

THE IMPORTANCE OF WIDTH IN ASBESTOS FIBER CARCINOGENICITY AND ITS IMPLICATIONS FOR PUBLIC POLICY

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Evidence from human epidemiology, experimental animal implantation and inoculation studies, and lung burden studies shows that fibers with widths greater than 1 μm are not implicated in the occurrence of lung cancer or mesothelioma. Furthermore, it is generally believed that certain fibers thinner than a few tenths of a micrometer must be abundant in a fiber population in order for them to be a causative agent for mesothelioma. These conclusions are fully consistent with the mineralogical characteristics of asbestos fibers, which, as fibrils, have widths of less than 1 μm and, as bundles, easily disaggregate into fibrils. Furthermore, the biological behavior of various habits of tremolite shows a clear dose-response relationship and provides evidence for a threshold between fiber width and tumor experience in animals. Public policy in regulating mineral fibers should incorporate this knowledge by altering the existing federal asbestos fiber definitions to reflect it.

Width and length of fibers are both important parameters in determining the carcinogenic potential of asbestos and other specific fibrous materials. Most

investigators who have examined this subject agree that there exists a minimum length and maximum width below which and above which fibers are not related to tumor induction. Although fiber dimension is linked to the pathogenic effects of asbestos and certain other fibrous materials, it is also recognized that fiber characteristics other than dimension (i.e., durability, harshness, surface chemistry, surface area or activity, etc.) likely play an important role in the pathogenetic process. Whatever fiber characteristics contribute to the pathogenicity of asbestos, however, it is important to ensure that size parameters used for regulatory purposes reflect those most closely associated with asbestos and known carcinogenic effects.

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Although it is common to see the dimensions of asbestos fibers discussed in terms of a ratio of length to width, or aspect ratio, the use of such a dimensionless parameter results in the loss of information about the size of fibers and, therefore, is of little use in the discussion of fiber carcinogenicity or exposure. While asbestos fiber length is recognized in federal regulatory policy, width is ignored entirely. It is the purpose of this paper to examine the relationship between asbestos fiber width and fiber carcinogenicity, to suggest how this parameter might be used to identify other potentially harmful mineral fibers and to enhance the specificity of existing asbestos regulations.

The National Institute for Occupational Safety and Health (NIOSH) has established the definitions and analytical methods for asbestos used to one degree or another by all asbestos regulatory bodies in the United States. Under the NIOSH scheme, asbestos is simply defined as any fiber of chrysotile, crocidolite, amosite, anthophyllite, tremolite, or actinolite. A "fiber" is defined as a particle with a length to width ratio (aspect ratio) of at least 3:1 and a length of 5 μm or more as determined by the phase-contrast optical microscope (PCM) at a magnification of 450X-500X.⁽¹⁻²⁾ In this paper a "NIOSH fiber" refers to any particle with these dimensional parameters as determined by any accepted analytical technique.

PREVIOUS WORK

Mesothelioma and Fiber Width

Several investigators have examined the question of what particle sizes are most likely associated with the induction of mesothelioma. Merle Stanton first proposed that a distinct relationship exists between the shape or dimensions of durable fibers and mesothelial tumors in rats.³ Stanton and co-workers concluded from these experiments that populations with abundant fibers longer than 8 μm and narrower

TABLE I. Bulk and Airborne Particles—Cleavage Fragments

Mineral and Reference	Instrumentation	Length Restriction	Percent less than stated widths or mean width (μm)
Wollastonite			
a. Bulk Samples (75) New York	TEM	None	90% < 2.3; 50% < 1.1 10% < 0.62
Tremolite			
a. Bulk Samples (78) New York	SEM	> 5 μm	9% < 1.0; 0% < 0.5
b. Airborne (58) New York	SEM	> 5 μm	0% < 0.25
Cummingtonite			
a. Airborne (61) S. Dakota	SEM	> 5 μm	22% < 1.0; 7% < 0.5; 0% < 0.25
(70) S. Dakota	SEM	> 5 μm	11% < 1.0; 2% < 0.5
Actinolite			
a. Airborne (75) Virginia	SEM	> 5 μm	15% < 1.0; 0.5% < 0.5 0% < 0.25
Grunerite and Actinolite			
a. Airborne (61) Minnesota	SEM	> 5 μm	1% < 1; 0% < 0.25
Antigorite			
a. Airborne (74) Vermont	TEM 400X	> 5 μm	22% < 1.0; 2% < 0.5
(74) Vermont	TEM 20KX	> 5 μm	37% < 1.0; 10% < 0.5
Hiebeckite			
a. Bulk Samples (75) California	SEM	> 5 μm	27% < 1.0; 5% < 0.5

than 0.25 μm were most closely linked to pleural tumor response irrespective of fiber type.⁽⁴⁾ Most other researchers who use the animal model support the position that only narrow fibers are capable of inducing tumors.⁽⁵⁻⁷⁾

— **Evidence for the importance of narrow fibers in regard to mesothelioma** also comes from human experience. Timbrell and co-workers observed that the differences in the incidence of mesothelioma among two groups of asbestos miners in South Africa noted by Harington is most likely related to width.^(8,9) In the northwestern Cape, where miners experienced elevated mesothelioma, the mean fiber diameter for crocidolite is 0.073 μm . In the Transvaal crocidolite and Transvaal arnosite regions, mean diameters of 0.212 μm and 0.243 μm , respectively, were noted and mesotheliomas are rare. Among vermiculite miners and millers in Libby, Montana who were exposed to tremolite-asbestos, mesothelioma was elevated.^(10,11) Studies by Atkinson and co-workers on bulk samples from the Libby vermiculite mine show that 87% of tremolite fibers longer than 5 μm have widths equal to or less than 1 μm and 54% have widths less than or equal

to 0.5 μm .⁽¹²⁾ The high incidence of mesothelioma in Turkey has been attributed to fibers of asbestiform (wooly) erionite that are on the order of 0.1 μm in width.⁽¹³⁻¹⁵⁾ In contrast, no evidence of mesothelioma has been found in mining environments where NIOSH fibers produced by cleavage of massive amphiboles are abundant.⁽¹⁶⁻²⁰⁾ In these mining environments 78% or more of the 5 μm long particles have widths greater than 1.0 μm while 93% or more have widths greater than 0.5 μm . Few, if any, show widths below 0.25 μm (Table I).

