Asbestos

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Chapter 27

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David M. Bernstein

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27.1 INTRODUCTION

Asbestos has been known for centuries. The Romans used it in cremation cloths and lamp wicks, the Greeks also wove the material into cloth, and, in the Middle Ages, asbestos was used for insulating suits of armor. Asbestos was used because it is strong, insulates well, and resists fire and corrosion (Browne and Murray, 1990; Ross and Nolan, 2003).

It was not until the late 1800s, however, that asbestos was mined commercially, starting in Italy and England. In Canada, the first mine was opened in 1879 at Thetford, in the Quebec province. This was followed shortly thereafter with commercial asbestos mining in Russia.

The term "asbestos" is a generic term used to describe a group of mineral families that have overlapping properties. The two most common mineral families that the term asbestos is used to describe are the serpentines and the amphiboles. Although the distinction between these two minerals has been well described mineralogically since the 1930s, until more recently, little if any differentiation has been made in addressing the potential health effects of these two types of minerals.

The toxicological differences between chrysotile asbestos, a serpentine mineral, and the amphibole asbestos, such as amosite, crocidolite, and tremolite, have more recently been debated extensively. Although many studies have shown that chrysotile is not of the same potency as the amphiboles and is cleared from the lung more rapidly than amphibole, this issue still is mired in controversy.

Both serpentine and amphibole asbestos are naturally occurring minerals that are extracted from the earth in surface, open-pit, or underground mines. The fibers are intertwined with adjacent rock and are separated through crushing and milling with subsequent filtration/separation steps. Although the production of chrysotile asbestos has probably always exceeded that of amphibole asbestos by more than 10:1, the amphibole minerals are more abundant and are frequently constituents of igneous rocks and often major components of metamorphic rocks; they are thought

to account for approximately 20% of the shield area of the earth. However, fortunately for us, most of these rock-forming amphiboles are not at all asbestos like (Whittaker, 1979).

The serpentine minerals mainly occur in serpentinized ultramafic rocks, which have a wide distribution throughout the world, occurring in mountain chains, in Precambrian shield areas, in island arcs, and in midocean ridges. Although concentrations of chrysotile asbestos large enough to mine are rare, serpentinized ultramafic rocks almost inevitably contain chrysotile and some of this will be chrysotile asbestos (Wicks, 1979).

Most asbestos fibers were formed under unique conditions when the rock formations were undergoing intense deformation characterized by folding, faulting, shearing, and dilation. These deformations were often accompanied by the intrusion of magmatic fluids forming dikes and sills. The fibers crystallized in these high-strain environments, such as within folds, shear planes, faults, and dilation cavities and at intrusion boundaries (Ross and Nolan, 2003).

27.2 CHRYSOTILE CHARACTERISTICS

In 1930, Pauling (1930) reported that if serpentine had a kaolinite-type crystal structure, it would have a tendency to curve because of the misfit of the octahedral tetrahedral layers of the unit cell.

The chrysotile fiber is a sheet silicate, monoclinic in crystalline structure, and has a unique rolled form. The chemistry of chrysotile is composed of a silicate sheet of composition $(Si_2O_5)_n^{-2n}$, in which three of the O atoms in each tetrahedron are shared with adjacent tetrahedra and a non-silicate sheet of composition $[Mg_3O_2(OH)_4]_n^{+2n}$. In chrysotile the distances between apical oxygens in a regular (idealized) silicate layer are shorter (0.305 nm) than the O–O distances in the ideal Mg-containing layer (0.342 nm), which may account for the curling of the layers that results in the rolling up like a carpet to form concentric hollow cylinders (Skinner et al., 1988). This structure is illustrated in Figure 27.1 (adopted from Skinner et al., 1988) and transmission electron micrographs of chrysotile are shown in Figure 27.2 (Kiyohara, 1991). The Mg molecule is on the outside of the curl and is thus exposed to the surrounding environment. This layered construction of chrysotile is illustrated in Figure 27.3.

Table 27.1 summarizes the chemical composition of typical serpentine and amphibole asbestos. The chemical composition and the structure of chrysotile are notably different from that of amphiboles such as tremolite or amosite (Hodgson, 1979).

Commercial chrysotile is usually subdivided into groups by using the Canadian Quebec Screening Scale (QSS). These groups are determined by using an apparatus with a nest of four rotating trays superimposed one above the other. A known quantity of fiber is placed on the top tray

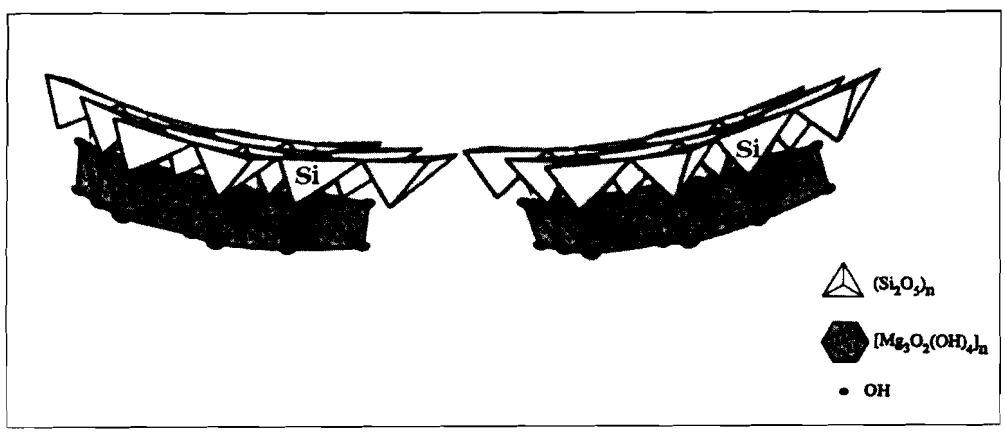

FIGURE 27.1 Schematic representation of the chemical structure of chrysotile showing the Mg molecule is on the outside of the curl. Adapted from Skinner et al. (1988).

and the trays are rotated for a fixed time to produce a sifting action. The longest/thickest fibers stay on the top screen (tray), which has the largest openings, and the shorter/thinner fibers fall though to lower screens. The grade is determined based on the weight fractions deposited on each screen and ranges from 3 to 9 with 3 being the longest (Cossette and Delvaux, 1979).

Nagy and Bates (1952), reporting on the stability of chrysotile, showed that it has a high solubility in hydrochloric acid. They also observed that chrysotile has a relatively low thermal stability compared with other hydrous silicate minerals. The heat of the electron beam of an electron microscope caused a very rapid change in the morphology of the fibers, and prolonged exposure to electron bombardment resulted in complete disintegration of the material (Noll and Kircher, 1952). Hargreaves and Taylor (1946) reported that if fibrous chrysotile is treated with dilute acid, the magnesium can be completely removed, and the hydrated silica remaining, though fibrous in form, completely lost the elasticity characteristic of the original chrysotile and gave an x-ray pattern of one or perhaps two diffuse broad bands, indicating that the structure is "amorphous" or "glassy" in type.

