



## Project Summary

# Asbestos Fiber Determination in Water Samples: Preparation Techniques, Improved Analytical Method, and Rapid Screening

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A three-year study was conducted to improve the analytical method for determination of asbestos fiber concentrations in water samples. The research produced an improved sample preparation and analysis methodology and an alternative method with the potential for routinely screening drinking water samples for asbestos.

Sample preparation techniques investigated included the "drop" and collapsed membrane filter techniques. When these two techniques were compared to the carbon-coated Nuclepore technique using a polycarbonate filter, the carbon-coated Nuclepore technique proved to be the superior method of sample preparation.

Compared with plasma ashing, ozone-ultraviolet light oxidation of water samples was found to be a simpler and superior technique for removal of organic materials. Experiments revealed that large proportions of the suspended asbestos fibers could become attached to the inside surfaces of sample containers. This effect was caused by trace organic materials of bacterial origin. Ozone oxidation, carried out inside the collection container, released the attached fibers into the water again. Initial experiments were carried out to determine the effectiveness of the attachment phenomenon as a fiber separation technique. Investigation of the nature of the scavenging effect of bacteria on container surfaces led to the

development of stable reference dispersions of asbestos fibers.

If bacteria and their products were excluded initially, and if absolute sterility was maintained thereafter, suspensions of both chrysotile and crocidolite appeared to be stable for long periods of time. Tests of reference suspensions in sealed glass ampoules stored for almost two years produced fiber concentration values statistically compatible with those obtained at the time of sample preparation.

An improved analytical method for determination of asbestos fiber concentrations in water samples was developed. In this method, the water sample is initially treated with ozone and UV light to oxidize suspended organic materials. The water sample is then filtered through a capillary-pore polycarbonate filter (0.1  $\mu\text{m}$  pore size), after which the filter is prepared by carbon extraction replication for examination in a transmission electron microscope (TEM). Fibers are classified using selected area electron diffraction (SAED) and energy dispersive X-ray analysis (EDXA). Measurement of characteristic features on a recorded and calibrated SAED pattern is specified for precise identification of chrysotile. Quantitative determination of the chemical composition and quantitative interpretation of at least one calibrated zone axis SAED pattern are specified for precise identification of amphibole.

**Mineral identification and generation of the standard reporting format specified for the fiber count results are achieved by using two computer programs that are integral to the analytical method.**

The high cost of asbestos fiber counting using the TEM led to the requirement for an inexpensive and rapid method by which water samples having low fiber concentrations can be excluded from further analysis. Alignment of asbestos fibers in magnetic fields, combined with measurements of the scattered light from the aligned dispersions, was investigated as a rapid analytical technique. A rapid, fixed-fiber alignment method and a dynamic method of fiber measurement were studied. The dynamic fiber method proved to be the more sensitive method. Detection limits of 0.5 million fibers per liter (MFL) and 5 MFL were achieved for crocidolite and chrysotile, respectively. These detection limits were achieved directly from the water sample without any preconcentration steps. The scattered light measurement techniques were applied to the determination of the fiber concentrations in drinking water samples from three sources, and the results were consistent with those obtained independently by transmission electron microscopy.

Using the dynamic fiber method, mineral species other than the asbestos varieties were examined to determine possible interferences. The results indicated that nonfibrous material that rotates with the magnetic field yields broad scattered light maxima at about 45 degrees to the magnetic field direction. Qualitative measurements showed that many other fibrous mineral species yielded alignment modes similar to those obtained with the asbestos varieties.

The analytical time required for a single measurement using the current instrumentation is less than 10 minutes. Labor requirements for a sample preparation are variable depending on the fiber concentration steps incorporated, but these need not exceed one man-hour per sample.

