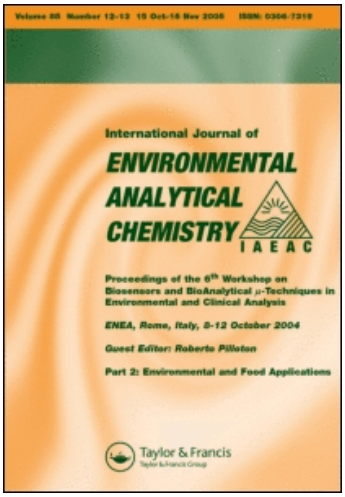

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Some Aspects of Analysis of Single Fibers in Environmental and Biological Samples

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Some Aspects of Analysis of Single Fibers in Environmental and Biological Samples†

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Nuclepore filters were used for sampling and evaluation of fibrous particles in ambient air, in liquids and in biological materials. The fiber counting and fiber size measurements were done by means of SEM-methods. The number of fibers and the distributions of fiber lengths and diameters were plotted. The specific identifications of asbestos, glass and other mineral fibrous particles were made by electron microprobe analysis. Certain elements proved to be approximative identification factors for different fibrous minerals in ambient air, in liquids, on material surfaces, or in biological materials.

For ambient air, asbestos, glass, and many other inorganic fibrous particles were found in the urban atmosphere as well as in the atmosphere of remote regions. Fibrous gypsum, fibrous ammonium sulfates, fibrous silicates, fibrous mica, and quartz were identified among these particles. Even in remote ambient air, relatively high concentrations of inorganic fibrous particles could be measured.

KEY WORDS: Asbestos fibers, ambient air, drinking water, electron microscopy, mass spectroscopy.

INTRODUCTION

Methods for a quantitative determination of small chrysotile fibers and fibrils in environmental samples are presently under development and an internationally standardized method does not yet exist. There is no doubt that, in the final analysis, electron microscopical methods (transmission-TEM, scanning-SEM, and scanning transmission-STEM electron

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microscopy) must be used for the sample evaluation. In general, fibers collected in environmental samples, mainly fibers collected in ambient air, are sufficiently small that identification in the optical microscope is not currently possible. All electron microscopical methods are useful for the evaluation of fibrous particles in environmental samples.

TEM or STEM should be considered as the basic methods for sample evaluation in the majority of cases and SEM has considerable importance in some special cases and as a complementary method for TEM and STEM. All three methods need a very careful standardisation of the quantitative procedure on an international level, before they can be used as a routine measurement assessement. Detection limits or detection ranges for the fiber length L_F, the fiber diameter D_F and the aspect ratio L_F/D_F have not yet been established. Biologists are not unanimous as to what standards are desirable or necessary to minimize the well-known health hazards associated with asbestos and other fibers. Values of $L_F \geqq 0.15\,\mu m$, $D_F \geqq 0.03\,\mu m$ (but $D_F \leqq 3\,\mu m$) will constitute the lowest fiber dimensions that could still be measured and counted by electron microscopical techniques.

During the last five years, all procedures—TEM, SEM and STEM—developed very intensively and we have sufficient international experience for standardisation of methods. Concerning the TEM, procedures developed by Sébastien *et al.*[1,2] and published later by Pooly *et al.*,[3] as well as the proposed ISO-procedure by Chatfield[4] seem to be the basis for the development of standard criteria. Furthermore experimental procedures for the application of SEM methods have already been published.[5] There is a need of standardizing filter types for the sampling of fibrous aerosols and fibrous liquid suspensions. Our previous investigations[6,7] have shown Nuclepore filters to be the most suitable material for sampling of fine fibers in ambient air (pore diam. of $0.4\,\mu m$) and in liquids (pore diam. of $0.1\,\mu m$).

The identification of asbestos and other fibers in SEM, TEM and STEM is mostly based on three methods: morphological identification of chrysotile fibrils, the energy-dispersive spectrometry (EDXA) and the selected-area electron diffration method (SAED).[1–5]

In spite of the progress achieved on the methodological field, during the past five years so far, relatively few field measurements have been made in the environment. Therefore data of asbestos concentrations in ambient air are scarce. While the analytical methods mentioned seem to be adequate, the data interpretation regarding both physico-chemical and ecotoxicological aspects is often very difficult.

This short communication deals with the problem of measurement of asbestos fibers in ambient air, in liquids and in biological material.

EXPERIMENTAL

Ambient air

Ambient fibrous aerosols were sampled by means of Nuclepore filter and evaluated by means of SEM and EDXA. Nuclepore filters with a mean pore diameter of 0.4 μm and a filter diameter of 47 mm were used for the sampling of aerosols of ambient air in remote and semiremote locations. The flow rates were between 7 and 12 liter/min. with total sample volumes per filter ranging from 5 to 50 cbm. Before sampling each filter was coated with two different materials. One half of the filter was coated with carbon, the other semi-circle was coated with metal (either Au of Cu). After sampling, each half of the filter was coated again with the same material.

