

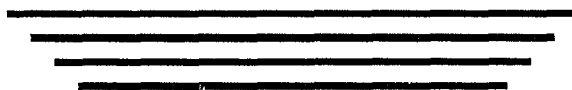
FILE NAME: RT Vanderbilt (RTV)

DATE: 2003 Apr

DOC#: RTV084

DOCUMENT DESCRIPTION: Report - The Asbestiform and Nonasbestiform Mineral Growth Habit and Their Relationship to Cancer Studies

THE ASBESTIFORM AND NONASBESTIFORM MINERAL GROWTH HABIT AND THEIR RELATIONSHIP TO CANCER STUDIES



A PICTORIAL PRESENTATION

The Asbestiform and Nonasbestiform Mineral Growth Habit and Their Relationship to Cancer Studies

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The recognition and regulation of asbestiform and nonasbestiform minerals is of critical concern to the entire mining and aggregates industry, to individuals exposed to these materials and to the economic vitality of the United States.

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INTRODUCTION

It has long been recognized that the inhalation of excessive asbestos fibers, over time, is associated with significant pulmonary disease in humans. The link between asbestos, lung cancer and mesothelioma is well established. Asbestos is perhaps the most feared mineral risk and certainly is among the most publicized, litigated and studied.

Despite this attention, a clear understanding of what asbestos actually is remains a source of confusion to many. This is often demonstrated when commercial asbestos is not known "a priori" to exist in a dust exposure. Nowhere is this problem better demonstrated than the decades old confusion over the difference between asbestiform and nonasbestiform crystal growth.

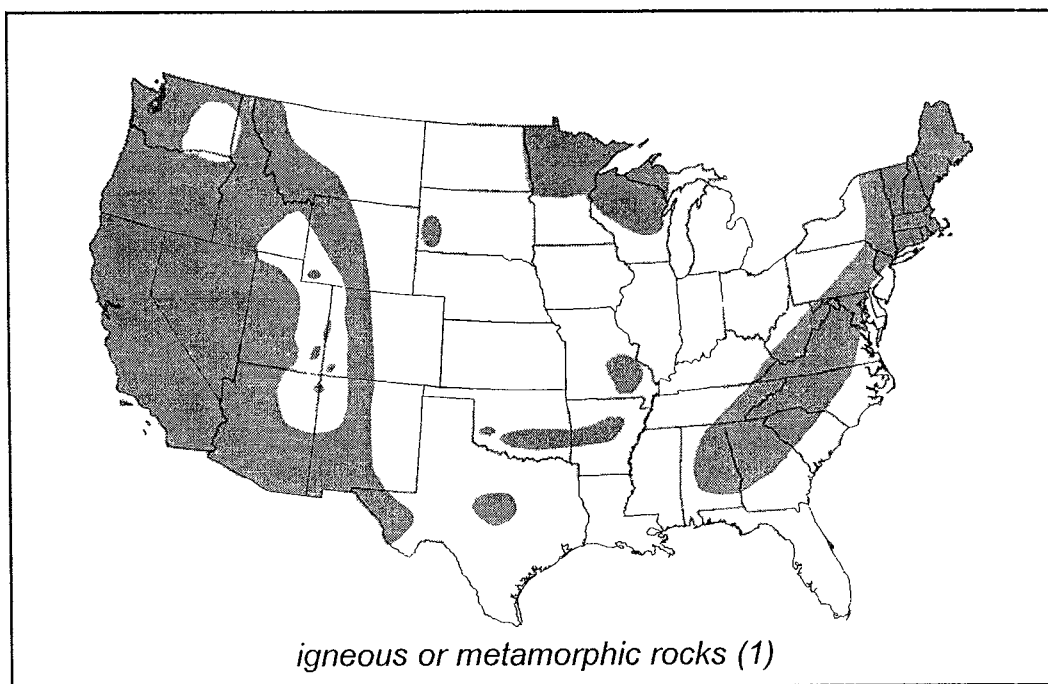
No federal regulatory agency treats elongated nonasbestiform mineral particulates as asbestos, yet some in the regulatory and health community believe that they should. These individuals mistakenly believe that the essential difference between nonasbestiform minerals and asbestos is not significant from both a mineralogic and biologic perspective.

This pictorial presentation demonstrates that important mineralogic and health differences do, in fact, exist. Health researchers who fail to understand these differences can assign and have attributed the carcinogenic effects of asbestos exposure to nonasbestiform minerals. Because these common, nonasbestiform rock-forming minerals make up so much of the earth's crust, it is important that this error be avoided.

WHY IS THIS DISTINCTION IMPORTANT?

The nonasbestiform minerals are common hard rock forming minerals found throughout the earth's crust. Unlike asbestos, they are not at all rare.

The map below shows the general areas in the continental United States where igneous and metamorphic rocks are likely to be found on or near the surface. Amphiboles and serpentine, the two mineral groups that contain mineral species that may form asbestos, are restricted in their occurrence to these types of rock. When amphiboles and serpentine form part of the bedrock, they may also be found in the overlying soil. All the rock and soil in the shaded areas, however, do not contain amphibole and serpentine, and the occurrence of the asbestiform habits of these minerals in the shaded areas is even more restricted. The shaded areas do not mean that every rock or soil mass in that area contains these minerals, but it does mean that they are often present in these areas.



The composition of the rock also affects the likelihood of finding asbestos. Asbestos is more likely to form during the metamorphism of limestone, mafic and ultramafic rocks and alkali igneous rocks than during the metamorphism of other common rocks such as granite and sandstone. Furthermore, many of the amphiboles, particularly those that contain a significant amount of aluminum, never form asbestiform fibers. Therefore, while the nonasbestiform habits of amphibole and serpentine are common throughout the shaded areas, asbestos occurrences are localized and uncommon.

The U.S. Bureau of Mines reports that the regulation of nonasbestiform minerals as asbestos would significantly impact the mining of important mineral commodities such as gold, copper, iron, crushed stone, sand, gravel and talc. Downstream users of these mineral commodities such as construction, refractories, smelters, ceramics and paint manufacturers, would be affected as well (2).

Therefore, it is important that these nonasbestiform minerals be properly assessed with respect to their health risk.

The goal of this document is to clearly and succinctly demonstrate that mineralogical and biological differences exist between asbestos and common nonasbestiform minerals. To accomplish this objective, this presentation:

- **DESCRIBES THE MINERALOGICAL DIFFERENCES BETWEEN ASBESTIFORM AND NONASBESTIFORM MINERALS.**
- **CLARIFIES THE MINERAL EXPOSURES CITED IN KEY HEALTH STUDIES.**
- **SUMMARIZES THE OUTCOME OF THIS COMPARISON.**

REFERENCE EXHIBIT 1

What is Asbestos?



In the *Glossary of Geology*, asbestos is defined as. . .

"A commercial term applied to a group of highly fibrous silicate minerals that readily separate into *long, thin, strong* fibers of sufficient flexibility to be woven. . ." (3).

This definition has been further expanded based on mineral-crystallographic studies over the last decade or so:

A. ASBESTOS - A collective mineralogic term that describes a variety of certain silicates belonging to the serpentine and amphibole mineral groups, which have crystallized in the asbestiform habit causing them to be easily separated into long, thin, flexible, strong fibers when crushed or processed. Included in the definition are: chrysotile, crocidolite, asbestiform grunerite (amosite), anthophyllite asbestos, tremolite asbestos and actinolite asbestos. The nomenclature and composition of amphibole minerals should conform with International Mineralogical Association recommendations (Leake, B.E., *Nomenclature of Amphiboles*. American Mineralogist. Vol. 82, 1019 - 1037, 1997).

B. ASBESTOS FIBERS - Asbestiform mineral fiber populations generally have the following characteristics when viewed by light microscopy:

1. Mean aspect ratios ranging from 20:1 to 100:1 or higher for fibers longer than 5 μm ,
2. Very thin fibrils, usually less than 0.5 μm in width,
3. Parallel fibers occurring in bundles, and
4. One or more of the following:
 - a) Fiber bundles displaying splayed ends,
 - b) Matted masses of individual fibers,
 - c) Fibers showing curvature

This definition represents the consensus of a group of mineral scientists, several of whom have published extensively in this area (see Appendix I).

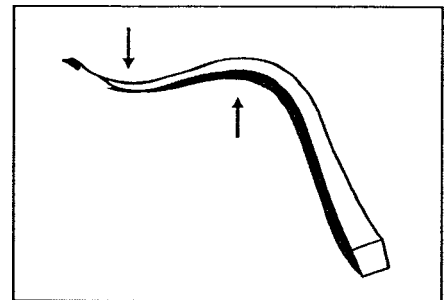
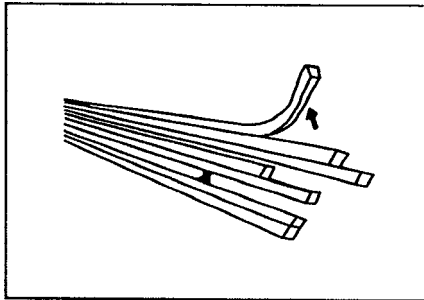
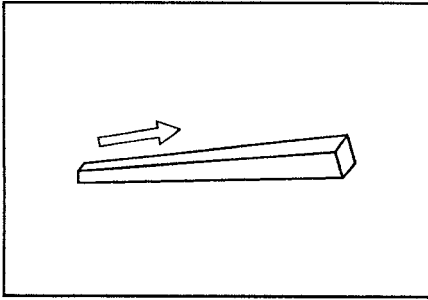
Morphological properties are difficult to apply to single particles when classifying them as a cleavage fragment or a fiber. Distinctions on morphology are most reliably made on populations. Furthermore, in air and water samples, in which particles are often less than 5 µm in length, the presence of asbestos should be verified in bulk material at the source before identification of particles as asbestos can be reliably made. Bulk materials display the full range of distinctive morphological characteristics, but in fibers collected from air and water, the range of morphological properties is more limited.

Asbestiform fibers normally exhibit anomalous optical properties that are distinctive. For example, under polarized light microscopy, asbestiform fibers may display parallel extinction in all orientations, they may display oblique extinction in some orientations at angles that are less than those characteristic of ordinary amphibole fragments in the same crystallographic orientation, they may have only two principal indices of refraction (as opposed to the expected three), or they may display orthorhombic optical properties when monoclinic optical properties are expected (79).

When asbestiform fibers are found in nature, there may be other habits of the same mineral inter-grown such as the brittle, fibrous nonasbestiform habit byssolite and fragments of the enclosing rock (cleavage fragments). Byssolite is characterized by wide, single glassy crystals usually > 1 µm in width. While asbestos is characterized by high tensile strength which results in difficulty on grinding with a mortar and pestle, byssolite and cleavage fragments will easily reduce to powder under the same circumstances (see page 16, Reference Exhibit #5).

Although asbestiform crystal growth is very rare in nature, under the right geologic conditions approximately 100 minerals may be formed in this manner - not just the six minerals we refer to as asbestos (76). Evidence on the carcinogenicity of asbestiform minerals that are not asbestos is mixed, but there is no compelling evidence that all asbestiform minerals are carcinogenic. Different minerals have different biodurabilities, surface chemistries, friabilities in vivo, and bioavailability differences that influence their biological activities (77). Asbestiform richterite, winchite and erionite are examples of fibers that appear to pose a risk similar to that of asbestos (74,78). In contrast, asbestiform talc (72) and minerals such as xonotlite (commonly found in an asbestiform habit but is water soluble) do not appear to pose the same risk.

ASBESTIFORM



In the asbestiform habit, fibers grow almost exclusively in one direction and exhibit narrow width (on the order of 0.1 μm). Fibers that are visible to the eye are bundles of individual crystal fibers known as “fibrils”. In some deposits, there is a range in fibril width, sometimes extending up to as much as 0.5 μm . Asbestiform fibers wider than 1.0 μm are always bundles of fibrils. Asbestiform minerals have fibrils that are easily separated, although variability exists. In populations of asbestiform fibers, the distribution of particle widths will reflect single fibrils as well as bundles of fibrils. Under the light microscope, this “polyfilamentous” characteristic of fibers is evident, and **is the single most important morphological characteristic of the asbestiform habit**. Asbestiform fibers are flexible and exhibit high tensile strength. The flexibility may be accounted for by the very narrow widths of fibrils and perhaps by the ability of fibrils to slide past one another on bending.

Six minerals have been regulated as asbestos. These are listed below:

ASBESTIFORM VARIETY (Asbestos, CAS No. 1332-21-4*)

SERPENTINE GROUP

chrysotile

(CAS No. 12001-29-5)

AMPHIBOLE GROUP

crocidolite

(CAS No. 12001-28-4)

grunerite asbestos (amosite)

(CAS No. 12172-73-5*)

anthophyllite asbestos

(CAS No. 77536-67-5*)

tremolite asbestos

(CAS No. 77536-68-6*)

actinolite asbestos

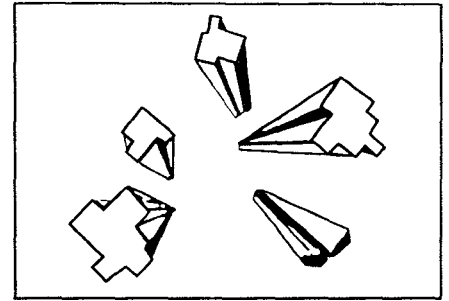
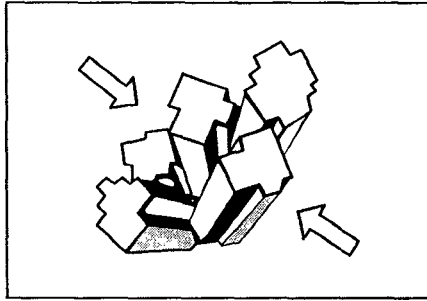
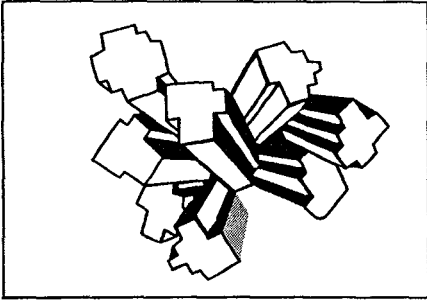
(CAS No. 77536-66-4*)

The presence of an asterisk (*) following a CAS Registry Number indicates that the registration is for a substance which CAS does not treat in its regular CA index processing as a unique chemical entity

For asbestiform fibers to grow, there must be mineral rich fluids that are either associated with regional metamorphism or contact metamorphism around crystallizing igneous bodies. The vast majority of the occurrences of asbestos are small because, in addition to metamorphic fluids, there must be open spaces into which the fibers can grow, a condition restricted to the upper portions of the earth’s crust in structurally specific environments such as faults, joints, the axes of folds, etc. Only rarely are large portions of a rock composed of asbestos.

The most common occurrence of asbestos is in cross-fiber or slip fiber veins. In the former, the fiber axes are perpendicular to the walls of narrow openings in the host rock; in the latter, they are parallel. Asbestos rarely occurs as mass fiber bundles in which fibrillar growth is in many directions. This growth pattern is not clearly related to planar structural features of the rock.

NONASBESTIFORM



In the nonasbestiform variety, mineral crystal growth tend not to grow with parallel alignment, but form multi-directional growth patterns instead. When pressure is applied, the crystals fracture easily, fragmenting into prismatic particles called cleavage fragments. Some particles or cleavage fragments are acicular or needle-shaped as a result of the tendency of amphibole minerals to cleave along two dimensions but not along the third. Stair-step cleavage along the edges of some particulates is common. Serpentine have a single cleavage direction and single crystals would form sheets when crushed. Serpentine rock, when crushed, will produce some elongated fragments.

Comminution of nonasbestiform amphibole produces particles that, although generally elongated, have widths larger than asbestos fibers of the same length. These wide widths are characteristic of all amphibole cleavage fragments, even those that have developed higher aspect ratios due to well-developed parting. Byssolite, the most acicular, needle-like nonasbestiform amphibole, will break perpendicular to the fiber axis during comminution because it is brittle, thereby producing particulates with low aspect ratios (See Reference Exhibit 5).

NON-ASBESTIFORM VARIETY

SERPENTINE GROUP

antigorite

(CAS No. 12135-86-3)

AMPHIBOLE GROUP

riebeckite

(CAS No. 17787-87-0)

grunerite

(CAS No. 14567-61-4)

anthophyllite

(CAS No. 17068-78-9)

tremolite

(CAS No. 14567-73-8)

actinolite

(CAS No. 13768-00-8)

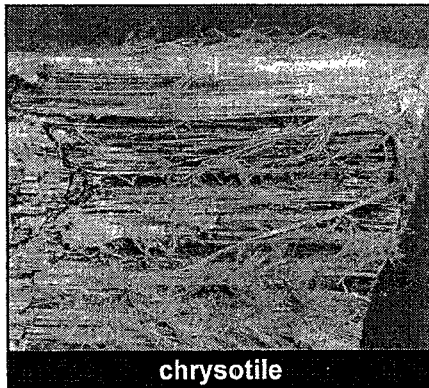
REFERENCE EXHIBIT 2

Macroscopic Raw Ore Comparisons

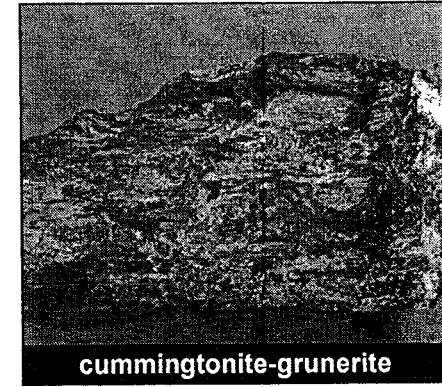
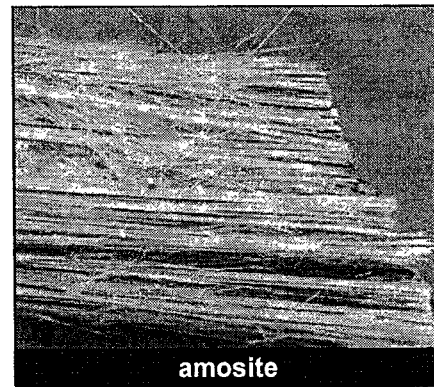
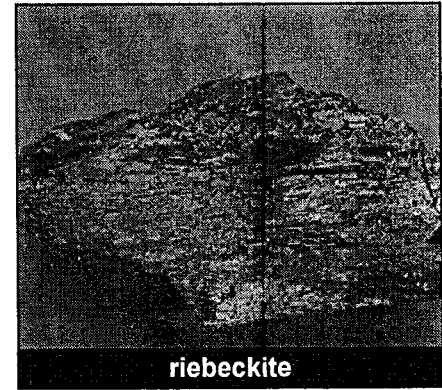
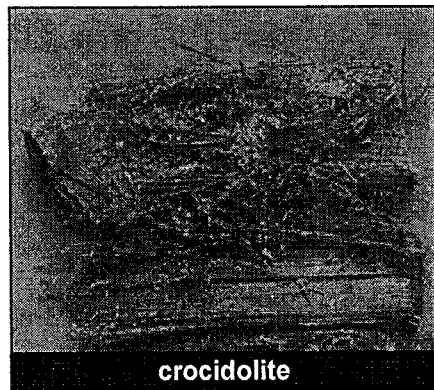
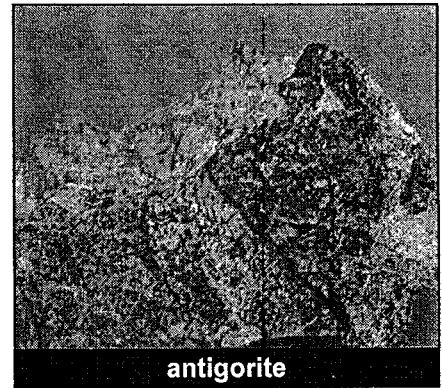
Each of these six minerals included in OSHA's asbestos standard occurs in both an asbestiform and a nonasbestiform variety.

Three of the six minerals have been given a different name for each of their two forms. *Chrysotile* is the asbestiform variety of the serpentine minerals group. In this group *antigorite* is a common nonasbestiform mineral. In the amphibole group, *crocidolite* is the asbestiform variety of *riebeckite*; *amosite* is the asbestiform variety of "cummingtonite"-grunerite.

Asbestiform

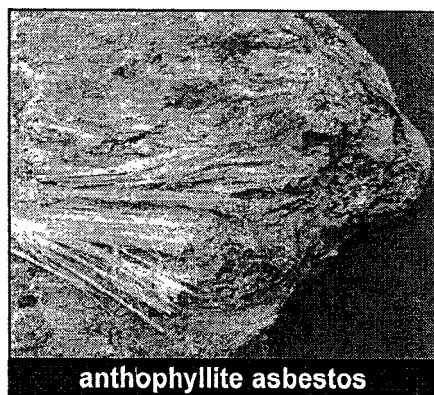


Nonasbestiform



Macroscopic Raw Ore Comparisons

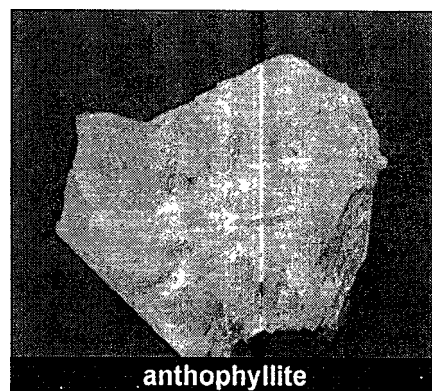
Asbestiform



g.

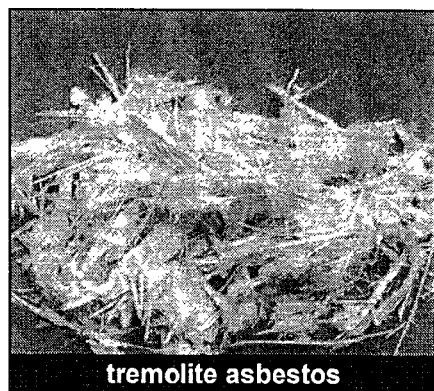
anthophyllite asbestos

Nonasbestiform



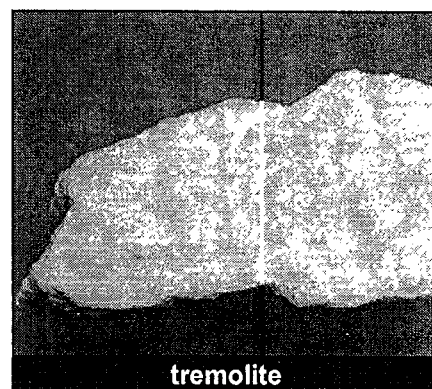
h.

anthophyllite



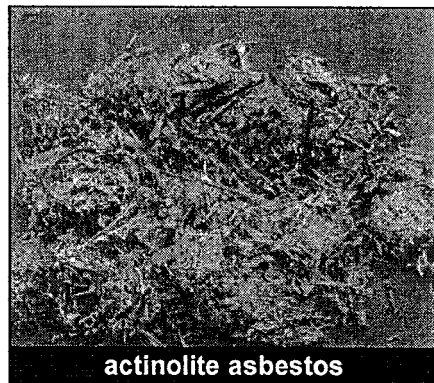
i.

tremolite asbestos



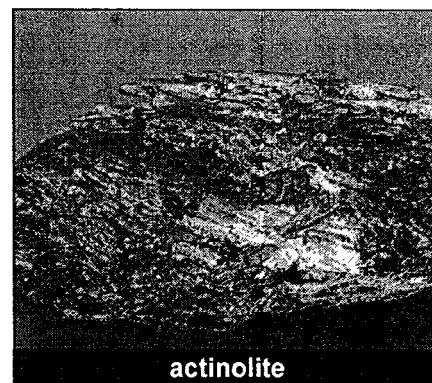
j.

tremolite



k.

actinolite asbestos



l.

