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**METHODOLOGY FOR THE MEASUREMENT OF AIRBORNE ASBESTOS
BY ELECTRON MICROSCOPY**

by

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FOREWORD

PREFACE

ABSTRACT

The provisional electron microscope methodology for measuring the concentration of airborne asbestos fibers was refined. The methodology is divided into separate protocols. The step-by-step procedures for each protocol are nearly identical, so that cumulative data can be obtained and uncertainties, especially in asbestos identification, can be clarified. The operational steps encompass (1) type of sample, (2) collection and transport, (3) sample preparation, (4) examination under the transmission electron microscope (TEM) and data collection, (5) data reduction and reporting of results, and (6) quality control-quality assurance.

The TEM analytical protocol is subdivided into three levels of analysis: Level I, for screening many samples; Level II, for regulatory action; and Level III, for confirmatory analysis of controversial samples. Because identification of asbestos structures is critical, the level of analysis is directly related to the information sought:

Level I—morphology and visual selected area electron diffraction (SAED) pattern recognition.

Level II—morphology; visual SAED; and elemental analysis.

Level III—morphology; visual SAED; a selected number of SAED micrographs of zone-axis patterns; and elemental analysis.

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LIST OF ABBREVIATIONS

| | |
|-------|--|
| AEM | analytical electron microscope |
| EDS | energy dispersive spectrometer |
| EM | electron microscope |
| JCPDS | Joint Committee on Powder Diffraction Standards |
| LTA | low-temperature ashing |
| NIOSH | National Institute of Occupational Safety and Health |
| PLM | polarized light microscopy |
| QC/QA | quality control/quality assurance |
| SAED | selected area electron diffraction |
| SEM | scanning electron microscope |
| STEM | scanning transmission electron microscope |
| TEM | transmission electron microscope |
| TSP | total suspended particulates |
| UICC | Union Internationale Contre le Cancer |
| XRD | x-ray diffraction |
| XRF | x-ray fluorescence |



SECTION 1

INTRODUCTION

Asbestos is recognized as a health hazard, especially if inspired into the alveolar region of the respiratory tract. Asbestos may be present in air samples, water samples, biological or clinical samples, and other miscellaneous bulk samples, such as ores and food. These various types of samples require different collection methodologies and diverse preparation techniques.

Asbestos analysis methodologies may be categorized as bulk-material analyses, or those providing concentration information, and single-fiber analyses, or those providing morphology, size distribution, and concentration. Bulk-material analysis techniques, which include infrared spectroscopy, differential thermal analysis, and x-ray diffraction analysis (XRD), are limited by an inability to analyze concentrations of less than 1 μg , and by an inability to differentiate between fibrous and nonfibrous forms of minerals.

Single-fiber analysis techniques include optical microscopy and electron microscopy. Optical microscopy employing phase contrast has been promulgated into a monitoring method for the workplace environment (NIOSH-P&CAM 239). In addition, promulgation of a monitoring method for bulk-material asbestos samples (building insulation) using polarized light microscopy (PLM) is presently being considered. However, optical microscopic techniques cannot determine fibers of less than approximately 1 μm in diameter, and phase contrast cannot differentiate between asbestos and nonasbestos fibers.

The electron microscope (EM) provides particle morphology and size, and a degree of identification. A comprehensive study of various EM procedures (Samudra et al., 1977) was conducted in development of a provisional methodology manual, Electron Microscope Measurement of Airborne Asbestos Concentrations (Samudra, 1978). Three EM methods are available: the scanning electron microscope (SEM), the transmission electron microscope (TEM), and the analytical electron microscope (AEM). The SEM, with an x-ray energy-dispersive spectrometer (EDS), permits visual characterization (analogous to reflection optical microscopy) and fiber identification by elemental analysis. The TEM, providing an increased data-acquisition capability, permits visual characterization (in the transmitted mode) and fiber identification by crystal structure analysis. The AEM is a TEM with an EDS, and with the added capability of SEM/STEM (scanning transmission electron microscope) operation, which permits visual characterization (morphology and size) as well as fiber identification using both crystal structure by selected area electron diffraction (SAED) and elemental analysis by EDS.

The original EM methodology was developed for the U.S. Environmental Protection Agency (EPA) for measuring airborne asbestos concentrations, specifically for ambient air and for use as a "screening" tool. Development guidelines included attainable precision and accuracy of results; relative rapidness in use; cost-effectiveness; applicability to a large number of laboratories possessing a TEM (at that time, very few laboratories had TEM's with x-ray analysis capability or an AEM); and procedural steps to be independent of unique or exceptional in-house capabilities of a single laboratory (that is, interlaboratory precision rather than intralaboratory).

