Understanding Chrysotile Asbestos: A New Perspective Based Upon Current Data.

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ABSTRACT: Recent publications have shown for synthetic mineral fibers that if a fiber dissolves rapidly and disappears from the lung, it does not cause a carcinogenic effect. With asbestos, chrysotile asbestos is often included with other asbestos materials. However, chrysotile is a serpentine mineral with markedly different mineralogical characteristics than amphibole asbestos (e.g. amosite, tremolite). These differences are mirrored in the differences in biopersistence between these two minerals. Chrysotile clears very rapidly from the lung with half-times ranging from 0.3 to 11 days and produces no inflammatory reaction. In contrast, the amphiboles clear with half-times in the range of 500 days or longer and produce a pronounced inflammatory response leading to mild interstitial fibrosis. These findings provide an important basis for substantiating both kinetically and pathologically the differences between chrysotile and amphiboles. In contrast to amphiboles, the toxicology of chrysotile can be understood in comparison to non-fibrous mineral dusts. These results fully support the differentiation of chrysotile from amphiboles reported in recent evaluations of available epidemiological studies.

Recent publications have clearly shown for synthetic mineral fibers the relationship of biopersistence to both chronic inhalation toxicity and chronic intraperitoneal injection tumour response in the rat. In essence, if a fiber dissolves rapidly and disappears from the lung, it does not cause a carcinogenic effect. This concept was incorporated in 1997 into the European Commissions Directive (regulation) on man made mineral fibers.

Asbestos fibers certainly are more controversial than synthetic mineral fiber. With asbestos, chrysotile asbestos is often included with other asbestos materials in evaluation and classification. However, chrysotile is a serpentine mineral with markedly different mineralogical characteristics than most other asbestos which are amphibole minerals (e.g. crocidolite, amosite, tremolite). In contrast to amphiboles which are solid rod-like fibers which usually only break transversally, chrysotile is composed like a rope of many fine fibrils which tend to unwind.

Chrysotile asbestos, which is of the serpentine mineral group, has been shown to be rapidly removed from the lung following inhalation (Bernstein et al., 2003a, 2003b, 2004, 2005a, 2005b). As serpentine is a naturally occurring mined fiber, there appears to be some slight differences in biopersistence depending on the commercial grade tested. 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 stone wools. It remains less biopersistent than ceramic and special-purpose glasses (Hesterberg et al., 1998) and more than 50 times less biopersistent than amphiboles.

In composition, the amphiboles such as amosite and crocidolite both have molecular structures in which iron comprises 25% to 36% by weight (Hodgson, 1979). The serpentine chrysotile has little iron and usually is found with 1% to 5% iron by weight (Skinner et al., 1988).

In addition, while magnesium is an important part of both chrysotile (~33%) and amphiboles (6–25%), in chrysotile the magnesium molecule is on the outside of the curled chrysotile structure. This is of particular importance in that magnesium is soluble in the lung fluids and can be readily leached from the surface. With amphiboles, the magnesium is locked within the I-beam type structure, which consists of corner-linked $(SiO_4)^4$ tetrahedra linked together in a double-

tetrahedral chain that sandwiches a layer with the Ca_2Mg_5.