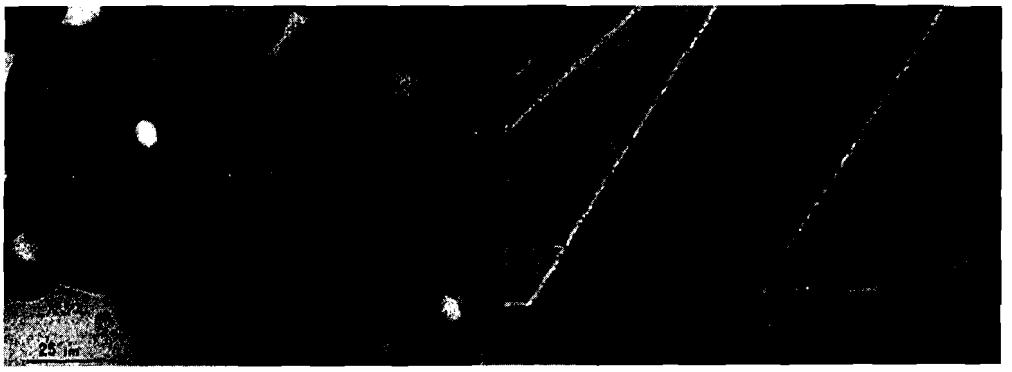

FIGURE 27.2 Transmission electron micrograph of chrysotile showing the curled sheet-like form of the fibers (Kiyohara, 1991).

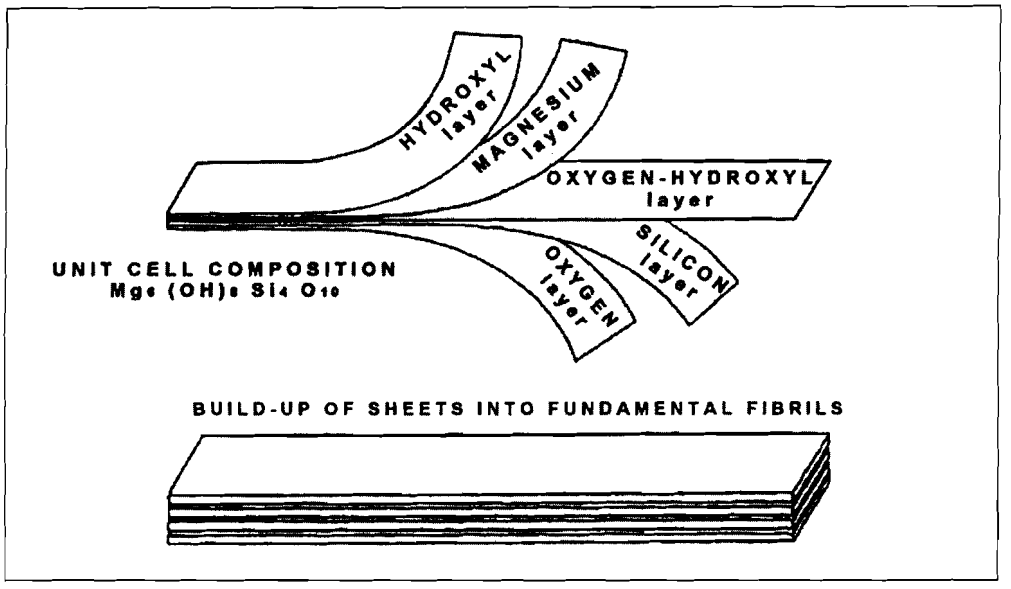

FIGURE 27.3 Illustration of the layered structure of chrysotile (reproduced from Speil and Leineweber, 1969).

TABLE 27.1 Typical Chemical Composition (Percent)

Compound	Chrysotile[a]	Tremolite[b]	Amosite[b]
SiO_2	40.6	55.10	49.70
Al_2O_3	0.7	1.14	0.40
Fe_2O_3	2.3	0.32	0.03
FeO	1.3	2.00	39.70
MnO	—	0.10	0.22
MgO	39.8	25.65	6.44
CaO	0.6	11.45	1.04
K_2O	0.2	0.29	0.63
Na_2O	—	0.14	0.09
H_2O^+	—	3.52	1.83
H_2	—	0.16	0.09
CO_2	0.5	0.06	0.09
Ignition loss	14.0	—	—
Total	100	99.93	100.26

[a] Typical chemical analysis of Canadian chrysotile from the Quebec Eastern Townships (LAB Chrysotile, Inc., Quebec, Canada).

[b] Hodgson (1979), pp. 80–81.

27.3 AMPHIBOLE CHARACTERISTICS

In contrast to chrysotile, with amphiboles, the basic structure is in the form of a double-silica chain, which appears as an I-beam with corner-linked $(SiO_4)^{-4}$ tetrahedra linked together in a double-tetrahedral chain that sandwiches a layer with the Ca_2Mg_5. These chains are paired, "back-to-back," with a layer of hydrated cations in between to satisfy the negative charges of the silica chains. The final structure is formed by stacking these sandwich ribbons in an ordered array (Speil and Leineweber, 1969). This is illustrated in Figure 27.4. Each of the blue boxes represents a double chain of tetrahedral (SiO_2). (The tetrahedra are illustrated in the middle chains.) With tremolite, the circles represent the magnesium and calcium cations that effectively glue one chain to its neighbor (Figure 27.4A).

Fewer shared cations bond the chains together along the broad sides of the chains than along the narrow sides, resulting in these broad surfaces being bonded less strongly. As shown in Figure 27.4B, it is along these weakly bonded surfaces, shown in red dashed lines, that the mineral will most likely break. With tremolite, these weak bonds are associated with the Mg. Figure 27.4C simplifies the picture and shows that the double-chain silicates can break into a set of fragments with a potentially regular shape.

Figure 27.4D shows the same situation in three dimensions. The potential breakages run along the chains and it can be seen how the fiber shape is formed. The chains themselves do not break easily because the bonds between the silica tetrahedra are very strong compared with the bonds gluing one chain to the next.

