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DISTINGUISHING ASBESTIFORM TREMOLITE FROM NON-ASBESTIFORM TREMOLITE

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SUMMARY

For more than a decade, high resolution transmission electron microscopy and selected area diffraction studies have been performed on the amphibole asbestos minerals and their non-asbestiform counterparts. Amosite, crocidolite, and asbestiform tremolite have been shown to possess unit fibrils with a high frequency of closely spaced twins on the (100) plane. Faults, or atomic displacements, were noted along many twin planes, which rendered adjacent twin individuals weakly bound. Chain-width defects were also noted to occur with frequency on (010) planes, which created compositional variation. Additionally, the asbestos unit fibrils which constituted the fiber appeared to be rotated with respect to each other which produced random orientation of fibril ab planes. This aggregate of submicroscopic defect-laden fibrils, with only their c-axes in common alignment, defined the structure and physical character of the asbestiform amphiboles. Comminution of asbestos produced preferred parting along the twin surfaces rather than the cleavage planes anticipated for amphibole minerals. These structural and the crystallographic anomalies allow the use of electron beam techniques to distinguish between asbestiform fibers and fibrils and non-asbestiform amphibole cleavage fragments of the same mineral.

Fibers of asbestiform monoclinic amphiboles, when made up of fine submicroscopic unit fibrils, display parallel extinction when examined by polarized light microscopy. The fibers, composed of polyfilamentous bundles of fibrils, are often splayed on the ends, and display curvilinear form along the fiber axis. Indices of refraction tend to be anomalous.

Twinning on large fibrils (>0.5μm diameter) results in gross structural modification producing an anomalous orthorhombic symmetry for each fibril. Three indices of refraction define each of these fibrils, but values are both unanticipated (n_α', n_β', n_γ'), and fiber extinction is parallel rather than oblique. In cases where unit fibrils are rotated with respect to each other and/or small, <0.5μm, and (100) twins are closely spaced, the resulting fiber bundles display uniaxial symmetry, i.e., two

indices of refraction (n_α', n_γ') and parallel extinction. The non-asbestiform monoclinic amphiboles display both the morphology and optical properties anticipated for the specific mineral regardless of twinning.

The (100) twin plane of the asbestiform fibril is the principal plane on which it lies on a flat surface. The fibril therefore generates reciprocal diffraction nets which are significantly different from those generated by cleavage fragments which lie at or near the (110) cleavage plane. Therefore, populations of fibrils/fibers may be separated as asbestiform or non-asbestiform on the submicroscopic level, by means of selected area electron diffraction study.

Comminution of asbestos produces a dust of fine particle size, with narrow diameter fibers/fibrils, high particle number per unit mass of dust, and high surface area. Asbestiform habit of a mineral produces an intrinsically more biologically active dust as compared to its crushed non-asbestiform variety. Both *in vitro* and *in vivo* studies support this assertion.

As an example of the ability to distinguish asbestiform and non-asbestiform varieties of the same mineral, the tremolite found in a New York play sand possesses both light- and electron-optical properties of cleavage fragments. It is non-asbestiform, and it therefore can carry no asbestos risk. Its biological potential, if based on the state of aggregation alone, would be orders of magnitude less than tremolite asbestos found elsewhere. The activity of the individual submicroscopic cleavage fragment is currently being studied.

Asbestos in Play Sand: The Issues

In October of 1986, a letter appeared in the New England Journal of Medicine which stated that 2-4% tremolite asbestos was present in a crushed marble, marketed in part as a sand to be used in children's sand boxes (Germine, 1986). We analyzed a specimen of this sand for the US Consumer Products Safety Commission and concluded that although tremolite was present in the amounts stated, it was not asbestiform but rather common tremolite which, upon crushing, yielded generally blocky, prismatic cleavage fragments (Langer and Nolan, 1987). The criteria we used to distinguish asbestiform fiber from non-asbestiform cleavage fragments included those data which appeared in the mineralogical literature over the past decade or more, such as anomalous optical properties (e.g., parallel extinction), fibril parting along (100) twin and (010) defect planes, and measurable selected area diffraction net differences between the particle types.

In his reply to our criteria, Germine (1987) suggested that the properties we assigned to asbestos were commercially constrained "mineralogic abstractions". He stated that the criteria used by us "...are not consistent with those used in (our) past work" (ca. 1975).

