An Evaluation of the Risks of Lung Cancer and Mesothelioma from Exposure to Amphibole Cleavage Fragments

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ABSTRACT

Amphiboles are hydrated mineral silicates five of which occur in asbestiform habits as asbestos [grunerite (amosite) asbestos, riebeckite (crocidolite) asbestos, anthophyllite asbestos, tremolite asbestos and actinolite asbestos] and non-asbestiform habits (grunerite, riebeckite, anthophyllite, tremolite and actinolite). The asbestiform varieties are characterized by long, thin fibers while non-asbestiform varieties such as cleavage fragments form short fibers with larger widths. The US regulatory method for counting asbestos fibers (aspect ratio $\geq$3:1, length $\geq$ 5 μm) does not distinguish between asbestos and cleavage fragments. The method biases toward increased counts of non-asbestiform cleavage fragments compared to long, thin asbestos fibers. One consequence of this regulatory approach is that workers can be erroneously classified as exposed to concentrations of asbestos (asbestiform amphiboles) above the U.S. 0.1f/mL exposure standard when in fact they are not exposed to asbestos at all but non-asbestiform amphibole cleavage fragments. Another consequence is that the known carcinogenic effects of asbestos may be falsely attributed to non-asbestiform amphibole cleavage fragments of the same mineral. The purpose of this review is to assess whether amphibole cleavage fragments pose the same risk of lung cancer and mesothelioma characteristic of amphibole asbestos fibers.

We identified three groups of workers exposed to non-asbestiform amphiboles: two groups exposed to grunerite (Homestake gold miners and taconite miners) and one group exposed to industrial talc containing non-asbestiform tremolite and anthophyllite in St Lawrence County, NY. In addition to assessing strength of association and exposure-response trends in the non-asbestiform amphibole cohorts, comparisons were also made with cohorts exposed to the asbestiform counterpart (positive control) and cohorts exposed to the mineral (e.g. talc) that does not contain amphiboles (negative controls).

The cohorts exposed to non-asbestiform amphiboles had no excesses of lung cancer or mesothelioma. Similar results were observed in the negative control groups, in stark contrast to the excess risks of asbestos-related disease found in the asbestos cohorts. The only possible exception is the two-fold increased risk of lung cancer where exposure was to industrial talc containing cleavage fragments of tremolite and anthophyllite. However, this risk is not considered attributable to the talc or amphibole cleavage fragments for several reasons. A similar increased risk of lung cancer was found in Vermont talc workers, studied in the same time period. Their exposure was to relatively pure talc. There was no relationship between lung cancer mortality and exposure measured as mg/m^3-years and years worked. A case-control study reported that all the lung cancer cases were smokers (or former smokers) and attributed the excess to smoking. There were two mesothelioma cases among the NY State talc workers exposed to cleavage fragments of tremolite and anthophyllite, but talc is not a plausible cause because of too short latency and potential for previous asbestos exposure. The positive controls of tremolite asbestos and anthophyllite asbestos exposed workers showed excess risks of both lung cancer and mesothelioma and positive exposure-response trends. St Lawrence, NY talc does not produce mesotheliomas in animals while amphibole asbestos does. In sum, the weight of evidence fully supports a conclusion that non-asbestiform amphiboles do not increase the risk of lung cancer or mesothelioma.

INTRODUCTION

Asbestos is a generic term applied to a group of hydrated fibrous mineral silicates. Their asbestiform habit permits them to be easily separated into long, thin, flexible, strong fibers and ultimately fibrils (single fibers). Included are the asbestiform serpentine (chrysotile) and the asbestiform amphiboles, riebeckite (crocidolite) asbestos, anthophyllite asbestos, grunerite (amosite) asbestos, tremolite asbestos and actinolite asbestos. These minerals also crystallize with non-asbestiform habits, their counterparts being lizardite or antigorite (chrysotile), riebeckite, anthophyllite, grunerite, tremolite and actinolite respectively. Crystal habit is a description of the shapes in which a certain

mineral is likely to occur, both in nature and when grown synthetically. Tremolite is a mineral in the tremolite-ferro-actinolite series that has fewer than 0.5 atoms of iron, and more than 4.5 atoms of magnesium per formula unit; actinolite has between 0.5 and 2.5 atoms of iron, and 2.5 atoms of magnesium per formula unit; ferro-actinolite has more than 2.5 atoms of iron per formula unit with the balance being magnesium.

By the early 1970's, airborne concentrations of asbestos fiber were being measured using "the membrane filter phase contrast method (PCM)". In many countries, including the U.S.A., this method was adopted for the regulatory control of asbestos. Fundamental to the method was the definition of a fiber as an elongated particle having a length: breadth ratio (aspect ratio) of at least 3:1 and a minimum length of 5 micrometers (μm). Such a definition does not allow the microcopist to distinguish between asbestos fibers and non-asbestos amphibole particles. Consequently, in work environments where there exist many elongated particles meeting the PCM fiber definition, they are counted as if they are "asbestos" even if they are neither asbestos minerals nor even amphiboles. This results in concern by workers and health professionals about health risks and potential economic impacts for companies mining ore deposits where amphibole minerals are present. This is because the amphiboles have cleavage planes such that when they are crushed they produce elongated prismatic particles called cleavage fragments.

All amphiboles that were once exploited commercially as asbestos have non-asbestiform counterparts. Hence, workers in industries where amphibole cleavage fragments are present, but not asbestos, are often erroneously reported as being exposed to asbestos based on current regulatory counting strategies and protocols. On the other hand, the evidence concerning the health consequences of exposure to cleavage fragments has never been widely understood. Industries involving exposure to cleavage fragments should not be exempt from similar controls to the asbestos industries, if elongated particles meeting the PCM definition of fibers pose qualitatively and quantitatively the same levels of health risk as their asbestiform counterparts. However, if cleavage fragments pose no or a lesser risk than the asbestos minerals, they should be regulated accordingly.

The purpose of this paper is to compare, as far as possible, the cancer risks (lung cancer and mesothelioma) for workers exposed to airborne amphibole cleavage fragments with those associated with exposure to amphibole analogues that formed asbestos fibers. Pneumoconiosis risk will not be compared because some of the minerals associated with the amphibole cleavage fragments are recognized in their own right as causing lung fibrosis (e.g.: talc and crystalline silica). However, pneumoconiosis is sometimes used to assess whether exposure is high enough and latency long enough to detect carcinogenic risk and to evaluate the exposure response.

METHODS

The extent to which the carcinogenic risks of exposure to cleavage fragments differ from those associated with exposure to asbestos was examined in several ways.

The potential of particles to cause health effects depends on the characteristics of the particles (e.g.: size, shape, respirability, solubility, toxicity, carcinogenic potential), the level and duration of exposure as well as host and other factors. It is important to determine whether amphibole cleavage fragments differ sufficiently from asbestos fibers for them to pose different levels of health risk than their asbestos counterparts. To do this requires examination of the characteristics of the particle such as dimensions, shape and density that influence fiber respirability, and fiber dimensions and biopersistence that influence carcinogenicity.

Mesothelioma and lung cancer are the health endpoints examined for comparison of the relative effects of non-asbestiform and asbestiform amphiboles. Mesothelioma is considered the more important indicator because it is both more specific and perhaps more sensitive than lung cancer. Mesothelioma is a rare cancer that acts as a marker or "signal" tumor, which is primarily associated with exposure to amphibole asbestos and has occurred in some situations after what appears to be exposure at quite low concentrations. Lung cancer is more subject to being caused by confounding exposures such as smoking, which is the primary cause of lung cancer. Thus while lung cancer might be caused by asbestos, it is an effect that is not specific to asbestos exposure.

If smoking prevalence is not known, the effects of dust exposure and smoking in the occurrence of lung cancer cannot readily be distinguished. Mesothelioma is a more sensitive and specific indicator of amphibole asbestos exposure than lung cancer in that pleural mesothelioma may occur following what are ostensibly brief exposures (Roggli 1990) and up to 80% of the cases in males may be associated with asbestos exposure (Price and Ware 2004). The exposure-response curve is thought to be non-linear for both mesothelioma and lung cancer. While the shapes of relationships are still subject to debate, pleural mesothelioma has been reported to increase less than linearly with cumulative dose. For peritoneal mesothelioma the risk is thought to be proportional to the square of cumulative exposure while for lung cancer the exposure-response lies between linear and square of cumulative exposure (Hodgson and Darnton, 2000). As some mesothelioma have been reported to occur after relatively low and perhaps brief exposures one might anticipate that if amphibole cleavage fragments act like asbestos in causing mesothelioma there might be some cases even if cleavage fragment exposures were low. For mesothelioma to be attributed to amphibole cleavage fragments the time since first exposure must be more than about 20 years and there should be no previous exposure to asbestos or other confounding etiological factors.

The mortality from lung cancer and mesothelioma are compared to that expected in age- and sex-adjusted external populations. The comparison measure is the standardised observed / expected mortality ratio or Standardized Mortality Ratio (SMR). When the incidence of lung cancer and mesothelioma are compared to that expected in age- and sex-adjusted external populations, the comparison measure is the standardised observed/expected cancer incidence ratio or Standardized Incidence Ratio (SIR). External comparisons for assessing lung cancer risk have inherent limitations such as differences in smoking and lifestyle between the study population and the external referent

population. It is generally not feasible to adjust for these differences. An SMR less than 1.5 or a statistically nonsignificant SMR is suggestive, but not conclusive, of no association. A deficit in the lung cancer SMR could be due to exposure levels below a no-effect threshold, or a few highly exposed workers diluted by many workers with low exposure, or negative confounding due to a low prevalence of smoking. A nonsignificant SMR might be due to the small size of the study population and the low power of the study to detect significant differences. Similarly, a positive finding of lung cancer could be due to differences in smoking prevalence between the study and reference populations rather than exposure to non-asbestiform amphiboles.

For mesothelioma, external comparisons using an SMR are often not possible because the expected number of cases is not known or not estimated. Therefore an internal proportional mortality ratio (PMR) is used to estimate risk of mesothelioma. PMR's have their limitations which must be taken into account when using them. For example, as a PMR can increase with length of follow-up of a cohort, attention must be given to the comparability of the follow-up period. Age differences in populations being compared are important as age determines the nature of diseases from which people die as well as the frequency of death. The ratio with total deaths to some extent adjusts for both differences in follow-up and age. Era of death may be important because of diagnostic trends. Never-the-less, comparison of PMRs between non-asbestiform amphibole-exposed and asbestos-exposed populations is a useful way to examine the question of whether non-asbestiform amphiboles cause cancer at the same rates as asbestiform amphiboles.

The actual measured risks of lung cancer and mesothelioma in persons exposed to amphibole cleavage fragments is compared to workers exposed to asbestiform amphiboles as follows:

- The lung cancer and mesothelioma experience of workers exposed to amphibole cleavage fragments is compared with the experience of workers exposed to their asbestiform equivalents. There are three main ore bodies containing non-asbestiform amphiboles where epidemiological studies have been conducted. These are a gold mine in South Dakota (grunerite-cummingtonite exposure), taconite mines in Minnesota (grunerite and other non-asbestiform amphiboles) and a talc mine in St Lawrence County, New York State (transition minerals, non-asbestiform anthophyllite and tremolite). Their experience was compared to that of workers exposed to asbestiform amphiboles. These "positive controls" were in amosite asbestos mines, mills and manufacturing facilities, anthophyllite asbestos mines and vermiculite mines (exposed to winchite asbestos also known as soda tremolite asbestos). In this report, winchite asbestos from the vermiculite mine in Montana, will be referred to as "tremolite asbestos" as this has been the terminology used in the medical literature.
- The mortality from lung cancer is examined in relation to estimated levels of exposure to "fibers" for workers exposed to asbestos and workers exposed to amphibole cleavage fragments. The existence of a positive gradient of

increasing risk with increasing exposure after taking account of potential confounders would be good evidence that the cleavage fragments were posing an increased risk of lung cancer. A negative gradient would be strong evidence against a causal association. The presence or absence of an exposure-response gradient is among the strongest evidence for or against a lung cancer association with cleavage fragment exposure because smoking is the major cause of lung cancer and rarely, if ever, can external comparisons be fully adjusted for smoking.

- The lung cancer and mesothelioma experience of workers exposed to dusts from an ore-body containing amphibole cleavage fragments is compared with that of workers exposed to dusts from a similar ore-body which does not contain asbestos or amphibole cleavage fragments. This is called a negative control. If the experience of the amphibole cleavage fragment exposed workers were worse than that of the negative control (non-cleavage fragment exposed workers), this would be suggestive of an increased risk due to the presence of asbestos cleavage fragments.
- In order to investigate this, the mortality for St Lawrence County talc miners is compared to that of talc miners where it is claimed amphiboles are not present. Also, the mortality of iron ore miners exposed to taconite rocks containing non-asbestiform grunerite and actinolite is compared to that of miners exposed to iron ore (hematite) which does not contain amphiboles.
- The biological plausibility of a difference in the potential of amphibole cleavage fragments to cause cancer compared to amphibole asbestos fibers was assessed by review of the results of toxicological studies involving asbestos and amphibole cleavage fragments. There is a clear pattern of an increased incidence of mesothelioma in animals exposed to amphibole asbestos. Observing a similar pattern for animals exposed to non-asbestiform amphiboles would be evidence supporting the hypothesis that non-asbestiform amphiboles pose a carcinogenic hazard similar to asbestos. The lack of an increased incidence of mesothelioma would be strong evidence against the hypothesis.

THE AMPHIBOLES

The crystallographic structure of amphiboles consists of double chains of silica tetrahedra. Their general chemistry incorporates $(Si, Al)_8 O_{22}(OH)_2$. The amphibole group of minerals is made up of a number of mineral series. These series result from the substitution of different elements in the structure. For example tremolite and actinolite are part of a homologous series of minerals – tremolite-actinolite-ferro-actinolite with chemistry $Ca_2 (Mg\ Fe)_5 Si_8 O_{22} (OH)_2$. Actinolite is $Ca_2 (Mg_{4.5}\ Fe_{0.5}) Si_8 O_{22} (OH)_2$ - $Ca_2 (Mg_{2.5}Fe_{2.5}) Si_8 O_{22} (OH)_2$. Ferro-actinolite is $Ca_2 (Mg_{2.5}\ Fe_{2.5}) Si_8 O_{22} (OH)_2$ - $Ca_2Fe_5 Si_8 O_{22} (OH)_2$. Actinolite with less than $Fe_{0.5}$ would be tremolite.

In practice, these minerals can have a fairly wide range of composition within the broad range of substitutions possible. The mineral names are defined where the ranges of the substituted elements fall within certain arbitrary boundaries.

Grunerite is a member of the mineral series cummingtonite-grunerite with chemistry $(Mg, Fe)_7 Si_8O_{22} (OH)_2$. As noted above, the asbestiform variety of grunerite is "amosite". As with the tremolite-ferro-actinolite series, the minerals in this series may display a range of compositions.

Anthophyllite occurs as asbestos and in a non-fibrous form and is an end member of the anthophyllite-ferro-anthophyllite series, which is chemically $(Mg\ Fe^{2+})_7\ Si_8\ O_{22}\ (OH)_2$. Anthophyllite is the name reserved for the orthorhombic Mg Fe amphibole where the ratio of Mg/ (Mg+Fe) is greater than 0.5; a lower amount of magnesium in the same type of amphibole requires the name ferro-anthophyllite.

Non-asbestiform riebeckite and crocidolite asbestos have the same chemistry which is $Na_2\ Fe_3^{2+}\ Fe_2^{3+}\ Si_8\ O_{22}\ (OH)_2$. Amphiboles exhibit prismatic cleavage, a property of nearly all samples of the amphiboles regardless of habit. There are two cleavage directions, both parallel to the length of the double-silicate chains. Cleavage across the crystal is usually poor so that the fracture of amphiboles produces long rods or prisms and repeated cleavage produces thinner rods with a rhombic outline consisting of bundles of I beams (i.e.: structural units of the amphibole) (Skinner et al. 1988). The presence of twinning or chain width errors may results in an additional direction of weakness parallel to the length, enhancing the aspect ratio of cleavage fragments (Langer et al. 1991).

PROPERTIES OF ASBESTIFORM AND NON-ASBESTIFORM AMPHIBOLES

While the chemical compositions of the asbestiform and non-asbestiform amphibole minerals are identical, the characteristics resulting from their differences in crystal habit are significant. The properties of the amphibole asbestos minerals include fibrous habit with parallel fibers occurring in bundles, fiber bundles with split or splayed ends, fibers showing curvature and fibers with high tensile strength. The high tensile strength and axial nature of asbestos means the diameters of asbestos fibrils are largely unaffected by milling. On the other hand, the low tensile strength of non-asbestiform amphiboles means that milling can reduce both particle length and width. The asbestos fibers have good heat insulation qualities, low electrical conductivity, fire resistance, and suitability for weaving. All asbestos minerals separate readily into long flexible fibrils with diameters less than about 0.5 μm and with aspect ratios (length: width ratios) ranging to well over 10,000 (Ross 1978).

In the hand specimen (that is a sample of the rock as it occurs in nature), the appearance of the non-asbestos minerals is distinctly different from that of the asbestos minerals. This difference persists when viewed by optical and electron microscopy where the non-asbestiform minerals appear as blocks, chunks or slightly elongated particles in contrast to the very evident fibrous nature of asbestos. The non-asbestiform counterparts tend not

to grow with parallel alignment. The crystals normally fracture when crushed forming cleavage fragments, some of which may appear as acicular or needle-like crystals because of the way in which amphibole minerals cleave. These cleavage fragments have diameters which on average, are much larger than those of asbestos fibers of the same length. Some asbestiform tremolite fibers with the majority of fiber diameters exceeding 0.25 μm, tested by intra-peritoneal injection in rats were found to be highly carcinogenic (Davis et al. 1991 Lee 1990). However, almost 70% of the fibers had aspect ratios greater than 10:1, 42% greater than 15:1 and 25% had aspect ratios more than 20:1. This contrasts with the observations that only about 6% of the aspect ratios of cleavage fragments exceed 15:1. The diameters of cleavage fragments appear to be rarely less than 0.25 μm (Table 1).

Fiber Diameters: The aerodynamic behavior of fibers is determined mainly by their diameter (Timbrell 1982). The majority of airborne asbestos fibers have diameters less than 0.25 μm making virtually all airborne fibers, respirable. In contrast, only very small percentages of non-asbestiform cleavage fragments have diameters less than 0.25μm (Table 1).

For the same length distribution, counting fibers by PCM will, based on fiber diameter differences, lead to higher counts of non-asbestiform cleavage fragments than asbestos fibers, because of their visibility by PCM. On the other hand, assuming the same density for fibers as for cleavage fragments, the respirability (i.e.: ability of particles to enter the alveolar regions of the lung) of the cleavage fragments will be less that that of asbestos fibers because of their larger diameters. Thus, the PCM method as presently formulated is more stringent for cleavage fragments than for asbestos fibers.

Fiber width is an important parameter determining the potential for causing both lung cancer and mesothelioma. The characteristics of non-asbestiform fiber populations are contrary to the hypothesis of carcinogenicity, while the abundance of thin asbestos fibers supports the hypothesis (Wylie et al. 1993). The evidence from experimental animal studies indicate fibers > 1 μm show no dose-response relationship with tumor incidence (<30% of population of non-asbestiform fibers ≥5 μm long are < 1 μm wide). For fibers < 1 μm (and > 5 μm long) there is an S-shaped dose-response curve with a threshold and then rapid increase in tumor incidence as the number of thin fibers increases. In populations of asbestos fibers >90% are < 1 μm wide and ≥ 5 μm long. Fiber width is also a major factor determining access to the lung. Even long, thin fibers (such as 200 μm long or more) are respirable and are found in lung tissue, while respirability decreases as width increases. Wide diameter cleavage fragments are more likely to be deposited in the upper airways and never gain access to the lower lung to cause disease. The potential for asbestos fiber bundles to disaggregate into increased numbers of even thinner fibers *in vivo* is one of their hazardous features and is not a characteristic of non-asbestiform minerals.

While it has been argued that a major determinant of carcinogenic potential is decreasing fiber width (Wylie et al. 1993), the precise role of the single parameter, diameter in carcinogenesis is still not clear (Addison and McConnell this volume).

Fiber Length: While the majority of asbestos fibers are in fact short (less than 5μm) there are airborne amphibole fibers which exceed 100 μm in length. Complete particle size data (length vs. diameter) on distributions of airborne cleavage fragments and asbestos fibers are extremely limited in number, making it difficult to compare length distribution differences. What data are available indicate that asbestos fibers are longer. For example, Dement et al. (1976) observed that the median length of "fibers" in the airborne dust in the South Dakota Homestake Gold mine was 1.10 μm as seen using scanning electron microscopy. This is less than the median length of airborne grunerite (amosite) asbestos fibers in South Africa mines and mills which were 1.83 μm and 2.53 μm respectively (Gibbs and Hwang 1980) and of grunerite (amosite) asbestos from a pipe insulation operation, 4.9 μm (Dement et al. 1976).

There is other evidence for a clear mineralogical difference between grunerite (amosite) asbestos and grunerite cleavage fragments. Virta et al. (1983) examined airborne particles of grunerite from the Homestake gold mine in South Dakota, particles of cummingtonite, hornblende and actinolite from the Peter Mitchell iron ore pit in Minnesota and particles of grunerite asbestos samples from a shipyard and an electric company. Hornblende is an amphibole that is similar to the tremolite-ferro-actinolite series but with aluminum substituted for some of the iron-magnesium as well as for some of the silicon in order to maintain the stoichiometric balance. There were two distinct particle size distributions. The non-asbestiform grunerite distributions from the mining sites were short, wide fibers (average length to width equal to 4.6 μm x 1.1 μm and 5.5 μm x 1.2 μm). The amosite fibers from the industrial sites were longer and narrower (average length to width equal to 8.2 μm x 0.4 μm and 15.6 μm x 0.5 μm respectively). Although the populations of grunerite cleavage fragment and grunerite asbestos are distinct, at the submicroscopic level it may be very difficult to be certain about the specific identity of an individual particle and may be extremely difficult, if not impossible to distinguish asbestos and non-asbestiform particles among the small number of fibers where the two fiber population overlap, especially when the source of the fiber is unknown (Langer et al. 1979).

The New York State talc deposit has been extensively studied for its mineralogy and presence of fibers and cleavage fragments. Commercially important deposits of zinc, lead, talc and wollastonite are found in the Grenville Series of sedimentary rock in St Lawrence County of NY. Three zinc mines and eleven talc mines have been worked in the area between Balmat Corners and Edwards, NY, which are about 8 miles apart. All of these holdings contain some non-asbestiform tremolite, encountered as either a gangue mineral or component of the recovered ore. Anthophyllite and transitional metals have also been identified in variable amounts both between and within mines. We will refer to the NY state talc as St Lawrence County talc.

Campbell et al. (1979) note that 5-10% of the earth's crust is amphiboles and therefore many mining industries have amphibole fragments in the gangue mineral tailings. There are at least 3 habits of non-asbestiform tremolite, none of which have the long, thin fibers characteristic of tremolite asbestos as shown in Table 2.

Long narrow fibers have been shown experimentally to be best capable of inducing mesothelioma when placed directly onto the pleura in experimental animals (Stanton et al. 1981). As there are likely to be fewer long fibers and fewer narrow diameter "fibers" in the case of exposure to amphibole cleavage fragments, compared to asbestos, it would be anticipated that cleavage fragments would pose lower carcinogenic risk.

Aspect Ratios: Asbestos fibers have thin diameters and do not readily break transversely. As a result, length/width ratios can be quite high. All "fibers" will by definition have aspect ratios >3:1. Around 30% of asbestos fibers will have aspect ratios >10:1 and nearly 20% greater than 20:1. There were very few cleavage fragments with aspect ratios greater than 10:1 The common blocky variety of non-asbestiform tremolite had less than 2% in the >10:1 class. The acicular and fibrous habits had more particles in the range between 10:1 and 20:1 category than did the blocky variety, but none of the non-asbestiform varieties had more than 0.5% particles in the range between 20:1 and 50:1 and none had any particles >50:1 Nearly 90% of the blocky and acicular habits did not meet the regulatory definition of a fiber. If only fibers that meet regulatory dimensions are counted, 1/100 of non-asbestiform particles have aspect ratios >20:1 while about 35/100 asbestiform tremolite particles have >20:1 aspect ratios (Table 2). A composite aspect ratio distribution reported in the Pictorial Atlas of Mineral Fibers (This Monograph) showed that for non-asbestiform particles with an aspect ratio of 3:1 or greater and length greater than 5um, 6% on average exceed an aspect ratio of 15:1 and for asbestiform particles, 80% on average exceed an aspect ratio of 15:1. The 3:1 aspect ratio is used principally to eliminate particulates and fiber clumps and improve the precision and accuracy of fiber counts. It is not a defining characteristic of asbestos fibers (Langer et al. 1991).

Wylie et al. (1993) points out that aspect ratio is not a useful parameter for sizing as it is dimensionless, provides no information on width, shows no association with risk of disease, and therefore is of little use in the discussion of risk or exposure.

Biopersistence: As far as we were able to ascertain, there have been no systematic studies of the biopersistence of cleavage fragments. It is known that for long amphibole asbestos fibers, the half-life is extremely long (Berry 1999). However, short fibers (i.e.: less than 20 μm in length) can be removed from the lung by macrophage action (Allison 1973; Bernstein et al. 1994). For later phases of lung clearance, particle solubility is a key factor. In the absence of data, there is no basis for concluding that cleavage fragments will be removed any faster than asbestos fibers during that phase. However, because of their shorter lengths, cleavage fragments are much more likely to be removed more rapidly than amphibole asbestos fibers during the early lung clearance phase. This will reduce their potential for carcinogenic action.

Ilgren (2004) notes dissimilarities that make cleavage fragments much less biopersistent than amphibole asbestos fibers. Surfaces of cleavage fragments have a high density of surface defects, which are preferred sites for dissolution from intracellular acidic enzymes of phagocytic cells that have engulfed them. Amphibole asbestos fibers are smooth and defect free and highly acid resistant. Cleavage fragments are weak, brittle

and inflexible because of there weak surface structure, which is further weakened by chemical dissolution. The tensile strength of amphibole asbestos fibers is 20-115 times greater than the non-asbestiform amphibole variety. This difference becomes greater as width decreases and biological relevance more pronounced. When long, thin biologically relevant cleavage fragments are deposited in the lung alveoli and engulfed by macrophages, the fragment begins to dissolve on all surfaces. They are already weak and inflexible and become thinner and weaker (greater surface area, more surface defects) with increasing susceptibility to chemical dissolution and breakage. The defect-free surface of the amphibole asbestos fiber is better able to resist acid attack. Many of the asbestos fibers are too long to be completely engulfed. Attempts at engulfment produce protein deposits that form an "asbestos body" and eventual death of the cell. In short, biopersistence is a characteristic of carcinogenesis. It is reasonable to conclude that cleavage fragments are likely to be far less bio-persistent than asbestos fibers.

Nolan et al (1991) compared activity of tremolite cleavage fragments with that of samples of tremolite-actinolite asbestos. For the same surface area, tremolite cleavage fragments had lower ability to alter the permeability of red blood cells than amosite and approximately the same membranolytic activity as anthophyllite and crocidolite. The surface charge of non-asbestos tremolite was about 70% less than asbestos analogues. Schiller et al (1980) reported that asbestos fibers and cleavage fragments of the same dimensions had the same net negative surface charge. Short fibers and cleavage fragments have a smaller net charge than highly elongated particles.

COMPARISON OF THE RISK OF HEALTH EFFECTS IN PERSONS EXPOSED TO ASBESTIFORM AND NON-ASBESTIFORM GRUNERITE

Grunerite Occurrence: Grunerite is the mineralogically correct name for amphiboles of the cummingtonite-grunerite series in which iron is at the 50% point in the 100 times Fe / (Fe+Mg)) ratio. Amosite (from the "Asbestos Mines of South Africa") is the commercial asbestiform product that was used in insulation and building materials. Grunerite asbestos is no longer mined.

The non-asbestiform variety of cummingtonite-grunerite (C-G) has no commercial use *per se* other than a aggregate but occurs in nature in conjunction with other asbestiform and non-asbestiform amphiboles and other minerals in ore deposits mined for other purposes. In the USA, ore containing C-G has been mined in at least 2 locations. One location is the Homestake gold mine in Lead, SD, where gold had been extracted since 1876. The other location is Mesabi Range where taconite has been mined since the 1950's and shipped to Silver Bay, Minnesota for extraction of iron. Because of its relationship to grunerite (amosite) asbestos, studies were initiated to determine if these minerals had similar pathogenicity. There have been four cohort studies of Homestake gold miners (Gilliam et al. 1976, McDonald et al. 1978, Brown et al. 1986, Steenland and Brown 1995) and two studies of taconite containing amphiboles; one of the Reserve iron

deposit (Higgins et al. 1983) and the other of the Erie-Minntac mine (Cooper et al. 1988, 1992) (Table 3).

Taconite iron ore contains actinolite and cummingtonite-grunerite (probably predominantly grunerite). In 1973, elongated grunerite particles, said to be similar to grunerite (amosite) asbestos, were found in the Duluth, Minnesota water supply. The source was mine tailings from the process plant at Silver Bay, Minnesota (MN) serving the Peter Mitchell Pit. In a suit against the Reserve Mining Company, the US Environmental Protection Agency (EPA) claimed that some of the particles were asbestos. This finding initiated a series of studies to determine if there were effects on the Duluth residents (Cook et al. 1974, Masson et al. 1974, Levy et al. 1976, Sigurdson et al. 1981). These studies of human health are not considered further because they are ecological studies without identification of individual exposures or responses, because the route of exposure is via ingestion and because experimental studies and the epidemiological studies described below have provided no evidence in support of any gastrointestinal cancer risk from ingestion. The other health studies are of taconite miners and millers (Clark et al, 1980, Higgins et al. 1983, Cooper et al. 1988, 1992).

A reasonably valid comparison can be made between the health risks of workers exposed to amosite asbestos in mining and manufacture and the health risks of workers involved in the extraction of minerals from ore bodies containing non-asbestiform grunerite.

GRUNERITE (AMOSITE) ASBESTOS

Amosite is the trade name given to a mineral that was previously mined in Penge region in the Transvaal of South Africa. The mineralogical name is grunerite asbestos. In the bulk specimen the fibers can be several inches long. The color, ranging grey to brown depends on whether the fiber was mined from a weathered or un-weathered zone. The size distribution of the airborne fibers in the mine and mill have been reported by Gibbs and Hwang (1980). In mining and milling 12.6% and 6.6% respectively of airborne fibers exceeded 5 μm in length when all particles with length to breadth ratios greater than 3: 1 were counted using transmission electron microscopy combined with light optical microscopy. The median lengths for mining and milling were 1.83 μm and 2.53 μm respectively. The median diameters were 0.20 to 0.26 μm depending on the process and there were no airborne fibers with diameters exceeding 3 μm.

Grunerite (Amosite) Asbestos Exposed Cohort Studies

The studies of cohorts of amosite-exposed workers include miners and millers in South Africa (Sluis-Cremer et al. 1992) and workers engaged in amosite insulation manufacture (Acheson et al. 1984, Seidman et al.1979, 1986, Levin et al. 1998). Cohorts where the exposure also included riebeckite (crocidolite) asbestos and/or chrysotile have been excluded from consideration as the ratios of the risks of mesothelioma associated with these various asbestos fiber-types have been reported to be in the ratio of 500:100:1 for riebeckite (crocidolite) asbestos, grunerite (amosite) asbestos and chrysotile respectively

(Hodgson & Darnton 2000). For lung cancer the differences are not as great or as clear-cut. Crocidolite and amosite pose similar exposure-specific risks for lung cancer (about 5% excess per f/mL-yr), while the risk from chrysotile is estimated as 0.1-0.5% of the risk of crocidolite and amosite. Thus the risk differentials between the amphibole asbestos (crocidolite, amosite) and chrysotile for lung cancer are about 10-50:1 (Hodgson & Darnton 2000). It should be noted that the chrysotile in these risk estimates included sources where the chrysotile contained traces of tremolite, the form of which was not investigated or reported.