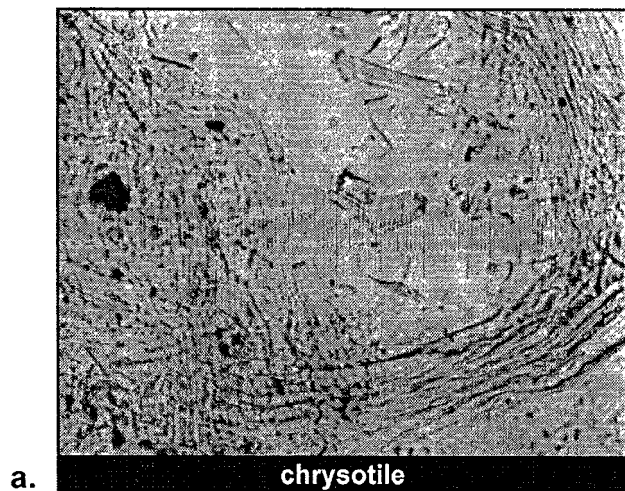
actinolite

REFERENCE EXHIBIT 3

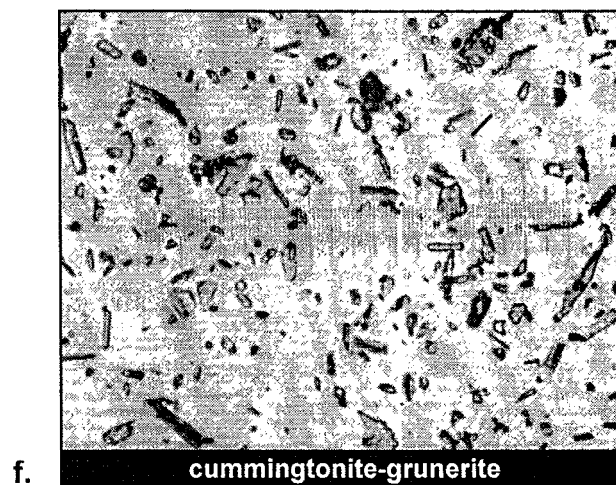
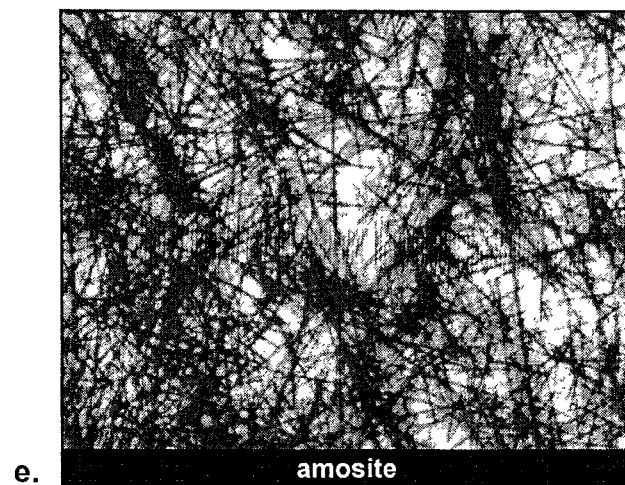
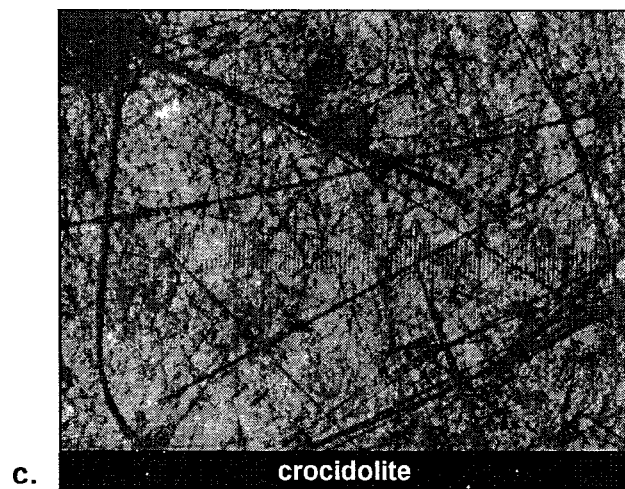
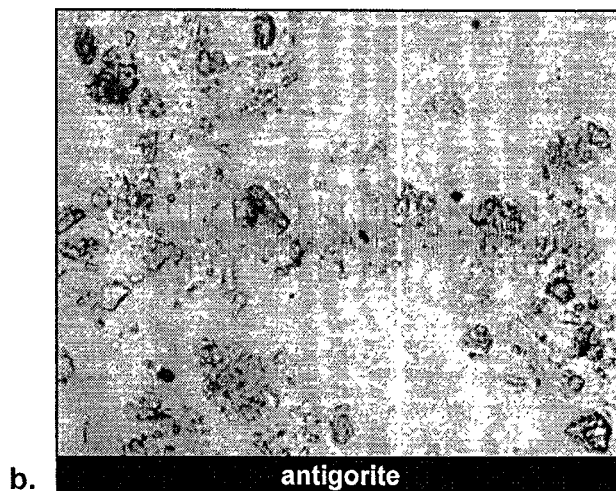
Light Microscopic Comparisons

(2.75 μm /divisions)

Asbestiform



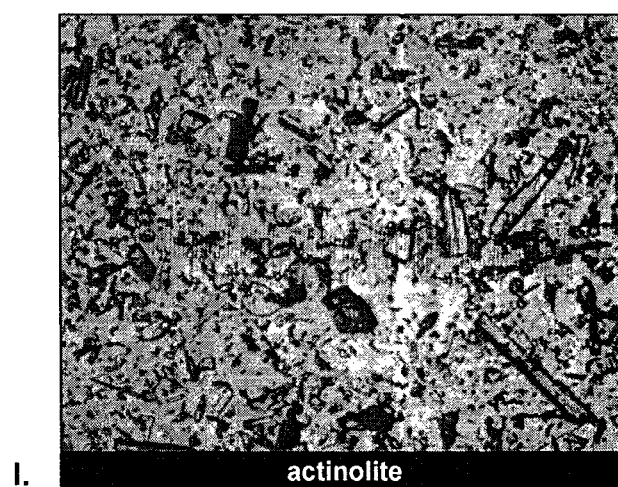
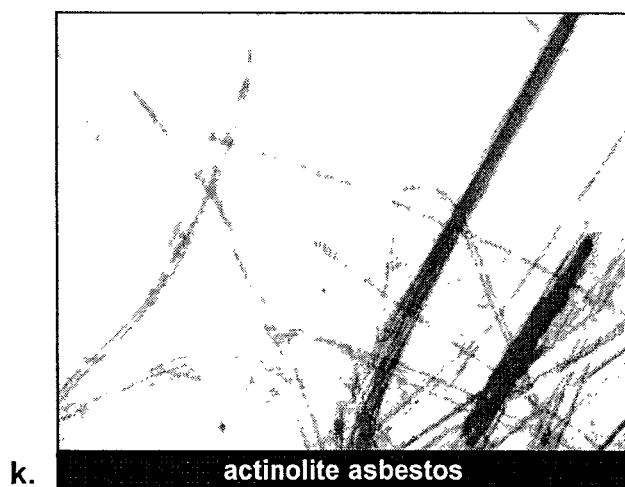
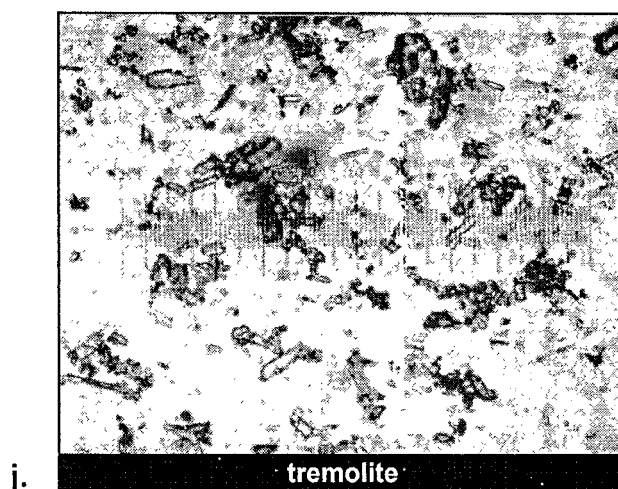
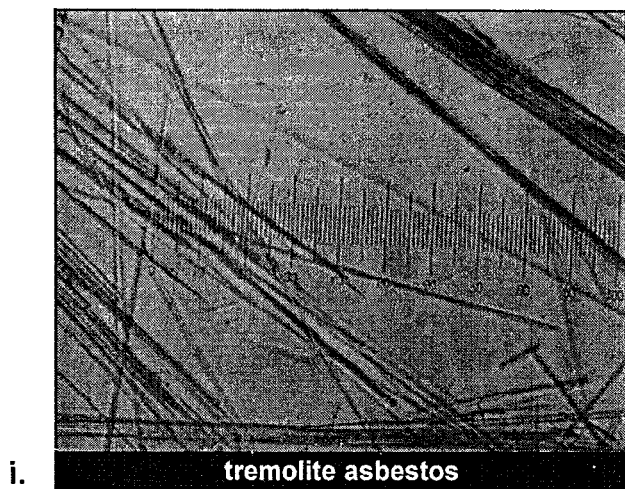
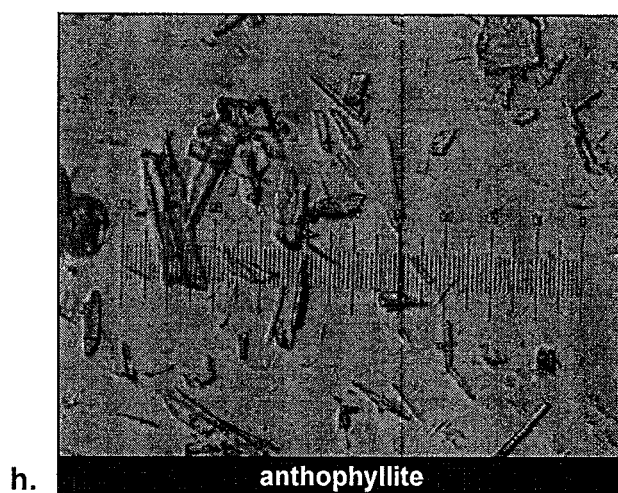
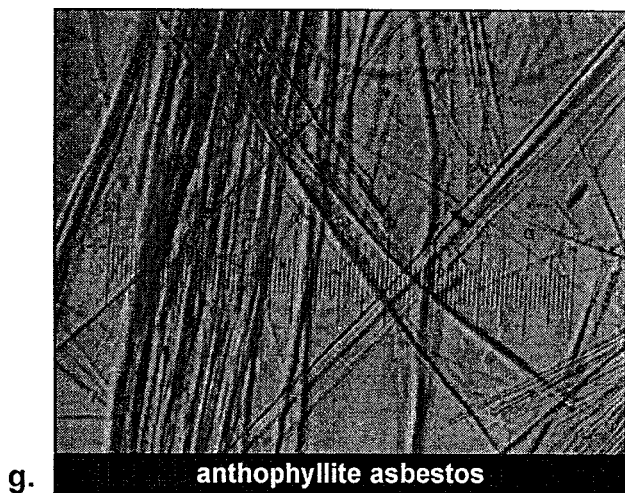
Nonasbestiform



(2.75 μm /divisions)

Asbestiform

Nonasbestiform



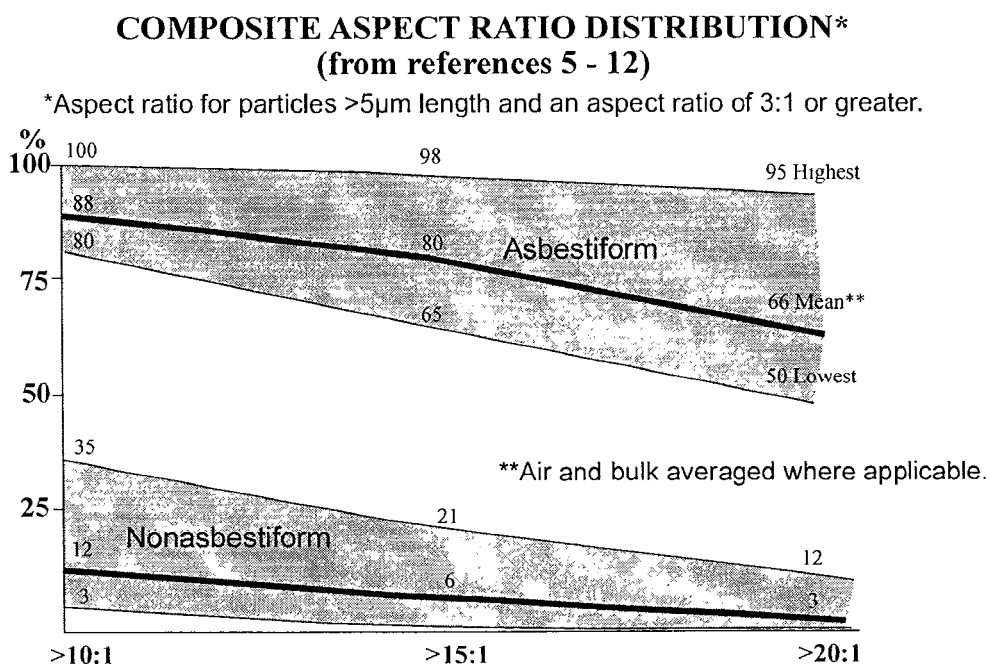
REFERENCE EXHIBIT 4

The Aspect Ratio

Existing regulatory standards for asbestos are based on a light microscopy analysis of airborne particles with a length-to-width ratio (aspect ratio) of 3:1 or greater and a length greater than 5 μm . This was arbitrarily set to obtain consistency among **asbestos** "fiber" counters. Unfortunately, this dimensionless parameter, adopted for asbestos quantification, has been misused by some as a means to "identify" asbestos. Since many other particles share these dimensions, it is improper to use the aspect ratio as a designator of asbestos.

However, the aspect ratio concept, when used with caution, can be useful in distinguishing the asbestiform or nonasbestiform nature of a given dust population. Due to the tendency of asbestiform fiber bundles to separate into thinner and thinner fibers when pressure is applied (i.e., ground), the aspect ratio tends to remain high. In contrast, because nonasbestiform minerals break or cleave in a more random fashion, few relatively long, thin particles are produced. Nonasbestiform dust populations will, therefore, generally retain low aspect ratio characteristics. This fundamental difference can be observed under the light microscope and used as one analytical parameter to distinguish an asbestiform dust population from a nonasbestiform dust population. It must be stressed, however, that this parameter is not a means to positively identify asbestos.

The following figure contrasts the typical aspect ratio difference between asbestiform dust populations and nonasbestiform dust populations. Starting with all particles that exceed a 3:1 aspect ratio ($> 5 \mu\text{m}$ length), the asbestiform dust population maintains an elevated percentage of high aspect ratio particles while the nonasbestiform population does not.



Example: Nonasbestiform particles with an aspect ratio of 3:1 or greater ($> 5 \mu\text{m}$ length), 6% on average exceed an aspect ratio of 15:1 while asbestiform particles, 80% on average exceed this ratio.

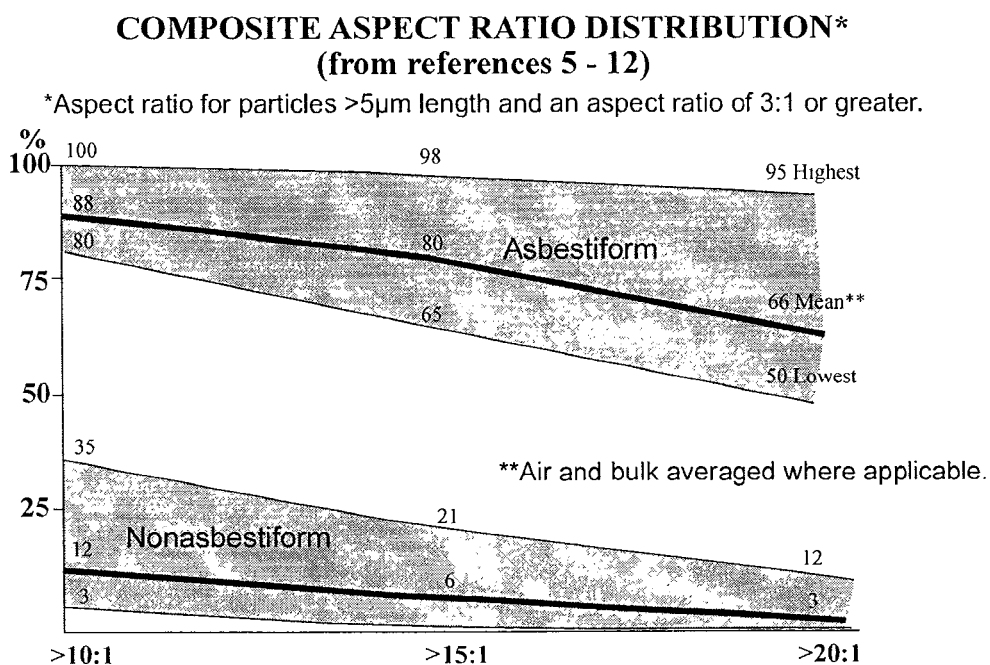
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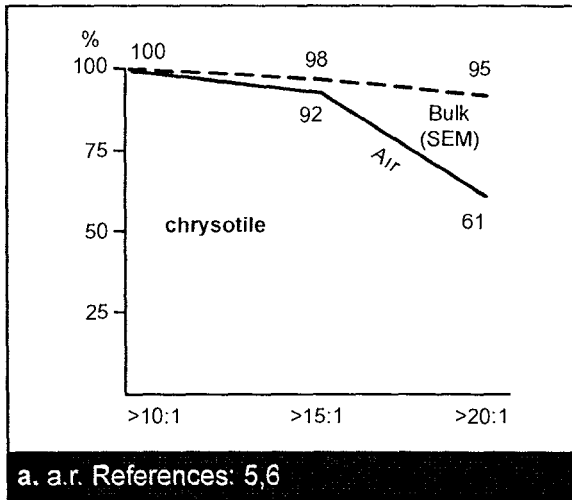


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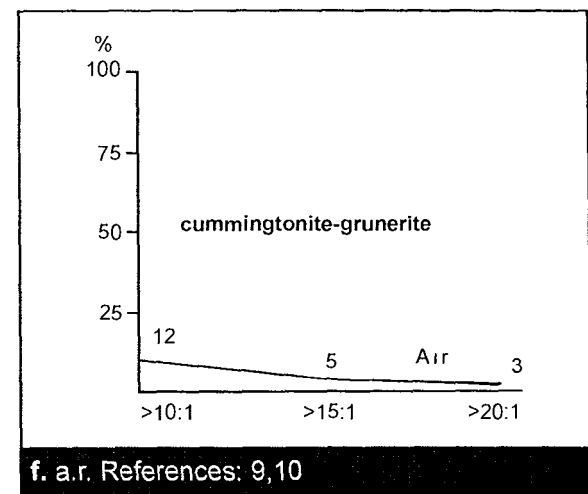
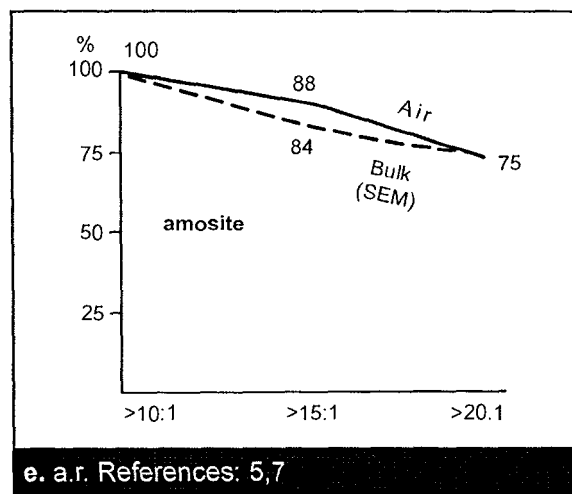
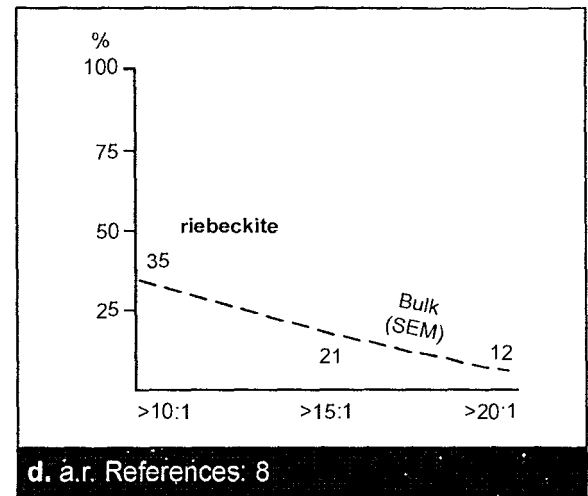
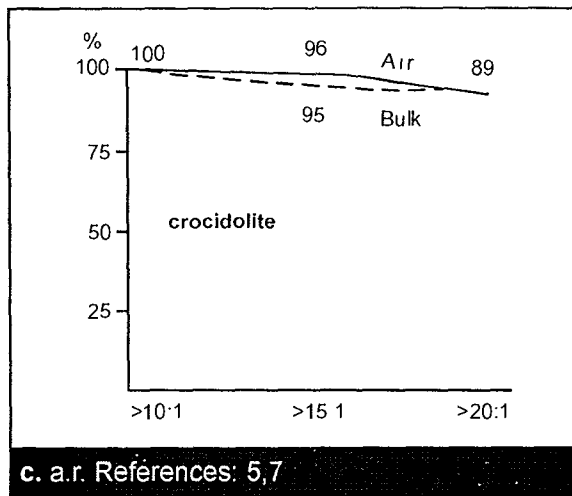
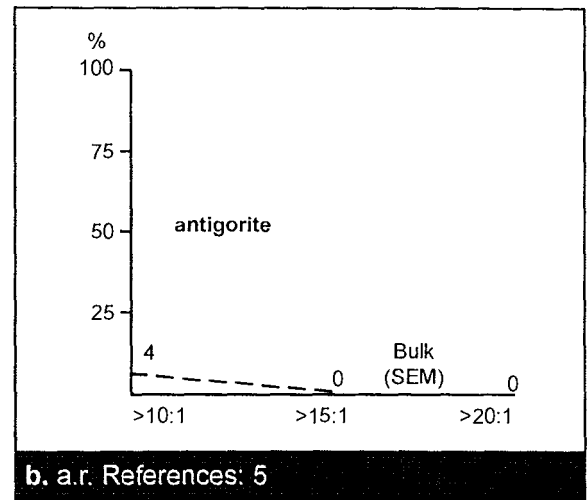
ASPECT RATIO COMPARISONS

Includes only particles with a 3:1 aspect ratio (a.r.) or greater and length > 5 μm .

Asbestiform



Nonasbestiform



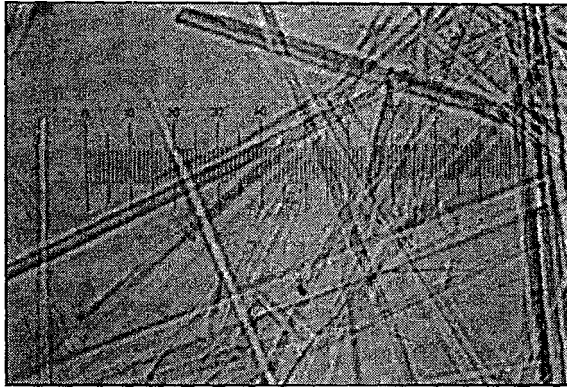
REFERENCE EXHIBIT 5

Byssolite Unusual Needle-like Nonasbestiform Mineral Growth

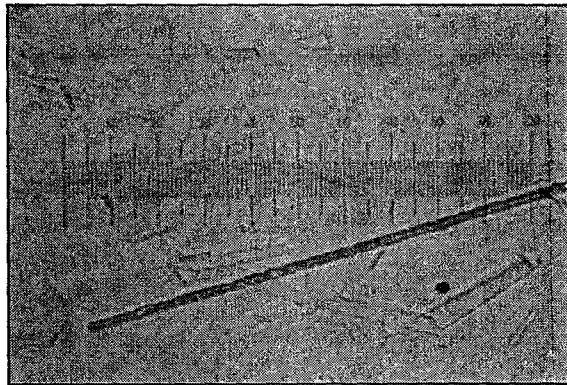
Although most nonasbestiform particulates appear as described and pictured in prior exhibits, nonasbestiform particles can appear in a very acicular or needle-like form. Although such particles do not exhibit characteristics unique to asbestos (fibrillar bundling, splayed terminations, extreme lengths, etc.), high length to width aspect ratios are possible. The Addison Italian and Dornie tremolite samples summarized in this pictorial exhibit (J and P respectively) reflect this rare particulate form. Byssolites, whose optical properties are often normal, sometimes exhibit their own distinctive optical property - a lack of optical extinction when oriented and viewed on the 010 crystallographic surface (79). This distinction, as well as a lack of other asbestiform morphological properties, allows one to distinguish the byssolite habit from the asbestiform habit.

Further comminution of these elongated nonasbestiform particles, as illustrated to the right, demonstrates the essential difference in mineral habit. Nonasbestiform minerals cleave to shorter prismatic particles, while asbestos continues to separate along crystal surfaces into smaller and smaller bundles of fibrils.