Depending on the type of amphibole, the principle cations are magnesium, iron, calcium, and sodium. The principle types are:

Crocidolite	$(Na_2Fe_3^{2+}Fe_2^{3+})Si_8O_{22}(OH)_2$
Amosite	$(Fe^{2+},Mg)_7Si_8O_{22}(OH)_2$
Tremolite	$Ca_2Mg_5Si_8O_{22}(OH)_2$
Actinolite	$Ca_2(Mg, Fe^{2+})_5Si_8O_{22}(OH)_2$
Anthophyllite	$(Mg, Fe^{2+})_7Si_8O_{22}(OH)_2$

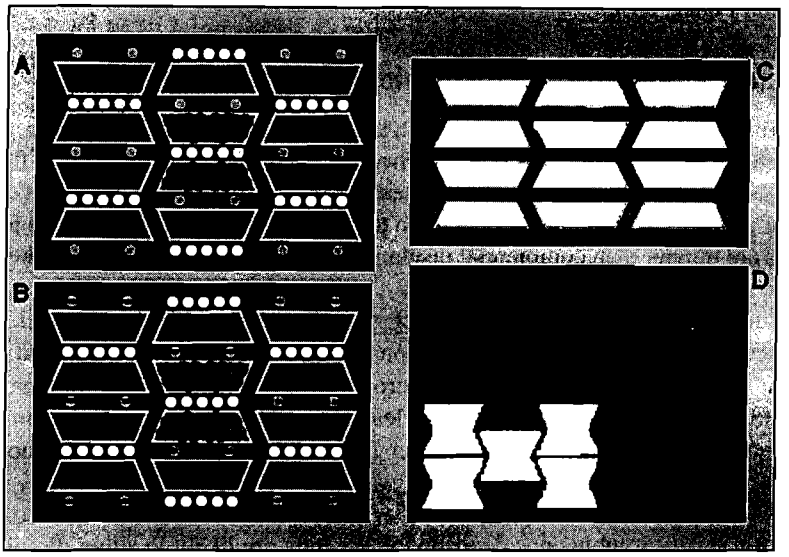

FIGURE 27.4 The structural formation of the double-chain silica tremolite asbestos is illustrated. (A) The amphiboles shown schematically with the slice directly across the chains. Each of the blue boxes represents a double chain of tetrahedral (SiO_2). (The tetrahedra are illustrated in the middle chains.) With tremolite, the circles represent the magnesium and calcium cations that glue one chain to its neighbor. Fewer shared cations bond the chains together along the broad sides of the chains than along the narrow sides. These broad surfaces are, therefore, bonded less strongly. (B) It is along these weakly bonded surfaces, shown in red dashed lines, that the mineral will most likely break. With tremolite, these weak bonds are associated with the Mg. (C) Simplification of the picture; the double-chain silicates can break into a set of fragments with potentially regular shape. (D) The same situation in three dimensions. The potential breakages run along the chains and it can be seen how the fiber shape is formed. The chains themselves do not break easily because the bonds between the silica tetrahedra are very strong compared with the bonds gluing one chain to the next.

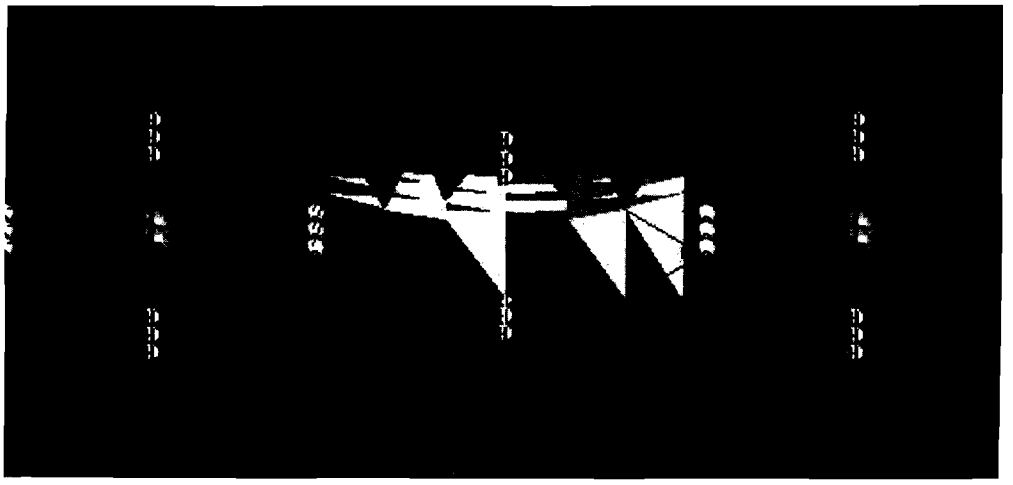

FIGURE 27.5 Schematic representation of the chemical structure of tremolite showing the Mg that is locked within the I-beam structure. Adapted with permission from: Department of Geology and Geophysics, University of Wisconsin, Crystal Structure Movies. Available at http://www.geology.wisc.edu.

The exterior surfaces of the amphiboles are tightly bound silica-based structures. This is illustrated with tremolite in Figure 27.5.

27.3.1 Factors Influencing Fiber Toxicology

Mineral fiber toxicology has been associated with three key factors: dose, dimension, and durability. The dose is determined by the fiber's physical characteristics/dimensions, how the fibrous material is used, and the control procedures that are implemented. In addition, the thinner and shorter fibers will weigh less and thus can remain suspended in the air longer than thicker and longer fibers. Most asbestos fibers are thinner than commercial insulation fibers; however, they are thicker than the new nanofibers that are currently being developed.

The fiber dimensions govern two factors, first, whether the fiber is respirable and, second, if it is respirable, whether the fiber will produce a response in the lung milieu once inhaled. Shorter fibers of the size that can be fully engulfed by the macrophage will be cleared by mechanisms similar to those for nonfibrous particles. These include clearance through the lymphatics and macrophage phagocytosis and clearance. It is only the longer fibers that the macrophage can not fully engulf, which if they are persistent will lead to disease.

This leads to the third factor, that of durability. Those fibers whose chemical structure renders them wholly or partially soluble once deposited in the lung are likely to either dissolve completely or dissolve until they are sufficiently weakened focally to undergo breakage into shorter fibers. The remaining short fibers can then be removed though successful phagocytosis and clearance.

These factors have been important determinants for synthetic mineral fibers (Hesterberg et al., 1998a, 1998 b; Miller et al., 1999; Oberdoester, 2000; Bernstein et al., 2001a, 2001b).

27.3.2 *In Vitro* Toxicology

In vitro toxicology studies are often very helpful in elucidating possible mechanisms involved in pathogenesis. However, as used in the assessment of fiber toxicology, they are difficult to interpret. This stems from several factors. The *in vitro* test system is a static system and thus is not sensitive to differences in fiber solubility. High doses of fibers are used to obtain a positive response and it is difficult to extrapolate from these large short-term cellular exposures to lower-dose chronic exposures that occur *in vivo*. In addition, the number of fibers and size distribution are often not quantified. Most important, however, is that these end points have not been validated as screening assays that are predictive of long-term pathological effects *in vivo*. Although *in vitro* tests may be useful tools to identify and evaluate possible mechanisms, with fibers, these *in vitro* test systems are of limited use in differentiating different fiber types (Olin et al., 2005).

27.3.3 Biopersistence

A fiber is unique among inhaled particles in that the aerodynamic diameter of fibers is largely related to three times the fiber diameter. Because of this, long thin fibers can penetrate into the deep lung, effectively bypassing the filtration that occurs for nonfibrous particles. Within the lung, fibers that can be fully engulfed by the macrophage can be removed as with any other particle. However, those fibers that are too long to be fully engulfed by the macrophage can not be cleared by this route.