Only one of the cohorts with pure grunerite (amosite) asbestos exposure was examined for a quantitative exposure-response relationship (Seidman et al. 1986). There was a clear increase in the risk of lung cancer with increasing exposure expressed in fibers/mL-years.

NON-ASBESTIFORM GRUNERITE COHORTS

Several groups of workers from Homestake gold mine and the Minnesota taconite deposits have been exposed to cleavage fragments of grunerite and studied to assess possible "asbestos-related" diseases (Table 3). The non-asbestiform amphiboles present in these mines generally crystallize in a prismatic habit with well-developed cleavage so breaks occur both perpendicular and parallel to particle length.

Taconite miners: There are several studies of workers who were exposed to cummingtonite-grunerite particles from the above deposits. These include the Reserve taconite miners (Higgins et al. 1983) and the Erie-Minntac taconite miners (Cooper et al. 1988, 1992). Another group of Iron ore (hematite) miners in Minnesota is included for comparison as a negative "control" since the hematite ore does not contain amphiboles (Lawler et al. 1985).

Taconite is an iron-bearing rock that by 1978 was supplying nearly 90% of the iron ore used in the US iron and steel industry. More than 60% of this came from the Mesabi Range that is 110 miles long and 1-3 miles wide extending east to west from Babbitt, Minnesota to Grand Rapids, Michigan. Iron ore has been mined along the Mesabi Range since about 1892 (Langer et al. 1979). Taconite contains 20-50% quartz and 10-36% magnetite with smaller amounts of hematite, carbonates, greenalite, chamosite, minnesotaite, stilpnomelane and amphiboles which are non-asbestiform minerals in the cummingtonite-grunerite series, actinolite and hornblende, (Nolan et al. 1999).

Taconite from the eastern end of the Mesabi Range contains non-asbestiform cummingtonite-grunerite (most probably grunerite) and actinolite with most elongated particles having aspect ratios greater than 3:1 and length less than 10 μm and are mostly acicular cleavage fragments. Respirable dust concentrations in the Reserve mining company ranged from about 0.02 mg/m^3 to 2.75 mg/m^3 at a crusher. The modal range in most jobs was 0.2-0.6 mg/m^3, with occasional concentrations of 1-2 mg/m^3 but mostly below 1 mg/m^3. Fiber concentrations were generally < 0.5 fibers/mL. Area samples

suggest no change in concentrations between 1952-1976 and exposure estimates were based on samples collected in the period 1975-8 (Higgins et al. 1983).

In the Reserve mining cohort (Higgins et al. 1983) there were no exposure-response relationships between lung cancer and cumulative exposure to silica dust or taconite (measured as mg/m^3-years) and no excess lung cancer based on the SMR. There were no cases of mesothelioma. Higgins et al. (1983) concluded that the lack of any increased risk of cancer is not surprising given the low silica and fiber exposure plus movement of miners to lower exposed jobs with increased seniority. The average and maximum latencies of lung cancer were 15 and 25 years. At high exposure levels the latency for pneumoconiosis has been as short as about 5 years or even less. As dust levels have declined latency is more in the range of 13-20 years. The cohort was also relatively young with 5% overall mortality and the number of cases was small with 15 lung cancer cases (17.9 expected), 8 with >15 years since hire (7.9 expected). Exposure-response functions were estimated using cumulative total dust exposure and cumulative silica dust exposure in mg/m^3-years as the exposure metrics. The relationship with total dust exposure, which is of interest from the standpoint of cleavage fragments, was not monotonic and the SMRs were at or below 1.0 in the three highest exposure categories. Higgins et al. (1983) concluded there was no suggestion of an association with lung cancer.

In the Eastern Mesabi district, west of the Reserve Mine are the Erie and Minntac operations. The Minntac ore has had a different metamorphic history and contains the lowest percentage of amphiboles. The Erie ore is a blend of the high and low amphibole ores with more amphiboles than Minntac but less than Reserve. Nolan et al. (1999) reported 28-40% quartz in dust from the Erie mine and 20% quartz from the Minntac mine. Concentrations of fibrous particulates were nearly always <2 fibers/mL. These particulates were >5 μm in length and included elongated cleavage fragments.

The Erie-Minntac cohort of taconite miners (Cooper et al. 1992) showed "no evidence to support any association between low-level exposure to non-asbestiform amphibole particles or quartz" and lung cancer. The Erie-Minntac cohort is older and larger than the Reserve cohort with 31% mortality and a minimum time since hire of 30 years. There were deficits in lung cancer SMRs for miners ever working in high or medium dust areas and no trend with years worked. There was no analysis by cumulative exposure.

There was one case of mesothelioma that had been reported in the initial study (Cooper et al. 1988). In this case, exposure to taconite began 11 years before death. Previous employment included work in the railroad industry as a locomotive fireman and engineer. Nolan et al. (1999) suggest it is unlikely that the mesothelioma is related to taconite because mesothelioma generally occurs after at least 25 years although latencies as short as about 18 years have been reported among insulation workers where asbestos exposure can be quite high. The more likely cause is from the railroad employment where there are opportunities for exposure to commercial amphibole asbestos from thermal lagging

used on steam locomotives. Also, the time since hire in the railroad jobs is more consistent with the long latency characteristic of mesothelioma.

Although deposits of grunerite asbestos large enough for commercial exploitation are very rare, small deposits are occasionally found as a gangue mineral in a limited area of a mine that is otherwise asbestos-free. Nolan et al. (1999) described the occurrence of such a localized seam of grunerite asbestos in a small portion of an iron ore mine otherwise free of asbestos. Samples from the seam revealed three kinds of morphological types or habits. One kind was the asbestiform habit with fibers occurring as parallel fibrils and forming polyfilamentous bundles. There were two non-asbestiform habits, namely splintery fibers and massive anhedral nodules, which when crushed may form elongated cleavage fragments that morphologically resemble some asbestiform fibers. To evaluate potential asbestos exposure, 179 personal air samples were collected for all relevant jobs associated with work on this localized seam. The mean concentration of fibers ≥ 5 µm in length and aspect ratio $\geq 3:1$ was 0.05 f/mL and the highest was 0.39 f/mL. All sample results were below the Mine Safety and Health Administration (MSHA) standard of 2 f/mL but 13% were above the Occupational Safety and Health Administration (OSHA) standard of 0.1 f/mL.

Nolan et al. (1999) estimated the potential lifetime risk of lung cancer and mesothelioma based on a worst case scenario. Lifetime lung cancer risks of 0.1 and 0.6 /100,000 for nonsmokers and smokers respectively were estimated using the EPA risk model and assuming a linear exposure-response relationship, age of 45 years at beginning of exposure and continuous exposure for 22 days to 0.05 asbestos fibers/mL. This was considered approximately equivalent to smoking 2 or 12 cigarettes over a lifetime.

Nolan et al. (1999) also estimated risk based on grunerite asbestos fiber content in the lungs of mesothelioma cases from a British grunerite (amosite) asbestos factory (Gibbs et al. 1994). Nolan et al. (1999) estimated it would take 75-265 years of daily 8-hour shifts to inhale the number of fibers found in the lungs of the mesothelioma cases, assuming no clearance. Fiber concentrations were about 45% higher in the lung cancer cases, suggesting about 100-380 years to reach similar fiber content in iron ore miner lungs.

Nolan et al. (1999) suggested concentrations were a minimum of 30 fibers/mL in the Paterson, NJ grunerite (amosite) asbestos factory (Seidman et al. 1986). No mesothelioma cases had less than 6 months employment and 20 years latency. Assuming breathing 0.05 fibers/mL from the gangue rock in the iron ore mine, Nolan et al. (1999) estimated it would take about 300 years to achieve the minimum exposures estimated for the mesothelioma cases in the Seidman et al. (1986) cohort.

Hematite Miners as Negative Control: Hematite from the Mesabi Range in Minnesota is a mixture of about 83% hematite (Fe_2O_3) and limonite ($HFeO_2$). The hematite deposit differs from taconite deposits in that there is the absence of all amphiboles. Some silica (about 8%) is present plus possibly low levels of radon.

Lung cancer mortality was not associated with years worked. Mesothelioma was not mentioned. Lawler et al. (1985) considered that the lack of an excess risk of respiratory disease was possibly due to strict prohibition of smoking while underground, apparent absence of significant radon daughter exposure and/or the aggressive silicosis control program. No estimates of dust exposure are available.

Gold Miners: There are several studies of miners at the Homestake gold mine in South Dakota (Gilliam et al. 1976, McDonald et al. 1978, Brown et al. 1986, Steenland & Brown 1995).

Ore containing cummingtonite-grunerite has been mined to extract gold in Lead, South Dakota, since 1876. An analysis of airborne "fibers" using electron diffraction and x-ray spectrometry was reported to show that it contained "80-90% amphiboles" with the amphiboles being "60-70% fibrous grunerite", "1-2% fibrous cummingtonite" and "10-15% fibrous hornblende" (Gilliam et al. 1976). The free silica content of the respirable airborne dust was reported to be 13.1%. Low concentrations of arsenopyrite were also reported. The NIOSH researchers identified the fibrous grunerite as grunerite (amosite) asbestos. Closer examination of the fiber population statistics suggests strongly that the fibrous grunerite particles are non-asbestos amphibole cleavage fragments as noted in the section on fiber length.

Measurements of airborne concentrations of "fibers" in the mine in 1974 showed concentrations to be about 0.25 f/mL greater than 5 μm with the highest concentration being 2.8 f/mL based on 200 samples (Gilliam et al. 1976). The mean total fiber concentration in the mine as determined by electron microscopy was 4.82 (±0.68) f/mL with the concentration of fibers greater than 5 μm being 0.36 (±0.08) f/mL. Approximately 94% of fibers were less than 5 μm in length, the mean fiber diameter was 0.13 μm and the mean "fiber" length was 1.1 μm. The US Bureau of Mines in 1960 reported average airborne dust concentrations of 1.7 million particles per cubic foot (mppcf) (Gilliam et al. 1976). This suggests a ratio of f/mL to mppcf of about 0.25/1.7 = 0.146 f/mL per 1 mppcf.

Exposure-response relationships were developed by several of these researchers. Only the results of the latest follow-up by Steenland and Brown (1995) will be considered. However, the exposure-response developed by McDonald et al. (1978) based on semi-quantitative exposure estimates is of interest because this cohort of 1,321 men with 21 or more years of service clearly had adequate latency to observe the occurrence of mesothelioma or increase in lung cancer. There were 17 deaths from respiratory cancer but no convincing evidence of an excess of respiratory cancer or grunerite related mesothelioma. This contrasts with the results of the earlier study by Gilliam et al. 1976, which involved 440 men who had worked more than 5 years underground. They reported 10 deaths from neoplasms of the respiratory system with 2.7 deaths expected. Conclusions from the study by Gilliam et al. (1976) are weakened by the fact that the study population is small, the SMR for men with latency less than 20 years (5.4) was greater than that for men with latency greater than 20 years (3.2) (McDonald et al. 1978), and the results are contradictory to later follow-up studies of the entire cohort (Brown et

al. 1986, Steenland and Brown 1995). While the reason for the high overall SMRs is not clear, selection bias is possible as the cohort was comprised of volunteers participating in a 1960 silica X-ray survey. The participation rate of workers from the mine was not reported.

The Homestake study comprises the largest and oldest cohort of workers exposed to non-asbestiform amphiboles with 47% mortality. In the Steenland and Brown (1995) study, there was a 2.6-fold excess of silicosis and a 3.5-fold excess of respiratory TB that were significantly associated with cumulative exposure and SMRs were significantly elevated in the highest exposure category for both dust-related diseases. Lung cancer was not associated with cumulative exposure in the SMR exposure-response analysis and there was a negative trend in the nested lung cancer case-control portion of this study, i.e., as exposure increased there was a trend for lung cancer risk to decrease. There were no mesothelioma deaths.

The mesothelioma and lung cancer experience of the grunerite (amosite) asbestos and non-fibrous amphibole workers will be compared separately below.

COMPARISON OF MESOTHELIOMA EXPERIENCE

One method of assessing whether non-asbestiform grunerite acts similarly to grunerite (amosite) asbestos is to compare the proportional mortality from mesothelioma in to grunerite (amosite) asbestos exposed workers and in non-asbestiform grunerite exposed workers. Mesothelioma is a cancer which can clearly be caused by amosite without known confounders such as smoking, although there are a small number of other potential causes (Pelnar 1988, Price and Ware 2004). Hodgson and Darnton (2000) argue that there is unlikely to be a threshold for asbestos-related mesothelioma, but that the exposure–response function may be non-linear. As previously discussed about 80% of mesotheliomas are asbestos related, mesothelioma is a more specific indicator of amphibole asbestos exposure and also more sensitive as there may be an excess mesothelioma risk in the absence of an excess lung cancer risk (Hodgson and Darnton 2000).

The measure of mesothelioma mortality used in this study is the percent of total mortality (labelled PMR in this context). To assume a work-related mesothelioma in the non-asbestiform grunerite cohorts there should be no previous asbestos exposure, no exposure to other potential etiological factors such as erionite or therapeutic radiation and the time of death should probably be 20 or more years since hire since exposure, or 15 or more years since hire if exposure was intense. Lanphear and Buncher (1992) estimated that for 1,105 mesothelioma cases meeting strict histological and exposure criteria, 99% had a latent period (time since first exposure) of 15 years or more and 96% of 20 years or more. The median latent period was 32 years with a range of 13 to 70 years. The probability was 0% for <10 years and 0.45% for 10-14 years.

Although there were only 19% of persons dead in the grunerite (amosite) asbestos cohorts combined, there was an overall proportional mortality from mesothelioma of 1.2%. In

contrast, 23% of persons were dead in the non-asbestiform cohorts combined and no mesothelioma linked to the exposures in the non-asbestiform cohorts (or 0.03% if the non-exposure related deaths are counted). It is well recognized that the proportion of mesothelioma increases with long follow-up as mesothelioma increases as a cubed function of the time since first exposure and so would increase as the percentage of deaths increase. Certainly on present evidence there is no increased risk of mesothelioma in non-asbestiform amphibole exposed workers at the levels of exposure encountered in these industries (Tables 3 and 4, Figure 1).

In view of the fact that there was no detected increase in mesothelioma, one would not anticipate an increased risk of lung cancer due to exposure to fibrous dust, as usually in amphibole-exposed workers the exposure necessary to produce an increased risk of lung cancer is much greater that that required to increase mesothelioma risk.

COMPARISON OF LUNG CANCER EXPERIENCE

There are statistically significant excesses of respiratory cancer in all the grunerite (amosite) asbestos industries (except mining). In contrast, it is very clear that, with the exception of the first small study of Homestake gold miners (Gilliam et al. 1976), there is no increased risk of lung cancer in the non-asbestiform amphibole exposed industries. The results from the study by Gilliam have not been reproduced in subsequent studies with complete ascertainment of the cohort and longer follow-up (Steenland and Brown 1995, McDonald et al. 1978). In the taconite-exposed miners there were some statistically significant deficits of respiratory cancer. This is in spite of the fact that workers in those industries are exposed to significant crystalline silica in addition to non-asbestiform grunerite (if silica increases lung cancer risk).

Another way to examine this question is to compare the exposure-response relationships for the various studies. In Table 5 the exposure-response relationships for the studies by Seidman et al. (1986) and by Steenland and Brown (1995) are compared. While both have limitations in their exposure estimates, there is clearly no increasing trend of lung cancer with increasing exposure to non-asbestiform grunerite (and other non-asbestiform amphiboles). The exponential increase in pneumoconiosis (silicosis) with increasing exposure suggests exposure produced fibrotic but not carcinogenic effects (ratio lung cancer/ silicosis mortality = 1.25) In contrast there is a steep and statistically significant slope for the lung cancer mortality in the grunerite (amosite) asbestos insulation manufacturing plant (lung cancer/asbestosis mortality ratio = 6.8) (Figure 2).

Acheson et al. (1984) reported concentrations of 30 fibers/mL in the late 1960s in the factory using grunerite (amosite) asbestos. Exposures were probably much dustier before 1964 with improved conditions after 1964. However, Acheson et al. (1984) did not attempt to assess exposure-response trends.

It seems clear that exposure to non-asbestiform grunerite cleavage fragments and/or "fibers" at cumulative exposures below about 30 f/mL-years has not resulted in an

increased lung cancer risk for workers. The risk for workers exposed to grunerite (amosite) asbestos was increased at cumulative exposures <6 f/mL-years.

OVERALL CONCLUSION CONCERNING ASBESTIFORM AND NON-ASBESTIFORM GRUNERITE

It is evident that the "fibers" to which the non-asbestiform amphibole workers were exposed were considerably shorter (and wider) than those to grunerite (amosite) asbestos workers were exposed. While both studies of grunerite (amosite) asbestos and non-asbestiform grunerite (plus other non-asbestiform amphiboles) may have limitations as far as estimates of fiber exposure are concerned, the results indicate very large differences in the mortality from mesothelioma and from lung cancer from both external and internal comparisons. It seems unlikely that errors in the exposure estimates are responsible for these very large differences as the grunerite (amosite) asbestos factory shows a definite increase in risk of lung cancer with increasing exposure while there is no statistically valid increase in trend with non-asbestiform grunerite. The results are consistent with cleavage fragments having no (or negligible or very low) apparent carcinogenic hazard for mesothelioma and lung cancer in contrast to the obvious carcinogenic hazard shown by their asbestiform counterparts.

The Evidence from Studies of Talc and Vermiculite Exposed Workers

The Mineral Talc: The term talc is used in two ways. First, it is a term applied to a commercial or industrial product that contains finely divided mineral or rock powder that usually, but not always contains the mineral talc as its main component. Second, it can refer to the mineral talc which is a phyllosilicate mineral with the chemical formula Mg_6 Si_8 O_{20} $(OH)_4$. Since talc is a metamorphic mineral it is often associated with other minerals and is rarely found in its pure form. Co-exposures are specific to each site. Tremolitic talc is a commercial product that contains a high proportion of the amphibole tremolite in addition to the mineral talc; it also can contain other minerals including anthophyllite, a transitional talc/anthophyllite mineral as well as antigorite, lizardite and quartz. Cosmetic and pharmaceutical talcs have strictly controlled mineral contents; industrial talcs may contain other minerals.

Structurally, talc occurs in sheets that can be separated by slight pressure, so that when milled, talc can form cleavage fragments or elongated talc platelets (Wild et al.2002).

THE NEW YORK AND NORWEGIAN TALC DEPOSITS

There are at least two talc deposits containing non-asbestiform tremolite and anthophyllite which have been studied, one in New York State and one in Norway (Table 6). The best known and best characterised is the industrial talc in New York. There has been considerable discussion in the literature concerning whether the tremolite and

anthophyllite present in this talc is asbestiform or non-asbestiform. However, the evidence is supportive of non-asbestiform amphiboles (Skinner et al. 1988). Norwegian talc contains tremolite and anthophyllite said to be in trace amounts. However, the mineralogy of this talc is less studied and the cohort of exposed miners/millers is much smaller.

The health experience (mesothelioma and lung cancer mortality) of these two cohorts of talc workers exposed to non-asbestiform amphiboles will be compared to 1) anthophyllite asbestos miners, 2) to workers exposed to vermiculite contaminated with tremolite asbestos; and 3) to workers exposed to talc that is not contaminated with amphiboles from Vermont, Italy, France and Austria.

New York Talc: The St Lawrence County, New York talc deposit has been extensively studied for its mineralogy and presence of fibers and cleavage fragments. The mineralogy is complex and there has been a long and ongoing debate about the amphiboles present in the Gouverneur, NY talc, which is the only mine currently operating in the region. Dement and Zumwalde (1980) concluded that bulk Gouverneur talc samples contained both amphiboles (4.5-15% anthophyllite and 37-59% tremolite) and serpentines (10-15% lizardite and antigorite) and less than 2.6% free silica as determined by X-ray diffraction and petrographic microscope analysis. It appears that the mineral identified as anthophyllite by Dement and Zumwalde (1980), is, at least in part, a mixed phase mineral with talc evolving from the anthophyllite (Kelse and Thompson 1989). The talc also contains talc fibers. Dement and Zumwalde (1980) considered the airborne dust 'fibers' greater than 5 μm long to contain upward of 70% amphibole asbestos. Based on electron microscopy, Dement and Zumwalde reported that: "In the mine 38% of all fibers were anthophyllite. 19% were tremolite and 39% were unidentified". In the mill 45 per cent of all fibers were anthophyllite, 12 per cent were tremolite and 38 per cent were unidentified. Three percent of the fibers in the mine and 2 percent in the mill reportedly gave chrysotile electron diffraction patterns. According to Thompson (1984) and Harvey (1979) all the amphibole minerals are cleavage fragments and in the non-asbestiform habit and it has now been shown that once the talc fibers are recognized, the talc does not contain asbestiform tremolite or asbestiform anthophyllite (Kelse and Thompson 1989, Dunn Geoscience Corp 1985, Langer and Nolan 1989, Virta 1985, Crane DT 1986, Wylie 1987, Wylie 1993, Nolan et al. 1991).

A survey of the many mortality studies of workers exposed to St Lawrence County, NY talc is summarised in Appendix 1. Most of these have been variations of the original NIOSH cohort study (Brown et al. 1979, 1980). We will focus on the nested case-control study, which addressed three of the hypotheses raised about reasons for the increased lung cancer, namely smoking, other work exposures, and short-term workers (Gamble 1993). Honda et al. (2002) added 6 more years update and estimated quantitative cumulative exposure to talc dust to address the question of exposure-response (Oestenstad et al. 2002).

Gamble (1993) conducted a case-control study nested in the Brown et al. (1990) cohort of NY talc workers. There were 22 cases and 66 controls matched on date of birth and date of hire. All cases were either smokers (91%) or ex-smokers compared to 27% nonsmokers, 73% smokers or exsmokers among controls. Negative trends were consistently observed by years worked after controlling for smoking, 20 or more years latency, and exclusion of short-term workers. Lifetime work histories suggested no apparent association with non-talc exposures or non-Gouverneur talc exposures. The author concluded that "after adjustment for...smoking and the postulated role of very high exposures of short-term workers, the risk ratio for lung cancer decreases with increasing tenure." The time occurrence of lung cancer was consistent with a smoking etiology, and was not consistent with a mineral dust relationship.

Honda et al. (2002) assessed cancer and non-cancer mortality among white male Gouverneur talc workers. The cohort analyzed for cancer endpoints consisted of 809 workers employed 1947-1989 and alive in 1950. The cohort analyzed for non-cancer endpoints consisted of 782 men employed during 1960-1989. The important additions in this study were 6 more years of follow-up (through 1989) and internal exposure-response analyses with cumulative exposure to talc dust as the exposure variable. Smoking status was not taken into account. The internal comparisons by cumulative exposure (mg/m^3-yrs) showed a significant monotonic decrease in lung cancer risk with increasing exposure. The RR was 0.5 (0.2-1.3) in the highest exposure category. Mortality from 'other NMRD' and pulmonary fibrosis showed monotonic increases in risk as exposure increased with 2-fold and 12-fold increased risks in the highest exposure categories. (Figure 3)

Honda et al. (2002) concluded that talc dust was unlikely to have a carcinogenic potency similar to asbestos for several reasons. First, there were negative exposure-response trends. Second, although lung cancer mortality was increased nearly 4-fold among miners (SMR of 3.94; 95% CI 2.33-6.22, 18 observed (obs)) it was not excessive among millers (SMR of 1.28; 95% CI 0.51-2.63; 7 obs) although exposure was similar in both groups (medians of 739 mg/m^3-years and 683 mg/m^3-years respectively). Third, the cumulative exposure was low for lung cancer cases compared to that of other workers. For example, if median cumulative exposure is set at 1.0 for lung cancer decedents, the relative median cumulative exposure is 1.1 for ischemic heart disease, 1.5 for all decedents, 3.5 for NMRD as underlying or contributory cause of death, and 10.8 for pulmonary fibrosis.

Honda et al. (2002) conclude that the lung cancer excess is unlikely to be due to talc dust *per se*. The reasons for the excess are unclear. Possible explanations for the excess include confounding by smoking or other risk factors or an unidentified constituent in the ore or mine environment that is poorly correlated with talc dust.

Norwegian Talc: Norwegian talc contains trace amounts of quartz, tremolite and anthophyllite; the main minerals are talc and magnesite. Small amounts of magnetite, chromite, chlorite, and antigorite are in the ore, while the surrounding rock contains small amounts of serpentine, mica, feldspar, calcite, and non-asbestiform amphiboles

(hornblende, tremolite). Personal air samples were collected 1982-4. Exposures were somewhat higher in the mine with a range for total dust of 0.94-97.4 mg/m^3 and peaks at drilling of 319 mg/m^3. The range in the mill was 1.4-54.1 mg/m^3 with peaks in the storehouse of 109 mg/m^3. Fibers of tremolite, anthophyllite and talc with aspect ratios >3:1 by optical microscopy ranged from 0.2-0.9 f/mL (Wergeland et al. 1990).

The Norwegian male talc cohort consisted of 94 miners employed at least 1 year in talc-exposed jobs 1944-1972 and 295 millers employed at least 2-years 1935-1972 (Wergeland et al.1990). In contrast to NY talc workers, this is a generally healthy work population with a significant deficit in all-cause mortality (SMR of 0.75; 0.62-0.89), which was below expected in both mine and mill. There were only 6 incident cases of lung cancer and 6.49 expected for an SIR of 0.92. There was a small positive trend with years worked because there were zero cases in the low tenure group but no significant excess (SIR) in the 2 groups with longer tenure. There were two lung cancer cases among miners (1.27 expected) and there were more expected (5.22) than observed (4) in the mill. There was no excess of NMRD cases (3 cases of pneumonia), but numbers were too small to make any conclusions. There were no cases of mesothelioma.

It is unclear why the mortality and incidence of cancer are so far below expected. There is no excess NMRD mortality and no cases of pneumoconiosis as a cause of death despite the apparently very high dust exposures. There were 3 cases of pneumoconiosis as a contributing cause of death: 2 cases with silicosis, 1 case with talcosis. In 1981, smoking histories were obtained from 63 of 94 miners. A reduced prevalence of smoking is an unlikely cause of the reduced mortality as only 8% were nonsmokers. In view of the small size of this cohort, interpretation is difficult.

NON-ASBESTIFORM AMPHIBOLES IN SOUTH CAROLINA VERMICULITE

There are several small vermiculite pits in South Carolina containing nearly 50% tremolite/actinolite but is believed to be virtually free of fibrous tremolite (McDonald et al. 1988). Mining and the first part of the milling process are carried out wet. Four types of elongated fibers were identified in air samples using analytical transmission EM and energy dispersive X-ray spectroscopy (EDSX): tremolite-actinolite (48%), vermiculite fragments (8%), talc/anthophyllite (5%), iron rich fibers (23%) and the rest unidentified. Mean fiber size was 1.1 μm diameter and 12.7 μm long. Mean fiber length seems to be quite large for the airborne fibrous dust cloud to be totally cleavage fragments. The mean exposure was 0.75 f/mL-yrs. Nolan et al. (1991) found tremolite cleavage fragments (some of which were >10:1 aspect ratio), but found no asbestos.

The mortality study comprises a small cohort of 194 men with 6 months or more tenure before 1971 and a minimum latency of 15 years. There were 51 total deaths and an all-cause mortality of 1.17 (0.87-1.51). There were 4 deaths from lung cancer and 3 from NMRD with SMRs of 1.21 and 1.22 respectively. There were no cases of mesothelioma and no deaths from pneumoconiosis. There was a negative exposure-response trend between cumulative fiber exposure and lung cancer (Figure 4). Three of the 4 cases were

in the lowest exposure category of <1 f/mL-yr (SMR = 1.71) and the 4th case was in the medium exposure category of 1-10 f/mL-yr (SMR = 0.73). Given the low fiber exposures (mean 0.75 f/mL-yrs) and the small sample size the authors concluded there was inadequate power to detect an adverse effect in this population (McDonald et al. 1988).

The health experience of workers at this mine would be of considerable interest for comparison with the miners in Montana where exposures involve asbestiform "tremolite" and other fibers. Exposure levels were so much higher in Montana and the study population is so small and exposures so low in South Carolina that comparisons are difficult. In the longer term, the population is too small for confident conclusions concerning lack of risk. On the other hand, the exposure-response trends (Figure 4) are suggestive that if tremolite asbestos were present instead of cleavage fragments there would likely have been an increase in lung cancer in the highest exposure category (and the work environment would have been more dusty with higher exposures).

Although the actual percentage of "non-asbestiform" anthophyllite in the airborne dust is not clear in these studies, we will assume that the airborne dust contains a proportion of non-asbestiform anthophyllite and non-asbestiform tremolite. In view of this, comparison of the risk of mesothelioma and lung cancer in the NY and Norwegian talc mining industry will be compared with other talc studies (negative control) and with asbestos-exposed workers in anthophyllite mining and workers exposed to vermiculite contaminated with tremolite asbestos(positive comparison). South Carolina vermiculite will be compared with Libby, Montana vermiculite.

OTHER TALC DEPOSITS

There are several mortality studies of talc where amphibole minerals are reported to be absent and the talc is relatively "pure" talc. These include studies of workers in the Vermont talc mines (Selevan et al. 1979), Italian talc mines (Coggiola et al. 2003), French and Austrian talc mines (Wild et al. 2002) (Table 6). According to Wild et al. (2002) "no asbestos contamination has ever been clearly documented in the talc deposits, at least not in the European sites."

LUNG CANCER IN NEW YORK AND VERMONT TALC MINERS AND MILLERS

In contrast to the high levels of amphibole cleavage fragments in New York's St Lawrence County talcs, geological studies conducted since the early1900's have shown no "asbestos" and little quartz in Vermont talc deposits (Boundy et al. 1979). Analyses of bulk samples collected in 1975/1976 from mines and mills of the three major Vermont talc companies showed talc and magnesite as major components (20-100%) and chlorite and/or dolomite as minor constituents (5-20%). There were trace amounts (<5%) of

dolomite, calcite, quartz, biotite, ankerite, chromite, phlogopite and oligoclase and no asbestos.

Sampling surveys conducted in summer/winter of 1975/1976 at the 3 talc mines/mills resulted in respirable geometric mean concentrations in the mines ranging from 0.5-5.1 mg/m^3 (median = 0.9) and in the mills from 0.5-2.9 mg/m^3 (median = 1.0). Two methods were used to count "fibers" with aspect ratios ≥3:1 and a "maximum width and minimum length" of 5 μm. Counts using phase contrast microscopy at a magnification of 437x ranged from 0-60 fibers/mL (median = 4.1). Parallel fibers counted by SEM at a magnification of 5000x ranged from 0-0.8 fibers/mL (median = 0). Cumulative exposures were not estimated, but past exposure levels commonly exceeded the MSHA and OSHA standards of 20 mppcf (Selevan et al.1979).

The Vermont talc study provides the best comparison with the New York talc because the original studies were conducted during the same time period using similar methods and some or the same investigators, and the mines were in adjacent US States (although different ore bodies).

