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The health effects of chrysotile: Current perspective based upon recent data ☆

David M. Bernstein [a,*], John A. Hoskins [b]

[a] *Consultant in Toxicology, Geneva, Switzerland*
[b] *Consultant in Toxicology, Haslemere, UK*

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Abstract

This review substantiates kinetically and pathologically the differences between chrysotile and amphiboles. The serpentine chrysotile is a thin walled sheet silicate while the amphiboles are double-chain silicates. These different chemistries result in chrysotile clearing very rapidly from the lung ($T_{1/2} = 0.3$ to 11 days) while amphiboles are among the slowest clearing fibers known ($T_{1/2} = 500$ days to ∞). Across the range of mineral fiber solubilities chrysotile lies towards the soluble end of the scale. Chronic inhalation toxicity studies with chrysotile in animals have unfortunately been performed at very high exposure concentrations resulting in lung overload. Consequently their relevance to human exposures is extremely limited. Chrysotile following subchronic inhalation at a mean exposure of 76 fibers $L > 20$ μm/cm^3 (3413 total fibers/cm^3) resulted in no fibrosis (Wagner score 1.8–2.6), at any time point and no difference with controls in BrdU response or biochemical and cellular parameters. The long chrysotile fibers were observed to break apart into small particles and smaller fibers. Toxicologically, chrysotile which rapidly falls apart in the lung behaves more like non-fibrous mineral dusts while response to amphibole asbestos reflects its insoluble fibrous structure. 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 have also differentiated between these two minerals. The most recent analyses also concluded that it is the longer, thinner fibers that have the greatest potency as has been reported in animal inhalation toxicology studies. However, one of the major difficulties in interpreting these studies is that the original exposure estimates rarely differentiated between chrysotile and amphiboles. Not unlike some other respirable particulates, to which humans are, or have been heavily occupationally exposed, there is evidence that heavy and prolonged exposure to chrysotile can produce lung cancer. The value of the present and other similar studies is that they show that low exposures to pure chrysotile do not present a detectable risk to health. Since total dose over time decides the likelihood of disease occurrence and progression, they also suggest that the risk of an adverse outcome may be low if even any high exposures experienced were of short duration.

Keywords: Chrysotile; Serpentine; Amphiboles; Asbestos; Biopersistence; Carcinogenicity; Epidemiology; Fiber

1. Introduction

Chrysotile asbestos is often included with other asbestos minerals in evaluation and classification. 'Asbestos' is not a mineral in itself. It is a collective term given to a group of minerals whose crystals occur in fibrous forms. The term 'asbestos' was adopted for the purposes of commercial identification alone.

The six minerals commonly referred to as asbestos come from two groups of minerals known as serpentines (chrysotile, *white asbestos*) and amphiboles (amosite, *brown asbestos*; crocidolite, *blue asbestos*; anthophyllite; tremolite; and actinolite). While they are all silicate minerals, the two groups are chemically and mineralogically distinct. In particular, their mineralogical structures are remarkably different and result in a notable difference in the manner they are processed by the lung once inhaled. Today, only one

☆ This study was supported by grants from the Government of Québec and The Asbestos Institute, Montréal, QC, Canada.
* Corresponding author. Fax: +41227351463.
E-mail address: davidb@itox.ch (D.M. Bernstein).

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mineral chrysotile, or white asbestos, is currently mined in any quantity. This serpentine mineral has always been the principal asbestos of commerce.

This review provides a systematic analysis and assessment of the available mineralogical, toxicological, and epidemiological data on those studies which differentiate the serpentine mineral chrysotile from amphiboles.

2. Mineralogical structure chemistry of chrysotile and amphibole

Chemically all of the asbestos minerals are silicates but mineralogically and crystallographically the serpentine and amphibole groups are quite different (Deer et al., 1966).

2.1. Chrysotile

Chrysotile is a sheet silicate and instead of forming into rods (fibers) as do the amphiboles, the mismatch in spacing between the magnesium ions and the silica ions causes chrysotile to curl into effectively a thin rolled sheet (Fig. 1A).

When chrysotile fibres are disaggregated as happens during milling, other comminution or just admixture with water, the chrysotile fibre structure breaks down to produce separated unit fibrils.

The external surface of a chrysotile fibril is the magnesium mineral brucite. 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 had a structure that was "amorphous" or "glassy" in type. Wypych et al. (2005) recently examined what happens to natural chrysotile fibers when acid-leached under controlled conditions. The authors reported that the leached products consisted of layered hydrated disordered silica with a "distorted" structure resembling the silicate layer existing in the original minerals. Extensive characterization techniques confirmed the removal of the brucite-like sheets, leaving silica with an eminently amorphous structure.

Removal of magnesium from the brucite layer by acid weakens the chrysotile fibrils and eventually destroys their dimensional stability. The sensitivity of chrysotile to acid dissolution is particularly important in the lung where the macrophages in the lung are capable of generating a milieu at a pH of ~4.5 (Fig. 1B). Chrysotile fibers which are cleared from the lung and swallowed would be readily attacked by the hydrochloric acid in the stomach which keeps the lumen of that organ below pH 2.

2.2. Amphiboles

The chemical composition of the amphiboles fibers is more complex and the idealized chemical formulae of the five