Fibers less than 5 μm in length are effectively no different than nonfibrous particles and are cleared with kinetics and mechanisms similar to particles. Although longer fibers may also be cleared effectively by the macrophage and as a result not be different kinetically than particles, the 5-μm cutoff was chosen to mirror the use by the World Health Organization (WHO) of a 5-μm cutoff in their counting schemes for fibers. As is discussed later, recent reviews of these size fibers have concluded that they present very little or no risk to human health (ATSDR, 2003).

Fibers between 5 and 20 μm in length represent the transition range between those fibers that are cleared as particles and the longer fibers that the macrophage can not fully phagocytize. The actual

TABLE 27.2 Inhalation Biopersistence Clearance Half-Times of Natural and Synthetic Fibers (Length > 20 μm)

Fiber	Type	Weighted $t_{1/2}$ Fibers l > 20 μm (days)	Reference
Calidria chrysotile	**Serpentine asbestos**	**0.3**	**Bernstein et al., 2005b**
Brazilian chrysotile	**Serpentine asbestos**	**2.3**	**Bernstein et al., 2004**
Fiber B	B01.9	2.4	Bernstein et al., 1996
Fiber A	Glasswool	3.5	Bernstein et al., 1996
Fiber C	Glasswool	4.1	Bernstein et al., 1996
Fiber G	Stonewool	5.4	Bernstein et al., 1996
MMVF34	HT stonewool	6	Hesterberg et al., 1998
MMVF22	Slagwool	8	Bernstein et al., 1996
Fiber F	Stonewool	8.5	Bernstein et al., 1996
MMVF11	Glasswool	9	Bernstein et al., 1996
Fiber J	X607	9.8	Bernstein et al., 1996
Canadian chrysotile	**Serpentine asbestos**	**11.4**	**Bernstein et al., 2005a**
MMVF 11	Glasswool	13	Bernstein et al., 1996
Fiber H	Stonewool	13	Bernstein et al., 1996
MMVF10	Glasswool	39	Bernstein et al., 1996
Fiber L	Stonewool	45	Bernstein et al., 1996
MMVF33	Special purpose glass	49	Hesterberg et al., 1998a
RCF1a	Refractory ceramic	55	Hesterberg et al., 1998a
MMVF21	Stonewool	67	Hesterberg et al., 1998a
MMVF32	Special purpose glass	79	Hesterberg et al., 1998a
MMVF21	Stonewool	**85**	Bernstein et al., 1996
Amosite	**Amphibole asbestos**	**418**	**Hesterberg et al., 1998a**
Crocidolite	**Amphibole asbestos**	**536**	**Bernstein et al., 1996**
Tremolite	**Amphibole asbestos**	**∞**	**Bernstein et al., 2005b**

limit as to what length fiber can be fully phagocytized has been proposed for the rat as ranging from 15 μm (Miller, 2000) to 20 μm (Luoto et al., 1995; Morimoto et al., 1994).

In the lung, extensive work on modeling the dissolution of synthetic vitreous fibers (SVFs) by using *in vitro* dissolution techniques and inhalation biopersistence has shown that the lung has a very large fluid buffer capacity (Mattson, 1994). These studies have shown that an equivalent *in vitro* flow rate of up to 1 ml/min is required to provide the same dissolution rate of SVF as that which occurs in the lung. This large fluid flow within the lung results in the dissolution of the more soluble fibers. Recent publications have shown that the biopersistence of the fibers longer than 20 μm is an excellent predictor of the pathological response to fibers following chronic inhalation studies and chronic intraperitoneal studies (Bernstein et al., 2001a, 2001b; Hesterberg et al., 1998a, 1998b). The value of 20 μm is used as an index for fibers that can not be fully phagocytized and cleared by the macrophage. The protocol used in these biopersistence studies was developed by a working group for the European Commission and involves a 5-d inhalation exposure followed by analysis of the lungs at periodic intervals up to 1 year postexposure (Bernstein and Riego-Sintes, 1999).

For synthetic vitreous fibers, the clearance half-time of fibers longer than 20 μm ranges from a few days to less than 100 days. This is illustrated in Table 27.2. Highlighted in this table are

those studies performed on chrysotile and amphiboles with the same protocol. For synthetic vitreous fibers, the European Commission has established a Directive which states that if the inhalation biopersistence clearance half-time of a fiber is less than 10 days then it is not classified as a carcinogen.

Clearly there is a large difference in biopersistence between serpentine asbestos and amphiboles. In addition, because serpentine is a naturally occurring mined fiber, there seem to be some differences in biopersistence, depending on where it was mined. However, chrysotile lies on the soluble end of this scale and ranges from the least biopersistent fiber to a fiber with biopersistence in the range of glass and stonewools. It remains less biopersistent than ceramic and special purpose glasses and more than an order of magnitude less biopersistent than amphiboles.

The rapid clearance of chrysotile is thought to be characterized not by congruent dissolution as with many SVFs but rather with the loss of structural integrity of the serpentine sheet silicate and the subsequent disintegration into smaller pieces.

This difference between chrysotile and amphiboles is better illustrated with the actual lung burden data for the fibers longer than 20 μm from the inhalation biopersistence studies. In Figure 27.6, the number of fibers remaining in the rat's lungs is shown as a function of the time in days following cessation of the 5-d exposure (Bernstein et al., in press; 2005). Included are the two amphiboles, tremolite and amosite, a SVF fiber, HT, which has a clearance half-time of 6 days and which showed no tumors or fibrosis in a chronic inhalation toxicology study, and the three chrysotile fibers from Brazil, the United States (Calidria), and Canada. The inhalation exposure aerosol in terms of the number of fibers longer than 20 μm was in the range of 150–200 fibers (length > 20 μm) per cm^3 for all fibers except the Brazilian chrysotile, which was 400 fibers (length > 20 μm) per cm^3.