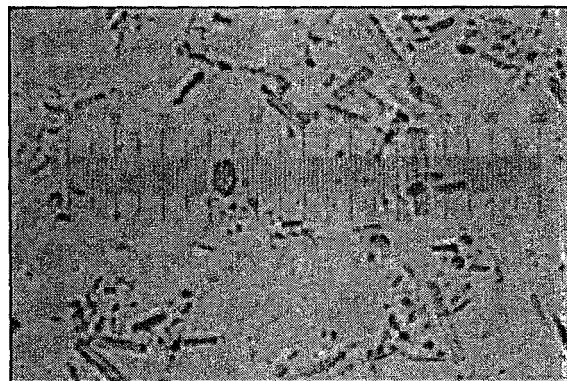
Comminution of Byssolite



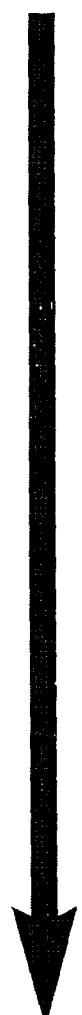
Photomicrograph - 265 X (2 μ m/Div.)



Minor Breaking
Photomicrograph - 265 X (2 μ m/Div.)



Commercial Grind
Photomicrograph - 265 X (2 μ m/Div.)



QUESTION

DOES THIS MINERALOGICAL (MORPHOLOGICAL)
DIFFERENCE = BIOLOGICAL DIFFERENCE?

A Review of Asbestiform and Nonasbestiform Cancer Studies

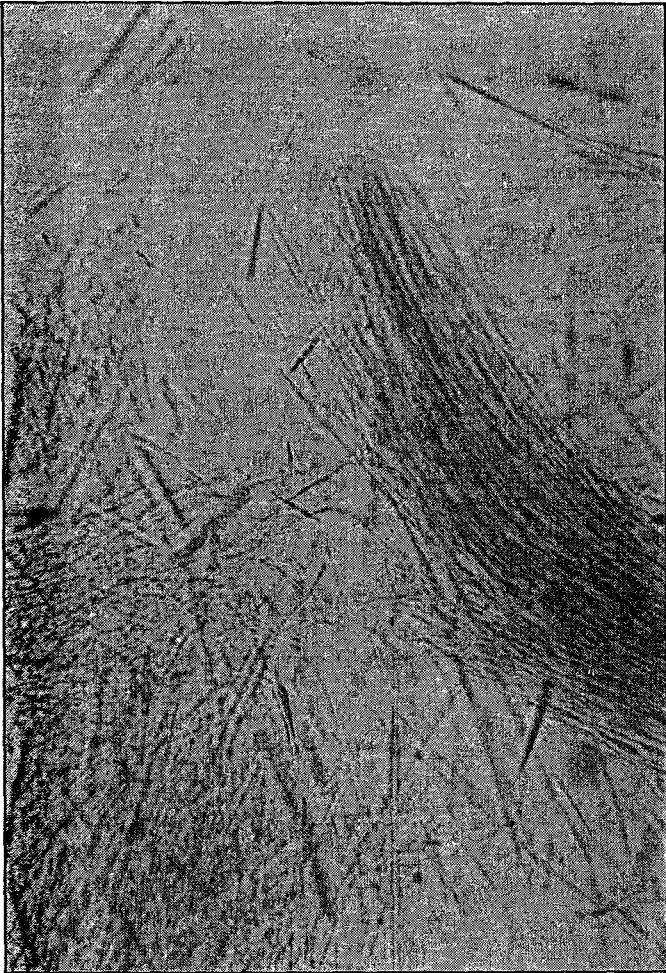
The following "EXPOSURE EXHIBITS" summarize human and animal studies relative to nonasbestiform amphiboles. The majority of studies available in this area involve tremolite.

A large body of literature amply addresses the most commonly encountered, commercially exploited asbestos minerals (*chrysotile*, *crocidolite*, and *amosite*). For the purpose of this presentation, further health review of these asbestos minerals is not considered necessary.

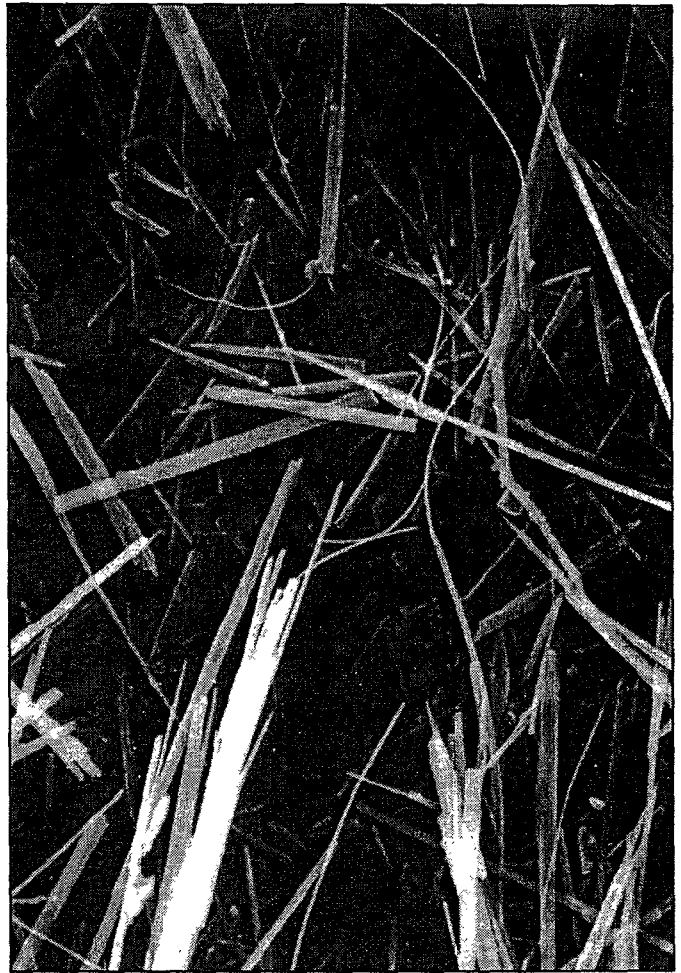
These asbestiform exhibits sufficiently demonstrate previously described mineralogical distinctions and provide the most appropriate contrast to nonasbestiform amphibole health studies.

Asbestiform Winchite — Human Mortality Study

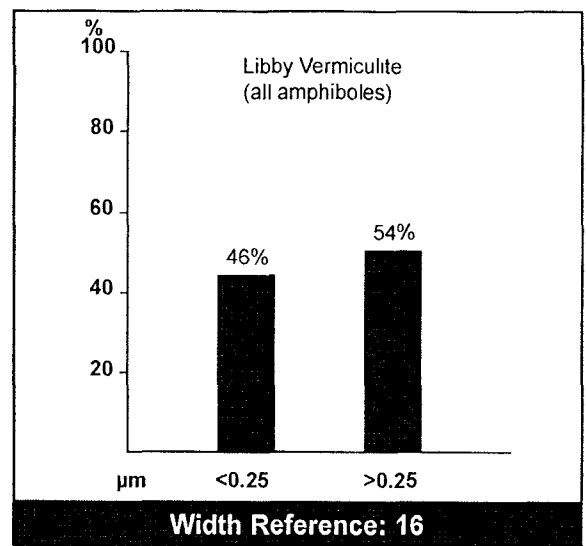
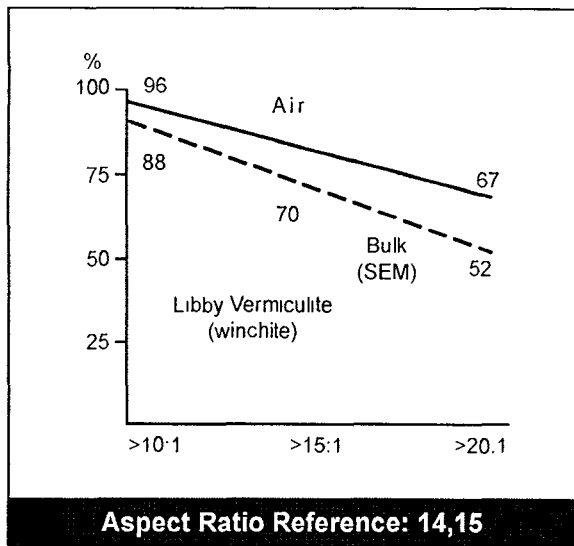
Light Microscopy: 320 X



SEM: 1180 X



ORE: "The vermiculite ore as fed to the mill contained 4-6% amphibole in the tremolite series" (13). More recent analysis of the Libby ore reports the asbestiform amphibole to be winchite asbestos (formally called soda tremolite) (74).



ADDITIONAL MINERAL PARTICLE DATA:

Range of: Diameters = 0.1 - 0.2 µm
 Length = 1 - 70 µm (62% > 5 µm)
 Aspect Ratio = 3:1 - 100:1 (13)

For fibers > 0.45 µm in width and > 5 µm in length, collected on air filters, 96% had aspect ratios > 10:1, 67% had 20:1 or greater aspect ratios and 10% were 50:1 or greater. (15)

HEALTH STUDIES:

Authors: McDonald, J.C., et al (13) Pub. 1986

Cohort: 406 men, >1 yr. exposure, hired prior to 1963

Vital Status Cut Off: July 1, 1983 **SMR** (resp. cancer) - 245

Conclusion: "The cohort studied was not large but sufficient to show that workers in this mine experienced a serious hazard from lung cancer, pneumoconiosis, and mesothelioma."

Authors: Amandus, H.E., et al (15) Pub. 1987

Cohort: 575 men, >1 yr. exposure, hired prior to 1970

Vital Status Cut Off: December 31, 1981 **SMR** (resp. cancer) - 223

Conclusion: "Results indicated that mortality from nonmalignant respiratory disease and lung cancer was significantly increased."

OVERALL CONCLUSION: **Asbestiform winchite in this mining operation is reasonably linked to excess lung cancer and mesothelioma.**

Asbestiform Tremolite — Human Mortality Study

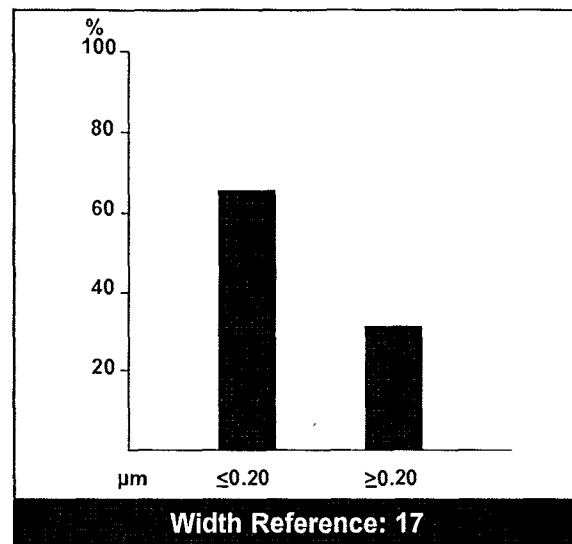
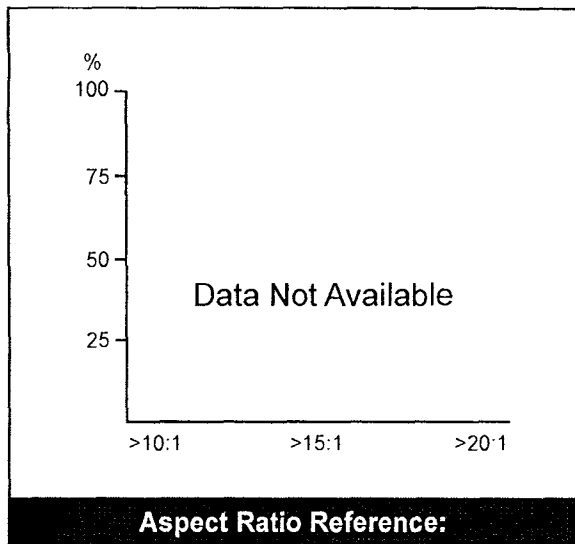
Light Microscopy: 320 X



SEM: 1900X



ORE: "This tremolite is linked to whitewash used in Greek villages. The villages involved Milea, Metsovo, Anilio and Votonosi (Metsovo area in North Western Greece)" (18).



ADDITIONAL MINERAL PARTICLE DATA:

"These fine fibers were unlike the usual tremolite laths, they had aspect ratios in excess of 100:1; they were curvilinear; they had parallel extinction, and they formed polyfilamentous bundles of fibers" (18). Only 6.7% of fibers exceeded a 0.61 μm width. Fifty-three percent of all fibers were < 1.0 μm in length while 6% exceeded 5 μm in length (17).

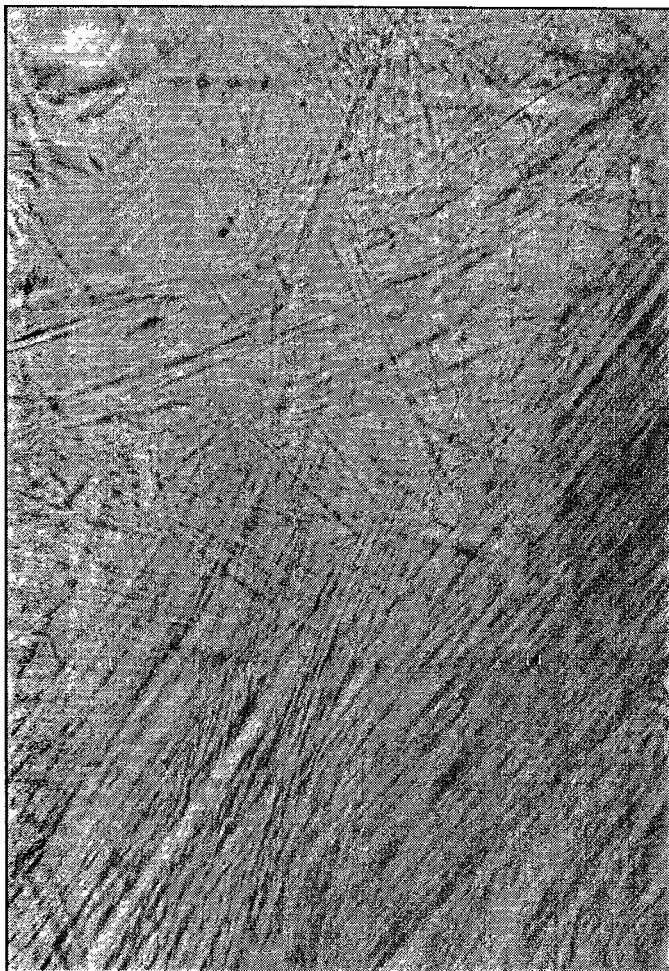
HEALTH STUDIES:

Authors: Langer, A.M., et al (18) Pub. 1987

Cohort: Population of Metsovo in Northwestern Greece

Conclusion: Substantial incidence of mesothelioma in certain towns is linked to tremolite asbestos found in whitewash and stucco.

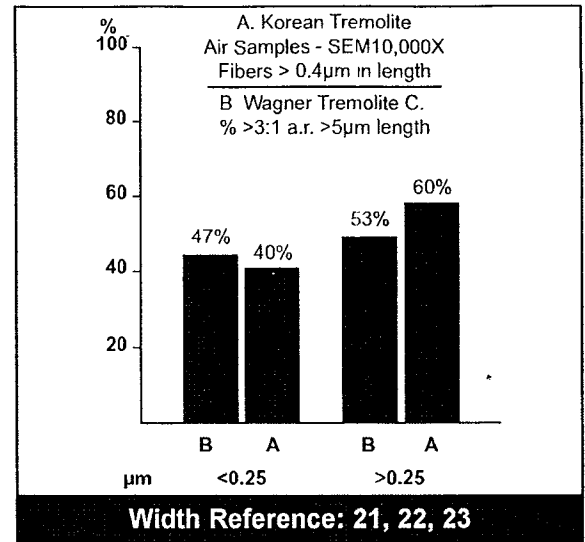
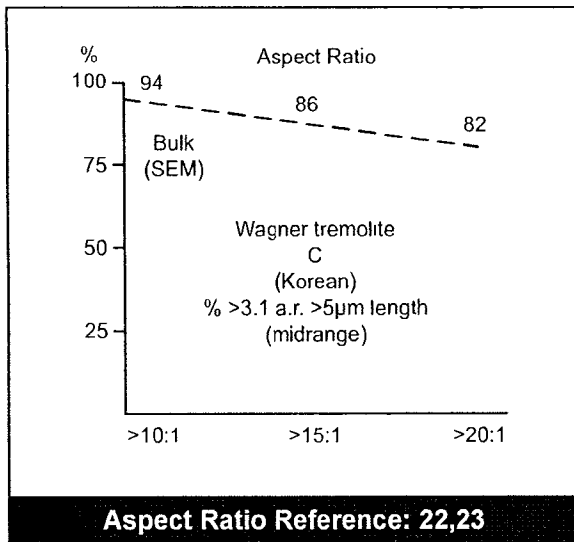
OVERALL CONCLUSION: **Asbestiform tremolite in whitewash has been linked to substantial incidences of mesothelioma.**

Asbestiform Tremolite — Animal Study**Light Microscopy: 320 X****SEM: 1900 X**

SAMPLE: Reported as commercial asbestos originating from S. Korea. Contains by mass approx. 95% asbestiform tremolite. It is reported this same material was used in three separate animal studies (19).

ADDITIONAL MINERAL PARTICLE INFORMATION

"In the optical microscopy and SEM examinations, the asbestos tremolites were found to be typical of that form in displaying polyfilamentous fiber bundles, curved fibers, fibers with splayed ends, and long, thin, parallel-sided fibers. Most of the fibers showed straight extinction when observed with polarized light under crossed polarizers, indicating the presence of multiple twinning of the crystals." "Samples did contain some elongated fragments of tremolite with oblique extinction, stepped ends, and nonparallel sides indicating that they were cleavage fragments." (20)



ANIMAL STUDIES:

Authors: Wagner, J.C., et al (22) Pub. 1982

Test Animals: Sprague-Dawley rats, 6-10 weeks old when injected.

Test Type: Pleural injection

Protocol: A single 20 milligram injection into the right pleural cavity of 48 rats. "The sample was prepared by milling in a small agate mill and ultrasonic dispersion, large particles being removed by sedimentation in water."

Findings: "Sample C produced 14 mesotheliomas in 47 rats."

Authors: Davis, J.M., et al (21) Pub. 1985

Test Animals: SPF male Wistar rats

Test Type: Inhalation and interperitoneal injection

Protocol: For inhalation, 48 rats were exposed for 7 hours each day, 5 days per week, over a 12 month period, to approx. 10 mg of respirable dust per cubic meter of air. For interperitoneal injection, a 25 mg dose of tremolite was collected from the inhalation chamber and injected (in saline) into the peritoneal cavities of rats.

Findings: For the inhalation study, a total of 16 carcinomas and 2 mesotheliomas occurred in 39 animals. None were observed in controls. For the interperitoneal study, a total of 27 animals out of 29 examined were found to have mesothelioma tumors. Mean survival time was 352 days.

Authors: Davis, J.M.G., Addison, J. (20) Pub. 1991

Test Animals: AF/Han strain rats

Test Type: Peritoneal injection

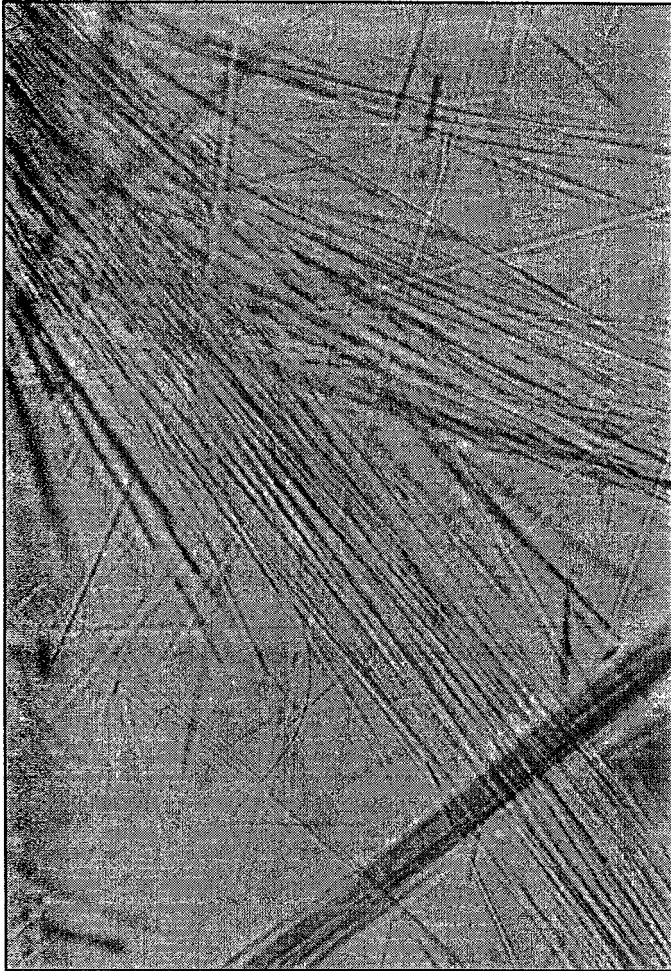
Protocol: Fractions of this sample were obtained by generating an airborne dust cloud in an experimental chamber (Timbrell dust dispensers) with fine fractions collected using a vertical elutriator. A single 10 mg dose was injected into the peritoneal cavities of the animals. All animals lived out of their full life span or were killed when moribund.

Findings: 32 mesothelioma deaths out of 33 animals were observed with a median survival time of 428 days.

OVERALL CONCLUSION: This asbestiform tremolite produced a strong carcinogenic response in the test animals.

Asbestiform Tremolite — Animal Study

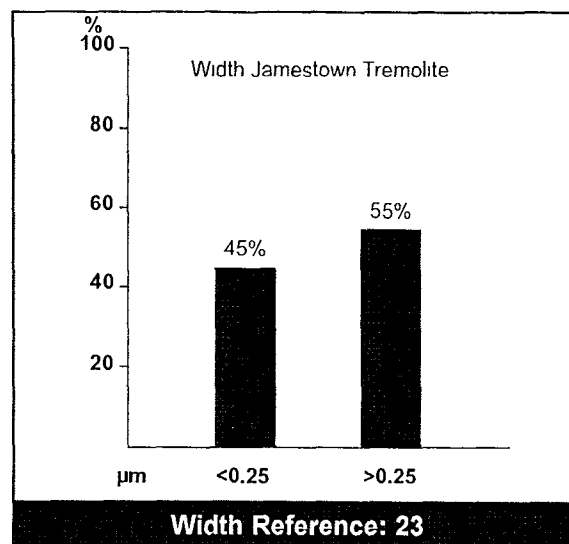
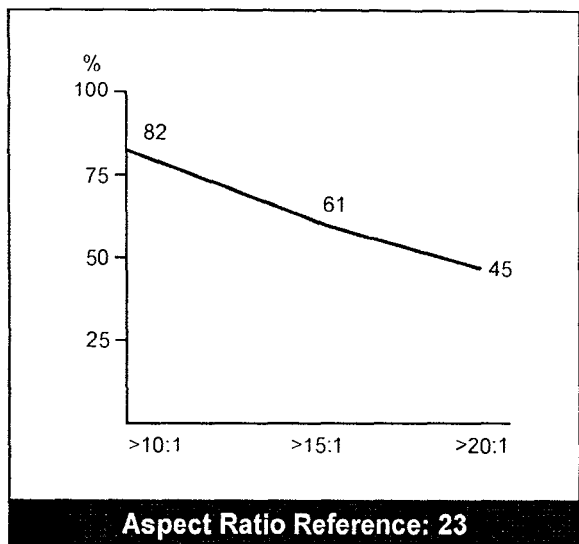
Light Microscopy: 320 X



SEM: 1900 X



SAMPLE: "Fine white tremolite asbestos, Jamestown, California" (20). (Above photomicrographs were taken from bulk material.)



