American Laboratory

Asbestos

By Walter C. McCrone and Ian M. Stewart

IN MAY 1968, Dr. Irving J. Selikoff of the Mount Sinai Hospital in New York established his position as the Rachel Carson of the industrial hygiene world. A series of *New Yorker* articles at that time was based largely on Dr. Selikoff's experiences in the study of asbestosis, lung and stomach cancer, mesothelioma, and other causes of death related to exposure to asbestos. His careful analyses of mortality of industrial workers in known asbestos fiber contaminated areas are convincing arguments that asbestos is one of the most deadly industrial hygiene hazards.

The reputation of large industrial concerns has not always been good when faced with evidence that their plant environments may be contributing to worker disabilities, if not death. However, largely through Dr. Selikoff's effort, followed up by the FDA, Department of Labor, and other interested parties, most responsible companies are now doing their best to minimize, if not eliminate, asbestos as a health hazard.

The microscopist has an important role in this program to control asbestos as a contaminant in the air we breathe, the fluids we drink, and the food we eat. He must be able to identify asbestos fibers and quantify their size and number. There are problems because there are other fibrous substances confusingly similar to asbestos, and there are several different types of asbestos (*Table I*), differing in optical properties and composition and possibly in the degree of their hazard to humans.

Analytical methods must be sensitive enough for the identification of single fibers. Of course, only a microscope and a trained microscopist can do this. There are methods, e.g., x-ray diffraction and DTA, that identify minute percentages of asbestos in

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Chrysotile fiber bundle.

Table 1

Asbestos

	Serpentine	Amphiboles				
	CHRYSOTILE	ACTINOLITE	TREMOLITE	ANTHOPHYLLITE	AMOSITE	CROCIDOLITE
Composition	$3MgO{\cdot}2SiO_2{\cdot}2H_2O$	$2CaO{\cdot}4MgO{\cdot}FeO{\cdot}8SiO_2{\cdot}H_2O$	$2CaO{\cdot}5MgO{\cdot}8SiO_2{\cdot}H_2O$	$7MgO{\cdot}8SiO_2{\cdot}H_2O$	$5{\cdot}5FeO{\cdot}1{\cdot}5MgO\ 8SiO_2{\cdot}H_2O$	$Na_2O{\cdot}Fe_2O_3{\cdot}3FeO{\cdot}8SiO_2\ H_2O$
Specific gravity	2.36–2.5	3.03–3.5	2.9–3.2	2.85–3.5	2.6–3.0	3.0–3.45
Crystal system	monoclinic	monoclinic	monoclinic	orthorhombic	monoclinic	monoclinic
Refractive indices	1.49–1.57	1.62–1.68	1.60–1.65	1.60–1.66	1.66–1.70	1.69–1.71

Table 2 **Dispersion staining colors for asbestos**

Asbestos type	Refractive index liquid	Dispersion staining color	
		Parallel	Perpendicular
Chrysotile	1.560	light blue	magenta
Anthophyllite	1.610	blue-green	golden yellow
Amosite	1.670	red magenta	golden yellow
Crocidolite	1.700	magenta	blue magenta

small samples, but only the microscopist can hope to identify individual fibers. Fortunately, the crystallographic properties of the different types of asbestos are sufficiently distinct to enable certain identification. If the fibers are thicker than about 0.5 μm, light microscopy suffices; smaller fibers require the electron microscope.

Identification of the larger fibers by light microscopy is based on refractive index measurement using a polarizing microscope. The easiest way to apply refractive index measurement to asbestos fiber identification is by use of dispersion staining, an optical staining procedure. This is based on annular or central screening of the objective aperture in common with specific refractive index media, such as the Cargille liquids. Under these conditions each type of asbestos shows two characteristic dispersion staining colors, one for the light vibration direction parallel, and another perpendicular, to the fiber length (but only in one particular refractive

index liquid). *Table 2* shows the colors obtained with several kinds of asbestos when mounted in appropriate Cargille refractive index liquids. Different colors or no colors are observed if other than the specified liquids are used for each particular asbestos sample. Finding small percentages of very fine asbestos fibers requires careful application of the method. In particular, intense Kohler illumination with carefully centered substage and objective apertures is essential. A careful microscopist should, however, be able to detect 0.01% of >0.5 μm-thick fibers by this technique. Light microscopy is ideal for rapid identification of asbestos in most powdered samples: dust, mortar, insulation or wallboard, mineral samples such as talc, etc. When the presence of asbestos in a liquid medium, such as a water supply, soft drink, beer, or parenteral solution is suspected, one must assume most of the fibers, if not all, will be too fine to observe by light microscopy. One turns, then, to the transmission electron microscope (*Figure 1*). The transmission electron microscope (TEM) is superior to the scanning electron microscope for two reasons. Smaller fibers can be observed and identified by TEM, and selected area electron diffraction (SAED) is more dependable for identification of asbestos than the elemental analysis capability of the SEM.