However, since that time, 1975, more data have become available on the nature of these minerals, more detailed than existed thirteen years ago. Often, mineral analyses undertaken by our medical and biological colleagues are not rigorous due to a lack of understanding of crystal structure. The federal standard and "definition" of asbestos is not mineralogically precise, and its criteria for distinguishing amphibole asbestos from non-asbestiform fibers are, in part, based on morphology, an important component of the regulatory fiber counting strategy for the workplace. The data presented here form the scientific basis for the distinction between asbestiform and non-asbestiform amphibole fibers.

The Basis of the Mineral Definition Used in the Asbestos Standard

The first US federal asbestos standard was promulgated in 1972. It included a broad definition of asbestos and specific criteria for determining which fibers in the workplace were to be assayed.

Asbestos, as defined in the mineralogical literature at the time of the first standard, was considered as one of several silicate minerals with the following characteristics:

- Fiber bundles are composed of "hair-like" (filiform) fibrils, each with a large length-to-width ratio;
- Fiber bundles are polyfilamentous, that is, composed of fibril strands which may be easily separated by hand;
- Fibers are chemically durable and flexible and may be woven like organic fibers;
- Fibers possess diameter-dependent tensile strength.

Fibers which exhibited these properties were said to display the asbestiform habit. The federal asbestos standard includes five amphiboles (Table 1). The morphological requirements in the standard were "fiber" length (>5μm) and aspect ratio (>3:1). The portion of the 1972 standard regarding length and aspect ratio was based on several data sets, some practical and some theoretical:

- The 5μm size length limit for asbestos fiber counting in a work environment was based on several factors. Firstly, it eliminated the requirement for the microscopist to distinguish short fiber from non-fibrous particles, especially important since the fiber standard (f/ml) replaced the total particle standard (mppcf). The prevailing wisdom was that the asbestos diseases were produced by asbestos and not total dust, and fiber was therefore a more accurate index for protection against asbestos disease in the workplace. Secondly, counting of fibers greater than 5µm in length improved precision (reproducibility) of counting, by a single microscopist, repeating examination of the same specimen, and especially among different microscopists analyzing the same specimen. Additionally, the fibers used in textile mills, the principal work site from which the standard was developed, were long, and they produced long motes of dust when carded and spun (by fiber ore standards). Short fiber, less than 5µm in length, constituted but a small component of the total airborne dust, as assayed by light microscopy in these plants (at 100x magnification utilizing phase-contrast microscopy). Lastly, the then existing theory of the etiology of asbestosis was based on the disintegration of asbestos bodies over time with the release of silicic acid. Asbestos bodies were noted to form on long fibers (>5µm).

- The 3:1 aspect ratio was also established as part of a counting strategy, principally to eliminate "particulates" and fiber clumps from environmental assays. Again, this form limitation improved both the precision and accuracy of fiber counting on air filters. The use of the aspect ratio was not introduced to define asbestos, but rather to define an accurate method to count fibers on an air filter.

These latter, important elements were stressed in the standard and remains currently. With time, many interpreted these counting criteria as part of the definition of what asbestos was mineralogically. This assumption was then, and is now, incorrect.

State of the Art in Identification of Asbestos 1988

Shortly after the publication of the asbestos standard in 1972, and up to the present time, new mineralogical and crystallographical studies of the asbestos minerals and their non-asbestiform analogues have contributed to the development of modern understanding of the nature of asbestos. These studies have helped to explain the anomalous optical properties of the asbestiform amphiboles and the relationship between asbestiform and non-asbestiform amphibole fibers.

THE NATURE OF ASBESTIFORM AMPHIBOLES

Recognition of Anomalous Properties

On the level of electron microscopic examination, the asbestiform amphiboles display anomalous characteristics which were described in the early 1970s. Fibers 0.20µm in diameter produce selected area electron diffraction patterns which display both reflections forbidden for its space group symmetry, and spacial periodicities which are not accountable for by the amphibole unit cell dimensions. Twinning and defects were suspected as the causes of these anomalies (Langer et al, 1974).

Nature of Twins

Twins, considered a form of crystal defect, are defined as differently oriented structural lattices (which form multiple individuals) contained within the same crystal. A number of specific symmetry operations produce these orientations and individuals. At the boundary of these differently oriented lattices, there exists a layer of atoms which is common to each lattice, and in which the atoms fit exactly into the structures of each individual. A single crystal containing these differently oriented lattices is said to be twinned.