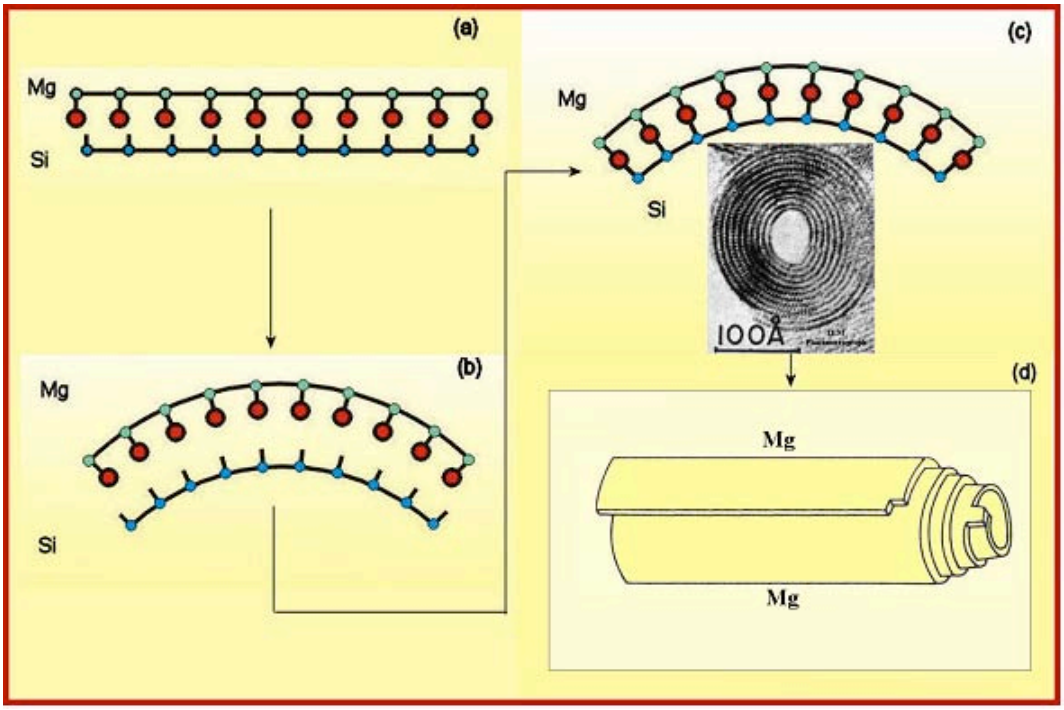
Fibrous chrysotile has been shown as well to be acid soluble in contrast to the amphiboles. Hargreaves and Taylor (1946) reported that if fibrous chrysotile is treated with dilute acid the magnesia can be completely removed. The hydrated silica which remains, though fibrous in form, had completely lost 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. This difference in characteristics is also important in the lung, where the macrophage is capable of generating a milieu at a pH of ~4.5.

Wypych et al. (2005) recently examined what happends to natural chrysotile fibers and pegmatitic phlogopite when they were acid-leached under controlled conditions. Extensive effort was put into the characterization of the materials using powder X-ray diffraction, Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, 29Si nuclear magnetic resonance, transmission electron microscopy, and selected area electron diffraction. The authors reported that the leached products derived of the two clays were similar, consisting of layered hydrated disordered silica with a "distorted" structure resembling the silicate layer existing in the original minerals. All characterization techniques confirm the removal of the brucite-like sheets, leaving silica with an eminently amorphous structure.

Chrysotile is also decomposed by water. Reimschussel (1969) studied the extraction of chrysotile with boiling water. He reported that after an initial rapid reaction, that magnesium and silica are removed from the chrysotile in amounts proportional to the chrysotile concentration. Speil & Leineweber (1969) reported that these results show that there is no doubt that chrysotile is slowly soluble in water under conditions of continuous extraction.

Most important, however, is that chrysotile is a sheet silicate. That is instead of forming in rods (fibers) as do the amphiboles, it forms in thin sheets. These sheets, however, have a mineral composition such that the spacing between the magnesium ions is greater than that between the silica ions. This mismatch in spacing causes the chrysotile to curl into effectively a rolled fiber. This is illustrated in Figure 1.

Figure 1 Structural formation of the sheet silica chrysotile asbestos



(Reproduced from Bernstein et al., 2005b)

