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Detection and Analysis of Asbestos in Environmental Samples Collected on Membrane-Filters: A Multiple-Choice Procedure Integrating the LM, the SEM and the EMP

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Visual methods remain the most adequate methods for detecting particulate pollutants present in trace amounts. Light microscopy (LM) and electron microscopy (EM) are widely used for this purpose, but none of these techniques gives the complete answer to the problem of the detection and identification of environmental asbestos. In addition to a number of problems inherent to asbestos analysis (BEAMAN & FILE 1976), the specific demands of environmental monitoring in terms of costs and statistical relevance have to be taken into account. LM,which is simple and cheap to use and enables the processing of many samples within a short time, is of no avail for the detection of submicronic fibers, and its analytical possibilities are limited. On the other hand, EM, which provides the resolution and analytical power necessary for full asbestos analysis down to elementary fibrils, is very costly in time and money and cannot but examine reduced fractions of samples.

Because LM- and EM-data are difficult to correlate, most existing procedures for the analysis of environmental asbestos recommend the use of either LM or EM, (TEM or SEM with coupled EMP, or TEM with SAED), but seldom of both, (ZUMWALDE & DEMENT 1977).

We believe however that if both efficiency, i.e. statistical relevance within short times, and reliability are to be achieved,LM and EM should be used together, in an integrated way, that takes advantage of either specificities. The SEM with coupled EMP is the instrument of choice to develop an integrated methodology, because of the possibility of bringing over directly to the SEM+EMP a preparation studied under LM.

The procedure presented here is devised to make the most of this possibility: the particulate sample is transferred from the membrane-filter onto a sample-mount specially chosen to ensure optimal conditions for the detection of fibers by both LM and SEM and their analysis by EMP. Moreover,provision is made for the recovery under SEM of the complete information recorded during LM-examination of the sample, so that the use of the SEM+EMP can be restricted to its most specific purpose: detect and identify what cannot be detected or identified by LM.
A further advantage of the integrated use of LM and EM is the possibility to bridge the gap between environmental asbestos monitoring and the analysis of biological tissue in medico-legal cases.

I. PRELIMINARY REMARKS

Before outlining the proposed procedure and explaining in detail all sample manipulations,we would like to comment on some features which

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we believe are original.

a) On the use of LM.

The traditional way to examine asbestos fibers is under phase-contrast with oil-immersion, (Joint AIHA-ACGIH Committee 1975). The reason for this is, among others, the fact that asbestos dust being usually collected on membrane-filters, a medium is needed to clear the membrane prior to LM-examination. From then on,phase-contrast is indeed the best mode to detect asbestos fibers. But when the inevitability of the presence of filter material is put aside, one finds out that much better conditions for the visual detection of asbestos fibers are obtained in the reflected light mode,with the fibers fixed dry on a smooth hard surface (glass,for instance). Under reflected light, careful adjustment of the diaphragms produces a relief-effect particularly favourable for the discrimination of elongated objects.
A hard smooth background is also very convenient for SEM. A single substrate, providing highly favourable conditions for detecting fibers under both LM and SEM can thus be chosen,enabling LM- and SEM-examination to be conducted in close sequence. We call such a double-purpose substrate a LM-SEM-EMP sample-mount.

b) The concept of conservative transfer and heat-fixing.

In order to benefit from the advantages of a hard smooth substrate for detecting asbestos fibers present in environmental samples by LM and/or SEM, some way had to be found to transfer the particles from the membrane-filter onto the chosen sample-mount. Several techniques exist for transferring particulate material collected on membrane-filters to various substrates,(FERREL et al. 1975, ORTIZ & ISOM 1974), but it was felt that none of them meets satisfactorily some essential demands of environmental studies; these demands being,according to us, that :
-the procedure be easy to perform so as to allow routine operation,
-the number of sample manipulations be kept minimal,
-accidental contamination be kept under complete control,
-no information pertaining to the origin, history or social behaviour of particles of interest be destroyed.

The concept of conservative transfer was developed as an attempt to meet these prerequisites. It means the transfer from the membrane-filter onto a chosen substrate of the totality of the particulate sample in its original distribution, without loss or denaturation of its components.The operation includes the fixing of the particles on the substrate by "heat-fixing",(see Ch III for details).

Here intervenes a remarkable and convenient property of asbestos fibers that is going to play a prominent role throughout the whole procedure : given the right substrate, silicate fibers exhibit outstanding properties of adherence after the very simple heat-treatment used for fixing the particulate sample on the sample-mount.