In monoclinic amphiboles, the twin is defined by the following symmetry operation: a mirror reflection of the same individual across a plane; the reflection plane is the (100), and it is referred to as the twin plane. It is important to add the following: the individual twins which are reflected across this plane may be offset ("faulted"), so that instead of the anticipated shared atom to bond these individual lattices, preserving crystal bonding, there may be a loss of bond strength. This will lead to loss of cohesion between twin individuals on either side of these planes. Faulted twin planes are therefore planes of structural weakness, and failure will tend to occur in this plane first. Failure of this type requires less energy than required by the cleavage process for a single, defect-free crystal.

Twinning accounts, in part, for the asbestos fiber's insensitivity to tilt in the electron beam, where change in crystal orientation, unexpectedly, does not produce new reciprocal net projections (new Laue zones). This behavior of amphibole asbestos fiber/fibril in the electron beam has been explained by the presence of these submicroscopic twin anomalies. These characteristics are not displayed by fragments of crushed nonasbestiform amphiboles.

Study of Asbestos Fibril Defects

Since the mid-1970s, electron beam study of amphibole asbestos varieties showed: the high frequency of twinning on amosite parallel to the (100) plane (Hutchison *et al*, 1975); the high frequency of (100) twins in amosite and crocidolite (Champness *et al*, 1976; Harlow *et al*, 1985); and the presence of "faults" along adjacent (100) surfaces (Seshan and Wenk, 1976). These later investigators compared the frequency of twinning in asbestiform amphiboles with their non-asbestiform analogues. Non-asbestiform tremolite and common hornblende showed the (100) twin only rarely.* Harlow *et al* compared the reflection twin (100) periodicity in amosite and grunerite. Amosite twin periodicity ranged from 0.004µm to 0.02µm (~40-200Å), whereas grunerite twin periodicity was rare and ranged from 1.0µm to 100µm. Differences in density of planes of failure produced thin, bladed, acicular fibrils of amosite on comminution as compared to low aspect ratio, thick fragments of grunerite.

Comparison of SAED Patterns

Harlow *et al* also showed that amosite generated selected area diffraction patterns corresponding to nets for particles lying on or near (100), whereas grunerite particles generally were either too thick to permit penetration of the electron beam (accelerated at 100Kv), or produced Kikuchi lines when penetrated, or showed patterns indicating most particles lay near or at (110), the prominent amphibole cleavage plane.

Chain-Width Errors

In the early 1970s, Chisholm noted the presence of anomalies in several amphibole chain structures. Rather than the anticipated "double chain" groups forming the *b* dimension of the unit cell (~18Å), the chain was formed of six planar-linked tetrahedra, producing a *b*-cell dimension of ~27Å. These were termed Wadsley defects, a crystallographical term for similar structural anomalies noted in other minerals (Chisholm, 1973). Wadsley defects, producing compositionally anomalous (010) planes, were noted with some frequency in specimens of both amosite and crocidolite by Champness *et al* (1976).

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*A recent report by Dorling and Zussman (1987) suggest that the (100) plane may also be a growth surface. See Wylie, this conference.

Random Orientation of Fibrils

Franco et al added to the understanding of the structural complexity of amphibole asbestos (shown for amosite, crocidolite, and asbestiform tremolite) by producing transmission micrographs which showed that unit fibrils of crocidolite were randomly oriented with respect to their ab planes. Only the c-axes of the fibrils were in common alignment (Franco et al, 1977). This azmithal, completely random orientation of unit fibrils which make up the asbestos fiber, was also shown to exist for asbestiform actinolite, based on a detailed single-crystal x-ray and polarized light microscopy studies by Wylie (1979).

The Amphibole Asbestos Fiber - 1988

Therefore, by the end-of-the-1970s, asbestiform fibers were found to consist of polyfilamentous bundles of disoriented, loosely held fibrils, with each of these fibrils complexly twinned on (100) surfaces, the twins offset by faults, and some fibrils possessing chain-width errors on (010) surfaces (Wadsley defects).