ADDITIONAL MINERAL PARTICLE DATA:

"In the optical microscopy and SEM examinations, the asbestos tremolites were found to be typical of that form in displaying polyfilamentous fiber bundles, curved fibers, fibers with splayed ends, and long, thin, parallel-sided fibers. Most of the fibers showed straight extinction when observed with polarized light under crossed polarizers, indicating the presence of multiple twinning of the crystals." "Samples did contain some elongated fragments of tremolite with oblique extinction, stepped ends, and nonparallel sides indicating that they were cleavage fragments." (20)

ANIMAL STUDIES

Authors: Davis, J.M.G., Addison, J. (20) Pub. 1991

Test Animals: AF/Han strain rats

Test Type: Peritoneal injection

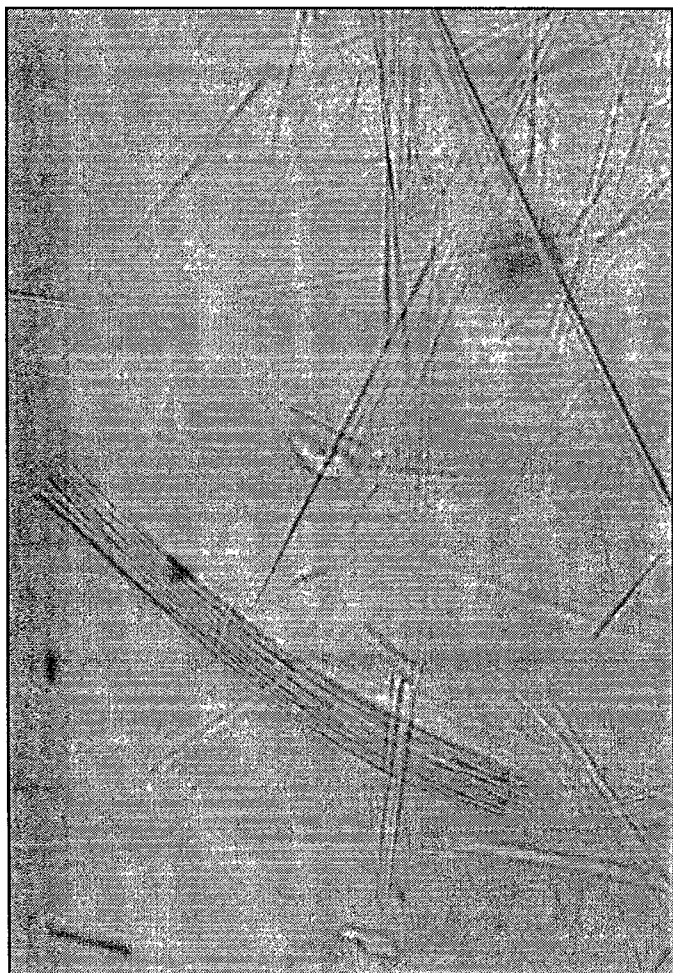
Protocol: Fractions of this sample were obtained by generating an airborne dust cloud in an experimental chamber (Timbrell dust dispensers) with fine fractions collected using a vertical elutriator. A single 10 mg dose was injected into the peritoneal cavities of the animals. All animals lived out of their full life span or were killed when moribund.

Findings: 36 mesothelioma deaths out of 36 animals were observed with a median survival time of 301 days.

OVERALL CONCLUSION: This asbestiform tremolite produced a strong carcinogenic response in the test animals.

Asbestiform Tremolite — Animal Study

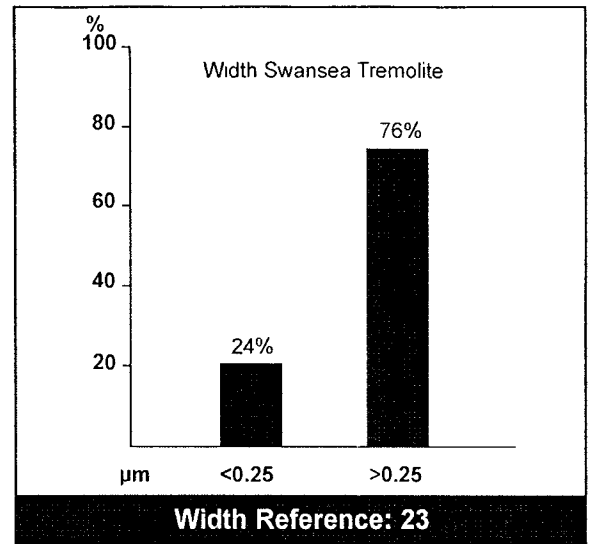
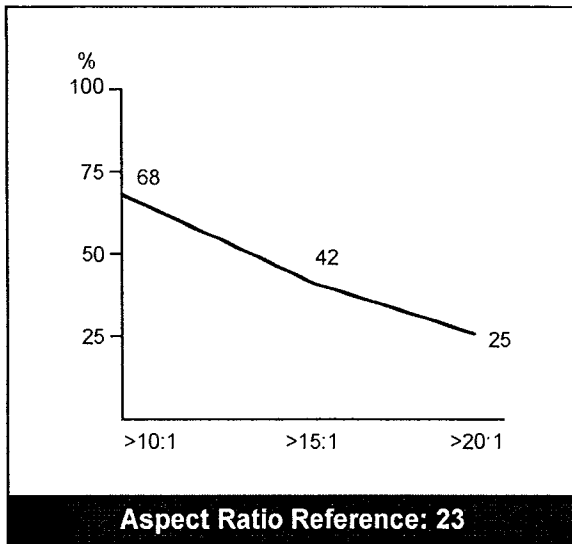
Light Microscopy: 320 X



SEM: 1900 X



SAMPLE: "Fine white tremolite asbestos, Swansea Laboratory" (20). (Above photomicrographs were taken from bulk material.)



ADDITIONAL MINERAL PARTICLE DATA:

"In the optical microscopy and SEM examinations, the asbestos tremolites were found to be typical of that form in displaying polyfilamentous fiber bundles, curved fibers, fibers with splayed ends, and long, thin, parallel-sided fibers. Most of the fibers showed straight extinction when observed with polarized light under crossed polarizers, indicating the presence of multiple twinning of the crystals." "Samples did contain some elongated fragments of tremolite with oblique extinction, stepped ends, and nonparallel sides indicating that they were cleavage fragments." (20)

ANIMAL STUDIES

Authors: Davis, J.M.G., Addison, J. (20) Pub. 1991

Test Animals: AF/Han strain rats

Test Type: Peritoneal injection

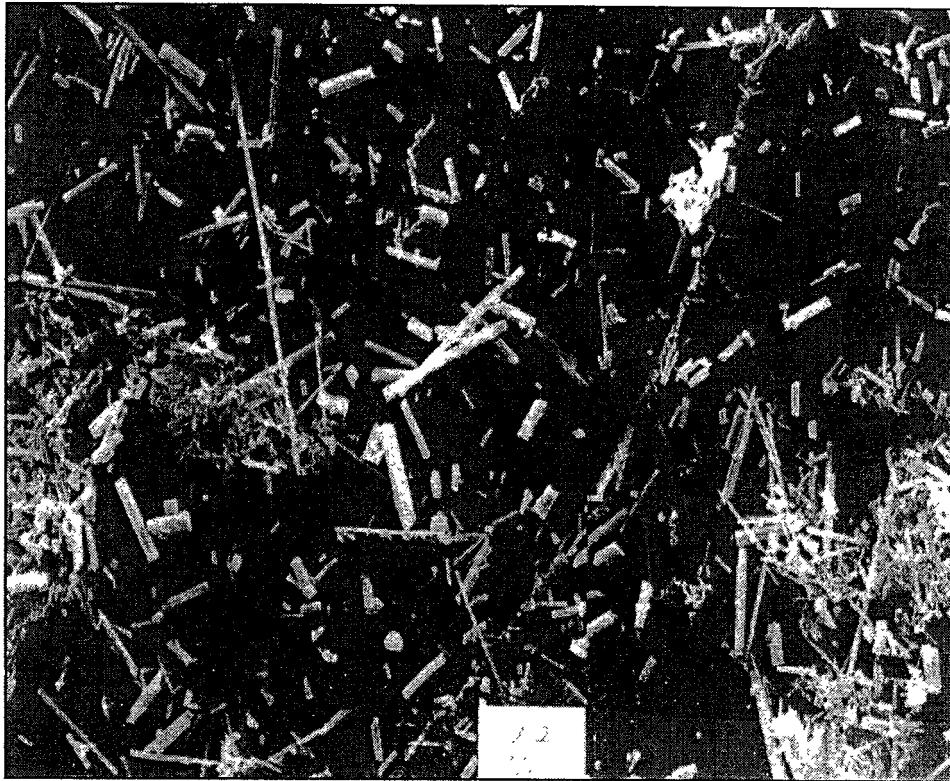
Protocol: Fractions of this sample were obtained by generating an airborne dust cloud in an experimental chamber (Timbrell dust dispensers) with fine fractions collected using a vertical elutriator. A single 10 mg dose was injected into the peritoneal cavities of the animals. All animals lived out of their full life span or were killed when moribund.

Findings: 35 mesothelioma deaths out of 36 animals were observed with a median survival time of 365 days.

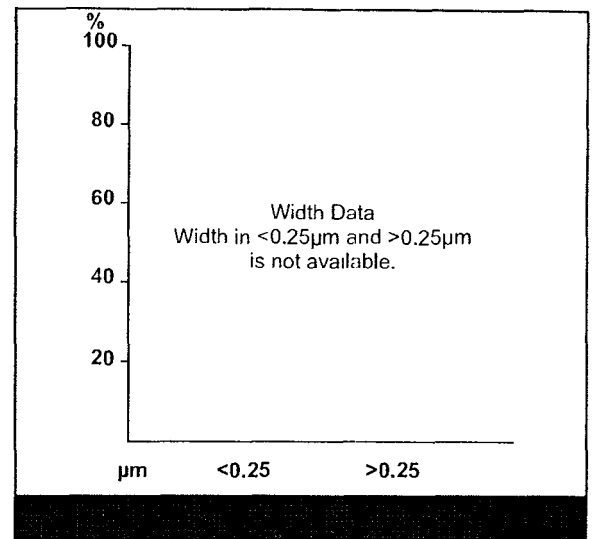
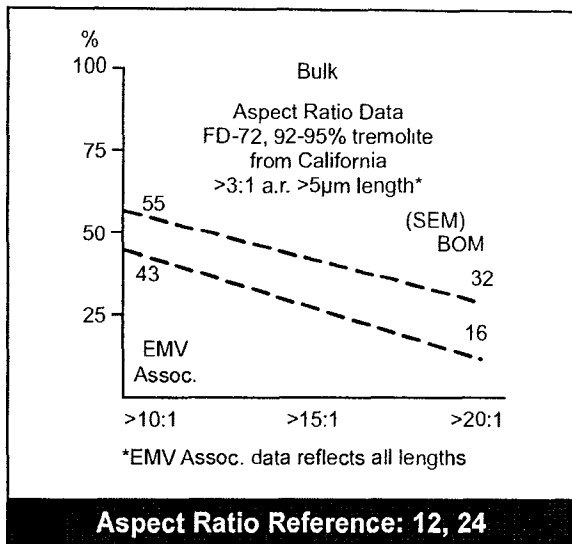
OVERALL CONCLUSION: This asbestiform tremolite produced a strong carcinogenic response in the test animals.

Asbestiform Tremolite — Animal Study

SEM: 1250 X



SAMPLE: FD-72 was supplied to Dr. Smith from Dr. Merle Stanton and indirectly from Johns-Manville. This material, reportedly from California, is described as asbestiform and may have been used by Dr. Stanton in his work (tremolite 1 and 2).



ADDITIONAL MINERAL PARTICLE DATA:

The sample preparation of FD-72 is unclear, although a portion of this sample was provided to the Bureau of Mines (BOM) for characterization. The sample was dispersed in water, ultrasonically agitated and filtered through a nucleopore filter for SEM preparation. Petrographic preparation required no such processing. There is some question as to how exact the BOM samples are to Dr. Smith's analysis (EMV Assoc), but major differences are not indicated. For FD-72, 9 particles with a length of >10 µm were observed in 200 total particles by SEM.

ANIMAL STUDIES

Authors: Smith, W.E., et al (25) Pub. 1979

Test Animals: Male LUG: LAK hamsters, injected at 2 months of age.

Test Type: Intrapleural injection

Protocol: Single intrapleural injection of two dosages (10 and 25 mg). The sample was suspended in saline and sterilized by autoclave. The occurrence of tumors (unspecified) was noted at necropsies for a starting group of 50 animals per dose. After short-term sacrifice of some animals and the loss of others through acute enteritis, the occurrence of tumors was noted in nonsurvivors up to 600 days.

Findings: Four tumors out of 13 animals were found at the 10 mg dose, and 13 out of 20 animals were found at the 25 mg dose.

OVERALL CONCLUSION: Asbestiform tremolite produced pleural tumors.

Asbestiform Tremolite — Animal Study

Light Microscopy: 320 X



SEM: 1800 X



SAMPLE: The exact origin of this tremolite asbestos from California, provided to Dr. Stanton by Johns-Manville, is unknown (26). "Both of these samples were from the same lot of asbestos and were in the optimal range of size for carcinogenesis" (27).

Aspect Ratio and Width Data

Aspect ratio and width data has not been developed due to concerns over the reliability of transcribing data presented in the literature (28). These difficulties result from questions over the accuracy (reproducibility) of size distribution data (especially for asbestiform samples — see discussion below). Size-data, however, does reflect a broad size distribution with many very long and very narrow fibers (i.e., < 0.25 width, > 20:1 aspect ratios).

ADDITIONAL MINERAL PARTICLE DATA:

Obtaining accurate dimensional data for these tremolite samples was difficult as reported by the investigators on page 965 of their report: "Of special interest are the data on the amphibole asbestoses: amosite, tremolite and crocidolite, though estimates of the dimensions of the asbestoses are especially liable to error." And on page 973: "In preparations of amphibole asbestos (which included the crocidolites and tremolites), we observed that both clumping and fragmentation of the particles were greater than those in other minerals, and estimates of particle size distribution in that the asbestiform characteristic of fiber bundles (reported as clumping), and the splitting of these bundles (reported as fragmentation), was the reason for the difficulty in obtaining accurate fiber size distributions.

ANIMAL STUDIES

Authors: Stanton, M.F., et al. (27) Pub. 1981

Test Animals: 20-week-old, outbred female Osborne-Mendal rats

Test Type: Pleural implantation

Protocol: A standard 40 mg dose of each tremolite asbestos sample was uniformly dispersed in hardened gelatin and applied by open thoracotomy directed to the left pleural surface. The animals were followed for 2 years, at which time the survivors were sacrificed and the tissue examined for pleural sarcomas.

Findings: Exposure to these tremolite asbestos samples resulted in tumor incidences in 22 out of 28 animals for Sample 1 and 21 out of 28 animals in Sample 2.

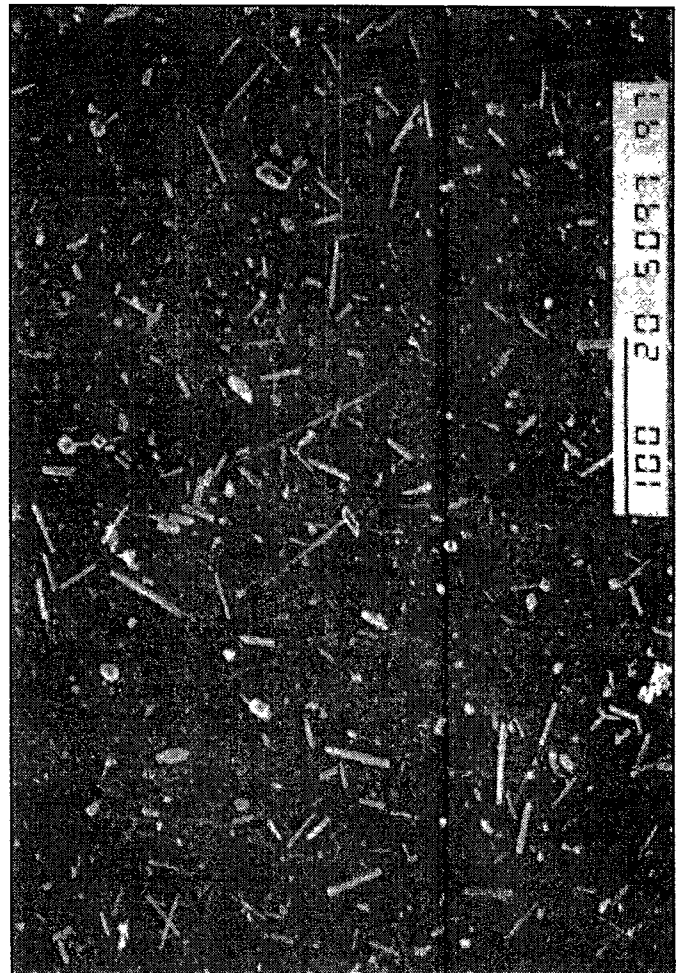
OVERALL CONCLUSION: **These asbestiform tremolites resulted in a significant carcinogenic response in the study population.**

Asbestiform Ferroactinolite — Animal Study

Light Microscopy: 400 X



SEM: 200 X



SAMPLE: “Test fibers were prepared from loose surface iron-formation rocks” (29).

NOTE: Although the reference photo-micrograph reflects actinolite asbestos, ferroactinolite is not a designated asbestos mineral. It appears, however, to be asbestiform.

Ferroactinolite Prior to Placement in the Animals				Ferroactinolite After Placement in the Animals			
				Mean After			
	Mean	Median	Range	1 Month	4 Months	12 Months	
Length	3.18	1.50	0.3 - 52.3	Length	2.10	2.00	1.77
Width	0.41	0.24	0.03 - 5.23	Width	0.19	0.17	0.11
Aspect Ratio	9.0	6.0	3.0 - 130.0	Aspect Ratio	17.1	22.3	30.1

ADDITIONAL MINERAL PARTICLE DATA:

"The estimated mineral particle content by volume was as follows: ferroactinolite fibers (50%), sheet silicate plates (20%), magnetite (5%), ferroactinolite and hornblende fragments (20%), and other minerals (5%)" (29). "Examination by transmission electron microscopy of low temperature ashed whole lung specimens of animals killed sequentially, indicated that the mineralogical characteristics of both ferroactinolite and amosite fibers changed in time. Longitudinal splitting of the fibers resulted in a greater number of thinner fibers with increased aspect ratio." "The ferroactinolite splitting reaction is more rapid and results in the formation of thinner and more numerous fibers than the amosite splitting reaction" (30).

ANIMAL STUDIES

Authors: Cook, P.M., Coffin, D.L., et al (29-30) 1982

Test Animals: Male Fischer - 344 rats

Test Type: Intratracheal instillation and intrapleural injection

Protocol: The intratracheal instillation experiment involved twelve week injections of 0.5 and 0.25 mg each in groups of 561 and 139 rats (ferroactinolite and amosite, respectively). For study of early pathological sequences and for the evaluation of clearance and fate of mineral fibers by electron microscopy, the animals were killed at various intervals up to 1 year, while others were allowed to live out their lives. The intrapleural injection experiment involved a single injection of 20 mg in groups of 135 and 137 rats. Animals were allowed to live out their lives.

Findings: "The data demonstrates that ferroactinolite produced neoplastic lesions through both routes of inoculation. On the basis of mass dose by intratracheal instillation on cogenic potency, it was greater for the ferroactinolite, whereas, by intrapleural inoculation, potency was greater for amosite, however, the difference was not statistically significant."

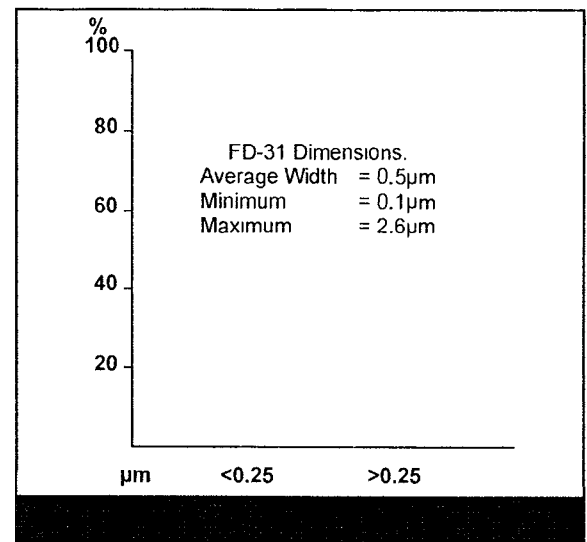
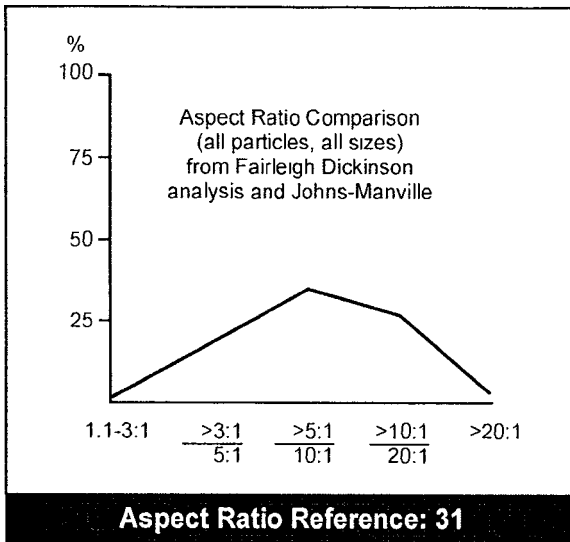
OVERALL CONCLUSION: **This study demonstrates a carcinogenic effect to asbestiform ferroactinolite.**

Asbestiform or Highly Fibrous Tremolite — Animal Study

SEM: 1250 X



SAMPLE: FD-31 was provided through Johns-Manville Corp. from a tremolitic talc in the Western United States (JM Sample 4368-31-3). The exact origin of this sample is unknown. This sample is generally considered a mineralogical curiosity.



ADDITIONAL MINERAL PARTICLE DATA:

The exact origin and preparation of this sample is unclear. Subsequent analysis of this sample suggests that: "The particle distribution in the sample is not typical of cleavage fragments of tremolite. The particles in Sample 31 appear to be composed of true fibers whose shape was attained by growth rather than cleavage." "Particles with a 20:1 aspect ratio are quite common." "There is at least one particle which appears to be a bundle of fibers although the photograph is too fuzzy to be absolutely sure, . . ." "This sample is probably not true asbestos, and would be more appropriately characterized as a stiff fibrous variety of amphibole, which is probably byssollite" (32).

ANIMAL STUDIES

Authors: Smith, W.E., et al (25) Pub. 1979

Test Animals: Male LUG:LAK hamsters, injected at 2 months of age.

Test Type: Intrapleural injection

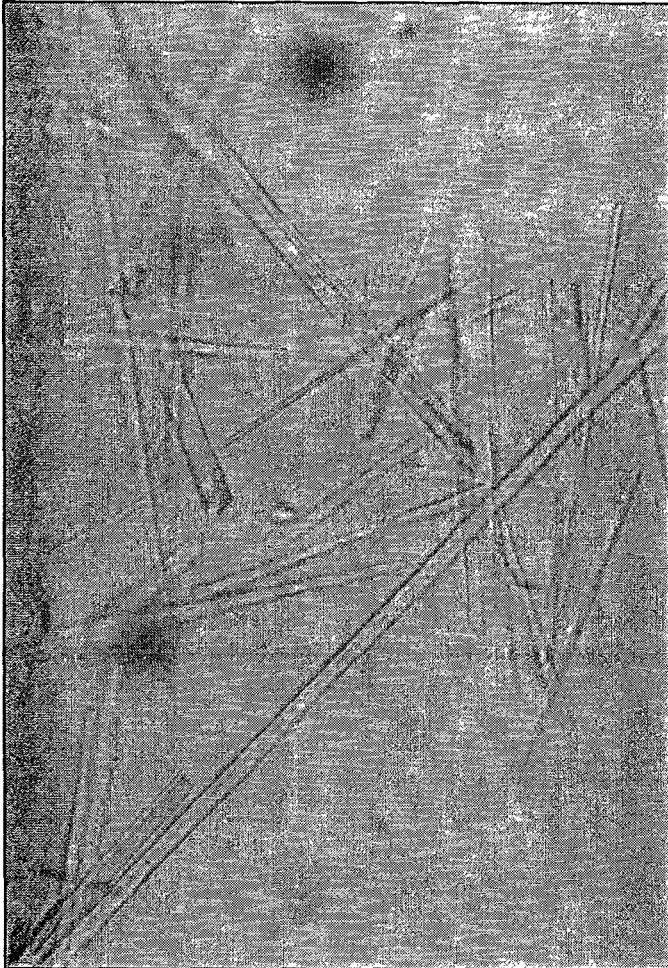
Protocol: Single intrapleural injection of two dosages (10 and 25 mg). The sample was suspended in saline and sterilized by autoclave. The occurrence of tumors (unspecified) was noted at necropsies for a starting group of 50 animals per dose. After short-term sacrifice of some animals and the loss of others through acute enteritis, the occurrence of tumors was noted in nonsurvivors up to 600 days.

Findings: Three tumors out of 41 animals were found at the 10 mg dose, and 12 out of 28 animals were found at the 25 mg dose.