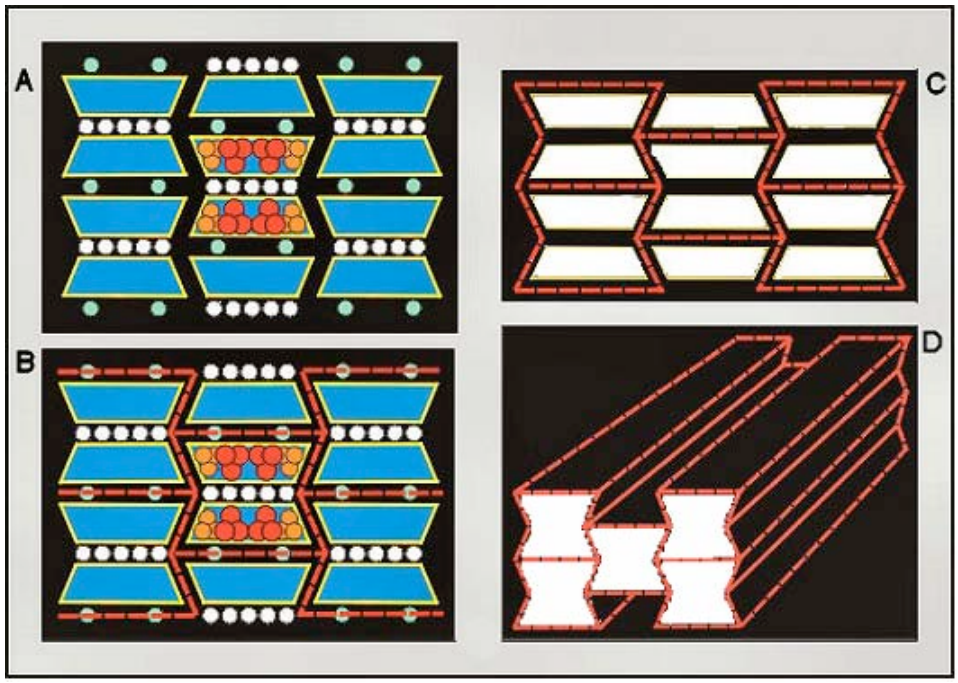
Amphiboles, such as tremolite, are double-chain silicates. Figure 2A shows the amphiboles schematically with the slice directly across the chains.

Each of the blue boxes represents a double chain of tetrahedral structures (SiO_2). (They are illustrated in the middle chains.) With tremolite, the white and green circles represent the magnesium and calcium cations that effectively glue one chain to its neighbour.

Fewer shared cations bond the chains together along the broad sides of the chains compared to the narrow sides resulting in these broad surfaces being bonded less strongly. As shown in Figure 2B 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 2C simplifies the picture and shows that the double-chain silicates can break into a set of fragments with potentially regular shape.

Figure 2D shows the same situation in 3 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 to the bonds gluing one chain to the next.

Figure 2 Structural formation of the double chain silica tremolite asbestos



(Reproduced from Bernstein et al., 2005b)

FIBER BIOPERSISTENCE:

A fiber is unique among inhaled particles in that the fibers aerodynamic diameter 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 which occurs for non-fibrous particles. Within the lung, fibers which can be fully engulfed by the macrophage can be removed as with any other particle. However, those fibers which 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 not different than non-fibrous particles and are cleared with similar kinetics and mechanism as particles. While longer fibers may also be cleared effectively by the macrophage and as a result not be different kinetically than particles, the 5 µm cut-off was chosen to mirror the use by the WHO of a 5 µm cut-off in their counting schemes for fibers. 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 which are

cleared as particles and the longer fibers that the macrophage can not fully phagocytise. The actual limit as to what length fiber can be fully phagocytised 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 (SVF) 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&b; Hesterberg et al. 1998a&b). The value of 20 µm is used as an index for fibers that can not be fully phagocytised and cleared by the macrophages. The protocol used in these biopersistence studies was developed by a working group for the European Commission and involves a 5 day inhalation exposure followed by analysis of the lungs at periodic intervals up to 1 year post exposure (Bernstein & 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 1. Highlighted in this table are those studies performed on chrysotile and amphiboles using 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, as serpentine is a naturally occurring mined fiber, there appears to be some differences in biopersistence depending upon from where it is 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.

Table 1: Comparative clearance half-times of fibers longer than 20 µm and fibers between 5-20 µm for chrysotile, synthetic vitreous fibers and amphiboles.

