



SLOVENSKI STANDARD

SIST ISO 13794:2002

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Ambient air -- Determination of asbestos fibres -- Indirect-transfer transmission electron microscopy method

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Air ambient -- Dosage des fibres d'amiante -- Méthode par microscopie électronique à transmission par transfert indirect
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INTERNATIONAL
STANDARD

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First edition
1999-07-15

**Ambient air — Determination of asbestos
fibres — Indirect-transfer transmission
electron microscopy method**

*Air ambient — Dosage des fibres d'amiante — Méthode par microscopie
électronique à transmission par transfert indirect*

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

International Standard ISO 13794 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

Annexes A to H form a normative part of this International Standard. Annex I is for information only.

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Introduction

This International Standard is applicable to the measurement of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for detailed evaluation of any atmosphere in which asbestos fibres are likely to be present. Because the best available medical evidence indicates that the numerical fibre concentration and the fibre size and type are the relevant parameters for evaluation of the inhalation hazards, a fibre counting and measuring technique is the only logical approach. Most fibres in ambient atmospheres are not asbestos, and therefore there is a requirement for fibres to be identified. Many airborne asbestos fibres in ambient atmospheres have diameters below the resolution limit of the optical microscope. This International Standard is based on transmission electron microscopy, which has adequate resolution to allow detection of small fibres and is currently the only technique capable of unequivocal identification of the majority of individual fibres of asbestos. The fibres found suspended in an ambient atmosphere can often be identified unequivocally, if sufficient measurement effort is expended. However, if each fibre were to be identified in this way, the analysis becomes prohibitively expensive. Because of instrumental deficiencies or because of the nature of the particulate, some fibres cannot be positively identified as asbestos, even though the measurements all indicate that they could be asbestos. Subjective and instrumental factors therefore contribute to this measurement, and consequently a very precise definition of the procedure for identification and enumeration of asbestos fibres is required.

In addition to single fibres and bundles, asbestos is often found in air samples as very complex, aggregated structures which may or may not be also aggregated with other particles. The number of asbestos fibres and bundles incorporated in these complex structures often exceeds the number of isolated fibres and bundles observed, and many of them may be completely obscured in direct-transfer TEM preparations. The method defined in this International Standard incorporates specimen preparation procedures that result in selective concentration of asbestos fibres, and removal of organic and water-soluble materials. These procedures have the effect of dispersing the majority of the complex clusters and aggregates of fibres into their component fibres and bundles so that the asbestos in the air sample can be more accurately quantified. All of the feasible specimen preparation techniques result in some modification of the airborne particulate. Even the collection of particles from a three-dimensional airborne dispersion on to a two-dimensional filter surface can be considered a modification of the particulate, and some of the particles in most samples are modified by the specimen preparation procedures. Although this method results in dispersal of complex clusters and aggregates, it minimizes other effects on the size distribution of fibres and fibre bundles.

This International Standard is necessarily complex, because the instrumental techniques used are complex, and also because a very detailed and logical procedure must be specified to reduce the subjective aspects of the measurement. The method of data recording specified in this International Standard is designed to allow re-evaluation of the fibre counting data as new medical evidence becomes available.

This International Standard describes the method of analysis for a single air filter. However, one of the largest potential errors in characterizing asbestos in ambient atmospheres is associated with the variability between filter samples. For this reason, it is necessary to design a replicate sampling scheme in order to determine the standard's accuracy and precision.

Comparison of results obtained using this indirect-transfer procedure with those from the direct-transfer procedure may not be done *a priori*. A site-specific intercomparison study must be done which takes into account the fibre size and type of asbestos, and also the nature of the source of the airborne asbestos.

