UNDERSTANDING CHRYSOTILE ASBESTOS:
A new understanding based on current data

Chrysotile at a Turning Point
Results and Scientific Perspectives
Montreal, Quebec, Canada
23 May 2006

Principles of Fiber Toxicology

- **Fiber size**
  - Is the fiber thin enough to be inhaled?
  - Is the fiber long enough to frustrate the macrophages ability to safely remove it from the lung?

- **Fiber / Particle bio-solubility**
  - Will the fiber / particle persist long enough to cause an effect or will it quickly dissolve or break apart?

- **Surface composition /effects**
  - If the fiber / particle is durable, can it cause cytotoxic effects.
Fiber Length Distribution

- WHO Fibers
- Long Fibers

![Fiber Length Distribution Diagram](image)
Pulmonary Alveoli

FIG. 3. Schematic diagram of a single alveolus emphasizing the movement of surfactant components through the type II cell and alveolar liquid. Components and compartments are not to scale. Key: 1, surfactant precursors such as glucose, amino acids, and fatty acids; 2, endoplasmic reticulum; 3, Golgi apparatus; 4, lamellar bodies; 5, tubular myelin; 6, surface film with adsorbed phospholipids; 7, vesicular and myelin forms of surfactant possibly derived from material desorbed from the film; 8 and 9, endocytotic compartments such as multivesicular bodies; 10, alveolar macrophage. (From ref. 122)
In the non exposed lung, one or two macrophages reside in each alveolus in a near sterile environment.


Immediately after Exposure many particles and short and long fibers are present.
Following Early clearance only long fibers remain

Bio-Soluble fibers disappear rapidly

rapid return to normal
With Durable fibers the long fibers remain

chronic inflammation

Glass Fibers following Intratracheal Instillation

Glass Fibers following Intratracheal Instillation


Presentation: D.M. Bernstein, May 2006
Macrophage Phagocytosis and Fiber Clearance
**Phagocytosis:**

1. Attachment of the bacterium to the long membrane evaginations, called pseudopodia.
2. Ingestion of the bacterium forming a "phagosome," which moves toward the lysosome.
3. Fusion of the lysosome and phagosome, releasing lysosomal enzymes into the phagosome.
4. Digestion of the ingested material.
5. Release of digestion products from the cell.

**Macrophage**
• All particles which reach the lower lung can be engulfed by the macrophages.
• Fibers, due to their aero-dynamic properties may enter the lower lung with lengths much longer than can be engulfed by the macrophage.

max particle diameter 10 microns

macrophage size 15 microns

fiber length up to 200 microns
Inhalation Biopersistence Study
Experimental Design


Inhalation Biopersistence of Long Fibers

Correlation of Fiber Biopersistence with Pathological Response

- Inhalation carcinogenicity studies
- Intraperitoneal Injection studies

References:

Correlation of Short Term Biopersistence to Chronic Toxicity

- Inhalation Biopersistence:
  \( T_{1/2} \text{ of Fibers } L > 20 \mu m \text{ correlates to}:
  \begin{align*}
    & \text{Number of fibers } L > 20 \mu m \text{ remaining at 24 months in the Chronic Inhalation Studies.} \\
    & \text{Early Pulmonary Fibrosis in the Chronic Inhalation Studies.} \\
    & \text{Number of Tumours in the IP studies when fiber length and number fibers injected are taken into account.}
  \end{align*}
European Commission


EC Directive 97/69/EC

- Refractory Ceramic Fiber (RCF) are classified as category 2. (The declaration to the directive states that RCF can move to category 3 with suitable long terms studies.)
- Synthetic Mineral fibers classified as category 3
- **Fibers in category 3 are exonerated if they meet one of the following criteria:**
EC Directive 97/69/EC
Nota Q

The classification as a carcinogen need not apply if it can be shown that the substance fulfils one of the following conditions:

- a short-term biopersistence test by inhalation has shown that the fibres longer than 20 µm have a weighted half-life less than 10 days, or
- a short-term biopersistence test by intratracheal instillation has shown that the fibres longer than 20 µm have a weighted half life less than 40 days, or
- an appropriate intra-peritoneal test has shown no evidence of excess carcinogenicity, or
- absence of relevant pathogenicity or neoplastic changes in a suitable long term inhalation test.