Some have suggested that the carcinogenic potential of mineral fibers extends to those with widths as large as 2 μm , and there is some evidence from animal experimentation to support this position. For example, Pott et al. have induced tumors in Wistar rats by intraperitoneal injection with basalt and ceramic fibers with median diameters close to or in a few cases greater than 1 μm .^(21,22) Hesterberg et al. report tumors in Syrian hamsters after inhalation of refractory ceramic fibers with an average diameter of 0.95 μm .⁽²³⁾ However, the effect of the wide

fibers in these studies is most evident when the fibers are very long (up to 50 μm) or when a significant number of narrow fibers are part of the population, a fact that may not be evident from reporting mean or median widths of the population. Many populations of shorter fibers (still longer than 5 μm) with widths predominantly greater than 0.5 μm , such as wollastonite, gypsum, and certain fibrous glasses, have been shown to produce no significant tumor responses after instillation in animals.^(22,24,25) Furthermore, the tumor potential of wide fibers has not been demonstrated by inhalation experiments, in part, at least, because such fibers deposit in the conductive airways in the head and lung and do not reach the lung alveoli.⁽²⁶⁾

An opportunity to examine in humans the carcinogenic potential of a naturally occurring population of relatively wide mineral fiber is provided by the experience of anthophyllite-asbestos miners and millers in Paakkila, Finland. Anthophyllite-asbestos from this locality has a mean width of approximately 0.6 μm , and in the fiber population, widths less than 0.1 μm are quite rare.⁽²⁷⁾ In his study of lung tissue

from four individuals exposed to Paakkila anthophyllite-asbestos. Timbrell reports one fiber of $4\ \mu\text{m}$ in width, some fibers between 2 and $4\ \mu\text{m}$ and more than 50% of the fibers with widths less than $0.7\ \mu\text{m}$. In fact, Timbrell has shown that the distribution of amphibole fiber widths in lung tissue closely resembles the distribution of fiber widths in air.⁽²⁸⁾ Among the occupational cohort of miners and millers exposed to Paakkila anthophyllite-asbestos, asbestosis is common and the incidence of lung cancer is elevated, primarily in smokers.⁽²⁹⁾ However, the incidence of mesothelioma is not elevated.⁽³⁰⁾ The fact that Paakkila anthophyllite-asbestos will induce malignant tumors in animals after intraperitoneal inoculation⁽²²⁾ and inhalation⁽³⁰⁾ demonstrates that it has a detectable carcinogenic potential in animals under certain experimental conditions. However, the human experience tells us that either because of aerodynamic characteristics and/or the body's defenses, a population of durable fibers with the dimensions of Paakkila anthophyllite-asbestos does not represent the same occupational or environmental mesothelioma risk as other types of asbestos. The best explanation for these observations, in conformity with the Stanton hypothesis, is that the most abundant fibers of Paakkila anthophyllite-asbestos are by and large too wide and the thin fibers are too scarce for the population to induce mesothelioma even with the high exposures associated with this occupational setting.

Lung Cancer and Fiber Width

There are fewer data on the relationship between fiber width and lung cancer than there are for fiber width and mesothelioma. However, studies of human populations exposed to asbestos and animal inhalation studies involving asbestos consistently show an association between asbestos exposure and lung cancer as well as between mesothelioma and asbestos exposure.^(8-11,29) In contrast, exposures to the nonasbestiform analogs of asbestos minerals (cleavage fragments) have not shown an elevated lung cancer risk in man.^(16,18-20) Lippmann has reviewed the literature in this area and concludes that lung cancer is associated with fibers with widths between 0.3 and $0.8\ \mu\text{m}$ (and length $> 10\ \mu\text{m}$).⁽³¹⁾ His conclusions rest in part on the work of Timbrell who has shown that lung retention is greatest for fibers with these widths and lengths.⁽³²⁾ Such dimensions are consistent with those commonly associated with asbestos fibers but not for common cleavage fragments (see Tables I and III). Thinner fibers migrate to the pleura and peritoneum. Thicker fibers are usually rare in an airborne population of asbestos, and when present, disaggregate into thinner fibrils.

Lung Burden Studies in Asbestos-Related Diseases and Fiber Width

During the past 15 years, there have been a significant number of lung burden studies of persons occupationally exposed to asbestos who have developed asbestos-related diseases. Numerous investigators have published information on the sizes of asbestos fibers found in these persons and

only rarely are fibers with widths greater than $1.0\ \mu\text{m}$ detected (See Table II). In fact, most asbestos fibers found in lung tissue have widths less than a few tenths of a micrometer. The data are summarized in Table II. While there may be a gradual transition in the carcinogenic potential of fibers from greater to lesser as fiber width increases, as suggested by Pott, wide fibers are not implicated in mesothelioma in humans because they appear to be incapable of translocating to pleural regions, and they are not found in the lungs of people who have developed this disease.⁽³³⁾

Fibers longer than $5\ \mu\text{m}$ with widths greater than $1\ \mu\text{m}$ are not often found in lung tissue of asbestos miners, millers, and fabricators for several reasons. First, wider fibers contain more mass than narrow fibers of the same length and are thus less likely to become airborne. Wider fibers are also likely to be intercepted in the upper respiratory tract before they reach the lung. Work by numerous investigators has shown that the penetrability of airborne fibers into the peripheral rat lung drops sharply with an aerodynamic diameter above two, which corresponds to a diameter of approximately $0.67\ \mu\text{m}$.⁽⁵⁾ Pott and co-workers assert that fibers with a diameter range of $1-5\ \mu\text{m}$ cannot be tested for carcinogenicity by inhalation because they deposit in the upper respiratory tract and do not reach the lung.⁽³⁴⁾

There are also two very important mineralogical reasons why wide fibers of asbestos are rare in lung tissue. First, populations of asbestos fibers of all types are composed of fibers that are less than $1\ \mu\text{m}$ in width, and, therefore, wide fibers are simply not readily available for inhalation (Table III). Second, asbestos fibers wider than $1\ \mu\text{m}$ are composed of bundles of fibrils that readily split longitudinally into individual fibers of much smaller width. Even if wider fibers were inhaled, because of the fibrillar structure of asbestos, the fibers disaggregate. Cook and co-workers demonstrated the effectiveness of this process in their animal intratracheal instillation experiments with ferroactinolite-asbestos.⁽³⁵⁾ In these experiments they showed that the number of fibers found in lung tissue increased following cessation of exposure and that the increase was due to longitudinal splitting of fiber bundles. Other natural fibers that have been shown to exhibit a significant carcinogenic potency such as asbestiform (wooly) erionite are also characterized by very narrow widths and the ability to split longitudinally. The fibrillar structure is the hallmark of asbestiform fiber. All asbestos minerals that have been implicated as carcinogens in humans exhibit this unique habit of crystal growth structure.