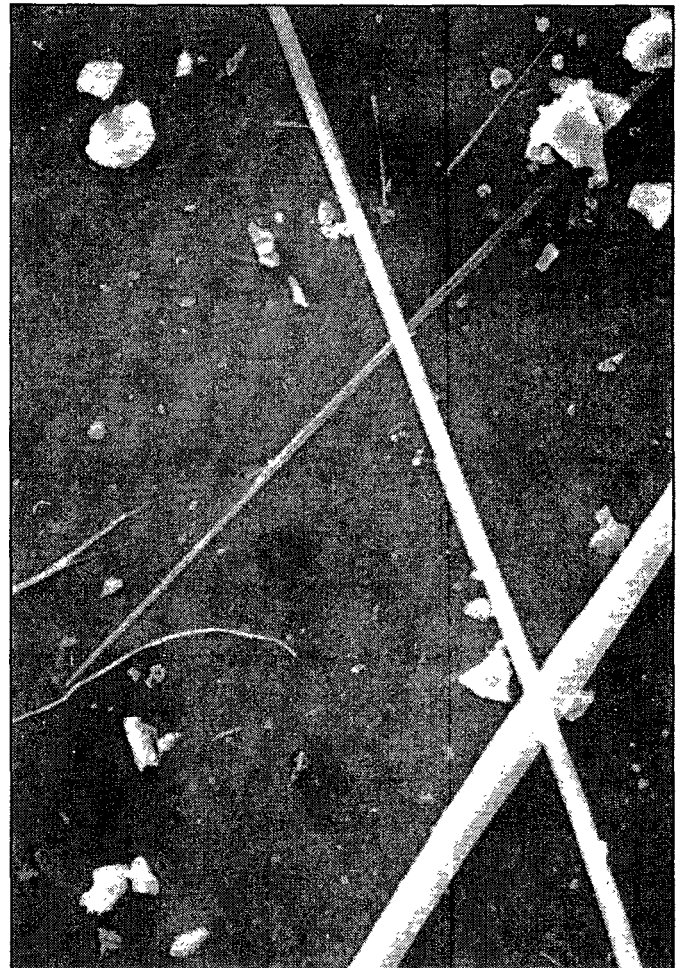
OVERALL CONCLUSION: **A highly fibrous, possibly asbestiform tremolite (or byssollite) produced pleural tumors.**

Nonasbestiform Tremolite
with Asbestiform Subpopulation — Animal Study

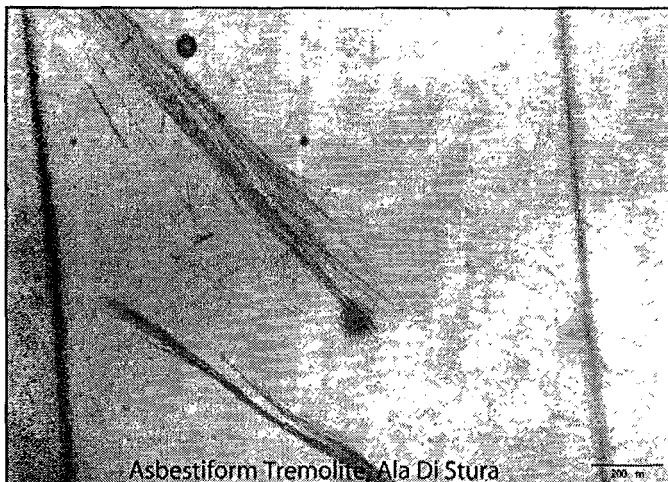
Light Microscopy: 320 X



SEM: 1800 X

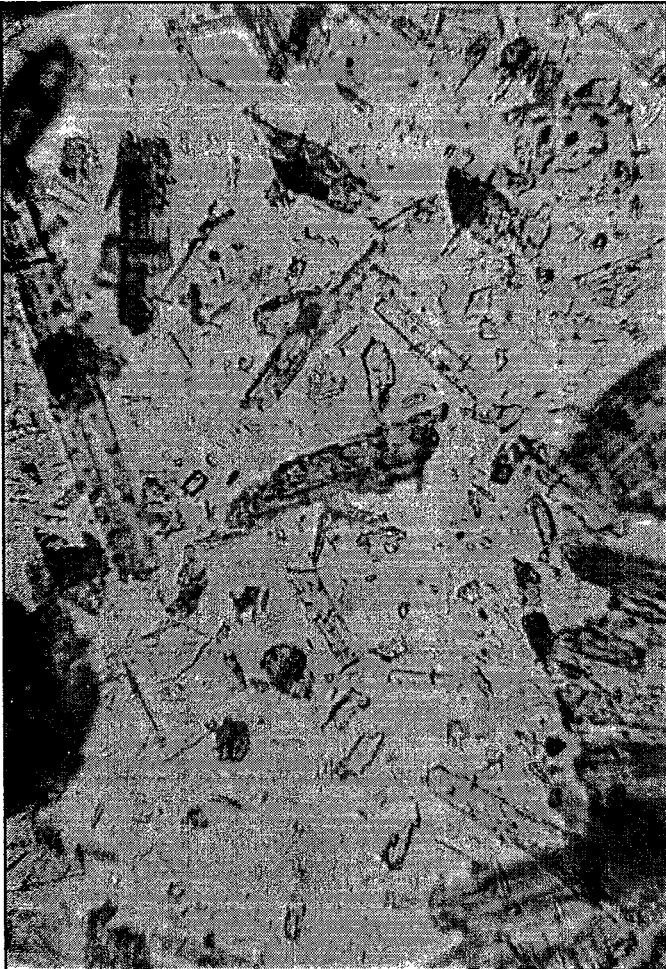


BULK MATERIAL



SAMPLE: The sample "consisted of large bundles of very long (often >5cm) needle-like fibers which were flexible and very elastic but quite brittle." "The tremolite from Italy contained mostly cleavage fragments, but some very long, thin fibers were observed." "The overall impression gained from dense SEM preparations, as shown in this paper, is that the Italian tremolite specimen did contain a certain amount of what observers would consider asbestiform fibers" (20).

Minerals have been characterized and verified as tremolite by x-ray diffractometry, optical microscopy, scanning electron microscopy and energy dispersive x-ray spectroscopy.

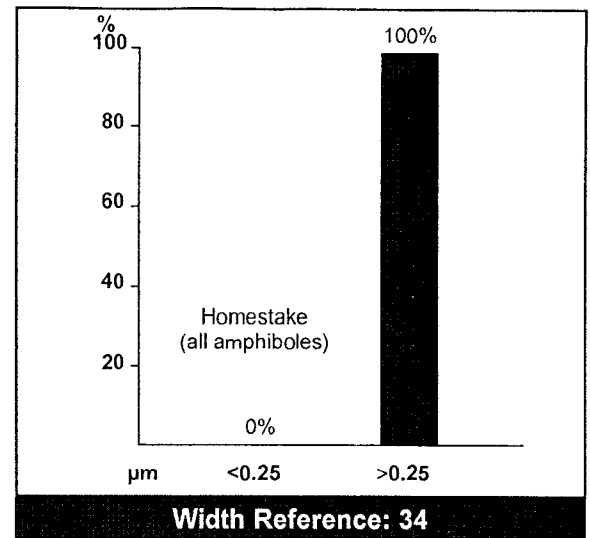
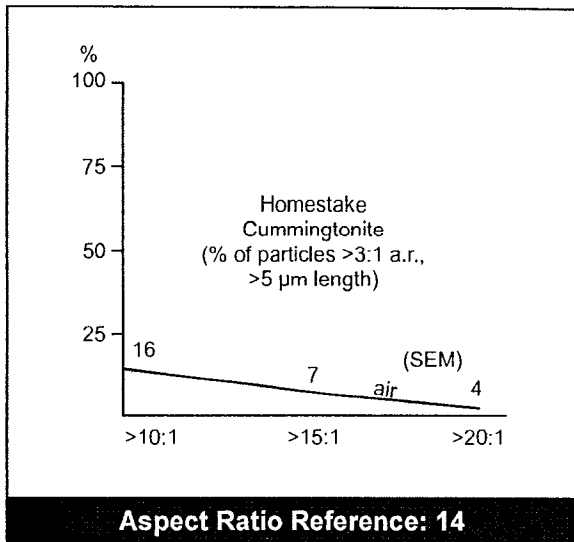
Nonasbestiform Grunerite — Human Mortality Study**Light Microscopy: 320 X****SEM: 1200 X**

ORE: The ore is a cummingtonite-grunerite (CG), quartz deposit mined for its gold in Lead, S. Dakota (33).

ADDITIONAL MINERAL PARTICLE DATA:

266 Fibers examined with aspect ratio of > 2:1 (air)			
Minimum Width =	0.3 μm	Minimum Length =	0.9 μm
Mean Width =	1.1 μm	Mean Length =	4.6 μm
Maximum Width =	4.8 μm	Maximum Length =	17.5 μm

“Eighty-four percent of the airborne fibers were identified as amphiboles.” “Sixty-nine percent of the amphiboles were characterized as CG, 15% as tremolite-actinolite, with the remaining 16% identified as fibrous hornblende minerals” (33). Note: tremolite-actinolite is reported as an atypical heterogeneous occurrence.



HEALTH STUDIES

Authors: McDonald, J.C., et al (35) Pub. 1978

Cohort: 1,321 men, worked > 21 years (in Co. Veteran's Assoc.)

Vital Status Cut Off: 1973

SMR (respiratory cancer): 103

Conclusion: "There was no convincing evidence of an increase in respiratory cancer." Relative to a high mortality from silicosis - "It is difficult to believe that deaths with so wide a distribution could systematically have blocked the appearance of respiratory cancer."

Authors: Brown, D.P., et al (33) Pub. 1986

Cohort: 3,328 men, > 1 year experience underground work between 1940 and 1965

Vital Status Cut Off: June 1, 1977

SMR (respiratory cancer): 100

Conclusion: "No association as measured by length of employment underground, by dose (total dust x time), or by latency was apparent with lung cancer mortality."

Authors: Steenland, K. et al (67) Pub. 1995

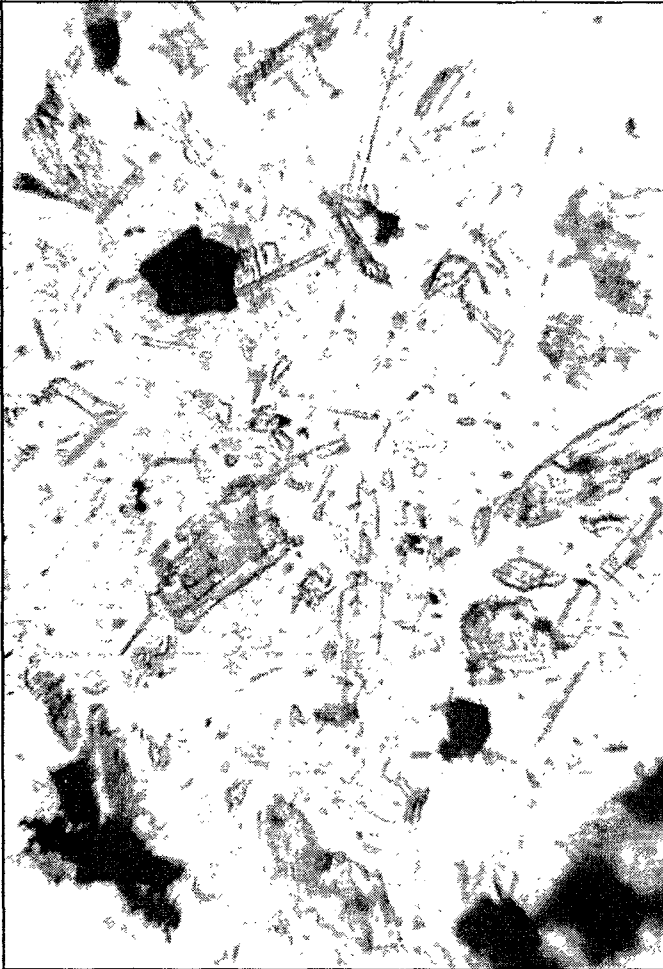
Cohort: 3,328 men, >1 year experience underground between 1940 and 1965

Vital Status Cut Off: Dec. 12, 1990

SMR (respiratory cancer): 115 (CI 94-136)

Conclusion: "Neither exposure to nonasbestiform amphiboles nor silica was likely to be responsible for the observed excess of lung cancer, at least not in a way related to quantitative exposure to dust." "There was only one death from asbestosis in this cohort -- it would therefore appear that the nonasbestiform fibers in this mine did not cause any marked excess of either asbestosis or lung cancer."

OVERALL CONCLUSION: **Nonasbestiform amphibole exposure in this mining operation is not linked to excess lung cancer or mesotheliomas.**

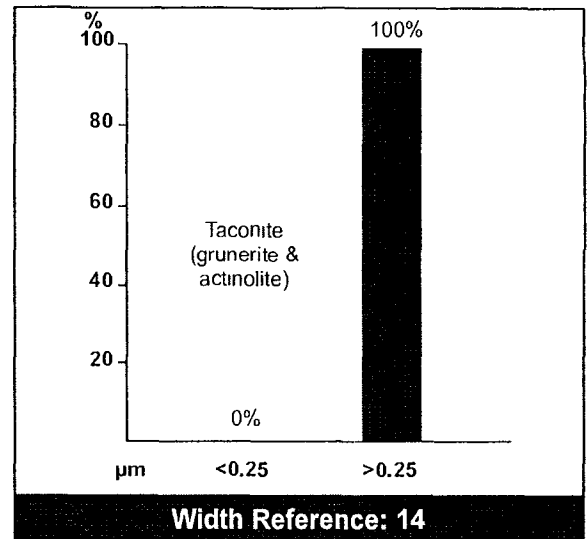
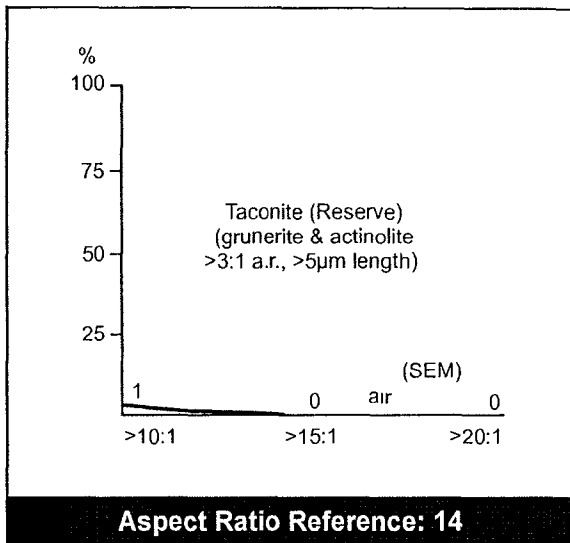
Nonasbestiform Grunerite — Human Mortality Study**Light Microscopy: 320 X****SEM: 1200 X**

ORE: Minnesota taconite contains cummingtonite-grunerite, actinolite and hornblende amphiboles. Trace amounts of riebeckite also occur (36).

ADDITIONAL MINERAL PARTICLE DATA:

464 Fibers characterized with aspect ratio of > 2:1 (air)			
Minimum Width	=	0.25 μm	Minimum Length = 1.0 μm
Mean Width	=	1.2 μm	Mean Length = 5.5 μm
Maximum Width	=	5.0 μm	Maximum Length = 32.4 μm

"Zoltai and Stout (1976) in a report prepared for the Minnesota Pollution Control Agency, concluded that the cleavage fragments of cummingtonite-grunerite found in the Peter Mitchell Pit (Reserve Mining) should not be referred to as asbestiform" (37). "The fibers of taconite are short in length, the vast majority being less than 10 μm " (14).



HEALTH STUDIES

Authors: Higgins, I.T.T., et al (38) Pub. 1983 (Reserve Mining Co.)

Cohort: 5,751 men, worked > 1 year, 1952 to 1976

Vital Status Cut Off: July 1, 1976

SMR (respiratory cancer): 84 (full cohort), 102 (> 15 years latency)

Conclusion: "This study does not suggest any increase in cancer mortality from taconite exposure."

Authors: Cooper, W.C., et al (39) Pub. 1988 (Erie & Minntac Miners)

Cohort: 3,444, worked > 3 months 1947 to January 1, 1959

Vital Status Cut Off: 1983

SMR (respiratory cancer): 61 (full cohort), 57 (> 20 years latency)

Conclusion: "Respiratory tract cancer deaths were 39% fewer than expected (U.S. comparison) and 15% fewer than expected for Minnesota white men. Even when analysis was limited to deaths 20 or more years after first exposure, which provided ample opportunity for the leading edge of any excess in latent tumors to appear, there was no excess."

Authors: Cooper, W. C. et al (68) Pub. 1992 (Erie & Minntac Miners)

Cohort: 3,341 men, worked >3 months 1947 to Jan. 1, 1959

Vital Status Cut Off: Dec. 1988 (update - minimum 30 yr. observation period)

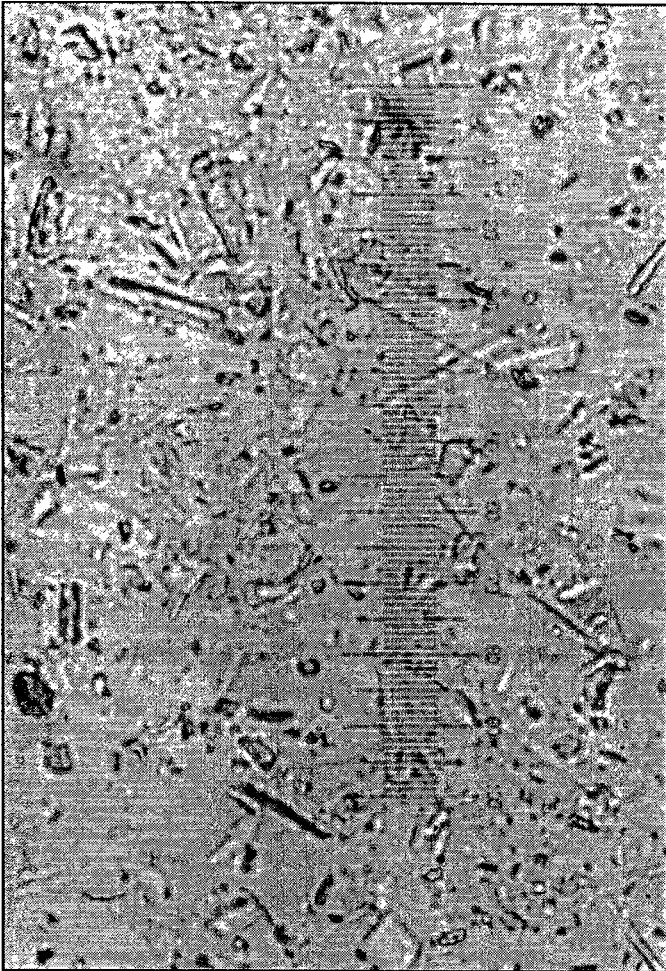
SMR (respiratory cancer): 67 (full cohort)

Conclusion: "no evidence to support any association between exposure to quartz or elongated cleavage fragments of amphibole with lung cancer, nonmalignant respiratory disease or any other specific disease."

OVERALL CONCLUSION: **Nonasbestiform amphibole exposure in this mining operation is not linked to excess lung cancer.**

**Nonasbestiform Tremolite — Human Mortality Studies
and Animal Studies**

Light Microscopy: 320 X

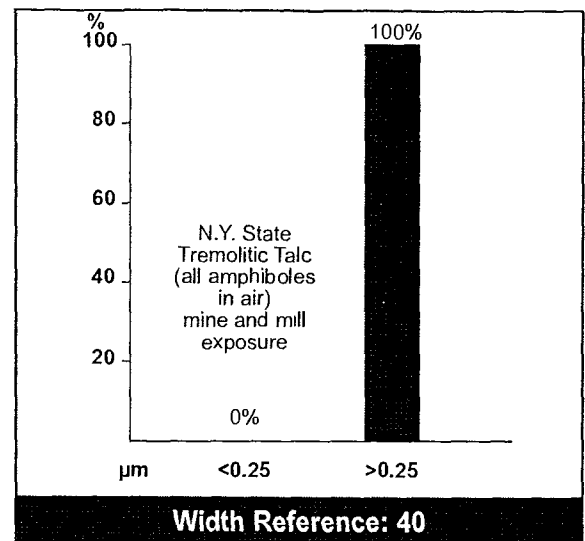
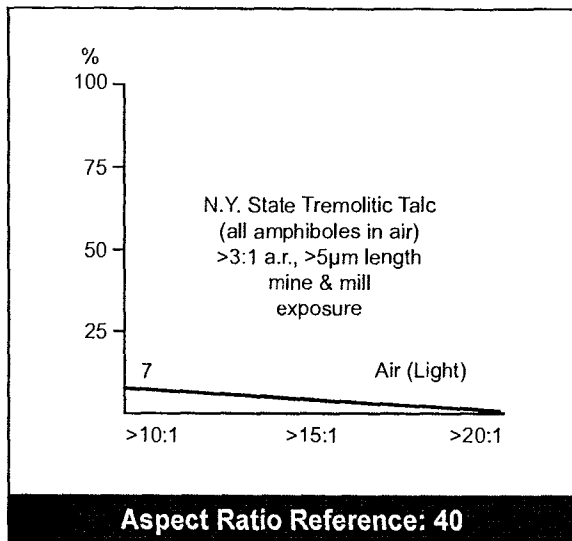


SEM: 1250 X



ORE: As mined and milled at the R. T. Vanderbilt Co., Gouverneur N.Y. mine: mainly talc (20-40%), and tremolite (40-60%) with minor antigorite and anthophyllite. Quartz trace, if detected at all (40).

Also contains minor but observable rod-like mixed talc/amphibole and ribbon-like talc fiber. (69).



ADDITIONAL MINERAL PARTICLE DATA:

R. T. Vanderbilt Mine: NIOSH reported upwards of 70% amphibole asbestos based upon % of all 3:1 aspect ratio or greater particles in air (41). However, the mining company states that **all** of the tremolite and anthophyllite in its talc products appear only in the nonasbestiform habit (42,43). Varying in concentration from one grade to another, fibers of the mineral talc and to a much smaller extent "transitional" particles (talc evolving from anthophyllite) may also be found in this ore deposit. Some of these fibers do exhibit gross morphological characteristics consistent with an asbestiform habit. Such fibers, however, are rare and possess certain physical-chemical properties very different from amphibole asbestos (i.e. harshness, surface properties, etc.). Once fibrous talc is recognized in the analysis, the absence of asbestos in this material is consistently confirmed (40,44-49).

Stanton-Tremolitic Talc Samples 6 and 7: These talcs were positively identified as N.Y. State tremolitic talcs (50), and described as "refined raw materials for commercial products" (27). Sample 6 contained some very elongated particles which are likely to be talc fibers (see discussion above). These fibers did satisfy Stanton's critical dimension range (< 0.25 µm width, > 8 µm length). Sample 7 was reported as containing no particles in this dimensional range but is likely to be another fraction of the same sample.

Smith-Tremolitic Talc FD-14: This sample was supplied by the R. T. Vanderbilt Company and represents a high fiber product grade known as IT-3X (as sold). Analysis reported 50% tremolite, 10% antigorite, 35% talc (of which 25% was fibrous), 2-5% chlorite. Median particle length was 8.5 µm. Diameters (2,000X): < 1 µm = 20%, 1-2 µm = 36%, 2-4 µm = 32%, 4-6 µm = 8%, 6-8 µm = 2%, 10 µm = 2% (51). Tremolite varied considerably in their size lengths, ranging from 1 µm to 40-50 µm. "Talc fiber is abundant in the specimens, occurring as finely fibrous material with high aspect ratio. The talc fibers are also mineral mixtures, structurally talc and a magnesium amphibole. These minerals are also mixtures compositionally. The tremolite contained within the talc occurs as cleavage fragments and is not asbestiform on any level of examination" (45). (Reference includes specific analysis of International Talc-3X product.) In this animal study, this sample was used without comminution or separation.

HEALTH STUDIES (R. T. Vanderbilt Company, Inc.)

Authors: Brown, D.P., Wagoner, J.K., (NIOSH) (41) Pub. 1980

Cohort: 398 men, any work period between 1947-1959

Vital Status Cut Off: 1979

SMR (resp. cancer): 270

Conclusion: "Exposures to asbestiform tremolite and anthophyllite stand out as the prime suspect etiologic factors associated with the observed increase in bronchogenic cancer. . ." No confirmed mesotheliomas.

Critique: Amphibole asbestos is not involved. Excess lung cancer was not reasonably shown to be casually associated with the dust exposure (52-58).

Authors: Stille, W.T., Tabershaw, I.R. (59) Pub. 1982

Cohort: 708 men, any work period between 1947-1977

Vital Status Cut Off: 1978

SMR (resp. cancer): 157

Conclusion: "Elevated mortalities but no significant increases in number of deaths from lung cancer. . ." ". . . workers with exposures in other jobs prior to work at the TMX were found to have excessive mortality from lung cancer. . ."

Critique: Inadequate latency analysis, small cohort and missing data (i.e., smoking) (60).