Therefore the sampled particles and fibers were located between two metal or two carbon films. For evaluation with SEM, both filter halves were used. The semicircle coated with metals was used for counting the fibers and measuring fiber size distributions, because of very good contrast (Figure 1). The section coated with carbon was used for fiber identification by means of a computer-based energy dispersive x-ray micro-analyzer. The section coated with carbon could also be easily prepared for TEM-evaluation, by means of the procedure developed by Sébastien.[1]

From 1 to 3 mm^2 of the filter surface with the ambient air particles were photographed at magnifications of 5000 to 30,000 x. All fiber-like particles on this surface were counted and measured. The number of fibers, and the three distribution curves—L_F, D_F and L_f/D_F—were plotted. Element spectra containing magnesium, silicon and iron in appropriate proportions (Table I) were the identification criterion for asbestos fibers. The analysis of individual fibers was carried out with a JEOL SEM (JMS 35), combined with an EDXA spectrometer.

Liquids

The sampling in liquids (drinking water, wines etc.) was done with a similar method. Nuclepore filters (pore diam. of 0.1 μm) were used for sampling. After filtering 100 to 1000 ml of liquids, the filter sample was dried and then ashed in a low temperature ashing furnace (4 hrs). The ash which contained inorganic fibers was resuspended in 10 ml of 1% acetic acid by ultrasonification (50 kHz, 0.1 W/ml). This suspension was filtered again with a Nuclepore filter (pore diam. of 0.1 μm) coated with metal and carbon layers. The filter preparation and evaluation were the same as in the case of the ambient air samples.

Using smaller sizes of Nuclepore filters (e.g. 25 mm) for the refiltration of the suspended ash, fibers could be concentrated on smaller surfaces

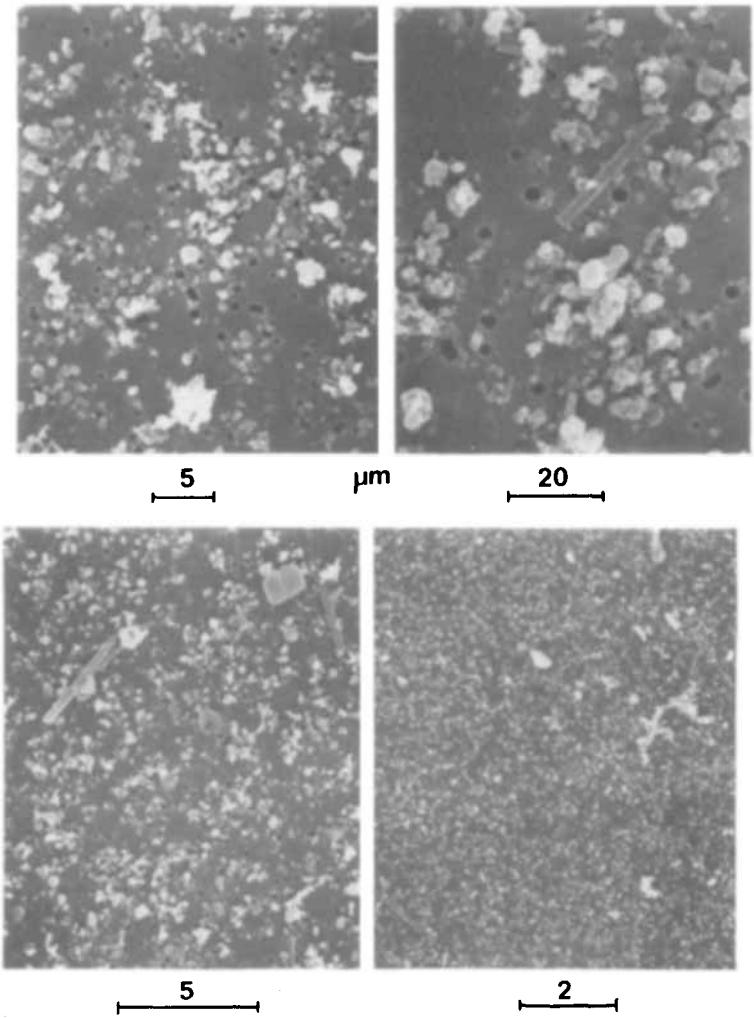

FIGURE 1 Scanning electron micrographs of an ambient air sample (Nuclepore filter 0.4 μm) at different magnifications.

(Figure 2). The fiber identification were also done using the EDXA-method (Figure 3).