IARC (Monograph 81, 2002)

- “This characteristic, known as high biopersistence, is correlated with the high carcinogenic potency of asbestos fibres. Some of these newer materials have now been tested for carcinogenicity and most are found to be non-carcinogenic, or to cause tumours in experimentals animals only under very restricted conditions of exposure.”
USEPA

- International Life Sciences Institute (ILSI) Working Group to provide guidelines to the EPA for the evaluation and testing of all fiber types.
- Recommended for non synthetic mineral fibers, biopersistence, followed by 90-day sub-cronic inhalation toxicology study.

Asbestos
‘Asbestos’
Refers to Two Different Mineral Types

- Chrysotile is a serpentine.
- Amosite, Tremolite and Crocidolite are amphiboles.

**What is the difference?**
- Amphibole fibers are single solid cylindrical shapes.
- Serpentine fibers are like ropes and are composed of many smaller fibrils.

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**Serpentine and amphibole fibers**

**Serpentine**
- Chrysotile

**Amphibole**
- Tremolite
- Amosite
- Crocidolite
Table 1 Typical chemical composition (percent)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chrysotile¹</th>
<th>Tremolite²</th>
<th>Amosite²</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂</td>
<td>40.6</td>
<td>55.10</td>
<td>49.70</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>0.7</td>
<td>1.14</td>
<td>0.40</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>2.3</td>
<td>0.32</td>
<td>0.03</td>
</tr>
<tr>
<td>FeO</td>
<td>1.3</td>
<td>2.00</td>
<td>39.70</td>
</tr>
<tr>
<td>MnO</td>
<td>--</td>
<td>0.10</td>
<td>0.22</td>
</tr>
<tr>
<td>MgO</td>
<td>39.8</td>
<td>25.65</td>
<td>6.44</td>
</tr>
<tr>
<td>CaO</td>
<td>0.6</td>
<td>11.45</td>
<td>1.04</td>
</tr>
<tr>
<td>K₂O</td>
<td>0.2</td>
<td>0.29</td>
<td>0.63</td>
</tr>
<tr>
<td>NaO</td>
<td>--</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>H₂O</td>
<td>--</td>
<td>3.52</td>
<td>1.83</td>
</tr>
<tr>
<td>H₂</td>
<td>--</td>
<td>0.16</td>
<td>0.09</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.5</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Ignition loss</td>
<td>14.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>99.93</td>
<td>100.26</td>
</tr>
</tbody>
</table>

1. Typical chemical analysis of Canadian chrysotile from the Quebec Eastern Townships (LAB Chrysotile, Inc., Quebec, Canada)
2. Hodgson (1979); pp. 80-81

Chrysotile structure

With chrysotile, the magnesium in the lattice is on the outside of the curved surface and is available for dissolution by the lung fluid.
How chrysotile forms:

Tremolite Structure
(amphiboles)
How amphibole splits:

A B C D

Biopersistence of Chrysotile

- To date 3 different commercial chrysotiles have been studied:
  - Canadian chrysotile (textile grade)
  - Calidria chrysotile (California, USA)
  - Brazilain chrysotile (Cana Brava Mine)
Biopersistence Study
Chrysotile Fiber Aerosol Exposure

- The exposure aerosol in the biopersistence study had 200 fibers/cm³ longer than 20 µm in length.
  - For mineral fibers (e.g. glass) the European Commission biopersistence protocol requires 100 fibers/cm³ longer than 20 µm in length.
- Total exposure for all fiber lengths was more than 14,805 fibers/cm³ (1,849 WHO fibers/cm³).
- Occupational exposure limits for chrysotile
  - TLV: 0.1 fibres/cc (as TWA) A1 (ACGIH 1998. For fibres longer than 5 um with an aspect ratio equal to or greater than: 3:1 as determined by the membrane filter method at 400-450X magnification (4-mm objective) phase contrast elimination).
  - 0.6 fibres/cm³ EC OELs