These structural characteristics helped explain a number of specific properties peculiar to the amphibole asbestos minerals which were not found in their non-asbestiform analogues:

- Asbestos fibrils are easily separable, in part, because of the faulted (100) twin plane surface having a much reduced cohesion;
- Mechanical manipulation of asbestos fiber produce many long, thin fibers/fibrils rapidly, as compared to identical manipulation of non-asbestiform analogues. (Note that more energy is required to size-reduce non-asbestiform amphiboles by comminution processes which induce failure along cleavage planes. Such manipulation also induces breakage across the fiber axis, producing short, equant fragments as compared to thin, elongate asbestos.);
- Asbestos fibrils express planar elements (100) > (010) > (110) > (hkl), produced by failure along the twin and defect planes (as well as cleavage), as compared to cleavage fragments (110) > (010) > (100) > (hkl), the more normal plane of cleavage dominating the expressed surfaces;
- The size distributions and aspect ratios for populations of asbestiform fibers/fibrils show them to be mostly smaller in diameter and greater in aspect ratio than non-asbestiform analogues;

- Optical properties of the amphibole asbestos fiber are "anomalous" (indices of refraction of asbestiform amphibole fibers which produce values with a slight variation to non-asbestiform amphibole single crystals or cleavage fragments);
- The amphibole asbestos fiber possesses a uniaxial indicatrix, displaying two refractive indices, rather than three; fiber displays *parallel extinction*, rather than characteristic inclined extinction, observable by polarized light microscopy (Wylie, 1979). (It should be noted that *tremolite asbestos* was originally misidentified as *anthophyllite* because of its *parallel extinction* (see Heinrich, 1965).);
- The differences in surfaces produced on comminution, imparts to amphibole asbestos fibrils a new crystallographic orientation to an electron beam which differs markedly from the orientations of cleavage fragments. The net effect is that different Laue zones of symmetry are generated during selected area electron diffraction. This results in monoclinic asbestiform amphibole fibrils, exhibiting reciprocal diffraction nets near or at, e.g., (001)x(010), (001)x(100), (103)x(010), etc., as compared to cleavage fragments which exhibit diffraction nets near or at (110)x($\bar{1}\bar{1}\bar{1}$), (020)x($\bar{1}\bar{1}\bar{1}$), (131)x($\bar{1}\bar{1}\bar{1}$), etc. (e.g., see Lee *et al*, 1979).

These above characteristics will vary as a function of mineral type and geological occurrence. We also note that, in some instances, single, isolated particles may be impossible to distinguish, i.e., acicular cleavage fragment from asbestiform fibril.

The Current Definition

The NIOSH-OSHA definition of asbestos is a composite of mineralogical terms enmeshed in a light microscopy strategy for particle counting at work sites. Phase contrast microscopy techniques and the current definition are insufficient to distinguish elongate prismatic or acicular cleavage fragments from asbestos.

The Play Sand

The play sand analysis is an illustrative example of how this broad definition led to the misidentification of cleavage fragments as asbestos. The analytical results are summarized in Table 2. The play sand consists of cleavage fragments rather than asbestiform fiber.

CONCLUSIONS

It is possible to distinguish amphibole asbestos fiber from its non-asbestiform acicular cleavage fragment, in large specimens, by both polarized light microscopy and transmission electron microscopy. TEM study also includes, in combination, selected area electron diffraction analyses of particles. Study of prevalent diffraction nets requires a population of particles and a determination of the principal Laue zones displayed by the diffracted objects. Because the asbestiform fibril tends to part along planes uncommon to its non-asbestiform counterpart, its diffraction nets generally differ. Submicroscopic respirable particles may thus be distinguished on a population basis. These data, relatively new, are not reflected in the scientific basis used to formulate the first regulatory standard in 1972.

If the asbestos standard of the United States is to focus only on asbestos minerals, then the standard should also regulate other asbestiform fibers which appear to be associated with human disease, e.g., asbestiform richterite (Na, trace Al, K) in tremolite- and vermiculite-exposed workers, and asbestiform winchite in talc-exposed workers. Other non-regulated asbestos minerals exist elsewhere.

In the mining-milling environment, crushing of rocks generate fragments of amphiboles which, on a cursory level, conform to the definition of asbestos. If the asbestos standard is to regulate asbestos only, then polarized light microscopy must supplant phase contrast microscopy as the analytical technique of the workplace environment. A change in aspect ratio, for the minerals industry only, may also help reduce "false positives". The mining industry, and its work environs, require such attention to detail.

If, however, the asbestos standard is to be the basis for a generic mineral "fiber" standard, which would include cleavage fragments, then some distinction should be made regarding relative fiber activity associated with dust exposure. A crushed hornblende-containing rock will carry significantly less biological potential than a similarly comminuted crocidolite ore specimen. Similarly, the relative dustiness of specimens will be related to crystallographic defects within the submicroscopic particle population. Increase in energy of comminution for asbestos produces long, thin, submicroscopic fibrils and much fibrous dust, whereas crushing of non-asbestiform amphibole-containing rocks produces thick, short fragments. A one fiber/ml standard, assayed by light microscopy, carries different biological potentials for these mineral forms.

