumes are unavoidable, increasing the area of filter examined may increase the number of fibres counted, but this should not be taken beyond 200 graticule areas since operator fatigue may affect the result.

Sampling

55 Attach the filter holder to the worker's upper chest (eg upper lapel, hood or shoulder) within 300 mm of the nose and mouth with the cowl pointing downwards.

56 You must take localised concentrations into account; in such cases, the sampling head should be positioned on the side expected to give the higher result (eg left- or right-handed operator). If a respirator is worn, the sampling head should be positioned away from the clean exhaust air.

57 When ready to begin sampling, remove the protective cap. If the pump is fitted with an integral timer, ensure that this is reset to zero and switch on the pump.

58 Adjust the flow rate if necessary using the calibrated flow meter and record the time.

59 Check the sampler and pump periodically during sampling to ensure that the equipment is still working and, if appropriate, remeasure the flow rate and record the new values.

60 For each sample, record the sample identity, time on and off and when flow rates were checked, as well as the volumetric flow rate and other relevant sampling information (eg the type of activity taking place and any environmental factors that may affect the results).

61 At the end of the sampling period measure the flow rate, switch off the pump, attach the protective cap, record the reading of the pump timer, or record the time. Carefully remove the sampling equipment without subjecting it to mechanical shocks.

Filter transport and handing

62 Transport the filters in the capped filter holders. Alternatively, if necessary, remove the filters from the holders in a clean area and place them in a clean tin (exposed face upwards). Sprays (eg cytology fixative) must not be used to 'fix' the dust to the filter. If a tin or container is used for transport, unless it can be guaranteed that it will be carefully handled and remain upright, adhesive tape should be used to secure the clean, unexposed edge of the filter to the container. The filter can be cut free for mounting using a surgical scalpel. Take care not to contaminate the filter at any stage or to dislodge any deposit.

Bulk sample for use in determination of refractive index (RI)

63 Wherever possible, a bulk sample should be taken of the MMF materials giving rise to the airborne dust for determination of the RI. The samples should be sealed in separate plastic bags, labelled and transported separately from the filter samples.

Analysis

Determination of RI of bulk samples

64 Two methods are described here, either of which may be impractical or inconclusive depending on the fibre being examined. You must bear in mind the possibility of mixed fibre types and the tests must indicate that all the fibres have a RI greater than 1.51 for the acetone/triacetin method to be used.

65 If any of the fibres have RI equal to or less than 1.51, the plasma etching method described must be used to make the fibre visible for counting. Most ceramic fibres, including rock and slag wool, have a RI >1.51. Superfine, special-purpose glasses or micro-quartz usually have RIs close to1.51 or lower, and the etching process should be used. Many man-made organic fibres (MMOFs) have RIs around 1.51 and may also not be sufficiently visible using acetone/triacetin and phase contrast microscopy, in which case the plasma etching method should be used.

Preparation

66 Place a small portion of the MMF bulk material into a drop of RI liquid (Cargille RI liquid 1.51, or immersion oil 1.515) on a slide, and add a cover slip on the top. When the fibres are a close match to the liquid, they may be difficult to find, but can be located if some easily visible and clearly distinct material, such as paper fibres, are added first.

67 A low NA objective (x10) should be selected on the microscope and then higher-power objectives, if necessary. The microscope should be set up with a total magnification of about x100 Koehler or Koehler-type illumination – focusing on

a specimen, closing the field iris, and adjusting the condenser position to a point just below the specimen so that the image of the field iris is brought into focus.

68 After centring the image with the condenser, the field iris is opened to the edge of the field of view. Partially closing the condenser aperture iris will often improve the visibility for these adjustments, although for subsequent use the condenser iris should be set so that it just enters the field of view in the back focal plane. These adjustments may vary with the type of microscope and the manufacturer's instructions should be followed. The principles are:

(a) the field iris must be in the same plane of focus as the specimen;
(b) the field iris must be centred in the field of view and opened just beyond it;
(c) the back focal plane must be fully illuminated;
(d) the lamp filament (if visible) and the condenser iris must be in focus in the back focal plane.

Method 1: Becke line and central illumination

69 This method comprises two phenomena, which enhance one another, and is suitable for fibres with diameter greater than 1 micron.

70 Bring the fibres into focus, select the bright field condenser position, and reduce the condenser aperture iris in size.