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Ambient air — Determination of asbestos fibres — Indirect-transfer transmission electron microscopy method

1 Scope

1.1 Substance determined

This International Standard specifies a reference method using transmission electron microscopy (TEM) for determination of the concentration of asbestos structures in ambient atmospheres. The specimen preparation procedure incorporates ashing and dispersion of the collected particulate, so that all asbestos is measured, including the asbestos originally incorporated in particle aggregates or particles of composite materials. The lengths, widths and aspect ratios of the asbestos fibres and bundles are measured, and these, together with the density of the type of asbestos, also allow the total mass concentration of airborne asbestos to be calculated. The method allows determination of the type(s) of asbestos fibre present. The method cannot discriminate between individual fibres of the asbestos and non-asbestos analogues of the same amphibole mineral.

1.2 Type of sample

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The method is defined for polycarbonate capillary-pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres.

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1.3 Range

The upper limit for the range of concentration that can be measured on the analytical filter is 7 000 structures/mm². The lower limit of the range that can be measured on the analytical filter corresponds to detection of 2,99 structures in the area of specimen examined. The air concentrations represented by these values are a function of the volume of air sampled and the degree of dilution or concentration selected during the specimen preparation procedures. The method is particularly applicable for measurements in areas with high suspended-particulate concentrations (exceeding 10 µg/m³), or where detection and identification of asbestos fibres are likely to be prevented or hindered by other types of particulate in direct-transfer TEM preparations. In theory, there is no lower limit to the dimensions of asbestos fibres which can be detected. In practice, microscopists vary in their ability to detect very small asbestos fibres. Therefore, a minimum length of 0,5 µm has been defined as the shortest fibre to be incorporated in the reported results.

1.4 Limit of detection

The limit of detection theoretically can be lowered indefinitely by filtration of progressively larger volumes of air, concentrating the sample during specimen preparation, and by extending the examination of the specimens in the electron microscope. In practice, the lowest achievable limit of detection for a particular area of TEM specimen examined is controlled by the total suspended particulate concentration remaining after the ashing and aqueous dispersal steps, and this depends on the chemical nature of the suspended particulate. For total suspended particulate concentrations of approximately 10 µg/m³, corresponding to clean, rural atmospheres, and assuming filtration of 4 000 litres of air, an analytical sensitivity of 0,5 structure/litre can be obtained, equivalent to a limit of detection of 1,8 structures/litre, if an area of 0,195 mm² of the TEM specimens is examined. Lower limits of detection can be achieved by increasing the area of the TEM specimen that is examined, or by concentration of the sample during specimen preparation. In order to achieve lower limits of detection for fibres and bundles longer than 5 µm, and for PCM-equivalent fibres, lower magnifications are specified which permit more rapid examination of larger areas of the TEM specimens when the examination is limited to these dimensions of fibre.

2 Terms and definitions

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

2.1

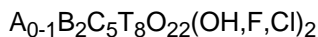
acicular

shape shown by an extremely slender crystal with cross-sectional dimensions which are small relative to its length, i.e. needle-like

2.2

amphibole

group of rock-forming ferromagnesium silicate minerals, closely related in crystal form and composition, and having the nominal formula:



where

A is K, Na

B is Fe²⁺, Mn, Mg, Ca, Na

C is Al, Cr, Ti, Fe³⁺, Mg, Fe²⁺

T is Si, Al, Cr, Fe³⁺, Ti

NOTE In some varieties of amphibole, these elements can be partially substituted by Li, Pb, or Zn. Amphibole is characterized by a cross-linked double chain of Si-O tetrahedra with a silicon:oxygen ratio of 4:11, by columnar or fibrous prismatic crystals and by good prismatic cleavage in two directions parallel to the crystal faces and intersecting at angles of about 56° and 124°.

2.3

amphibole asbestos

amphibole in an asbestiform habit

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2.4

analytical filter

filter through which an aqueous dispersion of ash from the sample collection filter is passed, and from which TEM specimen grids are prepared

2.5

analytical sensitivity

calculated airborne asbestos structure concentration, equivalent to counting of one asbestos structure in the analysis

NOTE 1 It is expressed in structures/litre.

NOTE 2 The method given in this International Standard does not specify an analytical sensitivity.