LM- and SEM-examination is carried out directly on the transferred sample, so that, simultaneously to the search for inorganic fibers, information about the sample as a whole is recorded. Subsequent elimination of the organic fraction by ashing will also be carried out conservatively, i.e. directly on the sample-mount, without disturbance to the distribution and general morphology of the inorganic particles. The problem of filter-material residues in ashed samples (FATEMI et al.

1977), is eliminated as a matter of course.

c) Integrated use of the LM and SEM+EMP.

The integrated use of the LM and the SEM+EMP is one of the factors ensuring efficiency and reliability to the proposed procedure.
It is accomplished through the choice as substrate for the conservative transfer of the collected sample of an adequate LM-SEM-EMP sample-mount, combined with a system ensuring the accurate recovery under SEM of an individual particle detected and documented under LM.

Choice of the LM-SEM-EMP sample-mount. The main requirement put on the LM-SEM-EMP sample-mount is to have such physical and chemical properties as to provide simultaneously optimal conditions for the detection of mineral fibers under LM (reflected light) and SEM, as well as for their subsequent analysis by EMP.
Our first experiments of conservative transfer were made onto glass-slides because of their ready availability. Glass,with its featureless surface and good heat-resistance,is actually ideally suited for this operation.But it is not at all adequate for the analytical stage,its EMP spectrum interfering heavily with that of asbestos.A substitute had to be found which would exhibit the surface qualities of glass, and on which asbestos fibers can be fixed permanently. Two materials were found suitable: pyrolitic graphite and electrolytic copper. Both can be treated to have glass-like surfaces. Their heat-resistance is good; although corrosion is liable to happen,it can be controlled by careful operation. Electrolytic copper should be preferred though, because the adherence on it of heat-fixed asbestos is better than on graphite; a sample-mount made of electrolytic copper can be rinsed with water or most organic solvents,even bathed for hours in organic solvents, without asbestos fibers being removed at all.
The copper discs currently available (Ernest F.FULLAM accessories for microscopy,cat n°1764) have also a very high-quality surface-polish, suggesting their use as substrates for automatic image-analysis .

Satisfactory substitutes for glass are thus available; nonetheless, we still recommend the use of glass-slides as sample-mounts in one option of our multiple-choice procedure,(see flow-chart). Two reasons for that: first, the high price of copper or graphite discs imposes that these expensive items be called for only when the presence of asbestos is being suspected in the course of the analysis. Second, the fact that glass has the specific advantage of being transparent, enabling thus the full use for particulate analysis of all LM implements: transmitted and/or polarized light,phase-contrast,dispersion-staining,reflected light. This can be very valuable in the first stages of an analysis.

Recovery of individual particles: From LM to SEM. A precision object-marker for LM has been devised in our Department (Belgian Patent n° 830.067). With the help of this instrument, any particle of interest detected under LM will be straightforwardly recovered under SEM for its immediate analysis by EMP.

d) Clearing of non-asbestos inorganic fibers and contamination control.

An environmental sample usually contains a huge variety of particles; many, both organic and inorganic, display a fibrous morphology, and can

be mistaken for asbestos or obscure its presence. The organic fraction is easily disposed of by ashing. As for the many inorganic particles mimicking asbestos,we propose a system of "selective rinsing": Indeed,as asbestos fibers exhibit an outstanding adherence on the LM-SEM-EMP sample-mount after heat-fixing, one can imagine a variety of chemical treatments which would remove all but asbestos particles. One such treatment is the removal of all calcium sulfate and calcium carbonate fibers often present in environmental samples (MIDDLETON 1978) by a short stay of the sample-mount in an ammonium chloride solution.

The same property of adherence provides a simple way to control any accidental contamination of the sample happening during analysis. After completion of conservative transfer, the particulate sample fixed on the sample-mount can be rinsed with distilled water or some organic solvent without risk of loosing asbestos particles.
The way in which conservative transfer is performed (see Ch III), also garantees minimal contamination risk.

II. OUTLINE OF THE PROCEDURE

The procedure, which will be described in detail in the next chapter, enables, at the cost of but a few simple sample manipulations, the implementation of the following steps :

- LM-examination of a representative piece of the original membrane-filter.
- LM- and SEM-examination of all particles present on the selected piece, in their original distribution, cleared of the membrane-filter material and transferred onto a LM-SEM-EMP sample-mount.
- LM- and SEM-examination of the same particulate material, cleared of its organic fraction.
- LM- and SEM-examination of the same material, cleared selectively of some of those inorganic fibers mimicking asbestos.

At each step, particles of interest are individually and permanently marked under LM for easy retrieval under SEM and immediate analysis by EMP.