71 As the microscope focus is raised, the bright line moves from the lower to the higher refractive index. If, when the position of focus is raised, ie the stage lowered or objective raised, the fibre becomes darker, with light lines along each side which move away from the fibres when the focus is altered, the fibre's RI is less than that of the mounting liquid. If the focus is moved in the other direction, the fibre then appears lighter with broad, ill-defined dark lines along each side.

72 These effects are reversed if the fibre RI is greater than the mounting fluid. Adjustment of the condenser aperture iris may improve visibility of these lines.

73 When the RIs match, these effects do not occur, and the fibres may be very difficult to see, or may show a transparent blue colour, possibly with colour fringing. In case of doubt, the fibres' RI should be taken as <1.51.

Method 2: Positive phase contrast

74 This is suitable for fibres with diameters less than about 1 micron. If a thin fibre is observed to be lighter than the background, the RI of the fibre is less than that of the liquid (larger diameter fibres can show an inhomogeneous yellow colour, depending on the RI difference between the fibres and mount.) If a thin fibre is observed to be darker than the background, the RI of the fibre is greater than the liquid (note than in this case, phase reversal can occur with larger-diameter fibres, and the fibre appears light with a thin, dark outline).

75 When the RIs match, the fibres appear either a transparent blue, sometimes with a red halo. The fibres, however, may be difficult to see, and in case of doubt, the fibres' RI should be taken as <1.51. If necessary other methods such as oblique illumination can be used.[8,9]

Filter preparation

76 If additional analytical work is required (eg transmission electron microscopy (TEM)), sample and blank filters can be cut in half with a scalpel using a rolling

action. Half of the filter can then be mounted using the acetone-triacetin procedure described below, and the other half kept for subsequent investigation if necessary.

77 The principle of the fibre clearing method is that the filter is immersed in hot acetone vapour, which condenses on the filter collapsing its pores and making it transparent and fixing it on to the slide. A liquid must be added to provide optimum contrast. If the fibre RI is greater than 1.51 the filter can be clarified by the acetone-triacetin technique, if the RI is equal or less than 1.51, the filter is collapsed, etched in the plasma etcher and then water used as the contrast liquid.

Acetone-triacetin hot block method

78 It is important that the filter is dry since water interferes with the clearing process.

79 Place the filter centrally on a clean microscope slide, sampled side upwards, and with grid lines parallel to the slide sides.

80 Place the slide under the outlet orifice of the acetone vaporiser.

81 Inject the acetone, 0.25 ml, steadily into the unit using a micropipette or syringe so that the vapour emerges in a steady stream over the filter, which should clear instantly. (A ring of metal or plastic may be used to form a 'well' around the filter, but not touching the exposed filter area. This helps to localise the spread of acetone and improves the efficiency of clearing, and should mean that 0.25 ml of acetone is sufficient to clear the filter.)

82 The acetone should be completely evaporated before proceeding.

When the refractive index of the fibres is greater than 1.51

83 When the acetone has evaporated, add a drop of triacetin (about 120 micro-litres) to the filter using a micropipette or syringe, just enough to cover the filter when the coverslip is in place without overflow around the edges.

84 Lower the coverslip gently onto the filter at an angle so that air is expelled (it should not be pressed onto the filter).

85 The filter becomes granular in appearance after mounting before clearing completely in about 24 hours; if a result is required quickly then the slide may be heated for a few minutes at about 50 °C and then counted.

When the refractive index of the fibres is less than or equal to 1.51

86 If the fibre RI is ≤1.51, is uncertain, or if no bulk sample is available, the following procedure should be used.

87 Use the acetone clearing procedure described above (paragraphs 78–82).

88 Place the cleared slide and film in a low temperature plasma etcher sufficiently to etch away the surface of the film, leaving the fibres exposed, but still attached to

the film. Experience will determine the parameters required to achieve the desired result. Normally this will involve adjusting the oxygen flow rate, power setting, and time exposed.

89 Add a drop or two of ultra-pure water onto the etched filter and place a cover slip on top, taking care not to trap any air bubbles. The amount of water should be sufficient to fill the space between filter and coverslip without excessive overflow. Water has an RI of 1.33 and provides a good contrast even with low RI fibres. As with all techniques, it is necessary to mount blank filters to ensure that there is no contamination.

Evaluation of samples

90 Place the slide with the mounted filter on the microscope stage. The sample should be examined with a low power objective to check uniformity of the deposit and that there is no gross aggregation of fibres or dust on the mounted filter. The filter should be discarded if badly non-uniform or overloaded with particles so that it is difficult to count.