Biological material

The aim of fiber analysis in biological material (e.g. lung, liver, kidney, stomac, spleen) is the estimation of fiber concentration (fibers/gram), fiber size distribution and the chemical composition of individual fibers. The

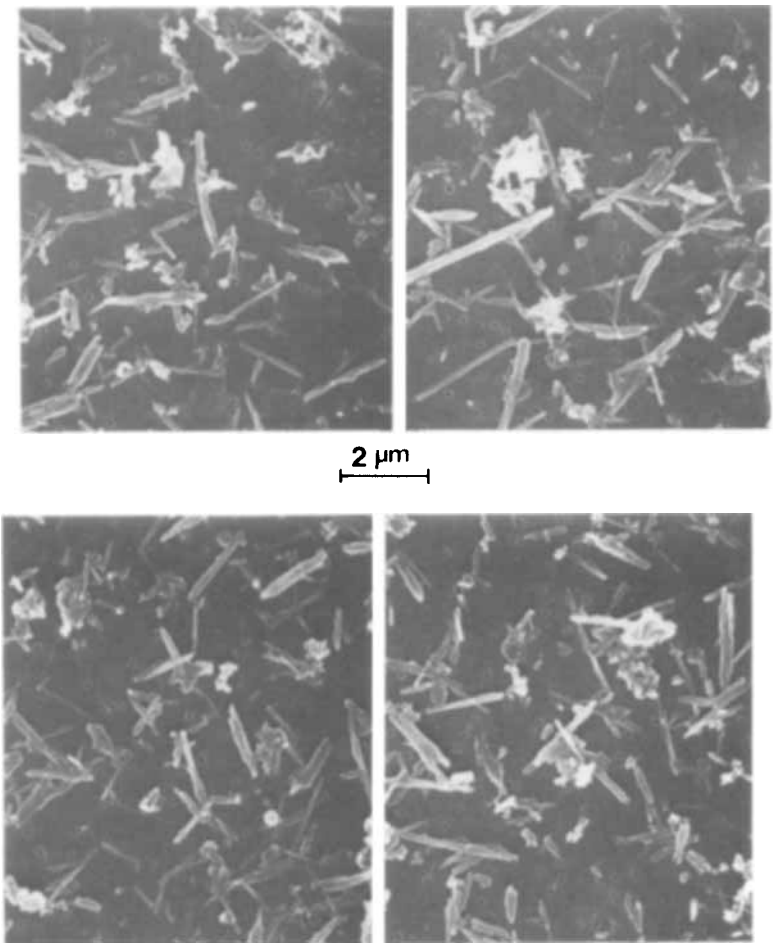

FIGURE 2 Scanning electron micrographs of fibrous particles sampled by filtration of white wine and concentrated on a Nuclepore filter (0.1 μm).

last data are very important for evaluating chemical changes of fibers in the human or animal tissue. During their residence time in the tissue, asbestos fibers can be leached of some elements (Mg, Fe, etc.).[8] This result can be verified by using very sensitive analytical procedures.

Before analysis the tissue has to be mineralized. Lung tissue can be mineralized in a liquid oxidizing suspension, e.g. in a saturated water solution of sodium hypochloride.[1] For other organs, and often also for lung mineralization, low temperature ashing is the most commonly used method. In our investigations the mineralization of human and animal

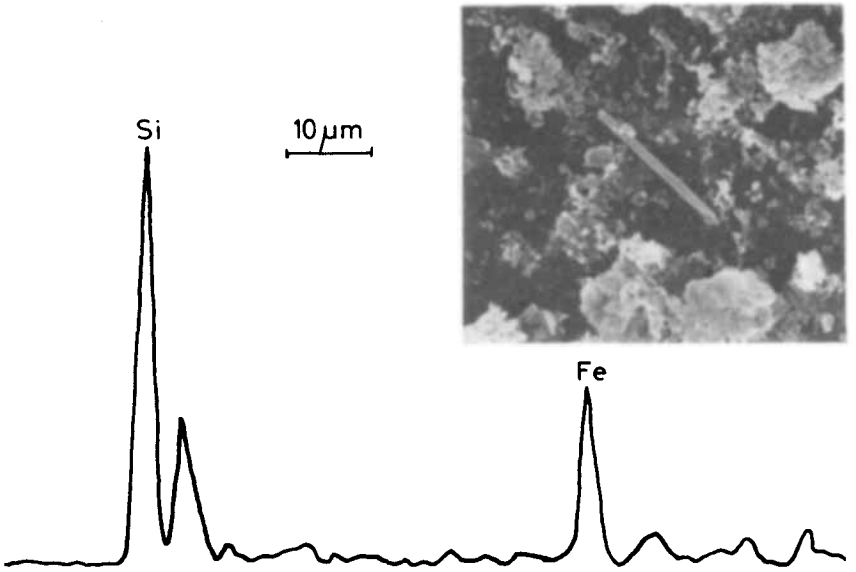

FIGURE 3 Scanning electron micrograph and EDXA-analysis of a single chrysotile fiber found in white wine.

organs had been done by low temperature ashing. The ash was resuspended again in 1% acetic acid solution. Otherwise the other preparation and analytical procedures were the same as described in the analysis of liquids.

Using SEM we have the possibility to analyze fibers directly *in situ*—in the tissue. The organs were dried at 105°C and then thin tissue slices were prepared. By examination with SEM fibers in the tissue could be found, measured and analyzed with the EDXA-method. Figures 4a, b and c show examples of such analysis.