In usage, the EM method was successful within its prescribed limitations--that is, the precision and accuracy of results between laboratories using the complete method was good. However, problems that had been recognized in the study developing the methodology (Samudra et al., 1977) arose in the areas of (1) interpretation of airborne, (2) sample collection, (3) need for more exacting identification of asbestos, especially of amphibole type, and (4) use of only part of the methodology.

The present study was undertaken to refine the methodology. The problem areas and related criticisms were addressed within the underlying goals and guidelines set for optimizing the methodology. Protocols similar to a cookbook were not possible since basic knowledge or training was required regarding (1) sample collection, (2) preparation of samples for EM, (3) use of the TEM-AEM, and (4) diffraction pattern analysis. The refined methodology is based on an assumption that each intended user of a particular level of analysis has the necessary background and training to use it.

SECTION 2

CONCLUSIONS AND RECOMMENDATIONS

The EM methodology for measuring the concentration of airborne asbestos fibers has been refined and specified, and is recommended for field evaluation. The methodology is based on a TEM analytical protocol that is divided into three levels of effort: Level I, for screening many samples; Level II, for regulatory action; and Level III, for confirmatory analysis of controversial samples. The three-level analytical methodology is cost-effective, and will provide the required results for proper assessment of asbestos.

SECTION 3

GUIDELINES FOR UNDERSTANDING THE METHODOLOGY

The methodology is divided into separate protocols. The step-by-step procedures for each protocol are nearly identical, so that cumulative data can be obtained and uncertainties, especially in asbestos identification, can be clarified. These operational steps are:

- (1) Type of Sample--Source
- (2) Sample Collection and Transport
- (3) Sample Preparation for Analysis--Grid Transfer
- (4) TEM Examination and Data Collection
- (5) Data Reduction and Reporting of Results
- (6) Quality Control/Quality Assurance (QC/QA).

The analytical protocol under the TEM examination and data collection procedure is subdivided into three levels of increasing analytical effort in terms of requiring an instrument of greater capability, an electron microscopist with greater expertise, and a longer analytical time. Level I, a monitoring or screening methodology, resembles the present EPA provisional methodology (Samudra et al., 1978; Anderson and Long, 1980). Level II is a regulatory method requiring additional analytical criteria to establish asbestos identification limits, and to provide guidance for Level I analyses by confirming or clarifying visual SAED patterns. Level III, the most sophisticated and the costliest of the methods, is intended for confirming asbestos identification, especially in judicial controversies and other special situations.

In Sections 4, 5, and 6, the protocols for each of the three levels of analysis are presented independently of each other, and thus procedures common to each are repeated. All figures are presented in Appendix A.

Section 7 describes modifications for using the methodology on archival samples, which are samples collected on nonprocedural filter substrates, or samples collected without regard to filter loading levels. Section 8 describes analysis of inorganic sources in bulk-air samples or in bulk form. Section 9 concludes the report with a discussion of analytical aids pertaining to the limits of detection, preparation of blanks, use of computers, magnification calibration, and statistical methodology.

General guidelines for understanding the methodology are discussed in the following paragraphs.

LEVEL OF ANALYSIS

Knowledge of the history, source, and location of the sample, and the purpose and objective of the analysis aids in selecting the correct level of analytical effort. Simply "grinding the samples out" neither is cost-effective nor produces the best results, especially for Level II and Level III analyses. Instead of all Level I, all Level II, or all Level III, the majority of the analyses may be Level I, followed by some Level II. Level III could be used in its entirety or only at the analytical phase. If the source is known to contain no amphibole-type interference, or if chrysotile is of interest, gold-coating can be eliminated.

If a legal proceeding is anticipated, Level III analysis will be required where a chain-of-custody record is kept from collection, transport to the laboratory, preparation, analysis, data reduction, and reporting of results. EM finder grids must be used for grid transfer. In addition, for quality assurance, a second laboratory must be available for analyzing a portion of the sample using the same degree of custodial care. QC/QA protocols must be observed and records kept.

Whenever possible, and especially for unknown source samples, 10 to 20% of each set of samples should be analyzed by Level II analysis prior to using Level I as a screening procedure.

Level I is a relatively rapid procedure, and can be used by many laboratories with access to a conventional TEM. However, Level I results should not be used in legal proceedings. If "positives" or "false positives" are found, especially in areas where asbestos is known to be absent, and the field blank and laboratory blank have been checked, Level II analysis, and possibly Level III analysis, should be performed.