In summary, human epidemiology, experimental animal studies, and the information on size distributions of fibers found in human lung tissue strongly suggest that fibers wider than $1\ \mu\text{m}$ are not likely to be a significant factor in the production of mesothelioma or lung cancer in man. To test the hypothesis that $1\ \mu\text{m}$ is a reasonable upper limit for critical width, we have examined data from tremolite-asbestos and nonasbestiform tremolite. This analysis will show that a clear dose-response relationship and evidence for a threshold exist between the abundance of fibers less than $1\ \mu\text{m}$ in width and carcinogenic response. While fibers wider than $1\ \mu\text{m}$ that are actually fiber bundles might also be important in

producing a carcinogenic response they are usually uncommon in terms of fiber number in an airborne asbestos population. We have therefore neglected these bundles in analyzing fiber abundance data although it may be inappropriate to ignore them for regulatory purposes.

MATERIALS AND METHODS

Tremolite occurs naturally as a gangue and as a component of ore at a number of mines producing industrial talc, vermiculite, play sand, marble, crushed stone, and chrysotile-asbestos. Health risks associated with tremolite have been the source of considerable debate in both the scientific community and regulatory arena for many years.⁽³⁶⁻³⁸⁾ Tremolite, in its massive and most common habit, when crushed, forms elongated cleavage fragments that are similar in size and shape to cleavage fragments of other common amphiboles. In this form, there are no epidemiological studies that clearly implicate tremolite as the cause of mesothelioma or lung cancer in man despite its prevalence in some mining environment. In its rare asbestiform habit, on the other hand, it appears to be the cause of both mesothelioma and lung cancer in man.^(10,11,39) In animals, mesothelioma has been observed after exposure to tremolite asbestos.^(4,40,41) One animal inhalation study involving tremolite asbestos showed elevated lung tumors as well as mesothelioma.⁴² There is a modern source of commercial tremolite-asbestos in Korea, and in the past tremolite-asbestos has been mined locally in Europe, Asia, and North America. Tremolite-asbestos possesses the characteristics that distinguish the more commercially important amphibole-asbestos types (crocidolite, amosite, anthophyllite-asbestos) including flexibility, thin fibrils, and a fibrillar structure.⁽³⁵⁾ Therefore, tremolite is an ideal mineral to study because it occurs naturally in the full range of amphibole habits, its asbestiform variety is known to cause mesothelioma and lung cancer in both man and animals, it is widely distributed, and it is known to occur in a number of important industrial mineral products.

TABLE II. Fibers in Lung Tissue of Humans Exposed to Asbestos

<i>Mineral and Reference</i>	<i>Instrumentation</i>	<i>Length Restriction</i>	<i>Percent less than stated widths or mean width (μm)</i>
Amphibole and Chrysotile			
⁽⁷⁶⁾ lung	TEM	None	mean = 0.13; range: 0.05-0.32
parenchyma			
⁽⁷⁶⁾ parietal pleura	TEM	None	
Amphibole			
⁽⁷⁶⁾	TEM	>4 μm	100% < 1.0; 63% < 0.25 mean width = 0.15 \pm 0.07 mean width = 0.19 \pm 0.21 mean width = 0.21 \pm 0.12
⁽⁷³⁾ pleura	TEM	None	
⁽⁷³⁾ parenchyma	TEM	None	
⁽⁷³⁾ node	TEM	None	
Crocidolite			
⁽²⁸⁾ mining	TEM	None	100% < 1.0 mean widths = 0.13, 0.09, 0.14, 0.15
⁽⁶⁸⁾	TEM	None	
⁽⁵²⁾ lung			96% < 0.375
parenchyma	TEM	>4 μm	
⁽⁵⁵⁾ shipyard and construction	TEM	>1 μm	
⁽⁴⁷⁾	TEM	None	25% < 0.07; 75% < 0.16 100% < 0.12 \pm 0.10
Amosite			
⁽²⁸⁾ mining	TEM	None	>95% < 1 mean widths = 0.27, 0.24, 0.35, 0.20
⁽⁶⁸⁾	TEM	None	
⁽⁵²⁾ lung			66% < 0.375
parenchyma	TEM	>4 μm	
⁽⁵⁵⁾ shipyard and construction	TEM	>1 μm	
⁽⁵⁵⁾ shipyard and construction	TEM	>4 μm	25% < 0.09; 75% < 0.29
⁽⁴⁷⁾ shipyard and construction	TEM	None	74% < 0.31
⁽⁴⁷⁾ shipyard and construction	TEM	None	100% < 0.43 \pm 0.29

A number of well-characterized samples of nonasbestiform tremolite and tremolite-asbestos have been used in animal experimentation and made available to us for study. The importance of these samples is that they represent a range in naturally occurring mineral habit that has not been evaluated for any other mineral. The tremolites include samples with numerous fibers of a fibrillar or asbestiform habit, samples in which only part of the tremolite is fibrillar, and samples lacking tremolite particles of a fibrillar habit altogether (nonasbestiform). We have examined the tumor response of these samples (established through animal experimentation by independent researchers) as a function of the dose of fibers longer than 5 μm with widths less than and greater than 1 μm . Only tremolite particles with a length to width ratio of 3:1 or greater (NIOSH fibers) were included.

Davis and co-workers have recently released the results of injection experiments that used six samples of tremolite: California tremolite-asbestos from Jamestown; Korean

TABLE II. Continued

<i>Mineral and Reference</i>	<i>Instrumentation</i>	<i>Length Restriction</i>	<i>Percent less than stated widths or mean width (μm)</i>
Chrysotile			
(54) referent	TEM	>5 μm	mean width = 0.15 ± 0.18
(54) environmental	TEM	>5 μm	mean width = 0.13 ± 0.25
(54) occupational	TEM	>5 μm	mean width = 0.13 ± 0.16
(53) textile plant	TEM	>5 μm	mean width = 0.10 ± 0.02
(53) mine	TEM	>5 μm	mean width = 0.07 ± 0.01
(68)	TEM	None	mean widths = 0.07, 0.07, 0.07, 0.07, 0.04, 0.11
(76)	TEM	>4 μm	100% < 0.25
(55) shipyard and construction	TEM	>1 μm	25% < 0.03; 75% < 0.06
(47)	TEM	None	100% < 0.07 ± 0.02
(73)	TEM	None	mean width 0.09 ± 0.15 mean width 0.07 ± 0.06 mean width 0.08 ± 0.06
Anthophyllite			
(28)	TEM	None	80% < 1
(551)	TEM	>1 μm	25% < 0.17; 75% < 0.44
(65)	TEM	None	50% < 0.67
Tremolite			
(57)	TEM	None	100% < 1.0; 80% < 0.5
(54) referent	TEM	>5 μm	mean width = 0.66 ± 0.48
(54) environmental	TEM	>5 μm	mean width = 0.62 ± 0.74
(54) occupational	TEM	>5 μm	mean width = 0.30 ± 0.25
(53) textile plant	TEM	>5 μm	mean width = 0.35 ± 0.04
(53) mine	TEM	>5 μm	mean width = 0.32 ± 0.02
(68)	TEM	None	mean widths = 0.24, 0.31
(55) shipyard and construction	TEM	>1 μm	25% < 0.23; 75% < 0.57
Actinolite			
(55)	TEM	>1 μm	25% < 0.15; 75% < 0.37