Authors: Lamm, S.H., et al (61) Pub. 1988

Cohort: 705, worked any time between 1947-1977

Vital Status Cut Off: 1978

SMR (resp. cancer): 220

Conclusion: "This increase in lung cancer mortality. . . has been shown to be concentrated in short term employees (in contrast with nonmalignant respiratory disease). This increase. . . is most likely due to risk acquired elsewhere, such as prior employments, or to differences in smoking experience or other behavioral characteristics." "The risk did not appear to be associated with either the magnitude or the duration of exposure of GTC and was not different from that of workers at talc plants where ores did not contain tremolite or anthophyllite."

Critique: "The findings of these analyses. . . are based on assumptions, small numbers and short latency" (62).

Authors: Brown, D. P. et al (NIOSH) (70) Pub. 1990. Health Hazard Evaluation Report: Update of original NIOSH 1980 study

Cohort: 710, worked any time between 1947-1978

Vital Status Cut Off: 1983

SMR (resp. cancer): 207

Conclusion: "Workplace exposures at GTC are, in part, associated with these excesses in mortality. Possible confounding factors, such as cigarette smoking and other occupational exposures from employment elsewhere, may have contributed to these risks as well."

Critique: "When stratified by smoking, the odds ratios decreased with tenure and the trend analysis were significant. In short, the analysis showed a strong association between lung cancer and cigarette smoking, and there appeared to be an inverse relationship between exposure and the development of lung cancer." (71).

Authors: Gamble, J., et al (71) Pub. 1993

Cohort: Case control applied to above NIOSH Cohort

SMR (resp. cancer): 207

Conclusion: "When stratified by smoking status, risk of lung cancer decreased with talc tenure and remained negative when excluding cases with <20 years latency and short-term workers. These data suggest that non-talc exposures are not confounding risk factors (for lung cancer) while smoking is, and that temporal and exposure-response relationships are consistent with a smoking etiology but not an occupational etiology for lung cancer."

Critique: No dust data and disagreement over whether the elevated smoking rates would or would not account for all the excess.

Authors: Honda, Y. et al (73) Pub. 2002

Cohort: 818 men, worked any time between 1947-1998 (Retrospective Mortality study update with exposure estimation study)

Vital Status Cut Off: January 1, 1990

SMR (resp. cancer): 254

Conclusion: "The results of this study are similar to those of earlier investigations. The cohort giving rise to the lung cancer was seen among subjects unexposed to GTC talc. These features suggest that some of the apparent increase is due to exposure to tobacco smoke. Mill workers and mine workers had similar estimated cumulative dust exposures, yet the excess of lung cancer was considerably stronger among miners than among millers. This indicates that GTC talc dust, per se, did not produce the excess. Most important, the presence of an inverse relationship between estimated cumulative exposure and lung cancer is inconsistent with the hypothesis that GTC talc dust is a carcinogen. The results of experimental animal studies also do not provide any support for this hypothesis."

ANIMAL STUDIES

Authors: Stanton, M.F., et al (27) Pub. 1981

Test Animals: 20-week-old outbred female Osborne-Mendel rats

Test Type: Pleural implantation

Protocol: A standard 40 mg dose of each sample was uniformly dispersed in hardened gelatin and applied by open thoracotomy directly to the left pleural surface. The animals (30-90 for each experiment) were followed for 2 years, at which time all surviving animals were sacrificed and the tissues examined for pleural sarcomas.

Findings: Exposure to these tremolitic talc samples resulted in no incidence of tumors. Similarly tested tremolite asbestos reflected a high tumor rate (see Exposure Exhibit G).

Authors: Smith, W. E., et al (25) Pub. 1979

Test Animals: Male LUG:LAK hamsters, injected at 2 months of age

Test Type: Intrapleural injection

Protocol: Single intrapleural injection of two dosages (10 and 25 mg). The sample was suspended in saline and sterilized by autoclave. The occurrence of tumors (unspecified) was noted at necropsies for a starting group of 50 animals per dose. After short term sacrifice of some animals and the loss of others through acute enteritis, the occurrence of tumors was noted in nonsurvivors up to 600 days.

Findings: No tumor development was noted. In contrast, tremolite asbestos similarly tested did produce tumors (see Exposure Exhibit F).

CELL STUDIES

Authors: Wylie, A. G., et al (72) Pub. 1997

Study: In vivo cytotoxicity and proliferative potential in HTE & RPM cells contrasting asbestos fibers to similar dose talc and transitional fibers (concentrate) from RTV talc.

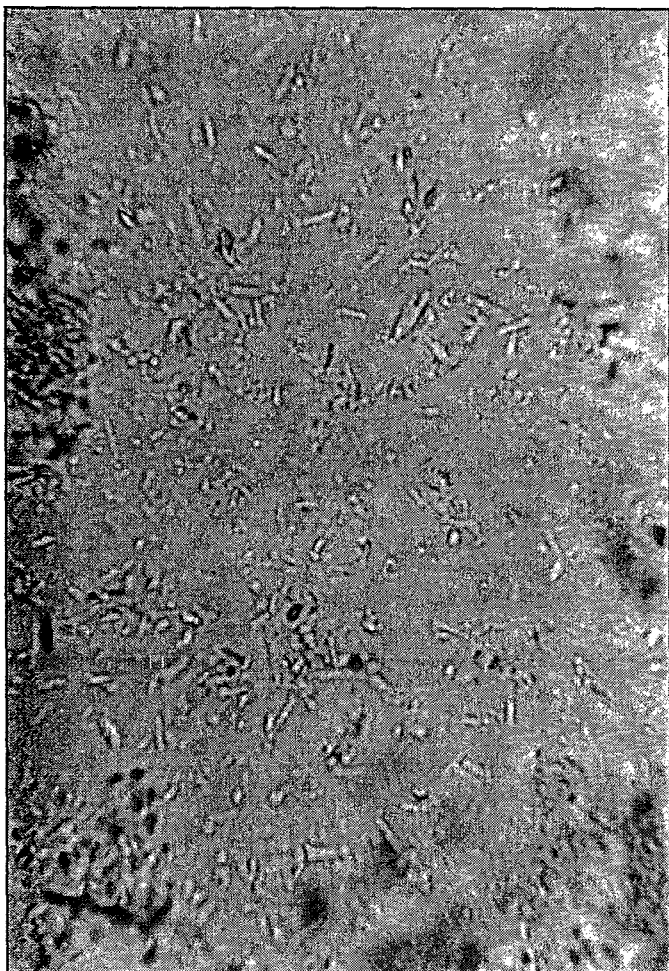
Conclusion: "Our experiments also show that fibrous talc does not cause proliferation of HTE cells or cytotoxicity equivalent to asbestos in either cell type despite the fact that talc samples contain durable mineral fibers with dimensions similar to asbestos. These results are consistent with the findings of Stanton, et al (1981) who found no significant increases in pleural sarcomas in rats after implantation of materials containing fibrous talc."

OVERALL CONCLUSION: **Human Studies - A definite link between nonasbestiform tremolite and respiratory cancer in the R. T. Vanderbilt Company talc mining population has not been demonstrated.**

Animal Studies - N. Y. State tremolitic talc containing a high nonasbestiform tremolite content produced no carcinogenic response in rats or hamsters.

Nonasbestiform Tremolite — Animal Studies

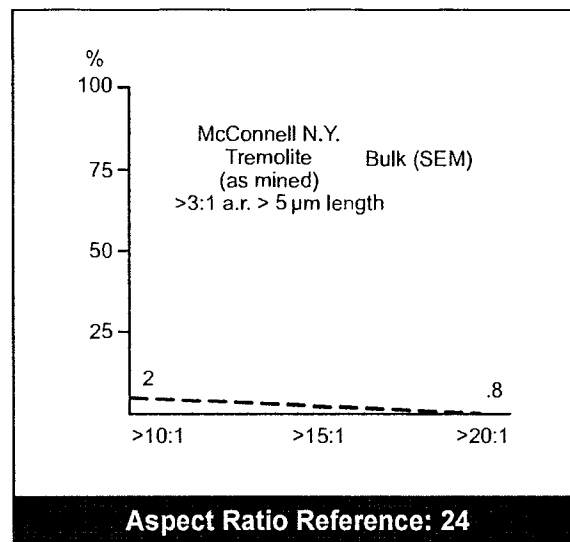
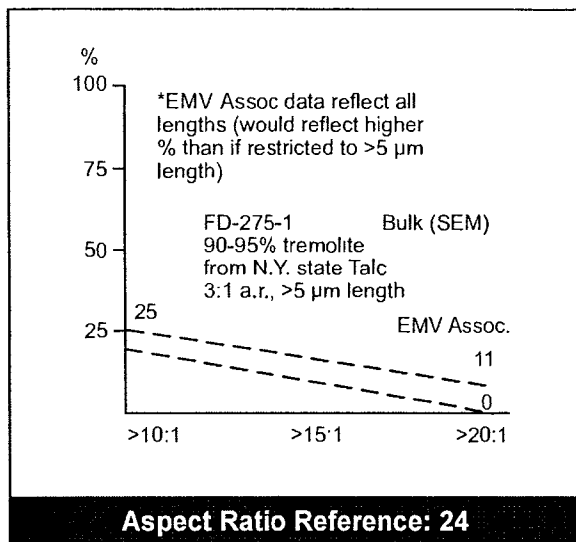
Light Microscopy: 320 X



SEM: 1250 X



SAMPLE: Both FD-275-1 and 275 originated from N.Y. State tremolitic talc ore. Both samples represent tremolite concentrates from this ore.



ADDITIONAL MINERAL PARTICLE DATA:

Tremolite 275 was selected from N.Y. tremolitic talc ore from an area rich in tremolite. This ore was provided to the Bureau of Mines (BOM) for mineral and elemental particle size characterization as well as use in an animal feeding study by Dr. E. McConnell (sample contained approximately 70% tremolite with the remainder talc and antigorite). Also, an aliquot of this sample was further processed to obtain a higher tremolite concentrate for use in another animal study by Dr. William Smith (approximately 95% tremolite).

The processing of FD-275-1 involved crushing, milling, separation via sedimentation and filtering to obtain only the respirable fraction. Particle size characterization of FD-275-1 was undertaken by Dr. Smith (via EMV Assoc. Inc.), and by the BOM.

For FD-275-1, no particles with a width < 1 µm and length of > 10 µm were observed (200 particles via SEM). For FD-275 (McConnell tremolite), a mean width of 3.4 µm for particles > 6 µm in length was recorded (for amosite similarly sized mean width = 0.4 µm).

ANIMAL STUDIES

Authors: Smith, W.E., et al (25) Pub. 1979

Test Animals: Male LUG:LAK Hamsters

Test Type: Intrapleural injection

Protocol: Single intrapleural injection of two dosages (10 and 25 mg). The occurrence of tumors (unspecified) was noted at necropsies for a starting group of 50 animals per dose. After short term sacrifice of some animals and the loss of others through acute enteritis, the occurrence of tumors was noted in nonsurvivors up to 600 days.

Findings: No tumor development was noted. In contrast, tremolite asbestos similarly tested did produce tumors (see Exposure Exhibit F).

Authors: McConnell, E.E., et al (64) Pub. 1983

Test Animals: Male and female Fischer 344 rats

Test Type: Ingestion

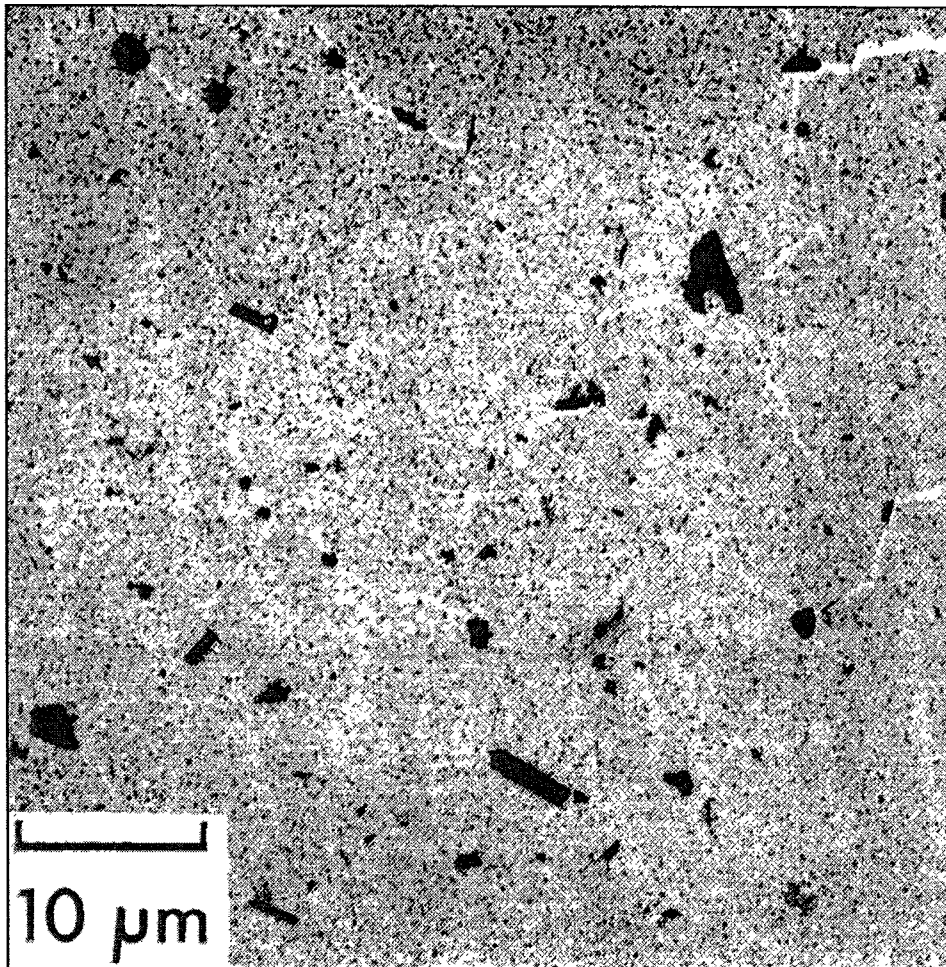
Protocol: Nonasbestiform tremolite and amosite were administered alone and in combination at a concentration of 1% in the daily diet of rats. Rats were sacrificed when exhibiting specified symptoms, or when less than 10% of the test group survived. Group size varied from 100 to 250 animals.

Findings: No toxic or neoplastic lesions were observed in the target organs - gastrointestinal tract, or mesothelioma for either the tremolite or the amosite.

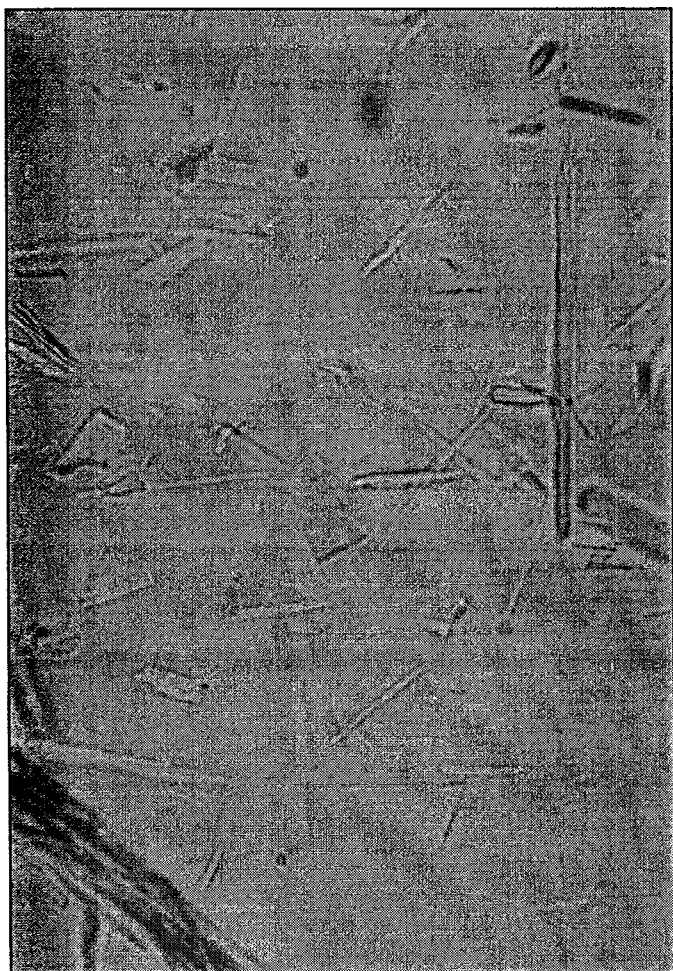
OVERALL CONCLUSION:

A concentrate of N.Y. State tremolite nonasbestiform produced no pleural tumors in hamsters and no gastrointestinal tract neoplastic lesions in rats.

Nonasbestiform Tremolite — Animal Study

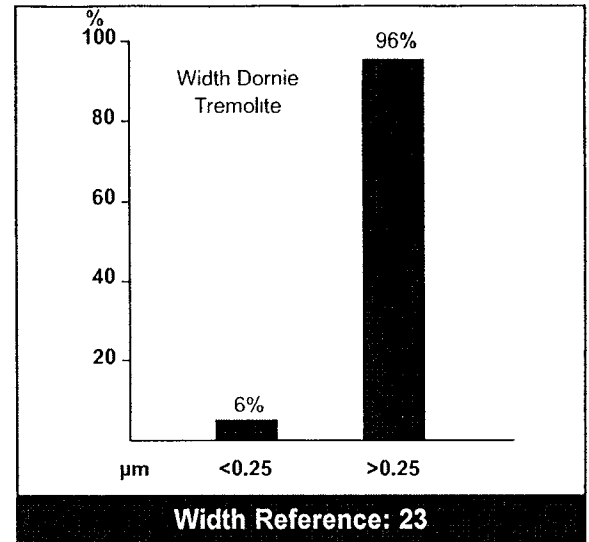
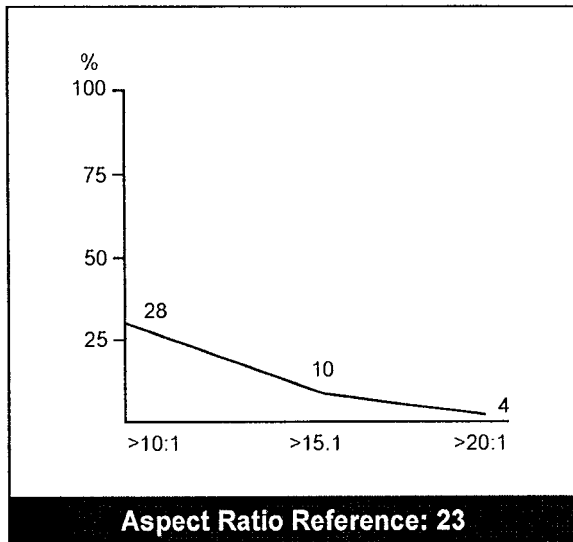


SAMPLE: Prepared from a rock specimen from Greenland. Referenced as tremolite "B" (22).

Nonasbestiform Tremolite — Animal Study**Light Microscopy: 320 X****SEM: 190 X**

SAMPLE: Like the tremolite from Italy (see exhibit J), this sample “contains mostly cleavage fragments, but some very long, thin fibers were also observed.” There are more fibers longer than 8 μm in this sample than in the Italian sample, but most were $>1 \mu\text{m}$ in diameter. A small amphibole asbestiform subpopulation may also exist in this sample as it does in the Italian sample (though this is less clear). “The material contains several populations of varying habits of a member of the tremolite-actinolite solid solution series. (65). Both this sample and the Italian sample are not typical of tremolite nonasbestiform cleavage fragment populations. Both exhibit the presence of byssolite in the samples.

Minerals were characterized and verified as a tremolite by x-ray diffractometry, optical microscopy, scanning electron microscopy and energy dispersive x-ray spectroscopy.



ANIMAL STUDIES

Authors: Davis, J.M.G., Addison, J. (20) Pub. 1991

Test Animals: AF/Han strain rats

Test Type: Peritoneal injection

Protocol: Fractions of this sample were obtained by generating an airborne dust cloud in an experimental chamber (Timbrell dust dispensers) with fine fractions collected using a vertical elutriator. A single 10 mg dose was injected into the peritoneal cavities of the animals. All animals lived out of their full life span or were killed when moribund.

Findings: 4 mesothelioma deaths out of 33 animals were observed with no median survival time published (too few tumors for median survival times to be calculated). It is important to note - as stated in the study - "The intraperitoneal injection test is extremely sensitive, and it is usually considered that, with a 10 mg dose, any dust that produced tumors in fewer than 10% of the experimental group is unlikely to show evidence of carcinogenicity following administration by the more natural route of inhalation - the material from Dornie is probably to be considered harmless to human beings."

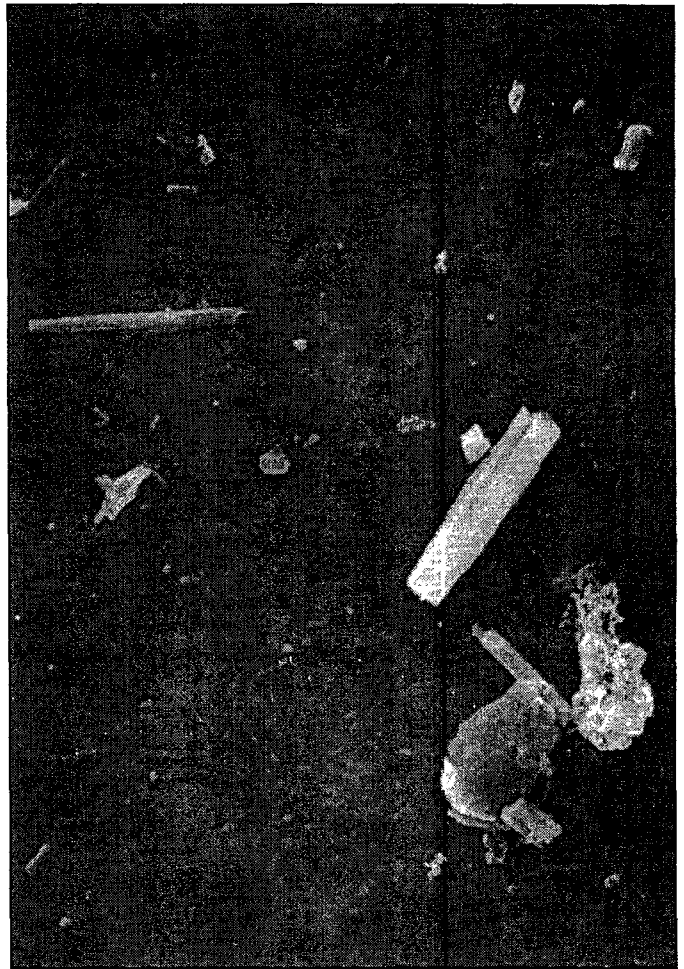
OVERALL CONCLUSION: This predominantly nonasbestiform tremolite produced no significant carcinogenic response in the test animals and is likely harmless to humans.

Nonasbestiform Tremolite — Animal Study

Light Microscopy: 45 X

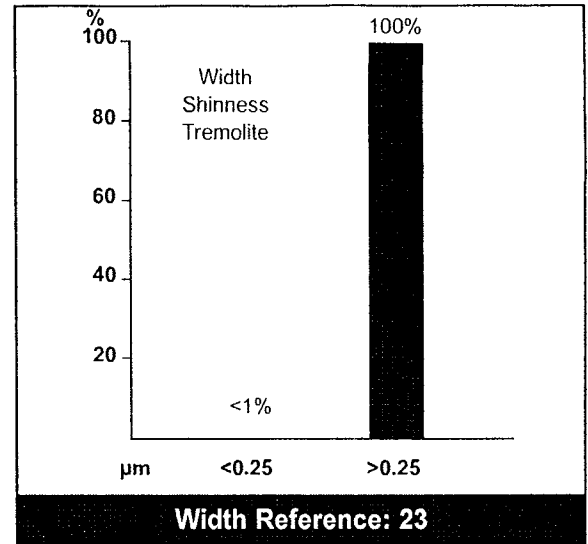
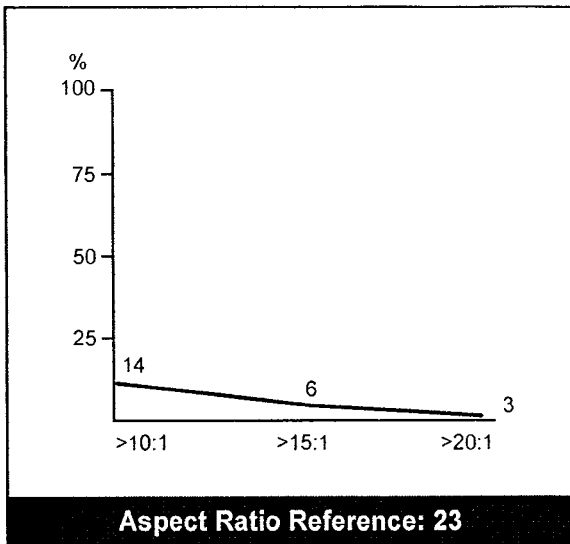


SEM: 1800 X



SAMPLE: "The Shinness tremolite dust was almost exclusively composed of cleavage fragments, only a small portion of which had an aspect ratio greater than 3:1."