The LM is used mainly to record the overall features of the sample, to make preliminary assessments of the presence of inorganic asbestiform particles and mark them for future identification by EMP, if needed.

The SEM+EMP is used for analyzing the previously marked and documented particles, and detecting possible submicronic fibers. As the original distribution of the particles on the filter has been investigated under LM, the use of the SEM for detecting and counting submicronic asbestos fibers can be reduced to a small number of representative fields.

At any time during analysis, provision is made for complete control of accidental contamination.

Several options are proposed in the procedure, to meet different situations. The main options are displayed in the included flow-chart, as 3 different sample-treatments performed on 3 separate segments of a same membrane-filter. The 3 options can be performed independently, or according to the logical order indicated in the flow-chart.The decision will depend on the type of environmental situation studied, on the

time and means available for the study, and its ultimate aim : are accurate ciphers wanted, or is a qualitative study sufficient ?
The complete treatment,involving the analysis of 2 or 3 separate segments of a same membrane-filter, should be reserved for the first sample of a new survey, for intensive controls, or when a full description of the particulate sample, including other particles besides asbestos, is wanted. The very fast treatment n°3, in which the different steps of sample preparation are performed in one go, prior to any visual examination, is particularly suited for routine analysis, when the overall features of the sample and the nature of the main particulate pollutants are already known. Treatment n°2, completely straightforward, will usually suffice in most cases where a heavy asbestos contamination is detected. In that option, the whole analysis is done on the original filter, cleared for proper LM-examination (reflected and transmitted light), then gold-coated for SEM+EMP analysis.
The fact that Millipore filters currently used in environmental monitoring have a tendency to bulge under the electron beam, does not matter here, as only big fiber bundles or aggregates are to be identified.

III. TECHNICAL DESCRIPTION OF THE PROCEDURE

The procedure which is depicted in the included flow-chart, is meant for samples collected on Millipore cellulose-ester membrane-filters. An identical procedure can be devised for polycarbonate Nuclepore filters. The main adjustment will be the use of 1,1,2,2-tetrachlorethane instead of acetone as solvent and clearing agent for the filter.

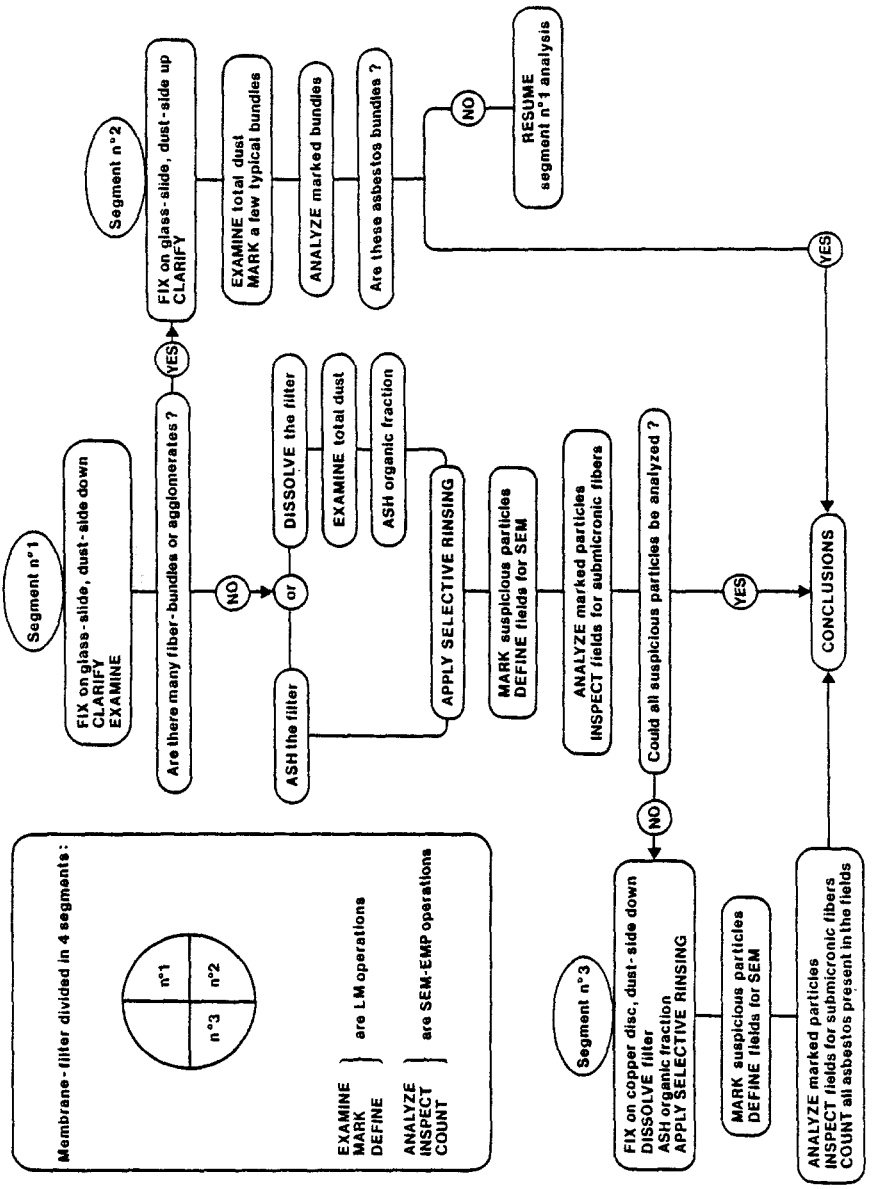
In the flow-chart, sample manipulations and LM or SEM operations are represented in capital letters; they are explained in detail hereunder together with a few other items, in the following order :

