UNDERSTANDING CHRYSOTILE ASBESTOS:

A new understanding based on current data

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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?
- Surface composition / effects
  - If the fiber / particle is durable, can it cause cytotoxic effects.

In the non exposed lung, one or two macrophages reside in each alveolus in a near sterile environment.

Respiratory Bronchiole

Macrophage

Alveolar Duct

Type II
Pneumocyte

Alveolar Sac

Reference: Bernstein, David M. Fiber Biopersistence, toxicity and asbestos. Journal of University of Occupational and Environmental Health (UOEH), volume 25 supplement 1, page 237-243, 2003.

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Immediately after Exposure
many particles and short and long fibers are present

Respiratory Bronchiole

Alveolar Duct

Type II
Pneumocyte

Alveolar Sac

Following Early clearance only long fibers remain

Respiratory Bronchiole

Alveolar Duct

Type II
Pneumocyte

Alveolar Sac

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Bio-Soluble fibers disappear rapidly

rapid return to normal

Respiratory Bronchiole

Alveolar Duct

Type II
Pneumocyte

Alveolar Sac

With durable fibers the long fibers remain

chronic inflammation

Respiratory Bronchiole

Alveolar Duct

Type II
Pneumocyte

Alveolar Sac

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Glass Fibers following Intratracheal Instillation

Reference: Bernstein, D.M., R.T. Drew and M. Kuschner. Pathogenicity of man-made mineral fibers and the contrast with natural fibers. In: Biological Effects of Man-made Mineral fibres. World Health Organization, Copenhagen (1983), ISBN 92 980 12471.

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Glass Fibers following Intratracheal Instillation

Intratracheal Instillation of Glass Fibers

References: Bernstein, D.M., R.T. Drew and M. Kushner. Pathogenicity of man-made mineral fibers and the contrast with natural fibres. In: Biological Effects of Man-made Mineral Fibres. World Health Organization, Copenhagen (1983), ISBN 92 980 12471.

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Glass fibers following Intratracheal Instillation

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- 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

Fiber Biopersistence Correlates with Pathological Response

- Inhalation carcinogenicity studies
- Intraperitoneal Injection studies

References:

Bernstein, D. M., Riego-Sintes, J.M., Ersboell, B.K., and Kunert, J., Biopersistence of Synthetic Mineral Fibers as a Predictor of Chronic Inhalation Toxicity in Rats. Inhalation Toxicology, Vol 13 (10), pp. 823-849, 2001.

Bernstein, David M., Riego-Sintes, Juan M., Ersboell, Bjarne Kjaer and Kunert, Joachim, Biopersistence of Synthetic Mineral Fibers as a Predictor of Chronic Intraperitoneal Injection Tumour Response in Rats. Inhalation Toxicology, Vol 13 (10), pp. 851-875 2001.

European Commission

- Commission Directive 97/69/EC of 5 December 1997
- Adopting for technical progress for the 23rd time Council Directive 67/548/EEC (classification and labeling of dangerous substances).

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 can be shown that the substance fulfils of the following conditions:

- a short-term Biopersistence test by inhalation has shown that the fibers 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 suitable long term inhalation test.

International Agency for Research on Cancer 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 tumors in experimental 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.

- Working Group: David Bernstein, Vince Castranova, Ken Donaldson, Bice Fubini, John Hadley, Tom Hesterberg, Agnes Kane, David Lai, Gene McConnell, Hartwig Muhle, Gunter Oberdorster, Steve Olin, David Warheit

‘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 composed of many smaller fibrils.

Serpentine and amphibole fibers

Serpentine
- Chrysotile

Amphibole
- Tremolite
- Amosite
- Crocidolite

Chrysotile Structure

Si
Mg

Si
Mg

With Chrysotile, the magnesium in the lattice is on the outside of the curved surface and is available for dissolution by the lung fluid.	(Si2O5)n (Mg3O2(OH)4)n ●OH

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How chrysotile forms:

(a) (c)

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Tremolite Structure (amphiboles)

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How amphibole splits:

Biopersistence of Chrysotile

- The EC Biopersistence Study is designed to measure how fast fibers are eliminated from the lung.
- To date 3 different chrysotiles have been studied:
  - Canadian chrysotile
  - Calidria chrysotile (California, USA)
  - Brazilian chrysotile (Cana Brava Mine)

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.