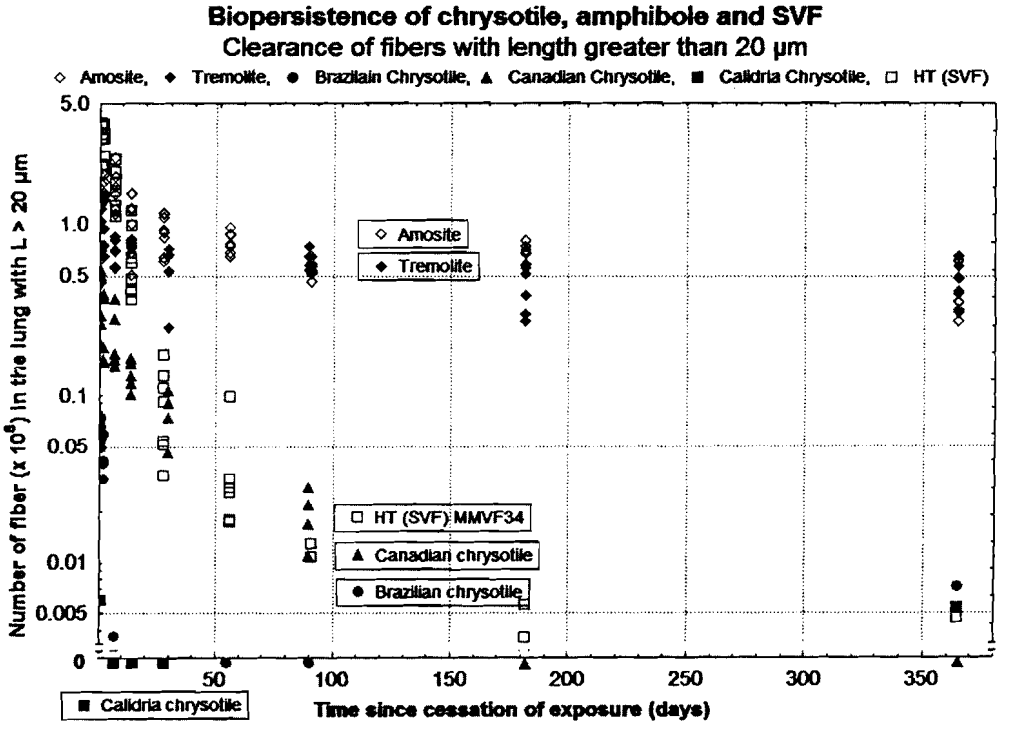

FIGURE 27.6 The number of fibers remaining in the rat's lungs is shown as a function of the time in days following cessation of the 5-day exposure (Bernstein et al., 2003a, 2005b). Included are the two amphiboles, tremolite and amosite, a soluble synthetic vitreous (SVF) fiber, HT, which has a clearance half-time of 6 days and which showed no tumors or fibrosis in a chronic inhalation toxicology study and the three chrysotile fibers from Brazil, the United States (Calidria), and Canada.

The amphiboles are very durable with only a small amount of clearance after cessation of exposure followed by virtually no further clearance. In the tremolite biopersistence study the histopathological response of the lungs was examined following the 5-day exposure. A pronounced inflammatory response with the rapid development of granulomas was seen at day 1 postexposure, followed by the development of fibrosis characterized by collagen deposition within these granulomas and by 90 days even mild interstitial fibrosis. In the same study, chrysotile showed no inflammatory or pathological response following the 5-d exposure (Bernstein et al., 2003b, 2005).

Although all the chrysotiles cleared relatively quickly, differences were observed among the three types studied. The Calidria chrysotile, which is known to be a short-fiber chrysotile, cleared the fastest with a clearance half-time for the fibers longer than 20 μm of 0.3 days.

The clearance half-time of the Brazilian chrysotile was 2.3 days. At the end of 12 months, two to three long fibers were measured following the lung digestion procedure. However, the exposure concentration for the Brazilian chrysotile was 400 fibers (length > 20 μm) per cm^3 rather than the 150 to 200 fibers (length > 20 μm) per cm^3 for the other fibers evaluated, thus resulting in a very high aerosol concentration of 7 million WHO fibers per cm^3 and more than 32 million total fibers per cm^3. It certainly is possible that this extremely high total exposure resulted in a response very different from what might be expected at lower exposure concentrations. Even so, the number of fibers observed at 12 months was not statistically different than that which was observed for the HT fiber, which had a 6-d clearance half-time for the long fibers.

For the Canadian chrysotile study textile-grade chrysotile was evaluated. This grade was chosen because it was specifically produced to have thin long fibers, which facilitated the production of textiles. The clearance of the Canadian chrysotile long fibers was 11.4 days. By 365 days there were no long Canadian chrysotile fibers remaining in the lung.

27.3.3.1 Clearance Mechanism of HT and Chrysotile

Kamstrup et al. (2002) described possible mechanisms that could account for the rapid clearance half-time of the long HT fibers. They stated that "The HT fiber is characterized by relatively low silica and high alumina content, with a high dissolution rate at pH 4.5 and relatively low rate at pH 7.4 (Knudsen et al., 1996). Apart from possible exposure to the acidic environment of the phagolysosomes within the macrophages (Oberdörster, 1991), measurements have shown that the microenvironment at the surface of activated macrophages is acidic with pH < 5 between attached macrophages and a non-porous glass surface (Etherington et al., 1981). It is therefore probable that long HT fibers, highly soluble at pH 4.5, are subject to extracellular dissolution and consequent breakage when exposed to the acidic environment of attached macrophages without being engulfed completely."

As mentioned above, at acidic pH chrysotile also becomes less stable and a similar mechanism may help accelerate the clearance/disintegration of the long chrysotile fibers.

27.3.3.2 Short-Fiber Clearance

For all fiber exposures, there are many more shorter fibers less than 20 μm in length and even more less than 5 μm in length. The clearance of the shorter fibers has in these studies been shown to be either similar to or faster than the clearance of insoluble nuisance dusts (Stoeber et al., 1970; Muhle et al., 1987). In a recent report issued by the Agency for Toxic Substances and Disease Registry entitled "Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length," the experts stated that "Given findings from epidemiological studies, laboratory animal studies, and in vitro genotoxicity studies, combined with the lung's ability to clear short fibers, the panelists agreed that there is a strong weight of evidence that asbestos and SVFs (synthetic vitreous fibers) shorter than 5 μm are unlikely to cause cancer in humans" (ATSDR, 2003; U.S. Environmental Protection Agency [EPA], 2003). In addition, Berman and Crump (2003) in their technical support document to the EPA on asbestos-related risk also found that shorter fibers do not appear to contribute to disease.

27.3.4 Chronic Inhalation Toxicology Studies

The studies presented above indicate that a large difference exists in the biopersistence between the serpentine chrysotile and the amphiboles, tremolite, and amosite. These differences appear to be related to the differences in chemical structure between the serpentines and amphiboles and possibly the influence of the acidic pH associated with the macrophage on the chrysotile fiber. Yet when the chronic inhalation studies that have been performed on chrysotile and amphiboles are examined these differences are not always apparent.

Berman et al. (1995) and Berman and Crump (2003), in an analysis of 13 inhalation studies that have been performed on nine different types of asbestos, concluded that:

- short fibers (less than between 5 and 10 μm in length) do not appear to contribute to cancer risk;
- beyond a fixed, minimum length, potency increases with increasing length, at least up to a length of 20 μm (and possibly up to a length of as much as 40 μm);
- the majority of fibers that contribute to cancer risk are thin with diameters less than 0.5 μm and the most potent fibers may be even thinner. In fact, it appears that the fibers that are most potent are substantially thinner than the upper limit defined by respirability;
- identifiable components (fibers and bundles) of complex structures (clusters and matrices) that exhibit the requisite size range may contribute to overall cancer risk because such structures likely disaggregate in the lung. Therefore, such structures should be individually enumerated when analyzing to determine the concentration of asbestos;
- for asbestos analyses to adequately represent biological activity, samples need to be prepared by a direct-transfer procedure; and
- based on animal dose–response studies alone, fiber type (i.e., fiber mineralogy) appears to impart only a modest effect on cancer risk (at least among the various asbestos types).