FIBER	TYPE	CLEARANCE HALF-TIME ($T_{1/2}$) (days)		REFERENCE
		FIBERS LENGTH >20 µm	FIBERS LENGTH 5 – 18 µm	
Calidria chrysotile	**Serpentine asbestos**	**0.3**	7	**Bernstein et al.,2005b**
Brazilian chrysotile	**Serpentine asbestos**	**1.3**	**2.4**	**Bernstein et al., 2004**
Fiber B (B01.9)	Experimental Glass wool	**2.4**	11	Bernstein et al., 1996
Fiber A	Glass wool	**3.5**	16	Bernstein et al., 1996
Fiber C	Glass wool	**4.1**	15	Bernstein et al., 1996
Fiber G	Stone wool	**5.4**	23	Bernstein et al., 1996
MMVF34 (HT)	Stone wool	**6**	25*	Hesterberg et al, 1998
MMVF22	Slag wool	**8.1**	77	Bernstein et al., 1996
Fiber F	Stone wool	**8.5**	28	Bernstein et al., 1996
MMVF11	Glass wool	**8.7**	42	Bernstein et al., 1996
Fiber J (X607)	Calcium magnesium silicate	**9.8**	24	Bernstein et al., 1996
Canadian chrysotile (Textile grade)	**Serpentine asbestos**	**11.4**	**29.7**	**Bernstein et al., 2005a**
MMVF 11	Glass wool	**13**	32	Bernstein et al., 1996

Fiber H	Stone wool	**13**	27	Bernstein et al., 1996
MMVF10	Glass wool	**39**	80	Bernstein et al., 1996
Fiber L	Stone wool	**45**	57	Bernstein et al., 1996
MMVF21	Stone wool	**46**	99	Bernstein et al., 1996
MMVF33	Special purpose glass	**49**	72*	Hesterberg et al, 1998
RCF1a	Refractory ceramic	**55**	59*	Hesterberg et al, 1998
MMVF21	Stone wool	**67**	70*	Hesterberg et al, 1998
MMVF32	Special purpose glass	**79**	59*	Hesterberg et al, 1998
Amosite	**Amphibole asbestos**	**418**	**900***	**Hesterberg et al, 1998**
Crocidolite	**Amphibole asbestos**	**536**	**262**	**Bernstein et al., 1996**
Tremolite	**Amphibole asbestos**	∞	∞	**Bernstein et al.,2005b**

- The $T_{1/2}$ for fibers 5-20 µm in length was not reported by Hesterberg et al. (1998); the values shown were calculated from the raw data by D. Bernstein.

CHRONIC INHALATION TOXICOLOGY STUDIES:

The difficulty of designing chronic inhalation toxicology studies with fibers:

While many chronic inhalation toxicology studies of fibers ranging from amphibole asbestos, to soluble glass fibers and to organic fibers have been performed their design and subsequent interpretation are often confounded by the fiber size distribution and the ratio of longer fibers to shorter fibers and non-fibrous particles. In some of these studies the exposures often approach and exceed that which has been shown to produce what is now termed 'lung overload' in the rat. Thus, it can become very difficult to compare the effects of such a study with those of another.

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).

This is illustrated in the following Table 2 which shows for the a number of studies performed with similar protocols the exposure concentrations and lung burdens for a series of synthetic vitreous fibers and serpentine and amphibole asbestos. The asbestos exposures in these studies were included as positive controls, however, as shown in Table 2, it would be very difficult to compare the chrysotile exposure and even the crocidolite exposure to those of the SVF on a comparative fiber number basis. The exposure concentration and resulting lung burden of chrysotile was so large that it is very likely that a lung overload effect did occur. It would have been much more useful and interesting in these studies if the exposure concentrations and fiber size distributions were comparable between the positive controls and the SVFs.

In a recent study, Bellmann et al. (2003) reported on a calibration study to evaluate the endpoints in a 90-day 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 had a large concentration of particulate material in addition to the fibers. After chronic inhalation of the fiber X607, which is similar to CMS but which had considerably fewer particles present, no lung tumours or fibrosis were detected (Hesterberg et al., 1998b). In the Bellmann et al., 90-day study, due to the method of preparation the aerosol exposure concentrations for the CMS fiber had 286 fibers/cm^3 length < 5µm, 990 fibers/cm^3 length > 5µm, and 1793 particles/cm^3, a distribution which is not observed in manufacturing. The total CMS exposure concentration was 3,069 particles & fibers/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." For the CMS fiber, the authors reported that the number of polymorphonuclear leukocytes (PMN) 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, the interstitial fibrosis persisted through 14 weeks after cessation of the 90-day exposure. This effect

attributed to particles was observed with an exposure concentration of 3,069 particles & fibers/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 at higher exposure concentrations.