tremolite-asbestos; tremolite-asbestos from a laboratory in Swansea; fibrous Italian tremolite (Ala de Stura); tremolite from Carr Brae, Dornie, Scotland; and tremolite from Shinness, Scotland.⁽⁴⁰⁾ In this paper, these samples are identified as tremolite A, B, C, E, F, and G, respectively. Tremolite A, B, and C are composed primarily of tremolite-asbestos. Fiber bundles, curved flexible fibers, and small fibril widths are evident from optical microscopic examination of the samples. Tremolite E (Italian) consists of very long, highly unusual, single, needle-like crystals, with limited flexibility. Many of these fibers are twinned and in subsequent analysis, an asbestos subpopulation was reported.⁽⁴⁰⁾ Sample F (Dornie) is composed primarily of tremolite cleavage fragments. However, a small portion of the sample contains fiber bundles of tremolite-asbestos. Tremolite G (Shinness) was obtained by crushing large prismatic crystals, and it is composed entirely of cleavage fragments. Davis and co-workers packed the samples into cylinders of Timbrell dust dispensers and airborne dusts were generated. They collected

the respirable fraction of these dusts and administered it to rats by using the intraperitoneal injection technique. Measurements of width and length for the fibers in the populations were collected by scanning electron microscopy after deposition on 0.2 μm pore-size polycarbonate filters. Davis and co-workers provide dimensional data for approximately 450 particles from each sample. The dose in terms of number of particles per milligram of dust was obtained directly from the data of Davis and co-workers.⁽⁴⁰⁾

In earlier work Stanton reported the results of 72 rat pleural implantation experiments involving approximately 30 different inorganic materials.⁽⁴⁰⁾ Among these materials, one tremolite-asbestos sample was implanted on two different occasions. The sample comes from California, but its exact origin is unknown. Like tremolite A, B, and C, this tremolite possesses all the characteristics of commercial asbestos. In this paper it is referred to as tremolite D. Another Stanton sample identified as Talc 6 is a commercial tremolitic talc from the state of New York identified from Stanton's laboratory notes as

Nytal 300. This sample contains 40–50% tremolite cleavage fragments. It is referred to as tremolite H in this paper.

Stanton and co-workers did not provide adequate dimensional data to evaluate width satisfactorily, and it was necessary to re-examine both tremolite D and H. Samples of tremolite-asbestos 1 and 2 (tremolite D) and of Talc 6 (tremolite H) were obtained from the National Cancer Institute and prepared for analysis by gentle sonication in distilled water and filtration on a polycarbonate filter. Portions of the filters were mounted on a polished SEM stub and carbon coated. For Talc 6, the filters were scanned at 5000X and the chemical composition of particles longer than 4 μm was established by energy dispersive spectroscopy (EDS). The particles were identified as tremolite or "other" from their chemical spectra, and their dimensions were measured and recorded. For tremolite D, the samples were photographed at 5000X. All particles in the photograph with lengths longer than 4 μm were measured. From each population 100–150 particles were measured.

Stanton provided estimates of the number of particles longer than 4 μm in a microgram.⁽⁴⁾ From our measurements of tremolite-asbestos D, we determined that 74% of the particles longer than 4 μm met the definition of a NIOSH fiber. We also determined that 99% of the NIOSH fibers of tremolite had widths less than or equal to 1.5 μm , 88% had widths less than or equal to 1.0 μm , and 52% had widths less than or equal to 0.5 μm . From these data we calculated the number of NIOSH fibers per total dose and the number of fibers within each of the width categories.

From Talc 6 (tremolite H), we used the number of particles per microgram longer than 4 μm provided by Stanton. From our analysis, we determined that 30% of those particles longer than 4 μm were NIOSH fibers of tremolite. Of these, 9% had widths less than or equal to 1.5 μm , 9% had widths less than or equal to 1.0 μm and 0% had widths less than or equal to 0.5 μm . As was the case for tremolite D, we calculated the number of tremolite NIOSH fibers per total dose and the number within each of the width categories.

Smith and co-workers reported the results of intrapleural injection of four tremolite samples into Syrian hamsters.⁽⁴⁴⁾ Only limited information on the size distributions of these samples was published, but one sample, FD-14, was available for additional analysis. Sample FD-14 was an off-the-shelf sample of tremolitic talc from the state of New York that contained approximately 50% nonasbestiform tremolite. The samples were examined by SEM at 2000X and the particles were identified as tremolite based on their chemical composition. Five hundred tremolite particles were measured of which 64 met the definition of a NIOSH fiber. Data regarding the number of tremolite NIOSH fibers in a microgram were not available for this sample. Interestingly, very long fibers of the mineral talc

TABLE III. Bulk and Airborne Particles — Asbestos and Other Fibers

<i>Mineral and Reference</i>	<i>Instrumentation</i>	<i>Length Restriction</i>	<i>Percent less than stated widths or mean width (μm)</i>
Crocidolite			
Cape Province			
a. Bulk Samples			
(50)	SEM	>5 μm	98%<1.0; 85%<0.5
(56)	TEM	None	>99%<1.0; >90%<0.5
(63)	TEM	None	mean width = 0.23 \pm 0.06
(64)	TEM	>2 μm	99%<1.0; 99%<0.5
(22)	TEM	None	median width = 0.20
(60)	SEM	None	mean width = 0.35 (2S.D. = 0.78 - 0.16)
(60)	TEM	None	mean width = 0.12 (2S.D. = 0.31 - 0.05)
b. Airborne			
(69)	TEM	None	98%<0.4
(69)	TEM	>5 μm	90%<0.3
(52)	TEM	>4 μm	88%<0.375
(45)	TEM	>5 μm	98%<1.0; 82%<0.5
(46)	TEM	>0.25 μm	99%<1.0; 88%<0.5
(9)	TEM	None	99%<0.5
Crocidolite			
Transvaal			
a. Bulk Samples			
(64)	TEM	>2 μm	89%<1.0; 65%<0.5
Crocidolite			
Australia			
a. Bulk Samples			
(64)	TEM	>2 μm	100%<1.0; 99%<0.5
Crocidolite			
Bolivia			
a. Bulk Samples			
(64)	TEM	>2 μm	85%<1.0; 60%<0.5
Amosite			
Transvaal			
a. Bulk Samples			
(50)	SEM	>5 μm	91%<1.0; 50%<0.5
(56)	TEM	None	98%<1.0; 80%<0.5
(72)	TEM	>5 μm	92%<1.0; 72%<0.5
(63)	TEM	None	mean width = 0.47 \pm 0.17
(60)	SEM	None	mean width = 0.55 (2S.D. = 1.29 - 0.23)
(60)	TEM	None	mean width = 0.35 (2S.D. = 1.22 - 0.10)

that have narrow widths and a fibrillar structure occur in this sample. This sample is referred to as tremolite I in this paper.