FIX
CLARIFY
DISSOLVE
ASH
SELECTIVE RINSING and contamination control
MARK
DEFINE FIELDS
ANALYZE
From LM to SEM

FIX and CLARIFY and DISSOLVE ⟶ conservative transfer

FIX : A Millipore membrane-filter is easily fixed on a glass-slide, or any other hard smooth surface, with the help of a few drops of a 1/1 mixture of amyl- and ethyl-acetates (JEDWAB 1971). The filter, or a segment of it, is laid down on the wet surface of the sample-mount, allowed to soak in the mixture for a few seconds; the sample-mount is then tipped up to let the excess of liquid be absorbed on a piece of tissue. Complete drying of the preparation takes about 1 h in a hot-air cabinet set at 60°C. Prior to the fixing of the filter, the substrate should be carefully cleansed using ethanol and thoroughly dried.
Conservative transfer: when conservative transfer is intended, the filter should be fixed on the sample-mount dust-side down. The preparation is then put for 1 h in a hot-air cabinet at 120°C to induce adherence on the substrate of the particles (GONI et al. 1975). This is what we

FLOW-CHART


Membrane - filter divided in 4 segments :
n°1
n°2
n°3
n°4
EXAMINE
MARK
DEFINE
are L.M operations
ANALYZE
INSPECT
COUNT
are SEM-EMP operations
Segment n°1
FIX on glass - slide, dust - side down
CLARIFY
EXAMINE
Are there many fiber - bundles or agglomerates ?
NO
YES
ASH the filter
or
DISSOLVE the filter
EXAMINE total dust
ASH organic fraction
APPLY SELECTIVE RINSING
MARK suspicious particles
DEFINE fields for SEM
ANALYZE marked particles
INSPECT fields for submicronic fibers
Could all suspicious particles be analyzed ?
NO
YES
Segment n°3
FIX on copper disc, dust - side down
DISSOLVE filter
ASH organic fraction
APPLY SELECTIVE RINSING
MARK suspicious particles
DEFINE fields for SEM
ANALYZE marked particles
INSPECT fields for submicronic fibers
COUNT all asbestos present in the fields
CONCLUSIONS
YES
Segment n°2
FIX on glass - slide, dust - side up
CLARIFY
EXAMINE total dust
MARK a few typical bundles
ANALYZE marked bundles
Are these asbestos bundles ?
NO
RESUME
segment n°1 analysis

call "heat-fixing" of the particulate sample.

Even if no LM-examination is intended, clarification of the filter (CLARIFY) should be carried out after the heat-fixing, as this has the effect of loosening the hold of the filter material on the particles, ensuring thus a smooth progress to the DISSOLVE step.

CLARIFY : A stay of a few minutes of the preparation, filter up, in acetone vapors will clarify completely the Millipore filter (ORTIZ & ISOM 1974). A box with a tight lid and a tissue impregnated with acetone is all that is needed. The effect of acetone vapor is to fuse slightly the filter material; the result is transparency and the homogenization of the filter-surface, enabling LM-examination also in the reflected light mode,(segment n°2). A clarified filter seems to stand better the electron beam.