*This Project Summary was developed by EPA's Environmental Research Laboratory, Athens, GA, to announce key findings of the research project that is fully documented in three separate reports (see Project Report ordering information at back).*

## **Introduction**

The Preliminary Interim Method for Determining Asbestos in Water was issued by the U.S. Environmental Protection Agency's Environmental Research Laboratory in Athens, Georgia, in July, 1976. The method was based on filtration of the water sample through a sub-micrometer pore size membrane filter, followed by preparation of the filter for direct examination and counting of the fibers in a TEM. Two alternative techniques were specified: one in which a cellulose ester filter was prepared by dissolution in a condensation washer and another known as the carbon-coated Nuclepore®\* technique that used a polycarbonate filter. In January 1980, the method was revised (EPA-600/4-80-005) to eliminate the condensation washer approach, and a suggested statistical treatment of the fiber count data was incorporated.

The first part of the research program described here was directed towards the development of improved techniques for the analysis of asbestos in water. Although the revised interim method had achieved substantial acceptance, other techniques of specimen preparation remained in use, including various "drop" methods and the collapsed membrane filter method. The most effective method was to be selected and used to study its analytical reproducibility in determining the levels of asbestos in drinking water and drinking water supplies. In addition, better techniques were sought for the removal of interfering organic materials because of the low temperature plasma ashing procedure had proved to be unsatisfactory in a number of ways.

Clearly defined, unequivocal methods for fiber identification, particularly of amphibole asbestos, were not incorporated in the Interim Method. It was recognized that adequacy of fiber identification procedures was a major issue when results were discussed, particularly if analyses were the subject of litigation. In other situations, where a large number of analyses were required, complete identification of each fiber was not economically possible. A substantial component of the research program was devoted to development of a systematic multi-level fiber classification and identification system. A standardized form of data reporting was also required.

\*Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Environmental Protection Agency.

The TEM fiber identification and counting procedure in the approved analytical method is labor-intensive, and the resulting high analytical costs have limited the extent to which water supplies can be monitored routinely for asbestos fibers. The cost factor has led to a requirement for a rapid method that would allow samples containing less than a pre-defined concentration of asbestos fibers to be eliminated from further analysis, allowing the TEM characterization to be confined to those samples that require a more detailed analysis. A rapid method is also required for routine monitoring of fiber concentrations in water sources where asbestos fibers are known to be present at concentrations of concern. To meet these needs, the research program investigated a rapid technique based on the measurement of the light scattered by magnetically aligned asbestos fibers.

The research project is described in three reports: *Development of Improved Analytical Techniques for Determination of Asbestos in Water Samples*, *Analytical Method for Determination of Asbestos Fibers in Water*, and *Rapid Screening Technique for Detection of Asbestos Fibers in Water Samples*. While supplies last, copies of *Analytical Method for Determination of Asbestos Fibers in Water* are available from ORD Publications, Center for Environmental Research Information, USEPA, Cincinnati, OH 45268.

## **Conclusions and Recommendations**

### ***Improved Sample Preparation***

The investigation of specimen preparation techniques for asbestos fiber counting by TEM showed that the carbon-coated Nuclepore method was superior to both the "drop" method and the collapsed membrane filter method. The "drop" method, in which a microliter volume of a concentrated dispersion is evaporated on a carbon-coated TEM grid, was shown to produce samples on which the fiber distribution was not sufficiently uniform to warrant its use in quantitative determinations. The collapsed membrane filter method was shown to display strongly size-dependent fiber losses relative to the Nuclepore preparation.

For chrysotile, the fiber losses increased with the pore size of the membrane filters used. For the 0.45  $\mu\text{m}$  and 0.22  $\mu\text{m}$  pore size filters, the losses were statistically significant at the 5%

level; for the 0.1  $\mu\text{m}$  pore size filters, fiber losses were not significant at the 5% level. For fibers shorter than 1.0  $\mu\text{m}$ , the fiber losses using the 0.45  $\mu\text{m}$  pore size filter were very high, and only 24% of these fibers were transferred to the TEM sample. The corresponding value for the 0.22  $\mu\text{m}$  pore size filter was between about 60% and 70%. The results were consistent with the postulate that the shorter fibers penetrated the filter structure more deeply and were engulfed during the collapsing procedure.