Minerals were characterized and verified as tremolite by x-ray diffractometry, optical microscopy, scanning electron microscopy and energy dispersive x-ray spectroscopy.



ADDITIONAL MINERAL PARTICLE DATA:

"In the optical microscopy and SEM examinations, the asbestos tremolites were found to be typical of that form in displaying polyfilamentous fiber bundles, curved fibers, fibers with splayed ends, and long, thin, parallel-sided fibers. Most of the fibers showed straight extinction when observed with polarized light under crossed polarizers, indicating the presence of multiple twinning of the crystals." "Samples did contain some elongated fragments of tremolite with oblique extinction, stepped ends, and nonparallel sides indicating that they were cleavage fragments." (20)

ANIMAL STUDIES

Authors: Davis, J.M.G., Addison, J. (20) Pub. 1991

Test Animals: AF/Han strain rats

Test Type: Peritoneal injection

Protocol: Fractions of this sample were obtained by generating an airborne dust cloud in an experimental chamber (Timbrell dust dispensers) with fine fractions collected using a vertical elutriator. A single 10 mg dose was injected into the peritoneal cavities of the animals. All animals lived out of their full life span or were killed when moribund.

Findings: 2 mesothelioma deaths out of 36 animals were observed (well below background for test method). There were too few tumors for median survival times to be calculated. Authors state: "Human exposure to a material such as that obtained from Shinness Scotland, whether as a pure mineral dust or as a contaminant of other products, will almost certainly produce no hazard."

OVERALL CONCLUSION: This nonasbestiform tremolite produced no carcinogenic response in the test animals.

Nonasbestiform Actinolite - Animal Study

No photograph available.

SAMPLE: Origin of sample unknown.

DIMENSIONAL DATA: Not provided by author.

ANIMAL STUDIES:

Authors: Pott, F. et al (66) Pub. 1974

Test Animals: Wistar rats

Test Type: Peritoneum injection.

Protocol: Assorted fibrous dust (chrysotile, anthophyllite asbestos, actinolite asbestos, wollastonite, glass fibers, gypsum, etc.) and granular dust (nonasbestiform actinolite, biotite, talc, etc.) were intraperitoneally injected (up to 12.5 mg/ml) into varying test groups of 40 rats at various dosages.

Findings: The "fibrous" dusts (with some exceptions such as gypsum, slag wool, and wollastonite), induced varying tumor development while the granular dusts reflected little to no tumors (nonasbestiform actinolite - no tumors). "Very low doses between 0.05 and 0.5 mg asbestos led to tumor incidences of about 20% to 80%."

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SUMMARY

MINERAL HABIT AND CARCINOGENICITY

**CLEAR AMPHIBOLE
ASBESTOS
EXPOSURES**
(amphibole asbestos)

Libby Vermiculite (H)
Greek Tremolite (H)
Smith FD-72 (A)
Stanton Tremolite #1 (A)
Stanton Tremolite #2 (A)
Wagner Korean Tremolite (A)
Davis Korean Tremolite (A)
Addison/Davis Jamestown Tremolite (A)
Addison/Davis Korean Tremolite (A)
Addison/Davis Swansea Tremolite (A)

**PREDOMINANTLY
ASBESTIFORM
AND/OR
HIGHLY FIBROUS**

Cook/Coffin-Ferroactinolite (asbestiform) (A)
Smith FD-31 (unique Tremolite/Byssolite) (A)
Addison/Davis Italian Tremolite (highly fibrous
with asbestos subpopulation) (A)

**COMMON
NONASBESTIFORM
AMPHIBOLE
EXPOSURES**

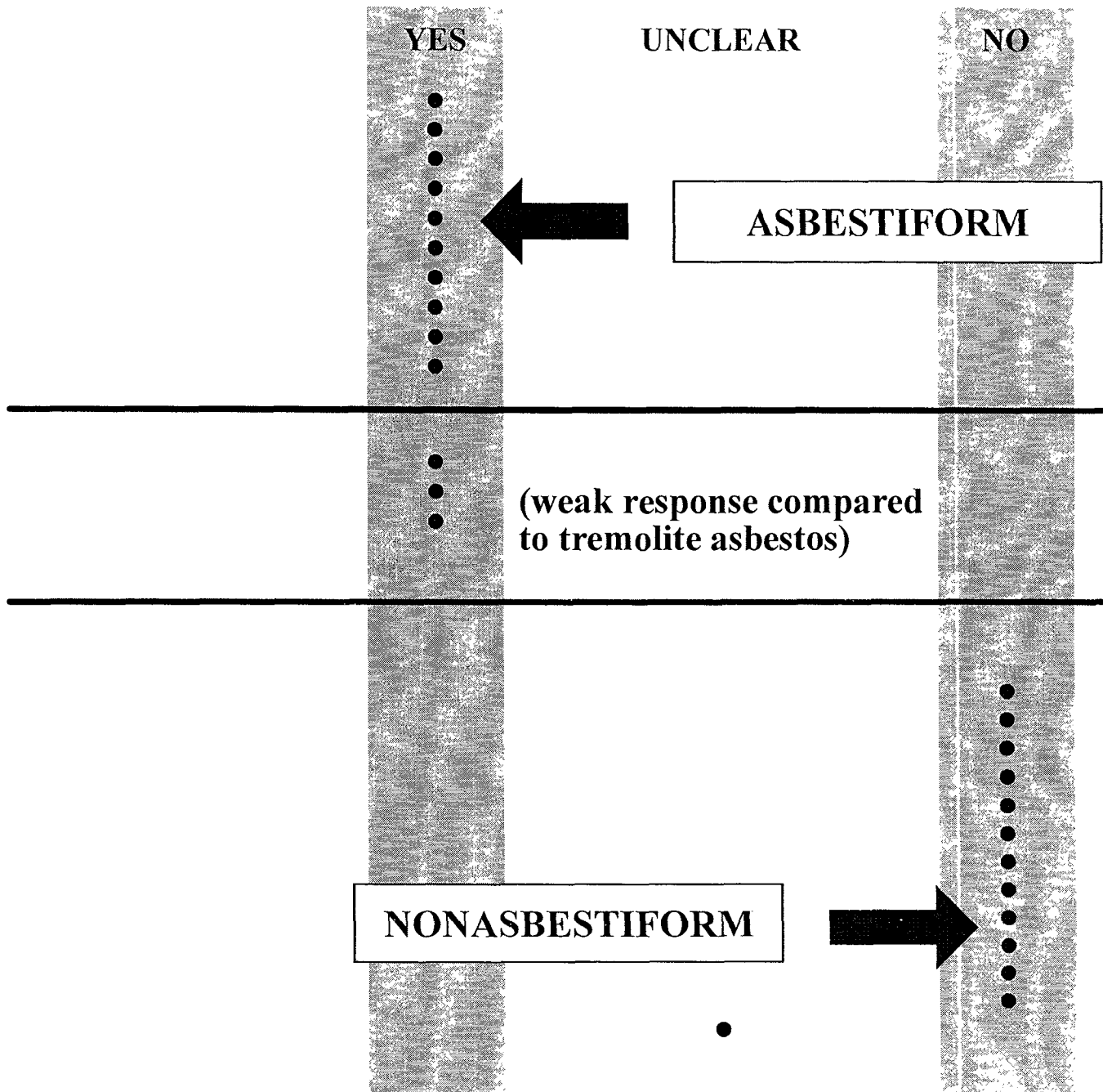
Homestake (C-G) (H)
Mesabi Range-Taconite (C-G, trace Actinolite) (H)
Smith FD-14 (Tremolitic Talc) (A)
Smith FD-275 (conc. Tremolite) (A)
McConnell Tremolite (conc. Tremolite) (A)
Stanton Talc #6 (Tremolitic Talc) (A)
Stanton Talc #7 (Tremolitic Talc) (A)
Pott-Granular Actinolite (A)
Wagner California Tremolite (A)
Wagner Greenland Tremolite (A)
Addison/Davis Dornie Tremolite (A)
Addison/Davis Shinness Tremolite (A)
N.Y. State Tremolitic Talc (neg. for animals) (H)

(H) = Human Studies

(A) = Animal Studies

C-G = Cummingtonite-grunerite

CARCINOGENIC RESPONSE



CONCLUSION

Difference Exists Mineralogically

AND

Biologically

In 1992, after many years of scientific review, the Occupational Safety and Health Administration (OSHA) specifically excluded elongated nonasbestiform cleavage fragments from the scope of their asbestos standard. OSHA's decision to recognize the key mineralogic and biologic distinctions reviewed in this pictorial presentation was instrumental in that decision.

Because this matter involves scientific issues ranging from geology, mineralogy and health, the authors believe it is important that these complex relationships be explained as simply as possible. This matter remains a source of confusion to many and the consequences of misunderstanding can be immense.

Sustaining confusion is an unfortunate array of overly broad asbestos analytical protocols and definitions now being applied in mixed dust environments. To address analytical ambiguities, appendix II is provided.

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Analytical Issues

INTRODUCTION:

As shown in this pictorial presentation, the properties of asbestos are unique. These properties include very long, thin, fibrillar fiber bundles that are flexible and strong. The ability of excessive exposure to asbestos to cause serious pulmonary disease has been extensively studied and documented.

Analytical procedures designed to identify and quantify asbestos must incorporate the unique characteristics of asbestos as fully as possible if the method is to be as specific to asbestos as possible. Minimizing mischaracterization (false positives and negatives) defines the value of any analytical protocol and is a key element to meaningful measurement of risk.

The most common analytical approach used for airborne asbestos fiber quantification is phase contrast microscopy (PCM). PCM methods typically measure airborne elongated particulate with a length to width ratio of at least 3 to 1 and a length 5 μm or greater (e.g. NIOSH 7400). Since there is little reason to measure airborne elongated particulates other than for asbestos, this relatively cheap, simple to apply method, is most often used to collect and count asbestos fibers. Although PCM will count all asbestos fibers observable under light microscopy (400X), it unfortunately also counts elongated nonasbestiform cleavage fragments, insect legs and any other elongated particulate collected on the air monitoring filter that meet the simple dimensional counting criteria. Consequently, the simple PCM method works well in an environment where commercial asbestos is known to be the predominate elongated particle in the air being sampled. In mixed dust environments, however, the PCM method must be enhanced to measure asbestos from the other particulate in the sample more selectively.

Fiber counting criteria employed in microscopy methods are often mistakenly viewed as the definition of an asbestos fiber. The fiber counting criteria employed in most PCM methods are, in fact, merely arbitrary parameters used to promote consistency in fiber counting. The 5 μm minimum length, and the 3:1 minimum aspect ratio criteria, originated in England's asbestos textile mills as a means to improve reproducibility of commercial asbestos fiber measurements. These counting parameters were **not** deemed to be the dimensions that corresponded to a specific health risk (Holmes, 1965).

The PCM method is unable to detect fibers below approximately 0.2 μm in width and has always been viewed as an **index of exposure** versus an absolute measure of all fibers present in a sample. It is also unable to characterize the mineral composition or crystal structure of the particles examined. Again, in an environment where it is known that the primary elongated particle present is commercial asbestos, these limitations become less important. In environments where there are mixed dusts and where asbestos may or may not be present, the PCM method, with its simple counting criteria, becomes wholly inadequate.

This inadequacy is clearly demonstrated in the 1986 OSHA asbestos standard preamble discussion of its quantitative risk analysis and its decision to exclude studies of Canadian asbestos miners. The asbestos miners were excluded because the fiber count dose-response relationship observed differed significantly from the fiber count dose-response observed for other asbestos exposed populations under review by OSHA.

OSHA found that the miners had been exposed to similar or higher "fiber" concentrations than textile or other commercial asbestos exposed populations but showed significantly less adverse health effects. The asbestos "fiber" exposure was based solely on 3 to 1 aspect ratio or greater, 5 µm or longer, light microscopy fiber counts.

In Canadian asbestos mines, asbestos often represents no more than 5% of the ore being mined with the remaining host rock predominantly being the nonasbestiform serpentine mineral, antigorite. The apparent "asbestos" fiber count in this mixed mineral dust environment therefore included antigorite cleavage fragments as well as chrysotile fibers. Inclusion in the fiber count of elongated nonasbestiform fragments which have never been shown to produce asbestos-like disease, significantly inflated the asbestos dose reported without a corresponding increase in response.

Had nonasbestiform cleavage fragments been properly identified and excluded from the asbestos fiber count, the asbestos risk observed for the Canadian asbestos miners may well have been comparable to that observed among the commercial asbestos exposed groups that were used in the OSHA risk analysis. In this example, analytical methods that failed to address what is and is not asbestos clearly impacted risk assessment (Wylie and Bailey, 1992).

Sub-light microscopic methods such as transmission electron microscopy (TEM) and scanning electron microscopy (SEM) present another analytical confounder when improperly applied. In contrast to the limitations of PCM, electron microscopic analytical methods such as TEM are capable of detecting asbestos fibers well below the resolution limit of the light microscope, identifying mineral type and can address crystal growth distinctions important to proper asbestos identification.

Despite the elevated costs associated with electron microscopic analyses, the desire to identify and quantify lower and lower asbestos levels in building materials and in asbestos abatement projects has contributed significantly to the proliferation of TEM laboratories across the country. These types of samples are typically limited to chrysotile, undergo highly prescriptive analytical protocols and require little to no mineralogical expertise in the analysis. For all its sophistication and sensitivity, electron microscopy presents a different set of analytical variables that will affect risk assessments when its results are improperly interpreted or improperly compared to health exposure standards.

The health literature on asbestos exposed populations overwhelmingly involves exposure to commercial asbestos. Asbestos exposure levels reported in epidemiological studies used to establish exposure limits have been obtained through light microscopy methods. Permissible exposure standards for airborne asbestos are based upon this light microscopy ***index of exposure***. Efforts to use electron microscopic analytical data for risk assessment purposes must include a means to correlate results to what would be observable under light microscopy.

Unfortunately, the difference between asbestos fibers observed under the light microscope and asbestos fibers observed by electron microscopy is highly variable. This variability is influenced by asbestos type, how the fibers become airborne and the nature of fiber bundle separation in each exposure setting. "One size fits all" correlations are difficult (if not impossible) to reliably establish. Electron microscopy views only a very tiny fraction of the sample being studied and is therefore a poor quantification tool. Unless coupled with other investigation techniques, electron microscopy does not adequately address populations of particles in a sample. In an unknown or mixed dust environment, this is an important indicator of the asbestiform or nonasbestiform nature of a given exposure.

Electron microscopy methods are unquestionably the best analytical tool for asbestos identification, but not for quantification unless coupled with other methodologies. The health significance of asbestos fibers observed only through electron microscopy and not correlated to PCM-observable exposure levels, is unknown at this time. The authors are not aware of any studies of asbestos-related disease where the asbestos exposure was not readily observable under light microscopy.

SOLUTIONS:

While the strengths and weaknesses of every asbestos analytical approach has not been addressed, most analysts would agree that there is no perfect, single asbestos analytical methodology. Certainly each approach is made more reliable in the hands of experienced, knowledgeable analysts. Effectively combining different analytical tools in a tiered approach can overcome individual method weaknesses, control costs and yield highly reliable results.

The following analytical guides reflect asbestos analytical approaches considered most reliable for asbestos identification and quantification. In each case, the unique characteristics of asbestos fibers and asbestos fiber populations are used to the fullest extent possible.

In the case of PCM, for example, dimensional fiber counting criteria that are more specific to asbestos are recommended as a more sensitive screening technique if standard PCM counts exceed established asbestos fiber permissible exposure limits. This additional PCM step significantly improves PCM as an inexpensive, easy to apply asbestos screening tool and assists the investigator in deciding if more specific, more costly analysis is warranted.

A polarized light microscopy method for bulk analysis is also provided. This method is designed with more guidance into what is and is not asbestos and, in the hands of a skilled analyst with mineral expertise, can be more informative than electron microscopic analysis.

The effective utilization of any asbestos analytical methodology, used singularly or in combination with others, does require a clear understanding of what asbestos is and what it is not. Methodologies that do not or can not recognize these distinctions should not be used.

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Differential PCM Fiber Counting Methodology for Air Samples

BACKGROUND:

In environments where the presence of asbestos is unknown or may be present as a mixed dust, the NIOSH 7400 PCM membrane analytical method must be supplemented with differential counting criteria to assist in determining what proportion of the dust is asbestiform and what part is not. This need for differential counting was recognized by the Occupational Safety and Health Administration (OSHA) in its final asbestos standard published in 1994 (Fed Reg. Vol. 59, No. 153, pp. 41073 - 41079 - Aug. 1994).

There is also concern among some researchers that abandonment of the traditional fiber counting criteria (fibers with a minimum length of 5 μm and a length to width aspect ratio of at least three to one) would forsake the historical database that has been created over many decades. The simplistic counting criteria alone, derived from an effort to improve analytical consistency in commercial asbestos textile exposure samples in the 1960s, is totally inappropriate for noncommercial asbestos exposure environments. Recognizing the fundamental morphological differences between asbestiform and nonasbestiform particle populations, the method must address those differences.

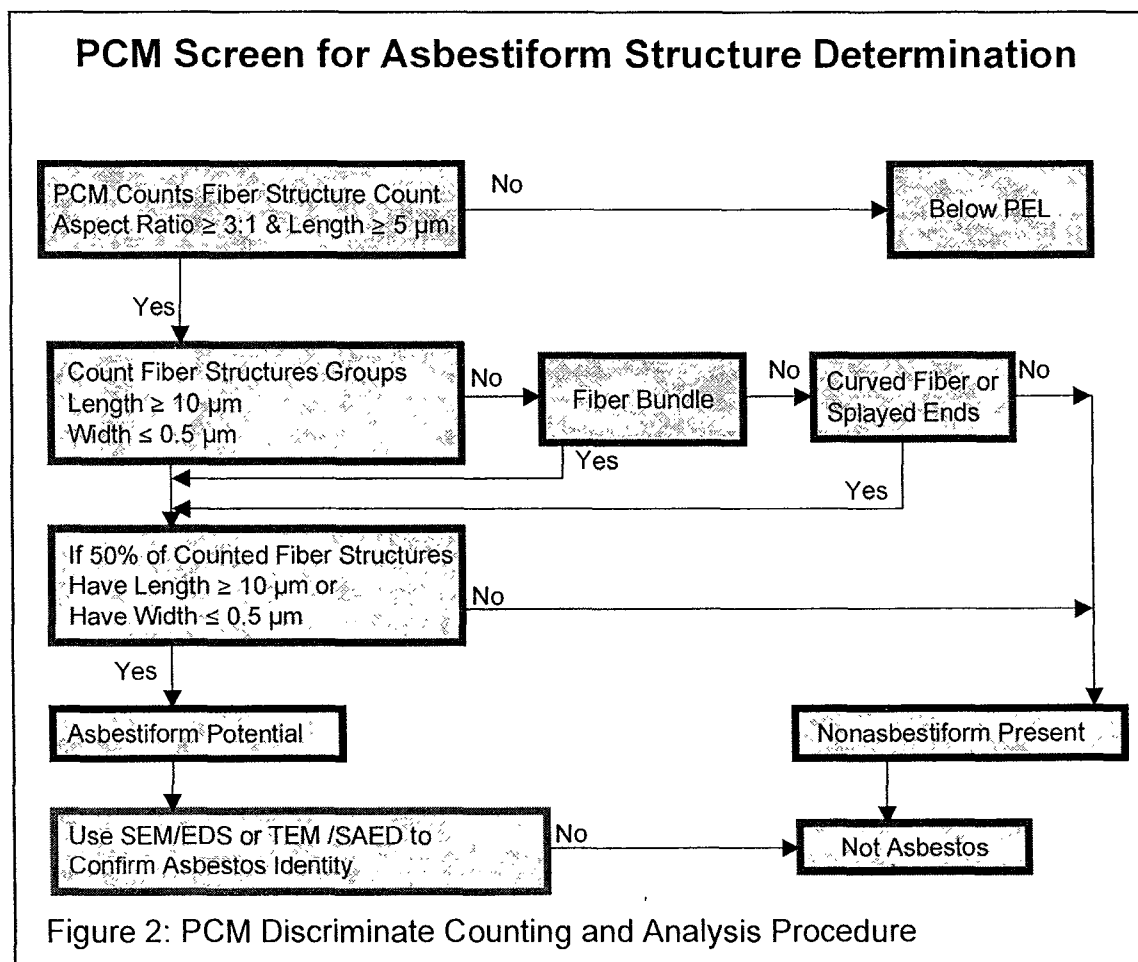
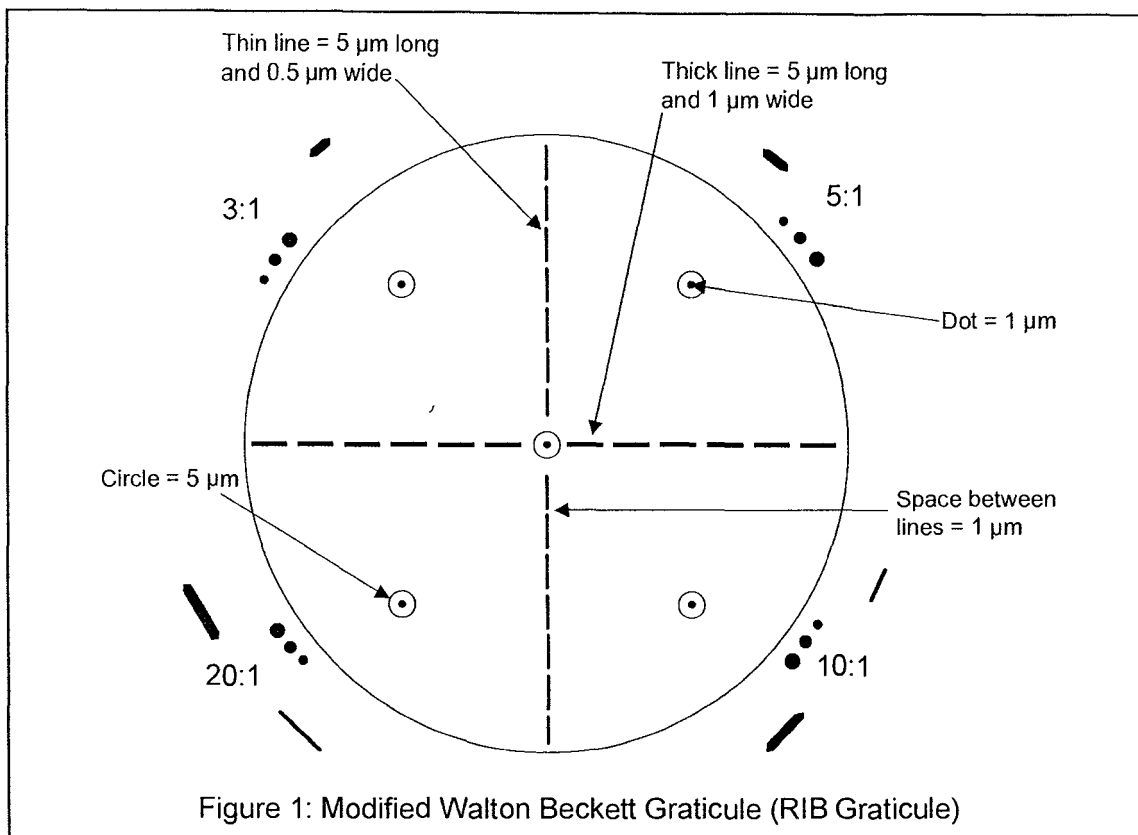
METHOD SUMMARY:

To satisfy historical preservation of exposure trends, the NIOSH 7400 method must be performed. Where the fiber count reaches or exceeds 0.1 fiber/cc (or the current exposure limit), supplemental measurements that allow a better characterization of the asbestiform nature of the sample must be done. These measurements will necessitate the use of a modified Walton Beckett graticule that assists in the measurement of those 3:1 or greater aspect ratio and 5 μm and longer particles that are equal to and longer than 10 μm and less than or equal to 0.5 μm in width. All fiber bundles need to be counted. This modified graticule is shown in Figure 1.

If the population of fibers has 50 % equal to or longer than 10 μm or if 50% of the fibers are equal to or less than 0.5 μm in width (unless a bundle), then the exposure can be considered to be asbestiform.