FIGURE 7. Photomicrographs of chrysotile fibers from an aerosol sample taken using scanning electron microscopy (SEM). SEM was used for these micrographs in order to provide a visual overview of the fiber size distribution. As described earlier, transmission electron microscopy (TEM) was used for all quantification of fiber size.
The Biopersistence of Amphibole Fibers

Presentation: D.M. Bernstein, May 2006

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Clearance of Chrysotile from the Lung Fibers with lengths > 20 µm

- Calidria Chrysotile L>20 µm
  - Open pit mine
- Brazilian Chrysotile L>20 µm
  - Extremely high exposure
- Canadian chrysotile L>20 µm
  - Textile grade
Chrysotile Asbestos Clearance Half-Times

<table>
<thead>
<tr>
<th>Fiber</th>
<th>Clearance Half-time ($T_{1/2}$) (days)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calidria chrysotile</td>
<td>0.3</td>
<td>Bernstein et al., 2003b</td>
</tr>
<tr>
<td>Brazilian chrysotile</td>
<td>1.3</td>
<td>Bernstein et al., 2004a</td>
</tr>
<tr>
<td>Canadian chrysotile</td>
<td>11.4</td>
<td>Bernstein et al., 2004b</td>
</tr>
<tr>
<td>(Textile grade)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing clearance of Calidria Chrysotile and Tremolite Fibers with length > 20 μm](image-url)
Chrysotile: Particle rather than Fiber Effect

- The rapid clearance of the long chrysotile fibers from the lung, that is those fibers which can not be effectively cleared by macrophages, provides an indication of what may happen when chrysotile is inhaled.
- While synthetic vitreous fibers (SVF) may dissolve congruently (all component elements dissolving at rates proportional to their mole equivalents in the fibre) or incongruently (leaching with enhanced release of specific elements) (Christensen et al., 1994)
- With chrysotile, the long fibers appear to break apart into small particles and smaller fibers.
In-vitro chemical dissolution

In-vivo (rat) dissolution in the lung
Lung at Day 1 – Confocal Microscopy

Lung at Day 90 – Confocal Microscopy
Alveolar surface
(covered with surfactant)
Rolled structure of chrysotile

Mg

Mg
Chrysotile in the lung – initially fiber which rapidly breaks up.

Chrysotile in the lung > particles
Chronic Inhalation Studies

- Nearly all previous studies involved exposure periods of from 3 to 12 mo and used a range of exposure concentrations from 2 to 10 mg/m³.
- With an exposure concentration of 10 mg/m³, the total fiber concentration was more than $1 \times 10^6$ fibers/cm³ (Mast et al., 1995).
- The number of non-fibrous particles was not reported, although from the current studies this could equal the number of fibers.
- With these very high exposure concentrations rat-specific lung overload occurred (Oberdorster, 1995a, 1995b, 2002).
- In addition, in these studies, the chrysotile was ground using a steel plate grinder and there was no reported investigation of the presence of other silicates in the aerosol and especially of amphibole fibers such as tremolite. Wagner et al. (1980) stated that “all materials contained impurities” in the chrysotile samples that he studied, although he did not identify these impurities.
Lung Overload  
(Brown et al., 2005)

- Unlike other laboratory animals and humans, rats appear susceptible to “overload”-related effects due to impaired macrophage-mediated alveolar clearance at high doses which are not observed at lower doses in rats.

- Oberdoerster (2002) 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.
90-day Sub-chronic inhalation toxicity study with Brazilian chrysotile

- The protocol was based upon that established by the European Commission for the evaluation of synthetic vitreous fibers.
- Wistar male rats were randomly assigned to an air control group and to two chrysotile exposure groups at mean fibre aerosol concentrations of:
  - 76 fibres L>20 µm/cm³ (3413 total fiber/cm³) or
  - 207 fibres L>20 µm/cm³ (8941 total fiber/cm³).
- The animals were exposed (nose only):
  - five consecutive days, 6 hours per day,
  - during 13 consecutive weeks (65 exposures)
  - followed by a subsequent non-exposure period lasting for 92 days.
Sub-chronic inhalation toxicity study with Brazilian chrysotile

- At each sacrifice sub-groups of rats were assessed for the determination of:
  - lung burden;
  - histopathological examination;
  - cell proliferation response (BrdU);
  - broncho-alveolar lavage with the determination of inflammatory cells and
  - Clinical biochemistry (LDH, Total Protein)
  - for analysis by confocal microscopy.