91 Fibres on the filter must be counted using at least X500 magnification (if higher magnifications are used they should not exceed 1000 times the numerical aperture of the objective lens). The fine focus must be adjusted upwards and downwards by several micro-metres at each new area to ensure that all fibres are seen. The counting should proceed according to the following rules:

(a) Graticule areas for counting must be chosen at random to avoid bias and to be representative of the exposed filter area. Fields lying between the filter edge and dust deposit (or 2 mm of a cutting line) should not be counted (the microscopist should determine under low power where the boundary is located, and then under higher power stay well clear of it). Fields should be rejected if:
    (i) a filter grid line obstructs all or part of the field of view;
    (ii) more than one eighth of the graticule field area is occupied by an agglomerate of fibre and/or particles, by discrete particles or by air bubbles;
    (iii) the microscopist judges that fibres are so obscured that they cannot be counted reliably.

    If the number of rejected fields exceeds 10% of the number accepted (counted separately from the numbers of 'ends'), or the microscopist judges the sample to be uncountable or biased, this should be noted in the final report.

(b) A countable fibre is defined as any object which is longer than 5µm, with an average width less than 3µm and having an aspect (length/width) ratio greater than 3:1 (fibres attached to particles are assessed as if the particle does not exist and are counted if the visible part of the fibre meets the above definition).

(c) The following recording rules apply:
    (i) a countable fibre with both ends within the graticule area is recorded as one fibre;
    (ii) a countable fibre with only one end in the graticule area is recorded as half a fibre;
    (iii) a countable fibre passing through the graticule area, and having no ends within that area, is not counted.

(d) 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.

(e) Loose agglomerates of fibres are counted individually if they can be distinguished sufficiently to determine that they meet the definition in (b).
(f) Fibres in a bundle and tight agglomerates of fibres, where no individual fibres meeting the definition of a countable fibre can be distinguished, are taken to be one countable fibre if the bundle or agglomerate as a whole meets the definition in (b).
(g) If the width of the fibre varies along its length, a representative average width should be considered.
(h) At least 100 fibres must be counted or 100 graticule areas must always be examined, whichever is reached first. At least 20 graticule areas need to be examined, even if these contain more than 100 fibres.

Calculation of results

92 The airborne concentration is given by the formula:

$$C = (1000 \times (N_1+N_2) \times D^2) / (V \times n \times d^2) \text{ fibres per millilitre (f.ml}^{-1})$$

Where N_1 = the number of fibres counted on the filter

N_2 = the number of fibres counted from the sampling head (if appropriate)

n = the number of graticule areas examined

D (mm) = the diameter of the exposed filter area

d (µm) = the diameter of the Walton-Beckett graticule

V (litres) = the volume of air sampled through the filter

93 When pooling two or more samples, V is the total volume sampled, N_1+N_2 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).

94 The reported concentration should not imply greater accuracy than can be justified by the limit of quantification, eg a 240 litre volume sample with 100 field counted should be reported as <0.4 f.ml^{-1} or rounded to two decimal places if >0.4 f.ml^{-1}.

Interpretation of results

95 The current WELs for MMF are summarised in Table 1. If it is not known whether the airborne fibres will present a respirable hazard or an inhalable one, then compliance should be determined to both fibre evaluations (this method) and gravimetric determination.[3]

96 If the type of fibre (MMF or ceramic) is not known then the fibre count result should be compared to the lowest WEL, currently 0.5 fml^{-1}.

Table 1 Workplace exposure limits for MMF (8-hr TWA)[2]

Fibre	Fibre count (fibre.ml^{-1})	Gravimetric assessment (mg.m^{-3})
MMF (rock and slag wool)	2	5
MMF (mineral wools)	2	5
Glass filaments	2	5
*Refractory ceramic and special purpose fibres	1	5
Para-Aramid	0.5	-
Silicon carbide/nitride whiskers	none	-

Para-Aramid determination

97 While para-Aramid determination can be carried out using the phase contrast microscope method described here, an alternative method can use fluorescence microscopy. When exposed to broadband ultra-violet light, para-Aramid fibres exhibit visible fluorescence.[10] This method has the advantage of making it possible to discriminate between fibres fluorescing at different wavelengths and, if phase contrast is available on the same microscope, other fibres.[1] An alternative evaluation method, if a fluorescence technique is not available, is to use slightly uncrossed polars; the very high birefringence of para-Aramid allows easy identification and counting to be carried out.[10]

Accuracy

98 It is not possible to compare the accuracy of this method in relation to an external standard because the method determines the numbers of fibres counted. However, information is available about the relative bias associated with sample evaluation.