RESULTS

There are several ways to examine the width data. First, the correlation between tumor incidence and the dose of NIOSH

TABLE III. Continued

<i>Mineral and Reference</i>	<i>Instrumentation</i>	<i>Length Restriction</i>	<i>Percent less than stated widths or mean width (μm)</i>
b. Airborne			
(69)	TEM	None	95% < 0.4
(69)	TEM	> 5 μm	45% < 0.3
(52)	TEM	> 4 μm	66% < 0.375
(62)	PCM	> 5 μm	99.4% < 1; 94.2% < 0.5
(61)	SEM	None	95% < 1.0; 80% < 0.5
(9) (inc.roc.)	TEM	None	95% < 1.0; 70% < 0.5
Chrysotile			
Quebec			
a. Bulk Samples			
(50)	SEM	> 5 μm	99% < 1.0; 94% < 0.5
(63) UICC	TEM	None	mean width = 0.17 ± 0.03
(22) UICC	TEM	None	median width = 0.15
(67)	PCM	82% > 5 μm	81% < 1.0
b. Airborne			
(69)	TEM	None	98% < 0.4
(69)	TEM	> 5 μm	61% < 0.3
Chrysotile			
California			
a. Bulk Samples			
(50)	SEM	> 5 μm	99% < 1.0; 94% < 0.5
(50)	TEM	> 5 μm	100% < 1.0; 98% < 0.5
Chrysotile			
Rhodesia			
a. Bulk Samples			
(63) UICC	TEM	None	mean width = 0.16 ± 0.04
Chrysotile			
Vermont			
b. Airborne			
(74)	TEM 400X	> 5 μm	63% < 1.0
(74)	TEM 20KX	> 5 μm	90% < 1.0; 71% < 0.5
Anthophyllite			
Finland			
a. Bulk Samples			
(56) UICC	TEM	None	90% < 1.0; 60% < 0.5
(22) UICC	TEM	None	median width = 0.61
b. Airborne			
(51)	TEM	None	70% < 1.0; 40% < 0.5

fibers wider than 1 μm is illustrated in Figure 1. It is clear that the dose of wide fibers (> 1 μm) shows no relationship to the likelihood of producing tumors. It is important to note that the number of wide (> 1 μm) NIOSH fibers in the dose of tremolite in the cleavage fragment samples (G, F, and H) is comparable to that in the tremolite asbestos samples. Thus, the argument that more tumors might have been observed if there had been more wide NIOSH fibers in these samples is not supported. In contrast, the correlation

between tumor incidence and the number of NIOSH fibers per total dose administered with widths less than or equal to 1 μm is shown in Figure 2. This figure shows a dose-response relationship in the form of an s-shaped curve suggesting a threshold and a rapid increase in tumor incidence as the number of these thin (< 1 μm) fibers increases. The curve in Figure 2 is derived from a least-squares linear regression of the form:

$$\text{logit} = m(\log \text{ of total dose } \leq 1 \mu\text{m}) + b$$

where

$$\text{logit} = \ln\left(\frac{\% \text{ tumor}}{1 - \% \text{ tumor}}\right)$$

The equation for the curve in Figure 2 is shown below and is highly significant (R² = 0.84, p < 0.005):

$$\text{logit} = 3.04(\log \text{ total dose } < 1 \mu\text{m}) - 6.25$$

A straight linear regression of the form below is also highly significant (R² = 0.90, p < 0.005). In the data in Figure 2, this equation is:

$$\% \text{ Tumor} = 49.3(\log \text{ total dose } \leq 1 \mu\text{m}) - 54.6$$

Another way to illustrate the importance of width relative to tumor response is to characterize the samples in terms of the percentage of NIOSH fibers that have widths of less than 1 μm. It has been shown in most cases that up to 30% of ordinary cleavage frag-

ments of amphibole longer than 5 μm have widths less than 1 μm, and more than 90% of asbestos fibers have widths less than 1 μm (all asbestos fibrils will be less than 1 μm). (See Tables I and III.) Therefore, the proportion of a fiber population with small widths is a measure of the asbestos-like nature of the population or of the abundance of the asbestiform components in a sample. Figure 3 shows the correlation between tumor incidence and the percentage of the tremolite NIOSH fiber population that has widths less than 1.0 μm. By

TABLE III. Continued

Mineral and Reference	Instrumentation	Length Restriction	Percent less than stated widths or mean width (μm)
Actinolite-asbestos			
a. Bulk Samples			
(34) Minnesota	TEM	None	mean width = 0.41 50% < 0.24
(75) South Africa	SEM	> 5 μm	96% < 1.0; 70% < 0.5
(49)	TEM	None	90% < 0.33; 50% < 0.06
(75)	SEM	> 5 μm	98% < 1.0; 90% < 0.5
(22) Fed. Rep. Ger.	TEM	None	median width = 0.17
Tremolite-asbestos			
a. Bulk Samples			
(12) Montana	TEM	> 5 μm	87% < 1.0; 54% < 0.5
(77) Montana	TEM	None	81% < 0.6; 67% < 0.4
(77) Metsovo	TEM	None	96% < 0.6; 85% < 0.4; 64% < 0.2
b. Airborne			
(66) Korea	TEM	> 0.4 μm	99% < 1.0; 90% < 0.5
(11) Montana	TEM	> 5 μm	for $w > 0.45$: 98% < 1.24; 93% < 0.88; 68% < 0.62
Tremolite-asbestos and tremolite			
a. Bulk Samples			
(12) S. Carolina	TEM	> 5 μm	81% < 1.0; 48% < 0.5
(75) India	SEM	> 5 μm	61% < 1.0; 34% < 0.5
Asbestos, mineral ID not specified			
b. Airborne			
(59)	TEM	None	80% < 0.43
Woolly Erionite			
a. Bulk Samples			
(22) Turkey	TEM	None	median width = 0.38
(22) Oregon	TEM	None	median width = 0.21
(71) Oregon	TEM	None	width range = 0.01–0.13 mean width = 0.03
Nemalite			
a. Bulk Samples			
+	TEM	None	mean width = 0.06