The cohort comprised 392 men who had had a chest radiograph administered by the Vermont Health Department since 1937 and had been employed for more than 1 year in the Vermont talc industry between January 1, 1940 and December 31, 1969. Workers were followed through December 31, 1975. As the inclusion of workers in the cohort required a radiographic examination, it was thought that long-term workers were more likely to have participated than short-term workers. In the 1960's the Health Department reported that 70% of those missing from their radiographic surveys had less than 1-year employment. While the overall effect is not known, the original authors concluded that selection bias could not explain the observed excess mortality.

There were a total of 90 deaths with an overall SMR of 1.16. There was a six-fold excess mortality (11 obs, 1.79 exp) from NMRD (excluding influenza and pneumonia). The largest excess was among millers (7 obs, SMR=7.87), but mortality was also increased among miners (2 obs, SMR = 3.6). Radiographic evidence of pneumoconiosis (80% > category 2/1) taken as part of the annual radiographic surveillance program of active workers, suggested to the authors that Vermont talc exposure was the causal agent. There was a non-significant 1.63-fold overall excess of lung cancer, which was significant among the miners (5 obs, SMR = 4.35) but not millers (2 obs, SMR = 1.02). There were no cases of mesothelioma (Selevan et al. 1979).

The most similar cohorts are Brown et al. (1979, 1980) and Lamm et al. (1988) (Table 6). Lamm et al. (1988) considered workers with >1 year tenure, which can be compared to Vermont. Brown et al. 1979, 1980 included all workers irrespective of tenure.

Risks of lung cancer were similar in Vermont and the NY talc workers with 1 or more year employment (1.63 versus 1.93 respectively) but elevated to 2.7 when all workers are included. The SMR for lung cancer among NY talc workers with less than 1 year tenure was 3.17 (6 obs) (Lamm et al. 1988). This supports the conclusion of Lamm et al. (1988)

that the risk of lung cancer in NY talc workers is concentrated in short-term workers and is most likely due to risks acquired elsewhere.

Risks of NMRD were increased 3-fold in all 3 cohorts. Risk of pneumoconiosis appeared to be higher in Vermont as non-infectious respiratory disease mortality (possible surrogate for pneumoconiosis) was increased 6-fold compared to about 4-fold for both studies of NY talc workers.

In the Vermont mills the mortality from NMRD was twice that in the mines. However, the risk of lung cancer was 4-times greater in the mine than mill. Exposures in both mine and mill in Vermont were above the then standard of 20 mppcf, but cumulative exposures were thought to be higher in the mill than the mine because mine operations were more sporadic. Selevan et al. (1979) concluded that for NMRD, "additional etiologic agent(s) either alone or in combination with talc dust affect mine workers" because exposures were higher in the mill than in the mines yet mortality was higher in the mines. If this same reasoning is used for lung cancer, one would also conclude that other etiological agents were involved since SMRs for lung cancer were near the null among millers in both Vermont (Selevan et al. 1979) and the updated NY talc cohort (Honda et al. 2002) (Table 6).

A clear limitation of the Vermont study is the small number of deaths; there were only 6 lung cancer deaths and 11 deaths from NMRD. Nevertheless, the increased risk of lung cancer in talc miners in Vermont where there is no evidence of exposure to asbestos or amphibole cleavage fragments is consistent with a conclusion that amphibole cleavage fragments are not responsible for the increased risk of lung cancer in the New York Talc miners. On the other hand the increased risk of Non-Malignant Respiratory Disease (Pneumoconiosis) appears to be related to both Vermont and NY talc dust exposure. Further follow-up and quantitative exposure-response analysis of the NY talc cohort tested these hypotheses and found that cumulative exposure to talc dust showed a strong association with pulmonary fibrosis mortality, a moderate association with other NMRD and no association with lung cancer (Honda et al. 2002, Oestenstad et al. 2002).

It is informative to think about the history of these two cohorts of similar size and similar risks and hopefully learn some useful lessons. There has been no further follow-up of the Vermont talc cohort. The NY cohort has been re-analyzed several times both with and without further follow-up (Stille and Tabershaw 1982, Lamm et al. 1988, Brown et al. 1990, Gamble 1993, Oestenstad et al. 2002, Honda et al. 2002). From the earlier studies has come the common (and current) perception that talc in the Gouverneur Talc District contains asbestos and that "exposures to asbestiform tremolite and anthophyllite stand out as the prime suspected etiologic factors associated with the observed increase in bronchogenic cancer" (Brown et al. 1980). We offer two possible reasons for this incorrect perception.

First is the difference between including and not including short-term employees. The evidence that lung cancer risk was concentrated in short-term workers appears to have been outweighed by the known risks associated with asbestos and the presumption that

NY talc workers were exposed to talc containing asbestos. The excess lung cancer among Vermont talc miners appears to have been discounted due to "talc free both of asbestiform minerals and significant quantities of free silica" and the potential for additional etiologic agents either alone or in combination with talc dust (e.g., radon).

Second, the most important limitation is with regard to the asbestos standard for regulating asbestos minerals. The OSHA-NIOSH definition of asbestos is inadequate for identifying and regulating non-asbestiform amphiboles. The crushing of rock containing non-asbestiform amphiboles (and other minerals) produces cleavage fragments that conform to the OSHA-NIOSH definition of asbestos (e.g., ≥3:1 aspect ratio, ≥5 μm length) but are not asbestos fibers.

Using this definition has produced errors regarding asbestos content of the ores that are the subject of this review, i.e., taconite tailings dumped into Lake Superior (see other presentations in this volume), asbestos exposure of Homestake gold miners (Gilliam et al. 1976) as well as talc. Other examples of the potential misuse of the federal fiber definition for asbestos include allegations of asbestos in play sand (Langer et al. 1991) and in crayons. The Agency for Toxic Substances and Disease Registry (ATSDR) in their Public Health Statement for Asbestos suggest that talc may contain asbestos. The Australian Government National Occupational and Health Commission say that industrial talc generally contains "asbestos fibers, notably tremolite." By this standard one might include all the negative control talc cohorts as positive controls of workers exposed to asbestiform amphiboles. More examples are readily available on the internet. While amphiboles are sometimes present in some talc, asbestiform amphiboles occur very rarely as a geological curiosity and not as far as we are aware using a mineralogical definition in any commercial or industrial talc.

The reasons for the increased risks of lung cancer in the New York and Vermont mining areas still remain speculative. Exposure to radon may be one reason as levels were apparently elevated in the Vermont Mines. The possibility that miners worked in areas of high asbestiform tremolite in the past cannot be totally excluded on present evidence as in one closed mine in Vermont "cobblestones of serpentine rock which were "highly tremolitic" have been reported, although workers in the Vermont cohort were considered unlikely to have had such exposure (Selevan et al. 1979). Whether this was asbestiform tremolite is not described although this appears to be inferred.

ITALIAN TALC

Italian talc is very pure and is used in the pharmaceutical and cosmetic industries. Miners and millers in this industry were studied for mortality (Rubino et al. 1976 1979, Coggiola et al. 2003). Miners were analyzed separately from millers because of silica exposure in the mine. The silica content of airborne dust in the mines was as high as 18% in drilling operations from footwall contact rocks, rock type inclusions, and carbonate, calcite and magnesite inclusions. The quartz content of the rock strata was inconsistent, ranging from 10-45%. Other minerals in the inclusions included muscovite, chlorite, garnet, and some

carbonate material. A small amount of (non-asbestiform?) tremolite was detected in the inclusions but not in the talc samples. Talc samples were commonly contaminated with chlorite. From 1920-1950 there was dry drilling and no forced ventilation so exposures were over 10 times the TLV (which appears to have been about 25 mppcf at that time) in the mines and a little over the TLV in the mills. Wet drilling and forced ventilation were introduced in about 1950 and dust concentrations dropped precipitously to about 1 mppcf and well below the TLV. Concentrations in the mills were reduced slightly and slowly and after about 1960 were higher than in the mines (Rubino et al. 1976).

Coggiola et al. (2003) updated the earlier talc studies by Rubino et al. (1976, 1979). The updated cohort comprised 1,795 men with at least 1 year of employment 1946-1995 and national rates were used for comparisons. There were 880 observed deaths with an overall SMR of 1.20 (1.12-1.28). There were slight deficits in observed lung cancer and total cancer and there were no mesotheliomas.

The SMR for lung cancer was 1.07 (0.73-1.50) for miners, while there was a deficit of lung cancer with an SMR of 0.69 (0.34-1.23) in millers. There was a 2-fold excess of NMRD due mainly to silicosis with the excess occurring among miners with a significant SMR of 3.05 (2.50-3.70) compared to 1.04 (0.65-1.57) among millers. Exposure-response was examined using duration of exposure. This showed that for miners the only lung cancer excess was in the <10-year exposure group while for NMRD the exposure-response trends were flat with all categories of duration of exposure showing about a 2-fold excess mortality.

The authors concluded there was no association between lung cancer or mesothelioma and exposure to talc containing no asbestos fibers. But there was an association in miners between NMRD (primarily silicosis) and talc containing quartz.

FRENCH AND AUSTRIAN TALCS

Wild et al. (2002) conducted cohort studies of talc workers in France and Austria with nested case-control studies of lung cancer and NMRD. The French ore was a talc chlorite mixture with quartz contamination ranging from undetectable to less than 3%. In Austria, three mines were studied. At one site the ore was a talc-chlorite mixture with 0.5-4% quartz. Rock containing about 25% gneiss was not milled. A talc-dolomite mixture of 25% medium talc and <1% quartz in the final product was the product at the second mine. The ore at the third site did not contain talc but was mixture of approximately equal proportions of quartz, chlorite and mica. Workers were stratified into semi-quantitative exposure categories. The non-exposed group consisted of office workers not exposed to talc and personal dust samples averaged 0.2 mg/m^3. The low exposure group was for workers with no direct contact to talc, such as maintenance workers, and concentrations were less than 5 mg/m^3. The medium exposure category included workers exposed to concentrations between 5-30 mg/m^3 for dustier areas such as bagging or milling and onsite maintenance. Quartz exposures occurred mostly in underground mining, tunneling and barrage building and milling products at site D. The highest exposure category was reserved for past production jobs (all before 1980) where concentrations were >30

mg/m^3. Some samples produced concentrations >50 mg/m^3 and higher. Three samples taken on workers wearing personal protective equipment were 73, 82 and 159 mg/m^3. To calculate cumulative exposures, values of 2.5, 10 and 40 mg/m^3 were assigned to the low, medium and high exposure jobs.

The French cohort consisted of 1,070 men with more than one year tenure between 1945 and 1995, with vital status follow-up through 1996. The Austrian cohort consisted of 542 men with >1-year tenure between 1972 through 1995 and vital status follow-up during this same period. Three controls per each case of NMRD and lung cancer from both the French and Austrian cohorts were matched on age and calendar year of employment.

Overall mortality was below expected. There were 294 deaths in the French cohort in the period 1968-1996 for an SMR of 0.93 (0.82-1.04). The Austrian cohort was smaller with 67 deaths and an SMR of 0.75 (0.58-0.95). In the French cohort SMRs were only slightly elevated for NMRD and lung cancer (1.06 and 1.23 respectively) but were increased over five-fold (SMR 5.56 CI 1.12-16.2) for the 3 cases with pneumoconiosis. There were zero mesotheliomas.

The case-control studies combined the French and Austrian cohorts. There were 40 combined deaths from NMRD: 10 from pneumoconiosis (including silicotuberculosis), 10 from chronic obstructive pulmonary disease (COPD, restricted to chronic bronchitis and airway obstruction), and 20 deaths from pneumonia and other diseases. When analyzed by exposure categories, the exposure-response trend for NMRD was not monotonic, with no apparent increased mortality below 400 mg/m^3-yrs and 2-fold and 2.5-fold increased risks in the 2 highest exposure categories respectively. When analyzed by conditional logistic regression there was a significant exposure-response trend with an 8% increased risk per 100 mg/m^3-yrs exposure. The slope was even higher for pneumoconiosis, 1.17 for pneumoconiosis versus 1.08 for NMRD. The slope was only 1.02 for COPD. Adjustments for covariates in the regression analyses had little effect on these trends. Smoking prevalences were similar between cases and controls with about 40% nonsmokers. (Figure 5)

There were 30 combined lung cancer cases. There was a negative exposure-response trend with odds ratios of 0.6 and 0.73 in the two highest exposure categories. The trend was unchanged when adjustments were made for smoking, quartz, working underground or when lagging the exposure estimates. Also, there were no trends when analyzed by maximum dose, latency, or duration of exposure (data not shown). About 40% of the controls were nonsmokers compared to about 8% (1/19) among cases although smoking classification was unknown on about half of the cases.

Wild et al. (2002) concluded that the small excess of lung cancer was not due to talc, despite follow-up of over 50 years, high exposures and mean duration of exposure >20 years.

The pattern of mortality of workers exposed to cleavage fragments in the New York talc mines and mills (Figure 3) is very similar to that of workers in the French and Austrian

mines and mills where there was no exposure to cleavage fragments (Figure 5). A limitation in these comparisons is the very large differences in cumulative exposures. If they are comparable, the dust to which the New York miners and millers are exposed is considerably more potent than that in the French and Austrian mines and mills from the standpoint of increasing lung fibrosis/pneumoconiosis. On the other hand, this "apparently highly potent pneumoconiosis producing dust" does not increase lung cancer risk.

These studies show that "pure" talc does not increase lung cancer risk. This is consistent with the observations for the New York millers, exposed to talc as there was no excess lung cancer in talc millers.

ASBESTOS-EXPOSED COHORTS FOR COMPARISON WITH TALC WORKERS

There are two ore deposits containing tremolite asbestos or anthophyllite asbestos potentially suitable for comparison with the talc cohorts exposed to non-asbestiform tremolite and asbestos. One site is the vermiculite mine located in Libby, Montana with significant contamination from tremolite asbestos. The other is an anthophyllite asbestos mine in Finland.

LIBBY, MONTANA VERMICULITE MINE CONTAMINATED WITH ASBESTIFORM AMPHIBOLE

Ore fed to the mill in Libby, Montana contains 4-6% asbestiform amphiboles (about half tremolite asbestos and the other half a mixture of winchite and richterite in the tremolitic series, Nolan et al 1991). The health concern is the asbestiform amphibole contamination in these ores and not the vermiculite itself.

The raw ore and vermiculite concentrate from the Libby mine contain both asbestiform and non-asbestiform tremolite-actinolite and non-fibrous anthophyllite. Atkinson et al. (1982) found 21-26% fibrous tremolite-actinolite in the raw ore and 2-6% in the concentrate. Company data taken several years later indicated 3.5-6.4% at the head feed of the mill and 0.4-1% in the concentrate (Amandus et al. 1987a). After removal of coarse rock the ore contained about 20% vermiculite, 21-26% fibrous tremolite-actinolite and the rest augite, biotite, calcite, diopside, hornblende, magnetite, quartz, sphene, and apparently non-fibrous tremolite-actinolite (McDonald et al. 1986).

Eight airborne samples from the mill and screening plant examined by phase contrast light microscopy indicated the asbestiform nature of the particles: 96% had aspect ratios >10, 67% >20 and 16% >50. In addition, 73% of the fibers were longer than 10 μm, 36% >20 μm and 11% >40 μm and width was < 2.5 μm in all instances (Amandus et al. (1987a).

Two independent mortality studies of the Montana vermiculite have been conducted. McDonald et al. (1986a, b) conducted a radiological survey and a cohort and nested case-control study of 406 persons employed for at least a year prior to 1963 with follow-up until 1983. The cohort study was subsequently updated with follow-up to 1999 (McDonald et al. 2002, 2004). We will primarily focus on the up-dated analysis. Exposure was estimated from first exposure (1945) to 1982 when work histories were no longer available. By this date most of the cohort was no longer employed and fiber concentrations were about 0.1 f/mL. The plant closed in 1990. Before wet milling processes were installed, fiber concentrations were very high (estimates of >100 f/mL). A wet mill was installed in 1955 and an entirely wet process replaced both wet and dry mills in 1974 so by 1980 nearly all concentrations were <1 f/ml. Exposure-response was estimated by both categorical and linear exposure-response (E-R) Poisson regression models and excluding those with <10 years latency. Average and cumulative exposure metrics showed similar relationships with mortality.

The overall all cause SMR was 1.27 (1.13-1.43). SMRs for lung cancer and NMRD were 2.40 (1.74-3.22) and 3.09 (2.30-4.06) respectively; the PMR for mesothelioma was 4.2%. Exposure-response trends were not linear, as risks of lung cancer, NMRD and mesothelioma increased steeply in the second quartile exposure category and showed less steep slopes in the third and fourth exposure quartiles (Figure 4, Table 8).

The other Libby cohort study was by NIOSH and published in 3 sections that included exposure estimates (Amandus et al. 1987a), cohort mortality study (Amandus et al. (1987b) and a cross-sectional radiographic study (Amandus et al. 1987c). Amandus et al. (1987b) also reported positive exposure-response trends for lung cancer with an almost 7-fold increased SMR in the high exposure category with more than 20-years latency. The PMR for mesothelioma was 2.2% considering only those with 20 years or more latency.

These results are a marked contrast to the decreasing trend of lung cancer with increasing exposure seen in the St Lawrence, NY talc workers. There is little doubt that the mesothelioma experience of the Montana work force is considerably worse than that of the talc miners. This is in spite of the fact that the New York talc workers are reported to have been exposed to dusts containing a very high percentage of non-asbestiform amphibole fibers (Kelse and Thompson, 1989).

The amphiboles in St Lawrence, NY talc are non-asbestiform while they are asbestos in the Libby deposit (Kelse and Thompson 1989, Langer and Nolan 1989, Thompson 1984, Dement and Zumwalde 1980).

Risk of pneumoconiosis, lung cancer and mesothelioma clearly increase as cumulative exposure to asbestiform tremolite increases (Figure 4). For the talc workers exposed to non-asbestiform tremolite, the risk of NMRD and pneumoconiosis increase as exposure increases, but the trends are reversed for lung cancer (inverse trend) and for mesothelioma (no cases so there is no trend) (Figure 3).

FINNISH ANTHOPHYLLITE ASBESTOS MINERS/MILLERS

Dement and Zumwalde (1980) mentioned the study of Finnish miners by Meurman et al. 1974 in the belief that both the NY talc and Finnish anthophyllite asbestos cohorts were exposed to asbestiform anthophyllite. They recommended that the risk of mesothelioma should be further studied by further follow-up of the NY talc workers. Both the NY talc (Honda et al. 2002) and anthophyllite asbestos cohorts have had further follow-up so the maximum latency in Finland is now about 40 years (Karjalainen et al. 1994; Meurman et al. 1994), which is about the same as for NY talc workers (Honda et al. 2002).

In the updated Finnish study there was a significant 2.9-fold excess incidence of lung cancer overall with a somewhat higher risk in the heavily exposed males (SIR 3.15) than in moderately exposed (SIR 2.35). There were four mesothelioma cases for a significant 46-fold increased SIR (95% CI = 12.2-115) overall (or a PMR of 0.7%, 4/593). All of the cases were in the heavy exposure group where there was a 67-fold excess (95% CI = 18.3-172) and all four had asbestosis. Asbestosis was mentioned on 20% of all death certificates (Karjalainen et al. 1994, Meurman et al. 1994).

MESOTHELIOMA COMPARISON

In the NY talc cohort, Honda et al. (2002) reported 2 deaths from mesothelioma. One was coded as benign neoplasm of the respiratory system and the other as malignant neoplasm of the lung and bronchus, unspecified. One man worked for 15 years and died 15 years after starting work at the talc facility. He had been a carpenter and millwright for 16 years, 8 years as a lead miner and 5 years as a repairman in a milk plant. The other man worked briefly at the facility as a draftsman during mill construction in 1947-8. He would have had minimal talc exposure. He had been employed on the construction of a previous talc mine, and then installed oil burning heating systems. Honda et al. (2002) concluded it is unlikely that either of these cases occurred as a result of talc exposure in the mine or mill. In essence, there are no mesothelioma cases that are plausibly related to occupational exposure to Gouverneur talc.

Vianna et al. (1981) reported a mesothelioma rate in Jefferson County twice that of New York State based on an incidence study of histologically confirmed mesothelioma cases. A total of six cases, four male and two female cases diagnosed between 1973 and 1978 were reported to have occurred in talc miners. Enterline and Henderson (1987) reported an excess mesothelioma incidence in Jefferson County from 1968 to 1981 with 4 female (0.6 expected) cases and 7 male (1.4 expected) cases for risk ratios of 6.7 and 5.0 respectively. These latter rates were the second and sixth highest in the USA and occur in the county next to the one where the talc mines are located.

Hull et al. (2002) drew attention to these elevated rates, added "five new mesothelioma cases," and concluded that New York talc exposure was associated with an increased risk

of mesothelioma. This conclusion is inconsistent with the limited available data as outlined in the following:

- The entire work histories of the "talc miners" with mesothelioma are apparently not known. Exposure to asbestos in other jobs is likely given the diagnosis of asbestosis and the smaller widths of the fibers in lung tissue.

- Hull et al. (2002) attempt to interpret the results of their tissue analyses of only two mesothelioma cases. This sample is too limited to reach any reliable conclusions. Available data do not support a talc etiology.

- Fiber dimensions are consistent with asbestos exposure as the mean fiber widths in the 2 mesothelioma cases examined are less than 0.25 μm, which are the dimensions characteristic of asbestos.

- The source of the fibers in the lungs is unlikely to be NY talc mines. The average width of the fibers in the mesothelioma lungs was 0.15 μm, which is considerably less than the average width of 1.3 μm of anthophyllite and tremolite in milled talc samples (Siegrist and Wylie, 1980). Kelse and Thompson (1989) reported that 0% of the fibers in NY talc samples had widths less than 0.25 μum.

- Asbestos-related employment occurs among residents of both St Lawrence and Jefferson counties. Fitzgerald et al. (1991) reported that 39% of workers with radiographic abnormalities of parenchyma and pleura had been employed for a year or more in asbestos-related industries (e.g., shipyard, construction, pipe and furnace insulation).

- Two of the five cases had worked only four years and two years in occupations likely to be linked to the mining industry. One of these persons died at age 72 and the other at age 53. There was no information concerning their employment during the rest of their lives.

- A non-talc etiology for mesothelioma is plausible. As noted above, females in the talc mining counties have a greater risk of mesothelioma than males (Enterline and Henderson, 1987). On the other hand, the cohort data on talc workers is based on men because less than 5% of those hired in the talc industry were women (Honda et al. 2002, Brown et al. 1990, Lamm et al. 1988).

- In the cohorts, the worker populations and exposures are well defined and no association is observed between talc or non-asbestiform amphibole exposure and mesothelioma in the absence of possible asbestos exposure. The cohort studies provide a more reliable estimate of risk than a small case report with limited information on exposure.

- Hull et al. (2002) indicate the "increased pleural mesothelioma mortality [is] in Jefferson County." Jefferson County stopped producing talc about 100 years ago and all talc over the past century has been mined in St Lawrence County.

- In the Libby cohort there were twelve mesothelioma cases. The PMR was 4.2 %. Exposure to tremolite asbestos in the Libby vermiculite clearly increased the risk of mesothelioma significantly (McDonald et al. 2004). The risk of mesothelioma among anthophyllite asbestos workers was less than the risk among crocidolite miners but almost as great as among amosite miners (Meurman et al. 1994). These comparisons show a clear excess incidence of mesothelioma for workers exposed to asbestiform tremolite and anthophyllite, but no mesothelioma attributable to exposure to non-asbestiform tremolite/actinolite or anthophyllite. These comparisons are graphically displayed in Figure 6.

LUNG CANCER COMPARISON

There was an overall 2-fold increased rate of lung cancer in the Gouverneur talc miners and millers compared to the surrounding counties in which the mine was located. This excess of lung cancer was not associated with dust exposure but was concentrated in miners with an SMR of 3.94 (CI 3.33-6.22) while millers had only a small increased risk with an SMR of 1.28 (CI 0.51-2.63). In contrast, non-malignant respiratory disease mortality was associated with dust exposure as it was increased in both miners (SMR 2.41, CI 1.16-4.44) and in millers (SMR 2.27 CI 1.13-4.07) to almost the same extent. Smoking was clearly a confounding exposure as 100% of cases were smokers or ex-smokers but only 73% among controls. When exposure-response relationships were examined, the rate ratio for the highest respirable dust exposed workers to the lowest respirable dust exposed workers was 0.5 (0.2-1.3) for lung cancer and 11.8 (3.1-44.9) for pulmonary fibrosis (Figure 3). One would expect that a respirable dust exposure index would reflect the respirable fractions of dust regardless of composition. Thus, the results indicate that the lung cancer excess in this industry is largely due to smoking and unlikely to be the result of exposure to the respirable fraction of dust (which would include talc and cleavage fragments of the various amphibole minerals). However the data suggest that the respirable dust did increase the risk of fibrosis.

In asbestos producing or using industries where midget impinger measurements were used as a basis for exposure estimates (Liddell et al. 1997), the risk of lung cancer increased with increasing levels of exposure. This illustrates the validity of exposure indices based on midget impinger measurements for assessing fiber-related risks, at least when exposures are high. However, in this talc mine, exposure estimates derived from midget impinger measurements (Oestenstad et al. 2002), showed no such relationship. If cleavage fragments were responsible for the lung cancer excess, an exposure-response relationship would have been anticipated.

To date a satisfactory explanation for the observation of an overall excess of lung cancer and for the concentration of the excess in miners rather than millers has not been found

for workers exposed to either NY or Vermont talc, although at least part of the excess among NY talc workers is due to smoking (Gamble 1993, Honda et al. 2002). If the airborne dust contained over 70% amphibole asbestos fibers as reported by Dement and Zumwalde (1980), there should an overall increased risk of lung cancer, which there is, but there should also be a logical increasing risk of lung cancer with increasing dust exposure, with a very high risk of lung cancer in highly exposed workers. This is clearly not the case.

In Finland where the incidence of cancer has been studied in anthophyllite miners, it was found that among heavily exposed male workers, the standardized incidence ratio (SIR) for lung cancer was 5.54 (CI= 3.90-7.63) and among moderately exposed workers it was 1.63 (0.20-5.89). The heavily exposed were those who worked in the mine or mill and the moderately exposed included all other personnel (Meurman et al. 1994). This exposure-response pattern is quite the opposite of that in the New York talc mines and mills.

There were consistent positive exposure-response trends for lung cancer risk as occurred with the increased asbestiform amphibole exposure in the Libby cohort. The slope of the exposure-response curve was steeper for lung cancer than for pneumoconiosis and for mesothelioma (Figure 4).

The clear exposure-response trends for lung cancer to increase with increasing exposure to asbestiform tremolite and anthophyllite is in marked contrast to the negative exposure-response trend for lung cancer risk to decrease with increasing exposure to non-asbestiform tremolite and anthophyllite present in industrial talc. The pattern of increasing risk of fibrosis is consistent with exposure to mineral dust with or without the presence of tremolite asbestos.

These lung cancer comparisons are graphically displayed in Figure 6.

BIOLOGICAL PLAUSIBILITY

Biological plausibility is not a necessary prerequisite to establishing a causal association, but it is considered "helpful" (Hill, 1965). Experimental evidence is available to consider whether or not cleavage fragments are more or less carcinogenic than asbestos fibers. These issues have been independently evaluated by Addison and McConnell and Mossman, elsewhere in this volume.

Experimental studies have the potential advantage of precisely defining the characteristics of the minerals and amount of exposure. However there are also difficulties that affect the studies and their interpretation. Hence it is important to examine the overall pattern of biological responses to asbestos fibers and cleavage fragments rather than the results of single studies. Feeding studies have been considered elsewhere (Wilson et al. this volume).

Many experiments in animals have been used to assess the potential of fibers to produce mesothelioma-type neoplasms. For example, Stanton et al. (1981) counted as a positive response, pleural sarcomas that resembled the mesenchymal mesothelioma of man. The observed response is a measure of potential hazard rather than risk. Nevertheless such studies have been helpful in suggesting the morphological characteristics of particles in relation to "mesothelioma" producing potency. "Index particles" have been derived from these experiments. For example, based on the work of Stanton and colleagues the index particle is >8 μm long and <0.25 μm wide and is the best predictor of tumors without regard to the chemical composition of the particle. As far as we were able to ascertain, few if any cleavage fragments have the combination of diameter less than 0.25 μm and length greater than 8 μm. This would suggest that cleavage fragments are not the most potent particles for the production of mesothelioma.

Different exposure techniques have been used, but most have not involved the inhalation route of exposure applicable to humans. Most experiments have involved placing fibers onto the pleural or into the peritoneal cavity or injections intratracheally, routes of exposure which are artificial. The incidence of tumors is therefore higher and the tests are likely to be more sensitive than by inhalation. However, these experiments ignore the factors which limit fiber passage to these sites and also the alterations to the particles during their passage to these sites if they get there at all. Nevertheless, these data are useful in hazard assessment, as the absence of "mesothelioma" occurrence when fibers are placed directly on the pleura or peritoneum in sufficient numbers, is strong evidence that human inhalation exposure is unlikely to be hazardous.

Samples used in experimental studies are not always related to the minerals to which workers are exposed. For example, no experimental studies of the Homestake gold ore were found. On the other hand, there are several studies of tremolitic talc samples from the Gouverneur mine in New York State (talc samples 6 and 7 used by Stanton et al. (1981); FD-14 used by Smith et al. (1979) and FD-275 (non-asbestiform tremolite) used by Smith et al (1979) and by McConnell et al. (1983)) in feeding studies. Wylie et al. (1997) used *in-vitro* cell studies to compare the effects of asbestos fibers to talc fibers and transitional fibers in NY talc.

Figure 7 shows the results of rat injection studies of asbestiform and non-asbestiform varieties of amphiboles, primarily tremolite. These data show a consistent pattern of high incidence of mesothelioma tumors with exposure to tremolite asbestos from South Korea, California, Swansea and Italy (Davis et al. 1985, Wagner et al. 1969 1982, Stanton et al. 1981). The mesothelioma incidence of both controls and samples was around 10%. The two Scottish tremolites studied contained relatively few asbestiform fibers and there was little difference between the control and exposed rats irrespective of whether the tremolite was asbestiform or not. Davis et al. (1991) noted that the intraperitoneal injection test used in their experiments is extremely sensitive so that any dust that produces fewer than 10% tumors is unlikely to show evidence of carcinogenicity by inhalation. Thus the non-asbestiform Scottish tremolite from Shinness was considered to pose no hazard.

The Scottish tremolite from Dornie was considered to be probably harmless as well. The latter sample was described as containing mostly cleavage fragments but also some very long, thin fibers, with a possible small asbestiform subpopulation. These results should be contrasted with those of asbestiform tremolite from Italy, California, Swansea and South Korea, which showed incidences of 70-100%. The Italian tremolite was described as a needle-like (byssolite) tremolite fiber but later shown to have an asbestiform component. For this fiber, the induction of tumors was much later than for the three asbestos types from California, Swansea and Korea. This is a normal response to a small dose of amphibole asbestos. Incidence was reduced to near zero for samples of non-asbestiform tremolite and talc fibers (Wagner et al. 1982, Stanton et al. 1981). Smith et al. (1979) assessed the incidence of tumors after injection of NY tremolitic talc and tremolite asbestos at two different doses. There were clear exposure-response trends for the asbestiform tremolite but no effect of non-asbestiform tremolite at either 10 or 25 mg exposures (Figure 8).