99 The UK MMF PT scheme is now amalgamated with the Regular Inter-laboratory Counting Exchanges (RICE) scheme. Participation in RICE can help determine the bias of an individual laboratory.

100 Counters have been shown, on average, to undercount dense deposits and over count sparse ones. In terms of the densities of fibres on a filter surface, results >1000 fibres.mm^{-2} may be underestimates, and results of <10 fibres.mm^{-2} may be overestimates. Any microscopical counting method is liable to systematic differences among operators and laboratories, which must be controlled by quality control checks.

Precision

101 Counting precision depends on the number of fibres counted and on the uniformity of the fibre distribution on the filter. The latter may be reasonably described by the Poisson distribution. Theoretically, the process of counting randomly distributed (Poisson) fibres gives a coefficient of variation (CV):

$$CV = 1/(N^{1/2}),$$ where N is the number of fibres counted.

102 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:

$CV = (N + 0.04N^2)^{1/2}/N$, where N is the mean number of fibres per evaluation.[11]

103 Typical CV values are given in Table 2 and Figure 4 for intra-laboratory counts.

104 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_{95} and M_{05} on 90% of occasions[11] where:

$$0.91\ M_{95}^2 - (2n + 2.25)M_{95} + n^2 = 0,$$

$$0.84\ M_{05}^2 - (2n + 4)M_{05} + n^2 = 0.$$

M = Confidence Interval (5% or 95%)

105 These equations have been used to calculate the upper and lower confidence limits shown in Table 2. It can be seen from this that counting more than 100 fibres gives only a small increase in precision. The method also 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.

Table 2 Intra-laboratory coefficient of variation (CV) associated with number of fibres counted

Number of fibres	Expected CV	Expected 95% confidence limits for the mean of repeat 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