Some preliminary chemical analysis of single asbestos fibers in different environmental samples and in biological materials were done by means of mass spectrometry. The laser microprobe mass analyser (LAMMA) developed by Kaufmann[9] was used for mass spectrometric analysis of single asbestos fibers. The sample preparation is the same as for TEM. Fibers were sampled on Nuclepore filters, which were coated before and after sampling with carbon. The filter substrate material was dissolved in chloroform. The procedure described by Sébastien[1] was used for specimen preparation. Fibers visible with light microscopy were irradiated with a Yag Nd-laser in the LAMMA instrument. After evaporation the material was analyzed in a mass spectrometer, giving the LAMMA-spectra of positive and negative ions. The positive ion spectra of

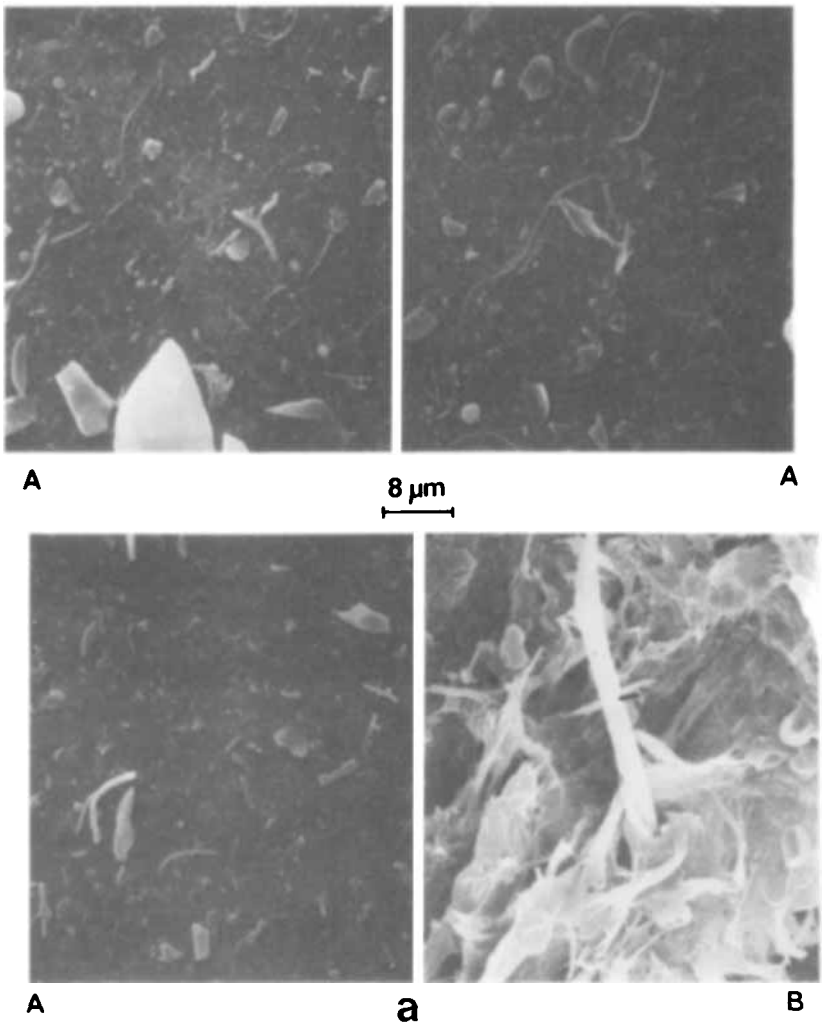

FIGURE 4a Scanning electron micrographs and analysis of single fibers *in situ*: a- chrysotile fibers in the liver ash (A) and in the liver tissue (B) of a rat; b- chrysotile fiber in the stomac tissue of a rat, and c- asbestos fiber in the human lung tissue.