STATISTICAL ANALYSIS OF POTENCY BY SIZE, SHAPE AND MINERALOGY

Berman et al. (1995) conducted a statistical reanalysis of inhalation studies using data from studies of AF/HAN rats exposed to different types of asbestos to identify the exposure metrics that best predicted the incidence of lung cancer or mesothelioma. New exposure metrics were first generated from samples of the original dust because of limitations in the original characterizations. This analysis provided more detailed information on mineralogy [i.e., chrysotile, grunerite (amosite) asbestos, riebeckite (crocidolite), tremolite asbestos)], type of structure (i.e., fiber, bundle, cluster, matrix), size (length, width) and complexity (i.e., number of identifiable components). In particular, transmission electron microscopy (TEM) was added to the descriptions so that asbestos structures less than 0.2 μm could be detected and identified and use in the statistical analysis of size distributions to evaluate combined effects of length and width.

Implantation and injection studies generally indicate long, thin fibers are most likely to induce mesothelioma. However, Berman et al. (1995) considered inhalation studies more relevant for assessing human risk because lung retention and transport from the lungs are likely to be important variables in potency but are bypassed in the implantation/injection studies. Also the exposure metrics from these studies are unable to satisfactorily predict tumor incidence (for example see Oehlert 1991).

The analysis by Berman et al. (1995) indicated that particles contributing to lung tumor risk are long (>5 μm) thin (<0.4 μm) fibers or bundles with the potency increasing as length increases. For example, thin fibers longer than 40 μm are about 500 times more potent than thin fibers 5-40 μm in length. Long and very thick particles (>5 μm) may pose some risk, but these appear to be complex structures rather than fibers. It is hypothesized that these structures with large widths may break down and release additional long thin fibers or bundles. Short particles less than 5 μm in length do not

appear to pose any lung cancer risk in this database. Thus in rats a particle length of 5 μm or less (or as Berman et al. suggest, 5-10 μm or less) appears to have zero potency.

The only other available data set for quantitatively assessing particle size is that of Stanton et al. (1981). The Berman et al. (1995) data set is considered more relevant because

1) It is based on an inhalation rather than implantation route of exposure;

2) It includes a range of representative samples of both asbestos fiber-types and particle sizes;

3) There is a more detailed characterization of long particles and complex structures than any other experimental study; and

4) The statistical analysis is more appropriate.

The analysis by Berman et al. (1995) is more appropriate as logarithms were not used, which avoided the problem of zero exposures in some size ranges and 0 tumors at some exposures. Also, an optimum exposure index was determined that provides a statistically adequate fit to the data. The models used by Stanton et al. (1981) do not fit the data well and therefore do not adequately describe the ranking of particle size potency.

In a statistical reanalysis of the Stanton et al. (1981) data, Oehlert (1991) confirmed the Stanton hypothesis that the primary ability of mineral particles to cause tumors are their dimensional properties, namely index particles that are long and thin (> 8 μm long and <0.25 um wide). Using improved models that fit the data better, Oehlert (1991) reinforced the idea that very long, very thin particles were the best predictors for tumors and that particles with dimensions outside the index class did not contribute to carcinogenicity. This is also in agreement with Berman et al. (1995) that non-index particles have essentially zero potency.

Oehlert (1991) disagreed with the Stanton hypothesis that dimensions alone determine carcinogenic potency. Model fit was significantly improved by assessing each mineral type separately, which indicates mineral type is also important. This disagreement was unfounded, as in fact, Stanton, himself noted that the solubility of the fiber was also important, a parameter that would be incorporated in any analysis by considering fiber type. Dimensions are necessary but are not alone sufficient to classify a substance as capable of inducing tumors. It is now well established that factors such as particle solubility and perhaps surface properties are also important. For example, fibrous talc from the Gouverneur talc deposit in New York is not equivalent (0% tumor probability) to grunerite (amosite) asbestos (93% tumor probability) in tumor producing potential although the dimensions are similar (Stanton et al. 1981).

In sum, the Oehlert (1991) reanalysis of the Stanton et al (1981) data is consistent with Berman et al (1995) that particles of certain dimensions are important predictors of tumor

incidence. Long and thin particles are the significant dimensions. Also, the minerals comprising sufficient particles in these size ranges to produce tumors included asbestos (crocidolite, amosite, and tremolite asbestos) but not the non-asbestiform amphibole mineral (tremolitic talc).

Given the importance of width and length from these experimental data, it is useful to summarize available data on dimensions of amphiboles in the epidemiological studies summarized in previous sections [Table 8].

This analysis indicates the low amounts or absence of long, thin particles in the size ranges that predict lung tumors or mesothelioma in the three ore bodies containing non-asbestiform amphiboles (NY talc, taconite and Homestake). A primary interest in studying these workers is the fact that they were exposed to non-asbestiform amphiboles. Steenland and Brown (1995) expressed the interest as follows: "Non-asbestiform amphibole fibers have not been shown to cause lung cancer, *but are suspect because of their similarity to asbestiform fibers* (emphasis added)." The data in Table 8 and noted above suggest that the similarity is applicable only to chemistry since there is no similarity in the occurrence of index particles. The long thin elongated particles (fibers) capable of inducing tumors are common in asbestiform amphiboles and absent in non-asbestiform amphiboles.

The absence of long thin particles in the size ranges identified by Stanton et al. (1981) and by Berman et al. (1995) as responsible for lung cancer and mesothelioma experimentally from ores containing non-asbestiform amphiboles detracts from the hypothesis that non-asbestiform particles have a carcinogenic potency similar to asbestos fibers. The other parameter which is now recognized as being important is biopersistence. As the cleavage fragments are in general shorter than the asbestos fibers they are likely to be more readily removed by macrophages than the asbestos. On the other hand, the solubility difference between cleavage fragments and fibers is not known, although Ilgren (2004) suggests greater solubility of cleavage fragments. However, it is possible that fibers, because they could split apart, would have greater surface areas and might be more soluble than cleavage fragments of the same dimensions. This would mean that they would have greater lung biopersistence than fibers. On this basis, long cleavage fragments would have the potential to pose a lung cancer/mesothelioma risk if cleavage fragments had the same biological potency as asbestos fibers of the same length.

In fact this is not a real problem because the biopersistence of the amphibole fibers is known to be very high. Even if there were long cleavage fragments, their large diameters would reduce the risk compared to asbestos and their retention would be highly unlikely to render them more hazardous than the asbestos fibers. In this regard, it should be noted that the sample FD14 from the NY deposit did contain elongated particles that ranged up to 50 um in length (Griegner and McCrone 1972) and did not produce mesothelioma.

Conclusions about cleavage fragments from some of the other experiments are somewhat limited because, for example, the sample of Greenland non-asbestiform tremolite studied by Wagner et al. (1982) had no fibers greater than 10 um in length and less than 0.25 um in width. The sample FD 275-1 did not contain any particles longer than 10 um in length

and no particles with a width less than 1 um. Stanton (1973) showed that riebeckite (crocidolite) asbestos, pulverized to the state where 80% of the mass of fibres was in the size range less than 10 μm in length, produced a "negligible incidence" of mesotheliomas in pleural implantation studies.

While it is reassuring that none of the samples of non-asbestiform tremolite have produced elevated rates of mesothelioma in experimental animals, it is unfortunate that systematic studies have not been done to determine whether cleavage fragments of the same lengths as asbestos fibers produce the same risks as doses have generally been measured on a mass basis and not on the basis of number of fibers or cleavage fragments of particular lengths. An obvious problem with cleavage fragment studies is that in order to achieve similar numbers of long thin fibers to the tremolite asbestos in the dose, there would have had to be a very much larger mass of cleavage fragments injected, and that alone would have produced difficulties in animal survival. There do not appear to be cleavage fragment-related increases in lung cancer or mesothelioma risk in the studies. The lack of risk may be related to the fact that workers in those industries are not exposed to high concentrations of long cleavage fragments and the fact that because of their diameters such fragments would carry a much lower carcinogenic potency than their equivalent asbestiform mineral.

Our review of the experimental literature did not reveal any findings which would indicate that cleavage fragments have the same or greater carcinogenic potential than asbestos. In fact, they indicated that amphibole cleavage fragments have a much lower carcinogenic potential than their asbestiform counterparts by many orders of magnitude. In conclusion, there are still many unanswered questions relating to the extent to which the asbestiform habit of a mineral influences its biological behavior relative to that of a cleavage fragment (size for size). But the experimental data do provide strong support for the epidemiological findings that the risks of lung cancer and mesothelioma are considerably less [or absent] for persons exposed to amphibole cleavage fragments when compared to persons exposed to amphibole asbestos fibers.

OTHER AMPHIBOLES AND OTHER MINERALS

A search of the literature for studies containing both health outcomes and descriptions of exposure to cleavage fragments failed to identify additional studies that would be of immediate assistance in examining the health risks associated with cleavage fragments. The review did identify studies such as that in Finland where the percentages of asbestiform tremolite and cleavage fragments and fibrous wollastonite and cleavage fragments of wollastonite were characterised in metamorphic limestone and dolomite mines (Junttila et al. 1996). However, epidemiological studies to relate to the environmental studies do not appear to be available. The exposure to "Federal fibers" in quarrying industries and coal mines with their large workforces would be of interest. There were experimental studies and health evaluations of arfvedsonite asbestos in Russia

(Kogan et al. 1970, Pylev and Iankova 1975). There were well described studies of crocidolite-exposed populations, but no health studies of workers exposed to non-asbestiform riebeckite have been identified.

There are potentially other populations of workers exposed to the hundreds of other minerals (e.g., erionite; fluoroedenite), which can occur with a fibrous morphology. There is some information on mesothelioma risks for some of these minerals, but no studies were found of populations exposed to the non-asbestiform fibers of these same minerals.

While the gaps in knowledge concerning the US studies need to be filled, a broader base of information would be helpful. In the absence of well defined occupational groups exposed to well- characterised cleavage fragments with well studied health outcomes, it may be useful to consider non-occupational settings. In some of these areas, there are definite concentrations of pleural calcification and definite areas of elevated rates of malignant mesothelioma. Perhaps mapping the geographical distribution of mesothelioma in various countries such as Southern Europe, New Caledonia and the Mediterranean region might identify clusters of cases which might be investigated for asbestiform amphibole exposure and non-asbestiform amphibole exposure in for example, case-comparison studies.

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TABLE 1

THE DIAMETERS OF ASBESTIFORM AND NON-ASBESTIFORM AMPHIBOLES

"FIBER"	REFERENCE	PERCENT DIAMETER > 0.25 μm
Amosite	Gibbs & Hwang [1980]	28% - 42% (> 0.3μm)
All amphiboles [Homestake Gold mine]	Virta et al [1983].	100%
Taconite – Grunerite & Actinolite [East Mesabi Range]	Wylie [1988]	100%
Asbestiform Tremolite [Swansea]	Lee [1990]	76%
Non-asbestiform tremolite, [Alada Stura, Italy]	Lee [1990]	98%
Non-asbestiform tremolite [Greenland]	Wagner & Berry [1969]	100%
All amphiboles [N.Y. State]	Kelse and Thompson [1989]	100%

TABLE 2

PROPORTION OF TREMOLITE PARTICLES LONGER THAN 10 μm AND NARROWER THAN 3 μm FROM MILLED BLOCKY (PRISMATIC), ACICULAR, FIBROUS, AND TREMOLITE ASBESTOS STRATIFIED BY ASPECT RATIO USING PETROGRAPHIC MICROSCOPY§.

Aspect Ratio	% <3:1 Non-regulatory	% 3:1 to 5:1	% >5:1 to 10:1	% >10:1 to 20:1	% >20:1 to 50:1	% >50:1
Non-Asbestiform Tremolite Particles (cleavage fragments)						
Blocky	87	6.5	5	1	0.5	0
Acicular	87	4	6	3	0.5	0
Fibrous	57	18.5	18.5	5.5	0.5	0
Asbestiform Tremolite						
Asbestos1	48.5	6.5	13	13.5	13.5	5
Asbestos2	53.5	3.5	14.5	12	13	4.5

Non-regulatory designates particles that do not meet the length >5 um, width <3 um, and aspect ratio >3 criteria

§ Modified from Table 2 of Campbell et al. 1979.

TABLE 3

MESOTHELIOMA/LUNG CANCER EXPERIENCE – NON-ASBESTIFORM GRUNERITE* WORKERS AND NEGATIVE NON-AMPHIBOLE CONTROLS

STUDY POPULATION	FOLLOW-UP PERIOD	Cohort N (% dead)	N mesothelioma / N DEATHS (PMR)	Lung cancer: O/E = SMR (95% CI)
Non-asbestiform Grunerite Cohorts (latest follow-up)				
Homestake Gold Miners (Steenland & Brown 1995)	Follow-up 1977-1990	3328 (46.6%)	0 / 1551= 0 7*#	115/101.8 = 1.13 (0.94-1.36)
Reserve Taconite Miners (Higgins et al. 1983)	More than 1 year in period 1952-1976	5751 (5.2%)	0 / 298	15 /17.9=0.84(0.47-1.38)
Erie Mining of taconite (Cooper et al. 1992)	> 3 months <1959, Erie-Minntac mine, 1947-1959	3431 (30.8%)	1# 0 / 1058 = 0	62/92.2 = 0.67(0.52-0.86)
TOTAL		12510 (23.2%)	0/2907=0	192/211.9 = 0.91
Negative Comparison: Hematite Iron Ore without amphiboles				
Hematite mining in Minnesota [Lawler et al. 1985].	> 1 year employment before 1966. Follow-up 1937-79.	Ugd 4708 (55%) Surface 5695 (36%)	0 / 2642 = 0 0 / 2057 = 0	117/117.6=1.00(0.83-1.20) 95/108 = 0.88(0.71-1.08)

* It is recognised that these workers were also exposed to non-asbestiform hornblende and actinolite

Exposure began only 11 years before death making it unlikely that this mesothelioma is related to work in the taconite mine. He was previously a locomotive fireman and engineer.

*# There were seven cases [4 cancers of the peritoneum and 3 other respiratory cancers] in categories that might include mesothelioma but no mention of mesothelioma on the death certificate or other evidence to support diagnoses of mesothelioma. No mention of mesothelioma was found in a review of deaths from lung cancer or other non-specified cancer, or "categories which at time include mesothelioma" [Steenland & Brown 1996].

TABLE 4

MESOTHELIOMA /LUNG CANCER EXPERIENCE–GRUNERITE (AMOSITE) ASBESTOS EXPOSED WORKERS

STUDY POPULATION	FOLLOW-UP PERIOD	No. IN COHORT (% mortality)	No. meso / No. DEATHS = PMR	Lung Cancer: Obs/exp = SMR (95% CI)
Amosite mining (Sluis-Cremer et al. 1992)	Miners 1945-1955. Follow-up to 1986	3212 (20.2%)	4 / 648 = 0.6%	26/18.8 = 1.38 (0.97-1.91)
Amosite Insulation manufacturing (Acheson et al. 1984)	1945-78: Follow-up to 1980.	4820 (6.9%)	5/333 =1.5%	61/29.1 = 2.10 (1.62-2.71)
Amosite insulation manufacturing (Seidman et al. 1986; follow-up of Seidman et al. 1979)	1941-1945; more than 5 year latency; follow-up to 1983	820 (72%)	6/593=1.01% (death certificates) 17/593=2.9% (Best evidence)	102 / 20.51 =4.97 (4.08-6.1)
Amosite insulation manufacturing (Levin et al. 1998)	1954-1972, >10 years latency; follow-up to 1994	755 (29.4%)	6/222=2.7%	35/12.6=2.77 (1.93-3.85)
TOTAL		9607 (18.7 %)	21/1796=1.2%	224/81=2.77

TABLE 5

LUNG CANCER SMRs BY CUMULATIVE EXPOSURE EXPRESSED AS Fiber/mL-yrs FOR NON-ASBESTIFORM GRUNERITE [Steenland & Brown 1995] AND ASBESTIFROM GRUNERITE EXPOSURES [Seidman et al. 1986].

Non-Asbestiform Grunerite [Steenland & Brown 1995]								
MPPCF-yrs *	<33.3	33.3 – 133.3	133.3 – 200	>200	-	--	--	--
Fiber/mL-yrs **	< 4.8	4.8 – 19.5	19.5 - 29.2	>29.2				
SMR	1.17	1.01	0.97	1.31				
Asbestiform Grunerite [Seidman et al 1986]								
Fiber/mL-yrs **	<6	6-11.9	12-24.9	25-49.9	50-99.9	100-149.9	150-249.9	250+
SMR	14/5.31 = 2.64	12/2.89 = 4.15	15/3.39 = 4.42	12/2.78 = 4.32	17/2.38 = 7.14	9/1.49 = 6.04	12/1.32 = 9.09	11/.94 = 11.7

* Dust days in table II of the paper by Steenland and Brown 1995 (i.e.: 1 day at 1 mppcf was converted to dust years by dividing by 240 days per year [i.e. 48 weeks x 5 day week).

** MPPCF-yrs converted to f/cc-yrs using a factor of 1mppcf = 0.146 f/mL. The conversion is based on the average concentration of "fibers" greater than 5μm and particles measured by the midget impinger and reported by Gilliam et al. (1976) i.e.: 0.25f/mL divided by 1.7mppcf

TABLE 6
LUNG CANCER AND NONMALIGNANT RESPIRATORY DISEASE (NMRD) MORTALTY (SMR) AMONG TALC WORKERS

AUTHOR	YEARS	Lung Cancer SMR	Lung Cancer Mine SMR	Lung Cancer Mill SMR	NMRD Overall SMR	NMRD Mine SMR	NMRD Mill SMR	mesoth
NY Brown et al(1979)	1947-59 Follow-up 1975 19% mortality	9/3.3=2.73 (1.25-5.18)			8/2.9=2.76 (1.19-5.13) Other 5/1.3 = 3.85(1.25-8.96)			
NY Lamm et al. (1988)	1947-78 >1-year tenure 14.8% mortality	6/3.1=1.93 (0.71-4.20)			7 / 2.5 = 2.78 (1.11-5.72)			
NY Honda et al.(2002)	>1 day tenure 1948-1989	31/13=2.32 (1.57-3.29)	18/46=3.94 (2.33-6.22)	7/5.5=1.28 (0.51-2.63)	28/13=2.21 (1.47-3.20)	10/4.2=2.41 (1.16-4.44)		
NY Brown et al. (1990)	1947-1978; follow-up 1983; 23% mortality	17 / 8.2 = 2.07 (1.20-3.31)			17 / 6.8 = 2.50 (1.46-4.01)			
	≥1 yr tenure	9/4.7 = 1.91 (0.88-3.64)			11/3.8 = 2.89 (1.45-5.18)			
Vermont Selevan et al.(1979)	1940-1975; >1-yr tenure before 1970; 23% mortality	6/3.69=1.63 (0.60-3.54)	5/1.15=4.35 (1.41-10.1)	2/1.96=1.02 (0.12-3.68)	11/3.67=3.0 (1.50-5.36) Other=11/1.79= 6.15 (3.07-11)	2/1.23= 1.63 (0.20-5.87) Other= 2/0.56 = (0.43-2.89)	7/1.72=4.07 Other =7/0.89 = 7.87 (3.15-16.2)	
Italy Coggiola et al. (2003)	>1 yr, 1946-1995 49% mortality	44 / 46.9 = 0.94 (0.68-1.26)	33 / 30.9 = 1.07 (0.73-1.50)	11/ 16 = 0.69 (0.34-1.23)	127 / 55.7 = 2.28 (1..9-2.72)	105 / 34.4 = 3.05 (2.5-3.7)	22 / 21.3 = 1..04 (0.65-1.57)	
France Wild et al. (2002)	1945-1995, >1- yr; 27.5% mortality	21/17=1.23 (0.76-1.89)			26/24.6= 1.06 (0.69-1.55) Pneumoconiosis 3/0.5=5.56 (1.12-16.2)			
Austria Wild et al. (2002)	1972-1996, >1- yr; 12.4% mortality	7/6.6=1.06 (0.43-2.19)			1/3.7=0.27 (0.01-1.52)			
Norway Wergeland et al (1990)	>1-yr: miners 1944 –1972; 28.7% mortality >2-yrs millers 1935-1972;	SIR: 6/6.49 = 0.92 (0.34-2.01)	SIR: 2 / 1.27 = 1.57 (0.19-5.69)	SIR: 4 / 5.22 = 0.77 (0.21-1.96)	Diseases of Respiratory System SMR: 3/10.9 = 0.28 (0.06-0.80)	SMR: 1/ 2.5 = 0.40 (0.01-2.23)	SMR: 2 /8.5 = 0.24 (0.03-0.85)	

	30.5% mortality.							

Table 7
MESOTHELIOMA/LUNG CANCER EXPERIENCE –NON-ASBESTIFORM ANTHOPHYLLITE AND ANTHOPHYLLITE ASBESTOS MINERS AND TREMOLITE ASBESTOS

STUDY POPULATION	FOLLOW-UP PERIOD	N in COHORT (% deaths)	PMR)Mesothelioma / total deaths)	Lung Cancer SMR (95% confidence intervals)
Talc workers, NY State. [Honda et al. 2002]	White men actively employed >1 day between 1948 and 1989 and alive in or after 1950. Follow-up 1950 thru 1989	809 (27%) Mill = 377 Mine = 311	2/209= 0.96%*	31/13=2.32 (1.57-3.29) Mill: 7/5.5=1.28 (0.51-2.63) Mine: 18/4.6=3.94 (2.33-6.22)
Norwegian talc workers (Wergeland et al, 1990)	Miners >1 yr 1944-1972; Millers >2 yrs 1935-1972; Follow-up 1953-1987	Total (M) 389 (30.1%) 94 miners (28.7%) 295 millers (30.5%)	0/117=0% 0/27=0% 0/90=0%	Incidence (SIR): 6/6.49=0.92(0.34-2.01) 2/1.27=1.57 4/5.22=0.77
Finnish anthophyllite asbestos miners Karjalainen et al. (1994) Meurman et al. (1994)	> 3 mos 1953-1967; Follow-up 1953-1991	999 (59.4%) M = 736 (68.3%) F = 167 (53.9%)	4 / 593 (0.7%) M = 4/503 (0.8%) F=0/90 (0%)	Incidence: SIR M: 76/26.4 = 2.88(2.27-3.6) Heavy Exp: 3.15(2.37-4.09) Mod Exp: 2.35(1.45-3.58)
Vermiculite miners, Libby, MN. [McDonald et al 2004]	> 1-year before 1963, followed to 1999	406 70.2% mortality	12 / 285 = 4.2%	44 / 18.3 = 2.40 (1.74-3.22)
South Carolina Vermiculite McDonald et al (1988)	<6 months 1971-1986, followed to 1986	194 51/194 = 27.8% (>15 yrs latency	0 / 51 = 0%	4/3.31 = 1.21 (0.33-3.09)

* See text. Cases were not considered to have resulted from work at the talc mine. One case had latency of 15 years and one was a draftsman during construction only.

TABLE 8

Dimensions of elongated particles associated with various amphibole exposure industries studied experimentally and/or epidemiologically.

Cohort	Width (μm)	Length(μm)	Reference
Libby vermiculite; tremolite asbestos	46% <0.25	62% >5	Langer et al. (1974)
Homestake gold mine (CG = cummingtonite-grunerite) (TA = tremolite-actinolite) (GM = geometric mean)	69% CG: GM= 0.43 15% TA: GM = 0.27	34% >5 32% >5	Brown et al. (1986)
	0% <0.25 minimum 0.3 mean 1.1	Mean 4.6 Max 17.5	Virta et al. (1983)
Taconite	0% < 0.25 min 0.25 mean 1.2	Mean 5.5 Max 32.4	Wylie (1988)
Vanderbilt tremolitic talc	0% <0.25		Kelse and Thompson (1989)
Experimental Studies			
Korean tremolite asbestos >5 um L	44.7% <0.25	11.8%>5 [1.9]	Addison (2004) Davis et al. (1985)
Californian white tremolite asbestos (Davis and Addison,1981)	50%<0.25	14.9%>5 [3.2]	Addison(2004)
Swansea tremolite asbestos (Davis et al. 1991)	8.2%<0.25	33.6%>5 [1.0]	Addison(2004)
Italian tremolite (Davis et al , 1991)	13.3%<0.25	9.7% >5 [0.27]	Addison (2004)
Greenland tremolite (Wagner, 1982)	0% <0.25	100% <10	Wagner and Berry (1969, 1982).
Dornie,Scotland tremolite Davis(1991)	13.7% <0.25	22.5% >5 [0.1]	Addison (2004)
Shinness tremolite, Davis (1991)	13.8%<0.25	10.6% >5 [0]	Addison (2004)
Ferro-actinolite asbestos	Median: 0.24 Range: 0.03-5.2	Median: 1.50 Range:0.3-52.5	Coffin et al. (1982)
UICC Amosite	Median: 0.22 Range: 0.02-4.1	Median: 1.8 Range:0.15-378	Coffin et al. (1982)

Figures in [] = % >5μm and less than 0.25μm.Addison(2004) provided figures from Davis et al. (1991), calculated from the fiber numbers in the doses used in the experiments by Davis et al.

FIGURE 2: Lung cancer SMRs by cumulative exposure (fibers/mL-years) and pneumoconiosis for non-asbestiform grunerite (Steenland and Brown1995) and grunerite (amosite) asbestos (Seidman et al. 1986)

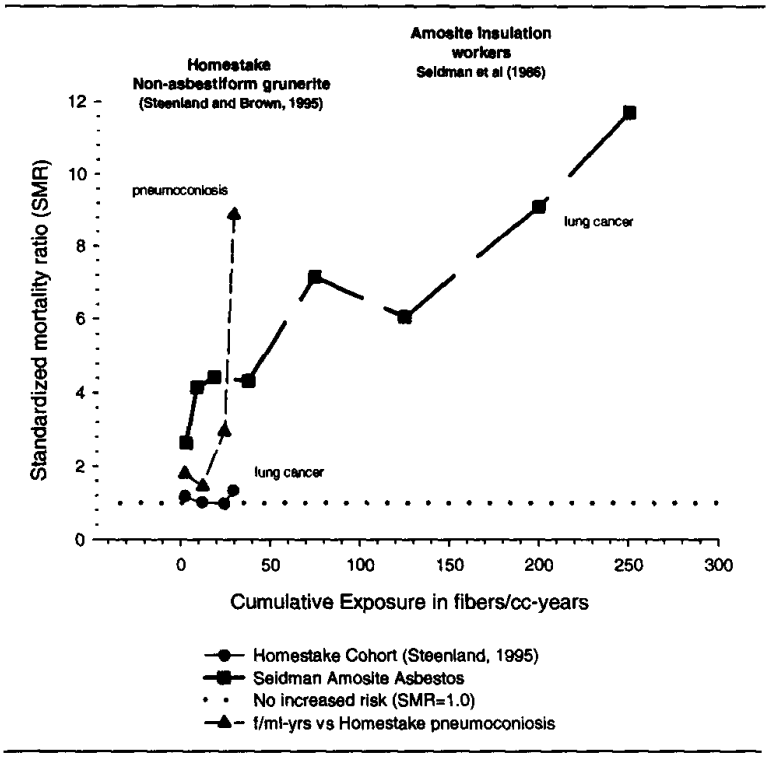


FIGURE 3: Exposure-response of lung cancer, other non-malignant respiratory Disease (other NMRD) and lung fibrosis by Cumulative exposure (mg/m^3-years) Honda et al. (2002)

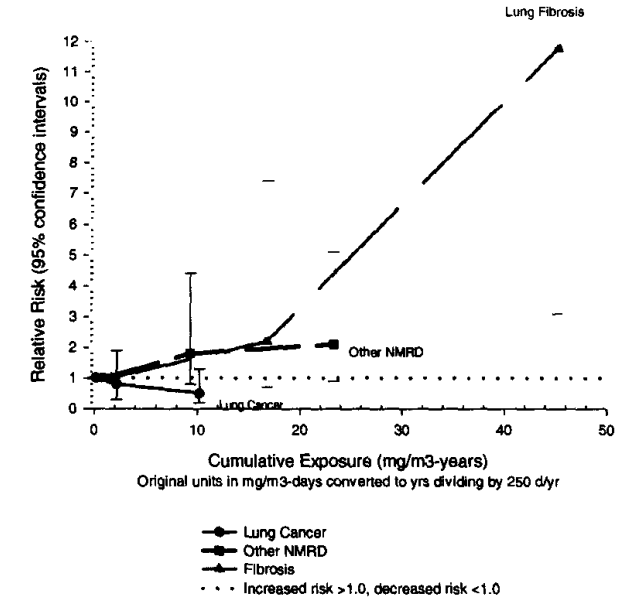


FIGURE 4: Exposure-response trends for lung cancer, mesothelioma and Pneumoconiosis among Vermiculite workers exposed to Vermiculite Ore contaminated with Tremolite asbestos In Libby, Montana (McDonald et al. 1986) Vermiculite with non-asbestiform amphiboles in South Carolina (McDonald et al. 1988)

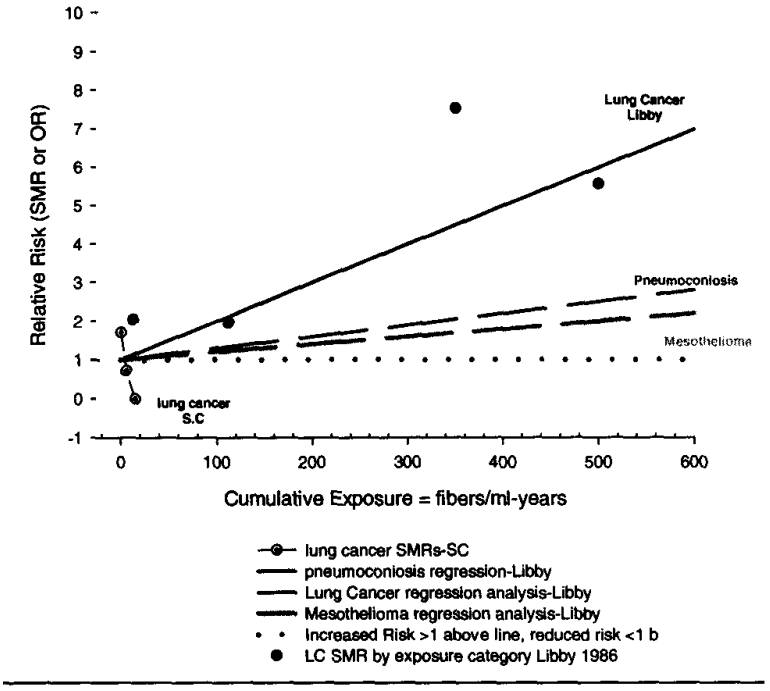


FIGURE 5: Exposure-response trends for lung cancer Non-malignant respiratory disease (NMRD) and Pneumoconiosis by cumulative exposure (mg/m3-years) to Talc not containing amphiboles Among French & Austrian Talc Workers Wild et al. (2002)

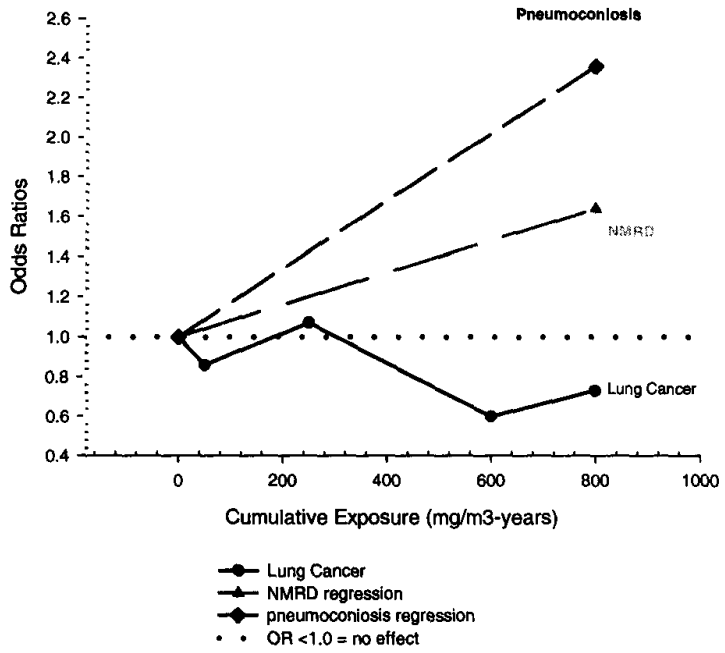


FIGURE 6; Lung cancer and mesothelioma mortality in workers exposed to Talc containing non-asbestiform amphiboles in New York and Norway (Honda et al. 2002, Wergeland et al. (1990) Talc without amphiboles (Vermont, Italy, France/Austria) Selevan et al. (1979), Coggiola et al. (2003), Wild et al. (2002) and Vermiculite containing tremolite asbestos (McDonald et al (1986) Anthophyllite Asbestos (Karjalainen et al. 1994, Meurman et al. 1994)

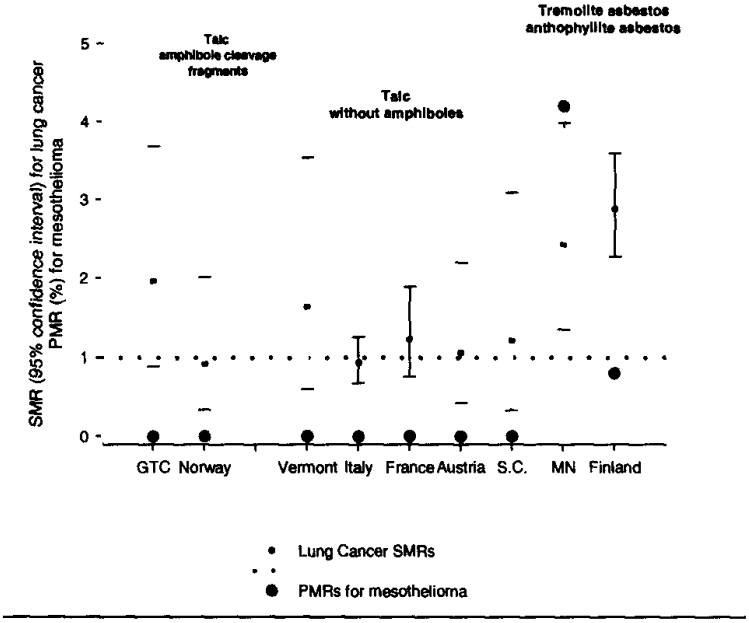


Figure 7: Experimental studies of injections into rats of asbestiform amphiboles and non-asbestiform amphiboles.