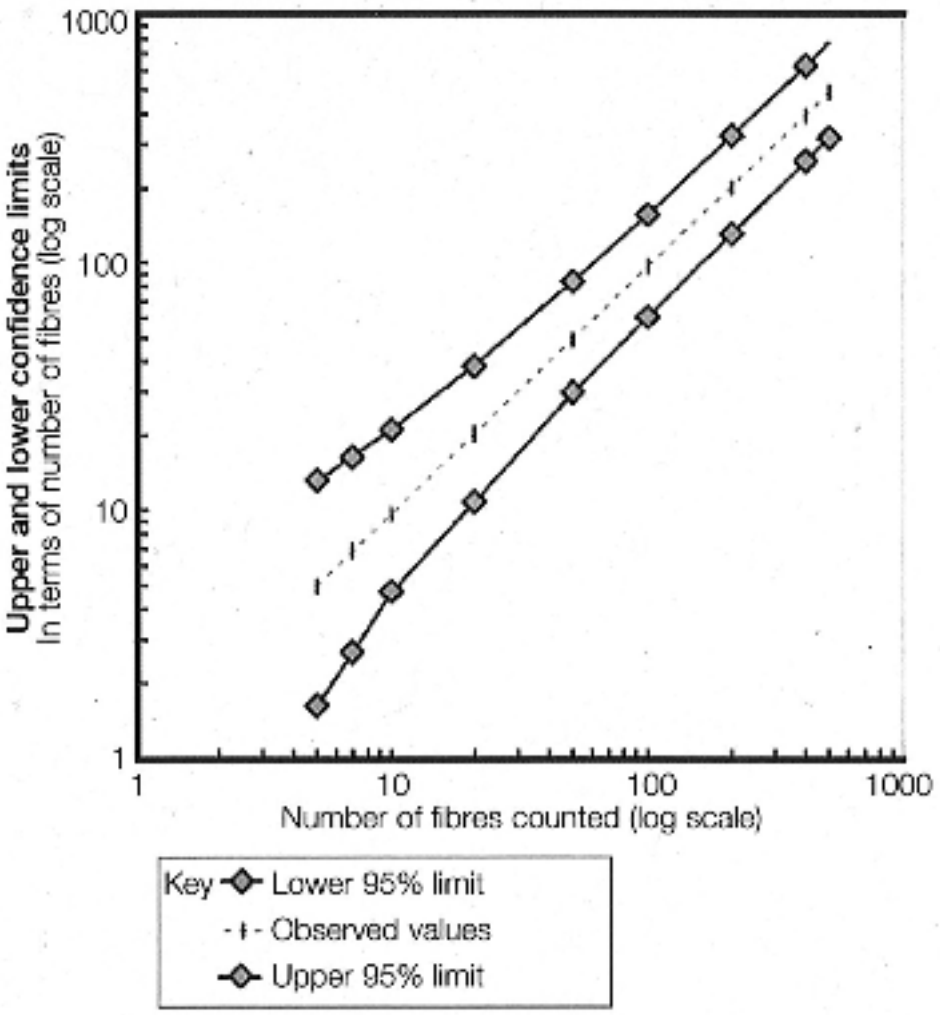

Figure 4 Graph of the calculated intra-laboratory confidence limits – this figure can be found in *Asbestos: The analysts' guide for sampling, analysis and clearance procedures* (HSG248)[1]

106 Table 2 shows that errors become very large when small numbers of fibres are counted, and decisions taken on air measurements should take this into account. So an average concentration on a filter of 10 fibres per 100 microscope graticule areas will sometimes give a result of 3 fibres per 100 microscope grids by chance variation. This is a blank count, so an average count of about 6 fibres per 100 grid areas should be regarded as the lowest measurable value. For a sample volume of 480 litres, this corresponds to a calculated airborne concentration of around 0.006 fm^{-1}.

107 Bias and inter-laboratory differences will seriously degrade the reliability of low-concentration results even further.

Quality assurance

108 As there are large differences in results within and between laboratories engaged in fibre counting, good quality assurance procedures are essential. It is recommended that laboratories do this work in accordance with the requirements of the ISO/IEC 17025,[12] as demonstrated in the UK through the United Kingdom Accreditation Service.

109 Laboratories should also participate in the UK RICE proficiency testing scheme, which distributes sample sets three times per year. 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.

110 The internal quality control scheme should incorporate the use of both reference samples (ie those that have a robust reference value) and routine samples. Participation and assessment of individual performances 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.

111 If it is suspected that the phase contrast test slide has deteriorated due to damage or wear, it should be re-evaluated. The manufacturer should be contacted for advice.

Reference standards

112 Laboratories should have typical bulk samples of appropriate fibres, for RI determination, or mounted in 'melt-mount', where the permanent mount matches the RI of the fibre, and where the optical effects can be observed. The Health and Safety Laboratory can be contacted for advice or for provision of reference materials (see 'Further information' at the end of the document).

Additional information

Dimensional analysis of MMF content

1 There is a requirement under REACH (Commission Regulation 761/2009)[13] for labelling of MMF products. The smaller the diameter of the bulk material, the more likely (other factors being equal) that respirable airborne fibres will be generated. In the UK this labelling requirement is carried out by determination of the Length Weighted Geometric Mean Diameter (LWGMD).[14]

2 This analysis is most easily accomplished by the use of a calibrated scanning electron microscope (SEM). This measurement method can be used to characterise the fibre diameter of bulk substances or products of MMF including refractory ceramic fibres (RCF), man-made vitreous fibres (MMVF), crystalline and polycrystalline fibres.

Method of determination

3 A number of representative core samples are taken from the fibre blanket or from the loose bulk fibre (see details in following paragraph). The bulk fibres are reduced in length using a crushing procedure and a representative sub-sample is dispersed in water. Aliquots are extracted and filtered through a 0.2 micron pore size polycarbonate capillary pore filter and prepared for examination in the scanning electron microscope (SEM). The fibre diameters are measured at an appropriate screen magnification using a line intercept method to give an unbiased estimate of the geometric mean diameter.

4 The method is designed to determine fibre distributions that have geometric mean diameters ranging between 0.5 to 6 microns. Larger diameters can be measured by using lower SEM magnifications but the method will be increasingly limited for finer fibre distributions and a TEM measurement is recommended if the geometric mean diameter is below 0.5 µm.

5 For blankets and bats, a 25 mm diameter core sampler or cork borer is used to take samples of the cross-section of a blanket. These should be equally spaced across the width of a small length of the blanket or taken from random areas where long lengths of the blanket are available. The same equipment can be used to extract random samples from loose fibre. Six samples should be taken when possible, to reflect spatial variations in the bulk material.