Even within the SVF exposures, as the long fibers are the most important in terms of potential toxicity, in the high dose exposures, the number of fibers longer than 20 µm/cm^3 varies from 84, 114, 99 to 101. Thus while the studies are relatively comparable, they may not be quantitatively. However, in consideration of the percentage of long fibers in the total aerosol, and the fact that the rat respirable fibers were pre-selected from the commercial bulk fiber and represented less than 2 % of the commercial product, these studies are still remarkable in the uniformity of exposures.

These discrepancies in study design put in question the value in terms of comparative analysis of especially the chrysotile study listed in Table 2 and perhaps as well the crocidolite study. McConnell, et al. (1999) reported on perhaps the only well designed multiple-dose study on any asbestos where amosite particle and fiber number and length chosen to be comparable to the SVF exposure groups. In this hamster inhalation toxicology study the amosite aerosol concentration ranged from 10 to 69 f/cm^3 longer than 20 µm and where chosen based upon a previous, multi-dose 90-day subchronic (Hesterberg et al., 1999).

Table 2: Fiber exposure and lung burden data for a series of fiber inhalation toxicology studies performed using a similar protocol.

FIBER TYPE	AEROSOL TOTAL (fibers/ cm^3)	AEROSOL WHO* (fibers/ cm^3)	AEROSOL NUMBER (fibers/cm^3 length > 20 µm)	LUNG TOTAL FIBER NUMBER (in lung after 24 months of exposure)	LUNG NO FIBERS L>20 µm (In lung after 24 months of exposure)	REFERENCE
MMVF11***	na	41	14	93,000,000	5,580,000	Hesterberg et al., 1993
MMVF11***	na	153	50	692,000,000	24,912,000	Hesterberg et al., 1993
MMVF11***	273	246	84	1,284,000,000	30,816,000	Hesterberg et al., 1993
MMVF21**	44	34	13	112,142,857	14,130,000	McConnell et al., 1994
MMVF21**	185	150	74	548,173,913	50,432,000	McConnell et al., 1994
MMVF21**	264	243	114	622,884,615	80,975,000	McConnell et al., 1994
MMVF22**	33	30	10	21,984,733	2,880,000	McConnell et al., 1994
MMVF22**	158	131	50	320,625,000	7,695,000	McConnell et al., 1994
MMVF22**	245	213	99	596,750,000	23,870,000	McConnell et al., 1994
RCF1***	36	26	13	99,900,000	12,787,200	Mast et al., 1994
RCF1***	91	75	35	233,120,000	31,238,080	Mast et al., 1994
RCF1***	162	120	58	578,240,000	58,402,240	Mast et al., 1994
RCF1***	234	187	101	1,017,500,000	132,275,000	Mast et al., 1994
Chrysotile	**102,000**	**10,600**	**na**	**54,810,000,000**	**na**	**Hesterberg et al., 1993**
Crocidolite(26w)	4,214	1,610	236	2,025,000,000	88,452,000	McConnell et al., 1994

* WHO Fibers: Fibers with length>5µm, a width<3µm; and an aspect ratio>3:1 (WHO, 1985 & NIOSH, 1994).

** The total number of fibers per lung was not reported in the publications and was calculated based upon counting data provided by Owens-Corning.

*** The number of fibers L>20 μm per lung was not reported in the publications and was calculated based upon counting data provided by Owens-Corning.

na: Data not available

CHRYSOTILE: FIBER OR PARTICLE EFFECTS

The rapid clearance of the long chrysotile fibers, that is those fibers which can not be effectively cleared by macrophages from the lung, provides an indication of what may happen when chrysotile is inhaled. While synthetic vitreous fibers (SVF) may dissolve congruently or incongruently (ref) chrysotile fibers appear to break apart into small particles and smaller fibers.

Kamstrup (2002) described possible mechanisms that could account for the rapid clearance half-time of the long HT fibers. He 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 nonporous 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 leads to the clearance/disintegration of the long chrysotile fibers. Wypych et al. (2005) showed that when chrysotile fibers were acid leached, the brucite-like sheets (magnesium) were removed leaving silica with "an eminently amorphous structure". That is, what remains are small glass particles. So in fact what appears to happen to the chrysotile fibers after inhalation is that the macrophages attack the fibers, and then the fibers effectively fall apart.