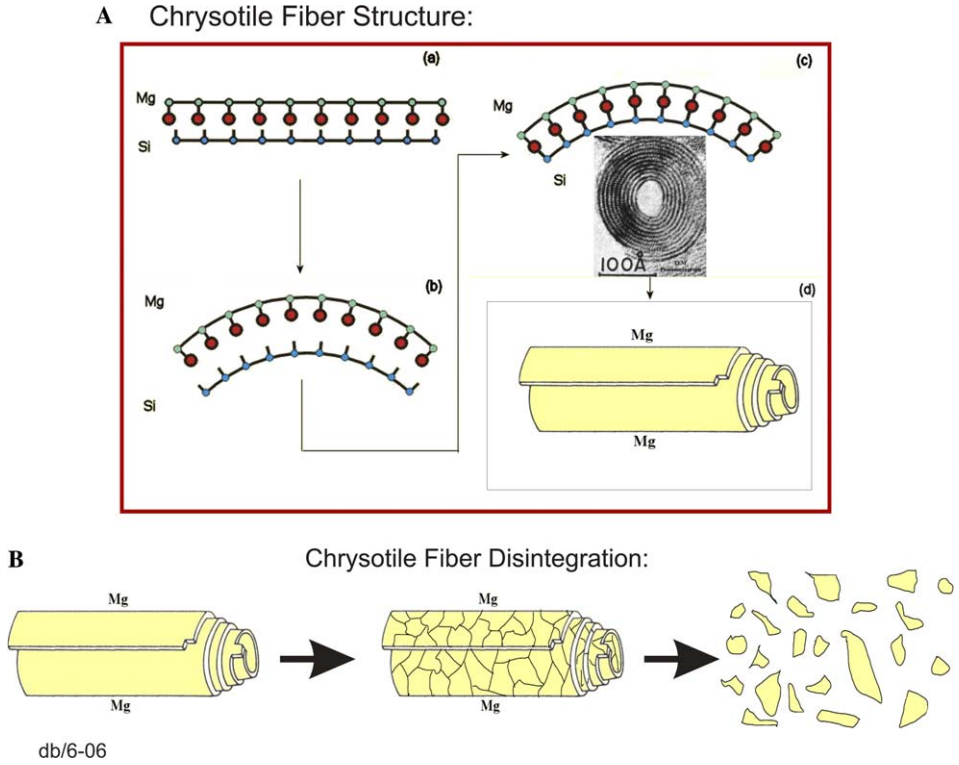

Fig. 1. A: Chrysotile Fiber Structure: Schematic representation of the structural formation of the sheet silica chrysotile asbestos showing the positioning of the Mg molecule on the outside of the curl. (a) The relative spacing of the Mg and Si sheets. (b) Illustrates how the sheets have to curl in order for the Mg and Si atoms to line up. (c) and (d) Show how this results in the chrysotile fiber being formed as a rolled thin sheet (~8 angstroms thick). (adopted with permission from: http://academic.brooklyn.cuny.edu/geology/powell/core_asbestos/asbestoshome.htm). B: Chrysotile Fiber Disintegration: The magnesium is dissolved at neutral pH and the silica matrix is broken up at acid pH (Pundsack, 1955; Wypych, 2005).

amphiboles are shown below. Although their structures are the same this variability in composition is a direct consequence of the fact that the silicate framework can accommodate a mixture of many different ions (as determined by the host rock) in the space between the silicate ribbons which form the fibers (Speil and Leineweber, 1969).

Crocidolite…$(Na_2Fe_3^{2+}Fe_2^{3+})\ Si_8O_{22}(OH)_2$
Amosite…$(Fe^{2+}, Mg)_7\ Si_8O_{22}(OH)_2$
Tremolite…$Ca_2Mg_5\ Si_8O_{22}(OH)_2$
Anthophyllite…$(Mg, Fe^{2+})_7\ Si_8O_{22}(OH)_2$
Actinolite…$Ca_2(Mg, Fe^{2+})_5\ Si_8O_{22}(OH)_2$

The external surface of the crystal structures of the amphiboles is quartz-like, and has the chemical resistance of quartz. This structure is illustrated for tremolite in Figs. 2(A,B).

Each of the blue fiber in Fig. 2A represents a double chain of tetrahedral silicate structures. With tremolite, the orange spheres represent the magnesium and calcium cations that effectively 'glue' one fiber chain to its neighbour. The chains bond the fibers weakly and it is along these surfaces that the mineral will likely break. This is illustrated in Fig. 2B which shows that the double-chain silicates can break into a set of fragments with fibrous shape. In the lung, the weak bonds holding the individual fibers together would quickly dissolve, however, the amphibole fibers themselves would not dissolve either at neutral or acid pH.

2.3. 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 endpoints have not been validated as screening assays that are predictive of long-term pathological effects in vivo. While 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 fiber types (ILSI, 2005).

2.4. Biopersistence

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 (Bernstein et al., 2001a,b). In essence, if the longer fibers which the macrophage cannot fully engulf dissolve or break rapidly and disappear from the lung, they do not cause a carcinogenic effect. This concept was incorporated, in 1997, into the European Commissions Directive on man made mineral fibers (European Commission, 1997).

Chrysotile has been shown to be rapidly removed from the lung following inhalation in experimental animals (Bernstein et al., 2003a,b, 2004, 2005a,b). In addition, lung analysis of workers exposed to predominately chrysotile

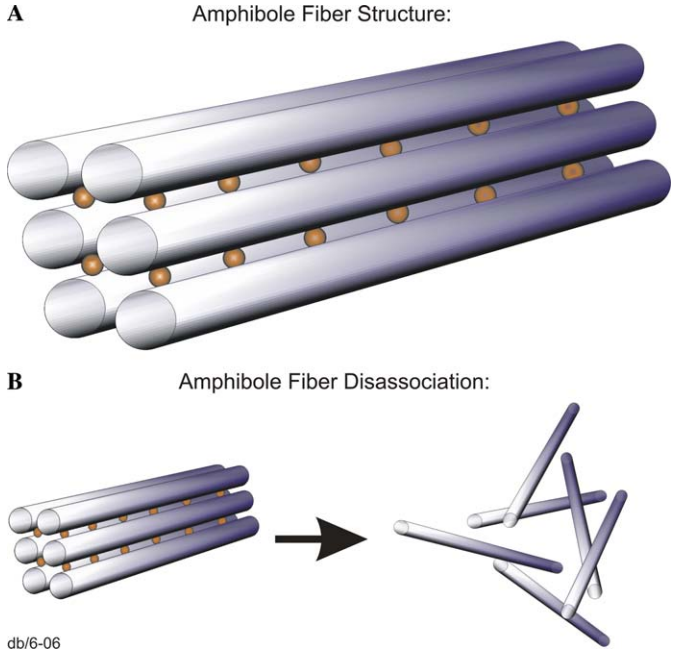

Fig. 2. A: Amphibole Fiber Structure: The structural formation of the double-chain silica tremolite asbestos is illustrated. The amphibole fibers are weakly bonded by the magnesium cations shown here as orange spheres between the fibers which are double chain tetrahedrals (SiO_2). B: Amphibole Fiber Disassociation: It is along these weakly bonded surfaces that the mineral will most likely break apart. With tremolite, these weak bonds are associated with the Mg. At neutral pH the Mg would dissolve releasing individual fibers. These fibers are highly resistant to either neutral or acid dissolution.

show low levels of chrysotile compared to amphiboles (Albin et al., 1994) even when amphibole exposure was only a trace impurity (Rowlands et al., 1982).

