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

*Air ambiant — 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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html. (standards.iteh.ai)

This document was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 3, *Ambient atmospheres*.

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This second edition cancels and replaces the first edition (ISO 13794:1999), which has been technically revised. The main changes compared to the previous edition are as follows:

- the use of electronic display systems with measurement software is permitted;
- the maximum particulate loading for TEM specimens is increased from 10 % to 25 %;
- a simplified fibre identification procedure for investigation of known sources of the regulated asbestos varieties and richterite/winchite asbestos is permitted;
- the reporting requirements have been changed to permit reporting of the concentrations of fibres and bundles longer than 5 µm and/or the concentrations of PCM equivalent fibres without the requirement to report the concentrations of structures equal to or greater than 0,5 µm;
- there is no requirement to report the 95 % confidence intervals of the fibre concentrations.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document is applicable to the measurement of airborne asbestos in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation of any atmosphere. 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 document is based on transmission electron microscopy, which has adequate resolution to allow for the 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 transmission electron microscope (TEM) preparations. The method defined in this document incorporates specimen preparation procedures that result in the selective concentration of asbestos fibres and the removal of organic, water-soluble and acid-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 document requires a very detailed and logical procedure is used to reduce the subjective aspects of the measurement. The method of data recording specified in the document is designed to allow re-evaluation of the fibre counting data as new medical evidence becomes available.

This document 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 cannot be done a priori. This can only be achieved by a site-specific inter-comparison study that 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

This document specifies a reference method using transmission electron microscopy for the determination of airborne asbestos fibres and structures in a wide range of ambient air situations, including the interior atmospheres of buildings, and for a detailed evaluation for asbestos structures in any atmosphere. 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 fibres present. The method cannot discriminate between individual fibres of the asbestos and elongate fragments (cleavage fragments and acicular particles) from non-asbestos analogues of the same amphibole mineral^[12].

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 4225, *Air quality — General aspects — Vocabulary*

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3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 4225 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

acicular

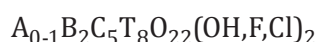
shape shown by an extremely slender crystal with cross-sectional dimensions which are small relative to its length, i.e. needle-like

[SOURCE: ISO 10312:1995, 3.1]

3.2

amphibole

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



where

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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 1 to entry: 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°.

[SOURCE: ISO 10312:1995, 3.2]

3.3

amphibole asbestos

amphibole in an asbestiform habit

[SOURCE: ISO 10312:1995, 3.3]

3.4

analytical filter

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

[SOURCE: ISO 13794:1999, 2.4]

3.5

analytical sensitivity

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

Note 1 to entry: It is expressed in structures/litre.

Note 2 to entry: This method does not specify a unique analytical sensitivity. The analytical sensitivity is determined by the needs of the measurement and the conditions found on the prepared sample.

[SOURCE: ISO 10312:1995, 3.4]

3.6

asbestiform

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

[SOURCE: ISO 10312:1995, 3.5]

3.7

asbestos

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 1 to entry: 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). Other varieties of asbestiform amphibole, such as richterite asbestos and winchite asbestos^[18], are also found in some products such as vermiculite and talc.

[SOURCE: ISO 10312:1995, 3.6]

3.8**asbestos structure**

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

[SOURCE: ISO 10312:1995, 3.7]

3.9**ashed filter blank**

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

[SOURCE: ISO 13794:1999, 2.9]

3.10**aspect ratio**

ratio of length to width of a particle

[SOURCE: ISO 10312:1995, 3.8]

3.11**blank**

structure count made on transmission electron microscope specimens prepared from an unused filter, to determine the background measurement

[SOURCE: ISO 10312:1995, 3.9]

3.12**camera length**

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

[SOURCE: ISO 10312:1995, 3.10]

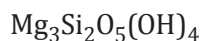
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3.13**chrysotile**

fibrous mineral of the serpentine group, which has the nominal composition:



Note 1 to entry: 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.

[SOURCE: ISO 10312:1995, 3.11]

3.14**cleavage**

breaking of a mineral along one of its crystallographic directions

[SOURCE: ISO 10312:1995, 3.12]

3.15**cleavage fragment**

fragment of a crystal that is bounded by cleavage faces

Note 1 to entry: Crushing of non-asbestiform amphibole generally yields elongated fragments that conform to the definition of a fibre, but rarely have aspect ratios exceeding 30:1.