This provides a basis for understanding the potential toxicity of chrysotile. Chrysotile does not act as a fiber but rather as a particle. The rapid disintegration of the chrysotile fibers results in exposure to a larger number of amorphous silica particles and shorter fibers. Amorphous silica has been categorized by IARC as Category 3 (IARC, volumes 42 and 68). This is illustrated in Table 2 where the high chrysotile exposure results in a huge number of particles/fibers in the lung most of them smaller than 5 μm in length. Like any mineral dust at high exposure concentrations, there is the potential for producing disease and eventually cancer with sufficiently high and long exposure with effectively lung overload conditions. This is what the animal inhalation toxicity studies of chrysotile have clearly demonstrated.

However, chrysotile at lower exposures levels leads to levels of exposure to amorphous silica particles which the lung can handle. This was illustrated following a 5 day short term exposure to chrysotile in which no inflammatory response was observed in the lung (Bersntein et al., 2003 & 2005). In contrast, a 5-day exposure to one-half the number of long fibers of tremolite produced marked inflammation, granulomas and even mild interstitial fibrosis.

In Figure 3, Wypych et al. (2005, Figure 4) showed how the chrysotile fibers were found to break apart into amorphous silica particles. It is interesting to compare this with Figure 4, which shows the confocal microscopy photomicrographs presented by Bernstein et al. (2004, Figure 10) of the remaining chrysotile in the lung at 6 and 12 months following a 5-day exposure to Brazilian chrysotile. As seen in Figure 4, what remains after the breakdown of the long fibers are short fibers and particles.

Figure 3: Reproduced from Wypych et al. (2005, Figure 4).
A) Chrysotile fibers before acid leaching. B) Silica derived from chrysotile after acid leaching.

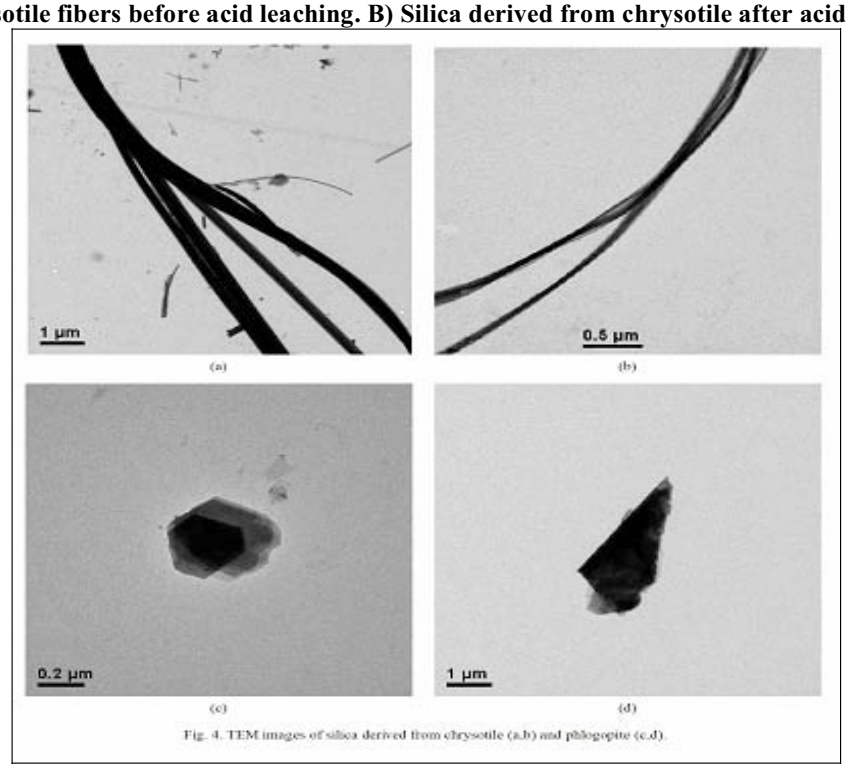


Figure 4: Reproduced from Bernstein et al. (2004, Figure 10) Confocal micrographs showing chrysotile particles (white arrows) at 6 & 12 months post exposure (inhalation biopersistence)

