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Air quality — Determination of the number concentration of airborne inorganic fibres by phase contrast optical microscopy — Membrane filter method

Qualité de l'air — Détermination de la concentration en nombre de fibres inorganiques en suspension dans l'air par microscopie optique en contraste de phase — Méthode du filtre à membrane

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Foreword

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The committee responsible for this document is ISO/TC 146, *Air quality*, Subcommittee SC 2, *Workplace atmospheres*.

This second edition cancels and replaces the first edition (ISO 8672:1993), which has been technically revised. This second edition provides additional quality assurance procedures.

Introduction

The concentration of optically visible airborne inorganic fibres can only be defined in terms of the results obtained with a particular measurement method. Moreover, experience has shown that different laboratories, using the membrane filter optical counting method, can obtain different results on the same sample, even when the laboratories appear to be working from a written version of the method which attempts to specify all variables.

Because of the unusual operator-dependence of the membrane filter method, it is important to apply this method with care and use it in conjunction with a quality control scheme. The second edition of this International Standard provides for additional quality assurance procedures.

Air quality — Determination of the number concentration of airborne inorganic fibres by phase contrast optical microscopy — Membrane filter method

1 Scope

This International Standard specifies the determination of the number concentration of airborne inorganic fibres by phase contrast optical microscopy using the membrane filter method in workplace atmospheres, as defined by the counting criteria given in 6.5.4.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 13137, *Workplace atmospheres — Pumps for personal sampling of chemical and biological agents — Requirements and test methods*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

NOTE Terms specific to this document are defined, in addition to those found in ASTM Standards D7200-12,[6] European Standard EN 1540.[7]

3.1
reference slide
slide prepared from a field sample by the acetone-triacetin method (Annex A) or the dimethyl formamide-Euparal method (Annex B) with a non-gridded cover slip that is to be used in a long-term quality control scheme

Note 1 to entry: For the inventory of reference slides, they should be selected from a previous prepared bank of samples for which the mean and variability have been historically established. They should also comprise of samples with varying fibre densities, and if available different fibre types. Reference slides should be checked for filter integrity periodically and replaced if necessary.

3.2
breathing zone
space around the nose and mouth from which a worker's breath is taken

Note 1 to entry: Technically, the breathing zone corresponds to a hemisphere (generally accepted to be a 30 cm in radius) extending in front of the human face, centred on the mid-point of a line joining the ears. The base of the hemisphere is a plane through this line, the top of the head and the larynx. This technical description is not applicable when respiratory protective equipment is used.[7]

3.3
countable fibre
any object having a maximum width less than 3 µm, an overall length greater than 5 µm and a length to width ratio greater than 3:1.

3.4
occupational exposure limit value
limit of time-weighted average of the concentration of a chemical agent in the air within the breathing zone of a worker in relation to a specified reference period

Note 1 to entry: Limit values are mostly set for reference periods of 8 h, but can also be set for shorter periods or concentration excursions. Limit values for gases and vapours are stated in terms independent of temperature and air pressure variables in ml/m^3 and in terms dependent on those variables in mg/ m^3 for a temperature of 20 °C and a pressure of 101,3 kPa. Limit values for airborne particles and mixtures of particles and vapours are given in mg/m^3 or multiples of that for actual environmental conditions (temperature, pressure) at the workplace. Limit values of fibres are given in numbers of fibres/m^3 or number of fibres/cm^3 for actual environmental conditions (temperature, pressure) at the workplace.[7]

4 General method description

A sample is collected by drawing a measured quantity of air through a membrane filter by means of a battery-powered sampling pump. The entire filter or a portion of the filter (wedge) is later transformed from an opaque membrane into a homogeneous optically transparent specimen. The fibres are then sized and counted using a phase contrast optical microscope. The result is expressed as fibres per cubic centimetre of air, calculated from the number of fibres on the filter and the measured volume of air sampled. The method is applicable for routine sampling and sample evaluation necessary to assess personal exposure to fibres and implement control measures of their presence in occupational environments. The method is applicable for routine static sampling and measurement of personal exposure to fibres.

4.1 Limitations of the method by particle type

This method cannot identify the composition or characteristics of particular fibre types and its use shall be restricted to workplace atmospheres where the predominant fibre types are inorganic.

The use of this method also has limitations when applied to samples containing platy or acicular particles and consequently it should not be implemented without prior knowledge of the fibres present in the workplace atmosphere. There are a variety of analytical methods which can be useful, e.g. polarizing light microscopy, electron microscopy.