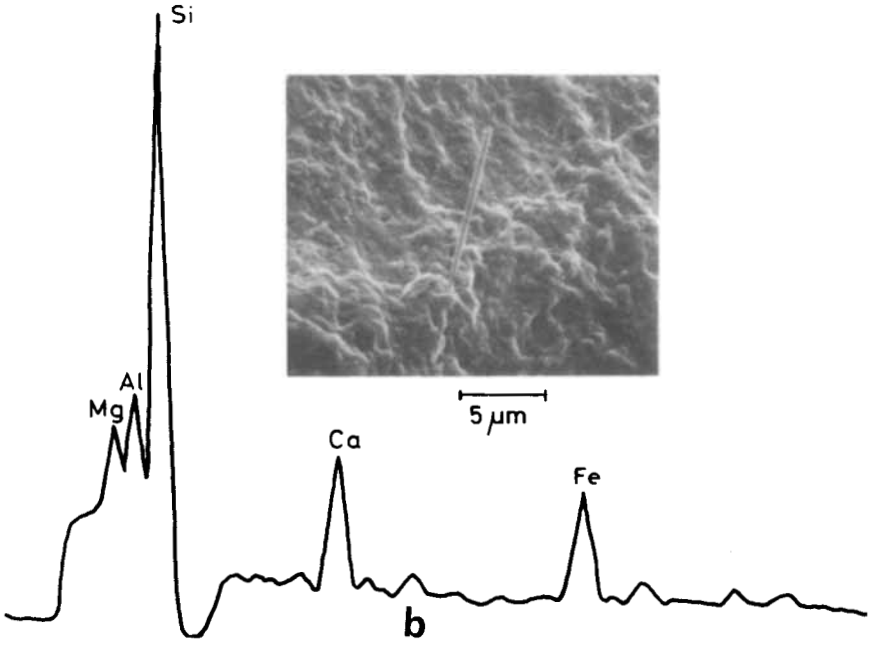

FIGURE 4b

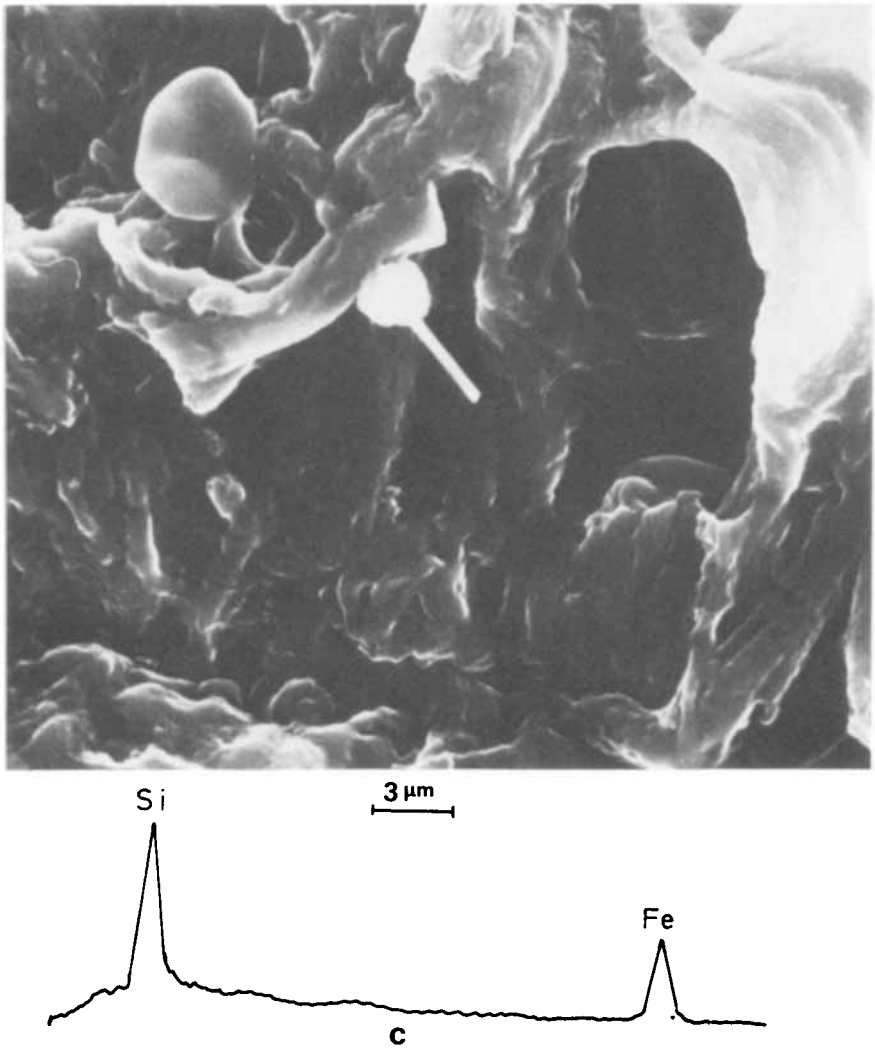

FIGURE 4c

asbestos fibers were relative simply and very reproducible (Figure 5). The mass spectrum of chrysotile is significantly different from the mass spectra of the amphibole asbestos (amosite and crocidolite). A differentiation among different amphibole asbestos types, primarily the differentiation between amosite and crocidolite, will probably be also possible. The application of this method for analysis of inorganic fibers in environmental samples and in biological material will continue.

RESULTS AND DISCUSSION

Samples were taken in an urban non-industrial area and in a clean non-urban areas (remote site) in West Germany. Total aerosol concentrations between 18 and 240 $\mu g/m^3$ and total fiber concentrations between 4 to 15 fibers per liter of ambient air were measured in these regions.[5] Estimated asbestos fiber concentrations were in the range of 0.03 to 0.90 fibers/liter. Thus, asbestos fiber concentrations were less than 6% of the total fiber concentrations and less than 1% in many cases. Furthermore, some of the 1 to 6% of the fiber content which was attributed to asbestos could only be considered as suspected asbestos.

Some of these "asbestos" fibers were either associated with other elements or void of some essential elements like Mg or Fe which may have been leached away. Silicon was often the only element that could be measured with sufficient accuracy. An identification of asbestos fibers or, even worse an identification of different asbestos types (chrysotile, amosite, crocidolite) based on the ratios of elemental intensities (Table I) was almost impossible. On the other hand, more than 90% of the fibrous minerals could be definitely assessed as non-asbestos material (Table II).