Figure 1 *Model 200KV transmission electron microscope.*

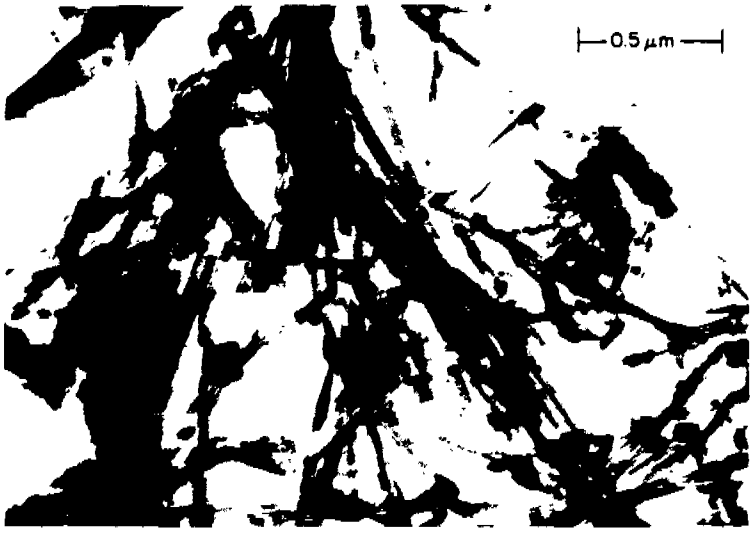


Figure 2 *Chrysotile fibers filtered from a water supply, 30,000X.*

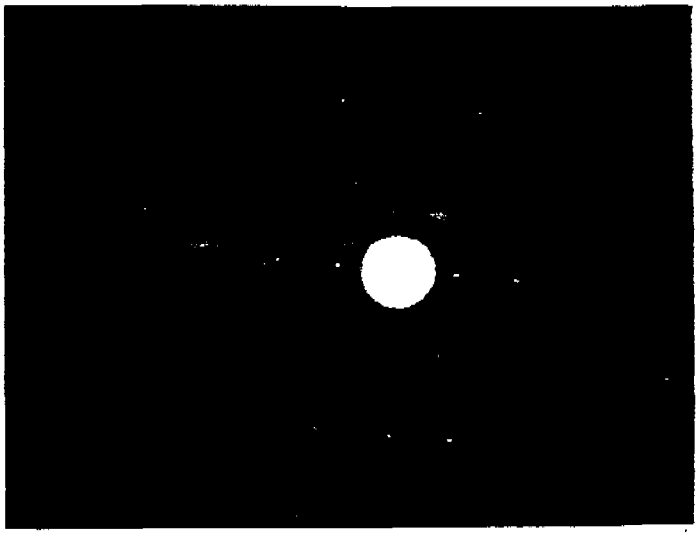


Figure 3 *Selected area electron diffraction pattern for a single chrysotile fiber.*

A sample of water of known volume is filtered through the finest membrane filter. A section of filter approximately 2–3 mm^2 is then placed particle side down on a previously carbon-coated electron microscope grid, and the membrane filter is dissolved using acetone in a Soxhlet extractor. The sample grids are examined in our laboratory with a JEM 200 electron microscope, using an accelerating voltage of 150 kv at a magnification of 17,000X on the M3 range of the instrument. This magnification is chosen to focus the intermediate lens aperture in line with the specimen plane; thus, a SAED pattern of the fiber is obtained with no other adjustments to the microscope. In this way it is possible to spot check the diffraction pattern of individual fibers rapidly (*Figures 2* and *3*).

The total number of asbestos fibers is related to a definite area of the filter, and hence a definite volume of sample, by counting fibers over a known number of TEM grid squares (having known areas). The length and width of each asbestos fiber is recorded. Interpolation from the screen scribed at half-centimeter intervals allows an accuracy of measurement on the screen of approximately ± 0.05 cm, or about ± 34 nm. Measurements of the individual fibers are read into a tape recorder; this permits the operator to devote full attention to the screen, precludes duplicate counting of single fibers, prevents missing fibers, and maintains the operator's dark accommodation.