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Clearance of Calidria Chrysotile and Tremolite Fibers with length > 20 µm

Number of Fibers L > 20 µm remaining in the lung

Time since cessation of exposure (days)

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Clearance of Chrysotile from the lung
Fibers with length > 20 µm

Percent remaining of fibers L > 20 µm (day 1 = 100%)

- ● **Calidria Chrysotile L > 20 µm**
  Open pit mine
- ❖ **Brazilian Chrysotile L > 20 µm**
  Extremely high exposure
- ▪ **Canadian Chrysotile L > 20 µm**
  Textile grade

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Chrysotile Asbestos Clearance Half-Times

FIBER	CLEARANCE HALF-TIME ($T_{1/2}$) (days)		REFERENCE
	FIBER LENGTH > 20 µm	FIBER LENGTH 5 - 20 µm	
Calidria chrysotile	0.3	7	Bernstein et al., 2003b
Brazilian chrysotile	1.3	2.4	Bernstein et al., 2004a
Canadian chrysotile (Textile grade)	11.4	29.7	Bernstein et al., 2004b

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.

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

Mg

Mg

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Chrysotile in the lung > particles

Chronic Inhalation Toxicology Studies

- In nearly all previous chronic inhalation toxicology studies the animals were exposed to huge concentration of fibers.
- The total fiber concentration was typically more than one million fibers/cm^3 of air (Mast et al., 1995)
- At such concentrations, even inert dusts such as Titanium di-Oxide have been shown to be carcinogenic in animals.
- These very high exposure concentrations result in *rat-specific lung overload* (Oberdorster, 1995a, 1995b, 2002) which invalidates the study

To correctly evaluate the inhalation toxicology of chrysotile:

- We performed what is termed a ‘Sub-chronic inhalation toxicology study’ of chrysotile following the protocol design recommended by the European Commission.
- A similar study is also recommended by the US EPA.
- Groups of animals were exposed to
  - Group 1: 76 fibres L> 20 µm/cm^3 (3413 total fiber/ cm^3) or
  - Group 2: 207 fibres L> 20 µm/cm^3 (8941 total fiber/ cm^3)
- For 6 hours/day, 5 days/week for 90 days

Results from the sub-chronic inhalation toxicity study with chrysotile:

- Though 90 days of exposure and 92 days of recovery without further exposure.
- Exposure to chrysotile at a mean concentration of 76 fibres L>20 µm/cm³ (3413 total fiber/cm³) resulted in no fibrosis (Wagner score 1.8 to 2.6) at any time and only a minimal increase in PMNs.
- 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, 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 et al., 2005).
- 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.

Epidemiology

- However, 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.

Lung at Day 1 – Confocal Microscopy

Lung at Day 90 – Confocal Microscopy

Comparison with other fibers

- Chrysotile:
  - $T_{½}$ (L>20µm) = 0.3 – 11.4 days
- Synthetic Mineral Fibers with European Commission exoneration from classification as a carcinogen:
  - $T_{½}$ (L>20µm) < 10 days
- Ceramic Fibers (RCF1)
  - $T_{½}$ (L>20µm) = 50 days
- p-aramid fibrils (after 90 days exposure with comparative cumulative dose of fibers with L >20µm as in 5 day biopersistence study):
  - $T_{½}$ (L>20µm) = 45 days
- Cellulose fibers:
  - $T_{½}$ (WHO fibers) = 1046 days to infinity
- Amphibole (e.g. amosite) Asbestos:
  - $T_{½}$ (L>20µm) = 466 days to ∞

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.

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Pulmonary Region (Human)

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Control lungs (exposed to filtered air)

At 90 day – inhalation toxicology study of chrysotile (Bersntein et al., 2005b)

Lung exposed to chrysotile for 90 days

90 day – inhalation toxicology study of chrysotile
(Bersntein et al., 2005b)

Lung exposed to tremolite for 5 days

Inhalation biopersistence study of tremolite
(Bersntein et al., 2005a)

Interstitial fibrosis

Interstitial fibrosis

Granulomas
with collagen

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 epidemiological studies, laboratory animal studies and in vitro genotoxicity studies, combined with the lung’s ability to clear short fibers, the panelists agreed that there is 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.

Conclusions

- Indeed, like other mineral dusts, there is evidence that humans can develop lung cancer from exposure to chrysotile asbestos, *when the exposure is very high and sustained for long periods*.
- The value of these studies is that they show that at low exposure pure chrysotile is probably not hazardous.
- They also suggest that the hazard may be low if even high exposures were of short duration.
- It would be most helpful if future studies on chrysotile and amphiboles whether in vitro or in vivo could be performed at doses approaching those which humans have been exposed.