4.2 Limit of visibility and detection limits

This procedure cannot enumerate thin fibres whose width is below the limit of visibilty by phase contrast optical microscopy. The limit varies according to the refractive index contrast between the fibres and the mounting medium, and the phase-shift of the microscope. The triacetin mounting medium proposed in this method has a refractive index of approximately 1,45, and the Euparal mounting medium has a refractive index of 1,48. In workplace atmospheres, fibres with refractive indices in the range of 1,4 to 1,5 might occur. As the relatively small refractive index difference between these fibres and the mounting media might not be sufficient for them to be visible, this mounting media might not be appropriate.

Previously published method limitations of 0,2 µm or 0,25 µm width limits are conservative consensus values. Practical studies have indicated the ability of a microscope properly adjusted to detect chrysotile fibres of 0,15 µm width[11] and Amosite fibres of 0,062 5 µm width.[12] These results suggest crocidolite fibres can be detectable at 0,05 µm width. Fibres with smaller widths can be detected under the electron microscope, but large differences in results sometimes observed between the two methods are more likely due to undercounting fine fibres under phase contrast microscopy (PCM) than to the presence of substantial numbers of fibres that can only be seen under the electron microscope. The quality assurance procedures in this International Standard are used to identify and resolve several types of counting errors under PCM.

With the parameters specified in this method, the theoretical lower detection limit for a sample of 480 l of air is 0,007 fibres/cm^3. However, the limit of practical use is often 0,1 fibres/cm^3 or higher. This is because blank filters can frequently give a reading of several countable fibres per 100 graticule areas. These “fibres” are contaminants on the filter, or artefacts from the clearing process which have the

appearance of fibres. Neither counting more fields nor increasing sampling duration overcomes the problem of background dust, when fibres are a minor constituent of the dust cloud. In relatively clean atmospheres, such as cleaned enclosure after asbestos removal (clearance sampling), the expected fibre concentration is < 0,01 fibres/cm^3, larger sample volumes (>480 l) are required to achieve quantifiable loadings.

4.3 Apparatus and equipment

4.3.1 Sampling equipment

4.3.1.1 Filters. Membrane filters (mixed cellulose ester or cellulose nitrate) of 0,8 to 1,2 µm or less pore size and a diameter of 25 mm are preferred with, or without printed grids (printed grids can allow the counter to focus easier on the plane containing the fibres, but the lines of the grid can obstruct all or parts of the fields of view and interfere with the counting so that these fields must be avoided).

In recent years, problems have been observed with portions of batches of mixed cellulose ester filters, where the porosity is not evenly developed over the filter. Areas of the filter without porosity can lead to a high pressure drop resulting in premature pump failure, areas of the filter without fibres deposited, and the appearance of cracking in acetone-triacetin mounts.[13] It is necessary to pay attention to the quality of filters in order to avoid these problems. In addition, each batch of filters should be tested for fibrous contamination as described in 5.4.

4.3.1.2 Open-faced filter holder fitted with a protective cowl.[14]

The distance between the cowl opening and the filter plane should be between one and a half times and two times the internal diameter of the cowl. The internal diameter of the cowl should be at least equal to the exposed diameter of the filter but not more than 2 mm greater.

The cowl helps to protect the filter from accidental contamination. A conducting cowl is preferred to a plastic one because of the possible risk of fibre loss due to electrostatic charge. Filter holders and cowls shall be thoroughly washed before re-use.

Due to the design of the filter support utilized in some filter holders, a supporting pad should be used. The purpose of this supporting pad is to ensure an even distribution of air passing through the primary membrane.

4.3.1.3 Sampling pump, capable of giving a smooth flow and having flow set to within ± 5 % of the required flowrate, and of maintaining this flowrate through the filter to within ±10 % for flowrate 2 l/min and ±5 % for flowrate > 2 l/min during the period of sampling.

Although some pumps are equipped with pulsation dampers, an external damper might have to be installed between the pump and the collecting media. Personal sampling pumps shall meet the criteria for a Type P pump as detailed in ISO 13137.

4.3.1.4 Connecting tubing, constriction-proof and the connections shall be leak-proof.

4.3.2 Microscope equipment

Because microscopes with identical "specifications" can give quite different performances, it is necessary that the performance of the proposed and existing microscopes be assessed by means of a detection limit test slide. Provided this criterion is met, small departures from the recommended specifications in items 4.3.2.4 and 4.3.2.5 are permitted. The necessary specifications are as follows.

4.3.2.1 Light source-Kohler or Kohler type illumination. It is preferable for the illuminator to be built-in with a variable light intensity control.

4.3.2.2 Substage assembly, Abbe or achromatic phase contrast condenser incorporated into a substage unit.

There shall be a means of centering each condenser annulus with respect to the phase plate in the corresponding objective, and also a means of focusing the condenser.