2.6

asbestiform

specific type of mineral fibrosity in which the fibres and fibrils possess high tensile strength and flexibility

2.7

asbestos

term applied to a group of silicate minerals belonging to the serpentine and amphibole groups which have crystallized in the asbestiform habit, causing them to be easily separated into long, thin, flexible, strong fibres when crushed or processed

NOTE The Chemical Abstracts Service Registry Numbers of the most common asbestos varieties are: chrysotile (12001-29-5), crocidolite (12001-28-4), grunerite asbestos (Amosite) (12172-73-5), anthophyllite asbestos (77536-67-5), tremolite asbestos (77536-68-6) and actinolite asbestos (77536-66-4).

2.8**asbestos structure**

term applied to an individual asbestos fibre, or any connected or overlapping grouping of asbestos fibres or bundles, with or without other particles

2.9**ashed filter blank**

fibre count made on TEM specimens prepared by the indirect procedure from a blank membrane filter of the type used for collection of air samples

2.10**aspect ratio**

ratio of length to width of a particle

2.11**blank**

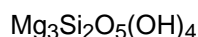
structure count made on TEM specimens prepared from an unused filter in order to determine the background measurement

2.12**camera length**

equivalent projection length between the specimen and its electron diffraction pattern, in the absence of lens action

2.13**chrysotile**

fibrous mineral of the serpentine group which has the nominal composition



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NOTE Most natural chrysotile deviates little from this nominal composition. In some varieties of chrysotile, minor substitution of silicon by Al^{3+} may occur. Minor substitution of magnesium by Al^{3+} , Fe^{2+} , Fe^{3+} , Ni^{2+} , Mn^{2+} and Co^{2+} may also be present. Chrysotile is the most prevalent type of asbestos.

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2.14**cleavage**

breaking of a mineral along one of its crystallographic directions

2.15**cleavage fragment**

fragment of a crystal that is bounded by cleavage faces

2.16**cluster**

structure in which two or more fibres, or fibre bundles, are randomly oriented in a connected grouping

2.17**direct-transfer blank**

structure count made on TEM specimens prepared by the direct-transfer procedure from a blank filter of the type used for filtration of aqueous dispersions of ash

2.18**d-spacing**

distance between identical adjacent and parallel planes of atoms in a crystal

2.19**electron diffraction**

technique in electron microscopy by which the crystal structure of a specimen is examined

2.20**electron scattering power**

extent to which a thin layer of substance scatters impinging electrons from their original directions

- 2.21**
empty beaker blank
fibre count made on TEM specimens prepared by the indirect procedure, using an empty beaker as the initial sample
- 2.22**
energy-dispersive X-ray analysis
EDXA
measurement of the energies and intensities of X-rays by use of a solid-state detector and multichannel analyzer system
- 2.23**
eucentric
condition in which the area of interest of an object is placed on a tilting axis, at the intersection of the electron beam with that axis, and is in the plane of focus
- 2.24**
field blank
filter cassette which has been taken to the sampling site, opened and then closed, and the filter subsequently used to determine the background structure count for the measurement
- 2.25**
fibril
single fibre of asbestos which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances
- 2.26**
fibre
elongated particle which has parallel or stepped sides
- NOTE** For the purposes of this International Standard, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0,5 µm.
- 2.27**
fibre bundle
structure composed of parallel, smaller-diameter fibres attached along their lengths
- NOTE** A fibre bundle may exhibit diverging fibres at one or both ends.
- 2.28**
fibrous structure
fibre, or connected grouping of fibres, with or without other particles
- 2.29**
funnel blank
structure count made on TEM specimens prepared by the direct-transfer method from a filter used for filtration of a sample of distilled water
- 2.30**
habit
characteristic crystal growth form, or combination of these forms, of a mineral, including characteristic irregularities
- 2.31**
limit of detection
calculated airborne asbestos structure concentration, equivalent to counting of 2,99 asbestos structures in the analysis

NOTE It is expressed in structures/litre.

