



Standard Test Method for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment by Transmission Electron Microscopy¹

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1. Scope

1.1 This test method covers the sampling methods and analysis techniques used to assess the airborne concentration and size distribution of single-crystal ceramic whiskers (SCCW), such as silicon carbide and silicon nitride, which may occur in and around the workplace where these materials are manufactured, processed, transported, or used. This test method is based on the filtration of a known quantity of air through a filter. The filter is subsequently evaluated with a transmission electron microscope (TEM) for the number of fibers meeting appropriately selected morphological and compositional criteria. This test method has the ability to distinguish among different types of fibers based on energy dispersive X-ray spectroscopy (EDS) analysis and selected area electron diffraction (SAED) analysis. This test method may be appropriate for other man-made mineral fibers (MMMF).

1.2 This test method is applicable to the quantitation of fibers on a collection filter that are greater than 0.5 μm in length, less than 3 μm in width, and have an aspect ratio equal to or greater than 5:1 (1).² The data are directly convertible to a statement of concentration per unit volume of air sampled. This test method is limited by the amount of coincident interference particles.

1.3 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:

D 1193 Specification for Reagent Water³

¹ This test method is under the jurisdiction of ASTM Committee D-22 on Sampling and Analysis and is the direct responsibility of Subcommittee D22.04 on Analysis of Workplace Atmospheres.

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² The boldface numbers in parentheses refer to a list of references at the end of this test method.

³ *Annual Book of ASTM Standards*, Vol 11.01.

D 1356 Terminology Relating to Sampling and Analysis of Atmospheres⁴

D 4532 Test Method for Respirable Dust in Workplace Atmospheres⁴

D 6057 Test Method for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment by Phase Contrast Microscopy⁴

D 6058 Practice for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment⁴

D 6059 Test Method for Determining Concentration of Airborne Single-Crystal Ceramic Whiskers in the Workplace Environment by Scanning Electron Microscopy⁴

E 691 Practice for Conducting Interlaboratory Study to Determine the Precision of a Test Method⁵

3. Terminology

3.1 Definitions:

3.1.1 *analytical sensitivity, n*—airborne fiber concentration represented by a single fiber counted in the TEM.

3.1.1.1 *Discussion*—Although the terms *fiber* and *whisker* are, for convenience, used interchangeably in this test method, whiskers is correctly applied only to single-crystal fibers whereas a fiber may be single- or poly-crystalline or may be noncrystalline.

3.1.2 *aspect ratio, n*—the ratio of the length of a fiber to its width.

3.1.3 *fiber, n*—for the purpose of this test method, an elongated particle having a minimum length of 0.5 μm , a width less than 3 μm , and an aspect ratio equal to or greater than 5:1.

3.1.4 *fibrous, adj*—composed of parallel, radiating, or interlaced aggregates of fibers, from which the fibers are sometimes separable. That is, the crystalline aggregate may be referred to as fibrous even if it is not composed of separable fibers, but has that distinct appearance. The term fibrous is used in a general mineralogical way to describe aggregates.

3.1.5 *man-made mineral fiber, n*—any inorganic fibrous material produced by chemical or physical processes.

3.1.6 *single-crystal ceramic whisker, n*—a man-made mineral fiber that has a single-crystal structure.

⁴ *Annual Book of ASTM Standards*, Vol 11.03.

⁵ *Annual Book of ASTM Standards*, Vol 14.02.

3.2 For definitions of other terms used in this test method, see Terminology D 1356.

4. Summary of Test Method

4.1 The sample is collected on a mixed cellulose ester (MCE) filter by drawing air, using a sampling pump, through an open-face 25-mm electrically conductive sampling cassette assembly (2-4). A section of the filter is prepared and transferred to a TEM grid and the fibers are identified, sized, and counted at a screen magnification in the range from 8000 to 12 000 \times in the TEM in Section 11. Results are reported as a fiber concentration per unit volume of air and a fiber loading per unit area of filter. The airborne concentration is expressed as fibers per millilitre (f/mL) and the fiber loading is expressed as fibers per square millimetre (f/mm²). Optionally, a supplementary low-magnification count in the range from 800 to 1200 \times may also be performed, using the criteria discussed in 11.1.5, to provide comparison with PCM data.

5. Significance and Use

5.1 The SCCW may be present in the workplace atmosphere where these materials are manufactured, processed, transported, or used. This test method can be used to monitor airborne concentrations of fibers in these environments. It may be employed as part of a personal or area monitoring strategy.

5.2 This test method is based on morphology, elemental composition, and crystal structure. The analysis technique has the ability to positively identify SCCW.

NOTE 1—This test method assumes that the analyst is familiar with the operation of TEM/EDS instrumentation and the interpretation of data obtained using these techniques.