A generic fiber standard should include fibrous zeolites and the various asbestos substitutes, e.g., wollastonite, fibrous clays, man-made vitreous fibers of all kinds, etc. Again, differences in fiber size distribution and biological potency will dictate choice of analytical tool for environmental assay of the workplace.

We have examined the New York State play sand which purportedly contained asbestiform tremolite. It did not, but rather was composed of tremolite cleavage fragments. We concluded there was no asbestos risk associated with its use. The amount of respirable tremolite dust in the specimen studied, of length and diameter considered biologically important, exists in the ppm range. Based on known properties thought to contribute to the biological potential of mineral fiber, the risk associated with the use of the sand is considered to be so quantitatively different from asbestos that the comparison cannot be justified.

Additionally, separate studies performed on several asbestiform amphibole minerals and their non-asbestiform analogues clearly showed marked differences in their biological potential:

- In systems which assayed for tumorigenesis, hemolysis, macrophage cytotoxicity, Chinese hamster ovary cytotoxicity, and V79 cytotoxicity, specimens of amosite (asbestiform grunerite) were all positive, whereas specimens of grunerite cleavage fragments failed to induce responses. All non-asbestiform varieties of amphibole, were not active (Palekar et al, 1988);
- Study of the ability of tremolite to induce mesothelioma in laboratory animals after intraperitoneal installation has shown a marked ability for asbestiform tremolite to induce mesothelioma, whereas tremolite cleavage fragments do not (Addison and Davis, this meeting).

A review of the federal asbestos standard in the United States to evaluate the merit of including some of the more recent data should be considered.

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Table 1: Asbestos Minerals Cited in 1972 Standard.

Asbestos Mineral Cited	Mineral Name	Counting Criteria; Analytical Problems
Amosite	Grunerite-Cummingtonite	Requirements for counting in workplace: >5μm length; aspect ratio >3:1; <3μm diameter; <100μm length. N.B.: Grunerite-cummingtonite is not regulated.
Crocidolite	Riebeckite	Requirements for counting are same as for amosite. N.B.: Riebeckite is not regulated.
Chrysotile	Chrysotile	Requirements for counting are same as for amosite. Is always asbestiform.
Actinolite	Actinolite	Requirements for counting are same as for amosite. Same name for asbestiform fiber and mineral, which may occur with non-asbestiform habit. Possible to count cleavage fragments as "asbestos", especially with phase contrast microscopy.
Anthophyllite	Anthophyllite	Requirements for counting are same as for amosite. Problems of distinguishing asbestiform and non-asbestiform varieties are same as for actinolite.
Tremolite	Tremolite	Requirements for counting are same as for amosite. Problems of distinguishing asbestiform and non-asbestiform varieties are same as for actinolite.

Table 2: Comparison of the Physical Characteristics Displayed by Asbestiform Tremolite from Metsovo, Greece and Tremolite Found in a New York Play Sand.

Property	Asbestiform Tremolite (Metsovo Whitewash)	Tremolite in Play Sand
Fiber, on Light Optical Level, by Polarized Light Microscopy	Polyfilamentous; Curvilinear with splayed ends; Parallel extinction; No characteristic amphibole cleavage observed; Some lath-shaped cleavage fragments present.	Optically continuous single fragment; Straight-edged length and termination; Angular extinction; Amphibole cleavage discernible; No polyfilamentous bundles observed.
Aspect Ratio	Avg. = 10.9:1, >20:1, 18%; Log-normal distribution towards high aspect ratios.	Avg. = 3.7:1, >20:1, 0%; Log-normal distribution towards low aspect ratios.
Fiber/Fibril on Electron Optical Level-Morphology	Tends toward long and thin, parallel sides to fibril, polyfilamentous.	Tends toward short and wide, irregular stepped-sides and ends.
Fibril Structure	Twins (100) frequent; closely spaced, <<1.0μm.	Twins rare; spacing >1.0μm.
Selected Area Diffraction	Common Diffraction Nets: (001) x (010) (001) x (100) (103) x (010) No Kikuchi lines.	Common Diffraction Nets:* (110) x (III) (131) x (III) Kikuchi lines common.
Material	Asbestos.	Cleavage fragment.

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*Most particles are too thick to permit passage of diffracted electrons. They are optically opaque on the TEM level of examination.