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

E.B. Ilgren

Bryn Mawr, Penna

**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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Dr E. B. Ilgren
Suite 503, 830 Montgomery Avenue,
Bryn Mawr, Penna, 19010, USA
Tel. (610) 525 5960, Fax (610) 520 1156, E-Mail dredilgren@aol.com

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, 2000 pers 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 | (a) % >5 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? | 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 $Si_4O_{11}$ double chains of the structure lie in planes parallel to {100} and are displaced relative to each other by approximately $\pm c/3$ 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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