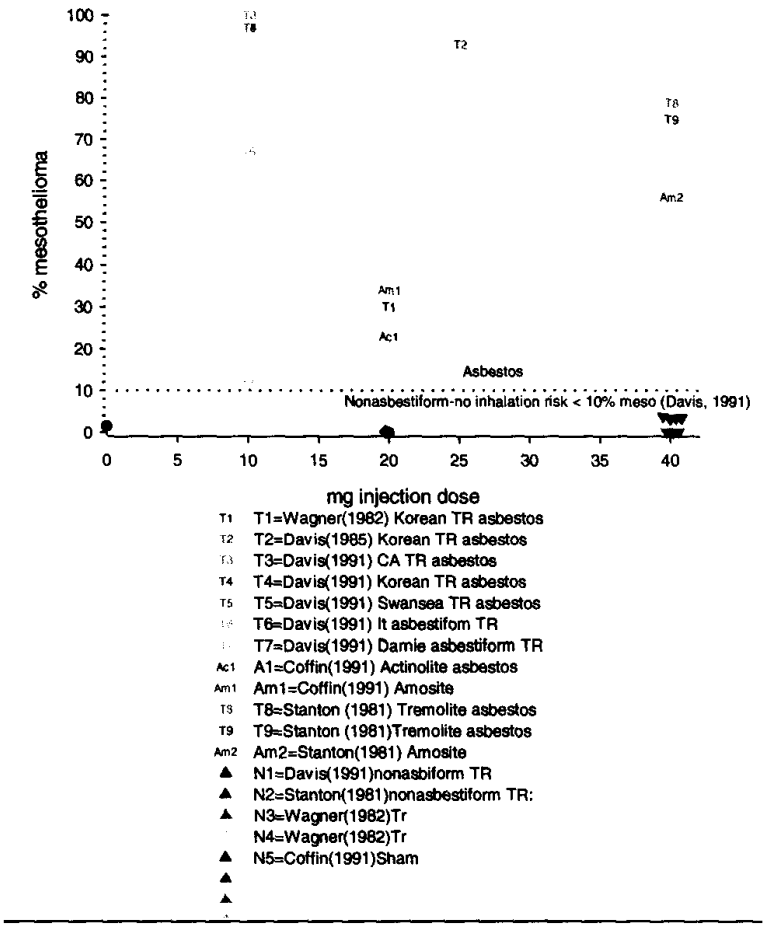
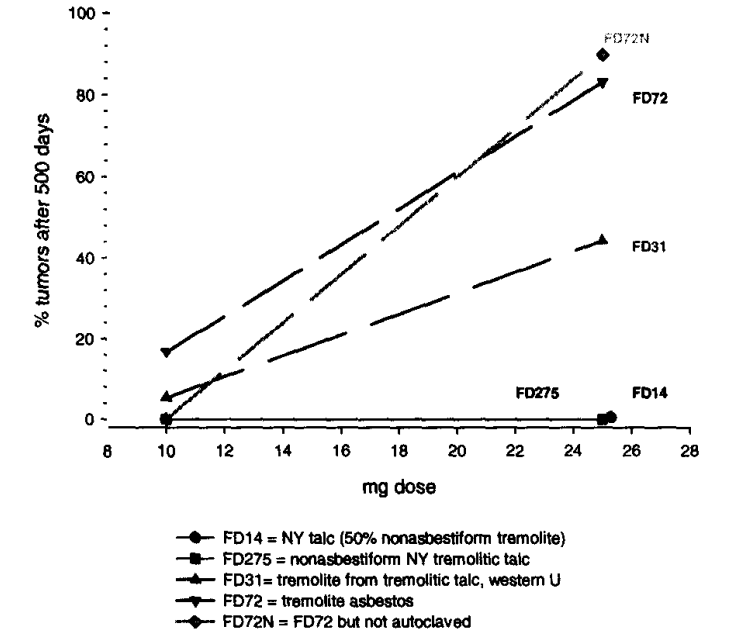


Figure 8: Mesotheliomas in hamsters after intrapleural injection of tremolite asbestos and talc containing non-asbestiform tremolite Smith et al. (1979)



APPENDIX

There is some overlap between this appendix and the main text in order to maintain the historical development of knowledge concerning the NY Talc deposit.

NEW YORK STATE TALC

Early NY Talc Studies: Kleinfeld et al. (1967) conducted a PMR mortality study among 220 talc miners/millers with 15 or more years of exposure in 1940, with follow-up to 1965. There were 28 deaths (31%) attributed to pneumoconiosis and complications and a PMR of 3.44 for 9 deaths from lung cancer and 1 from fibrosarcoma of the pleura. Kleinfeld et al. (1967) also reported that in a small group of asbestos insulation workers with similar years of exposure, the asbestos workers had about twice the proportion of lung cancer deaths (24% Vs 11%) and the significant excess was in both the 40-59 and 60-79 year age groups. This is "at variance" with the talc workers where the excess was only in the 60-79 year age group (PMR = 4.36) and a deficit (PMR = 0.96) in the 40-59 year age group. Overall, lung cancer mortality among the asbestos insulators was 2.5 times higher than among the talc workers, 8.43 versus 3.44

Kleinfeld et al. (1974) added 4 more years of follow-up (to 1969), 40 more workers in the cohort (for a total of 260), 17 more total deaths (for a total of 108) and 3 more respiratory cancers (for a total of 13). Similar results to the 1967 study were obtained with the only significant excess of respiratory cancers in the 60-79 age range (PMR = 4.61) and not in the 40-59 year age group (PMR = 1.63). The authors thought it was noteworthy that the significant excess respiratory cancer mortality was in the years 1945-1959 (PMR = 3.37) and not in the years 1960-69 (PMR = 1.35) when dust counts were appreciably reduced but fiber counts (fibers/mL >5 um) remained high. Ten of the 13 respiratory cancer deaths occurred in workers exposed 15-24 years (and about the same latency). The authors suggested a more susceptible group develops cancer between 15-24 years leaving a less susceptible group in spite of more years of exposure. The size of the cohort is too small to confirm this hypothesis. There was one case of peritoneal mesothelioma but no information regarding latency or other work exposures.

Exposure was characterized as predominantly talc admixed with silicates such as serpentine, tremolite, carbonates and a small amount of free silica. Exposures were quite high before 1945 when both pneumoconiosis and lung cancer cases began working. Wet drilling began after 1945, which reduced mine levels from 818 to 5 mppcf. Exposures were lower in the mill than the mine prior to 1945, but after 1945 were not reduced as much as in the mine and were now 5 times (or more) higher than in the mine. Workers with lung disease had initial exposures prior to 1945 before wet drilling began and when average dust counts in the mine were 818 (83-2800) mppcf for drilling and 120 (2-475) for mucking. In the mill, averages were 180, 69, 92 and 151 mppcf for crushing, screening, milling and bagging. After 1945 (1946-1965) average dust counts were reduced to about 5 mppcf in these jobs in the mine and in the mill averages were generally below 50 mppcf.

Kleinfeld et al. (1973) studied 39 workers exposed to commercial talc dust where tremolite and anthophyllite were the major fibrous components. They also examined 16 talc samples from different mining and milling operations as well as finished products from NY State. Analyses included polarized LM, TEM with selected area diffraction, X-ray diffraction and electron microprobe analysis. No data are provided on distribution by fiber sizes. The point is made that there was no correlation between fiber count (fibers > 5 um) and mean dust counts (mppcf). Particles observed included "true talc, talc fibers, serpentine minerals and after fragments, and amphibole fibers and fragments." Fiber counts "may not provide a true picture of exposure to asbestiform minerals because the fiber counts include talc fibers but exclude many small asbestos fibers and 'aggregate fibers' which may contain substantial amounts of asbestiform minerals." The electron micrographs of amphibole fibers present in talc suggested amphibole cleavage fragments.

NY Tremolitic Talc

Brown et al. (1980) reported the dimensions of fibers determined by electron microscopy. Only 3% of tremolite fibers and 8-10% of anthophyllite fibers were longer than 5 μm; median lengths were about 1.5 μm. Median aspect ratios of 7.5 and 9.5 were reported for *all* fiber lengths of tremolite and anthophyllite. Data were not provided on aspect ratios for fibers > 5 um counted using phase contrast microscopy.

There then began a series of mortality studies of workers at the Gouveneur talc mine and mill in NY state (GTC) (Brown et al. 1979 1980 1990, Stille and Tabershaw 1982 Lamm et al.1988, Gamble 1993, Honda et al. 2002, Oestenstad et al. 2002). The extensive literature on GTC talc centers on three major issues that started with the first NIOSH mortality and industrial hygiene study of GTC workers.

Is the reported excess SMR for lung cancer due to the alleged asbestiform amphiboles in the talc or due to confounding? Confounding factors could include other work exposure (primarily in the surrounding mines/mills), from life-style factors such as smoking or short-term employees.

Is the tremolite and anthophyllite content of the talc non-asbestiform cleavage fragments or is the talc contaminated with tremolite asbestos and anthophyllite asbestos?

Is there biological plausibility that the tremolitic talc acts like asbestos producing asbestos-like effects in animal studies?

Epidemiology of Health Effects of GTC Talc: Brown et al. (1979, 1980) studied 398 white males first employed 1947-1959 with vital status determined as of 1975. There was a 2.73-fold excess risk of lung cancer. Risk increased with increasing latency with SMRs of 2.00 and 4.62 at 10-19 and 20-28-yrs latency, which was said to be "consistent with an occupational etiology." There was no analysis by years worked although 4 / 9 cases had worked less than 1 year. Smoking was considered unlikely to account for all the increased

risk by Brown et al. (1979, 1980). Exposures in surrounding mines and mills were higher but all were said to involve exposures to "asbestiform amphiboles." Exposures to "asbestiform tremolite and anthophyllite stand out as the prime etiologic factors associated with the observed increase in bronchogenic cancer."

Stille and Tabershaw (1982) studied 655 white males employed 1948-1977 with vital status determined at the end of 1978. Lung cancer was only significantly elevated among employees with any prior employment history . There was no analysis by years worked and latency was not taken into account.

Because of these conflicting findings, Lamm et al. (1988) reanalyzed these data. They studied 725 male talc workers who had ever worked at Vanderbilt since the plant opened in 1947 through the end of 1977 with follow-up through 1978. Previous employment obtained from job applications were classified as posing a prior risk, no prior risk or unclassifiable (no indication of prior work history) with regard to risk of lung cancer. Among those with more than 1-year employment the SMRs for lung cancer and non-infectious, non-neoplastic respiratory diseases were 1.93 and 3.70 respectively, compared to 3.00 and 0 for those with less than 1-year duration. Adding prior exposure history to the analysis showed that lung cancer risk appeared to be related to prior employment. The SMRs were similar for all job risk categories, although the number of cases was too small to be definitive. Mean latency was 20.8 years (12-25) and all those with less than 20 years latency since being hired at GTC had worked elsewhere. Five of the 12 cases had 3 months or less employment. The authors conclude the increased risk of lung cancer in this cohort of talc workers is concentrated in short-term workers, probably due to prior employment, smoking or other differences in behavioral characteristics.

At the request of RT Vanderbilt and Company, NIOSH conducted a health hazard evaluation (HHE) of the GTC cohort (Brown et al. 1990). Eight years of follow-up (through 1983) and an analysis by latency and tenure were added to the retrospective cohort study. Nearly a third (27%) of the cohort had died, with 161 total deaths and 17 lung cancer deaths with an overall SMR of 2.07. About 50% of the cohort had worked less than 1 year. Among the 13 lung cancer cases with 20 or more years latency, there was a 3.6-fold excess in the 8 cases with less than a year tenure Vs. a nonsignificant SMR of 1.79 among the 5 cases with >1-year tenure. There were also 17 NMRD deaths with an overall SMR of 2.50 (1.46-4.01). Six of the cases had worked for less than 1 year with an SMR of 1.94 (0.72-4.28). There was a 3-fold excess (SMR 2.89; 1.45-5.18) among those with more than 1-year tenure. This pattern for NMRD is "more consistently associated with an occupational exposure at GTC." Principal limitations in this study were small size (especially those with long tenure), inability to precisely characterize past occupational exposures at GTC or elsewhere, and lack of reliable smoking history. The authors concluded it is unlikely these potential confounders alone could account for the observed excess risks.

Gamble (1993) conducted a case control nested in the Brown et al. (1990) cohort. Information was collected on smoking, time exposed to talc plus a risk ranking on non-talc exposure. There were 22 cases and 66 controls matched on date of birth and date of hire. There were zero nonsmokers among the cases (91% smokers, 9% ex-smokers) compared to 27% nonsmokers, 73% smokers or ex-smokers among controls. Inverse trends were consistently observed by years worked for different subsets of the study

population; e.g., all cases and controls, smokers only, those with >20-years latency, total tremolitic talc years. The author concluded that "after adjustment for...smoking and the postulated role of very high exposures of short-term workers, the risk ratio for lung cancer decreases with increasing tenure." The time occurrence of lung cancer was consistent with a smoking etiology, and was not consistent with an occupational relationship.

Finally, Honda et al. (2002) assessed cancer and non-cancer mortality among white male GTC talc workers. The cohort analyzed for cancer mortality consisted of 809 workers employed 1947-1989 and alive in 1950. The cohort analyzed for non-cancer mortality consisted of 782 men employed during 1960-1989. The important additions in this study were 6 more years of follow-up (through 1989) and internal exposure-response analyses with cumulative exposure to talc dust as the exposure variable. Overall mortality continued to remain elevated at 1.31 ((209/160) due largely to 2.32-fold excess from lung cancer (31/13) and 2.21-fold excess in NMRD (28/13). The patterns are consistent with previous results, in particular with the inverse lung cancer trends from the nested case-control study (Gamble, 1993) and the inverse relationships for NMRD and lung cancer reported by Lamm et al. (1988). Honda et al. (2002) reported that among workers with >20-years latency, there was a 3.3-fold excess lung cancer for <5-years tenure and 1. 9-fold excess for >-5 years tenure. For other NMRD (COPD + pneumoconiosis and excluding pneumonia, influenza, asthma, emphysema and bronchitis) the SMRs were 2.71 and 3.02 respectively. The internal comparisons by cumulative exposure (mg/m^3-yrs) and adjusted for age and latency, showed a significant monotonic decrease in lung cancer risk with increasing exposure with a RR of 0.5 (0.2-1.3) in the highest exposure category. Mortality from 'other NMRD' and pulmonary fibrosis showed monotonic increases in risk as exposure increase. Risks were increased 2-fold and 12-fold increased risks in the highest exposure categories (Figure 3).

There were 2 cases of mesothelioma, but because of too short latency in one case and minimal exposure for a short time, Honda et al. (2002) considered it unlikely that exposure to talc ore was the cause.

Because of too short latency, Honda et al. (2002) concluded that the cause of the increased lung cancer mortality in the cohort is unclear, but speculated that it could be due in part to smoking or "other unidentified risk factors." They suggest it is unlikely to be related to talc ore dust per se. Other NMRD (and in particular fibrosis) were considered causally related to talc ore dust, other dusts in other work environments and smoking. This conclusion is supported by the differences in years worked and median cumulative exposures among decedents with these three causes of death and the inverse E-R trend for lung cancer (Table A1).

TABLE A1

Exposure differences between cases of lung cancer, Other NMRD and Fibrosis in NY talc workers (Honda, 2002)

	Lung Cancer	Other NMRD	Fibrosis
Median Yrs worked	1.0	8.3	11.8
Median Cumulative Exposure (mg/m^3-days)	347	1199	3759

These results are not at all consistent with the dust causing fibrosis being responsible for the lung cancer excess.

Summary of Results from studies of NY Talc Workers

The cohorts studied before 1979 by Kleinfeld and colleagues worked in talc mines in St Lawrence County, NY. After 1978 the cohorts were comprised of workers at the Gouverneur mine and mill, some of whom had previous employment in other mines in St Lawrence County, NY (Table A2).

The authors of the two NIOSH studies of GTC talc (Brown et al. 1979, 1980, 1990) concluded that the tremolite and anthophyllite were the most likely etiological agents. This conclusion is based on the following logic.

The excess risk of lung cancer and NMRD were consistent with the findings of Kleinfeld et al. (1967, 1973) among NY talc workers and Meurmann et al (1974, 1979) among anthophyllite asbestos miners.

The etiological agents were considered to be "asbestiform tremolite and anthophyllite," which were said to be in both talc ores at concentrations well above standards. Smoking could not account for the excess lung cancer risk. Short-term workers may have had "very high exposures, especially in the early years of the mining operation," which might account for their excess risk (Brown et al. 1990). There was an increased risk of developing pleural changes (including pleural thickening and pleural calcification), and the prevalence is higher when there is exposure to anthophyllite (Dement et al. 1980).

The lack of an association with years worked could be due to a combination of factors above plus work in other talc operations and/or other work-related exposure to lung carcinogens.

Many of these arguments have been contradicted by further analyses.

Kleinfeld et al. (1967) compared lung cancer risk patterns of talc workers with (apparently) their own data for a similar group of asbestos insulation workers. The asbestos PMRs were 2-3 times higher among the asbestos workers for lung cancer and GI cancers. Kleinfeld et al. commented that a major difference was the increased risk of lung cancer in age groups of 40-59 and 60-79 among asbestos workers, but excesses for talc workers were among only the 60-79 age group. In addition, longevity of talc miners was longer than the national average. Age at death among the talc lung cancer cases was 3-years greater than the average of all deaths and 10-years greater than the U.S. average. The talc lung cancer cases occurred in persons exposed before wet drilling was introduced. Wet drilling reduced mean exposures 164-fold from an average of 818 mppcf to 5. Kleinfeld et al (1967) suggested part of the reason for the earlier deaths of asbestos cases compared to talc cases "may be partly due to the greater carcinogenicity of asbestos dust or to an increased level of exposure to asbestos or both."

There was excess mortality among the NY talc workers, but considerably less than the risk of asbestos workers exposed in the same time period. It is not possible to directly compare risks from the Kleinfeld et al. (1974) cohort with that of the GTC cohort. The Kleinfeld et al. cohort et al is older, had worked decades earlier than the GTC cohort, and consisted of workers with more than 15-years tenure and 40+ years tenure. Vanderbilt workers included many short-term workers with 26-years as the maximum possible years worked and no analysis by years-worked (Brown et al. 1979, 1980). In addition, overall mortality was over twice as great in the Kleinfeld et al cohort, i.e., 42% Vs. 19%. *When stratified by years worked in subsequent follow-ups there were 2 cases with >20-years tenure (SMR = 1.82) and 5 cases with >10-years tenure (SMR = 2.17) (Brown et al, 1990). Gamble (1993) reported risk ratios less than 1.0 for lung cancer cases with >15-years tenure and adjusted for smoking. These data are suggestive of a different mortality pattern of GTC talc workers compared to the Kleinfeld talc cohort.*

Smoking. *Further updates of the GTC cohort revealed that all of the lung cancer cases were or had smoked cigarettes, while only 73% of controls had ever smoked. Also, smoking latencies for GTC cases was consistent with latency from studies of smokers. Talc latencies were too short to attribute lung cancer etiology to talc exposure or work (Gamble 1993). This is particularly true for short-term workers where the risk of lung cancer was highest and talc exposure (or most any work exposure including asbestos) too short to be plausible. Risk among workers with more than 1-year exposure was increased about 2-fold compared to the US population. This degree of increased risk is in large part plausibly attributable to smoking.*

High Exposure of Short-Term Workers. *Gamble (1993) matched on date of hire in the nested case control study of lung cancer. Thus, cases and controls had equivalent opportunities for very high exposures. Six of the lung cancer cases had less than 3-months tenure, several with only a few days, so there were very few opportunities for excessive cumulative exposure. Honda et al (2002) showed that lung cancer cases had lower exposures than other subgroups. For example, median cumulative exposure of lung cancer decedents was 347 mg/m^3-days, which was less than all decedents (520), ischaemic heart disease decedents (376), all NMRD decedents (888), other NMRD decedents, pulmonary fibrosis decedents (3,759). Thus there is no evidence to support the*

speculation that excessively high exposure in short-term workers could explain their increased risk.

Pleural Changes. *Gamble et al. (1979a, b, 1982) showed that the prevalence of pleural changes in GTC talc workers was essentially the same among other workers exposed to talc containing no measurable quantities of amphiboles. Thus it would appear that the pleural thickening observed in NY talc workers and other talc workers is likely due to factors other than exposure to amphiboles.*

Exposure-response (E-R): *The inverse exposure-response trends with duration of exposure were present when adjustments were made for other talc exposures and potential exposure to other work-related carcinogens (Gamble 1993). The inverse E-R trends for lung cancer and cumulative exposure are strong arguments against attributing increased risk of lung cancer to talc exposure. This argument is further strengthened by the very strong exposure-response relationship between fibrosis and cumulative talc exposure as well as the higher exposure of NMRD and fibrosis cases compared to lung cancer cases (Honda et al. 2003).*

TABLE A2

Summary of results for Lung Cancer and Mesothelioma from studies of NY Talc workers. All but two of the studies (Kleinfeld et al. 1967, 1974) were the same cohort of GTC workers.

Reference	Study Characteristics	Lung Cancer	Mesothelioma
Kleinfeld et al. (1967)	220 NY Talc Miners ≥ 15 yrs tenure in 1940; 1965 follow-up, 91 total deaths, PMR	PMR=3.44 (1.65-6.3) (11 deaths)	1 peritoneal mesothelioma (1.1%)
Kleinfeld et al. (1974)	260 NY Talc Workers ≥ 15 yrs in 1940 or between 1940-1969; 108 total deaths, PMR, follow-up of Kleinfeld et al (1967)	PMR resp cancer =3.24 (1.72-5.54) (12 lung cancer, 1 fibrosarcoma of pleura)	1 peritoneal mesothelioma (0.93%)
Brown et al. (1979,, 1980)	398 WM employed GTC 1947-1959, follow-up 1975; 18% < 1month, 24% 1mos-6 mos, 50% < 1 yr; 44% <1950;	9/3.3 = 2.73 (1.25-5.18) (p<0.05); 4 <1-yr tenure	1/74 = 1.4% (16-y talc tenure, 11 yrs construction)
Stille & Tabershaw 1982)	655 WM employed GTC 1948- 1978, vital status 1978;	10/6.4 = 1.57 (10 obs) Prior employment=2.14 (8 obs)) No prior work = 0.76 (2 obs))	
Lamm et al. (1988)	705 men employed GTC 1947-end 1977, vital status 1978	12/5=2.40(1.24-4.19) <u>≥1 yr</u> 6/3.1=1.93(0.71-4.20) prior risk = 3.08(6/2) <u>≤1 yr</u> 6/1.9=3.160.16-6.88) prior risk=3.33 (3/0.9)	1 electrician 15-yr latency; 20-yrs prior as miner, miller, construction
Brown et al. (1990)	710 WM employed at GTC 1947-1978 with vital status 1983;	17/8.2=2.07(1.20-3.31) <u>≥20-yrs latency</u> <1-yr = 3.64(1.54-7.04) 1-9-yrs = 0.83(0.02-4.57) 10-19-yrs = 4.0(0.54-16.1) 20-36-yrs = 1.820.21-6.36)	Not reported,
Gamble (1993)	22 lung cancer cases at GTC 1947-1978 matched 3:1 on data of birth and date of hire.	Tenure / OR lung cancer Smokers >20-y latency < 5 yr / 1.0 5-15 yrs / 0.63 15-36 yrs / 0.42	
Honda et al. (2002)	809 WM talc workers employed GTC 1948-89 follow-up Cancer: 1950-1989 Non-cancer mortality = 1960-1989	mg/m3-d RR (n) <95 1.0 (11) <987 0.8 (9) 987 + 0.5(9) Hired : < 1955 SMR 2.86 (0.9-4.1) Hired >1955 SMR: 0. (0.2-2.4)	2 cases not considered causal due to short latency, Case 1 & very low exposure, Case 2 (3.7%)

Pn = pneumoconiosis

Assessment of the Pathogenic Potential of Asbestiform vs. Nonasbestiform Particulates (Cleavage Fragments) in *In Vitro* (Cell or Organ Culture) Models and Bioassays

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Abstract

Asbestos fibers are highly fibrous silicate fibers that are distinguished by having a large aspect (length to diameter) ratio and are crystallized in an asbestiform habit that causes them to separate into very thin fibers or fibrils. These fibers are distinct from nonasbestiform cleavage fragments this may appear as thick, short fibers which break along cleavage planes without the high strength and flexibility of asbestiform fibers. Because cleavage fragments of respirable dimensions have generally proven nonpathogenic in animal studies, little data exists on assessing well-characterized preparations of cleavage fragments in *in vitro* models. The available studies show that cleavage fragments are less bioreactive and cytotoxic than asbestiform fibers.

Introduction and Definition of Asbestiform vs. Non-asbestiform Particulates

'Asbestos' is a commercial and regulatory designation for a family of naturally occurring asbestiform fibers. Asbestos fibers are recognized as human carcinogens and also cause pleural and pulmonary fibrosis, i.e., asbestosis in occupationally exposed individuals (Mossman *et al.*, 1990; Mossman & Churg, 1998; Mossman & Gee, 1989). Mineralogical and biological differences exist between various types of asbestos fibers, and much research has focused on the characteristics of fibers that are associated with the causation of lung disease. The different types of asbestos include chrysotile [$Mg_6\,Si_4\,O_{10}\,(OH)_8$] , the only asbestos in the serpentine family of minerals, and other types of asbestos classified as amphiboles. These include crocidolite [$(Na_2\,(Fe^{3+})_2(Fe^{2+})_3\,Si_8\,O_{22}\,(OH)_2$], asbestiform grunerite or amosite [$(Fe,Mg)_7\,Si_8\,O_{22}\,(OH)_2$] , anthophyllite [$(Mg,Fe)_7\,Si_8\,O_{22}\,(OH)_2$], tremolite [$Ca_2\,Mg_5\,Si_8\,O_{22}\,(OH)_2$], and actinolite [$(Ca_2\,(Mg,Fe)_5\,Si_8\,O_{22}\,(OH)_2$]. These formulae are indeed ideal, and natural amphiboles differ to varying degrees from these as the chemical environment, pressure and temperature at the time of formation control the mineral chemistry. Other factors such as shear stresses and directed pressures determine whether or not an amphibole that crystallizes is asbestiform. Although various types of asbestos are different chemically, structurally and biologically, they are common in that they are highly fibrous silicate minerals that are crystallized in an asbestiform habit, causing them to separate into thin fibers or fibrils (Klein, 1993; Veblen & Wylie, 1993). In addition, asbestos fibers are distinguished by having large aspect (length to diameter) ratios, generally from 20:1 or higher for fibers > 5 microns in length. Smaller fibers (<0.5 microns in width) appear by microscopy as very thin fibrils as defined by the American Society of Testing Materials in 1990. In contrast, nonasbestiform cleavage fragments, although sometimes elongated with aspect ratios of >3:1 which can be defined as fibers, have widths much larger than asbestos fibers of the same length. Though the more common nonasbestiform analogs of asbestos share the same, or essentially the same chemical composition, they do not share the same crystal structure (the crystals form or grow differently).

Cleavage fragments of amphiboles lack the tensile strength of asbestos amphiboles and are traditionally regarded by mineral scientists as distinctly different from asbestos fibers, primarily based on their morphology, and lack of strength or flexibility. For example, in the report of the Committee on Nonoccupational Health Risks of Asbestiform Fibers commissioned by the

National Research Council (National Research Council, 1984), cleavage fragments were categorized as distinctive from asbestiform fibers, i.e.:

"CLEAVAGE refers to the preferential breakage of crystals along certain planes of structural weakness. Such planes of weakness are called cleavage planes. A mineral with two distinct cleavage planes will preferentially fracture along these planes and will produce ACICULAR fragments. Minerals with one cleavage plane produce PLATY fragments and those with three or more cleavage planes yield POLYHEDRAL fragments..... Cleavage cannot produce the high strength and flexibility of asbestiform fibers" (National Research Council, 1984).

These definitions were also recognized by the members of the panel of the Health Effects Research-Asbestos Research in their report on Asbestos in Public and Commercial Buildings (Health Effects Institute-Asbestos Research, 1991). Because epidemiologic and animal studies have not suggested that nonasbestiform amphiboles or cleavage fragments are pathogenic or biologically active, they have not been used in many *in vitro* models, except as negative or nonpathogenic controls for testing of asbestos fibers. Moreover, the results of numerous epidemiologic, animal, and *in vitro* studies, have led scientists to conclude that short asbestos fibers (< 5 microns in length) are inactive or much less active biologically than long, thin asbestos fibers (ATSDR, 2003; Health Effects Institute-Asbestos Research, 1991). Thus, it is unlikely that cleavage fragments of respirable dimensions (i.e., less than 3 microns in diameter) will be pathogenic or targeted extensively for *in vitro* fiber testing in the future. The results of limited work with these minerals from our laboratory and others are summarized below.

