# FIBER DURABILITY AND BIOPERSISTENCE – ASSESSMENT AND ROLE IN ASBESTOS TOXICOLOGY

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With Thanks to David M. Bernstein
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# CONTENTS

# BIOPERSISTENCE - BACKGROUND

- European Commission Directive 97/69/EC

  Adopted in December of 1997

- "Biopersistence of fibers > 20µm in length is a good predictor of lung burden and early pathological changes in chronic inhalation studies with synthetic mineral fibers" (Bernstein, et al., 2001).

# BIOPERSISTENCE - BACKGROUND

- Biopersistence – the ability of a material (fiber) to persist in the lung in spite of the lung's physiological clearance mechanisms and environmental conditions.
  - Fibers L>20µm
- Clearance of Fibers L>20µm as well as other fibers and particles.
  - Dissolution
  - Mucociliary Clearance via Tracheobronchial Tree
  - Lymphatic Clearance
  - Fragmentation/Disintegration (long fibers)

# BIOPERSISTENCE - BACKGROUND

EC Protocol for Biopersistence of Fibers. Short-Term Exposure by Inhalation

- Rats exposed nose-only 6h/day for 5 consecutive days (1 Fiber Group and 1 Control Group/sacrifice time)

- Well characterized fiber aerosol with a mean aspect ratio of at least 3:1 and at least 100 fibers/$cm^3$ L>20µm and diameter of about 0.8 µm and <40mg/$m^3$, approximate mass lung burden 0.5 to 1mg

- Sacrifice animals at 1 day, 2 days, 3 days, 14 days, 4 weeks, 3 months, 6 months, and 12 months.

# EC PROTOCOL (Continued)

- Freeze drying followed by low temperature plasma ashing of lungs of a minimum of 5 animals/group

- TEM evaluation of fibers at 10,000X
  - Classify fibers
    >20μm
    5-20μm
    <5μm
    WHO fibers (L>5μm, D<3μm)
    Non fibers (Aspect ratio <3:1)

- Clearance $T_{1/2}$

  Single exponential ($T_{1/2}$)

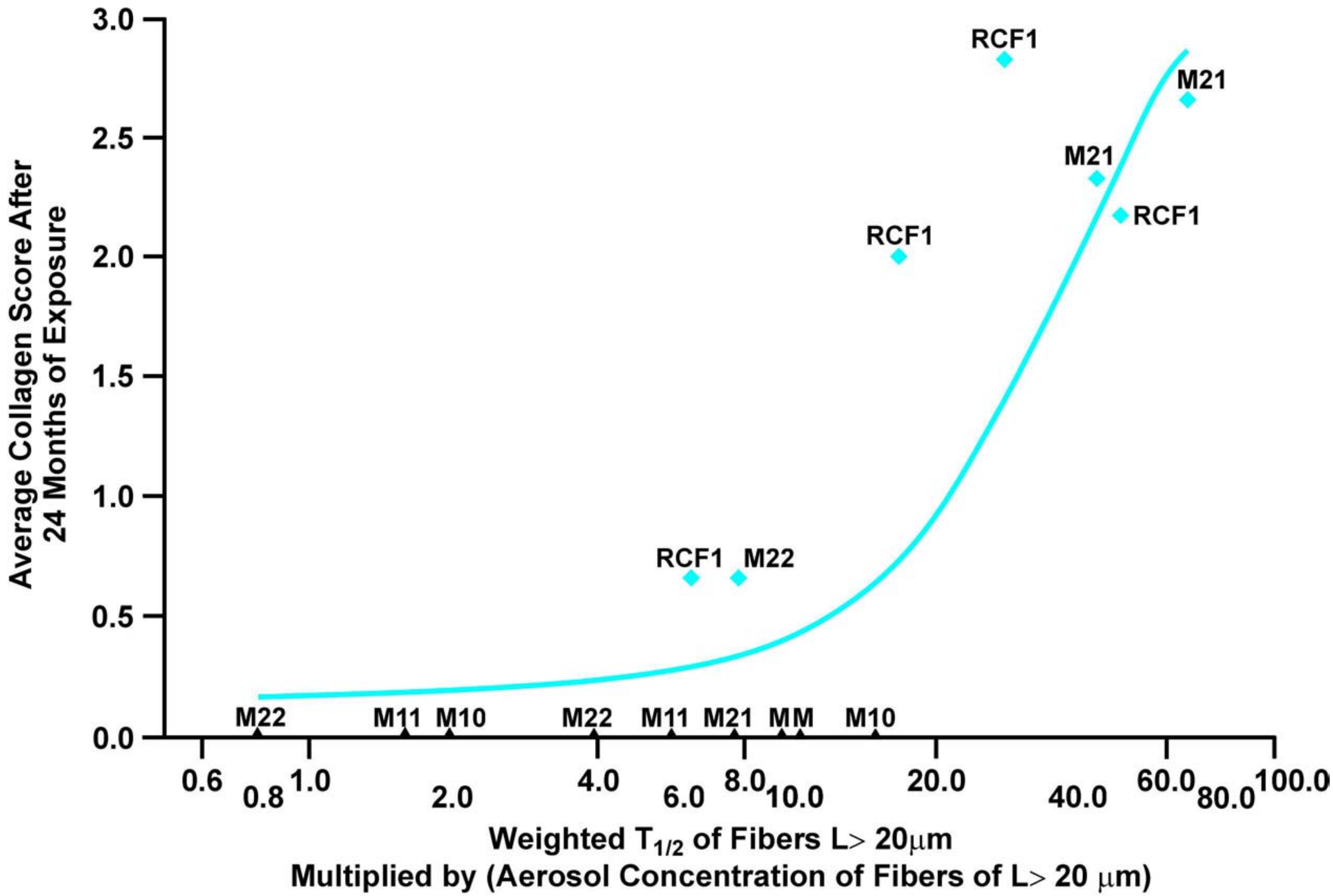
  Double Exponential with weighted half life ($WT_{1/2}$)