2.32 matrix

structure in which one or more fibres, or fibre bundles, touch, are attached to or partially concealed by, a single particle or connected group of nonfibrous particles

2.33 Miller index

set of either three or four integer numbers used to specify the orientation of a crystallographic plane in relation to the crystal axes

2.34 PCM-equivalent fibre

fibre of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

NOTE For the purposes of this International Standard, PCM is the abbreviated term for phase-contrast optical microscopy.

2.35 PCM-equivalent structure

fibrous structure of aspect ratio greater than or equal to 3:1, longer than 5 µm, and which has a diameter between 0,2 µm and 3,0 µm

NOTE For the purposes of this International Standard, PCM is the abbreviated term for phase-contrast optical microscopy.

2.36 primary structure

fibrous structure that is a separate entity in the TEM image

2.37 replication

procedure in electron microscopy specimen preparation in which a thin copy, or replica, of a surface is made

2.38 selected area electron diffraction

technique in electron microscopy in which the crystal structure of a small area of a sample is examined

2.39 serpentine

group of common rock-forming minerals having the nominal formula



2.40 structure

single fibre, fibre bundle, cluster or matrix

2.41 twinning

occurrence of crystals of the same species joined together at a particular mutual orientation, and such that the relative orientations are related by a definite law

2.42 unopened fibre

large diameter asbestos fibre bundle which has not been separated into its constituent fibrils or fibres

2.43 zone-axis

line or crystallographic direction through the centre of a crystal which is parallel to the intersection edges of the crystal faces defining the crystal zone

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3 Abbreviated terms

DMF	Dimethylformamide
ED	Electron diffraction
EDXA	Energy dispersive X-ray analysis
FWHM	Full width, half maximum
HEPA	High efficiency particle absolute
MEC	Mixed esters of cellulose
PC	Polycarbonate
PCM	Phase-contrast optical microscopy
SAED	Selected area electron diffraction
SEM	Scanning electron microscope
STEM	Scanning transmission electron microscope
TEM	Transmission electron microscope
UICC	Union Internationale Contre le Cancer

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4 Principle

A sample of airborne particulate is collected by drawing a measured volume of air through either a capillary-pore polycarbonate (PC) membrane filter of maximum pore size 0,4 µm or a cellulose ester (either mixed esters of cellulose or cellulose nitrate) membrane filter of maximum pore size 0,8 µm by means of a battery-powered or mains-powered pump. A portion of the filter is ashed in an oxygen plasma, and the residual ash is dispersed in distilled water with adjustment of the pH to between 3,0 and 4,0 using acetic acid. Analytical filters are then prepared by filtration of known volumes of this aqueous dispersion through either capillary-pore PC membrane filters of maximum pore size 0,2 µm or cellulose ester membrane filters of maximum pore size 0,22 µm.

TEM specimens are prepared from PC analytical filters by applying a thin film of carbon to the filter surface by vacuum evaporation. Small areas are cut from the carbon-coated filter, supported on TEM specimen grids, and the filter medium is dissolved away by a solvent extraction procedure. This procedure leaves a thin film of carbon which bridges the openings in the TEM specimen grid, and which supports each particle from the original filter in its original position.

Cellulose ester analytical filters are chemically treated to collapse the pore structure of the filter, and the surface of the collapsed filter is then etched in an oxygen plasma to ensure that all particles are exposed. A thin film of carbon is evaporated onto the filter surface and small areas are cut from the filter. These sections are supported on TEM specimen grids and the filter medium is dissolved away by a solvent extraction procedure.

The TEM specimen grids from either preparation method are examined at both low and high magnifications to check that they are suitable for analysis before carrying out a quantitative fibre count on randomly-selected grid openings. If the selected TEM specimen grid has too high a particulate or fibre loading, another specimen grid with a lower filtered aliquot shall be selected for analysis. In the TEM analysis, electron diffraction (ED) is used to examine the crystal structure of a fibre, and its elemental composition is determined by energy-dispersive X-ray analysis (EDXA). For a number of reasons, it is not possible to identify each fibre unequivocally, and fibres are classified according to the techniques which have been used to identify them. A simple code is used to record, for each fibre, the manner in which it was classified. The fibre classification procedure is based on successive inspection of the morphology, the selected area ED pattern, and the qualitative and quantitative EDXAs. Confirmation of the identification of chrysotile is only by quantitative ED, and confirmation of amphibole is only by quantitative EDXA and quantitative zone-axis ED.