As chrysotile is a naturally occurring mined fiber, it is not surprising that there are some slight differences in biopersistence depending on the origin and commercial grade tested. However, across the range of mineral fiber solubilities chrysotile lies towards the soluble end of the scale and ranges from the least biopersistent fiber to a fiber with biopersistence in the range of glass and stone wools. It is less biopersistent than the ceramic fibers tested or the special-purpose glasses (Hesterberg et al., 1998a) and considerably less biopersistent than amphiboles.

2.4.1. Biopersistence of fiber structure

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 cannot be cleared by this route.

Fibers less than 5 μm in length are effectively not different from non-fibrous particles and are cleared with similar kinetics and mechanism as isomorphous particles. While longer fibers may also be cleared effectively if the macrophage can fully phagocytise them, 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 the shorter fibers 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 cannot 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; Zeidler-Erdely et al., 2006). The value of 20 μm has been used in animal studies as an index for fibers that cannot be fully phagocytised and cleared by the macrophages.

In the lung, modelling 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). 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.

To standardise the evaluation of the biopersistence of fibers a protocol has been developed by a working group for the European Commission which involves a 5 day inhalation exposure followed by analysis of the lungs at periodic intervals up to 1 year post-exposure (Bernstein and Riego-Sintes, 1999). For mineral fibers, the clearance half-time of fibers longer than 20 μm ranges from a few days to more than 100 days (Table 1).

The chrysotile fiber is physically a very thin rolled sheet. This thin sheet is much more fragile than the silicate double chains of the amphiboles and can break. The brucite (Mg^{2+}) layer can dissolve in water or the lung fluid, and the remaining structure is attacked in an acid environment such as is encountered with the macrophage. Deterioration of the chrysotile surface can result in the loss of the structural integrity of the fiber and disintegration.

Earlier studies have shown chrysotile to clear less rapidly (e.g., Coin et al., 1992; Kauffer et al., 1987). In the Coin et al. (1992) study, the NIEHS chrysotile used was derived from a chrysotile product called Plastibest-20 which was a chrysotile used in the plastics industry. The sample was ground three times using a "hurricane pulveriser" which is a commercial device designed to grind material under steel. In the Coin et al studies, an exposure concentration of 10 mg/m^3 was used. Although not stated in the publications, due to the extensive grinding of the Plastibest-20, many more short fibers would be expected. As presented below, in chronic studies at a concentration of 10 mg/m^3, this exposure meets the criteria presented by Oberdörster (2002) for lung overload. In the Kauffer et al. (1987) study, a single mass concentration of 5 mg/m^3 UICC chrysotile was reported with no indication of the fiber number or distribution in the aerosol. In this study, the aerosol was generated by a fluidized bed generator which would preferentially aerosolise the shorter/lighter fibers, thus it is possible that a similarly high number of total particles/fibers were present in the aerosol. The authors reported that this was "a level sufficiently high to produce cell responses in BAL fluids, and in which lung fibres were examined for their diameter and length distribution at different post-exposure times, suggests that lung fibres may be progressively separated into individual fibrils and that their number can increase in the lung." The fact that macrophage mediated clearance could not remove the shorter fibers suggests as well that "lung overload" could have occurred in this study as well. Besides the biopersistence studies cited above, there have been no other studies examining the clearance of commercial chrysotile products at exposure concentrations even a few orders of magnitude higher than the concentrations found in the workplace.

2.5. Chronic inhalation toxicology studies

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 present in the exposure aerosol. It is now recognised that high concentrations of insoluble nuisance dusts will compromise the clearance mechanisms of the lung, cause inflammation and a tumorigenic response in the rat, phenomena which taken together

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

Fiber	Type	Clearance half-time ($T_{1/2}$) (days)		Reference
		Fibers length >20μm	Fibers length 5–20 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[a]	Hesterberg et al. (1998a)
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[a]	Hesterberg et al. (1998a)
RCF1a	Refractory ceramic	55	59[a]	Hesterberg et al. (1998a)
MMVF21	Stone wool	67	70[a]	Hesterberg et al., 1998a
MMVF32	Special-purpose glass	79	59[a]	Hesterberg et al. (1998a)
Amosite	Amphibole asbestos	418	900[a]	Hesterberg et al. (1998a)
Crocidolite	Amphibole asbestos	536	262	Bernstein et al. (1996)
Tremolite	Amphibole asbestos	∞	∞	Bernstein et al. (2005b)

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

are referred to as lung overload (Bolton et al., 1983; Muhle et al., 1988; Morrow, 1988, 1992; Oberdörster, 1995).

In Table 2 the exposure concentrations and lung burdens are shown for a series of chronic inhalation toxicology studies performed using similar protocols with synthetic vitreous fibers and serpentine and amphibole asbestos. The asbestos exposures in these studies were included as positive controls, however, it can be seen that 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 a lung over-

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

Fiber type	Aerosol			Lung		Reference
	Total (fibers/cm^3)	WHO[a] (fibers/cm^3)	Number (fibers/cm^3 length > 20 μm)	Total fiber number (in lung after 24 months of exposure)	No. fibers $L > 20$ μm (in lung after 24 months of exposure)	
MMVF11[c]	Na	41	14	93,000,000	5,580,000	Hesterberg et al. (1993)
MMVF11[c]	Na	153	50	692,000,000	24,912,000	Hesterberg et al. (1993)
MMVF11[c]	273	246	84	1,284,000,000	30,816,000	Hesterberg et al. (1993)
MMVF21[b]	44	34	13	112,142,857	14,130,000	McConnell et al., 1994
MMVF21[b]	185	150	74	548,173,913	50,432,000	McConnell et al., 1994
MMVF21[b]	264	243	114	622,884,615	80,975,000	McConnell et al. (1994)
MMVF22[b]	33	30	10	21,984,733	2,880,000	McConnell et al., 1994
MMVF22[b]	158	131	50	320,625,000	7,695,000	McConnell et al. (1994)
MMVF22[b]	245	213	99	596,750,000	23,870,000	McConnell et al. (1994)
RCF1[c]	36	26	13	99,900,000	12,787,200	Mast et al. (1994)
RCF1[c]	91	75	35	233,120,000	31,238,080	Mast et al. (1994)
RCF1[c]	162	120	58	578,240,000	58,402,240	Mast et al. (1994)
RCF1[c]	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)	4214	1610	236	2,025,000,000	88,452,000	McConnell et al. (1994)

Na, data not available.