Advantages and Caveats of *In Vitro* Mineral Studies

In vitro studies have been used historically to compare the effects of different types of minerals on cells or organ (explant) cultures (Mossman & Begin, 1989). Regardless of cell type, asbestos fibers, in comparison to a variety of other nonpathogenic, synthetic or naturally occurring fibers (glass, cellulose, etc.) or particles, have been most biologically active in these models. In addition to elucidating the properties of minerals (size, fibrous morphology, surface charge, chemical composition, etc.) that are associated with toxicity (cell injury or death), DNA damage, proliferation and/or alterations in cell function that may be predictive of their pathogenic potential, *in vitro* studies have shed light on the complex features of bioreactive minerals that may be important in reactions with cells and their ability to cause disease. Cell and organ culture models are also much more inexpensive than animal testing. Thus, they have been suggested as screening tools for new synthetic fibers developed for industry.

However, there are also caveats that must be recognized in *in vitro* work with minerals. First, dependent upon the cells used in these models, cell type and species-specific responses may exist. Thus results from lab to lab working with the same mineral might be inconsistent. Although the most appropriate *in vitro* cell types to use in these models are normal cells of respiratory tract origin, i.e., epithelial or mesothelial, these are notoriously difficult to isolate and maintain in a differentiated state for prolonged periods of time. It also should be acknowledged that concentrations of minerals used in short term *in vitro* assays, where weighed amounts of fibers or particles are precipitated on cells, do not mimic normal clearance patterns and long-

term dissolution patterns after inhalation into the human lung, factors that are important in dosimetry and disease causation (Mossman *et al.*, 1990). Lastly, different minerals are generally evaluated in *in vitro* studies on an equal weight basis, which might be misleading based on the facts that different weights of dissimilar fiber types or particles may reflect vastly different total numbers of fibers and surface areas. Regardless of these caveats, however, *in vitro* studies have helped to establish mechanisms of fiber carcinogenesis and differentiated between responses to asbestos fibers and nonasbestiform particles.

Studies Using Tracheal Explants

In comparison to cell cultures, tracheal explant cultures can be maintained for weeks in a differentiated state in which the respiratory epithelium is maintained in a normal, mucociliary phenotype. We have used this model to show that crocidolite and chrysotile fibers (asbestos) and long glass fibers cause squamous metaplasia, a reversible but often premalignant lesion, and increased DNA synthesis, a signature of injury and proliferation of fibers that might be important in tumor promotion and progression and/or repair (Woodworth *et al.* 1983). In contrast, the non-fibrous mineral analogs of these asbestos types, riebeckite (similar in chemistry to crocidolite) and antigorite (similar in chemistry to chrysotile) failed to induce these changes at a range of concentrations and exposure times. Though a number of these riebeckite and antigorite particles were elongated, they were thick, short single crystal cleavage fragments. These studies highlight the importance of fibrous geometry, crystal growth and aspect ratio in bioreactivity.

Studies Using Cell Types of Lung or Pleural Origin:

The antigorite and riebeckite preparations used in the Woodworth et al. 1983 study above were also evaluated in cell cultures of hamster tracheal epithelial cells (HTE) for their ability to induce ornithine decarboxylase (ODC), an enzyme associated with cell proliferation and tumor promotion in mouse skin models of cancer, with asbestos fibers (Marsh & Mossman, 1988). These studies showed that crocidolite and chrysotile (fibers> 10 microns in length) fibers stimulated ODC, but neither of the two nonasbestiform (cleavage fragment) preparations were bioreactive. Subsequent studies revealed that both antigorite and riebeckite were less potent than crocidolite (asbestos) in stimulating survival or proliferation of HTE cells in a colony-forming assay (CFE) in which proliferation was measured directly over a 7 day period in low-serum containing medium (Sesko & Mossman, 1989). Experiments in HTE cells also revealed that antigorite and riebeckite were less cytotoxic than crocidolite or chrysotile to these cells when release of radioactive chromium, a marker of cell damage, was measured (Mossman & Sesko, 1990).

Another exciting development in our laboratory was the observation that crocidolite (asbestos) generated Reactive Oxygen Species (ROS) which have been linked to cell injury, inflammation, mutagenesis, and the development of many cancers, (Shukla *et al.* 2003). In a study in which we isolated alveolar macrophages (AMs) from rodents and measured release of the ROS, superoxide, after addition of crocidolite and riebeckite (nonasbestiform analog of crocidolite) to these cells, as well as nonasbestiform mordenite (note that all particle diameters and/or fiber

lengths were measured by scanning electron microscopy), the nonasbestiform particles were taken up, i.e., phagocytized, by cells, but were much less bioreactive than crocidolite at comparable concentrations, only causing release of superoxide at concentrations 5- to 10-fold higher than asbestos in the rat cells and never causing significantly increased release in the hamster macrophages (Hansen & Mossman 1987). It should be emphasized that lung epithelial cells, mesothelial cells and fibroblasts are target or progenitor cells of lung cancers, mesotheliomas and pulmonary fibrosis, respectively, and that alveolar macrophages are inflammatory cells that first encounter asbestos and may contribute to and/or alternatively, be important in lung defense from pathogenic minerals. This is an important question that has yet to be resolved by scientists. However, alveolar macrophages are studied because these cells accumulate in the lung at sites of deposition of inhaled particles or fibers and responses of alveolar macrophages to dusts are known to produce ROS after phagocytosis of minerals.

In recent years, we have used riebeckite and antigorite preparations as nonasbestiform control minerals to determine whether early response proto-oncogene (*fos/jun* cancer- causing genes) (Janssen *et al.* 1994) or signaling pathways leading to activation of these genes (Janssen *et al.* 1997; Zanella *et al.* 1996; Zanella *et al.* 1999) are selectively induced by asbestiform, cancer-causing fibers (crocidolite and chrysotile asbestos, erionite) in HTE cells, rat lung epithelial cells (RLE) and isolates of normal rat pleural mesothelial cells (RPM). These studies have consistently revealed that these nonasbestiform minerals are inactive, regardless of endpoint. Moreover, they are incapable, in contrast to asbestos fibers, of causing alterations in cell proliferation or death in RPM cells (Goldberg *et al.* 1997).

Comparative studies in HTE and RPM cells with well-characterized mineral samples of crocidolite and chrysotile (asbestos) and 3 mineral samples containing various proportions of fibrous talc have also been useful in illustrating fundamental differences in response to asbestos fibers and fibrous talc preparations based on various dose parameters including equal weight concentrations, equivalent surface areas and numbers of fibers > 5 microns in length (Wylie *et al.* 1997). Using the CFE assay described above to document proliferative potential (increased numbers of colonies as compared to untreated control cells) or cytotoxicity (decreased numbers of colonies as compared to untreated control cells), exposure of RPM cells to both asbestos types, but not fibrous talcs, elicited cytotoxicity in RPM cells that was more striking at higher weight concentrations of asbestos. In contrast, HTE cells proliferated in response to asbestos at nontoxic lower concentrations, but not to fibrous talcs. Since cell responses could not be correlated directly with the presence of mineral fibers > 5 microns in length or aspect ratios, mineral type rather than fiber length *per se* appeared to be a more important determinant of bioreactivity. This study suggests that while fiber morphology is important, it is not the only factor important in biologic responses. This has also been noted by critics of Stanton's famous pleural implantation studies in rats (Oehlert, 1991) (Wylie *et al.* 1987).

Studies Using *In Vitro* Models of Non-Respiratory Cells:

As detailed above, cytotoxicity testing in cells of non-respiratory origin was used decades ago to determine differences in fiber-cell interactions and the ability of asbestos fibers to induce cell

death or lysis. Since dead cells can not give rise to cancers, the extrapolation of these results, especially to mechanisms of cancer causation, is questionable. However, studies by Palekar and colleagues (Palekar *et al.* 1979) used sheep red blood cells (RBC) and Chinese Hamster Ovary (CHO) cells to test the hemolytic potential and cytotoxicity of 4 samples of cummingtonite-grunerite including amosite asbestos fibers, and 3 other samples of various crystallization habits, predominantly asbestiform cummingtonite, acicular cummingtonite ,and acicular grunerite. At the same surface areas of dose, these minerals were found to be hemolytic and cytotoxic in this same order, again showing the increased potency of amphibole asbestiform fibers.

Summary and Conclusions

The results summarized above represent a large body of work showing that nonasbestiform minerals are less potent than asbestos fibers in a number of *in vitro* bioassays. In most assays, these cleavage fragments or non-fibrous minerals are virtually inactive. These observations have been incorporated into the conclusions of several panel reports that should be recognized by regulatory agencies. For example, the HEI-Asbestos Research Panel (page 6-75, 1991) concluded:

"Good evidence exists that thick fibers (>2 to 3 microns in diameter) are less harmful than thin fibers".

"Support for the importance of fiber length in the production of biological effects has been obtained from the use of non-fibrous analogues of asbestos and other fibers. In general, these materials produce no detectable biological effects, or do so only at high dose levels"

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A Review of Carcinogenicity Studies of Asbestos and Non-Asbestos Tremolite and Other Amphiboles.

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ABSTRACT

Experimental animal studies comparing asbestos and non-asbestos varieties of tremolite indicate tremolite asbestos is markedly more carcinogenic. By direct analogy, the differences in carcinogenicity between tremolite asbestos and non-asbestos prismatic tremolite should be the same for the other types of amphibole that also crystallize in the asbestos and non-asbestos habits. The earliest of the experiment animal studies, done more than 25 years ago, have design limitations by modern standards including the use of injection or surgical implantation as the route of administration rather than the more relevant route of inhalation. However the differences in the carcinogenicity of amphibole asbestos and non-amphibole asbestos are sufficiently large to be clearly discernable even with the study limitations. Together with later studies on these and related minerals, there is strong evidence of a much lower hazard associated with the shorter, thicker fibers of the non-asbestos amphiboles, than is found for the asbestos analogues of the same mineral. It is possible that the non-asbestos amphiboles are no more hazardous than other silicate minerals widely considered nuisance dusts.

INTRODUCTION

We will define some basic asbestos terminology to clarify the terms used. The glossary in 'The Health Effects of Mineral Dusts' produced by The Mineralogical Society of America (Guthrie & Mossman 1993) has the following definition: "Asbestos is a term applied to asbestiform varieties of serpentine and amphibole, particularly chrysotile, 'crocidolite', 'amosite', asbestiform tremolite, asbestiform actinolite, and asbestiform anthophyllite. The asbestos minerals possess asbestiform characteristics". The Mineral Society's glossary goes on to define asbestiform as: 'an adjective describing inorganic materials that possess the form and appearance of asbestos. When applied to a mineral, the term 'fibrous' is applied when it 'gives the appearance of being

composed of fibers, whether the mineral actually contains separable fibers or not' (Veblen and Wylie 1993). Asbestiform is a subset of fibrous, where asbestiform implies relatively small fiber thickness and large fiber length, flexibility, easy separability, and a parallel arrangement of the fibers in native (unprocessed) samples. Often, asbestos fibers occur in bundles, i.e., they are often polyfilamentous. From the definition it is clear that not all fibers or fibrous minerals are asbestiform and not all fibrous minerals called asbestiform are asbestos.

A convention has developed that a fiber is any particle with an aspect ratio equal to or greater than 3:1. This stems from the fiber definition in the early UK and US fiber counting methods (Asbestosis Research Council 1969, Asbestos Textile Institute 1971, Langer et al. 1991); it could just as easily have been 5:1 or 10:1. In using these methods, the microscopist had to make a decision to count or not count a particle depending on whether the shape and size met certain size criteria. The decision was more easily and consistently made for particles with aspect ratios just higher or lower than 3:1, and much more difficult with the higher aspect ratio thresholds. Similarly, a minimum fiber length of 5 μm was arbitrarily introduced for a fiber to be counted by these methods.

The inclusion of the abundant short fibers (less than 5μm length) in the count would have made it much less consistent or reliable. Since the aim of the fiber counting rules was to differentiate between asbestos and total particles aspect ratio and length cut-off chosen was one that produced consistency and not the ratio or length that might have had greater toxicological significance. By convention then, for a fiber to be counted it has to have an aspect ratio equal to or greater than 3:1 and a length equal to or greater than 5μm (and in some rules a diameter less than 3 μm, e.g. WHO 1985).This counting strategy has nothing to do with a definition of asbestos per se; it is simply helpful to microscopists doing fiber counting method. Since fiber counting analysis is performed using a phase-contrast light microscope at a magnification of 400-450x, the minimum width that can be counted is 0.2-0.25 μm.

Many non-asbestos particles, including non-asbestos amphiboles and other minerals can have aspect ratio greater than 3:1, but that does not make them 'asbestos' even though they are technically fibers. However, it does mean that they would be counted *as if they were an asbestos fiber* when seen in the course of a count of fibers in a membrane filter sample of airborne dust. In addition, asbestos will produce asbestos dust particles that mostly have aspect ratios equal to or greater than 3:1, but it will also produce particles that have lower aspect ratio. That does not mean that these low aspect ratio particles are not asbestos, but simply that they would not be counted as asbestos in the membrane filter method. The same is true for asbestos fibers with lengths shorter than the 5 μm minimum specified in the fiber counting method.

The adoption by some scientists and regulatory agencies of the fiber counting protocol using a 3:1 aspect ratio and a length of 5 μm or greater as being in some way a definition of asbestos has no scientific basis. It has been useful an improved metric when compared to just counting particles for assessing workplace exposure to airborne fiber dust leading to better epidemiological correlations between asbestos exposures with disease.

MINERALOGY

Tremolite is one member of the calcic amphibole group of minerals that all possess similar crystal structures, basic chemical formula, although the various crystal forms have profoundly different physical properties. The group is characterized by a crystal structure described as a double chain of silicon oxide tetrahedra that is common to all members of the group. Within this chain structure are between 7 and 8 metal cations allowing wide range in elemental composition that still maintains the basic crystalline form (Deer et al. 1997). This has produced the large number of named variants or species within the amphibole group (Leake et al. 1997, 2004) Actinolite and ferro-actinolite are part of a solid solution series with tremolite and differ only in the amount of substitution of magnesium by iron.

All of the amphibole minerals, and particularly tremolite, are very resistant to chemical attack by strong acids and bases (Addison & Davies 1990) so that their biopersistence when inhaled would be expected to be very high. In addition to the chemical variability there is further variability in what is known as the crystal habit of the minerals that may arise independent of chemistry (Dorling & Zussman 1987). The habit of a mineral is a description of the way that the crystals are commonly formed, and might otherwise be described as morphology.

The most common crystal habit for any amphibole is that called prismatic; elongate prisms with a lozenge shaped cross section that grade one way into short stocky prisms and in the other way into fine needle-like crystals or ultimately fine hair-like crystals (sometimes known as byssolite). The prismatic habit is the normal form for amphiboles in igneous and metamorphic rocks and is very widespread throughout the continental crust of the planet. Some amphiboles are also found in the habit that is termed asbestiform; this means that they have crystallized as bundles or matted masses of extremely fine fibers. The appearance of these forms usually implies some sort of secondary modification such as shearing and faulting or hydrothermal alteration. These may be found in three types of geological situations; 1) cross-fiber veins where the fibers have filled planar fissures, such as in the riebeckite (crocidolite) asbestos and grunerite (amosite) asbestos mines of South Africa; 2) in shear planes where slip fiber has formed in the plane of movement of a fault or shear plane; or 3) as disseminated fiber formed by hydrothermal alteration, such as in Libby, Montana (Meeker et al. 2003).

The differences in the manner of the formation of asbestos amphiboles, compared to the prismatic and other forms, have led to subtle differences in the details of the crystal structure that, while not sufficient to warrant a different mineral name, nevertheless lead to profound differences in physical properties (Langer et al. 1991). The commercial exploitation of the asbestos amphiboles depended upon these properties, including their capacity to be readily split into long, thin fibers with high tensile strength. These physical differences also lead to

differences in the size distributions of dusts formed when the minerals are crushed, and arguably properties which impact the pathogenic potential of the material, especially their carcinogenic properties when these dusts are inhaled. Cleavage planes are planes of relative weakness along which certain minerals tend to fracture and are determined by the crystal lattice geometry. Mica, for example, is described as having a single perfect cleavage because it splits easily along the silicate sheet structure. Calcite has three perfect cleavages that form perfect rhombohedra when the mineral is crushed. Amphiboles have two sets of cleavage planes at 126° to each other and parallel to the long axis of the crystals (and parallel to the dominant prismatic crystal faces). In addition they also have a cleavage plane on (100).

These are not perfect cleavages; they are not persistent across or along the crystals and tend to be more widely spaced than the separations between the fibers of the asbestos amphiboles. The prismatic amphiboles, including byssolites, have relatively low tensile strength and the thin needle-like crystals fracture easily across the length. They also fracture along cleavage planes that are parallel to the length of the crystals. When prismatic amphiboles are crushed a relatively small proportion of the fragments formed are elongate with faces determined by the cleavages along which the crystal fractures. These elongate particles will often meet the regulatory size criteria for an asbestos fiber within the asbestos permissible exposure limits, but differ from the asbestos fibers in critical ways. The cleavage fragment fibers often show the typical lozenge shape cross section as determined by the cleavage faces, at 126° degrees to each other. The cleavage fragment fibers tend to be thicker than asbestos fibers because of the spacing of the cleavage planes, and for any given length the cleavage fragment fibers are roughly twice as thick as asbestos fibers. Very few, if any, of the cleavage fragment fibers longer than 10μm will have diameters less than 1μm. With cleavage fragment fibers the width distribution is much broader and width increases with length so aspect ratios tend to be lower and of narrower distribution. In overall size distributions the asbestos fibers have a very narrow width distribution and the width

of fibers is largely independent of length. As a result, the aspect ratio of fibers increases with length.

Since the cleavage fragments and asbestiform fibers tend to be morphologically defined by somewhat different crystal surfaces it is tempting to speculate that this may go some way to explaining the apparent differences in toxicological properties as described below.

EXPERIMENTAL ANIMAL STUDIES

Five *in vivo* experimental animal studies provide information on the variation in carcinogenicity of dusts derived from prismatic or non-asbestos tremolite and tremolite asbestos. Davis et al. (1985) remains the only inhalation experiment to be carried out using tremolite asbestos. Previously, Smith et al. (1979) used a variety of tremolite types for intrapleural injection in hamsters; Stanton et al. (1981) used two different tremolites for intrapleural implantations in rats, while Wagner et al. (1982) report on three different tremolites for intrapleural injection in rats. Later, Davis et al. (1991) used six tremolites of different morphology for intraperitoneal injections in rats. If the actinolite and ferro-actinolite amphiboles are included the number of studies increases slightly but is still small. Coffin et al. (1978, 1982, 1983) and Cook et al. (1982), used a fibrous ferro-actinolite in intrapleural injection and intratracheal instillation into rats. Pott et al. (1974, 1989, 1991) reported results from intraperitoneal injection of a granular actinolite and (later) an asbestiform actinolite. A lifetime (including exposure to the dams and gavage during the neonatal period) oral ingestion study (1% in the diet) in rats of 'blocky' tremolite did not to show evidence of carcinogenic activity (NTP 1990, McConnell et al. 1983).

Other studies might also be considered as contributing to the debate about the relative carcinogenicity of amphiboles and their asbestiform varieties. Berman et al. (1995) reviewed the size distributions of all of the asbestos dust exposures used in the Institute of Occupational Medicine inhalation studies over many years, including the Korean asbestos tremolite, and

concluded that, while no univariate measure of exposure could be found to predict lung tumor incidences, the concentration of total structures longer than 20 μm provided the best fit. Furthermore the best estimate for the carcinogenic potency of fibers greater than 0.5 μm in width was zero. The inhalation and intraperitoneal injection experiments of Davies et al. (1986) with long and short fiber amosite, the inhalation studies of various sized chrysotile (Ilgren and Chatfield 1998, McConnell et al. 1984, Wagner et al. 1984), and the cell studies of Donaldson et al. (1989, 1991), Donaldson and Golyasnya (1995), Brown et al. (1986) were aimed at understanding the relative importance of fiber length in carcinogenicity and fibrogenicity. Other mechanistic studies such as those by Kane (1991), and reviews such as those by Oberdorster & Lehnert (1991) and Jaurand (1991), among others also have a bearing on the understanding of the different reactions observed between asbestos particles and other particles with the same mineral chemistry but different morphology.

Inhalation Experiments

Davies et al. (1985) exposed rats (SPF male Wistar, whole body exposure) to a commercially mined tremolite asbestos form South Korea at concentrations of 10 mg/m^3, around 1600 f/mL, (>5 μm) for 12 months. Having produced very high levels of pulmonary fibrosis as well as 16 carcinomas and two mesotheliomas (rarely found in rat inhalation experiments) among the 39 treated animals the tremolite asbestos was described by them as the most dangerous mineral ever studied at the Institute of Occupational Medicine, UK. The Korean tremolite asbestos is the same one used later in the intraperitoneal injection experiments (Davis et al. 1991) for which full size distributions of the respirable dust were given, as shown in Figure 2.

The important feature of the size distribution of the Korean tremolite asbestos is that the vast majority of fibers are less than 0.5 μm in diameter and shorter than 5 μm in length, which is typical of asbestos amphiboles. The geometric mean diameter for Korean tremolite asbestos

fibers longer than 0.4 μm was 0.24 μm (SD 1.6) and the mean length was 1.97 μm (SD 2.11) which are somewhat longer and thicker than airborne fibers in crocidolite mining (GM diameter 0.076 μm, GM length 0.98 μm, Hwang & Gibbs 1981).

The high carcinogenicity of the Korean tremolite asbestos was attributed to the much higher airborne fiber concentration for fibers longer than 5 μm (1600 f/mL) which was almost twice that of the UICC amphiboles at the same 10 mg/m^3 dust mass concentration used grunerite (amosite) asbestos 550f/mL and riebeckite (crocidolite) asbestos 860 f/mL, Davis et al. 1978). This also is a reflection of the finer diameter of the Korean tremolite asbestos.

Injection and Implantation Experiments

Smith et al. (1979) injected a range of tremolites and tremolitic talcs intrapleurally into hamsters (of unspecified type) at doses of 10 mg and 25 mg. The samples were identified as follows:

The animals were allowed to survive up to 600 days after which the final survivors were sacrificed for necropsy. No tumors were found in the final survivors. The samples used by Smith et al. 1979 and described as asbestos or asbestiform produced higher levels of fibrosis and numbers of mesotheliomas in the hamsters than those described as tremolite or tremolitic talc. Most of the tumors were diagnosed as mesotheliomas.

Campbell et al. (1979) examined some of the tremolites used by Smith et al. 1979 and described two of the tremolites (275 and FD72) in more detail. The images of the fibers clearly show FD72 (tumor rate 5/23 and 3/13) to be asbestos and 275 (tumor rate 0/31 and 0/34) to be a prismatic amphibole. This is reflected in the numbers of fibers of length > 10 μm and diameters less than 1μm in the tremolite asbestos, and their absence in the non-asbestos minerals. Similarly, in tremolite FD72 many more of the fibers longer than 5μm had aspect ratios greater than 10:1 than in tremolite 275 (23 – 0, and 19 – 1 using the petrographic microscope and the Scanning Electron Microscope respectively).

Non-asbestos tremolite 14 (FD 14, tumor rate 0/35) was later evaluated by Wylie et al. (1993) and confirmed to be a tremolitic talc with very few tremolite fibers in the size ranges longer than 5 μm and less than 1μm diameter.

This study was criticized for being deficient in a number of ways (Federal Register, 1992). In particular, the fiber size measurements and fiber characterizations were found to be inadequate for the purposes of identification of the materials as tremolite asbestos or prismatic tremolite. The later characterizations by Campbell et al. (1979) and by Wylie et al. (1993) improved on the original ones and the classification of the mineral types appears established. The higher carcinogenicity of those materials described as asbestiform compared to those of tremolitic talc or non-asbestos tremolite is without doubt.

Wagner et al. (1982) used a tremolite from the California talc deposits (A), a prismatic tremolite from Greenland (B) and a tremolite asbestos from Korea (C, probably from the same source as the one in Davies et al. 1985) for a series of intrapleural injection experiments with SPF Sprague-Dawley and Wistar rats and a range of *in vitro* tests. The rats were 8 –10 weeks old when injected and were allowed to live out their lives. Median survival times after injections were 644, 549, and 557 days respectively for samples A, B and C.

The value of the Wagner et al. (1982) injection experiments was impaired by the poor survival rates as a result of infection of the positive control animals injected with riebeckite (crocidolite) asbestos. Nevertheless, the tremolite (C) asbestos was the only one the three tremolites that showed carcinogenic activity producing mesotheliomas in 14 of 47 rats (30%). Neither of the other non-asbestos tremolites produced any tumors in the 31 and 48 rats used. The fiber size data as presented are not amenable to numerical evaluation, but measurements taken from the published diagrams show that in the tremolite (C) asbestos about 25% were longer than 10 μm and less than 0.6 μm in width. The non-asbestos forms had no fibers at all in that size range

(Sample A California, or Sample B, Greenland). Table 2 shows Wagner's figures for the numbers of particles, fibers longer than 1 μm, and fibers longer than 8 μm with widths less than 1.5 μm; the differences are obvious with Tremolite C containing many more long fibers.

The *in vitro* tests used by Wagner et al. (1982), including mouse peritoneal macrophage lactic dehydrogenase (LDH) and B-glucuronidase (BGL) release, cytotoxicity to V79-4 cells and giant cell stimulation with A549 cells confirmed the relative toxicity of the different tremolite morphologies *in vivo*. So, while the study remains limited by the poor survival of the positive controls, it is nevertheless useful in that it reproduces the general findings of Smith et al. (1979).

Stanton et al. (1981) described a series of 70 experiments where a wide range of different fibers were implanted at doses of 40 mg in hardened gelatin on to the left pleural surface of Osborne-Mendel rats by thoracotomy. It should be noted that in contrast to intrapleural or intraperitoneal injection, the use of the "hardened gelatin" exposure technique literally holds the fibers in contact with the target tissue (pleura) and does not allow for potential macrophage phagocytosis and clearance of the particles. Therefore this technique may create the highest effective dose of all of the exposure methods used for assessing the potential carcinogenicity of fibers. Stanton et al. 1981 reported on two tremolite asbestos samples from the same lot, described as "in the optimal range of size for carcinogenesis" and "distinctly smaller in diameter than the tremolite fibers used by Smith et al. (1979)". As they anticipated the two tremolites produced mesotheliomas in 21 and 22 animals out of the 28 used, with a 100% tumor probability. The tremolites contained very high numbers of fibers in the Stanton size range (>8 μm in length and <0.25 μm diameter) with 1.63×10^{8} and 2.76×10^{7} respectively *in each dose* for tremolites 1 and 2. In addition, the talc (No 6), which produced no tumors in the Stanton study, was actually New York State tremolitic talc (Wylie et al. 1993) with 40-50% non-asbestos tremolite and talc fibers, in fact the same material as used by Smith et al. (1979) and identified as FD 14. The general relationship between the probability of developing a tumor in these experiments and the

common logarithm of the number of fibers > 8μm in length and less than 0.25 μm in diameter per microgram of implanted dust was highly significant (Figure 3).

There were however a number of problematical experiments in the Stanton series where tumors developed for test materials with no fibers in the critical size range, and one where no tumors had developed even with large numbers of critical fibers present. Some of these results were attributed to large numbers of fibers with sizes close to the critical range, and others to problems of clumping and fragmentation in the fiber preparations for transmission electron microscopy analysis

Figures 3 and 4 show the general relationships developed, and described by Stanton as highly significant, between the numbers of fibers per microgram in the dose and the probability of tumor development. The statistical relationships between the fiber numbers in the different sets and probabilities of tumor development have not been evaluated but the diagrams show that the correlation for the shorter classes of fiber is much weaker than that for the longer fibers. It is reasonable to suggest that there must be more short fibers per microgram in the short fiber dusts than in the longer fiber dusts so the poorer correlation for short fibers is, if anything, even more indicative of their lack of importance in tumor development.

The size distributions given in Stanton et al. (1981) do not make it easy for full comparison with other size distributions of known asbestos minerals because the size classification was relatively crude and the method of exposure (hardened gelatin) was unique. The two tremolite samples however have sufficient numbers of long fibers with diameters less than 0.5 μm to indicate that their identification as asbestos is reasonable. The size distributions are somewhat unusual for pure asbestos as is seen in Figure 5 which shows Tremolite 2 to have a bimodal distribution which suggests that it is actually a mixture of tremolite asbestos and prismatic

tremolite. Such an occurrence in poor commercial quality tremolite asbestos formations is common.

Wylie et al. (1993) re-examined Tremolite 1 and 2 as well as Talc 6 that were used in the Stanton studies. They state that Tremolites 1 and 2 are the same material, tremolite asbestos from California, with all the characteristics of commercial amphibole asbestos. The two size distributions given by Stanton differ somewhat but they are similar and have the appearance of a mixed asbestos – prismatic fiber assemblage.

In contrast, the size distribution of Stanton's Talc 6 shows the much thinner, shorter distribution (Figure 6) not typical of a prismatic tremolite fiber population even though it consists of 40-50% tremolite. Talc 6 produced no tumors despite containing more fibers in the "Stanton fiber" range than Tremolite 2, and almost as many as Tremolite 1, both of which had a 100% probability of producing tumors.

This talc (6), or tremolitic talc, was reported by Wylie et al. (1993) as being identified in Stanton's laboratory notes as Nytal 300. Pure talc is a specific mineral with a closely defined chemical composition and crystal structure. Commercial producers however often named their products as 'talc' even though they contained less than 50% of the mineral talc.

Davies et al. (1991) used six tremolites of differing morphologies in a series of intraperitoneal fiber in saline injection experiments with male SPF Wistar rats. These were identified as follows:

1. Tremolite asbestos from Jamestown, California, United States;
2. Tremolite asbestos from Korea;
3. Tremolite asbestos from National Coal Board Laboratory, Swansea, Wales, Great Britain;

4. Tremolite, long needle-like crystals from Ala di Stura, N. Italy;
5. Tremolite, short needle-like crystals from Dornie, NW Scotland, Great Britain;
6. Tremolite, prismatic crystals from Shinness, N. Scotland, Great Britain.

The tremolite from Korea was the same material as was used in the earlier tremolite inhalation and injection experiments by Davis et al. (1991). The fiber size distributions were assessed by counting and measuring 300 fibers of all sizes in a known weight of sample deposited on to a polycarbonate filter using Scanning Electron Microscopy. At 10,000 times magnification the effective minimum diameter that is visible is 0.1 μm, so the effective minimum length of a counted fiber was 0.4 μm. This was followed by the counting and measurement of a further 100 fibers longer than 5 μm. The data were combined to calculate the numbers of fibers in a series of length and diameter classes in the 10 mg dose administered to the rats. In addition, the numbers of particles (Aspect ratio less than 3:1) were also counted and estimated for each dose.

The rats were allowed to live out their full life span or until they showed signs of debility or tumor formation. Statistical analysis of the times at which death from mesothelioma occurred was used to calculate survival curves and these were correlated with the fiber doses received by each animal.

Table 3 shows the relative hazard ranking, the numbers of mesotheliomas and the fiber numbers in the doses. The relative hazard was derived from Cox's proportional hazards model (Cox & Oakes 1984) and is a function of the numbers of animals developing mesothelioma and their median survival times. The values given in the table differ from those shown in Davis et al. (1991) only in that the hazard is expressed arithmetically as a multiple of the lowest hazard, and the fiber numbers are expressed as those in the dose.