A straight linear regression of the data using the equation below is also highly significant ($R^2 = 0.93$, $p < 0.005$):

$$\% \text{ Tumors} = 1.2(\% \text{ fibers} \leq 1 \mu\text{m}) - 14.4$$

DISCUSSION

Figures 1 and 2 contrast the pleural and peritoneal tumor response (mesothelioma) produced by wide and thin tremolite NIOSH fibers. For wide NIOSH fibers alone, there is no regular dose-response relationship, whereas for thin fibers, the s-shaped curve indicates a strong relationship between dose and carcinogenicity. Furthermore, as illustrated by Figure 3, as the proportion of tremolite NIOSH fibers with widths greater than 1 μm increases, the tumor incidence produced by the sample decreases. Complicating this somewhat simple picture is the fact that as the width of fibers increases, the number of fibers per microgram must decrease. Hence, the number of wide fibers will always be less than the number of narrow fibers in samples of equal weight. Notwithstanding this reality, however, is the observation that without thin fibers, tremolite NIOSH fiber populations are not associated with the induction of pleural or peritoneal tumors in animals. It is also made clear in these figures that characterization of populations of nonasbestiform tremolite by

this measure an increase in tumor incidence is again observed as the proportion of tremolite fibers < 1.0 μm in width increases in the population. The curve in Figure 3 is derived from a least-squares linear regression of the form:

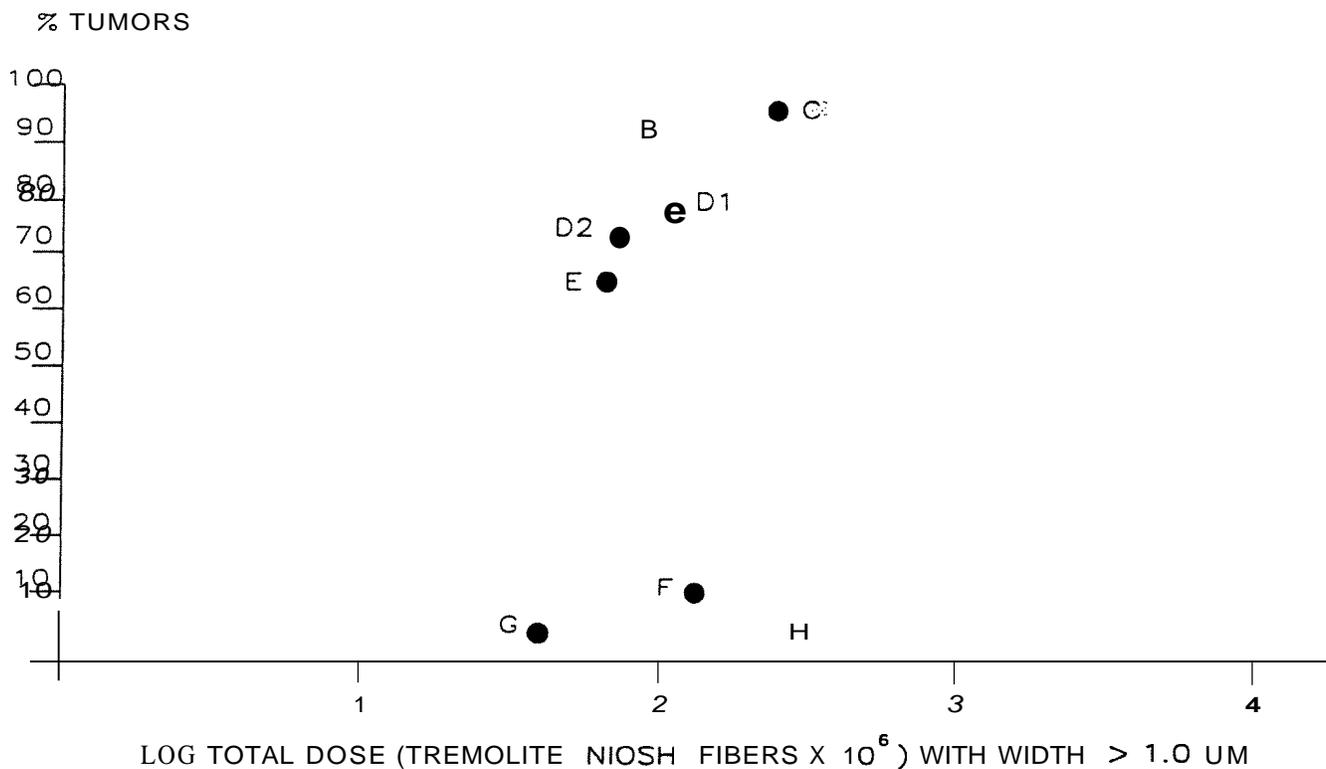
$$\logit = m(\% \text{ fibers} \leq 1 \mu\text{m}) + b$$

The equation for the curve in Figure 3 is shown below and is highly significant ($R^2 = 0.85$, $p < 0.005$):

$$\logit = 0.008(\% \text{ fibers} \leq 1 \mu\text{m}) - 4.6$$

the NIOSH aspect ratio criterion for fibers produces an index that shows no relationship to mesothelioma risk.

The tremolite samples can be divided into three groups based on their carcinogenic potential: those without significant response, those with intermediate responses, and those that produce tumors in almost all the experimental animals. Criteria for a "significant" response varies according to the experimental animal, the level of total dose, the method and location of sample introduction of the fibers, latency, and experience of controls. Davis and co-workers indicate that by, intraperitoneal injection, tumor responses in less than 10% of the animals are insignificant. For Stanton and



- A: Addison-Davis California Tremolite Asbestos
 B: Addison-Davis Korean Tremolite Asbestos
 C: Addison-Davis Swonseo Tremolite Asbestos
 D1: Stanton Tremolite Asbestos 1
 D2: Stanton Tremolite Asbestos 2
 E: Addison-Davis Italian Tremolite Asbestos/Cleavage Fragments
 F: Addison-Davis Dornie Tremolite Cleavage Fragments/Asbestos
 G: Addison-Davis Shinness Tremolite Cleavage Fragments
 H: Stanton Talc 6 Tremolite (Non-asbestiform)

Figure 1. Percentage of tumors observed in experimental animals after exposure to tremolite as a function of the total dose of tremolite (number of fibers) equal to or longer than 5 μm , wider than 1 μm , and with an aspect ratio equal to or greater than 3