Equipment

6 Press and dyes (capable of producing 10 MPa).

7 Polycarbonate capillary filters 0.2 µm pore size and 25 mm diameter.

8 Cellulose ester membrane filters 5 µm pore size for use as a backing filter.

9 Glass filtration apparatus (or disposable filtration systems) to take 25 mm diameter filters, for example Millipore glass microanalysis kit, type No XX10 025 00.

10 Freshly distilled water that has been filtered through a 0.2 µm pore size filter to remove micro-organisms.

11 Sputter coater with a gold or gold/palladium target.

12 Scanning electron microscope capable of resolving down to 10 nm and operating at X10 000 magnification.

13 Miscellaneous: spatulas, type 24 scalpel blade, tweezers, SEM stubs, carbon glue, silver dag.

Sample preparation

14 It has been found that six individual core samples should be representative of the range of fibre dimensions found in slab materials. These should be individually crushed in a 25 mm (±2.5 mm) diameter die at 10 MPa (±50%). The resulting material is mixed together with a spatula and re-pressed at 10 MPa. The material is then removed from the die and stored in a sealed glass bottle.

15 If necessary, organic binder can be removed by placing the fibre inside a furnace at 450 °C for about one hour.

16 The sample should then be coned and quartered to produce a subdivided sample (this procedure should be done inside an extracted enclosure).

17 A small amount (<0.5 g) is added to 100 ml of freshly distilled water filtered through a 0.2 µm membrane filter, into a wide necked flask. This is dispersed thoroughly by the use of an ultrasonic probe operated at 100 W power and tuned so that cavitation occurs. (If a probe is not available use the following method: repeatedly shake and invert for 30 seconds; sonicate in a bench top ultrasonic bath for five minutes; repeatedly shaking and inverting for 30 seconds).

18 A wide-mouthed pipette (2–5 ml capacity) is then used to remove three different aliquots of the dispersion (eg 3, 6, and 10 ml).

19 Vacuum filter each aliquot through a 0.2 µm polycarbonate filter with a 5 µm pore size MEC backing filter. A Millipore 25 mm glass filter funnel with a cylindrical reservoir is used for filtration. Approximately 5 ml of filtered distilled water should be placed into the funnel and the aliquot should be slowly pipetted into the water with the pipette tip below the meniscus.

20 Carefully remove the filter and separate from the backing filter and place in a container to dry.

21 Cut a quarter or half filter section of the filtered deposit using a rocking action with a scalpel blade and carefully place on an SEM stub which has been coated with carbon glue. In addition, use silver dag to improve the contact at the edges of the filter to the stub in at least three positions. Alternatively, the whole filter can be attached to a 25 mm pin stub using carbon glue.

22 When it is dry, place the SEM stub into the splutter coater with a gold or gold/palladium target and coat with approximately 50 nm of gold.

The SEM should be operated at an appropriate magnification using conditions that give good resolution with an acceptable image at slow scan rates of 5 seconds per frame. Generally, to obtain best visibility with materials of relatively low atomic weight, an accelerating voltage of 5–10 keV should be used with a small spot size setting and short working distance to give best resolution. The exact conditions will vary between different SEMs. As a linear traverse is being conducted, a tilt of 0º should be used to minimise refocusing or if the SEM has a eucentric stage, the eucentric working distance should be used. Lower magnifications may be used if the material does not contain small (diameter) fibres and the fibre diameters are large (>5 µm).

Low magnification examination to assess sample

23 The sample should be initially examined at low magnification (x10 objective) to look for evidence of clumping of large fibres and the fibre density assessed. The optimum fibre density should give an average of about one or two countable fibres per field of view at the operating magnification (or about 200 fibres. mm^{-2} for a linear traverse). Fibre concentrations which exceed 100 fibres. mm^{-2} have been found to bias the result towards larger diameters. Low-fibre concentrations will increase the time of analysis and it is often cost-effective to prepare a sample with a fibre density closer to the optimum.

Data analysis

24 Each fibre touching the line should be measured and the dimensions recorded in relevant software, and once done the field of view moved, a field width at a time, in a horizontal direction using the step scanning controls (if available).

25 The ends of long fibres should be checked at low magnification to ensure that they do not curl back into the measurement field of view and are only measured once.

Calculation and decision

Method 1:

26 Fibre diameters are far from being normally distributed. However, by performing a log transformation it is possible to obtain an approximate normal distribution:

(a) Take logs (to base e) of the diameters.
(b) Calculate the arithmetic mean and standard deviation of the log values.
(c) Divide the standard deviation by the square root of the number of measurements to obtain the standard error.
(d) Subtract two times the standard error from the mean.