The main conclusions of the study were: 1) that all of the materials appeared to have some potential to cause mesothelioma by intraperitoneal injection in rats; 2) that fiber numbers alone were not sufficient to explain the differences in response, nor were the fiber numbers in the 'Stanton' fiber class able to fully explain the response; and 3) that the Dornie and Shinness material would be unlikely to pose a risk of mesothelioma to humans from inhalation of the dust. The spontaneous occurrence of peritoneal mesothelioma in male rats of this strain may account for the small numbers of tumors found in the animals injected with the latter two dusts (Pott et al. 1991).

Coffin et al. (1978, 1982, 1983) and Cook et al. (1982) confirmed that ferro-actinolite asbestos has a high potency for generating mesothelioma in rats. In each case the ferro-actinolite asbestos had large numbers of fibers in the 'Stanton' range. The papers by Coffin and his colleagues were based on experiments using intratracheal instillation and intrapleural injection of an actinolite asbestos from the Mesabi Range (USA) iron ores in comparison to UICC amosite. The results were problematical in that the response from the amosite was lower than expected from previous experiments (Stanton et al. 1981). In Coffin et al. (1983) 33.6% of F344 rats injected intrapleurally with 20 mg of UICC amosite developed mesothelioma. The response to the actinolite asbestos was lower than that from the UICC amosite or amosite in general in terms of the mass dose used, but the response relative to the numbers of Stanton fibers was higher. Cook et al. (1982) explained the relatively high response from the ferro-actinolite as resulting from shortening and splitting of the fibers in the lungs and on the pleural surface of the rats.

Pott et al. (1988) reported more than 80% of rats with tumors two years after intraperitoneal injection of 0.3 mg of a German actinolite although the given size distribution of the actinolite is not provided. Pott et al. (1989) then reported 56% of rats with tumors after an injection with 0.25 mg of (presumably) the same German actinolite. The size distribution is not detailed but shows 90% of the fibers as less than 0.2 μm in length and 10% longer than 4.2 μm. In contrast,

when a dose of 4 x 25 mg of 'granular' actinolite was used in similar experiments (Pott et al. 1974) no tumors were found.

Grunerite (Amosite) Asbestos Studies

The inhalation and intraperitoneal injection experiments of Davies et al. (1986) used long and short fiber amosite asbestos. These were produced from the same bulk batch of amosite, the short form by ceramic ball milling and the long by elutriation. Importantly, TEM examination showed no loss of crystallinity in the milled short fiber sample. In the inhalation studies rats were exposed for one year (224 days in 12 months) to 11.9 and 11.6 mg/m^3 of respirable dust for the long and short fiber types respectively. The aerosol contained 2,060 and 70 f/mL for fibers longer than 5 μm, and 1,110 and 12 f/mL for fibers longer than 10 μm. In the injection studies two batches of rats received doses of 10 mg and 25 mg of the respirable dust collected from the inhalation experiment chambers using a vertical elutriator.

The results showed that rats exposed to the long fiber grunerite (amosite) asbestos developed significantly higher levels of pulmonary fibrosis and more lung tumors than rats exposed to the short fiber grunerite (amosite) asbestos. In fact the animals exposed to the short fiber developed no more fibrosis than did the control animals, no pulmonary tumors and only one peritoneal mesothelioma that was considered to be unrelated to the dust exposure as the type had previously been reported in untreated rats. The animals exposed to the short fiber had significantly higher burdens of asbestos in their lungs immediately after the inhalation period, and they remained higher throughout the following six months of clearance. The injection experiments produced mesothelioma in 88% and 95% of rats treated with 10 and 25 mg respectively of the long grunerite (amosite) asbestos, while the short fiber grunerite (amosite) asbestos produced 0% and 4% (1 animal) tumors with the same respective doses (mass) (Table 4). The short fiber grunerite

(amosite) asbestos contained about 0.1% of fibers longer than 10 μm and about 2% longer than 5 μm while the long fiber grunerite (amosite) asbestos contained more than 11% longer than 10 μm and 3% longer than 25 μm. The diameter distributions were very similar with about 50% less than 0.5 μm in width.

These results were taken as an indication that the short fiber grunerite (amosite) asbestos showed a much lower relative pathogenicity than the long fiber grunerite (amosite) asbestos.

In Vitro CELL STUDIES

The cell culture studies of Donaldson et al. (1989, 1991, and 1992) Brown et al. (1986) and Hill et al. (1995) have generally confirmed the impression that fibers shorter than 5 μm, and indeed possibly less than 10 μm, have little pathologic effect other than what might be expected from a general respirable silicate mineral dust. Tumor necrosis factor released from macrophages was shown to be dependent on fiber length as demonstrated by the long and short fiber grunerite (amosite) asbestos (Donaldson et al. 1992). The same minerals showed that release of superoxide anions by macrophages differed significantly (Hill et al. 1995). Since such factors are associated with the development of inflammation, pulmonary fibrosis, and tumor formation, this supports the view that fiber length is an important element in determining the pathogenicity of fibers.

OTHER RELEVANT STUDIES

The studies at IOM (Miller et al. 1999a, b and Searl et al.1999) confirm that biopersistence was a significant factor controlling the pathogenicity in animals of a wide range of different synthetic mineral fibers, but for durable fibers the most important factor was fiber length. The fibers used were: glass microfiber, JM 100/475; MMVF 10, 21, 22 and Refractory ceramic Fibers 1, 2, and 3, from the Thermal Insulation Manufacturers Association repository of size selected fibers; a

silicon carbide whisker fiber and the long fiber grunerite (amosite) asbestos as used by Davis et al. (1986). In the intraperitoneal injection studies the best correlation with capacity to produce mesothelioma was with the *in vivo* biopersistence factor (derived from measurement of fibers before and after intratracheal instillation) and the number of fibers longer than 20μm with diameters less than 0.95 μm. In the inhalation studies with the same suite of fibers the pulmonary tumor production (lung cancer) was best predicted by a function of the dissolution rate (measured in continuous flow through with simulated physiological saline solution) and the numbers of fibers in the length range greater than 20 μm with diameters less than 0.95 μm.

DISCUSSION

The main question that has been asked of these studies is to what extent they support the hypothesis that the carcinogenicity of fibers depends upon morphology. A second question that is being debated to what extent the short mineral fibers contribute to the carcinogenicity in humans. There are limitations to the injection or implantation assessments of carcinogenicity that reduce their ability to predict the outcome of inhalation of the same materials by humans (US EPA 1986). These include the avoidance of normal defence mechanisms of the inhalation process, the unnatural introduction of large doses to sensitive tissue sites, possible clumping of dusts introducing even higher doses at some sites, and the reduction of normal lung clearance mechanisms. However, the net result of these limitations is to over-estimate carcinogenicity by these methods, so that a negative finding is a strong indication that a given mineral dust is unlikely to be carcinogenic when inhaled by humans.

The early studies of Wagner et al. (1982) and Smith et al. (1979) are limited by poor survival and uninformative size distribution measurements. However, both experiments showed no potential for prismatic amphibole fibers to cause tumors by inhalation or by injection. So while limitation do exist, they ought not to be seen as grounds for disregarding the results and general concepts

derived from the experimental animal studies indicating that amphibole asbestos minerals are carcinogenic while the prismatic amphiboles or cleavage fragments are markedly less active.

Some questions have been raised about the interpretation of the Davis et al. (1991) study which we will answer. For example, the authors stated that the response from the Shinness fiber was no more than would be expected from control animals, and that the non-asbestos tremolites were unlikely to pose a specific mesothelioma risk to humans by inhalation. It was suggested that these two tumors, with the non-asbestos Shinness dust, were significant since there were no tumors among animals in many other experiments from the same laboratory (IOM) (Federal Register 1992). The experiments referred to in the Federal Registry were inhalation experiments with other asbestos fibers, and that, other than with the Korean tremolite, these have rarely produced mesotheliomas in rats. The background mesothelioma incidence is higher when the route of administration is by injection. Furthermore, Stanton et al. (1981) implantation studies have shown a percentage of animals with tumors in the range of 0 to 10% may well be within the expected range for a 40 mg dose of mineral particles of any type not introduced by inhalation.

The size distributions of the fiber types show that the tremolite asbestos from different geological locales as exemplified by the Californian (Jamestown) sample, are dominated by very much thinner fibers than the prismatic tremolites, as exemplified by the Shinness sample, which contain almost no fibers longer than 8 μm and less than 1 μm in diameter. While it is true that the response could be explained simply as a dose response to the numbers of Stanton fibers, yet this fails to explain all of the variance in the results between the various growth habits in which tremolite naturally occurs. It is a distinct possibility that, as with Stanton's experiments, the low responses from the non-asbestos Shinness fibers and the Dornie fibers are inert dust responses.

A second criticism in the interpretation of these results stems from the high tumorigenicity of the Italian (Ala di Stura) tremolite (Davis et al. 1992) This was described in the paper as a spicular

(the same as acicular, a sub-type of prismatic) non-asbestos variety of tremolite which would not be expected to produce tumors; so the high tumor rate has been used to suggest that acicular and byssolite amphiboles do indeed have a similar carcinogenicity to the asbestos amphiboles. It has been shown that the Ala di Stura tremolite sample contains a sub-set of asbestiform tremolite fibers that appear as extremely long and fine fibers but which, because of the limitations of fiber sizing methodology, are not fully expressed in the fiber numbers as reported in the study (Figure 1b).

The tumor response from the Ala di Stura tremolite was unusually high compared to the number of Stanton fibers in the sample, but an important factor in the response was the timing of the mesotheliomas in the life spans of the animals. Two thirds of the rats exposed to the Ala di Stura tremolite developed mesothelioma, but very late in life (median survival time was 755 days). In contrast the three asbestos samples had much shorter median survival times ranging from 301 days to 428 days. (The Korean tremolite asbestos had a median survival time of 428 days compared to 325 days in the earlier study with a 25 mg dose). The median survival time for those animals that develop mesothelioma appears to be inversely related to dose, as seen in Davis et al. (1991), so the response from this dust could be simply that which might be expected from a trace asbestos component in a dust injected into animal at high concentrations.

It was also pointed out in the original report that the tremolite asbestos from Swansea had produced a response that was much higher than expected given the number of Stanton fibers in the dose. Both the Swansea tremolite asbestos and the Korean tremolite asbestos produced the maximum response in mortality but the high Hazard Index of the Swansea asbestos, calculated in the statistical analysis, was the result of the much faster tumor induction. It was suggested that this may have been the result of a masking of the response to simple fiber numbers by the overdose of asbestos forms, and that a multi-dose-response experiment might produce a clearer picture of the relative potencies of these types.

The Stanton studies confirmed the high tumorigenicity of tremolite asbestos and identified the Stanton Fiber range, fibers > 8 μm with diameters < 0.25 μm, for which the correlation between fiber numbers and mesothelioma generation was highly significant. Had the size classes and instillation method been different, the 'Stanton Fiber' critical size may well have been different. The authors stated that shorter and thicker size classes also correlated with mesothelioma potency, and that it should not be assumed that they had no potency. However, as can be seen in Figure 7, the numbers of fibers in the different classes are strongly correlated.

So it is to be expected that if the tumorigenicity is correlated strongly with numbers in the long, thin class of fibers it will also correlate with the fiber numbers in the shorter classes. That does not necessarily imply a causal relationship, and these short fibers may indeed have insignificant tumorigenicity. Even particulates that are considered relatively innocuous, e.g. FeO, magnetite can produce tumors by injection techniques if the dose is high enough (Pott et al. 1991).

As can be seen in Figure 8 many of the mineral and glass fibers in the experiments had less than 10 % probability of generating mesothelioma despite having huge numbers of fibers in the administered dose in the size range of 4 –8 μm length with no fibers in the longer classes. It is noteworthy; the fibrous talc minerals (5 and 7) produced no tumors despite having large numbers of short, thin fibers. The halloysites produced only 5 and 4 tumors despite having among the highest numbers of short fibers. Halloysite has the same tubular morphology as chrysotile asbestos despite having a little thicker fundamental diameter (0.07 μm). The attapulgites (palygorskite) produced few (2/29) tumors with similarly high numbers of fibers shorter than 8 μm. However, one long fiber attapulgite has been found by Wagner et al. (1987) to be capable of producing large numbers of mesotheliomas in rats by intraperitoneal injection. Both halloysite and attapulgite have been described as asbestiform but neither fiber-type is asbestos.

The size distributions of the various fibers used by Stanton et al. (1981) are in many cases highly unusual but a detailed discussion of all their full fiber size distributions is beyond the scope of this paper; some contained no long fibers, some contained no short fibers, some contained no fibers thinner than 0.5 μm, and others contained no fibers thicker than 0.5 μm. The tremolites however were unusual in having bimodal distributions consistent with a mixture of tremolite asbestos and prismatic non-asbestos tremolite (Figure 5).

One important factor in the Stanton studies that has implications for many other injection and implantation experiments is the range and distribution of the results found. There are a large number of dusts producing between 0 and 10% mesothelioma in experimental animals even though many of these samples contained more than 100,000 fibers per microgram of implanted dust. In a 40 mg dose implanted there are 40,000 times more fibers present than in a microgram. It is reasonable to conclude that this range of tumor production may be the "normal" background for his mineral dust implantation technique. In addition, Stanton's implantation controls had a 2.8% incidence of pleural sarcomas and all controls had an age-adjusted rate of $7.7 \pm 4.2\%$. Also, Pott et al. (1991) using intraperitoneal injection stated that tumor rates of below 10% in small groups should be regarded as spontaneously occurring or induced non-specifically. The background rate of his non-injected controls is 0%, but up to 10% for saline injection controls, which is highly significant when compared to non-injected animals.

One implication of this observation would be that the testing of materials by the implantation or injection of unrealistically high doses might be useful a screening test for mesothelioma potency in humans by inhalation. In addition, both routes of exposure do not allow for normal physiological removal as would be expected after inhalation (McConnell, 1995). The Stanton method is particularly problematic in this regard because the fibers are 'held in place', i.e. in contact with the mesothelium in the gelatin vehicle. For these reasons the methods is very useful when a negative result is obtained for the assessment of fundamental differences between fiber-

types and concepts of carcinogenic activity. But positive results are of limited use as predictors of the risk to humans from inhalation of more general dusts. Furthermore, the doses to which the animals are exposed are probably many orders of magnitude higher than would be expected from exposure of humans to airborne dust.

CONCLUSIONS

The conclusion that should be drawn from the evaluations of this set of studies is that there is very little evidence of carcinogenicity from exposure of animals to mineral fragments or short fibers formed from normal prismatic amphibole minerals. No positive carcinogenicity has been found with any experiment using non-asbestos amphibole dust (Ilgren 2004). Furthermore, when genuinely short fiber amphibole asbestos has been used in inhalation or injection experiments they have also been shown to have no carcinogenic properties. Evidence from experiments with other mineral fibers suggests those fibers in excess of 20 μm and with diameters less than 1 μm are necessary to cause cancer. This is probably because such long fibers cannot be phagocytized by resident macrophages and therefore, cannot be removed from the lung (Lippmann et al. 2000). This explains the lack of carcinogenicity of cleavage fragment fibers of amphiboles since these rarely if ever contain fibers of these critical dimensions.

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Review Article

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The Biology of Cleavage Fragments: A Brief Synthesis and Analysis of Current Knowledge

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Key Words

Amphibole • Chrysotile • Cleavage fragment • Biopersistence • Animal studies • *In vitro* studies

Abstract

Asbestos is a commercial term referring to 6 fibrous minerals from 2 mineralogical classes: serpentine and amphibole. Chrysotile, or white asbestos, is the only serpentine mineral. The asbestiform habit of amphibole asbestos is far more toxic than chrysotile. However, most amphibole minerals are found in the "non-asbestiform" state that pose few, if any, health risks. Comminution, whether deliberate during crushing or grinding, or incidental in usage may produce structures known as "cleavage fragments" from a wide variety of sources. A considerable body of evidence, gathered over the last 30 years, demonstrates that amphibole cleavage fragments do not show the same toxicity as their asbestiform analogues. Since there still continues to be confusion and controversy on this point, this review is aimed at resolving a major portion of this controversy. It has done so by bringing together the supporting mineralogical, animal and human evidence from many sources. These observations demonstrate that cleavage fragments and amphibole asbestos fibers have fundamentally different properties and these differences are biologically relevant. Indeed, the toxicity of respirable cleavage fragments is so much less than that of the fibrous amphiboles that by any reasonable measure they are not biologically harmful.

Introduction

Asbestos is a commercial term referring to 6 fibrous minerals from 2 mineralogical classes: serpentine and amphibole. Chrysotile, or white asbestos, is the only serpentine mineral. As fibrous asbestiform minerals amphiboles are far more toxic than chrysotile (see Ilgren and Chatfield for review) [1]. However, most amphibole minerals are found in the "non-asbestiform" (non-fibrous) state that pose few, if any, health risks [2]. Amphiboles may be associated with a variety of very common industrial minerals such as serpentine, talc, vermiculite and certain marbles [3,4], and may also be a component of many rocks used as aggregate, road stone, or building materials [5]. Comminution, whether deliberate during crushing or grinding, or incidental in usage may produce structures known as "cleavage fragments". Some elongated cleavage fragments are difficult to distinguish from amphibole asbestos fibers using counting criteria routinely employed for regulatory purposes. It is very

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important to distinguish whether the amphibole in a sample is, or is not, asbestiform not only for regulatory reasons but also because, without this knowledge, it would be impossible to assess properly any health risks associated with exposure to respirable particles released from the materials being used. A considerable body of evidence, gathered over the last 30 years, demonstrates that amphibole cleavage fragments do not show the same toxicity as their asbestiform analogues. The evidence in support of this was summarized previously in the voluminous hearings that led up to the OSHA regulations [6]. In spite of much evidence to support the lack of toxicity of cleavage fragments, there continues to be confusion and controversy both in the USA [6–10] and Europe, see [5] as cited by Chisholm, on this point.

This review is thus aimed at resolving a major portion of this controversy. To do so, it has brought together evidence from a wide variety of sources. These demonstrate that the toxicity of respirable cleavage fragments is so much less than that of the fibrous amphiboles that by any reasonable measure they are not biologically harmful.

Cleavage Fragments and Amphibole Asbestos Fibers have Fundamentally Different Properties

Amphibole minerals make up as much as 6% of the earth's crust and are major constituents of approximately 30% of the rocks in the continental United States [11,12]. Tremolite is a particularly common form of non-commercial amphibole. Thus, given their ubiquity, tremolite cleavage fragments are, not surprisingly, "the most commonly encountered amphibole in the lungs of urban dwellers in North America" [3,9,13]. Indeed, the vast majority of amphiboles in nature are "non-asbestiform" [11,14] (also frequently called 'massive') a term that refers to an amphibole's growth habit.[1] The precise determinants of the growth habit of a mineral are not known (Zussman, 2000 pers comm) but, very specific conditions of temperature and pressure are required to form asbestos fibers (Addison, 2003 unpub.) [15]. "[T]he appearance of [asbestiform fibers] usually implies some sort of secondary modification such as shearing, faulting, or hydrothermal alteration" (Addison, 2003 unpub.). Such conditions rarely occur in nature and, thus, the asbestiform habit is very rare [16,17]. Non-asbestiform amphiboles may also be found in areas where asbestos occurs. The rocks around Libby, Montana provide a good example of this since a large percentage of the dust aerosols from this area is composed of cleavage fragments [18]. Cleavage fragments have also been found, for example, in the ore from the Libby vermiculite mines [19].

Non-asbestiform and asbestiform amphiboles are chemically indistinguishable.[2] The "classification of minerals in the amphibole group is based on the general formula A0–1B2C5T8O22 (OH,F)2 in which A=Na, K; B=Na, Ca, Fe(II), Mg, Mn; C=Fe(II), Mg, Al, Fe(III), Mn; and T=Si, Al" [21]. The main difference between them is their morphology.[3] However, "Subtle differences in their crystal structure can lead to profound differences in physical properties" (Addison, 2003 unpub.).

Geology governs morphology [25].[4] The asbestiform and non-asbestiform habits thus reflect vastly different modes of origin. The asbestiform habit arises through unidirectional crystalline growth which produces exceedingly long, thin fibrils [26]. Each fibril is a single crystal "the structure [of which] consists of SiO_4 tetrahedra linked into double chains or ribbons with a strip of cations sandwiched between pairs of double chains" [4,5]. Individual asbestiform amphibole fibers, in turn, contain fibrils that run parallel to one another. Asbestiform minerals are thus highly fibrous and fibrillar.

"Only specimens which occur as bundles of fibres (commonly having splayed ends) which readily split into still finer sub-microscopic units (fibrils), are referred to and are classed as asbestos" [16]. Thus, "fiber bundles are the hallmark of asbestos" [25]. Non-asbestiform amphiboles are not naturally fibrous. They are not composed of fibers or fibrils. Their crystalline growth is not unidirectional; instead, it occurs along two or three planes. This most commonly gives rise to tiny "prisms" or irregularly shaped crystals by prismatic or acicular growth [16] (Addison, 2003 unpub.).[5]

"The way a mineral sample breaks is determined by its crystal structure and geological history" [27]. Breakage generally occurs along cleavage planes. These are "planes of relative weakness along which certain minerals tend to fracture and are determined by their crystal lattice geometry" (Addison, 2003 unpub.). Since such planes are pre-determined, "you cannot make fibers out of non-fibrous material by mechanical manipulation" [27]. The US Agency for Toxic Substances and Disease Registry (ATSDR) [9] thus wrongly contends that "tremolite asbestos can cleave into short, squatty cleavage fragments". Asbestiform minerals never form cleavage fragments. Conversely, non-asbestiform (massive) amphibole minerals never separate into fibers or fibrils.

Instead, when non-asbestiform amphiboles are crushed, fragments are cleaved or "torn" away from the main rock mass and structures called "cleavage fragments" may be formed. Such "cleavage fragments were thus once part of a larger (non-fibrous) crystalline lattice split apart due to the application of force". Cleavage fragments attain their shape by breakage, not by fibrous growth [25]

Non-asbestiform and asbestiform amphiboles have fundamentally different physical properties [14,16,26,28, 29]. Even though they are inter-related, these properties can be discussed in terms of those that relate primarily to a fiber's "surface structure" or to its "internal structure".

Surface Properties

Surface properties are probably the most important factor distinguishing asbestiform from non-asbestiform amphibole fibers and reflect "differences in their origins" [14]. The geological forces that produce the asbestiform habit make the outer surfaces of asbestos fibers largely smooth[6] and defect free [4,14,16,26,29,30]. Asbestiform fibrils have smooth surfaces with "relatively well satisfied chemical bonds" [29]. The surface of a cleavage fragment is created by external force, and consequently, is not expected to be as stable as an asbestos fiber, since "the stresses have created a high density of surface defects" [14], "steps, and cracks" [29]. "A strong surface structure, with relatively few defects, can only develop when a crystal grows in one direction" [26] as is characteristic of asbestiform fibers. Since the surfaces of asbestos fibers are "growth faces", not mechanical breakage planes, their surfaces are therefore radically different from those of cleavage fragments. Macroscopically, "many asbestos fibers have the shiny luster indicative of a surface structure that is relatively free of defects" [26]. This is not the case for cleavage fragment-derived materials.

At least 3 pieces of evidence suggest that the outer surface of an asbestiform fiber is stronger than its inner surface (and that the opposite is true for non-asbestiform cleavage fragments). These include studies of tensile strength, grinding and acid dissolution.

Tensile Strength

Tensile strength is "the most important and most commonly quoted physical property of an asbestos fiber" [31]. It provides flexibility, the hallmark of an asbestos fiber [14,26,28]. Such properties have enabled asbestos fibers to be exploited widely for the many commercial purposes they are uniquely suited to. The lack of defects in the outer surface of an asbestos fiber largely accounts for its great strength since it allows the integral "linear silicon–oxygen structures" to continue uninterrupted [31] throughout the length of the fibril. Moreover, the outer surface needs to be stronger than its internal structure for a fiber to be flexible [14]. Thus, as each fiber is made up of a discrete number of fibrillar units, the greater outer surface strength of the fiber enables the fibrils within to "slide" past one another without causing the fiber to disintegrate. Their ability to slide past one another within the fiber enables the fiber to bend and therefore serves as the basis of its unique flexibility. Such sliding is also known as interplanar "parting" or "slip" and this occurs at sites called twinning planes [4,14,25,32]. Twin planes,[7] common in amphibole asbestos fibers, are rare in non-asbestiform amphiboles and may be an important microstructural feature in differentiating the one from the other [5,16,25,32,33,34; Seshan and Wenk, 1976, op. cit. 5; Chisholm, 1995; Whittaker, 2000pers comm]. A high frequency of partings across multiple twinning planes {100} and possibly multiple chain disorders {010} within the crystals and fiber bundles may thus lead to the development of extreme fibrosity (Addison, 2003 unpub.). By contrast, a high frequency of dislocation networks and sub-grain boundaries in prismatic crystal forms (but not in asbestos) may reduce tensile strength (Addison, 2003 unpub.). In fact, "The frequency of {100} twin boundaries (high in amphibole asbestos, very low in prismatic amphiboles) seems to offer the most reliable means of distinguishing the two types [5]"[8]

By contrast, non–asbestiform cleavage fragments are weak, brittle and inflexible largely because their outer "surfaces are weaker than their internal structure" [26,28] (also Addison, 2003 unpub.). Cleavage fragments "cannot be bent more than a few degrees" [26,35] which makes them more susceptible to physical stress than the asbestiform varieties of the same mineral" [26]. Numerous defects and cracks make cleavage fragments inherently weak and brittle, "the density of these defects [Griffith cracks] being inversely proportional to [the fiber's] tensile strength" [4]. "Surface defects also propagate brittle fracture" enabling physical and chemical forces to proceed internally to cause secondary structural faults and failure zones that can weaken the already brittle cleavage fragment even further [4].

Direct measurements of tensile strength demonstrate that cleavage fragments are much weaker and less flexible than asbestos fibers of the same size [36]. The tensile strength of amphibole asbestiform fibers is between 20 to 115 times stronger than non-asbestiform varieties of the same amphibole mineral [26,28,29,31]. The difference in strength between asbestos fibers and cleavage fragments

becomes greater as they get progressively thinner [4,26,28,29,31]. The difference is therefore probably greatest for fibers and fragments thin enough to meet the minimal width (<0.5 μm) and length (>5 μm) criteria of a biologically relevant structure (see below). Asbestos fibers are therefore unique in displaying diameter-dependent strength. Thus, as an asbestos fiber becomes thinner, it gets stronger [26,28,29]. By contrast, as a cleavage fragment gets thinner, it gets weaker [28,36].

Grinding Studies

Simple grinding studies provide additional evidence to support the proposition that the outer surface of a cleavage fragment is weaker than its inner surface. Such studies demonstrate that cleavage fragments can be easily reduced to a powder by hand grinding [17,26] to yield short equant fragments [16,32,37] (Addison, 2003 unpub.). Simple manipulation of asbestos can cause large numbers of very long, thin fibers and fibrils to separate [16,17,32]. By contrast, whilst the simple manipulation of asbestos fibers may cause them to split into large numbers of very long thin fibrils [16,17,32], bundles of asbestiform amphibole fibers can only be ground with great difficulty often causing the asbestos fibers to mat in the mortar [16,17,26].[9] The greater resistance of an asbestos fiber's surface to such physical stress reflects the greater surface strength of the asbestiform over the non-asbestiform habit. Paoletti et al. [39] have also demonstrated that the response of fibrous and non-fibrous tremolite to comminution is very different.

Dissolution Studies

Dissolution studies provide further evidence to support the notion that a cleavage fragment's surface is weaker than its internal structure. Indeed, the unique ability of amphibole asbestos fibers to survive the harshest forms of chemical attack has formed the basis of many vital industries Thus, the defect-free outer surface of an amphibole asbestos fiber is highly acid resistant [28,29]. By contrast, the numerous cracks and defects on the surface of a cleavage fragment serve as "etch pits" that can allow acid to penetrate into the interior of the structure [21,26,28,29] (also Zoltai, 2000 pers comm). In such cases, grunerite (known as amosite when in a fibrous form) cleavage fragments will begin to dissolve on all surfaces when soaked in acid. By contrast, asbestiform grunerite fibers start to dissolve at the ends of the fibers and also require a stronger acid to commence the dissolution process [26,29]. As dissolution proceeds, solid asbestiform fibers become partially hollow cylinders long before their surfaces have dissolved. By this time, many cleavage fragments have undergone complete dissolution [26,28,29]. Surface defects are thus "preferred sites for chemical attack" [29] through which fractures may be propagated. If this occurs, a cleavage fragment may be weakened along its length so reducing its resistance to fracture even further [4] (Wylie, 2000 pers comm). Additional experimental data from chemical "weathering" studies [40,41,42] further demonstrate that surface defects cause massive non-asbestiform amphiboles to dissolve more readily than asbestiform amphiboles.

Surface Charge Studies

The surface charges of asbestiform and non-asbestiform amphiboles may also differ [14,43,44]. Such differences may be biologically important since surface charge has been shown to be related to cationic exchange and particle absorption [29] as well as fibrogenic and tumorigenic potential [45] (also see [30,46,47]).

Internal Micro-Structural Features

A detailed discussion of the internal micro-structural features that differentiate cleavage fragments from amphibole asbestos fibers is beyond the scope of this review but has been detailed by others [5,16,21]. By TEM, prismatic non-asbestiform specimens have been found to contain "extensive sub-grain boundaries and dislocation networks". "Fine multiple twinning" has been observed in asbestos but is less common in non-asbestiform amphiboles. Microscopically, the "crystallographic orientation to an electron beam of an asbestos fiber differs markedly from that of a cleavage fragment" [32]. "The behaviour of cleavage fragments of amphibole should be different as their most strongly developed faces are {110}" [5]. This is reflected in differing polarizing, x-ray diffractometric and infrared spectrophotometric patterns due to preferred orientation and preferential alignment of the crystals [5] (Addison, 2003 pers comm; also see [37]).

The Differences in the Properties of Cleavage Fragments and Amphibole asbestos Fibers are Biologically Relevant

Cleavage Fragments Do Not Possess the Extreme Dimensions of asbestos Fibers

Because non-asbestiform amphiboles are brittle they typically fracture "horizontally" across their length rather

than along it and in so doing produce shorter fragments. These are, for the most part, much thicker, for the same length, than their asbestiform analogues [16] (Addison, 2003 pers comm). Asbestiform amphiboles, however, don't typically break horizontally to produce short fibers when crushed. Instead, they tend to separate into fibrils of their original length [16]. The typical manner in which cleavage fragments fracture is unable to generate uniform long, thin fibrils and fibers (also see [5]; Addison, 2003 pers comm). The extremely high percentage of "short fibers" in dusts generated by those working with ores contaminated with massive amphibole (e.g. Homestake Gold and Minnesota Taconite miners) noted by the ATSDR [9] strongly supports this idea. Only a very small proportion of cleavage fragments conform to the dimensions of asbestiform fibers.[10] An even smaller percentage ever resembles a biologically relevant structure longer than 5 μm and less than 0.5 μm in width.