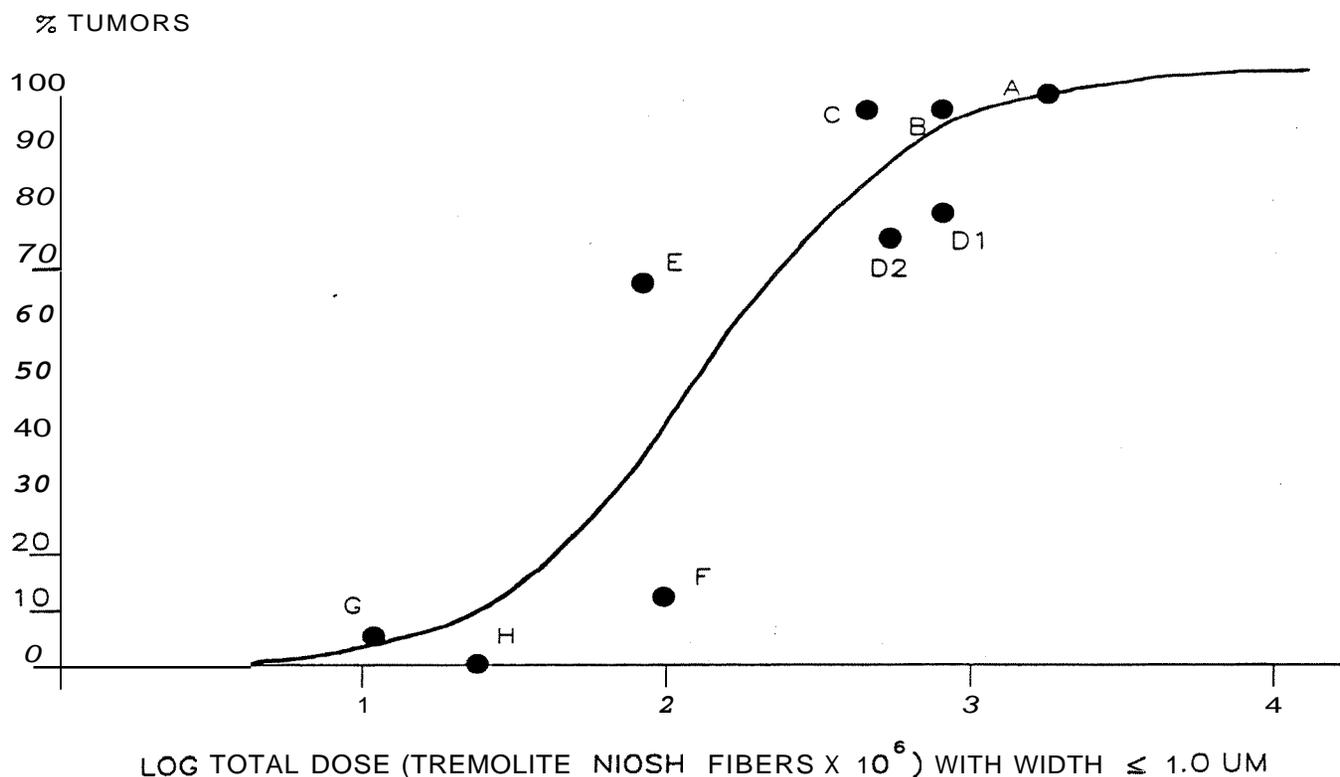
co-workers, 30% tumors were necessary for a significant response by pleural implantation.

Samples G, H, and I fall into the first category. Two of these samples are tremolitic talc from the state of New York (H and I). The high proportion of wide tremolite fibers in these samples is a clear indicator that the tremolite is nonasbestiform.

Samples E and F produced intermediate tumor responses in the animals. However, while sample E produced a high proportion of tumors, the mean survival time of the animals was almost twice that of the animals injected with tremolite A, B, and C, leading Davis and co-workers to conclude that tremolite E represented one-fortieth the hazard of tremolite C, a relationship not evident from the total tumor response. The intermediate responses might be expected from these two samples based on their mineralogical characteristics. Sample E contains a large proportion of highly unusual mineral fibers that lack a recognizable fibrillar structure. Other researchers, employing higher resolution electron microscopic techniques, report an asbestos subpopulation in

this sample.''' The long latency observed in the animals injected with this material might reflect a slow disaggregation of twinned or possibly asbestiform fibers. This hypothesis is further supported by the fact that the number of fibers per microgram, whether defined as total NIOSH fibers or as NIOSH fibers with widths less than 1 μm , in sample E is less than in sample F. The inverse correlation between dose and response could be explained by sample alteration in vivo as well as by several other mechanisms resulting from differences in surface properties. A comparison between the width distribution of the sample and the width distribution of the fibers found in the lung is necessary to evaluate the hypothesis that disaggregation occurs in vivo.

Sample F contains a small proportion of asbestiform fibers. The limited response of the animals to this material is most likely due to a low dose of asbestos. Davis and co-workers characterize this sample as unlikely to be carcinogenic to man given the marginal biological response observed in what is generally regarded as the most sensitive animal tumor induction technique (intraperitoneal



- A: Addison-Davis California Tremolite Asbestos
 B: Addison-Davis Korean Tremolite Asbestos
 C: Addison-Davis Swonseo Tremolite Asbestos
 D1: Stanton Tremolite Asbestos 1
 D2: Stanton Tremolite Asbestos 2
 E: Addison-Davis Italian Tremolite Asbestos/Cleavage Fragments
 F: Addison-Davis Dornie Tremolite Cleavage Fragments/Asbestos
 G: Addison-Davis Shinness Tremolite Cleavage Fragments
 H: Stanton Talc 6 Tremolite (Non-asbestiform)

Figure 2. Percentage of tumors observed in experimental animals after exposure to tremolite as a function of the total dose of tremolite (number of fibers) equal to or longer than 5 μm , less than or equal to 1 μm wide, and with an aspect ratio equal to or greater than 3

injection).'''' Tumors have been induced with this test through the introduction of substances as benign as saline solution.''''