Though 90 days of exposure and 92 days of recovery,

- NOEL: Exposure to chrysotile at a mean concentration of 76 fibres L>20 µm/cm³ (3,413 total fiber/cm³) resulted in no fibrosis (Wagner score 1.8 to 2.6) at any time point.
- MTD: At the high dose of 207 fibres L>20 µm/cm³ (8,941 total fiber/cm³), minimal fibrosis was observed.
- The long chrysotile fibers were observed to break apart into small particles and smaller fibers.
**Sub-chronic inhalation toxicity study with Brazilian chrysotile**

- In comparison with other studies, chrysotile produced less inflammatory response than the biosoluble synthetic vitreous fiber CMS.
- As predicted by the recent biopersistence studies on chrysotile, this study clearly shows that at an exposure concentration 5000 times greater than the US-Threshold Limit Value of 0.1 (WHO) f/cm³, chrysotile produces no significant pathological response.

**Epidemiology**

- 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 (Berman & Crump, 2003; Hodgson, & Peto et al., 2005).
- The Berman & Crump analyses also concluded that it is the longer, thinner fibers that have the greatest potency as has been reported in animal inhalation toxicology studies.
Epidemiology

- Hodgson, & Peto et al., 2005 reported that in the UK, all mesothelioma can be accounted for by exposure to amphibole alone.
- Berman & Crump presented that one of the major difficulties in interpreting these studies is that the original exposure estimates rarely differentiated between chrysotile and amphiboles.
- Lung burden analysis has confirmed in the important South Carolina cohort that amphiboles were used in textile production and as a result could account for much if not all of the ascribed effect (Case, 2000).

Comparison with other fibers

- **Chrysotile:**
  - $T_{1/2} (L>20 \mu m) = 0.3 – 11.4$ days
- **Synthetic Mineral fibers with European Commission exoneration from classification as a carcinogen:**
  - $T_{1/2} (L>20 \mu m) < 10$ days
- **Ceramic Fibers (RCF 1):**
  - $T_{1/2} (L>20 \mu m) = 50$ days
- **p-aramid fibrils (after 90 days exposure with comparative cumulative dose of fibers with L>20 \mu m as in 5 day biopersistence study):**
  - $T_{1/2} (L>20 \mu m) = 45$ days
- **Celullose fibers:**
  - $T_{1/2} (WHO fibers) = 1046$ days to infinity
- **Amphibole (e.g. amosite) Asbestos:**
  - $T_{1/2} (L>20 \mu m) = 466$ days to $\infty$
Histopathological comparison of chrysotile and tremolite:

- In the biopersistence study of Calidria chrysotile asbestos, the lungs were examined histopathologically.
  - Inflammation
  - Fibrosis
- In addition, for amphibole Tremolite asbestos the biopersistence and histopathology were also evaluated.
Control Lungs (exposed to filtered air)
At 90 day - inhalation toxicology study of chrysotile
(Bernstein et al., 2005b)

Lung exposed to exposed to chrysotile for 90 days
90 day - inhalation toxicology study of chrysotile
(Bernstein et al., 2005b)
Lung exposed to tremolite for 5 days
Inhalation biopersistence study of tremolite
(Bernstein et al., 2005a)

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Short Fibers

- Report of the Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length, issued recently by the Agency for Toxic Substances and Disease Registry (ATSDR), it was stated that:

  “Given findings from epidemiologic 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)
Conclusions

- Taken in context with the scientific literature to date, these studies provide new robust data that clearly support the difference seen epidemiologically between chrysotile and amphibole asbestos.

- The studies reviewed today confirm that at the current workplace standards, exposure to chrysotile would not be hazardous.

- They also suggest that the hazard would be low if even high exposures were of short duration.

- Indeed, like other mineral dusts, there is evidence that as used 30 – 40 years ago, humans could have developed lung cancer from exposure to chrysotile asbestos, when the exposure was very high and sustained for long periods.

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