5.3 This test method is applicable for the measurement of the total population of SCCW fibers including fibers with diameters $\leq 0.1 \mu\text{m}$.

5.4 Results from the use of this test method shall be reported along with 95 % confidence limits for the samples being studied. Individual laboratories shall determine their intralaboratory coefficient of variation and use it for reporting 95 % confidence limits (2,5,6).

6. Interferences

6.1 This test method has been designed to filter air for the determination of SCCW concentration. However, filtration of air also involves collection of extraneous particles and other fibers that may not be of interest. Extraneous particles may obscure fibers by overlay or overloading of the filter. This situation can be managed by regulating the air volume sampled and thus the filter loading. Fibers should appear separated from other particles to ensure an adequate opportunity for their recognition as separate entities in the TEM and accurate counting. Some coincident particulate agglomeration does occur even with these guidelines. Analyze an alternate filter with a reduced loading if the obscuring condition appears to exceed 15 % of the filter area (7). Redeposition of a portion of an overloaded filter is permitted only in circumstances where an alternate filter is not available and cannot be obtained through resampling (see 10.1.12).

7. Apparatus and Reagents

7.1 *Sampling Cassette*—Use a 25-mm electrically conduc-

tive cassette assembly such as a three-piece cassette with an extension cowl or retainer ring containing a 0.45- μm pore size MCE filter and a support pad. Seal the cassette assembly with shrink tape. Reloading of used cassettes is not permitted.

7.2 *Personal Sampling Pump*—Use a portable battery-operated pump for personal sampling. Each pump must be capable of operating within the range from 0.5 to 4 L/min and continuously over the chosen sampling period (2,3). The flow must be free from pulsation. All pumps shall be calibrated prior to use (8).

7.3 *Area Sampling Pump*—Use a personal sampling pump or a non-portable high-volume pump for area sampling. Each pump shall be capable of operating within the range from 0.5 to 16 L/min and continuously over the chosen sampling period (2,3). The flow shall be free from pulsation. All pumps shall be calibrated prior to use (8).

7.4 *Vinyl tubing or equivalent.*

7.5 *Plasma Asher*, a low-temperature asher (LTA) is required to plasma-etch the collapsed MCE filter.

7.6 *Oxygen*, used as a bleed gas for plasma asher.

7.7 *Vacuum Evaporator*, for vapor deposition of conductive layers of carbon.

NOTE 2—Sputter coaters and carbonaceous fiber coaters are not appropriate.

7.8 *Specimen Grids*, copper 200-mesh TEM grids for mounting the specimen for TEM examination.

7.9 *Transmission Electron Microscope*—A TEM capable of operating using an accelerating voltage of at least 80 kV. The TEM must also be capable of performing EDS and SAED analyses. A light-element X-ray analyzer capable of detecting carbon, nitrogen, and oxygen is recommended. Use of a tilt-rotation holder as well as a double-tilt stage is also recommended. The TEM must have a fluorescent screen inscribed with calibrated gradations. It must be capable of producing a spot less than 250 nm in diameter at crossover under routine analytical conditions. Scanning transmission electron microscope (STEM) mode is allowed for this purpose.

7.10 *Sample Preparation Area*, consisting of either a clean room facility or a room containing a positive pressure HEPA-filtered hood.

7.11 *Tweezers.*

7.12 *Scalpel Blades.*

7.13 *Large Glass Petri Dishes* (approximately 90 mm in diameter).

7.14 *Jaffe Washer.*

7.15 *Lens Tissue.*

7.16 *MCE Filters*, 25 mm, 0.45 μm , and 0.22 μm .

7.17 *Funnel/Filtration Assembly*, 25 mm.

7.18 *Acetone.*

NOTE 3—**Precaution:** Acetone is a flammable liquid and requires precautions not to ignite it accidentally.

7.19 *Specification D 1193 Type II Water* (particle free).

7.20 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where

such specifications are available.⁶ Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

8. Sample Collection

8.1 Collect samples of airborne SCCW on MCE filters using sampling cassettes and pumps in accordance with Section 7.

8.2 Remove the outlet plug from the sampling cassette and connect to a sampling pump by means of flexible, constriction proof tubing.

8.3 Perform a leak check of the sampling system by activating the pump with the closed cassette and rotameter (or other flow measurement device) in line. Any flow indicates a leak that must be eliminated before starting the sampling operation.

8.4 Remove the inlet plug from the sampling cassette to eliminate any vacuum that may have accumulated during the leak test; then remove the entire inlet cap.

8.5 Conduct personal and area sampling as follows:

8.5.1 For personal sampling, fasten the sampling cassette to the worker's lapel in the worker's breathing zone and orient face down. Adjust the calibrated flow rate to a value between 0.5 and 4 L/min (2,3). Typically, a sampling rate between 0.5 and 2.5 L/min is selected (4-7). Also see Test Method D 4532.