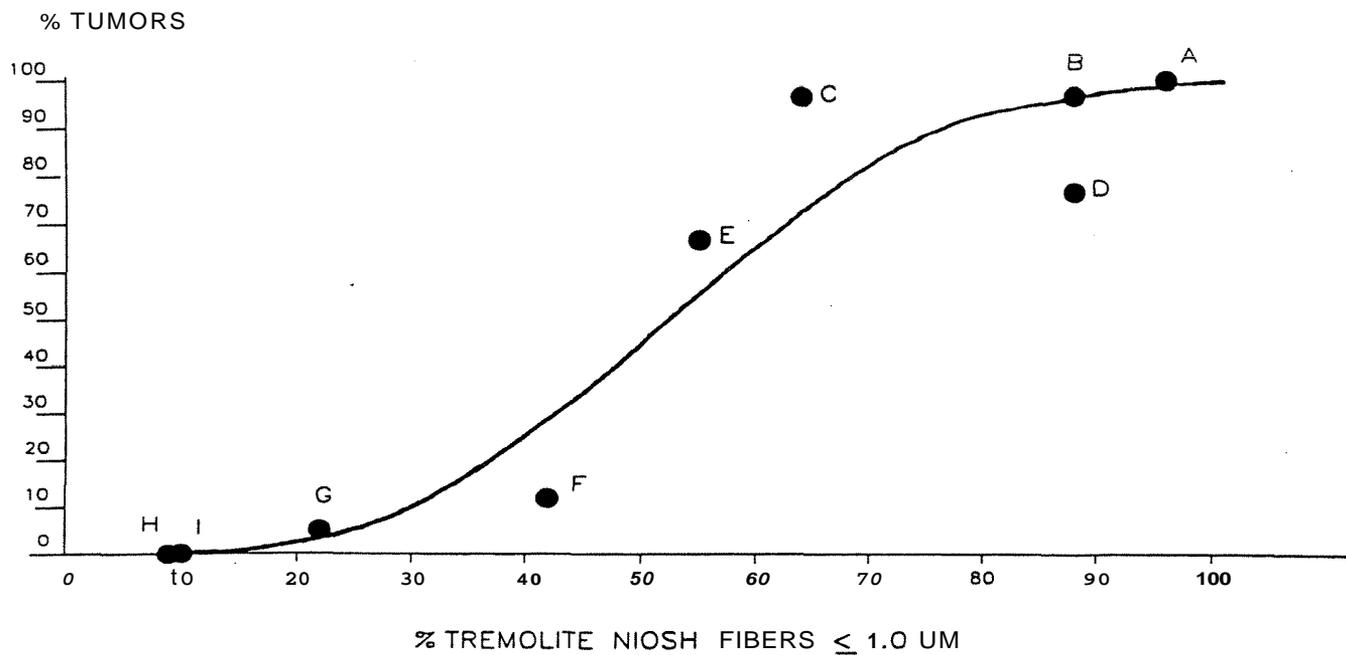
Tremolite-asbestos samples A, B, and C produced pleural tumor incidences in excess of 95% with very short ratancy periods. While tremolite-asbestos samples D1 and D2 produced 75% and 79% tumor incidences, respectively, Stanton considered this response equivalent to a 100% tumor probability.

Because the tremolite studies did not involve inhalation exposures in either man or animals, they do not directly test carcinogenic potential relative to lung cancer. However, human epidemiology, lung burden data, and animal experimentation previously discussed support the hypothesis that as the number of asbestos and certain other fibers with widths below 1 μm (and 5 μm or longer) increases, the risk of both lung cancer and mesothelioma increases as well. Therefore, to the extent mesothelioma tumor experience observed in these tremolite animal studies is consistent with both fiber-size observations and biological response reported

elsewhere, reasonable assumptions about lung cancer can be made with respect to the tremolite samples discussed here. It has already been established that excess lung cancer and mesothelioma are not evident in human populations exposed only to amphibole cleavage fragments but they are evident for human populations exposed to tremolite asbestos.

CONCLUSIONS

Combining tremolite samples that have been administered in different ways and to different animal groups such as we have done may appear to overlook important distinctions among these approaches. Despite this simplification, the data show a systematic relationship between dose based on width and mesothelioma tumor response in animals. It should also be noted that by using only the Addison and Davis data, the relationship of tumor response to fiber width remains strong. Furthermore, the fact that asbestos, with its unique dimensions, is known to cause lung cancer and pneumoconiosis suggests that width is related to respiratory



- A Addison-Davis California Tremolite Asbestos
- B Addison-Davis Korean Tremolite Asbestos.
- C Addison-Davis Swansea Tremolite Asbestos
- D Stanton Tremolite Asbestos
- E Addison-Davis Italian Tremolite Asbestos/Cleavage Fragments
- F Addison-Davis Dornie Tremolite Cleavage Fragments/Asbestos
- G Addison-Davis Shinness Tremolite Cleavage Fragments
- H Stanton Talc 6 Tremolite Cleavage Fragments
- I Smith FD-14 Tremolite Cleavage Fragments

Figure 3. Percentage of tumors observed in experimental animals after exposure to tremolite as a function of the percentage of tremolite equal or longer than $5 \mu\text{m}$ with an aspect ratio equal to or greater than 3 that have widths less than or equal to $1 \mu\text{m}$.

diseases other than mesothelioma. It seems clear that width is an extremely important variable that to date has been overlooked in regulatory policy. While fibers from a tenth to $200 \mu\text{m}$ long have been found in human lung tissue, it is the narrow width of these fibers that has given them access. A fiber $15 \mu\text{m}$ long and $5 \mu\text{m}$ wide meets the NIOSH criteria for a fiber, but such a particle is highly unlikely to cause disease in humans because it cannot gain access to a human lung. Not only is width a useful indicator of mesothelioma tumor induction, but a dose-response with a threshold is indicated as well.

We propose that NIOSH fiber size parameters used in the quantification of asbestos be modified to include only particles longer than $5 \mu\text{m}$ with widths less than $1 \mu\text{m}$ and that the use of the aspect ratio criterion be abandoned. Furthermore, in monitoring airborne asbestos particles or in determining the weight percentage of asbestos in bulk mineral samples, all $5 \mu\text{m}$ or longer particles that exhibit a fibrillar structure should be included as possible asbestos regardless of width. The potential of fiber bundles to disaggregate, in the air or in vivo, appears to be one of the most hazardous aspects of asbestos. The observation of fiber bundles should be included as part of the asbestos identification procedure. Electron and/or polarized light microscopy of the bundles would be necessary to determine the mineral composition.

Regulatory policy should also recognize that there exists a natural background of mineral particles that are longer than $5 \mu\text{m}$ and have widths less than $1 \mu\text{m}$, which are not asbestos and which, from all evidence, are not associated with any carcinogenic risk. Nonasbestiform amphiboles, pyroxenes, feldspar aluminosilicates, and even phyllosilicates may form elongated fragments when they are crushed, and some will be of this size. However, populations of these elongated mineral fragments are easily distinguished from populations of asbestiform mineral fibers and vice versa. By establishing thresholds and meaningful definitions, asbestos regulations will not be extended to harmless rock fragments unnecessarily. While we may advocate asbestos regulation based on specific widths, we fully recognize that the scientific basis for regulation comes from populations of mineral fibers and that if asbestos is present in a population of mineral particles, the full range of its dimensions will also be present. It must be stressed that our recommendations have been derived from data and literature references on minerals whose asbestiform variety is known to be carcinogenic. Given the broad range and complexity of physio-chemical properties typically associated with mineral dusts, it is not reasonable to assume similarly sized particles of different minerals will act the same way once in the human lung. Therefore, the authors do not advocate the untested application of dimensional

observations addressed in this paper to all elongated particles. Rather, their application should be restricted to asbestos until such time that their relevance to other materials can be empirically demonstrated.

It is clear, however, that dimensional parameters can be effectively applied to distinguish asbestos dust populations and other fibrous dust exposures from common cleavage fragment dust exposures. This distinction appears to be both dose and risk dependent as well. It is also reasonable to conclude that all fiber populations of similar width, length, and crystal morphology as asbestos should be viewed with caution and perhaps given deference with respect to biological testing.

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