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

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

[c] 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.

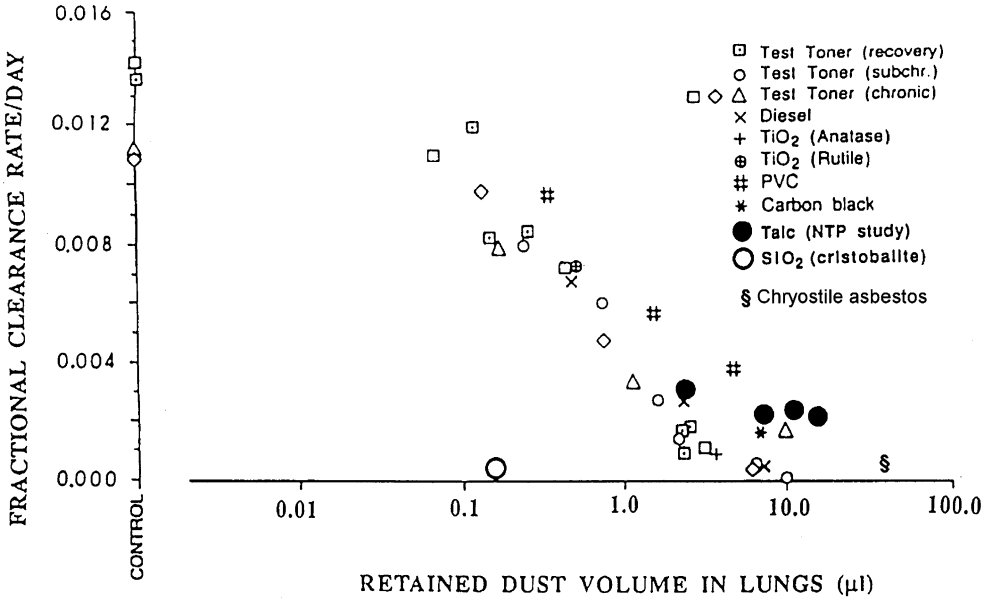

Fig. 3. Retained dust volume in lungs and fractional clearance rate (adapted from Oberdörster, 2002, Figure 3). Added to the Figure is the point which corresponds to the retained lung volume (µl) for chrysotile. This value was calculated from measurements of total chrysotile volume retained in the lung as determined in a 90 day inhalation toxicity study of chrysotile (see text).

load effect likely occurred based upon the number of shorter fibers present. It would have been much more useful in these studies if the exposure concentrations and fiber size distributions were comparable between the positive controls and the SVFs.

More recently, Oberdörster (2002) reviewed the toxicokinetics and effects of fibers and of non-fibrous poorly soluble particles and related how high exposure concentrations of poorly soluble particles can induce lung particle overload in the rat which can result in the induction of lung tumours. He proposed that high-dose effects observed in rats may be associated with two thresholds.

1. The first threshold is the pulmonary dose that results in a reduction in macrophage mediated clearance.
2. The second threshold, occurring at a higher dose than the first, is the dose at which antioxidant defences are overwhelmed and pulmonary tumours develop.

The reduction in macrophage mediated clearance was plotted against retained dust volume in the lung as shown in Fig. 3 (reproduced from Figure 3, p. 34 of Oberdörster, 2002 adding a data point for chrysotile (see below)). The author stated that a threshold for the retained dust burden seems to exist above which the clearance rate begins to decrease.

Neither the retained dust volume nor the clearance rate were presented by Hesterberg et al. (1993)[1] for the chronic inhalation study of chrysotile listed in Table 2. It was, however, possible to estimate this from measurements of total chrysotile volume retained in the lung as determined by Bernstein et al. (2006). In this study the total chrysotile lung volume was found to be 28 µl at one third the exposure concentration used in the chronic studies. This value has been added to Fig. 3 and suggests that at the retained dust volume used in the chronic inhalation of chrysotile that the macrophage mediated clearance would be strongly decreased. If macrophage activity is inhibited, then their interaction in creating an acid environment which would break apart the chrysotile fibers would also be affected.

The second threshold postulated by Oberdörster occurs at a higher dose than the first, and is the dose at which antioxidant defences are overwhelmed and pulmonary tumours develop. This relationship was illustrated by Oberdörster (2002, Figure 6, p. 37) adapted as Fig. 4 (with the addition of a data point for chrysotile) with the surface area of retained dusts as a function of the percent lung tumours in chronic inhalation studies. The surface area was calculated using the same dataset from the 90 day inhalation toxicity study extrapolated to the chronic studies of chrysotile. The particle and short fiber chrysotile lung dose in the chronic inhalation study falls within the "overload" range shown in Fig. 4 for poorly soluble low toxicity particles.

While this comparison shows that the results of the chronic inhalation toxicology studies of chrysotile could have occurred as a result of the number of short fibers and particles alone, it cannot exclude the possibility that the small percentage of longer fibers present could have caused the tumorigenic response. However, the biopersistence results indicate that fewer if any long fibers would have been present if overload conditions were not present in these studies.

[1] The same study reported by Hesterberg et al. (1993) was also reported in Mast et al. (1994); Hesterberg et al. (1994) and Hesterberg et al. (1998b).