- Other End Points – Histopathology and Confocal Microscopy

http://ecb.jrc.it/DOCUMENTS/Testing-Methods/mmmfweb.pdf

# BIOPERSISTENCE BACKGROUND

## Logistic Regression to Predict the Collagen Score from the Inhalation Biopersistence Weighted $T_{1/2}$ of Fibers of L>20 μm




Bernstein et al., 2001

5075-1

# BIOPERSISTENCE - BACKGROUND

EC Directive: Fiber Not Classified as Carcinogen if:

- Inhalation Biopersistence: Fibers $L>20\mu m$ have $WT_{1/2}$ <10 days
- Intratracheal Biopersistence: Fibers $L>20\mu m$ have $WT_{1/2}$ <40 days
- Intraperitoneal Carcinogenicity: Not Carcinogenic
- Chronic Inhalation: Not Carcinogenic and absence of relevant pathogenicity

(Bernstein, et al., 2001; Hesterberg and Hart, 2001)

# The biopersistence of Canadian chrysotile asbestos following inhalation

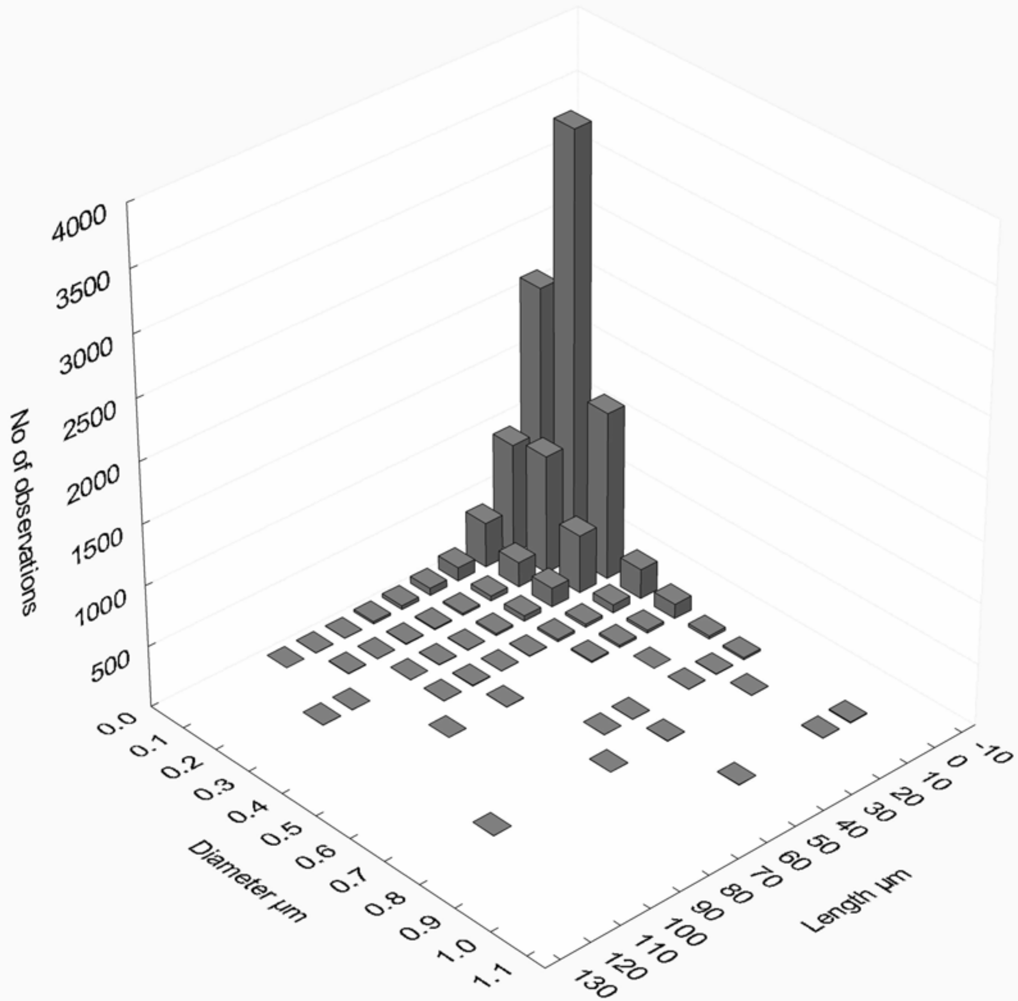
David M. Bernstein, Rick Rogers, Paul Smith

# CANADIAN CHRYSOTILE (CONTINUED)

- Wistar Rats Exposed 6h/day for 5 consecutive days
- Chrysotile – Canadian, Commercial Textile Grade, which is longest grade for Textile use
- Exposure Atmosphere
  - 200 fibers L>200μm/cm$^3$
  - 4.32 mg/m3
  - 14,800 Total Fibers/m$^3$
  - 1850 WHO Fibers/m$^3$
  - GMD 0.12μn (99% L>20 had D<0.8μm)

**Chrysotile Fibers in the Exposure Atmopshere**

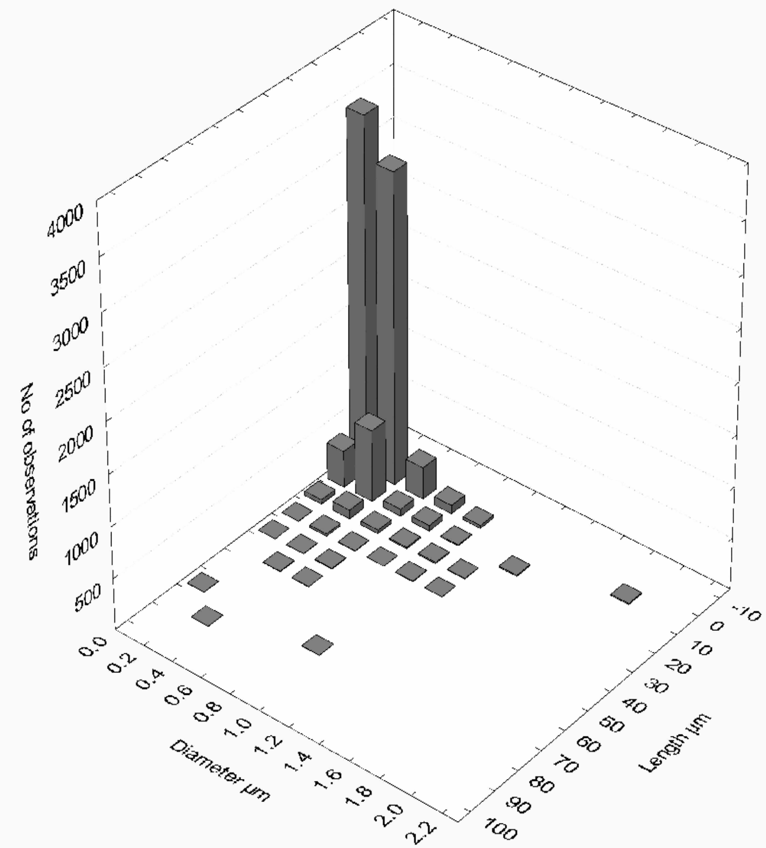
Bivariate Length-Diameter Histogram of WHO Fibers