Therefore, the fiber dimensional distributions of equivalent numbers of cleavage fragments and their asbestiform analogues differ greatly [5] (Addison, 2003 pers comm). The dimensional differences are so great that Chisholm [5] concluded that "A criterion based on particle dimensions is left as the only quick and simple option for a routine method of quantitative analysis" and that "it should be possible to set criteria such that there is very little risk of failing to count an asbestos fibre through wrong identification as a cleavage fragment". Furthermore, "there is relatively little overlap between the width and aspect ratio distributions for the two particles types" [11] so "good quality size distribution data should provide a satisfactory basis for distinguishing between asbestos particles and cleavage fragments" [5].[11] Indeed

> "The distinction between asbestos particles and mineral fragments emerges most clearly in their width: virtually no cleavage fragments are <0.25 μm in width and almost none are <0.5 μm (if >5 μm in length) [49,52]. In examining a single fibre <0.5 μm wide, or a small population of such narrow particles, it is reasonable to conclude that they are asbestos" [5] (see Table 1).[12]

This is related to the fact that, as cleavage fragments get longer, their widths increase, so that nearly all cleavage fragments that are longer than 5 μm are also greater than 0.3 μm in width (Chatfield, pers comm. also see [5]). By contrast, as asbestos fibers get longer, they remain uniformly thin [53] so significant quantities of asbestiform fibers longer than 5 μm and thinner than 0.25 μm are commonplace. Cleavage fragmentation *cannot* therefore generate appreciable quantities of extremely long, thin structures so the majority of airborne cleavage fragments are not biologically relevant (see above).

Cleavage fragments thinner than 0.3 μm and longer than 15–20 μm are very rare, if they exist at all [5,9–11]. Amongst asbestos fibers thinner than 0.3 μm, those longer than 40 μm are 500 times more potent than those shorter than 40 μm [54]. Cleavage fragments of these dimensions do not exist. Fibers less than 5 μm have little or no potency [9,10] and those in the 5–10 μm range have a mesothelioma potency 1/300th of fibers longer than 10 μm [10]. Cleavage fragments greater than 10 μm long are, in fact, very uncommon [5].

Regarding width, cleavage fragments >5 μm long are generally too thick to be respired (they would need to be *c.* <1.5 μm) [10], too wide to penetrate into the deep lung (they would need to be *c.* <0.6 μm) [10], or too thick to comport with a pathogenic width (*c.* <0.15–0.3 μm) [55,56]. Various researchers have demonstrated width

Table 1. SEM characterization of bulk samples of asbestos and cleavage fragments

Asbestos	% of (a) with Widths <0.25 μm	% of (a) with Widths <0.5 μm	% of (a) with Aspect Ratio >3:1	% of (a) with Aspect Ratio >10:1	% of (a) with Aspect Ratio >15:1	% of (a) with Aspect Ratio >20:1
Fibers						
Croc, SA	48	85	100	99	95	89
Amosite, SA	73	50	100	98	84	75
Cleavage fragments						
Tremolite, NY	30	1	47	3	2	2
Riebeckite, Calif.	50	5	78	35	21	12

From [38] as cited by [5], Table 2.

cut-offs for mesothelioma formation on the basis of animal studies [57]; also see criticisms in [10] where this [57] was refuted in discussion, and human observations in relation to attendant fiber size measurements made in air, ore, and lung tissue, e.g. [50,58–63] (Karjalainenen, 1997 pers comm and Wagner, 1999 pers comm). Therefore, cleavage fragments cannot have the same mesothelioma-inducing potential as asbestos fibers since the vast majority do not conform to the physical dimensions that pose a mesothelioma risk (also see [10]).

Biopersistence Strongly Determines Carcinogenicity and Cleavage Fragments are Far Less Bio-persistent than Asbestos Fibers

Biopersistence strongly determines carcinogenicity [64]. This is largely a macrophage-mediated phenomenon. Macrophages can physically clear a fiber depending on its length and/or dissolve it depending largely upon its durability and surface strength.

The Ability of the Macrophage to Clear and/or Dissolve Asbestos Fibers and Non-asbestiform Cleavage Fragments from the Lung is Very Different

Long, thin durable asbestiform amphibole fibers are extremely difficult for the lungs to clear and can 'biopersist' long enough to produce severe adverse biological effects. The critical length for fiber clearance approximates the diameter of an alveolar macrophage [63]. This is species-dependent with the critical length cut-off being significantly longer for humans than rodents (rat: 10–15 μm [63]; 5–10 μm [54]; 8 μm [65,66]: humans: 10–15 μm [54]; 24 μm [65,66]; 17 μm [67,68]; 18–20 μm [9]). Human alveolar macrophages are also better able to clear fibers than those of rodents due to their vastly greater surface areas and because the number of macrophages per alveolus in humans is much greater than in rodents; a 600-fold difference [66]. Since risk assessments generally ignore such comparative clearance considerations, animal data usually overestimate human risk.

Any long, thin cleavage fragments that exist are almost certainly brittle and weak and "cannot bend more than a few degrees" [26]; also see [44]. Physical stresses may cause them to break as they enter, remain within, and/or leave the body. The forces experienced during alveolar collapse and expansion may impose bending forces on cleavage fragments causing them to break. After phagocytosis, the muscular strands of a macrophage's cytoskeleton (that enable it to change shape and size dramatically so it can enter lymphatic vessels or squeeze through tiny pores between epithelial cells), may impose forces on the phagocytosed cleavage fragments that cause them to break. By contrast, asbestos fibers are extremely strong and flexible. Thus, "The relatively high flexibility of asbestiform fibers enables them to bend without breaking and may facilitate their passage through the respiratory tract" [26].

Fibers thin enough to reach the deep alveolar lung may be engulfed by phagocytic cells such as macrophages and neutrophils. Although phagocytes cannot "digest" mineral particulates as they might, say, bacteria, the acid milieu produced by release of intracellular acidic enzymes does cause some mineral dissolution. Dissolution is greatest within surface defects [69]. The exceedingly strong, defect-free surface of an amphibole asbestos fiber enables it to resist acid attack better than a cleavage fragment [26,28,29]. If fibers are too long to be completely engulfed, the cell will eventually die in an attempt to clear it. Repeated attempts by cells to engulf a long fiber result in deposits of glycoprotein/hemosiderin along its length giving it an appearance, under the microscope, of a beaded 'drumstick'. This is known as an 'asbestos body'. Asbestos body formation takes place primarily on long amphibole structures. Partial dissolution of the fiber can eventually weaken the asbestos body so that its breaks at "internodal" points along its length. This disintegration continues until the fragments are short enough to be phagocytosed and can then be cleared from the body.

The difference in biopersistence between cleavage fragments and asbestos fibers may be most pronounced for the very small proportion of cleavage fragments with 'biologically relevant' dimensions, i.e. those longer than 5 μm and thinner than 0.5 μm. As discussed above, cleavage fragments become weaker as they become thinner which follows in part from the inverse relationship between diameter and surface area. As the surface of an asbestos fiber is largely defect free, this increase in surface area with decreasing diameter does not particularly increase defect frequency. The converse is true for cleavage fragments; the thinner they are, the greater their surface area, and the greater the number of surface defects [28]. This would make thin cleavage fragments far more susceptible to the effects of macrophage attack than amphibole asbestos fibers of the same width.

Animal Studies Demonstrate Cleavage Fragments are not Carcinogenic

The effects of asbestos fibers and non-asbestiform cleavage fragments on animals have been assessed in the same

studies to compare their carcinogenic potential.[13] Indeed, some of the "most compelling evidence that their effects are very different comes from animal studies" [5]. All such studies have used either intrapleural injection, intrapleural implantation, or intraperitoneal injection. Each delivers massive doses directly to the mesothelium. This can only be accomplished by artificial exposure methods that bypass host defense mechanisms that normally prevent all but a small fraction of fibers from reaching the mesothelium following inhalation. Despite the extreme sensitivity of these injection test methods and the massive doses employed, cleavage fragments still fail to produce any tumors or a tumor response exceeding background [70–72]. This concept is ignored by some such as the Final Report [10]. By contrast, asbestos fibers in these injection studies produce high tumor rates not infrequently reaching 100%. The negative carcinogenic responses noted with cleavage fragments therefore provide very strong evidence that cleavage fragments are not carcinogenic to humans, particularly when the sensitivity of the assay and the large doses used are taken into consideration. OSHA [6] concluded that "virtually all participants agreed" that the animal studies clearly demonstrate qualitative differences in the carcinogenic potential of asbestos and cleavage fragments.

The following summarizes the most relevant studies.

Wagner et al. [62], Stanton et al. [57] and Smith et al. [73] intrapleurally injected rodents with large [10–40 mg] doses probably containing up to 80 million cleavage fragments longer than 5 μm and less than 0.5 μm wide (also see [74,75]). The rats either failed to develop mesotheliomas or the resultant tumor rates did not exceed background [70–72].

Davis et al. [76] intraperitoneally injected rats with 10 mg doses [49 million cleavage fragments longer than 5 μm; 2 million longer than 5 μm and thinner than 0.5 μm] of two tremolite cleavage fragment samples. The Shinness tremolite sample, "almost exclusively composed of very brittle cleavage fragments" [76], (Addison, 2000 pers comm) and not a "mix" as suggested by Lockey (cited in [10]), produced mesotheliomas in only 5.6% (2/36) of rats, an incidence well below background [76–78]. The same number of asbestos fibers of similar dimensions would have produced a very high incidence of mesotheliomas (see Table 2) [77]. Davis et al. [77,78] said that asbestos fibers longer than 8 μm were the most carcinogenic in intraperitoneal injection studies. He stated further that "tumours may be expected regularly at dose levels of between 150,000 and 200,000 fibres (>8 μm) and will develop in at least 25% of animals if more than about 600,000 fibers are injected". However, the intraperitoneal injection of 17 million cleavage fragments longer than 8 μm [77] failed to produce mesothelioma rates above background (Table 3). By contrast, much smaller numbers of asbestos fibers produced mesothelioma rates up to 95% [77]. The second cleavage fragment sample from Dornie, Scotland contained 24 million fibers longer than 5 μm and this also failed to produce tumor rates greater than background (data not shown). Davis et al. [76] concluded that human exposure to materials such as those obtained from Shinness or Dornie, Scotland, whether as a pure mineral dust or as a contaminant of other products, "will almost certainly produce no hazard".

In Vitro Studies

In vitro studies have also demonstrated that non-asbestiform tremolite [61,79], grunerite [43,80] and riebeckite [81–83]; also see [84], cleavage fragments are far less biologically active than asbestiform amphibole fibers tested in the same study as measured by a great variety of cellular endpoints.

Table 2. Comparison of Shinness tremolite "fibers" (>5 μm) and asbestos fibers (>5 μm)

Type	Mass Dose (mg)	No. Fibers >5 μm Length	Meso. Incidence	Above Background?	The Study
Shinness Tremolite (cleavage fragments)	10	49,000,000	5.6%	No	Davis et al. [76]
Amosite	0.05	1,700,000	25%	Yes	Davis et al. [78]
Crocidolite	0.05	2,075,000	25%	Yes	Davis et al. [78]
Actinolite	0.01	4,000,000	23%	Yes	Pott [137]
Actinolite	0.05	20,000,000	42%	Yes	Pott [137]

Table 3. Comparison of Shiness tremolite "fibers" > 8 μm and asbestos fibers > 8 μm

Type	Mass Dose (mg)	No. Fibers >8 μm Length	Meso. Incidence	Above Background?	Study
Shinness Tremolite (cleavage fragments)	10	17,000,000	5.6%	No	Davis et al. [76]
Amosite	2.5	153,000	60%	Yes	Davis et al. [138,139]
Amosite	0.05	305,000	28%	Yes	Davis et al. [138,139]
Amosite	5.0	305,000	78%	Yes	Davis et al. [138,139]
Crocidolite	0.05	420,000	25%	Yes	Davis [140]
Amosite	7.5	458,000	65%	Yes	Davis et al. [138,139]
Amosite	10	610,000	72%	Yes	Davis [141]
Crocidolite	0.05	745,000	25%	Yes	Davis et al. [78]
Amosite	0.05	765,000	25%	Yes	Davis et al. [78]
Amosite	15	915,000	76%	Yes	Davis et al. [138,139]
Crocidolite	0.5	4,200,000	31.3%	Yes	Davis [140]
Amosite	10	6,100,000	88%	Yes	Davis [140]
Amosite	25	1,525,000	95%	Yes	Davis [138,139]

Epidemiological Studies Show No Association Between Exposure to Amphibole Cleavage Fragments and Asbestos-Related Disease

Homestake Gold Miners

Steenland and Brown [85] performed the most recent study of the Homestake gold miners (n=3,328). Although these workers were exposed to significantly elevated levels [86] of grunerite and tremolite cleavage fragments, there were no deaths due to mesothelioma. The one "mediastinal" mesothelioma was "unconfirmed" [9,87,88] and there was no lung cancer excess (SMR 1.13) (also interpreted as "negative" by the ATS [7], Chisholm [5] and the ATSDR [9]).

Ontario Gold and Nickel Miners

Kusiak et al. [89] conducted the most recent study of the Ontario gold and nickel miners (n=54,128) exposed to non-asbestiform amphibole fibers. A lung cancer excess was thought to be related to arsenic and radon, not to cleavage fragments (also see [90, 91] (Kusiak, 2003 pers comm). Two cases of mesothelioma occurred in gold miners but neither case "was known to be exposed to the komatiite rocks that sometimes contain fibrous amphiboles" [89].

Minnesota Taconite Miners

Higgins et al. [92] studied the Reserve Mining Company taconite miners and millers (n=5,751). These workers were exposed to elevated levels of grunerite cleavage fragments but displayed no attributable asbestos-related disease.

Cooper et al. [93] conducted the latest update of the Erie and Minntac Company taconite miners and millers (n=3,444) exposed to elevated levels of grunerite cleavage fragments (as estimated from Higgins et al. [92]). One mesothelioma was found but it was not thought to be attributable due to insufficient latency and significant alternative exposure, i.e. from long-term work with boiler insulation on locomotives [93]. A recent mesothelioma case control study by the Minnesota Department of Health [94] also failed to find any attributable cases. There was no lung cancer excess (SMR<100) (interpreted as "negative" by others [5,9]).

New York State Gouverneur Talc Company [GTC] Talc Miners

Honda et al. [95] conducted the most recent study of the GTC talc miners and millers (n=818) exposed to significant levels of tremolite cleavage fragments [49,96]. A lung cancer excess was observed. However, this was not felt to be attributable due to a lack of dose response, smoking (see [5,90,91,97–104] and pers comm from Delzell, 2003 and Beall 2003) and alternate causation (e.g. see data for individual lung cancer cases in [103–106]). Two mesotheliomas noted by Honda et al. [95] and Delzell et al. [105] were not thought to be attributable on the basis of insufficient latency, inadequate exposure and/or alternative causation. Hull et al. [107] claimed that there were at least 8 mesotheliomas, citing their own work and that of others [108–110]. Again most, if not all, of those cases did not appear to be attributable on diagnostic and/or causation grounds. A radiographic survey of the counties surrounding the GTC mines failed to find attributable asbestos-related disease [111].

US Paint Plant Production Workers Exposed to GTC Talc

Morgan [112] did the only study of paint and coating production workers ($n=16{,}000$) from 32 plants in the United States and these workers, in particular sub-cohort 2 (pigment) (Sides, 2003 pers comm) had a very high, ongoing use of and presumed exposure to GTC talc. No lung cancer excess was found (also see [103,104,113]). No mesotheliomas were reported.

UK Ceramics Pottery Workers

Thomas and Stewart [114] noted that pottery workers exposed to tremolitic talc displayed no lung cancer excess (also see [7,103, 104]

Norwegian Talc Miners and Millers

Wergeland et al. [115] studied Norwegian talc miners and millers probably exposed to trace amounts of tremolite cleavage fragments (see [115], p. 506). No lung cancer excess was found. No mesotheliomas were recorded.

Italian Talc Miners and Millers

Rubino et al. [116] studied Italian talc miners and millers probably exposed to trace amounts of tremolite cleavage fragments [7,117–119], and see the Pooley Report cited by [116]. No attributable cancer excess was found.

Vermont Talc Miners and Millers

Wegman et al. [120] and Selevan et al. [121] performed the latest studies of the Vermont talc miners and millers probably exposed to trace amounts of tremolite cleavage fragments [121]. No cancer excess was found.

Swedish Dolomite Limestone Miners and Millers

Selden et al. [122] studied Swedish dolomite limestone miners exposed to low concentrations of tremolite cleavage fragments. No cancer excess was found.

Enoree Vermiculite Miners and Millers

McDonald et al. [123] studied the Enoree South Carolina vermiculite workers ($n=194$) exposed to "trace" amounts of cleavage fragments [124]. There were no attributable deaths due to lung cancer, pneumoconiosis or mesothelioma.

New York Hard Rock Tunnel Diggers

Selikoff [125] studied 932 tunnel workers in New York City exposed from 1955 to 1972 to cleavage fragments from the massive, non-asbestiform amphibole, known as hornblende. There were 294 deaths but no evidence of asbestos-related disease [126].

Kennicott Copper Miners

The Kennicott Copper mine is one of the largest mining operations in the world. Workers have been exposed to cummingterite–tremolite–actinolite cleavage fragments for many years [4] with no suggestion of attributable asbestos-related disease (Kennicott management, 2000 pers comm).

The "Central European Arc of Pleural Pathology"

Endemic pleural plaques, not associated with any occupational exposure, occur from Finland in the north southwards through the former Soviet Union, Czechoslovakia, Austria, Yugoslavia, Bulgaria and Greece [127]. The plaque excess has been attributed to exposure to soils naturally contaminated with "coarse" (>1 μm in diameter) tremolite (or anthophyllite) fibers [62,127] that are probably cleavage fragments. Such asbestos-related plaques are thought to be due to largely non-fibrous, "blocky" [128], thick [55] amphibole [129,130].

Sparta Marble Quarry Workers and Residents

The Sparta New Jersey marble quarry has been in operation for almost 100 years and the workings are associated with very low exposures to tremolite cleavage fragments. There is no evidence to indicate that these exposures are associated with an attributable risk of asbestos-related disease in either the workforce or the residents of the town of Sparta several miles from the quarry.

Nephrite Jade Workers

Nephrite jade is a form of massive tremolite–actinolite amphibole (see, for example, [16]) mined in various parts of the world. One of the world's largest deposits is in British Columbia and the removal, wedging and slicing of nephrite boulders can be a source of dust exposure (Ward, 2003 pers comm). Whilst formal epidemiological studies of the Canadian nephrite jade miners have not been performed, mesotheliomas do not appear to have occurred in these workers (Ward, 2003 pers comm). Canadian nephrite is also purchased by the Chinese who work the stone on a lathe. This can be a source of considerable dust exposure (Ward, 2003, pers comm.). To date, there do not appear to have been formal studies of the health of the Chinese jade factory workers.

Quebec Chrysotile Miners and Millers

The Quebec chrysotile miners and millers have almost certainly been exposed to considerable airborne concentrations of tremolite cleavage fragments since a substantial proportion of the tremolite contaminating the ore is non-asbestiform [15]. However, detailed review of the Quebec chrysotile miner and miller lung burden studies for which relevant data are available failed to provide evidence that the predominant form of tremolite retained in these lung tissues is non-asbestiform.[14] In fact, the only study that appears to have addressed this issue [131] concluded that most of the tremolite was asbestiform. This observation would provide further support that non-asbestiform tremolite amphiboles are, for the most part, short enough to be cleared or, if initially longer than the macrophage, fragile enough to be rapidly broken down in the body and thus readily removed. Case [3] remarked "on the long tremolite fibers in miners and millers with asbestosis" and suggested that these could "produce increased levels of shorter fibers due to fiber breakage into shorter fragments" and thus contribute to a "possible increasing composition of the tremolite mass by cleavage fragments". This could only happen if the long tremolite fibers were actually long tremolite cleavage fragments since asbestiform fibers cannot produce non-asbestiform structures. Moreover, Dufresne et al. [131] did not find increased numbers of cleavage fragments making it very unlikely that cleavage fragments, contributed to the pathology found in the Quebec chrysotile miners and millers.

Conclusions

Cleavage fragments are not asbestos ("non-asbestiform"). There are fundamental differences in the properties of cleavage fragments and asbestos fibers. Cleavage fragments lack the strength, durability, flexibility and acid resistance of asbestos. They are therefore unable to persist in the body largely because they are short and are readily cleared. They also fail to persist since the few that are long break into short fragments due to their lack of strength, durability, flexibility and acid resistance. Moreover, those that would be long enough to thwart the macrophage are almost always too wide to be inhaled. Therefore, physical properties related to respirability and clearance and, probably to a lesser extent, chemical characteristics related to dissolution directly and clearance indirectly, account for their observed differences in carcinogenic potential.

OSHA [6] determined that the scientific evidence was insufficient to regulate cleavage fragments. Nonetheless, the California Geological Survey [134] still does not recognize the difference between asbestiform fibers and cleavage fragments saying there is "no general consensus on the health effects of cleavage fragments in the scientific community". This conclusion is contradictory since the California Geological Survey has said that "cleavage cannot produce the high strength and flexibility of asbestiform fibers" and that acicular crystals, "special types of prismatic (non-asbestiform) crystals", do not have the "strength, flexibility, or the other properties of asbestiform fibers" [134].

The scientific evidence that demonstrates that cleavage fragments are non-carcinogenic in animals and humans is robust. The methods used to assess tumor production in these animal studies are extremely sensitive and discriminatory even when the doses employed are vastly greater than humans would ever encounter even under worst-case scenario exposure conditions. This is particularly relevant to allegations of low dose risk where the levels of exposure are exponentially lower than those employed in such animal studies. The fact that cleavage fragments are non-carcinogenic in such animal tests demonstrates that cleavage fragments, even at extremely high doses, do not pose a carcinogenic risk to humans. Epidemiological studies of many tens of thousands of workers in various primary and secondary industries exposed to cleavage fragments fail to reveal evidence of an attributable cancer excess.[15] Moreover, amphiboles are ubiquitous throughout the earth's crust and clearly permeate numerous mineral deposits of potentially high commercial value, e.g. gold, silver [135], nickel [89], copper [4], sulphide [136], talc [95], vermiculite [123], marble [4], crushed stone, and a variety of gemstones such as jade (Ward 2003, pers comm). Many thousands of workers exposed to dusts containing cleavage fragment do not appear to display an attributable excess of mesothelioma. Similarly, the permeation of numerous residential areas by non-fibrous amphiboles has not resulted in a "pandemic" of mesotheliomas which again attests to the inability of cleavage fragments to produce asbestos-related disease.

Notes

1 The most common habit for an amphibole is an elongated prism, lozenge-shaped in cross section, ranging from short stocky prisms to fine needle-like crystals or ultimately fine hair-like crystals (sometimes known as byssolites). The prismatic habit is the normal form of igneous and metamorphic rocks and is very widespread throughout the continental crust of the earth (Addison, 2003 unpub.).

2 Very subtle chemical differences may influence growth habit. For example, the presence or absence of traces of aluminum may determine whether an asbestiform or a non-asbestiform habit exists. [16,17,20]. Since a fiber is composed of highly aligned and oriented chemical units, there is no room to accommodate larger atoms such as aluminum. [4]. Substitution of aluminum for silicon will lead to structural distortions that cause the development of prismatic crystals rather than asbestos fibers. [4]. This substitution also increases the Z–O bond distance and therefore reduces the strength of bonding within, and parallel to, the length of the amphibole chain. Although substitution is thought to occur mostly with aluminum, other metals have been proposed such as calcium [4], manganese [4], iron [4], titanium [16] and chromium [16] (and also see [5,17]) to be important substituents.

3 Elongated amphibole structures known as "transitional fibers" also exist but these are very rare [6]. They are thought to display features of both the asbestiform and the non-asbestiform condition. Their rarity puts them beyond the scope of this review (but see [22] for discussion) and they cannot materially affect the overall conclusions reached herein. Some [23] incorrectly claim that it is very difficult to distinguish between asbestiform and non-asbestiform amphiboles inferring that "transitional structures" are actually commonplace. Such claims do not comport with their data and may be related to a certain degree of "litigation bias" [24].

4 This might be reflected for asbestos in geological environments that favor "relatively rapid multi-nucleation and growth in a low temperature stress free environment", "the opposite conditions applying to most prismatic specimens" [16].

5 Some problems exist in distinguishing asbestos particles from cleavage fragments. The main difficulties arise from uncertainty over the features used to define asbestos, from the effect which processing has on those characteristic features and from the limited applicability of the defining characteristics to the small particles observed in the TEM [5].

6 Dorling and Zussman [16] refer to the surface of a cleavage fragment as "smooth" but "broken up by steps in the {110} cleavage plane" and the surfaces of growth faces [of asbestos fibers] as "usually roughened and striated due to the presence of vicinal faces" and small irregularities". The use of the term "smooth" in this review denotes the large scale absence of steps, dislocations, and large irregularities from asbestos fibers. Vicinal faces are also probably "metastable" disappearing as growth continues [16].

7 A "twinning plane [may also be regarded] as a stacking fault: the Si4O11 double chains of the structure lie in planes parallel to {100} and are displaced relative to each other by approximately ±c/3 along the chain axis in order to provide octahedral co-ordination for the cations between the double chains". ... twinning planes are points of weakness in the crystal structure and fracture is likely to occur along the {100} planes as a result, producing bladed or lath-like particles. This process may contribute to the observed morphology of asbestos particles and their tendency to have {100} faces as well as or in preference to {110} [5]

8 Chisholm [5] describes the many problems encountered in developing a reliable quantitative method and these include selection of the correct microscopic method, the degree of overlap between the size and aspect ratio ranges for the two types of particle; the lack of reliable, independent, systematically derived data in the literature; the use of potentially atypical reference samples; and the availability of data from different measuring techniques. Chisholm [5] also discusses the limitations of using diffraction to differentiate cleavage fragments from amphibole asbestos fibers. Also, "The frequency of {100} twin boundaries may offer the most reliable means of distinguishing the two types" on a quantitative basis but it "may not be easily determinable for all particles."

9 The main dimensional characteristics of the material are retained unless the grinding is extremely severe [38]. Grinding opens the asbestos fibers, i.e. separates them into their component fibrils, whose cross-section dimensions are established during their formation. The width of cleavage fragments will depend more on the degree of grinding. The width distribution does however depend on whether the measurements are made using TEM or SEM (see above): TEM tends to 'see' the smaller fibers better compared to SEM. So comparisons between width distributions should ideally be made using the same type of instrument. TEM gives by far the most accurate size data for thin fibers [5].

10 The NIOSH definition covered particles >5 µm long with an aspect ratio >3:1; the limit on the aspect ratio was intended to exclude non-fibrous mineral fragments but was otherwise arbitrary. It subsequently emerged that many particles derived from non-asbestiform amphiboles nevertheless came within the scope of this definition. Measurements on the particle dimensions of asbestiform and non-asbestiform amphiboles have shown that the 3:1 aspect ratio criterion bears little relation to the differences between the two. Many proposals have been made to change the definition of a fiber but the original definition still stands [11,48–51]:

> "the definition of a 'fibre' usually adopted for optical microscopy, i.e. a particle >5 µm in length and with aspect ratio >3:1, is not a satisfactory criterion for distinguishing asbestos particles from cleavage fragments. Alternatives have been proposed (length >5 µm and aspect ratio >20:1, [11=]; length >5 µm and width <1 µm, [50] which are certainly more realistic."
>
> "A distinction based on size and aspect ratio is the only practical way of [classifying a fibre or a fragment] whatever uncertainties it may introduce. To set up a quantitative method whose results have some practical meaning will require great care in setting the size and aspect ratio criteria which define asbestos fibres and cleavage fragments ... it should be possible to set criteria such that there is very little risk of failing to count an asbestos fibre through wrong identification as a cleavage fragment. However, the overlap of the size and aspect ratio distributions is such that there will always be some risk of wrongly counting a cleavage fragment as an asbestos fibre. The key to a successful quantitative method lies in minimising this latter risk by careful setting of the defining criteria for an asbestos fibre" [5].

11 Whilst Chisholm [5] said "no conclusion on a fibre-by-fibre basis can be drawn for particles >0.5 µm wide unless their aspect ratio is <3:1 in which case they lie outside the conventional definition of asbestos fibres and would be taken to be cleavage fragments", the data he provides "for particles >0.25 µm wide, >5 µm long and with aspect ratio >3:1" clearly demonstrate that "the greater the aspect ratio, the more likely the particle is to be an asbestos fibre". This is evident from the percentage of particles with aspect ratios >10:1, >15:1 and >20:1" (cf: Fig. 8 from [5]). Therefore, whilst "the possibility that one particular particle is an unusually long cleavage fragment can never be completely eliminated", "The aspect ratios of a small population of particles >0.25 µm wide may give a valid indication of their type" [5].

12 Some have suggested that the potencies of equi-dimensional tremolite fibers or cleavage fragments from different sources, e.g. vermiculite, marble, chrysotile, talc, may differ and that such differences may be biologically important, thus lowering the comparability of some of the animal studies. These differences, however, do appear to be minor (Zussman, 2003 pers comm), e.g. see cell parameter and chemical microprobe results for Gouvenour Talc, Shiness, Jamestown, Korean, and Ala d' Stura tremolites [16,17]. The observed chemical and morphological variations have also been described as "slight" (Zussman, 2003 pers comm).

13 Some panelists of the Final Report [10] "cautioned against inferring too much from this animal study" since they said it was not peer reviewed, the fiber measurements were difficult to reproduce, and the mesotheliomas could have reflected the use of the intraperitoneal injection model". However, the study was peer reviewed (by Case according to Addison, 2003 pers comm); there was no problem with fiber measurement reproducibility (Addison, 200 0 pers comm); and the model, as indicated above, could be used reliably to interpret such data.

14 The ATSDR report [9] states that the tremolite found in the lungs of the Quebec chrysotile workers is "relatively short, low aspect ratio" which seems to contradict the findings of Dufresne et al. [131]. However, the geometric mean (GM) of these fibers is 8:1–10:1. Since this is based on all fiber lengths, it could still include large numbers of high aspect ratio asbestos fibers. Moreover, according to the ATSDR [9] citing both the ATS [7] and Case (unpublished), the GM AR of fibers longer than 5 µm is said to be greater than 20:1. This may certainly contain significant numbers of fibers with much higher aspect ratios and thus be compatible with the findings of Dufresne et al. [131] (also see Langer's testimony in [6]). Thus, there is little evidence to support the ATSDR's [9] view that "high concentrations" of "lower" aspect ratio tremolite (i.e. cleavage fragments) can cause mesothelioma". Magee et al. [132] is often cited to support this notion, e.g. [7] but this paper is simply a case report and the data have been misinterpreted (e.g. by the ATSDR [9]). Wagner et al.

[62] says that "there are irregular deposits of a coarse fibered tremolite in the massive chrysotile ore bodies in Quebec" [which are] "found in the lungs of miners with pulmonary fibrosis and pleural plaques" [133]. Nonetheless, Pooley [133] actually fails to provide diameter distribution and aspect ratio data for these tremolite fibers. Only the pictures of the tremolite fibers in the lungs are given and, whilst these suggest that some may be "thick" or "coarse" in nature [133], they obviously cannot substitute for actual data.

15 Some may criticize cross comparison of studies based on exposures to different types of amphibole fiber, i.e. those derived from grunerite, taconite or cummingtonite. However, as the Final Report [10] states: "The potency of regulated and unregulated amphibole fibers should be considered equal based upon the reasoning that similar durability and dimension would be expected to result in similar pathogenicity." Uncertainties are also expressed about some of the conclusions reached by the ATS [7] panel (e.g. Lockey, 2003) but these are surely overridden by the fact that OSHA [6] concluded that there was not enough evidence to say that cleavage fragments posed a risk to workers. The Final Report [10] also said it was "prudent to assume an equivalent potency for cancer" (for cleavage fragments and fibers) despite that fact that most panelists acknowledged that the epidemiology and animal studies were negative.

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