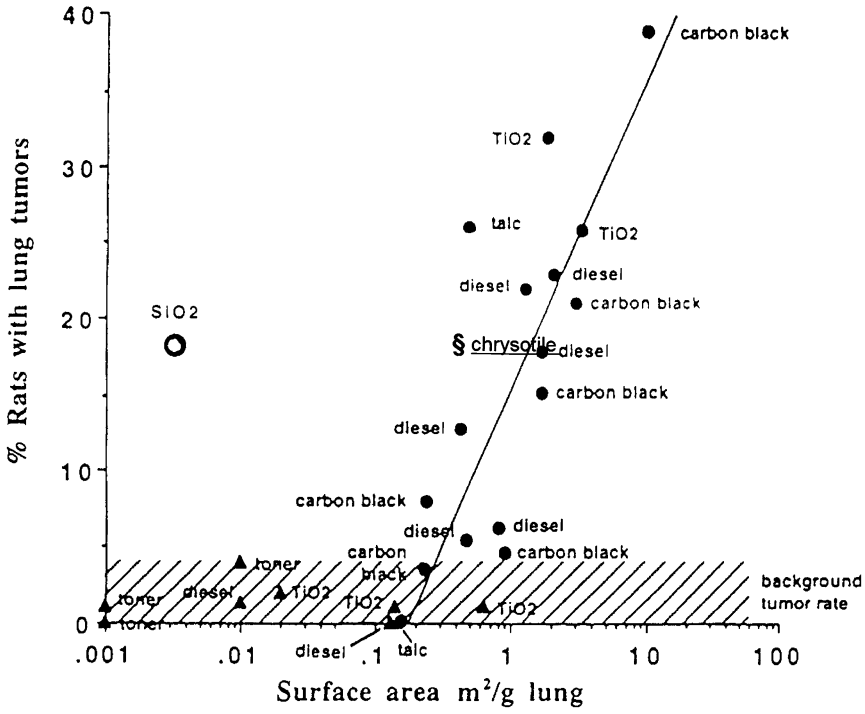

Fig. 4. Particle surface area as a function of percent rats with tumours (adapter from Oberdörster, 2002). Added to the Figure is the point which corresponds to the surface area m^2/g lung for chrysotile as was calculated from the Mast et al. (1995) study which had 18% lung tumours. Note: For chrysotile, Ono-Ogasawara and Kohyama (1999) have shown that the surface area as determined by nitrogen adsorption is 2.3 times the calculated surface area. In addition, there were 200 times more chrysotile fibers remaining in lung at 24 months in the Mast et al. (1995) chronic inhalation study than at 90 days in the 90 day study.

2.6. Subchronic inhalation toxicology studies with chrysotile

The available chronic inhalation studies with chrysotile are difficult to interpret due to this overload effect. A recent working group convened by ILSI in conjunction with the U.S. EPA recently proposed testing strategy for prioritizing fibers for chronic testing (ILSI, 2005). The proposed strategy has three fundamental components: preparation and characterization of an appropriate fiber sample, testing for biopersistence in vivo, and assessment of toxicologic endpoints in a subchronic rodent study.

The working group also noted when specifying the parameters that should be assessed in a subchronic inhalation study that "The European Commission guideline for subchronic inhalation toxicity testing of synthetic mineral fibers in rats (Bernstein and Riego-Sintes, 1999) specifies similar parameters."

To assess the cellular and pathological response in the rat lung to a well characterized aerosol of chrysotile asbestos, a 90-day subchronic inhalation toxicology study was performed using a commercial chrysotile (Bernstein et al., 2006). The protocol was based on that established by the European Commission for the evaluation of synthetic vitreous fibers and met the criteria recommended by the ILSI working group (ILSI, 2005).

In this study, Wistar male rats were exposed to an air control group and to two chrysotile exposure groups at mean fiber aerosol concentrations of 76 fibers $L > 20\,\mu m/cm^3$ (3413 total fibers/cm^3; 536 WHO fibers/cm^3) or 207 fibers $L > 20\,\mu m/cm^3$ (8941 total fibers/cm^3; 1429 WHO fibers/cm^3) for 5 days/wk, 6 h/day, during 13 consecutive weeks followed by a non-exposure period lasting for 92 days. Animals were sacrificed after cessation of exposure and after 50 and 92 days of non-exposure recovery. At each sacrifice, subgroups of rats were assessed for the determination of the lung burden; histopathological examination; cell proliferation response; bronchoalveolar lavage with the determination of inflammatory cells; clinical biochemistry; and for analysis by confocal microscopy.

Through 90 days of exposure and 92 days of recovery, chrysotile at a mean exposure of 76 fibers $L > 20\,\mu m/cm^3$ (3413 total fibers/cm^3) resulted in no fibrosis (Wagner score 1.8–2.6), at any time point and no difference with controls in BrdU response or biochemical and cellular parameters. The long chrysotile fibers were observed to break apart into small particles and smaller fibers. At an exposure concentration of 207 fibres $L > 20\,\mu m/cm^3$ (8941 total fibers/cm^3) slight fibrosis was observed. The authors reported that as predicted by the recent biopersistence studies on chrysotile, that at an exposure concentration 5000 times greater than the U.S. threshold limit value of 0.1 f(WHO)/cm^3, chrysotile produces no significant pathological response.

In contrast, the amphibole tremolite produced, following only 5 days of inhalation exposure as part of a biopersistence study, granuloma, interstitial fibrosis, and numerous

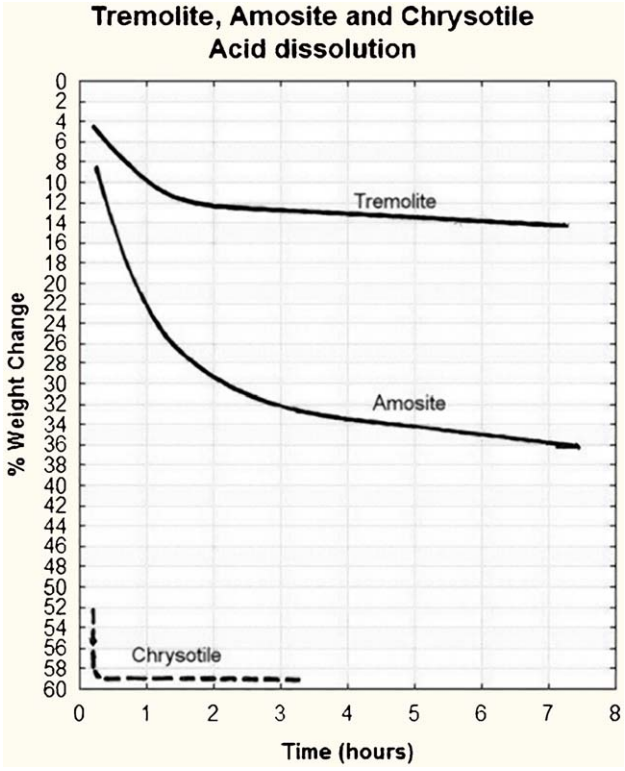

Fig. 5. Acid dissolution studies of tremolite, amosite and chrysotile (adapted from Speil and Leineweber, 1969). Shown are the rates of decomposition of different asbestiform fibers in boiling hydrochloric acid.

macrophage aggregates as well as multinucleated giant cells (Bernstein et al., 2005a).

Bellmann et al. (2003) reported a similar 90-day subchronic inhalation toxicity study of SVF 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 comparison with the results from the subchronic inhalation study of chrysotile discussed above (Bernstein et al., 2006), chrysotile produced less inflammatory response than the biosoluable synthetic vitreous fiber CMS.