X-ray element analysis of individual fibers helps to distinguish between organic and inorganic fibers and between asbestos and non-asbestos fibers. This method cannot give an exact identification of different mineral and chemical compounds, but in some cases, the chemical composition can be estimated. Fibrous and non-fibrous calcium sulfate (Figure 6) as well as ammonium sulfates were very often found in the remote ambient air.

As seen in Table II, more than 90% of the mineral fibrous material in clean ambient air could be assessed as non-asbestos material. After this experience, we think that the asbestos fibers are only a special part of a broad total spectrum of natural and man-made mineral fibers in the atmosphere. Perhaps also the biological and health effects of these non-asbestos fibers in the atmosphere have yet been underestimated.

The methods described above were used for preliminary analysis of fibers in German wines and German drinking waters. Asbestos fibers were counted using the SEM-method in 10 white wines and in 20 drinking

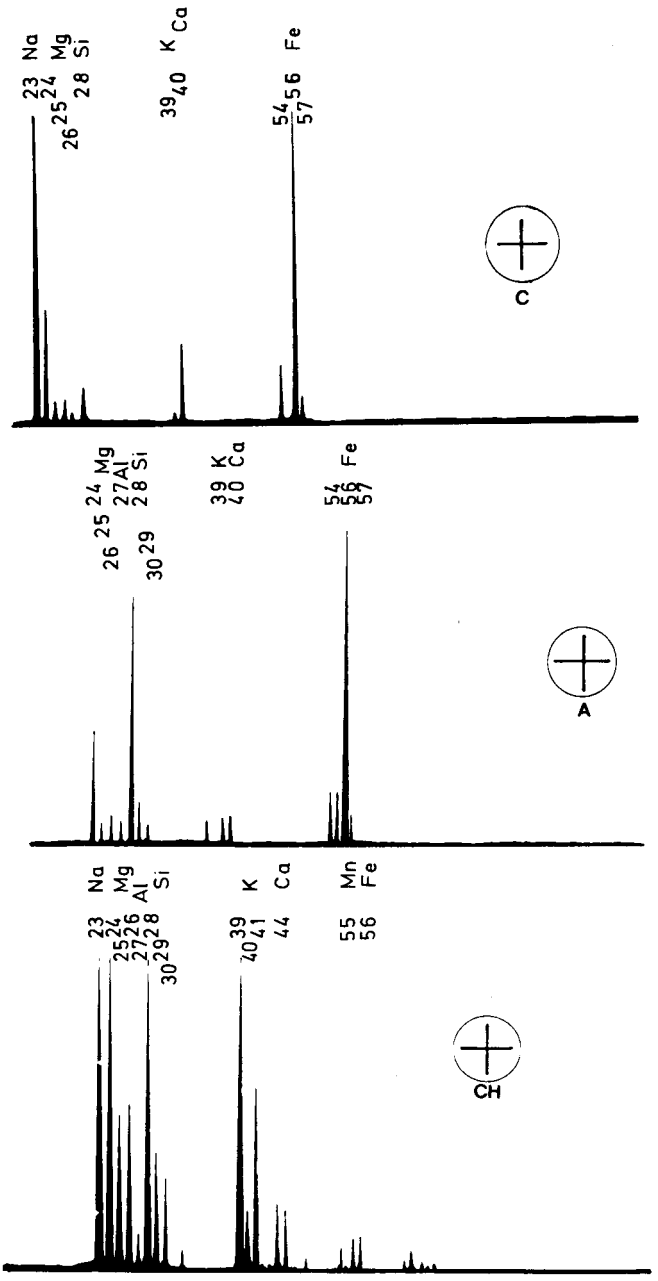

FIGURE 5 Mass spectra of single standard asbestos fibers: crocidolite (C), amosite (A), and chrysotile (CH).

TABLE I

Bulk concentration, calculated intensity correction factors k_{xSi}†, and intensity ratios for different asbestos minerals[9]

Asbestos mineral	% Mg	% Si	% Fe	k_{MgSi}	k_{FeSi}	(Mg/Si)	(Fe/Si)
Amosite	2.4– 4.0	22.4–23.1	28.4–34.2	–	1.12–1.57	0.023–0.181	0.917–1.008
Anthophyllite	12.7–17.2	21.5–27.1	4.3– 8.4	1.30–1.58	1.09–1.61	0.410–0.489	0.123–0.202
Chrysotile	24.0–25.9	18.3–19.6	1.4– 2.4	1.29–1.42	–	0.946–1.036	0.021–0.104
Crocidolite	0.6– 1.6	22.7–23.8	27.9–29.5	–	1.22–1.59	0.010–0.129	0.822–0.916
Talc	18.3–19.2	28.1–29.6	0.3– 1.0	1.31–1.71	–	0.402–0.511	0.006–0.051
Tremolite	14.4–14.8	26.5–29.6	1.0– 3.3	1.31–1.65	–	0.307–0.413	0.018–0.111

†k_{xSi} is a factor for the calculation of concentrations by means of known x-ray peaks intensities for a metal and for Si:

$$\frac{\text{Conc}_x}{\text{Conc}_{Si}} = k_{xSi}\frac{\text{Inten}_x}{\text{Inten}_{Si}}.$$

(k_{xSi} is not a constant. It depends to some extent on particle size, orientation and matrix composition.)