Concerning the lack of differentiation seen in the dose–response studies, the authors stated that this may be due at least in part to the limited lifetime of the rat relative to the biodurability of the asbestos fiber types evaluated in these studies.

Perhaps more important in understanding these results are the specifics in the study design of theses studies in light of the more recent understanding of the effect of high concentrations of insoluble particles on the rat lung.

The chronic inhalation studies that have been performed on asbestos are summarized in Tables 27.3 and 27.4. The exposure regime was similar in most studies and ranged from 5 to 7 h/d, 5 days per week for either 12 months or 24 months. Although it is difficult to determine how this was derived the exposure concentration was set for most studies based on mass concentration at 10 mg/m^3 Davis et al. (1978) referencing Wagner et al. (1974) states that 10 mg/m^3 was considered to be high enough to cause significant pathological change; however, no rationale is given in the Wagner paper as to why 10 mg/m^3 was chosen.

The issue of using equivalent fiber numbers for exposure was approached in a study reported by Davis et al. (1978) where chrysotile, crocidolite, and amosite were compared on an equal-mass and equal-number basis, but the fiber number was determined by phase-contrast optical microscopy (PCOM) and thus the actual number of especially the chrysotile fibers was probably greatly underestimated. As an example, by PCOM, the 10 mg/m^3 exposure to chrysotile was reported by PCOM as approximately 2000 fibers/cm^3 (length greater than 5 μm), whereas when a similar mass concentration of another chrysotile was measured by scanning electron microscopy (SEM) 10,000 fibers/cm^3 (length greater than 5 μm) were reported with a total fiber count of 100,000 fibers/cm (Mast et al., 1995). There are few quantitative data presented in these publications on the nonfibrous particle concentration of the test substances to which the animals were exposed. Pinkerton et al (1983) presented summary tables of length measurements of Calidria chrysotile by SEM in which the number of nonfibrous particles counted is stated; however, from the data presented, the aerosol

exposure concentration of nonfibrous particles can not be extracted. In all studies, the asbestos was ground prior to aerosolization, a procedure that would produce a lot of short fibers and dust. Most of the studies prior to Mast et al. (1995) used for aerosolization of the fibers an apparatus in which a rotating steal blade pushed/chopped the fibers off a compressed plug into the airstream. As some of the authors state, the steal used in the grinding apparatus and the aerosolization apparatus often wore, resulting in sometimes considerable exposure to the metal fragments as well. These factors contribute significantly to the difficulty in interpreting the results of the serpentine chrysotile and the amphibole inhalation exposure studies.

In these studies a tumorigenic response to amphiboles response is observed as would be expected from the biopersistence results; however, as mentioned, there is also a tumorigenic response to some of the chrysotile exposures even though the biopersistence results would suggest otherwise. Eastes and Hadley (1996) developed a model that related the dose of fibers in the lung to potential pathogenicity.

However, as many studies have now shown, in the rat another factor can also influence the inflammatory and pathological response. High concentrations of insoluble nuisance dusts have been shown to compromise the clearance mechanisms of the lung, cause inflammation and a tumorigenic response in the rat, a phenomenon often referred to as lung overload (Bolton et al., 1983; Muhle et al., 1988; Morrow, 1988; Oberdorster, 1995).

The biopersistence studies elucidate two kinetic patterns with chrysotile. They show that the long fibers are not biopersistent. From the fiber chemistry, the longer fibers are likely falling apart or disintegrating into the smaller pieces. The biopersistence studies also show that these smaller pieces clear at a rate that is similar to the rate of clearance of insoluble nuisance dusts. Chrysotile has also been shown to split longitudinally. In most of the chronic inhalation studies the total aerosol concentration was probably on the order of 10^6 particles and fibers per cm^3 and if the fibers upon contact with the lung begin to split and break apart, the effective dose in terms of the total number of particles would be increased even further.

With such a breakdown of chrysotile into shorter particles, the question remains as to whether the resulting concentration of particles can result in a nonspecific inflammatory reaction and an overload effect in the rat lung. In a recent study, Bellmann et al. (2003) reported on a calibration study to evaluate the end points in a 90-d subchronic inhalation toxicity study of man-made vitreous fibers with a range of biopersistence and amosite. One of the fibers was a calcium-magnesium-silicate (CMS) fiber for which the stock preparation, due to the method of preparation, had a large concentration of particulate material in addition to the fibers. The aerosol exposure concentration for the CMS fiber was 286 fibers/cm^3 (length $< 5\mu m$), 990 fibers/cm^3 (length $> 5\ \mu m$), and 1793 particles/cm^3, a distribution that is not observed in manufacturing. The total CMS exposure concentration was 3069 particles and fibers per cm^3. The authors point out that "The particle fraction of CMS that had the same chemical composition as the fibrous fraction seemed to cause significant effects." The number of polymorphonuclear leukocytes (PMNs) in the bronchoalveolar lavage fluid (BALF) was higher and interstitial fibrosis was more pronounced than had been expected on the basis of biopersistence data. In addition, interstitial fibrosis persisted through the 14-week recovery period following the 90-day exposure. In a separate study on X607, a fiber chemically similar to CMS, but with considerably fewer particles present in the aerosol, was evaluated in a chronic inhalation toxicity study and produced no lung tumors or fibrosis at any time point (Hesterberg et al., 1998b).

This effect attributed to particles in the rat CMS study was observed with an exposure concentration of 3069 particles and fibers per cm^3, 50% of which were particles or short fibers. It would follow directly from this and the many publications on overload to expect that a dramatically more pronounced effect would occur if the exposure concentration was 1,000,000 particles and fibers per cm^3, 90% of which were particles or short fibers, as was the case with chrysotile.