2.7. Chrysotile: fiber or particle effects

The rapid clearance of the long chrysotile fibers from the lung, that is those fibers which cannot be effectively cleared by macrophages, provides an indication of what may happen when chrysotile is inhaled. While synthetic vitreous fibers (SVF) may dissolve congruently or incongruently (Christensen et al., 1994) chrysotile fibers appear to break apart into small particles and smaller fibers.

At acidic pH chrysotile becomes less stable which leads to the clearance/disintegration of the long chrysotile fibers. Kamstrup et al. (2001) described a similar process for long HT fibers which are highly soluble at pH 4.5. Wypych et al. (2005), showed that acid leaching the chrysotile fibers removes the brucite-like sheets leaving essentially amorphous silica particles. That is, what remains are small glass-like particles. Speil and Leineweber (1969, Figure 12, p. 182) summarized studies on the rate of decomposition of different asbestiform fibers in boiling hydrochloric acid. Their results for tremolite, amosite, and chrysotile have been replotted in Fig. 5. The acid dissolution kinetics are remarkably similar to the biopersistence kinetics of the fibers longer than 20 μm shown in Fig. 6.

This two stage process by which chrysotile disassociates has been described as early as 1955. Pundsack (1955)

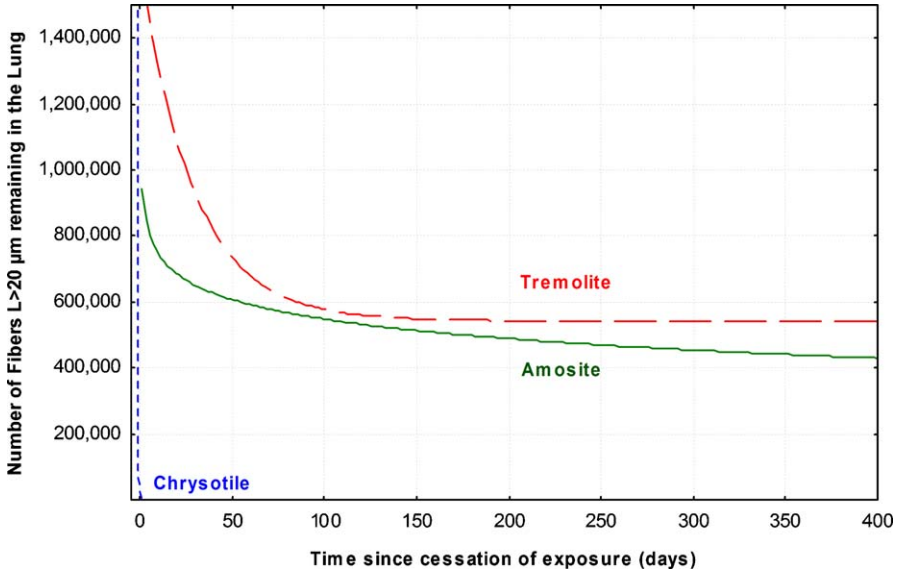

Fig. 6. Clearance of tremolite, amosite and chrysotile fibers longer than 20 μm from the lung in inhalation biopersistence studies (adapted from Bernstein et al., 2004, 2005a,b).

explained that: "From a chemical point of view chrysotile behaves in certain aspects as if it were magnesium hydroxide. This is not unexpected when one considers that the structure generally ascribed to the mineral consists of fundamental layers made up in terms of a unit cell of O_6–Si_4–$O_4(OH)_2$–Mg_6–$(OH)_6$ planes."

He found that the behavior of chrysotile fibers can be understood as a magnesium hydroxide layer on a silica substrate. He explained as well the two step process in which initially at neutral pH such as would be found in the lung surfactant "in contact with relatively pure water the fiber surface dissociates partially until an equilibrium of the order of that attained by pure magnesium hydroxide is reached."

The second step in the lung is associated with the acid environment created by the macrophage. In an acid environment Pundsack (1955) stated that "It is important to note that chrysotile reacts with strong acids to form eventually a hydrated silica residue. Therefore, the particles suspended in initially acid solutions are not chrysotile in the strict sense, but they represent instead intermediate reaction products of the acid and the fiber." In an acid environment Pundsack found that dissociation of the surface is more pronounced because of the interaction of surface hydroxyl groups with hydrogen ions. Disintegration of the fibers provides a basis for understanding the potential toxicity of chrysotile. The rapid disintegration of chrysotile fibers results in exposure to a larger number of amorphous silica particles and shorter fibers. 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.

However, chrysotile at lower exposures levels leads to levels of exposure to shorter fibers and particles which the lung can handle. The contrast in the response between the serpentine chrysotile and amphiboles is most clearly illustrated by the histopathological response in the inhalation studies. Fig. 7 (reproduced from Bernstein et al., 2006) shows the histopathological response in the subchronic study with chrysotile presented above (90-days of exposure and 92 days recovery) in which no inflammatory response was observed in the lung. In contrast, Fig. 8 (reproduced from Bernstein et al., 2005a) shows the histopathological response following a 5-day exposure to tremolite which produced marked inflammation, granulomas and even mild interstitial fibrosis.

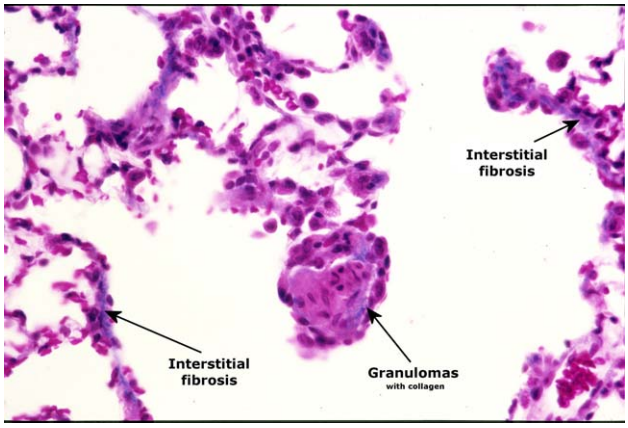

Fig. 8. (Reproduced from Figure 7, Bernstein et al., 2005a) Photomicrograph of a lung histopathological section from rats exposed 5-days to tremolite (at 90 days following cessation of exposure). The severity of the fibrosis in the granulomas was increased from earlier time points, and the granuloma can be seen interlaced with collagen. By this time the collagen had progressed into the interstitium and interstitial fibrosis is seen as well. Numerous macrophage aggregates are also observed, as well as multinucleated giant cells.