The results for crocidolite were more difficult to interpret. Although the total fiber losses using the 0.22  $\mu\text{m}$  and 0.1  $\mu\text{m}$  pore size filters were not significant at the 5% level, significant losses were observed in some fiber size ranges.

On the basis of the results, the collapsed membrane method was found to be unsatisfactory for quantitative analyses. It is recommended, however, that if cellulose ester membrane filters must for some reason be used, plasma etching of collapsed membranes should be investigated as a means of increasing the transfer efficiency of short fibers to the TEM specimens.

Samples from water sources contaminated by chrysotile fibers were collected from Sherbrooke, Quebec, and prepared using the carbon-coated Nuclepore technique. It was shown that 10 replicate measurements from each of these samples were statistically compatible. The same conclusion was drawn for samples contaminated by amphibole fibers collected near Duluth, Minnesota. This indicated that for a series of subsamples filtered at the same time, intralaboratory measurements by a single operator using the same instrument were repeatable.

A method of oxidation of organic materials in water samples, based on the use of ozone and short wavelength (254 nm) uv light was found to be successful. This oxidation technique was found to remove those organic components of drinking water samples that inhibit filtration and to be an effective and more convenient replacement for oxidation by two-step filtration and low-temperature ashing. When the ozone-uv light technique was used, no changes in either the electron diffraction behavior or the chemical compositions of chrysotile and amphibole fibers were detected. A second oxidation technique, based on the use of oxygen at pressures up to about 13.8 MPa and temperatures of up to 300°C, was found to be effective for removal of organic materials, but some

degradation of chrysotile fiber morphology was observed after treatment at the most extreme conditions. It was also found that containers made of polytetrafluoroethylene were required, because both glass and silica were attacked under the extreme conditions used. Because of the success of the ozone technique, no further investigation of the more involved high-pressure method was conducted. For samples containing large concentrations of refractory organics, such as sewage or plant effluents, the high-pressure technique warrants further consideration.

Studies of the stability of asbestos fiber dispersions yielded some surprising results. Initial experiments indicated that mechanical shaking of polyethylene bottles containing chrysotile fiber dispersions in double-distilled water reduced the suspended fiber concentrations to very low values. This effect did not occur if the bottles were exposed to continuous ultrasonic agitation for a similar period of time. The behavior was unaffected by either ionic or pH conditions. The effect was also observed for dispersions of crocidolite.

Apparently, the presence of the trace organic materials of bacterial origin in some way promoted the attachment of asbestos fibers to the inside surfaces of the containers. This effect could seriously compromise the results of routine sample analyses. Container and storage studies indicated that the effect was a consequence of an organic product of bacteria, rather than a mechanism involving direct interaction with the bacteria themselves, and that the organic material was probably a variety of polysaccharide. The effect of this phenomenon on routine sample analyses could be eliminated by ozone treatment carried out inside the original sample container. This treatment was found to perform the double task of oxidation of interfering organic materials and release of fibers attached to the container.

The observation of the interaction of asbestos fibers with the trace organic materials had two other consequences: the development of stable reference fiber suspensions and the development of a separation technique that was at least partially specific for chrysotile. Reference fiber suspensions have been required for some time in order to facilitate analytical suspensions have been required for some time in order to facilitate analytical quality assurance programs, but their stability has always been in question. If the reference dispersions were prepared

so as to exclude all bacteria and their organic products, they were then stable for long periods of time, provided that absolute sterility was maintained. It is recommended that a standards agency maintain a supply of these reference dispersions, with appropriate certification of their contents, so that analytical quality assurance of future sampling programs can be established by incorporations of control samples. Also, the separation method should be developed further and the mechanism that gives rise to the attachment phenomenon should be investigated. The observation of this strong interaction between asbestos fibers and organic materials of biological origin may have a significance in other fields unrelated to analytical method development.