Samples that reflect an asbestiform nature must have PCM observable fibers (widths between 0.15 and 0.5 μm or bundles) analyzed by electron microscopy. Analysis by electron microscopy will evaluate morphology, chemistry and crystal structure if using TEM. The percentage PCM fibers that are regulated asbestiform fibers is then calculated and compared to the permissible exposure limit. The procedure is shown diagrammatically in Figure 2.

Mineralogical expertise is needed for those samples requiring electron microscopy and the standards for classifying amphibole minerals must conform to the International Mineralogical Association recommendations (Leake, B.E., Nomenclature of Amphiboles. American Mineralogist. Vol. 82, 1019 - 1037, 1997).



Standard Method of Testing for Asbestos Containing Materials by Polarized Light Microscopy

1. SCOPE

- 1.1 The method describes the procedures for the determination of the presence or absence of six types of asbestos: chrysotile-asbestos, grunerite-asbestos (amosite), crocidolite (riebeckite-asbestos), anthophyllite-asbestos, tremolite-asbestos and actinolite-asbestos and for the determination of a quantitative estimate of the percent of asbestos. This method may be applied to bulk materials other than building materials, but the accuracy of the method under these circumstances is not characterized. For non-building materials, there may be more interference with a greater possibility for false positives or fibers may be dispersed below the resolution of the light microscope, yielding a higher possibility of false negatives. When the content of asbestos in a sample is close to the 1% level, other more precise methods of quantification may be necessary if it is important to determine whether or not asbestos content is more or less than 1% by weight. This distinction may be important because the EPA defines asbestos-containing materials as those materials containing greater than 1% asbestos (Ref. 2 and 3).

2. APPLICABLE DOCUMENTS

- 2.1 U.S. Environmental Protection Agency, "Interim Method for the Determination of Asbestos in Bulk Insulation Samples," EPA 600/M4-82-020, Dec. 1982.
- 2.2 U.S. Environmental Protection Agency, "Guidance for Controlling Asbestos-Containing Materials in Buildings," EPA 560/5-85-024, 1985.
- 2.3 U.S. Environmental Protection Agency, "Asbestos-Containing Materials in School Buildings: Guidance for Asbestos-Analytical Programs," EPA 560/13-80-017A, 1980 (under revision).
- 2.4 ASTM STD 834, Definitions for Asbestos and Other Health-related Silicates, B. Levadie, ed., ASTM, 1916 Race Street, Philadelphia, PA 19103, 1984.

3. TERMINOLOGY

- 3.1 Asbestos: A commercial term applied to a group of highly fibrous silicate minerals that readily separate into long, thin, strong fibers of sufficient flexibility to be woven, are heat resistant and chemically inert, and possess a electric insulation properties, and therefore, are suitable for uses (as in yarn, cloth, paper, paint, brake linings, tiles, insulation, cement, fillers, and filters) where incombustible, nonconducting, or chemically resistant material is required. Federal regulation of asbestos is restricted to chrysotile-asbestos, grunerite-asbestos (amosite), crocidolite (riebeckite-asbestos), anthophyllite-asbestos, tremolite-asbestos and actinolite-asbestos.

- 3.2 Asbestiform: said of a mineral that is like asbestos, i.e., crystallizes with the habit of asbestos. Some asbestiform minerals may lack the properties which make asbestos commercially valuable such as long fiber length and high tensile strength. All asbestos exhibits a fibrillar structure, i.e., parallel growth of fibrils in bundles. Under the light microscope, the asbestiform habit is generally recognized by the following characteristics:
- 3.2.1. mean aspect ratios ranging from 20:1 to 100:1 or higher for fibers longer than 5 μm .
 - 3.2.2. very thin fibrils, usually less than 0.5 μm in width, and
 - 3.2.3. two or more of the following:
 - a. parallel fibers occurring in bundles
 - b. fiber bundles displaying splayed ends
 - c. matted masses of individual fibers, and
 - d. fibers showing curvature
- 3.3 Fiber: an elongated single crystal or similarly elongated polycrystalline aggregate.
- 3.4 Fibril: the smallest unit fiber in a bundle of fibers characteristic of the asbestiform habit.

4. SUMMARY OF THE METHOD

- 4.1 Bulk samples of building materials taken for asbestos identification are first examined with a low-power binocular microscope for homogeneity, the presence or absence of fibrous constituents, preliminary fiber identification, and an estimate of fiber content. Possible identification of fibers or the confirmation of the absence of fibers is made by analysis of subsamples with the polarized light microscope.

5. SIGNIFICANCE AND USE

- 5.1 This method of testing is applicable to building materials including insulation, ceiling tiles, surface coatings, asbestos board, pipe coverings, etc. It is not recommended for floor tiles. However, if fibers can be liberated from a non-friable matrix, they can be identified by this method.
- 5.2 If the estimate of the percentage of asbestos in a sample is close to the 1% by weight level, other methods of quantification may be necessary if it is important to determine whether or not asbestos content is more or less than 1% by weight. This distinction may be important because the EPA defines asbestos-containing materials as those materials containing greater than 1% by weight asbestos (Ref. 2 and 3).
- 5.3 The details of the methods used to determine the optical properties of minerals are not included in this method. The method assumes that the analyst is proficient in making these measurements.

6. INTERFERENCES

- 6.1 Cellulose may have approximately the same index of refraction as chrysotile-asbestos. For this reason, it is frequently confused with chrysotile. However, cellulose fibers frequently pinch and swell along their length, exhibit internal cellular structure, and lack splayed ends: they are not composed of bundles of smaller fibers.
- 6.2 Cleavage fragments of many natural minerals including amphiboles, talc, gypsum, wollastonite and vermiculite may appear as elongated anisotropic particles. The aspect ratio of these particles may be as great as 20:1. Therefore, aspect ratio alone is not sufficient for the identification of asbestos. Other properties of the asbestiform habit, such as curved fibers, fiber bundles exhibiting splayed ends, and fibers with aspect ratios in excess of 20:1 must be observed in order to be sure asbestiform material is present in the sample. However, these properties need not be characteristic of every fiber or fiber bundle in the sample. Therefore, once asbestos is known to be present, other properties such as index of refraction and aspect ratio can be used to identify asbestos and determine which particles will be counted in making a quantitative estimate of the amount of asbestos in the sample.
- 6.3 Sprayed-on binder materials may coat fibers and affect color or obscure optical characteristics. Fine particles of other materials may also adhere to fibers. Occasionally, procedures other than those described in this test method may be helpful if the analyst is unable to observe fibers clearly. Some of these are described in Reference 1.
- 6.4 Vermiculite may be confused with chrysotile because it has a similar index of refraction and, while it is not fibrous, its extinction characteristics under crossed polars may give the impression that the particles are composed of masses of matted fibers. The problem is compounded by the fact that chrysotile and vermiculite are a common mixture in sprayed-on coatings.
- 6.5 Certain materials may be found in construction materials, which are fibrous or asbestiform but which are not asbestos. Those include but are not limited to fibrous talc, fibrous brucite (nemalite), zeolites and dawsonite.
- 6.6 Man-made fibers such as carbon, aluminum oxide, polyamides (nylon), polyester (Dacron) and polyolefins (polyethylene), and rayon are occasionally encountered in building materials.
- 6.7 Fibrous glass including both mineral wool and fiberglass is very common in building materials. Its isotropic character makes it readily distinguishable from asbestos.
- 6.8 Animal hair is occasionally encountered.
- 6.9 Heat and acid treatment may alter the index of refraction of asbestos and change its color. Heat can cause chrysotile and amosite to turn brown and may raise the indices of refraction significantly.

- 6.10 Moisture can interfere with the determination of optical properties. Wet samples should be dried at a temperature less than 150°C before examination.

7. EQUIPMENT

- 7.1 A magnifying glass or a low power binocular microscope, approximately 10-45x, with built-in or separate light source
- 7.2 Forceps, dissecting needles and probes
- 7.3 Glassine paper or clean glass plate
- 7.4 Polarized light microscope complete with a port for wave retardation plate, 360 degree graduated rotating stage, substage condenser, lamp and lamp iris
- 7.5 Objective lenses: low power (10x); high power (40-50x). Medium power (20-25x) and very low power (2-4x) lenses are optional.
- 7.6 Dispersion staining objective lens (optional)
- 7.7 Ocular lens: 8x minimum
- 7.8 Eyepiece reticle: cross hair
- 7.9 Compensator (wave retardation plate): 550 nanometer (first-order red or gypsum)
- 7.10 Microscope slides
- 7.11 Coverslips
- 7.12 Mortar and pestle: agate or porcelain

8. REAGENTS

- 8.1 Index of refraction liquids: $N_D = 1.490-1.720$ in increments of 0.002 or 0.004.
- 8.2 Index of refraction liquids for dispersion staining: high dispersion series, $N_D = 1.550, 1.605, \text{ and } 1.680$. (Optional. Required only if dispersion staining will be used to measure the index of refraction.)
- 8.3 Reference materials:
 - 8.3.1 Asbestos Materials
 - a. Commercial asbestos, including amosite, chrysotile, crocidolite, and anthophyllite asbestos. (UICC Asbestos Reference Sample Set available from UICC MRC Pneumoconiosis Unit, Llandough Hospital, Penarth, Glamorgan, CF6 1XW UX and commercial distributors.)

- b. Tremolite-asbestos: available from commercial distributors, such as Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, New York, 14692-9012.
- c. Actinolite-asbestos: source to be determined (very rare; not used commercially).

8.3.2 Suggested Matrix and Non-asbestos materials.

- a. Cellulose
- b. Vermiculite: source to be determined.
- c. Non-asbestiform amphiboles: available from commercial distributors, such as Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, New York 14692-9012.
- d. Other silicates, such as fibrous talc, wollastonite, gypsum, nemalite (brucite): available from commercial distributors, such as Ward's Natural Science Establishment, Inc., P.O. Box 92912, Rochester, New York 14692-9012.
- e. Synthetic fibers, such as fiberglass and mineral wool.

9. PRECAUTIONS

- 9.1 This method involves the analysis of material (asbestos), which may be hazardous if inhaled. It does not address the safety problems associated with its use. In addition, it should be noted that some immersion oils manufactured prior to 1978 might contain Polychlorinated Biphenols (PCB). PCB's have been identified as hazardous materials. It is the responsibility of whoever uses this method to establish appropriate safety and health practices to ensure that asbestos is not inhaled and exposure to PCB does not occur.

10. SAMPLING

- 10.1 Samples should be taken in the manner prescribed in Reference 2. Information on design of sampling and analysis programs may be found in Reference 3. If there are any questions about the representative nature of the sample, another sample should be requested before proceeding with the analysis.

11. GENERAL METHOD DESCRIPTION

- 11.1 Bulk samples of building materials are first examined with a low power binocular microscope or magnifying glass for homogeneity, the presence or absence of fibrous constituents, preliminary fiber identification and an estimate of fiber content.

- 11.2 Positive identification of fibers or the confirmation of the absence of fibers is made by analysis of subsamples with the polarized light microscope according to the outline presented in Table I. The optical properties of six types of asbestos are given in Table II. The use of plane polarized light allows the determination of index of refraction parallel to elongation. Morphology and color are observed. Orientation of the two polarizers such that their vibration directions are perpendicular (crossed polars) allows the distinction between anisotropic and isotropic materials to be made. It also allows observation of the birefringence and extinction characteristics of anisotropic particles. When a compensator is inserted into the optical path, the sign of elongation of the particle can be determined. Also, the fibrillar structure of asbestos is most evident under crossed polars.
- 11.3 Identification of the fibrous constituents is facilitated by comparison of the unknowns to materials in the reference collection.
- 11.4 A quantitative estimate of the amount of asbestos present is derived from the combination of the estimate made from slide preparations and the estimate of total fiber made from examination of the bulk sample.

12. SAMPLE PREPARATION

- 12.1 For initial observation, the sample should be placed on a clean glass plate or glassine paper and placed under the binocular microscope or examined with a magnifying glass. Color, the presence or absence of fibers, and homogeneity should be observed and recorded. If only an occasional fiber is observed, one or two should be isolated with forceps and prepared for examination by polarized light microscopy. A preliminary estimate of total fiber content can be made at this time.
- 12.2 Subsamples for polarized light microscopy are usually best prepared by using forceps to sample at several places from the bulk material. These subsamples are immersed in a refractive index liquid on a microscope slide, teased apart and covered with a cover glass. At a minimum, two slide preparations should be made.
- 12.3 If the material is obviously layered or comprised of two or more materials that differ in color or texture, slide preparations of each component should be made.
- 12.4 If the sample is not readily friable or if the sample consists of a coarse-grained matrix, a mortar and pestle can sometimes be used to crush the sample.
- 12.5 Other methods of sample preparation for homogenization and to remove interferences, such as milling, acid and sodium metaphosphate treatment and ashing, are not normally necessary. They are described in Reference 1.

13. IDENTIFICATION OF ASBESTOS

- 13.1 Positive identification of asbestos requires the determination of the following optical properties: morphology, color and pleochroism, index of refraction parallel to elongation, birefringence, extinction characteristics and sign of elongation. Techniques

for determining these properties are described in References 4 through 8. Characteristics of the asbestiform habit (morphology) are described in References 9 and 10. The sign of elongation is determined by use of a compensator and crossed polars. Index of refraction may be determined by the Becke line method (Reference 4) or by dispersion staining (Reference 8). The optical properties are given in Table II. General optical properties of silicates other than asbestos are found in References 4-7.

14. QUANTIFICATION OF ASBESTOS CONTENT

- 14.1 A quantitative estimate of the amount of asbestos present is most readily obtained by visual comparison of the bulk sample and slide preparations to other slide preparations and bulk samples with known amounts of asbestos present in them. Reference samples containing known amounts of asbestos will be available in the future from the National Institute of Standards and Technology, Office of Standard Reference Materials. Until these standards are available, laboratories should make their own standards for training and intra-laboratory comparison.
- 14.2 Point counting of slide preparations is not generally recommended. Point counting only produces accurate quantitative data when the material has uniform thickness. In practice, the thickness of asbestos-containing materials placed on a glass slide for petrographic analysis is often highly variable, rendering quantitative volume estimates inaccurate. However, the method recommended by the EPA for determining the amount of asbestos uses point counting techniques. It is described in Reference 1.
- 14.3 Estimates of the quantity of asbestos obtained by the method described in 14.1 above are neither volume nor weight-percent estimates. They are based on estimating the projected area from observation of the distribution of particles over the two-dimensional surface of the glass slide and on an observation of the bulk material. A basis for correcting to a weight or volume percent basis has not been established. However, the error introduced by assuming that the estimates are equivalent to weight percent is probably within the precision of the visual estimate techniques.

15. DATA PRESENTATION

- 15.1 The following information should be reported for each sample: color, presence or absence of asbestos, type or types of asbestos present, estimate of the area percentage of each type of asbestos present, area percentage of other fibrous materials present, and identity of other fibrous materials if known.
- 15.2 If the sample submitted for analysis is inhomogeneous and subsamples of the components were analyzed separately, the data for each subsample should be recorded separately. However, the separate components should be combined in proportion to their abundances and a single analysis should be provided for the sample as a whole.

15.3 Example Sample Analysis Sheet

Analysis of Asbestos in Bulk Materials	
Sample Identification	
Analyst:	
Date:	
Macroscopic Examination:	
1. Size and Condition of Sample:	
2. Texture: (occurrence of fibrous and other components)	
3. Color:	
4. Homogeneity:	
5. Comments	
Microscopic Examination:	
1. Number and Size of Subsamples:	
2. Preparation: (incl. Grinding, ashing, acid washing, ...)	
3. Method of estimation if other than visual estimation:	
4. Standards used for quantitation (if any):	
5. Index of refraction of the immersion medium	
Sample Identification:	
Analysis of fibrous component:	
a. Morphology	Component 1 Component 2
b. Color	
c. Birefringence	
d. Extinction characteristics	
e. Indices of refraction (dispersion characteristics)	
f. Sign of elongation	
g. Estimated range (percent area) of fibrous component	
Comments: (Describe any unusual characteristics or problems with analysis and if possible, briefly describe non-fibrous matrix components.)	
Sample Summary	
Sample Identification:	
Conclusions	
1. Asbestos present: yes no	
2. Fibrous-nonasbestos component present: yes no	
3. Number of distinct fibrous components:	
4. Types of fibers:	
5. Estimated range (percent area) of each fiber type:	
6. (Optional information on nonfibrous components).	

16. QUALITY ASSURANCE

- 16.1 Laboratories performing this test method should have demonstrated proficiency in the method. This would include adequate training of the analyst, an internal quality assurance program and participation in the EPA's Bulk Sample Analysis Quality Assurance Program or the National Institute of Standards and Technology Laboratory Accreditation Program for the Analysis of Asbestos. The laboratory should have a complete set of reference materials.
- 16.2 In order to obtain the accuracy indicated in 17.3, it is suggested that the analyst have completed a college-level course in mineralogy, had formal training in polarized light microscopy and its application to crystalline materials including instruction in the measurement of the index of refraction by the immersion method through Becke line technique and/or dispersion staining, and have experience analyzing asbestos samples. If this training is lacking, two years of participation in the EPA's Bulk Sample Analysis Quality Assurance Program with a 100% success rate is a good indication of proficiency in the application of this method.
- 16.3 An internal quality assurance program should involve blind samples and replicate analyses. It is also necessary to analyze blank samples to check for contamination of immersion oils, probes, slides and general sample preparation.
- 16.4 A record of the sample analyses should be kept that includes all the sample and analysis data. An example analysis recording form can be found in section 15.3. While the format of the record is not required, all the information detailed in the sample should be recorded for each sample.

17. PRECISION AND BIAS

- 17.1 The upper detection limit is 100%. The lower detection limit is less than 1%.
- 17.2 A preliminary evaluation of a method similar to that outlined in this document is found in Reference 11.
- 17.3 If used by a properly trained and experienced analyst, the accuracy in the determination of the presence or absence of greater than 1% asbestos is greater than 99%. If the analyst does not have the training specified in 16.2, the accuracy may be considerably reduced.
- 17.4 The error associated with the quantitative estimate of weight or area percent asbestos may be quite large. When the percentage of asbestos in the bulk sample is small, the error in the estimate may exceed 100% relative. Relative errors are particularly large in estimates near 1%. When the percentage of asbestos is large, however, the error is significantly reduced and may be as low as 10% relative or less. The precision and accuracy of the quantitative estimate are highly dependent on the training and experience of the analyst.

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TABLE I: Flow Chart for Qualitative Analysis of Bulk Samples by Polarized Light Microscopy

Polarized light microscopy qualitative analysis: For each type of material identified by examination of sample at low magnification, mount spatially dispersed sample in 1.550 RI liquid. (If using dispersion staining, mount in 1.550 ND.) View at approximately 100x with both plane polarized light and crossed polars. More than one fiber type may be present.

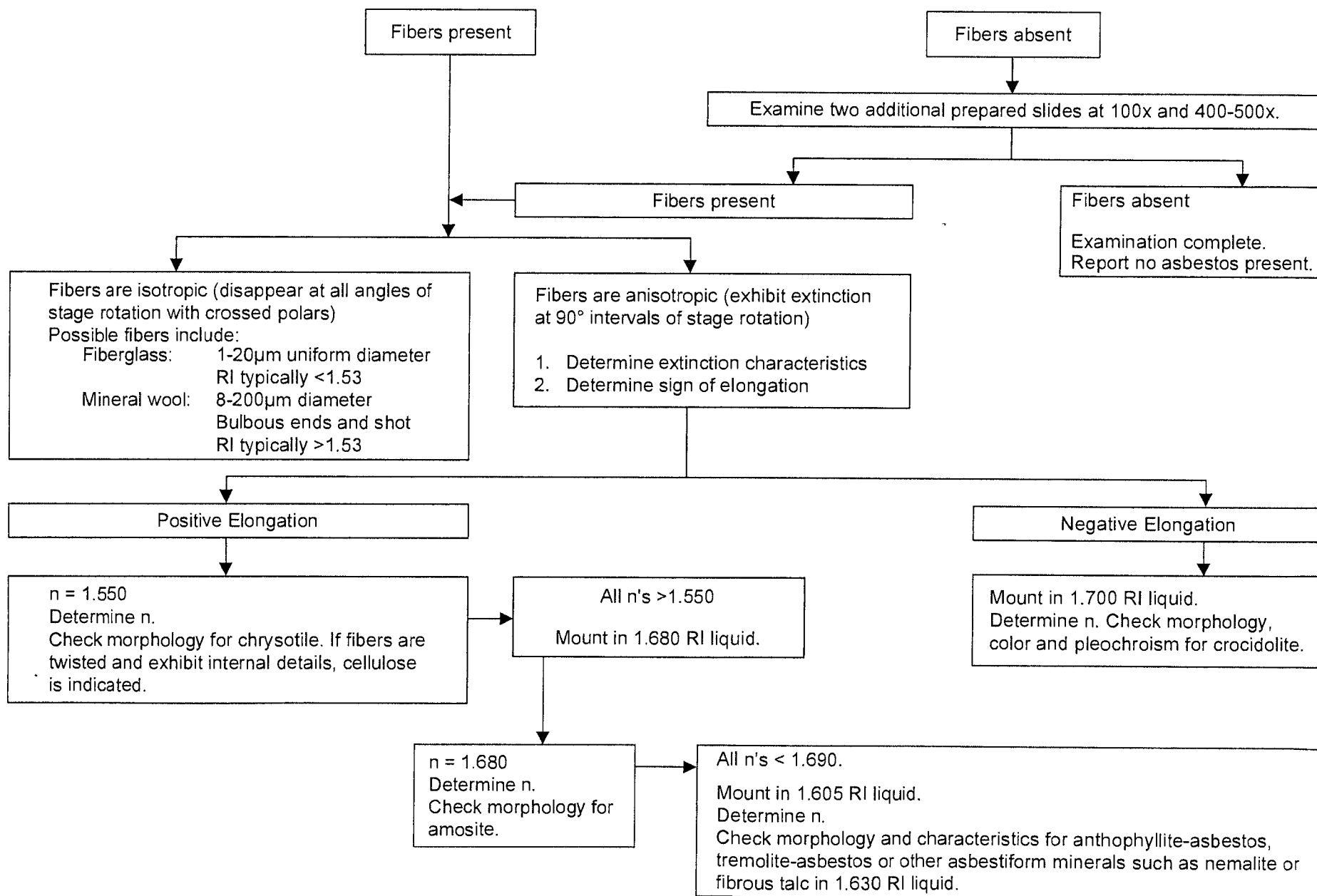


TABLE II

Mineral	Morphology and Color	Refractive Indices (Approximate Values)		Birefringence	Extinction	Sign of Elongation
		Parallel to Elongation	Perpendicular to Elongation			
Chrysotile-asbestos	Wavy fibers with "kinks" common. Large fiber bundles may show splayed ends. Colorless and nonpleochroic. Very common in building materials.	1.55	1.54	0.002-0.014	Parallel	Positive (length slow)
Cummingtonite-grunerite-asbestos (Amosite)	Straight fibers and fiber bundles. Only long fibers show curvature. Fiber bundles usually show splayed ends. Colorless to brown; may be weakly pleochroic. Common in building materials.	1.70	1.67	0.02-0.03	Parallel	Positive (length slow)
Crocidolite	Straight and curved fibers showing splayed ends are common. Blue color characteristic. Pleochroism marked. Uncommon in building materials.	1.70	1.71	0.014-0.016 Interference colors may be masked by blue color	Parallel	Negative (length fast)
Anthophyllite-Asbestos	Straight fibers and fiber bundles showing splayed ends. Colorless to light brown. Pleochroism absent. Rare in building materials.	1.63	1.61	0.013-0.028	Parallel	Positive (length slow)
Tremolite-asbestos and actinolite asbestos	Straight and curved fibers and fiber bundles. Large bundles show splayed ends. Tremolite is colorless. Actinolite is green and weakly to moderately pleochroic. Both actinolite and tremolite are extremely rare in building materials.	1.62-1.64 (tremolite) 1.64-1.68 (actinolite)	1.60-1.62 (tremolite) 1.62-1.67 (actinolite)	0.02-0.03	Parallel in most fibers. Narrow fibers may show oblique extinction (cΔZ up to 20°) in some samples	Positive (length slow)

FINAL REPORT ON BIOLOGIC TESTS
ON SAMPLE CPS-275-1*

Report submitted to R. T. Vanderbilt Co., Inc.

Report prepared by: William E. Smith, M.D.
Director, Health Research Institute
Fairleigh Dickinson University

*CPS-275-1 is a sample of the tremolite portion of a typical New York State talc ore normally containing 40 to 60 percent tremolite. This material was ground to a nominally respirable size (less than 10 micrometers) for test purposes. The sample is approximately 90 to 95 percent tremolite, the remainder being antigorite.



H-DYK-28