2.8. Epidemiology

While it is clear that exposure to 'asbestos' has resulted in lung cancer and mesothelioma the epidemiological studies which have quantified these relationships have had to attempt to deal with sometimes important limitations in the available data. These limitations would be less important if as considered many years ago all asbestos mineral types were of equal potency. However, as presented above, the animal

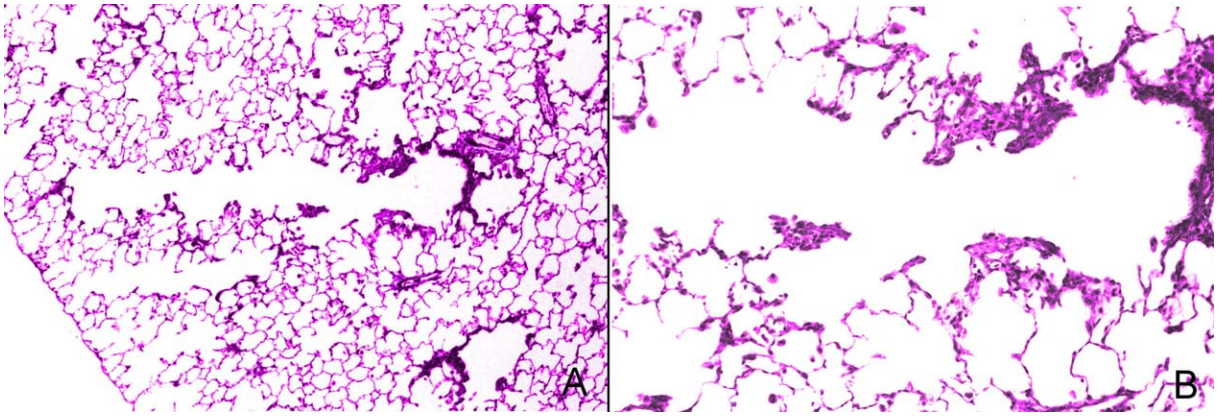

Fig. 7. (Reproduced from Figure 5, Bernstein et al., 2006) Photomicrographs showing of histopathology of the medium dose lungs after cessation of the 90 day exposure. Trichrome stain for collagen specific analysis. Frame A is at 63× and from B at 160× magnification. A few small microgranulomas with slight collagen and a macrophages are seen.

Table 3
Capabilities and limitations of analytical techniques used for asbestos measurements[a] (reproduced from Berman and Crump, 2003)

Parameter	Midget impinger	Phase contrast microscopy	Scanning electron microscopy	Transmission electron microscopy
Range of magnification	100	400	2000–10,000	5000–20,000
Particles counted	All	Fibrous structures[b]	Fibrous structures[b]	Fibrous structures[b,c]
Minimum diameter (size) visible	1 μm	0.3 μm	0.1 μm	<0.01 μm
Resolve internal structure	No	No	Maybe	Yes
Distinguish mineralogy[d]	No	No	Yes	Yes

[a] The capabilities and limitations in this table are based primarily on the physical constraints of the indicated instrumentation. Differences attributable to the associated procedures and practices of methods in common use over the last 25 years are highlighted in Table 4–2 in Berman and Crump, 2003.
[b] Fibrous structures are defined here as particles exhibiting aspect ratios (the ratio of length to width) greater than 3 (see Walton, 1982).
[c] TEM counts frequently resolve individual fibrous structures within larger, complex structures. Based on internal discussion of methods presented below.
[d] Most SEM and TEM instruments are equipped with the capability to record selected area electron diffraction (SAED) spectra and perform energy dispersive X-ray analysis (EDXA), which are used to distinguish the mineralogy of structures observed.

studies indicate that there is an important difference between chrysotile, a serpentine, and amphibole asbestos. For chrysotile, this is often a pivotal issue and the limitations and associated errors merit a more complete understanding.

In a recent analysis of available epidemiological data on the different asbestos types, Berman and Crump (2003) have summarised the various limitations that could influence the epidemiological evaluations and that had to be addressed. These included:

- limitations in air measurements and other data available for characterizing historical exposures;
- limitations in the manner that the character of exposure (i.e., the mineralogical types of fibers and the range and distribution of fiber dimensions) was delineated;
- limitations in the accuracy of mortality determinations or incompleteness in the extent of tracing of cohort members;
- limitations in the adequacy of the match between cohort subjects and the selected control population; and
- inadequate characterization of confounding factors, such as smoking histories for individual workers.

In addition, the authors reviewed the capabilities and limitations of the analytical techniques used for asbestos measurements (Table 3). Midget impinger (MI) and phase contrast microscopy (PCM) were the two analytical techniques used to derive exposure estimates in the majority of epidemiology studies from which the existing risk factors were derived. However, the manner in which asbestos was quantified in the available epidemiology studies (i.e., MI and PCM) may not have adequately reflected the characteristics of the inhaled aerosol that relate to biological activity.

With few exceptions little or no sampling was conducted prior to the 1950s when exposure concentrations were thought generally to be higher than those monitored more recently, due to lack of use of dust control equipment at the time and procedures to reduce dust levels that were introduced only later. For many studies, therefore, early exposures had to be estimated by extrapolation from later measurements.

In particular, as a result of the measurement techniques there was often little quantitative mineralogical exposure information on the types of fibers to which workers were exposed. The nature of the industrial process dictated in part what type of fiber was used, however, in the past there was little attempt to differentiate serpentine from amphibole asbestos, and as a result amphibole was often substituted or mixed with serpentine without detailed documentation. The use of amphibole in place of serpentine resulted from such factors as availability, cost, and effectiveness in the process. In addition, work histories of employees were not always as well documented as might occur today. While all uncertainty factors are important in assessing the difference between chrysotile and amphiboles, the differentiation of the fiber type in the exposure atmosphere is obviously critical in determining possible effects associated with each type of fiber.

As an example, in Berman and Crump (2003), retroactive exposure indices were determined using TEM analyses of samples conducted in the same environment in which an epidemiological study was conducted or from an environment involving a similar operation (e.g., mining, textile manufacture, etc.). However, herein lies a significant difficulty in interpretation. If, for example, pure chrysotile was used or the worker was exposed to amphibole elsewhere during the time the TEM samples were taken and if during the time of the epidemiology study amphibole was also used, then the TEM exposure indices will attribute any effect to chrysotile when in fact amphibole was present.