The recorded data are transcribed onto punch-tape and computer processed to give listings of the length, width, and aspect ratio of the fibers, together with a computed mass of each fiber calculated on the basis of density D and dimensions L and W ($D \times L \times W^2$). Density values for the different asbestos types are given in Table 1. Also presented in the computer print-out is the number of fibers per liter of water, the size distribution of the fibers based on length and width, and the distribution of fibers by aspect ratio. The program automatically assigns the longest dimension to the fiber length and excludes all fibers with an aspect ratio below three.

This procedure, designed by Ian Stewart of our laboratory, has been applied with excellent success to a number of water samples containing large numbers of amphiboles per liter but very low weight percentages. Most of these samples show no asbestos fibers by light microscopy. It is therefore important to specify the method of analysis when stating analytical results. The same sample of talc could be analyzed by different methods with very different results, e.g.:

1. X-ray diffraction shows no chrysotile or amphiboles.

2. A phase-contrast method (similar to the NIOSH procedure to determine the number of fibers per milliliter of air) shows 1500 fibers greater than 5$\mu m/m^3$ of sample.

3. The scanning electron microscope with energy dispersive x-ray analyzer shows about 10^4

fibers/liter suspected of being amphibole asbestos.

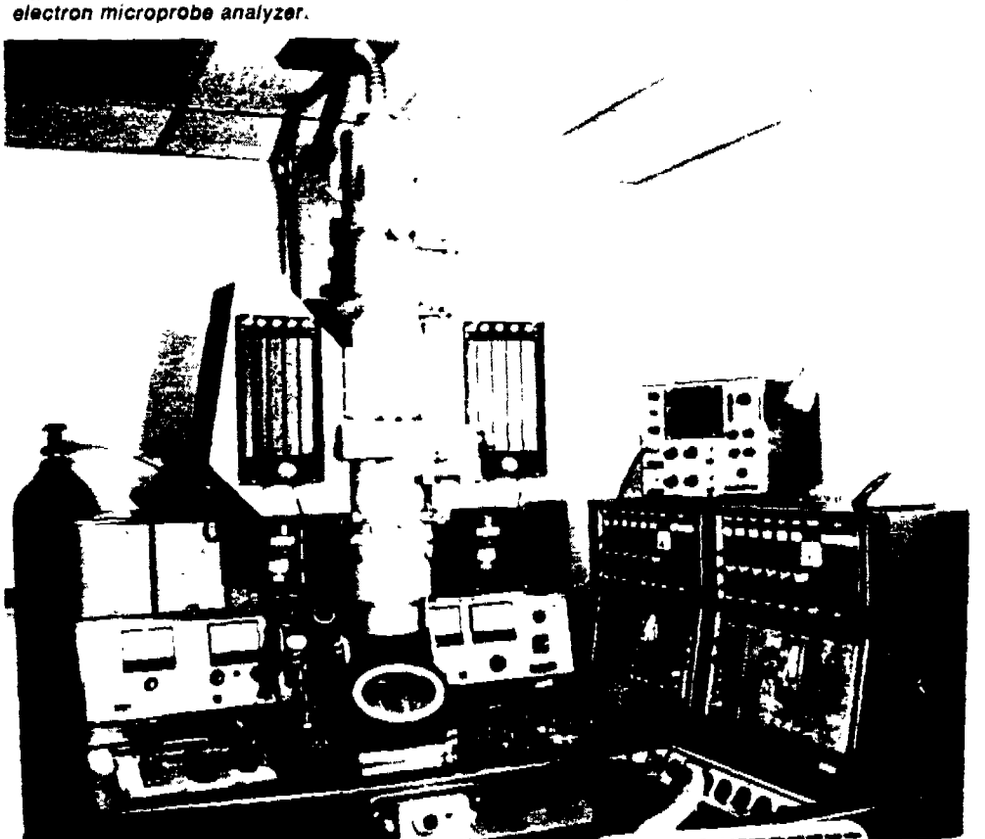
4. Dispersion staining shows 150 anthophyllite fibers/gram of sample.

5. Transmission electron microscopy shows 5×10^6 anthophyllite fibers/liter of sample.

The AEI Scientific Apparatus, Ltd. EMMA-4 (*Figure 4*) only recently installed in our laboratory will undoubtedly be the ideal instrument for the detection and identification of very fine asbestos fibers. A combination transmission electron microscope and electron microprobe analyzer (EMA), it enables the microscopist to see the smallest fiber and to identify it by diffraction pattern and elemental analysis. Although a given sample mounted on a TEM grid can be examined successively by TEM and EMA, the advantage of being able to examine many particles in a sample with assurance of one-to-one correspondence of particle size, shape, diffraction pattern, and elemental analysis is obvious. We have already applied this instrument to some asbestos samples with excellent results, and expect it will be the instrument of choice for such samples in our laboratory.