(e) Calculate the exponential of this value (mean minus two standard error) to give the geometric mean minus two standard errors.
(f) If the value calculated (geometric mean minus two standard errors) is below 1 µm the material is classified as a category 2 carcinogen.

Method 2: (for statistical comparisons only)

27 To determine the result to the required precision, at least 300 counts are ranked in size order (this can be easily carried out on a computer spread sheet). The value of the 150th fibre is the median value reported, the value of the 137th ranked fibre is the lower 95% confidence limit (median diameter – two standard deviations).

Reporting

28 Report the geometric mean and lower 97.5% confidence limit for method 1.

29 Report the median (150th ranked measurement) and the lower 95% confidence interval 137th ranked measurement for method 2 for statistical comparisons only. Error bands: The LWGMD is measured and the lower 95% confidence limit of this value is reported.

References

1 *Asbestos: The analysts' guide for sampling, analysis and clearance procedures* HSG248 HSE Books 2005 ISBN 978 0 7176 2875 9 www.hse.gov.uk/pubns/books/hsg248.htm

2 *EH40/2005 Workplace exposure limits: Containing the list of workplace exposure limits for use with the Control of Substances Hazardous to Health Regulations (as amended)* Environmental Hygiene Guidance Note EH40 (Second edition) HSE Books 2011 ISBN 978 0 7176 6446 7 www.hse.gov.uk/pubns/books/eh40.htm

3 *General methods for sampling and gravimetric analysis of respirable, thoracic and inhalable aerosols* MDHS14/4 HSE Books 2014 www.hse.gov.uk/pubns/mdhs

4 *Determination of Airborne Fibre Number Concentrations; A Recommended Method by Phase-Contrast Microscopy (Membrane Filter Method)* World Health Organisation ISBN 92 4 154496 1

5 *Monitoring strategies for toxic substances* HSG173 (Second edition) HSE Books 2006 www.hse.gov.uk/pubns/books/hsg173.htm

6 *MMMF Deposition in Sampling Cowls* HSE IR/L/MF/94/

7 BS EN ISO 13137:2013 *Workplace atmospheres. Pumps for personal sampling of chemical and biological agents. Requirements and test methods* British Standards Institution

8 Hartshorne NH and Stuart *A Crystals and the Polarising Microscope* (Fourth edition) Edward Arnold, London ISBN 0 7131 2256 0

9 McCrone WC and Delly JC *The Particle Atlas* (Second edition) pp 39,40, 72–75 Ann Arbor Science Ann Arbor, Michigan 1973 ISBN 0 250 40008 1

10 *Measurements of Airborne Kevlar Fibrils; Final Report; Discrimination, Reproducibility and an Outline Method* HSE IR/L/MF/95/7

11 Ogden *The reproducibility of asbestos counts* HSE Research Paper 18 1982 ISBN 0 7176 0101 3

12 ISO/IEC 17025:2005 *General requirements for the competence of testing and calibration laboratories* International Standards Organisation

13 Commission Regulation (EC) No 761/2009 http://eur-lex.europa.eu/homepage.html?locale=en

14 *The Size Classification of MMMF: An Investigation Into Proposed Methods Of Classifying Bulk Fibres Using The Length-weighted, Geometric Mean Fibre Diameter* HSE IR/L/DI/93/08

You should use the current edition of any standards listed.

Further information

For information about health and safety, or to report inconsistencies or inaccuracies in this guidance, visit www.hse.gov.uk/. You can view HSE guidance online and order priced publications from the website. HSE priced publications are also available from bookshops.

British Standards can be obtained in PDF or hard copy formats from BSI: http://shop.bsigroup.com or by contacting BSI Customer Services for hard copies only Tel: 020 8996 9001 email: cservices@bsigroup.com.

This guidance is issued by the Health and Safety Executive. Following the guidance is not compulsory, unless specifically stated, and you are free to take other action. But if you do follow the guidance you will normally be doing enough to comply with the law. Health and safety inspectors seek to secure compliance with the law and may refer to this guidance.

This MDHS is available at: www.hse.gov.uk/pubns/mdhs.htm.

For further information about this method or other MDHS methods, please visit HSL's website: www.hsl.gov.uk or email: hslinfo@hsl.gov.uk