8.5.2 Place area samples on an extension rod facing down at a 45° angle. Adjust the calibrated flow rate to a value between 0.5 and 16 L/min (2,3). Typically, a sampling rate between 1 and 10 L/min is selected (1).

8.5.3 Set the sampling flow rate and time to produce an optimum fiber loading between 100 and 1300 f/mm² (2-4). The time of sampling can be estimated by using the following equation:

$$t = \frac{(A_c)(F_L)}{(Q)(C_e) 10^3} \quad (1)$$

where:

A_c = active filter collection area (~385 mm² for 25-mm filter),⁷

t = time, min,

F_L = fiber loading, f/mm²,

Q = sampling flow rate, L/min,

C_e = estimated concentration of SCCW, f/mL, and

10^3 = conversion factor.

8.5.4 At a minimum, check the flow rate before and after sampling. If the difference is greater than 10 % from the initial flow rate, the sample shall be rejected. Also see Test Method D 4532.

8.6 Carefully remove the cassette from the tubing at the end of the sampling period (ensure that the cassette is positioned upright before interrupting the pump flow). Replace the inlet cap and inlet and outlet plugs, and store the cassette.

⁶ *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

⁷ The active collection area (A_c) should be measured periodically, especially if different types of cassettes are used.

NOTE 4—Deactivate the sampling pump prior to disconnecting the cassette from the tubing.

8.7 Submit at least one field blank (or a number equal to 10 % of the total samples, whichever is greater) for each set of samples. Remove the cap of the field blank briefly (approximately 30 s) at the sampling site, then replace it. The field blank is used to monitor field sampling procedures. Field blanks shall be representative of filters used in sample collection (for example, same filter lot number).

8.8 Submit at least one unused and unopened sealed blank which is used to monitor the supplies purchased as well as procedures used in the laboratory. The sealed blank shall be representative of filters used in sample collection (for example, same filter lot number).

9. Transport of Samples

9.1 Ship the samples in a rigid container with sufficient packing material to prevent jostling or damage. Care shall be taken to minimize vibrations and cassette movement.

NOTE 5—Do not use shipping material that may develop electrostatic forces or generate dust.

NOTE 6—Shipping containers for 25-mm sampling cassettes are commercially available and their use is recommended.

9.2 Include in the container a list of samples, their descriptions, and all other pertinent information.

10. Specimen Preparation

10.1 The objective of the specimen preparation technique is to produce a thin carbon film (sufficiently clear for the TEM analysis) containing the particles from the filter surface. This requires four separate preparation steps: (1) partially fuse or collapse the filter to obtain a more continuous surface for the evaporated carbon layer, (2) in a lowtemperature asher, lightly etch the filter surface to uncover any fibers that may have been covered in the collapsing step, (3) evaporate a thin carbon film over the collapsed and etched filter, and (4) dissolve the MCE filter and retain the carbon film with particles for TEM analysis. Procedures described as follows or other equivalent methods (for example, dimethyl formamide (DMF) procedure (9)) may be used to prepare samples.

10.1.1 Wipe the exterior of the cassettes with a damp cloth before taking them into the clean preparation area to minimize the possibility of contamination.

10.1.2 Perform specimen preparation in a clean area.

NOTE 7—At a minimum, the clean area must include a positive pressure HEPA-filtered hood.

10.1.3 Carefully cut a wedge of the filter area (for example, 25 %) with a curved, steel surgical blade using a rocking motion to prevent tearing.

NOTE 8—Use care not to disturb the particles on the filter surface.

10.1.4 Place the section, particle side up, on a clean microscope slide. Affix the filter section to the slide with a gummed page reinforcement or other suitable means. Label the slide with a glass scribing tool.

10.1.5 Place the slide in a petri dish which contains several paper filters soaked with acetone (1). Cover the dish and wait for the filter to fuse and clear completely (typically, 2 to 4 min).

10.1.6 Place the slide containing the collapsed filter into a low-temperature plasma asher, and etch the filter.

NOTE 9—Because plasma ashers vary greatly in their performance, both from unit to unit and between different positions in the asher chamber, it is difficult to specify the conditions that shall be used. Insufficient etching will result in a failure to expose embedded fibers, and too much etching may result in loss of particles from the surface. It is recommended that the time for etching of a known weight of a collapsed filter be established and that the etching tare weight be calculated in terms of micrometres per second. The actual etching time used for a particular asher and operating conditions will then be set such that a ~1 to 2- μm layer (not more than 10 %) of collapsed surface will be removed (1,10).

10.1.7 Place the slide inside the bell jar of a vacuum evaporator. Evaporate a section (1 mm in diameter by 8 mm in length) of graphite rod onto the etched filter. Remove the slide to a clean, dry, covered petri dish.