These discrepancies in study design put in question the value of especially the chrysotile studies listed in Table 27.3. The only well-designed multiple-dose study that was performed on any asbestos where particle and fiber number and length were controlled was that for amosite in the hamster (McConnell et al., 1999) as shown in Table 27.4. In this study the aerosol concentration ranged from

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Chrysotile NIEHS	6, 5, 24	n-o 10	1.02×10^5 SEM 1.06×10^4 >5μm	F, 69	13	18	1	Mast et al., 1995
Chrysotile	7, 5, 12	w.b. 10	1950 >5μm pcom 360 >20μm pcom	W, 40	15	38	0	Davis et al., 1978
Chrysotile	7, 5, 12	w.b. 2	390 >5μm pcom 72 >20μm pcom	W, 42	8	19	1	Davis et al., 1978
Crocidolite	7, 5, 12	w.b. 10	860 >5μm pcom estimated figure 34 >20μm pcom	W, 40	1	3	0	Davis et al., 1978
Crocidolite	7, 5, 12	w.b. 5	430 >5μm pcom 17 >20μm pcom	W, 43	2	5	1	Davis et al., 1978
Amosite	7, 5, 12	w.b. 10	550 >5μm pcom 6 >20μm pcom	W, 43	2	5	0	Davis et al., 1978
Chrysotile Calidria	7, 5, 12	w.b. 10	Nd	F, 51	2	4	0	Ilgren & Chatfield 1997, 1998; Pinkerton et al., 1983
Chrysotile Jeffrey	7, 5, 12	w.b. 10	Nd	F, 49	11	22	0	Ilgren & Chatfield 1997, 1998; Pinkerton et al., 1983
Chrysotile UICC/B	7, 5, 12	w.b. 10	Nd	F, 54	13	24	0	Ilgren & Chatfield 1997, 1998; Pinkerton et al., 1983
Tremolite Korean	7, 5, 12	w.b. 10	1600 pcom	39	18	46	2	Davis et al.
Amosite UICC	7, 5, 24	w.b. 10	Nd	W, 21	13	62	0	Wagner et al., 1974

(*Continued*)

TABLE 27.3 Chronic Inhalation Toxicology Studies with Chrysotile and Amphibole Asbestos in Rats[a] (Continued)

Fiber type	Exposure Time h/d, d/wk, total months	Exposure type, Exposure Concentration mg/m³	Fiber Concentration f/cm³ (determined by electron microscopy unless otherwise noted)	Type & Total no. of rats	Number Pulmonary Tumours	% Pulmonary Tumours	Noumber of meso-theliomas	References
Amosite long	7, 5, 12	w.b. 10	2060 > 5μm pcom 70 > 10 μm pcom	W, 40	11	28	3	Davis et al., 1986
Amosite short	7, 5, 12	w.b. 10	70 > 5μm pcom 12 > 10 μm pcom	W, 42	0	0	1	Davis et al., 1986
Amosite	—	w.b. 300	Nd	SD, 16	3	19	0	Lee et al., 1981
Crocidolite UICC	7, 5, 24	w.b. 10	Nd	W, 18	13	72	0	Wagner et al., 1974
Crocidolite	5, 5, 12	w.b. 2.2	2011 162 > 5 μm	W, 50	1	2	0	Muhle et al., 1987
Crocidolite UICC	6, 5, 24	w.b. 7	3000 90 > 10 μm	OM, 60	3	5	1	Smith et al., 1987
Crocidolite exposure truncated	6, 5, 10	n-o 10	1.6×10^4 > 5 μm SEM	F, 106	15	14	1	McConnell et al., 1994

[a] Exposure types: w.b., whole body; n-o, nose only. Type of rat: F, Fisher 344; OM, Osborne Mendel; SD, Sprague–Dawley; W, Wistar. ND, not determined; pcom, phase-contrast optical microscopy; SEM, scanning electron microscopy.

TABLE 27.4 Chronic Inhalation Toxicology Studies with Chrysotile and Amphibole Asbestos in Hamsters[a]

Fiber Type	Exp Time h/d, d/wk max months	Exposure Concentration mg/m³	Fiber Concentration f/cm³	Total Number of Hamsters	Number Pulmonary Tumours	% Pulmonary Tumours Adenoma, Carcinomes	Number of Meso-Theliomas	References
Amosite	—	w.b. 300	ND	7	0	0	—	Lee et al., 1981
Amosite –low	6, 5, 18	n-o 0.8	36 > 5 μm 10 > 20 μm	83	0	0	3	Hesterberg et al., 1999; McConnell et al., 1999
Amosite –mid	6, 5, 18	n.o 3.7	165 > 5 μm 38 > 20 μm	85	0	0	22	Hesterberg et al., 1999; McConnell et al., 1999
Amosite –high	6, 5, 18	n-o 7.1	263 > 5 μm 69 > 20 μm	87	0	0	17	Hesterberg et al., 1999; McConnell et al., 1999
Crocidolite UICC	6, 5, 18	w.b. 7	3000 90 > 10 μm	58	0	0	0	Smith et al., 1987
Chrysotile NIEHS	6, 5, 18	n-o 10	1.02×10^5 $1.06 \times 10^4 > 5\mu m$	??	0	0	0	Mast et al., 1994

[a] Exposure types: w.b., whole body; n-o, nose only.

10 to 69 fibers/cm^3 (longer than 20 μm) and was chosen based on a previous, multidose 90-day subchronic inhalation toxicology study (Hesterberg et al., 1999).

27.3.4.1 Fiber Length

In an analysis that provided the basis for the European Commission's directive on synthetic mineral fibers, Bernstein et al. (2001a, 2001b) reported that an excellent correlation exists for SVF between the biopersistence of fibers longer than 20 μm and the pathological effects following either chronic inhalation or chronic intraperitoneal injection studies. This analysis showed that it was possible using the clearance half-time of the fibers longer than 20 μm as obtained from the inhalation biopersistence studies to predict the number of fibers longer than 20 μm remaining after 24 months of chronic inhalation exposure. These studies, however, only included synthetic mineral fibers.

As mentioned above, Berman et al. (1995) analyzed statistically nine different asbestos types in 13 separate studies. Because of limitations in the characterization of asbestos structures in the original studies, new exposure measures were developed from samples of the original dusts that were regenerated and analyzed by transmission electron microscopy. The authors reported that although no univariate model was found to provide an adequate description of the lung tumor responses in the inhalation studies, the measure most highly correlated with tumor incidence was the concentration of structures (fibers) greater than or equal to 20 μm in length. However, by using multivariate techniques, measures of exposure were identified that do adequately describe the lung tumor responses.

The potency appears to increase with increasing length, with structures (fibers) longer than 40 μm being about 500 times more potent than structures between 5 and 40 μm in length. Structures <5 μm in length do not appear to make any contribution to lung tumor risk. As discussed above, this analysis also did not find a difference in the potency of chrysotile and amphibole toward the induction of lung tumors.

27.3.4.2 Purity of the Samples

In most inhalation studies on both amphiboles and serpentines, there was no analytical confirmation reported that the fibers that were aerosolized were uniquely of the type stated.

In addition, an issue that has been discussed at length is whether the presence of tremolite in the chrysotile samples can account for some of its carcinogenic potential as well. This is especially pertinent to the mesotheliomas that have been observed in some of the rat inhalation studies (Churg, 1994; McDonald et al., 1999; Roggli et al., 2002). Using microscopic analysis, Frank et al. (1998) reported the absence of tremolite in the UICC chrysotile sample that has often been used in the chronic studies. However, when present with chrysotile, tremolite is usually found in very low concentrations that could be missed when using microscopic analysis.