In addition to isolated fibres, ambient air samples often contain more complex aggregates of fibres, with or without other particles. Some particles are composites of asbestos fibres with other materials. Individual fibres and these more complex structures are referred to as "asbestos structures". The indirect specimen preparation procedure permits the majority of these complex structures to be dispersed into their constituent fibres and fibre bundles, allowing more precise quantification than is possible using the direct-transfer procedure.

A coding system is used to record the type of fibrous structure, and also to provide the optimum morphological description of each structure. The two codes remove from the microscopist the requirement to interpret the fibre counting data, and allow this evaluation to be made later without the requirement for re-examination of the TEM specimens. Several levels of analysis are specified, the higher levels providing a more rigorous approach to the identification of fibres. The procedure permits a minimum required fibre identification criterion to be defined on the basis of previous knowledge, or lack of it, about the particular sample. Attempts are then made to achieve this minimum criterion for each fibre, and the degree of success is recorded for each fibre. The lengths and widths of all classified structures are recorded.

The number of asbestos structures found on a known area of the microscope sample, together with the volume of air filtered through the sample collection filter, the proportion of the sample collection filter that was ashed, the proportion of the aqueous dispersion that was filtered, and the area of the analytical filter are used to calculate the airborne concentration of asbestos, expressed in asbestos structures/litre of air. The mass concentration of asbestos is calculated using an assumed density for the asbestos variety, and the widths and lengths of the fibres.

5 Apparatus

5.1 Air sampling

5.1.1 Filter cassette, for sample collection.

Field monitors, comprising 25 mm to 50 mm diameter three-piece cassettes with cowls which project less than 2 cm in front of the filter surface, shall be used for sample collection. The cassette shall be loaded with either a capillary pore PC filter of maximum pore size 0,4 µm or a cellulose ester [mixed esters of cellulose (MEC) or cellulose nitrate] filter of maximum pore size 0,8 µm. When the filter is in position, a shrink cellulose band or adhesive tape shall be applied to prevent air leakage. Suitable precautions shall be taken to ensure that the filters are tightly clamped in the assembly so that significant air leakage around the filter cannot occur.

Representative filters from the filter lot shall be analyzed as described in 8.8 for the presence of asbestos structures, and also tested for suitability as described in annex H, before any are used for air sample collection.

NOTE This method permits the use of larger pore size cellulose ester filters for sample collection than the maximum pore size permitted in the direct-transfer method in ISO 10312. The smaller maximum pore size is specified in ISO 10312 in order to ensure that collected particulate and fibres are retained close to the surface of the filter, which is required if the particulate and fibres are to be transferred to the TEM grid with high efficiency by the direct-transfer procedure. In this indirect-transfer method, the depth of penetration of particulate and fibres into the filter medium during sample collection is unimportant, provided that they do not pass through the filter.

5.1.2 Sampling pump, capable of a flowrate sufficient to achieve the desired analytical sensitivity. The face velocity through the filter shall be between 4,0 cm/s and 70 cm/s. The sampling pump used shall provide a nonfluctuating air flow through the filter, and shall maintain the initial volume flowrate to within $\pm 10\%$ throughout the sampling period. A constant-flow or critical-orifice controlled pump meets these requirements. Flexible tubing shall be used to connect the filter cassette to the sampling pump. A means for calibration of the flowrate of each pump is also required.

NOTE The sampling efficiency for a particular particle size varies with the face velocity. Depending on the size distribution of the airborne particles, the analytical result may vary with face velocity.

5.1.3 Stand, to hold the filter cassette at the desired height for sampling, isolated from the vibrations of the pump.

5.1.4 Variable area flowmeter, calibrated, with a range suitable for determination of the selected flowrate as required for calibration of the air sampling system.