ORDER OF ANALYSIS

The order of analysis is (1) field blanks, (2) laboratory blanks (if needed), and (3) field samples.

COLLECTION AND REPORTING

The counting rule, "minimum 100 fibrous structures per known area (complete grid opening) or 10 grid openings, whichever is first," is a minimum rule for cost limitation. For very low asbestos presence, or for asbestos contamination studies, where particulate loading is high and asbestos presence very low, counting 20 grid openings from each of 2 grids (10 per grid) is recommended.

The EM magnification factor is very high or, conversely, the area of deposit examined is very small. Therefore, although the electron microscopist may report a zero count, the notation "Below Detectable Level" is the appropriate in the sample report. Along the same lines, the electron microscopist

should report observations, measurements, and conclusions as objectively as possible, realizing the subjective nature of his decision-making, such as parallel-sided, 3:1 aspect ratio, number count, size measurements, recognition-discrimination of SAED patterns, and categorizing of asbestos structure.

Data reduction and reporting of results must be consistent and stated. Dimensions of X-fibers (unknown length since complete fiber is not visible) may be doubled, not counted at all, or presented separately. Doubling of the visible portion is recommended, and should be so stated in the report.

Mass or conversion of size measurements to an assumed shape-volume-density relationship, is calculated, and thus is the least reliable of the data, especially for X-fibers, bundles, clusters, and matrices.

Although morphology, SAED, and XRF either singly or in combination will provide identification of asbestos, not all structures will be identified. The nature of the asbestos structure prevents analysis of all structures by SAED and/or by elemental analysis with EDS. Such factors as specimen thickness, orientation, and proximity to other particulates or to the grid wire will prevent attainment of good SAED patterns and limit the effectiveness of chemical analysis.

COSTS

Levels I, II, and III analyses are estimated to require 200, 400, and 1200 min per analysis, respectively. Additional costs will result from collection, preparation, and reporting of results. The equivalent monetary costs will depend on the laboratory rates of the personnel involved.

APPLICATION TO NONAIRBORNE SOURCES

Although the methodology has been developed for airborne asbestos, other types of samples from different sources can be analyzed if the samples are finely divided and placed with proper loading and uniform distribution either on a polycarbonate membrane filter or on a carbon-coated EM grid. Of course, the limitations of the collection and preparation steps must be known and accounted for to prevent inaccuracies in comparing results.

GEOGRAPHICAL CONSIDERATIONS

In some parts of the country, such as the Upper Great Lakes area, the possibility of misidentification is much greater because some nonamphibole minerals have visual SAED patterns that closely resemble those of amphiboles. Gold-coating and Level II analysis will help in differentiating between these minerals.

LABORATORY CONDITIONS

Asbestos analysis involves sustained microscopy for periods of 3 to 7 hours with unscheduled rest breaks. Subjective decisions regarding such factors as morphology, size measurement, visual identification, and possible

EDS make it difficult to break a manipulative physical routine or rhythm. Therefore, a professional environment for the microscopist is essential for effective asbestos analysis. In particular, such factors as unnecessary or redundant procedural steps, lack of personal recognition, and unreasonable deadlines may contribute to poor precision.

SECTION 4

LEVEL I ANALYSIS

SUMMARY OF PROTOCOL

Level I analysis is a monitoring or screening technique. It assesses the amount and type of asbestos structures in the atmosphere through the following steps:

- (1) A known volume of air is passed through a polycarbonate membrane filter (pore diameter, 0.4 μm ; filter diameter, 37 or 47 mm) to obtain approximately 5 to 10 μg of particulates per cm^2 of filter surface.
- (2) The particulate-laden filter is transported in its own filter holder.
- (3) The filter is carbon-coated in the holder.
- (4) The particulates are transferred to an EM grid using a refined Jaffe wick washer.
- (5) The EM grid, containing the particulates, is gold-coated lightly.
- (6) The EM grid is examined under low magnification (250X to 1000X) followed by high-magnification (16,000X on the fluorescent screen) search and analysis.
- (7) A known area (measured grid opening) is scanned, and the fibrous structures (fibers, bundles, clusters, and matrices) are counted, sized, and identified as to asbestos type (chrysotile, amphibole, ambiguous, or no identity) by morphology and by observing the SAED pattern.
- (8) The observations are recorded—a minimum of 100 fibrous structures or 10 grid openings, whichever is first.
- (9) The data are reduced and the results reported.

EQUIPMENT, FACILITIES, AND SUPPLIES

The following items are required for Level I analysis:

- (1) An 80 or 100-kV TEM with a fluorescent viewing screen inscribed with graduations for estimating the length and width of fibrous particulates.