Berman and Crump summarised that the residual inconsistency in both the lung cancer and mesothelioma potency values is primarily driven by those calculated from Quebec chrysotile miners and from South Carolina chrysotile textile workers. While the present review will not resolve this, it is still interesting to note that amphiboles have been found in the lungs of the few South Carolina chrysotile textile workers that have been examined (Case et al., 2000). Unfortunately, due to the few fibers analysed per sample, the statistical power of this study is low.

It is interesting to note as well that Berman and Crump (2003) reported that the optimal exposure index[2] that best

reconciles the published literature assigns equal potency to fibers longer than 10 μm and thinner than 0.4 μm and assigns no potency to fibers of other dimensions.

Hodgson and Darnton (2000) reviewed 17 epidemiology studies that were referenced in reports by Peto et al. (1985), HEI (1991) and INSERM (1996) with the goal of differentiating effect by asbestos fibre type. The authors state that "Not only are there the inevitable problems of extrapolating earlier exposures on the basis of more recent measurements; there are also problems of converting the most usual historic measurements (in terms of particle counts) to the more relevant measure of fibre counts. Direct fibre counting only became generally used in the 1970s."

For the Carolina cohort they stated that "Very small amounts of crocidolite yarn were used, but raw crocidolite fibre was not processed. The quantity of crocidolite used was about 0.002% of the total." However, no reference was cited as to where this was derived. Even so, Hodgson and Darnton (2000) did differentiate chrysotile from amphiboles. It is interesting to note that there were 47% amphiboles in the total fiber count in the lungs of the Carolina cohort.

In a more recent analysis, Hodgson et al. (2005) modelled the expected burden of mesothelioma mortality in Great Britain, male mesothelioma deaths from 1968 to 2001 as a function of the rise and fall of asbestos exposure during the 20th century taking account of the difference between fibre types. Two models were fitted to the data and the predicted exposure patterns compared with the actual exposure patterns based on imports of amosite and crocidolite. The authors state that chrysotile had zero weight in both (sic) models. Thus the mesothelioma occurring in Great Britain since 1920 was explained by a combination of amosite and crocidolite reversing the earlier explanation of this as due to chrysotile (Peto et al., 1999). Weill et al. (2004) have recently examined the temporal pattern and change in trend of mesothelioma incidence in the United States since 1973. They concluded that mesothelioma risk was prominently influenced by exposure to amphibole asbestos (crocidolite and amosite) which reached its peak usage in the 1960s and thereafter declined.

More recent studies not included in these analyses also indicate that chrysotile produces little if any effect. Rees et al. (1999, 2001) reported that while South Africa is noted for amphibole mining it has also mined about 100,000 tons of chrysotile per year. Cases of mesothelioma have not been found in the South African chrysotile miners and millers despite decades of production. The authors suggest one possible explanation for the scarcity or absence of the cancer may be the relative lack of fibrous tremolite, an amphibole that may occur with chrysotile ores Yarborough (2006) reviewed 71 asbestos cohorts exposed to free asbestos fibers and reported that the hypothesis that chrysotile, uncontaminated by amphibolic substances, causes mesothelioma was not supported. Non-occupational studies such as that reported by Camus et al. (1998) examined the effect of chrysotile on non-occupationally exposed woman in two mining areas in the province of Quebec. Mean ambient exposure concentrations were estimated to have peaked around 1945 at approximately 1–1.4 f/cm^3. Average total cumulative life-time exposure was estimated as 25 fiber-yr/ml. The authors reported that there was no measurable excess risk of death due to lung cancer among women in two chrysotile-asbestos–mining regions.

A large number of studies have been conducted on workers occupationally exposed to chrysotile in manufacturing industries. Paustenbach et al. (2004) recently reviewed workers employed in the manufacture of friction materials and mechanics who fitted and serviced such products. The review included a meta-analysis of studies made over a century of use. The authors reported that brake mechanics were not at increased risk of adverse health effects due to exposure to chrysotile. They had no increased risk of mesothelioma or asbestosis and no evidence of lung cancer that could be attributed to exposure to chrysotile during brake repair.

3. Conclusions

This review provides an important basis for substantiating both kinetically and pathologically the differences between chrysotile and amphiboles. The toxicology of chrysotile which rapidly falls apart in the lung into many small particles can best be understood in comparison to other non-fibrous minerals, while that of amphibole asbestos is clearly a response to the insoluble fibrous structure of this mineral.

Chrysotile is mineralogically distinct from the amphiboles with a very different chemical structure. This structure leads to the ability of the lung to decompose the chrysotile fibers once inhaled as seen in the biopersistence studies of commercial chrysotiles.

The chronic inhalation toxicity studies that have been performed on chrysotile in animals have unfortunately been performed at very high exposure concentrations and so under conditions of lung overload. In consequence their relevance to human exposures is extremely limited.

Chrysotile following subchronic inhalation at a mean exposure of 76 fibers $L > 20$ μm/cm^3 (3413 total fibers/cm^3) resulted in no fibrosis (Wagner score 1.8–2.6) at any time point and no difference with controls in BrdU response or biochemical and cellular parameters. The long chrysotile fibers were observed to break apart into small particles and smaller fibers.

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 and have also differentiated between these two minerals. The most recent analyses

[2] Berman et al. (1995) derived an optimal exposure index from an analysis of rat inhalation studies involving exposures to different types of asbestos and fibrous structures of differing dimensions. The optimum index consists of a weighted sum of the air concentrations of structures between 5 and 40 μm in length and >40 μm in length (all thinner than 0.4 μm).

also concluded that it is the longer, thinner fibers that have the greatest potency as has been reported in animal inhalation toxicology studies. However, one of the major difficulties in interpreting these studies is that the original exposure estimates rarely differentiated between chrysotile and amphiboles.

Not unlike some other respirable particulates (e.g., silica, diesel fume particles, etc.), to which humans are, or have been heavily occupationally exposed, there is evidence that heavy and prolonged exposure to chrysotile can produce lung cancer.

The value of the present and other similar studies is that they show that low exposures to pure chrysotile do not present a detectable risk to health. Since total dose over time decides the likelihood of disease occurrence and progression, they also suggest that the risk of an adverse outcome may be low if even any high exposures experienced were of short duration.

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