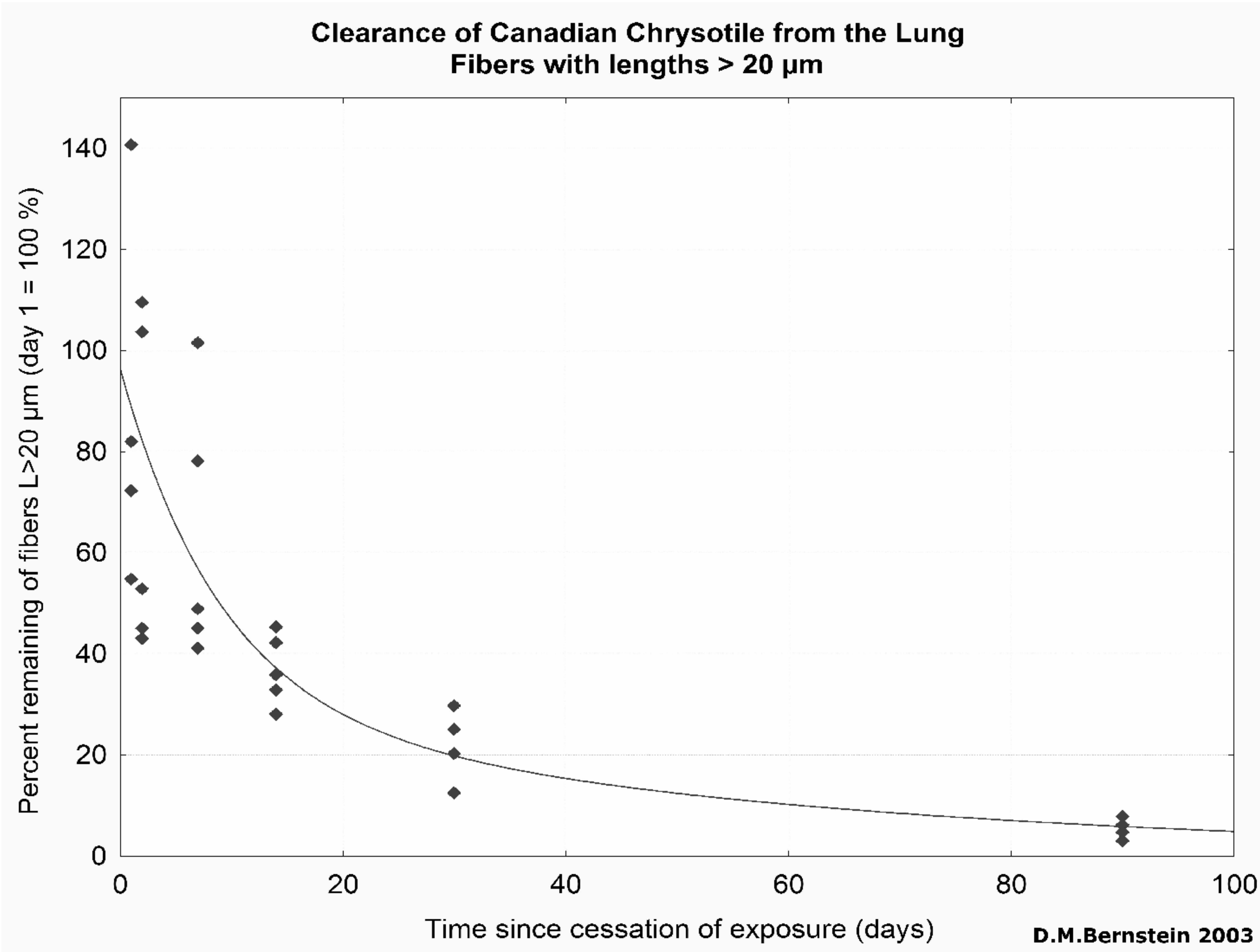

**D.M.Bernstein 2003**

## Chrysotile Fibers in the Lung at 1 Day after Cessation of Exposure

Bivariate Length-Diameter Histogram of WHO Fibers

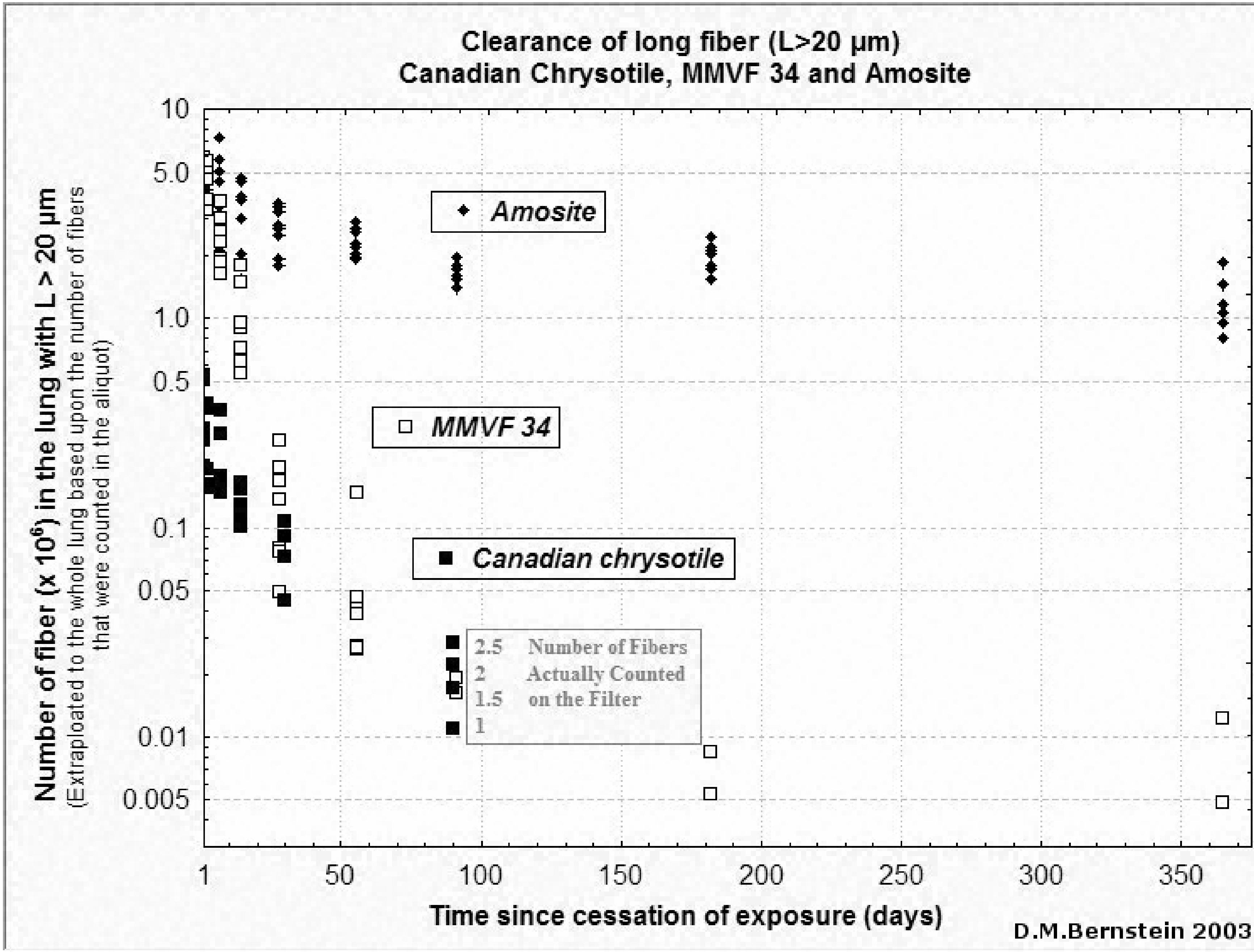



D.M.Bernstein 2003


Clearance of Canadian Chrysotile from the Lung
Fibers with lengths > 20 µm
Percent remaining of fibers L>20 µm (day 1 = 100 %)
0
20
40
60
80
100
120
140
0
20
40
60
80
100
Time since cessation of exposure (days)
D.M.Bernstein 2003