The monitoring of asbestos as a contaminant in our environment is an excellent example of one important problem solved by microscopy. The importance of choosing the proper analytical tool and having a well-trained operator is also emphasized by this application to asbestos detection and identification.

Figure 4 *EMMA-4, a combined transmission electron microscope and electron microprobe analyzer.*



Asbestos
A Family of Minerals

SUMMARY:
This paper sets forth some of the reasons why the term "asbestos" should not be used loosely in discussing medical questions.

There is no single mineral known as asbestos. "Asbestos" is a name given to a family of minerals. It includes two major groups, containing a total of six varieties of asbestos. Each of these six differs from the others, physically, chemically and in biological effect. Any study of the effects of asbestos is complicated by this diversity.

Studies of both people and animals show differing types of reaction to the various types of asbestos. Animal studies of excessive exposure made with three of the major commercial types of asbestos indicate that commonly-used chrysotile is least likely to produce a reaction.

Because different varieties of asbestos produce different reactions, scientists are working on better methods for identifying the types of asbestos involved in their studies on human health. Other complications arise because trace amounts of mineral impurities are often found in samples of raw asbestos ore and refined fiber.

TYPES OF ASBESTOS

Any attempt to assess the effect of asbestos on health is complicated by the fact that asbestos is not a single chemical or physical entity. "Asbestos" is a generic name given to a group of hydrated silicate minerals that have one common attribute – namely, the ability to be separated into relatively soft, silky fibers.

The known varieties of these minerals can be divided into two main classes on the basis of their crystalline structures: **serpentine** asbestos and **amphibole** asbestos. The lone member of the serpentine class is **chrysotile** asbestos, which is by far the most common of the asbestos minerals. About 95 per cent of the asbestos used in this country is chrysotile, principally from mines in Canada.

There are five recognized varieties of amphibole asbestos: **crocidolite, amosite, anthophyllite, tremolite and actinolite.** Of these, crocidolite and amosite have the greatest commercial significance, although the use of anthophyllite is increasing.

Each of the six types of asbestos differs from the others, chemically and physically. To add to the complexity, chrysotile asbestos from one Canadian mine, for example, will differ in chemical impurities and physical properties from the chrysotile taken from another Canadian mine.

CHARACTERISTICS OF THE FOUR MAJOR TYPES

Each of the four types of asbestos that are most important commercially – chrysotile, crocidolite, amosite and anthophyllite – exhibits distinguishing characteristics.

Chrysotile

By far the leading producers are Canada and the Soviet Union. Smaller deposits are found in South Africa, Rhodesia, China, the United States and Italy. Chrysotile is a *white* serpentine asbestos and occurs in areas where serpentine rock was cracked by earth movement thousands of years ago and subjected to intense pressure and the flow of volcanic waters. This action transformed some of the granular serpentine

into fibrous chrysotile. Chemically, chrysotile is hydrous magnesium silicate with a magnesium hydroxide surface. It has a positive electrical charge. It can be attacked by acids. It is a flexible fiber and does not pulverize readily. Commercial chrysotile contains small and varying amounts of iron and calcium compounds, depending upon its origin.

Crocidolite

The bulk of the world's supply comes from South Africa; there are also small deposits in Australia, but they are no longer mined commercially. Crocidolite is an amphibole asbestos occurring in iron-rich sedimentary rock. Chemically, it is a ferrous sodium silicate with a silica surface. It has a negative electrical charge. It is acid-resistant. It is characterized by a deep *blue* color. Crocidolite is less flexible than chrysotile.

Amosite

The Transvaal district of South Africa is the only place in the world where amosite is mined commercially. It is a member of the amphibole asbestos family, occurs in the same type of rock as does crocidolite, has the same negative electrical charge, but is brown in color. Chemically, amosite is a ferrous magnesium silicate with a silica surface. It is acid-resistant, but less so than crocidolite. It is brittle and pulverizes easily.

Anthophyllite

The least used of the commercial fibers, it comes primarily from Finland but small amounts are mined in the United States. It is an amphibole asbestos, has a negative electrical charge, and is white in color. Chemically, anthophyllite is a magnesium silicate with a silica surface. It is acid resistant like the other amphiboles. It is brittle and pulverizes easily.