To resolve this issue of method sensitivity, Addison and Davies (1990) developed a method of chemical digestion of chrysotile in which the chrysotile is dissolved away by using an acid digestion, leaving behind the amphiboles such as tremolite. This method was applied to a sample UICC chrysotile obtained from Dr. Fred Pooley who has a repository of the original UICC preparation. In conjunction with Gesellschaft fuer Schadstoffmessung und Auftragsanalytik GmbH (GSA, Neuss, Germany), 2.13 g of UICC chrysotile were digested in acid after a procedure similar to that of Addison and Davies (1990). Following digestion, the bivariate size distribution was determined for all residual fibers by transmission electron microscopy and the chemical composition of each fiber determined by energy-dispersive analysis of x-rays (EDAX) to clearly identify it as amphibole, chrysotile, or other.

In the 2-mg sample analyzed, the results indicated that there were 3400 tremolite fibers per mg of UICC chrysotile. These fibers ranged in length from 1.7 to 14.4 μm and had a mean diameter of 0.65 μm. Forty-one percent of the fibers were longer than 5 μm with 1394 WHO tremolite fibers per mg of UICC chrysotile. These results indicate that tremolite is present in the UICC sample at low concentrations. Because no dose–response studies have been performed at low amphibole concentrations, quantification of the effect of these fibers is not possible in the rat. However, as discussed above,

amphibole asbestos fibers are very biopersistent in the lung and will persist once inhaled. Davis et al. (1985) performed a chronic inhalation toxicity study on tremolite to determine the effect of commercial tremolite in comparison with other asbestos types. The authors reported that tremolite was the most dangerous mineral that they have studied, producing 16 carcinomas and 2 mesotheliomas in a group of 39 animals. As described above, even short exposure to tremolite produces a notable response in the lung. Bernstein et al. (2003b) reported that after a 5-d exposure to tremolite, a pronounced inflammatory response was observed with the rapid development of granulomas, collagen deposition within these granulomas, and, by 90 days, even mild interstitial fibrosis.

27.3.5 Epidemiology

Many studies have shown that chrysotile is not of the same potency as the amphiboles and is cleared from the lung more rapidly than amphibole (Howard, 1984; Churg and DePaoli, 1988; Mossman et al., 1990; Morgan, 1994; Churg, 1994; McDonald et al., 1995, 1997, 1999, 2002, 2003; McDonald, 1998; Rodelsperger et al., 1999; Hodgson and Darnton, 2000; Berman and Crump, 2003). Still, other studies have stated the opposite.

Two studies have provided a quantitative review of the potency of chrysotile and amphiboles based on the statistical analysis of currently available epidemiology studies.

Hodgson and Darnton (2000) conducted a comprehensive quantitative review of potency of asbestos for causing lung cancer and mesothelioma in relation to fiber type. They concluded that amosite and crocidolite were, respectively, on the order of 100 and 500 times more potent for causing mesothelioma than chrysotile. They regarded the evidence for lung cancer to be less clear cut, but concluded nevertheless that amphiboles (amosite and crocidolite) were between 10 and 50 times more potent for causing lung cancer than chrysotile.

Berman and Crump (2003) have recently reviewed and analyzed as part of a technical support document for the U.S. EPA an epidemiology database consisting of approximately 150 studies of which approximately 35 contained exposure data sufficient to derive quantitative exposure/response relationships.

Using this database of epidemiological studies, the authors determined estimates of lung cancer or mesothelioma from asbestos exposure with mathematical models that express risk as a function of exposure. The models contain parameters (K_L for lung cancer and K_M for mesothelioma) that gauge the potency of asbestos for causing these health effects. Using their reanalysis of this dataset, they determined that for disease in humans the important fibers are longer than 10 μm and thinner than 0.4 μm. The authors concluded that the corresponding coefficients for pure fiber types were, for lung cancer, 0.6 and 3 for chrysotile or amphibole, respectively, and for mesothelioma, 0.04 and 30 for chrysotile or amphibole, respectively. They also noted that, without adjustments for fiber size, the lung cancer exposure–response coefficients (K_L values) estimated from 15 studies vary by a factor of 72 and these values are mutually inconsistent (based on nonoverlap of uncertainty intervals). However, when the studies were adjusted for fiber size and type, the overall variation in K_L values across these studies was reduced to a factor of 50. Similarly, without adjustments, the mesothelioma exposure–response coefficients K_M values varied by a factor of 1089, and that these values are likewise mutually inconsistent. However, when the studies were adjusted for fiber size and type, the overall variation in K_M values was reduced to a factor of 30.

Direct comparison between these two analyses is difficult because the Hodgson and Darnton (2000) potency estimates are based on WHO fibers, whereas the Berman and Crump (2003) potency estimates are based on fibers longer than 10 μm and thinner than 0.4 μm, which they found as the determinant fibers for disease. Berman and Crump (2004) also stated in their report that, based on their review, the supporting literature suggests that the optimum cutoff for increased potency occurs at a length that is closer to 20 μm than to 10 μm (the latter of which is the cutoff in the exposure index provided in this study). However, they found that not enough data currently exist to improve quantitatively on this latter cutoff.

27.4 SUMMARY

The mineralogy of the serpentine chrysotile fibers and amphiboles fibers shows distinct differences in the structure and chemistry of these two minerals. In contrast to the curled layered construction of the sheet silicate chrysotile, which appears to result in greater susceptibility to degradation, the amphibole fibers are rigid impermeable structures that are resistant to degradation. These differences are reflected in the inhalation biopersistence studies that clearly differentiate chrysotile from the amphiboles and show that longer chrysotile fibers rapidly disintegrate in the lung whereas the longer amphiboles, once deposited, remain. There is an excellent correlation between the biopersistence of the longer synthetic vitreous fibers and chronic toxicity data. Because of the difficulties in study design and the large particle/fiber exposure concentrations used, the chronic inhalation studies with asbestos are difficult to interpret due in part to the nonspecific effects of the very large particle concentrations in the exposure aerosols.

Recent quantitative reviews that analyzed the data of available epidemiological studies to determine potency of asbestos for causing lung cancer and mesothelioma in relation to fiber type also differentiated between chrysotile and amphibole asbestos. The most recent analysis also concluded that it is the longer, thinner fibers that have the greatest potency. The quantitative experimental results provide additional support for this differentiation.

There is no question that amphibole asbestos is highly carcinogenic. Both animal studies and epidemiology studies indicate the potency of amphibole asbestos. Recent studies on tremolite show that even short exposure can produce a pathogenic response in the lung.

With chrysotile asbestos, indeed, there is evidence that humans can and do develop lung cancer when the exposure is high and sustained for long periods. The weight of evidence suggests that at low exposure pure chrysotile is probably not hazardous. It also suggests that the hazard may be low if even high exposures were of short duration.

It would be most helpful if future studies on chrysotile and amphiboles, whether *in vitro* or *in vivo*, could be performed using size distributions and at doses approaching those to which humans have been exposed.

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