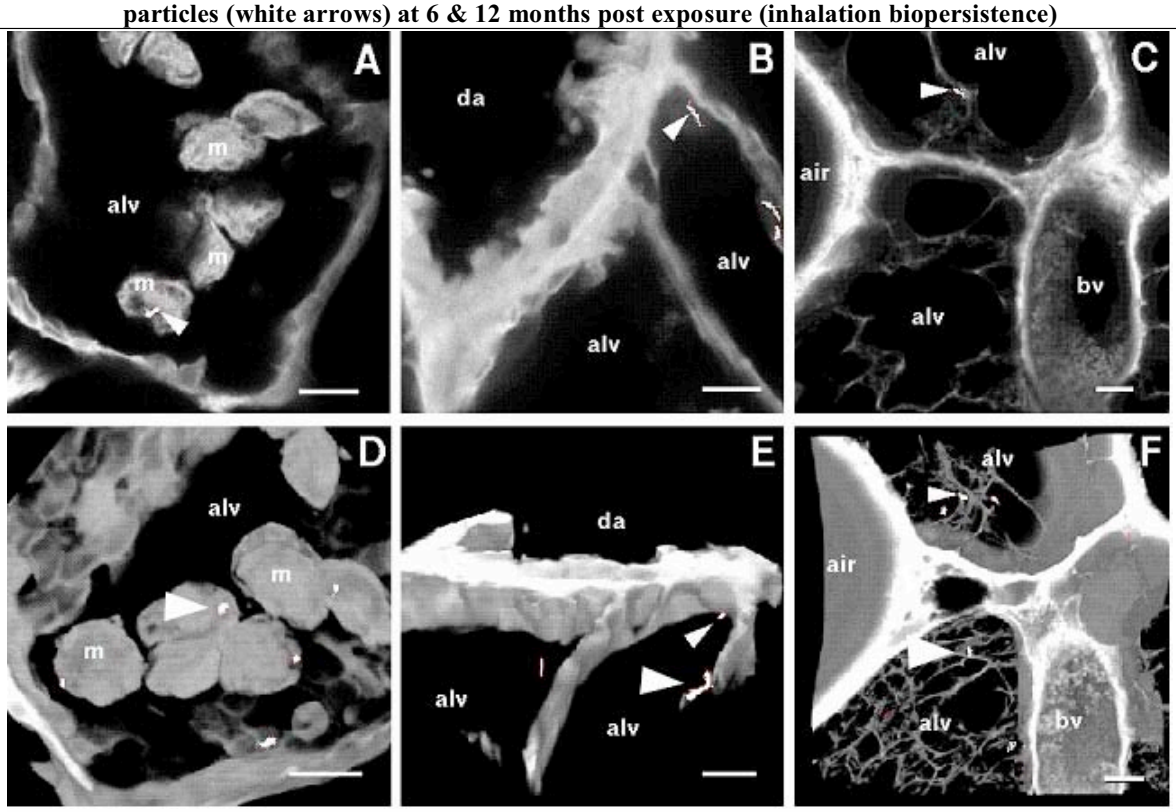


FIG. 10. Confocal micrographs of the lung at 6 mo (plates A–D and B–E) and at 12 mo (plates C and F) after cessation of exposure. (A), (B), (C) show an original single-scan image of the region. (D), (E), (F) show the reconstructed three-dimensional images of the same regions, which are composed of 70 serial sections. The length bars for (A), (B), (C), and (E) are 10 μm. For (C) and (F) the length bars are 50 μm.

CONCLUSIONS:

These findings provide an important basis for substantiating both kinetically and pathologically the differences between chrysotile and amphiboles. The toxicology of chrysotile can best be understood in comparison to other non-fibrous minerals, while that of the amphiboles is clearly a response to the insoluble fibrous structure of this mineral.

Recent quantitative reviews of epidemiological studies of mineral fibers have determined the potency of chrysotile and amphibole asbestos for causing lung cancer and mesothelioma in relation to fiber type also differentiated between these two minerals (Hodgson & Darnton, 2000; Berman & Crump, 2003). The most recent analyses also concluded that it is the longer, thinner fibers that have the greatest potency.

Like other mineral dusts, there is evidence that humans can and do develop lung cancer from exposure to chrysotile, when the exposure is high and sustained for long periods. The value of this and other similar studies is that it shows 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.

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