4.3.2.3 Stage, a built-in mechanical specimen stage fitted with slide clamps and x -y displacement.

4.3.2.4 Objectives, a rotating nose-piece fitted with 10X and 40X parfocal phase contrast achromatic objectives.

The 40X objective shall have a numerical aperture (NA) between 0,65 and 0,75. It shall have a phase ring of absorption not less than 65 % and not greater than 85 %.

4.3.2.5 Binocular eyepieces, chosen to give a total magnification of 400X to 500X.

At least one eyepiece shall permit the insertion of a graticule. The compensating and focusing type is recommended.

4.3.2.6 Graticule (Walton-Beckett or RIB),[15] the diameter of the graticule in the object plane, when using the 40X phase objective and an appropriate eyepiece, shall be 100 ± 2 µm.

4.3.3 Accessories

4.3.3.1 Centering telescope or Bertrand lens, for checking that the phase rings in the condenser are centred with respect to those in the objectives.

4.3.3.2 Green filter, to ensure the best phase contrast conditions because the optics are designed for this wavelength.

4.3.3.3 Stage micrometer, with 1 mm divided into 0,01 mm divisions.

4.3.3.4 Scalpel holder and Disposal blades, #10 or #22 surgical steel, curved blade.

4.3.3.5 Tweezers, fine point.

4.3.3.6 Acetone vaporizer, to clear mixed cellulose filters.

4.3.3.7 Hypodermic syringe, with 22 gauge needle or disposable micropipette.

4.3.3.8 Pre-cleaned microscope slides, of approximately 76 mm x 25 mm and 0,8 to 1,0 mm thick.

4.3.3.9 Cover slips (without grids), 22 mm x 22 mm, 0,16 to 0,19 mm thick, e.g. No. 1-1/2 or as specified by microscope manufacturer. Larger cover slips are necessary to cover a whole 25 mm diameter filter.

4.3.3.10 Phase contrast test slide, HSE/NPL Mark II or HSE/ULO Mark III where the certificate includes reference to at least one block of lines that should not be visible (see 6.4).

4.3.3.11 Relocatable cover slips, each cover slip has 2 grids and 2 logos which help to orient the cover slip.

Each grid has 140 viewing fields, each of which is approximately 100 µm in diameter. The viewing fields are arranged into 14 columns and 10 rows. With proper orientation, a letter appears on the top and

bottom of each column and a number appears on either side of the rows. Thus, each viewing field is identified for relocation.[9]

4.3.3.12 Standard relocatable test slides, prepared from different types of asbestos and inorganic fibres with different matrix background by the dimethyl formamide/ Euparal method.[9]

There should be no fibre migration observed in these slides for more than 5 years.[8] Other clearing and mounting procedures can be used if no filter migration is observed over the term of use.

They can be prepared from a proficiency test filter from the Proficiency Analytical Testing program (PAT) of the American Industrial Hygiene Association's (AIHA) Laboratory Quality Programs.[8][9] The filter or filter wedge is cleared and mounted by the dimethyl formamide-Euparal method with a relocatable gridded cover slip (Annex B). The fibres visible in each grid opening have been identified and their locations marked on a drawing of each opening. The identity, number and position of each fibre have been verified by a second counter.

4.3.3.13 Disposal gloves.

4.3.3.14 Thermostat-control hotplate or drying oven.

4.3.3.15 Thermometer, 0 °C to 100 °C.

4.3.4 Reagents

4.3.4.1 Dimethyl formamide, reagent grade.

4.3.4.2 Glazier acetic acid, reagent grade.

4.3.4.3 De-ionized water.

4.3.4.4 Euparal resin.

4.3.4.5 Acetone, reagent grade.

4.3.4.6 Triacetin, reagent grade.

4.3.4.7 Lacquer or nail polish.

4.4 Mounting media

Acetone-triacetin is the mounting medium most often used (see Annex A). However, fibre migration can occur over time when excess triacetin is used. While this does not affect the analysis of routine samples and it might not affect the count concentration over time, it does restrict the ability to perform quality checks by re-examining the same areas. This problem can be controlled by using an appropriate amount of triacetin. However, the visual quality of the slides made with triacetin also deteriorates in about 12 months. Therefore, for permanent slides, the dimethyl formamide-Euparal mounting method (see Annex B) should be used. No fibre migration or visual quality deterioration has been observed in slides more than 5 years old.[8] Fibre counts are not affected by using Euparal in place of triacetin,[9] [16] and have also been shown to be equivalent to fibre counts using the dimethyl phthalate-diethyl oxalate method which was used previously for samples that were instrumental in the development of risk assessments.[16]