Because of the basic chemical and physical differences among chrysotile, crocidolite, amosite and anthophyllite, it would be expected that over-exposure to the four varieties would show varying biological effects. And scientific observation confirms this expectation. There seems to be broad agreement that chrysotile, the type of asbestos most widely used in the United States, is least likely of the three most commercially significant fibers (chrysotile, crocidolite, amosite) to produce asbestosis, a non-malignant lung disease brought on after inhalation of excessive concentrations of asbestos dust over a period of many years.

ANIMAL EXPERIMENTS SHOW DIFFERENT EFFECTS

Dr. J. C. Wagner experimented with three types of asbestos (chrysotile, crocidolite and amosite) on various laboratory animals, and found that –

crocidolite produced the most severe asbestosis, and

amosite was more than five times as likely to produce a reaction as chrysotile. He reported[1] in 1963:

"With chrysotile dust it was possible to produce severe lesions in the lungs of guinea pigs, slight fibrosis in monkeys, while no significant effect was observed in two rabbits. Amosite dust causes marked asbestosis in all three kinds of animals. Lesions occur more rapidly in guinea pigs exposed to this dust than in those exposed to chrysotile. There is an indication that the disease is progressive in monkeys and rabbits. Similar pathology to that which occurred in a monkey exposed to chrysotile for 22 months was observed in a monkey exposed to amosite for four months. The impure crocidolite dust caused severe disease in guinea pigs, and the rate of respiratory infection among animals exposed to this dust was more marked than with the other types. It is possible that this may be due to the high quartz content of the dust.

"Finally, asbestos bodies could be demonstrated in the lungs of animals exposed to all three types of asbestos dust. These were scanty in the animals exposed to chrysotile and usually segmented. Only occasional fibers were observed. In contrast to this, the asbestos bodies in the animals that were dusted with amosite were plentiful, and segmentation was rare: fibers were extremely numerous and far outnumbered the bodies."

Following further investigation, Dr. Wagner offered an explanation[2] for this observation at a medical symposium in 1965:

"A method of producing asbestos dust clouds has been devised and an animal inhalation experiment carried out to test it... The elimination rate of Rhodesian chrysotile has been found to be three times greater than that of the amosite and crocidolite, which suggests an explanation for the previously observed reduced fibrogenicity of this dust. The reason for the difference in the elimination rate remains to be determined."

COMMERCIAL ASBESTOS CONTAINS OTHER MATERIALS

Asbestos ore and refined asbestos fiber contain mineral impurities, trace elements, and trace quantities of organics, which vary from type to type and from mine to mine. In addition, the ore may pick up additional trace organic impurities when in transit.

These trace materials are also the subject of medical study to see if they may have a role in any of the health effects observed in animals. Trace impurities of nickel, chromium and manganese, for example, were found by Cralley, et al.[3] in samples of chrysotile used in the asbestos textile industry. Other impurities in commercial asbestos fiber include iron, aluminum, calcium, sodium and potassium.

WORLD ASBESTOS BANK

Much scientific research has focused on studying the varying biological effects of different types of asbestos and on developing techniques for identifying asbestos fibers of unknown origin. In 1967, a "World Asbestos Bank" was established in Johannesburg, South Africa, under the direction of the British Medical Research Council. The purpose of the "bank" is to provide standardized samples of asbestos from regions all over the world to be supplied to medical researchers for chemical, physical and biological investigation and for comparison purposes in clinical studies.

CHARACTERISTICS OF MAJOR COMMERCIAL TYPES OF ASBESTOS

NAME	Chrysotile	Crocidolite	Amosite	Anthophyllite
CLASS	Serpentine	Amphibole	Amphibole	Amphibole
COLOR	White	Blue	Brown	White
CHEMICAL COMPOSITION	Hydrous Magnesium Silicate	Ferrous Sodium Silicate	Ferrous Magnesium Silicate	Magnesium Silicate
SURFACE COMPOSITION	Magnesium Hydroxide	Silica	Silica	Silica
ELECTRICAL CHARGE	Positive	Negative	Negative	Negative
REACTION TO ACID	Susceptible	Resistant	Resistant	Resistant
TEXTURE	Flexible	Medium Flexibility	Brittle	Brittle

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[3] **Cralley, L. J., Keenan, R. G. and Lynch, J. R.,** "Exposure to Metals in the Manufacture of Asbestos Textile Products," *AMERICAN INDUSTRIAL HYGIENE ASSOCIATION JOURNAL*, September/October, 1967.

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