DISSOLVE : After fixing and clarification of the filter, and heat-fixing of the particles on the sample-mount, the preparation is put horizontally, filter down, in a bath of pure acetone, without agitation. The sample-mount should not be moved before the dissolution of the filter material is completed, which takes about 2 h. The result is the faithful transfer of the collected dust on the LM-SEM-EMP sample-mount.
Reliability of the operation: as far as small particles are concerned, specially those with fibrous morphology -and most of all silicate fibers- we have checked that the risk of loss is extremely low, provided the porosity of the filter is 0.4 microns or less. A risk of loss does exist however, and understandably, for bulky particles; big asbestos bundles may leave the sample-mount during the dissolution. This is why examination under LM of the clarified membrane is recommended before processing (see flow-chart).

There are cases where the DISSOLVE operation does not work properly; one obvious case is when the filter is overloaded, but other cases happen, probably because of the presence in the collected sample of some compound preventing the good adherence of the particles on the sample-mount. In these cases, ASH should be carried out instead.

ASH :

ASH the filter: after fixing of the filter and heat-fixing of the particles on the glass-slide or sample-mount,the preparation is left for 8 to 12 h in a LTA oven (THOMAS & HOLLAHAN 1974). (The reason for such a long time is the hardening effect on the membrane of the acetate-mixture used for fixing it).
As far as inorganic particles are concerned, the result of ashing the filter in this way is a conservative transfer: the original distribution is respected and most inorganic particles remain unaffected because of the low temperature at which the ashing takes place (80°C). Residual ashes from the filter are gently washed away with water.

ASH the organic fraction: in the option DISSOLVE, ashing of the organic fraction is carried out after elimination of the filter-material. Any means are thus applicable, provided they do not destroy the sample-mount or denature the asbestos possibly present (t° should not exceed 500°C). We have found that the use of a simple Bunsen-burner,held at the right distance, is adequate for glass or copper sample-mounts.

SELECTIVE RINSING and contamination control:

After completion of conservative transfer, be it onto a glass-slide or an electrolytic copper disc, the sample can be cleared of any accidental contamination by simply rinsing it with distilled water. Immersion oil (LM) is readily eliminated by rinsing with carbon tetrachloride. In the same way, fibers mimicking asbestos can be washed away or dissolved by using the proper solvents; for instance,a stay of 10 min in a saturated solution of ammonium chloride will clear the sample of all calcium sulfate and calcium carbonate fibers,without removing the asbestos fibers possibly present.

MARK : The marking of interesting particles during LM-examination, with micron-precision, has been made possible by the development of a special object-marker. This instrument prints a straight mark of about 0.3 mm at each side of the particle. The whole mark is less than 1 mm long. The object marker can be used on membrane-filters, but also on any hard smooth surface, by simply dipping first its tip in indelible ink (stamp ink is adequate).

DEFINE FIELDS : Selected fields for SEM are easily defined on most hard smooth substrates with the help of a Vickers microdurometer.

ANALYZE : Analysis and identification of particles are performed with the help of an EMP coupled to the SEM, in the energy-dispersive mode. With the copper LM-SEM-EMP sample-mount, substraction of the background is hardly necessary for the identification of mineral fibers, the copper peaks being narrow and well-defined, with practically no background noise. If the sample-mount is a piece of glass-slide, the proper identification of asbestos will usually not be possible, except for chrysotile: in many instances,the morphology of chrysotile is so characteristic and the Mg peak so intense that little doubt is left as to the nature of the fiber.

From LM to SEM : After conservative transfer of the particulate sample from the membrane-filter onto the LM-SEM-EMP sample-mount, no further treatment is needed for preparing the sample for SEM besides carbon coating in a vacuum evaporator. In case of direct transfer of a segment of filter from LM to SEM (segment n°2), the segment is stripped from the glass-slide, glued with colloidal silver on an aluminium stub, and gold-coated.

CONCLUSIONS

- The procedure proposed here is meant for systematic monitoring of asbestos in the environment.
  It is simple to perform, reliable, and leads to a qualitative as well as quantitative assessment of the presence of asbestos fibers.
  The investment in time remains within reasonable limits thanks to the integrated use of the LM and the SEM+EMP, and to the technique of conservative transfer. The latter allows in addition for an objective description of each sample as a whole, and for the recording of any information pertaining to the origin, history and social behaviour of the asbestos particles possibly present.

- The procedure is readily adaptable to other particulate pollutants.
- The choice of electrolytic copper discs as LM-SEM-EMP sample-mounts, with their very high surface qualities, together with our technique of selective rinsing, can be of use in the development of an automated LM-SEM image-analysis system for particle counting.

It is hoped that this work will contribute to encourage collaboration between the "LM and EM people".
Not only does the integrated use of both techniques provide a tentative way to overcome the cost vs. accuracy dilemma inherent to environmental studies, but it also hints, we believe, to a whole field of analytical practices not yet explored for most of it.

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