### ***Improved Analytical Method***

The improved analytical method developed in the study represents the best available technology for determination of asbestos fibers in water. A number of new features were incorporated. These include the introduction of ozone-uv oxidation for all samples, a fiber classification system, a minimum fiber length for reporting, a standardized reporting format, quantitative interpretation of fiber identification data, and a fibrosity index that appears to permit discrimination of fibrous and nonfibrous species. The fiber classification system that was developed recognizes instrumental limitations, and if required, permits later reevaluation of the raw data using different fiber identification criteria. A computer program was written that permits fiber identification on the basis of EDXA and zone axis SAED patterns. The identification procedure operates by selection of minerals that are consistent with the measurements, using a library of data from 226 minerals. A computer program for reporting of fiber counting data in a standardized format was also established. The fiber identification protocol based on zone axis SAED and quantitative EDXA is capable of more specificity than had previously been provided by TEM analysis. The identification procedure permits determination of approximate chemical composition, which is adequate for the general classification of amphibole fibers but is not sufficiently precise for the incorporation of adjectival modifiers in the mineral description. The study recommends that the identification procedure be reviewed on a regular basis and that more precise X-ray analytical

procedures be developed and applied as they become available. The changes and additions introduced into the basic analytical method should eliminate the problems of poor interlaboratory reproducibility that have been observed in the past.

### **Rapid Screening**

The measurement of scattered light from magnetically aligned asbestos fibers has been demonstrated as a suitable method for detection of asbestos fibers in water samples. Two techniques were investigated.

A rapid, fixed fiber alignment method was studied in which an aqueous fiber dispersion is filtered through a membrane filter located in a strong magnetic field. This results in a filter on which the asbestos fibers are permanently aligned in preferred directions. When the filter is exposed to solvent vapor, the structure collapses and the filter becomes transparent. Rotation of the filter in a collimated beam of light yields maxima in the intensity of the scattered light; the positions of these maxima are related to the alignment direction of the fibers.

A dynamic method of fiber measurement also was investigated in which the behavior of aqueous asbestos fiber dispersions in a rotating magnetic field is observed. A spectrophotometer cell containing the fiber dispersion is placed between the poles of a rotating magnet and illuminated by a collimated beam of light. The fibers rotate in synchronism with the magnetic field, and maxima in the intensity of the forward scattered light are observed. Because light is scattered more strongly in directions normal to the lengths of fibers, a maximum in intensity of the scattered light occurs for every 180 degrees of fiber rotation. When the scattered light is monitored and the intensity displayed as a function of magnet position, the areas under the peaks are proportional to the fiber concentration.

For the fixed fiber alignment method, the lowest detection level was limited by the residual structure in the collapsed membrane filter. For the UICC crocidolite and UICC amosite, the detection level was about 0.1 ng/mm<sup>2</sup>, and for chrysotile about 1 ng/mm<sup>2</sup>. If 25-mm-diameter filters were used, these detection levels correspond to filter loadings of about 20 ng and 200 ng, respectively. In order to detect a concentration of 0.2 MFL of chrysotile, filtration of about 100L of water through an active filter area of 200

mm<sup>2</sup> would be required. Signal enhancement techniques such as RF plasma etching of the filter and shadowing of the particulate by evaporated metal films failed to improve the detection limits significantly. The dynamic fiber method achieved much lower detection limits of 0.5 MFL (180 ng/L) and 5 MFL (30 ng/L) for crocidolite and chrysotile, respectively. These detection limits apply to the 5mL volume of aqueous fiber dispersion in the spectrophotometer cell and correspond to detection of 0.9 ng of crocidolite and 0.15 ng of chrysotile. The required detection limits of 0.2 MFL or 1 ng/L can be achieved with the incorporation of a selective fiber concentration technique. A limited study was made of the high gradient magnetic separation (HGMS) technique for amphibole fibers. A new method was also devised for separation of chrysotile fibers by selective adhesion to organic materials.