TABLE II

Some results concerning evaluation in ambient air samples

Fibre type	Evaluated samples† (% of fibres) A	B	C	D	E
Potential non-contaminated asbestos fibres	1.02	0.82	1.94	0.52	—
Potential contaminated asbestos fibres	1.51	1.22	6.80	1.04	0.41
Potential asbestos fibres leached of Mg or Fe	1.02	0.82	5.83	0.52	0.41
Other fibrous silicates	2.54	1.22	9.71	1.04	—
Fibrous gypsum	24.37	38.36	46.60	53.88	27.06
Contaminated fibrous gypsum	15.74	20.40	2.91	3.11	1.22
Fibrous ammonium sulfate	48.22	3.66	0.16	15.02	30.74
Unidentified fibre-like‡ particles	5.58	33.50	26.05	24.87	40.16

†Sampling sites A represent urban non-industrial ambient air, sites B are non-urban low-mountain areas, and C, D, C are different places in remote locations (at a distance of more than 100 km from the industrial Ruhr valley region in West Germany).

‡Fibre-like mineral particles, that were too small to be identified by the EDXA method.

waters in Germany. All wine and drinking water samples were found to be significantly positive for chrysotile asbestos. Concentrations in wines were in the range of 0, 4 to 12.10^6 fibers/liter. Concentrations in drinking water lay between 0,3 to $3,2.10^6$ fibers/liter. In the majority of cases the concentrations in drinking water were $<10^6$ fibers/liter (e.g. $0,3.10^6$ in well water and $0,2.10^6$ in mineral water). These estimations were also in agreement with measurements in wines[10] and in drinking waters [11] in other countries.

These methods were, also, used for the analysis and identification of asbestos fibers in biological material. By means of the mass spectrometric method as well as the SEM and the EDXA methods a collection of chrysotile fibers were analyzed in human lung tissue and after a one year treatment with dilute sulfuric and hydrochloric acids. The agreement of the analytical results for both methods was good. By analyzing a larger collection of fibers the results of both methods have shown a distribution of chemical composition. In the majority of the chrysotile fibers only