NOTE 10—Rotation of the sample at an angle (~45°) is recommended during the coating process.

10.1.8 Prepare a second petri dish as a Jaffe washer with the wicking substrate prepared from filter or lens paper (1). The wicking substrate shall fit into the petri dish without touching the lid.

10.1.9 Identify the sample by labeling the petri dish or filter paper. In a fume hood, fill the petri dish with acetone until the wicking substrate is saturated. The level of acetone shall be just high enough to saturate the wicking substrate without creating puddles.

10.1.10 Remove a 3-mm square section of the carbon-coated filter from the glass slide using a surgical knife and tweezers. Carefully place the section of the filter, carbon side up, on the shiny side of a TEM grid. Cover the petri dish. Elevate one side of the petri dish by a few millimetres. This allows drops of condensed solvent vapors to form near the edge rather than in the center where they would drip onto the grid. Allow the sample to remain in the Jaffe washer until total dissolution of the MCE filter. Typically, a minimum of several hours is required to dissolve the MCE filter.

10.1.11 Three or more grids shall be prepared for each sample.

10.1.12 *Indirect Sample Preparation*—Resuspension of particulate matter collected on an overloaded filter and subsequent filtering onto another substrate may result in loss or breakup of the sample materials. Therefore, redeposition is permitted only in circumstances where an alternate filter is not available and cannot be obtained through resampling (for example, evaluation of a prototype procedure where the operational parameters cannot be duplicated). If indirect sample preparation procedures are employed, it must be clearly noted in the report. Furthermore, it must be clearly stated that results were obtained from the use of indirect sample preparation techniques and used only as an estimate of SCCW concentrations in the workplace environment. The following procedures are appropriate for this purpose.

10.1.12.1 Carefully remove the filter from the sampling cassette and cut a wedge (for example, one half or one quarter of the area of the original filter) as accurately as possible from the filter with a curved, steel surgical blade using a rocking motion to prevent tearing.

NOTE 11—Use care not to disturb the particles on the filter surface.

NOTE 12—The size of the wedge will depend on filter loading. If the sample is very heavily loaded, then a smaller wedge (for example, one eighth or one sixteenth of the area of the original filter) may be more appropriate.

10.1.12.2 Place the section of filter into a 100-mL beaker.

10.1.12.3 Add approximately 80 mL of filtered Type II distilled water to the beaker.

10.1.12.4 Place the beaker into the ultrasonic bath. Sonicate for approximately 1 min.

10.1.12.5 Remove the section of filter and rinse it using filtered distilled water. The rinse shall be collected in the 100-mL beaker. Add enough distilled water to result in a 100-mL suspension.

10.1.12.6 Filter the suspension using a funnel through a 25-mm, 0.22- μm MCE filter using vacuum filtration techniques. Rinse the interior of the beaker into the funnel using filtered distilled water.

NOTE 13—It is recommended that disposable funnels be used to reduce the potential for contamination.

NOTE 14—Use of a 47-mm funnel/filter assembly is permissible provided the active filter area is accounted for in the calculations provided in 13.1.2.

NOTE 15—If the resuspended filter is too heavily loaded with particles to permit analysis, then reprepare the sample using a smaller portion of the original filter as discussed in 10.1.12.1.

NOTE 16—The MCE filters used for resuspension must have an average blank level less than 18 f/mm².

10.1.12.7 Remove the funnel from the vacuum system. Place the deposited filter in a desiccator for approximately 2 h to remove moisture.

10.1.12.8 Cut a wedge of the filter (for example, 25 %) with a curved, steel surgical blade and continue to follow the procedures outlined in 10.1.4-10.1.11.

NOTE 17—Account for the area of the filter used in the resuspension process in the equations provided in 13.1.2 when calculating the estimated airborne concentration. For example, if 25 % of the original filter area was redeposited onto a 25-mm filter, then a dilution factor of 4 is used in the calculations.

11. Analysis Method

11.1 The objective of this method is to determine the concentration of SCCW fibers per cubic millilitre of air sampled based on the number of SCCW fibers observed during the TEM analysis.

11.1.1 Place the specimen in the TEM and select an accelerating voltage of at least 80 kV.

11.1.2 To ensure representative analysis, one half of the sample area to be analyzed shall be analyzed on one sample grid preparation and the remaining half on the second sample grid preparation.

11.1.3 Examine specimens at low magnification (300 to 500 \times) for integrity and selection of grid openings for analysis.

11.1.3.1 Individual grid openings with greater than 5 % openings (holes) or covered with greater than 15 % particulate matter or obviously having nonuniform loading shall not be analyzed (7). Reject the grid if:

(1) Less than 50 % of the grid openings covered by the replica are intact,

(2) The replica is doubled or folded in >50 % of its area,