In the investigation of light scattering from liquid suspensions, it was found that particles of random shape that rotate with the magnetic field produce a broad maximum of scattered light intensity corresponding to alignment at an angle of 45 degrees to the magnetic field direction. This effect was observed, for example, with borosilicate glass fragments, and is in contrast with the simple increase in constant scattering obtained from particulate that is unaffected by the magnetic field. In general, however, the presence of other particulate degrades the detection limit, and therefore specific fiber separation techniques were investigated. HGMS was successful in extracting UICC crocidolite and amosite, having a 95% numerical collection efficiency for dispersions of amosite. Because they are not strongly magnetic, fibers of chrysotile were not retained by the magnetic separator. Therefore HGMS is a useful technique for separating chrysotile asbestos from amphibole asbestoses that contain high concentrations of iron.

A new separation technique based on scavenging of fibers by organic materials was successful for specific separation of chrysotile. The same technique also appears to allow concentration of crocidolite and amosite fibers, but separation specificity has not yet been established. The recovery of separated chrysotile was between 87% and 100% for standard dispersion, falling to about 45% in the case of drinking water samples.

Three municipal water supplies were analyzed directly by the rotating fiber

method. The particulate of random shapes yielded prominent, broad peaks at 45 degrees and 225 degrees and it was necessary to perform profile subtractions in order to extract the signal originating from the fibers present. The residual peaks after this procedure agreed with the known asbestos fiber levels. For a water sample from Beaver Bay, Minnesota, the 45 degree component was subtracted and this resulted in residual peaks at 0 degrees and 180 degrees and at 90 degrees and 270 degrees. This agrees with the known asbestos content of the water: cummingtonite is known to align parallel and grunerite normal to the magnetic field direction. It was possible to measure directly the chrysotile fiber concentration in a municipal water that had a concentration of 40 MFL. Application of the fiber separation technique to the same sample yielded a concentrated suspension for analysis that contained chrysotile.

Development of computer profile subtraction techniques will permit the separation of the components corresponding to mineral fibers from the total scattered light profile. This refinement will reduce the amount of sample preparation required for separation and pre-concentration. Variation of the rotation rate and strength of the magnetic field may provide additional information by which particle species may be differentiated. In routine use, it is estimated that water samples could be analyzed directly in five to ten minutes, whereas samples requiring separation or pre-concentration would require less than one man-hour for preparation and analysis.

The alignment modes of a number of fibrous mineral species in a magnetic field were investigated qualitatively. Some yielded broad scattered light profiles similar to those from chrysotile; others displayed sharper peaks from fibers aligned in directions parallel or normal to the magnetic field. If the primary purpose is the detection of "asbestos" then there is some potential for interference by fibrous species other than those normally considered to be asbestos. Assuming that the purpose of the technique is to determine whether any fibrous mineral species are present, then it is highly successful, extremely sensitive, and allows for some discrimination between mineralogical species.

Assuming some pre-concentration of the sample, the magnetic alignment technique has the required detection level and sensitivity for measurement of

fiber concentrations in water. It is capable of significant further development, particularly for the determination of fiber dimensions. More extensive fiber characterization could also be achieved on the basis of iron content and alignment mode. Additional research is also required to optimize the specific fiber separation techniques.

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*J. M. Long is the EPA Project Officer (see below).*

*This Project Summary is based on the three reports listed below:*

*"Rapid Screening Technique for Detection of Asbestos Fibers in Water Samples," (Order No. PB 83-262 915; Cost: \$11.50)*

*"Development of Improved Analytical Techniques for Determination of Asbestos in Water Samples," (Order No. PB 83-261 651; Cost: \$14.50)*

*"Analytical Method for Determination of Asbestos Fibers in Water," (Order No. PB 83-260 471; Cost: \$23.50)*

*The above reports are available only from: (costs subject to change)*

*National Technical Information Service*

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*The EPA Project Officer can be contacted at:*

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