[SOURCE: ISO 10312:1995, 3.13, modified — Note 1 to entry added.]

**3.16
cluster**

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

[SOURCE: ISO 10312:1995, 3.14]

**3.17
direct-transfer blank**

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

[SOURCE: ISO 13794:1999, 2.17]

**3.18
d-spacing**

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

[SOURCE: ISO 10312:1995, 3.15]

**3.19
electron diffraction**

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

[SOURCE: ISO 10312:1995, 3.16]

**3.20
electron scattering power**

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

[SOURCE: ISO 10312:1995, 3.17]

**3.21
empty beaker blank**

fibre count made on transmission electron microscope specimens prepared by the indirect procedure using an empty beaker as the initial sample

[SOURCE: ISO 13794:1999, 2.21]

**3.22
energy dispersive X-ray analysis**

measurement of the energies and intensities of X-rays by use of a solid state detector and multi-channel analyser system

[SOURCE: ISO 10312:1995, 3.18]

**3.23
eucentric**

condition when 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

[SOURCE: ISO 10312:1995, 3.19]

**3.24
field blank**

filter cassette that has been taken to the sampling site, opened and then closed and which is used to determine the background structure count for the measurement

[SOURCE: ISO 10312:1995, 3.20]

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3.25**fibril**

single fibre of asbestos, which cannot be further separated longitudinally into smaller components without losing its fibrous properties or appearances

[SOURCE: ISO 10312:1995, 3.21]

3.26**fibre**

elongated particle which has parallel or stepped sides

Note 1 to entry: For the purposes of this document, a fibre is defined to have an aspect ratio equal to or greater than 5:1 and a minimum length of 0,5 µm.

[SOURCE: ISO 10312:1995, 3.22]

3.27**fibre bundle**

structure composed of parallel, smaller diameter fibres attached along their lengths

Note 1 to entry: A fibre bundle may exhibit diverging fibres at one or both ends.

[SOURCE: ISO 10312:1995, 3.23]

3.28**fibrous structure**

fibre, or connected grouping of fibres, with or without other particles

[SOURCE: ISO 10312:1995, 3.24] (standards.iteh.ai)

3.29**funnel blank**

structure count made on transmission electron microscope specimens prepared by the direct-transfer method from a filter used for filtration of a sample of distilled water

[SOURCE: ISO 13794:1999, 2.29]

3.30**habit**

characteristic crystal growth form or combination of these forms of a mineral, including characteristic irregularities

[SOURCE: ISO 10312:1995, 3.25]

3.31**limit of detection**

calculated airborne fibre concentration in structures/l, equivalent to the upper 95 % confidence limit of 2,99 structures predicted by the Poisson distribution for a count of zero structures

[SOURCE: ISO 10312:1995, 3.26]

3.32**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 non-fibrous particles

[SOURCE: ISO 10312:1995, 3.27]

3.33

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

[SOURCE: ISO 10312:1995, 3.28]

3.34

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

[SOURCE: ISO 10312:1995, 3.29]

3.35

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

[SOURCE: ISO 10312:1995, 3.30]

3.36

pixel

smallest image-forming element to which a grey level is assigned

[SOURCE: ISO 23900-6:2015, 2.10]

3.37

primary structure

fibrous structure that is a separate entity in the transmission electron microscope image

[SOURCE: ISO 10312:1995, 3.31]

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3.38

replication

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

[SOURCE: ISO 10312:1995, 3.32]

3.39

selected area electron diffraction

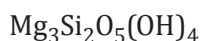
technique in electron microscopy in which the crystal structure of a small area of a sample is examined

[SOURCE: ISO 10312:1995, 3.33]

3.40

serpentine

group of common rock-forming minerals having the nominal formula:



[SOURCE: ISO 10312:1995, 3.34]

3.41

structure

single fibre, fibre bundle, cluster or matrix

[SOURCE: ISO 10312:1995, 3.35]

3.42**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

[SOURCE: ISO 10312:1995, 3.36]

3.43**unopened fibre**

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

[SOURCE: ISO 10312:1995, 3.37]

3.44**zone-axis**

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

[SOURCE: ISO 10312:1995, 3.38]

4 Symbols and abbreviated terms

eV electron volt

kV kilovolt

l/min litres per minute

µg microgram (10^{-6} grams)

µm micrometre (10^{-6} metre)

nm nanometre (10^{-9} metre)

W Watt

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