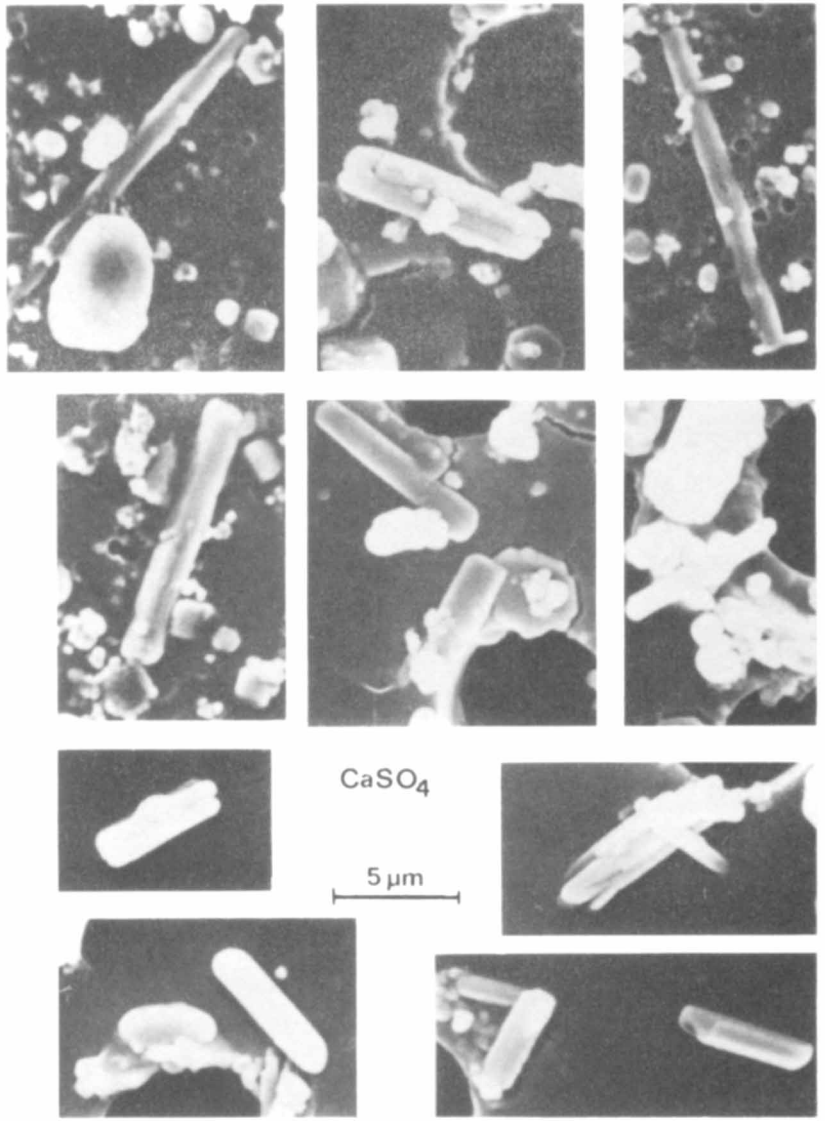

FIGURE 6 Scanning electron micrographs of fibrous $CaSO_4$ sampled in clean ambient air.

silicon could be identified. But many chrysotile fibers were without significant chemical change and some fibers contained only silicon and

iron. Of course, in many fibers the Mg and Fe were partially leached. In Figure 4c it can be observed that Mg was leached in the human lung tissue. In Figure 7 is an example of changes in chemical composition of 3

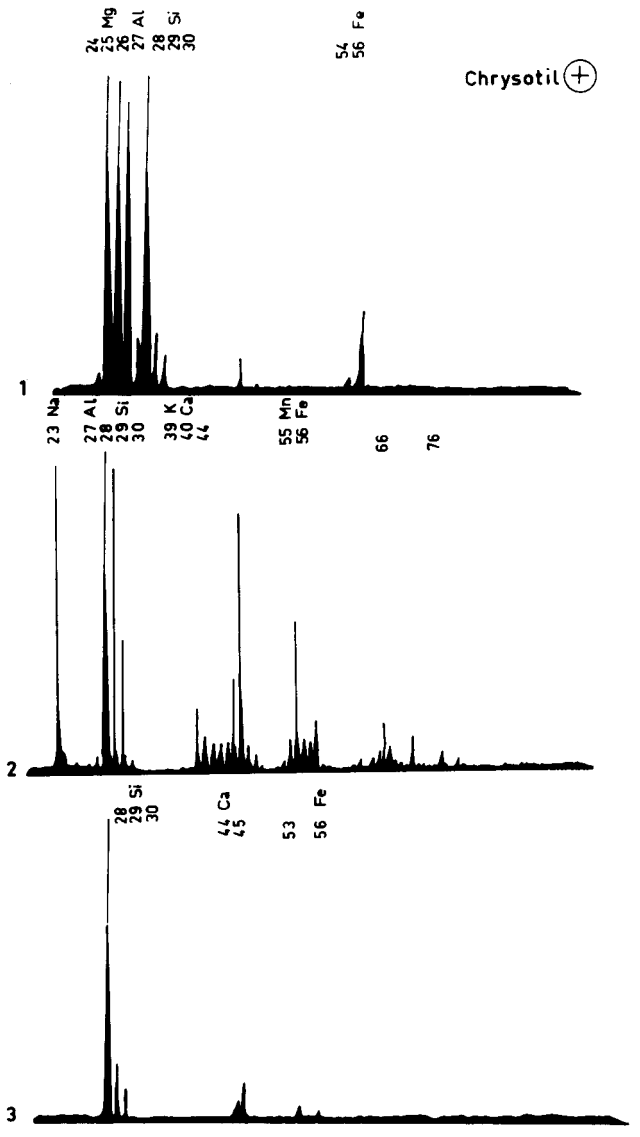

FIGURE 7 Mass spectra of 3 single chrysotile fibers digested 1 year in 2 N H_2SO_4.

chrysotile fibers after a one year treatment with diluted sulfuric acid. The analysis of the third fiber shows that after digestion for one year the silicon is practically the only element which remained in this "chrysotile" fiber. These results are in good agreement with other published data.[8]

CONCLUSIONS

An inherent difficulty is the physical and chemical interpretation of the measured data in the atmospheric environment. Much experience is needed for a correct estimate of an asbestos fiber content. The fibers are often contaminated with other elements, they may be corroded or weathered. As a consequence, the health risk is the most difficult parameter to be evaluated. In general, fibers in ambient air from remote locations are much shorter than 5 μm and thinner than 0.5 μm. It remains to be investigated as to whether the contaminated, corroded, weathered or leached asbestos fibers will be biologically less active than perfectly identified asbestos fibers, such as the fibers in the ambient air of asbestos mines. There is also the aspect of a biological activity of other, non-asbestos mineral fibers. Unfortunately, these and many other questions concerning ambient air cannot be resolved beyond reasonable doubt at this time. Therefore, more ambient air data and more biological animal experiments are needed to obtain more and better information on the ecotoxicological importance of fibrous particles in ambient air.

Similar effect of chemical changes in asbestos fibers were also observed by analyzing liquids (waters and wines) as well as by analyzing biological materials. The mass spectrometric method (LAMMA) used for analysis of single fibers seems to be a promising technique in some special cases, but it needs still more investigation.

Acknowledgement

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