# CANADIAN CHRYSOTILE

| Fiber length | Clearance Half-time $T_{1/2}$ |
|---|---|
| > 20 µm | $WT_{1/2}$ = 16 days |
| 5 – 20 µm | $T_{1/2}$ = 29.4 days |
| < 5 µm | $T_{1/2}$ = 107 days |



Clearance of long fiber (L>20 µm)
Canadian Chrysotile, MMVF 34 and Amosite
Amosite
MMVF 34
Canadian chrysotile
2.5 Number of Fibers
2 Actually Counted
1.5 on the Filter
1
10
5.0
1.0
0.5
0.1
0.05
0.01
0.005
1
50
100
150
200
250
300
350
Number of fiber (x 10^6) in the lung with L > 20 µm
(Extrapoloated to the whole lung based upon the number of fibers that were counted in the aliquot)
Time since cessation of exposure (days)
D.M.Bernstein 2003

# DISTRIBUTION OF CHRYSOTILE FIBERS FROM CONFOCAL MICROSCOPY

- Short fibers not clumped together – separate fine fibrils with occasional unwinding at ends
- Short free fibers observed at corners of alveolar septa and within alveolar macrophages
- Fibers in lymphatics were free or within phagocytes
- Absence of neutrophil inflammatory response at times examined

# EC BIOPERSISTENCE PROTOCOL
# FIVE DAY INHALATION STUDY IN RATS

- U.S. CHRYSOTILE - 200 Fibers L>20µm/$cm^3$
- Tremolite (IOM) - 100 Fibers L>20µm/$cm^3$

| | |
|---|---|
| Chrysotile: | $T_{1/2}$ – less than 1 day |
| Tremolite: | no Clearance |
| Chrysotile | No histopathological changes through 90 days |
| Tremolite | 1 Day - nodules and granulomas |
| | 14 Days - focal fibrosis |
| | 90 Days - interstitial fibrosis |

(David M. Bernstein – Personal Communication, 2003b)

# BIOPERSISTENCE AND EFFECTS OF CHRYSOTILE CHRONIC STUDY WITH EVALUATION OF BIOPERSISTENCE

CHRYSOTILE - Jeffrey Mine Canada

10 mg/m$^3$

0 fibers L>20μm

10,600 WHO fibers/cm$^3$

Exposure for 104 weeks

14% of WHO fibers retained after 104 weeks exposure and 23 weeks of recovery, still 216x10$^6$ WHO fibers/lung

Lung Tumors – 12/69 (17.4%)

Mesotheliomas – 1/69 (1.4%)

Lung Overload ??

Hesterberg, et al., 1998

# BIOPERSISTENCE OF CROCIDOLITE (NIEHS)

Biopersistence Protocol: 287 Fibers L>20µm/$cm^3$
11 mg/$m^3$

**Fiber Half Times (Days)**

| Fiber | <5µ | >5µ WHO | >10w | >20µ |
|---|---|---|---|---|
| MMVF10 | 111 | 89 | 83 | 44 |
| MMVF1 | 46 | 35 | 22 | 6 |
| MMVF22 | 118 | 75 | 18 | 5 |
| Crocidolite | 44 | 234 | 142* | 986* (817) |

In previous studies MMVFs 10, 11, 22 produced no fibrosis, lung tumors, or mesotheliomas while crocidolite caused fibrosis by 3 months, and mesotheliomas and lung tumors.

*Data scatter and poor curve fits.

Hesterberg, et al., 1996;
Hesterberg, et al., 1998

# BIOPERSISTENCE OF AMOSITE

Biopersistence Protocol: 235 fibers L>20μm/cm$^3$
17mg/m$^3$
GML – 7.7μm
GMD – 0.48μm

| Fiber | WT1/2 (Days) for L<20μm |
|---|---|
| Amosite | 418 |
| RFC1a | 55 |
| MMVF21 | 67 |
| MMVF34 | 6 |

Short Amosite fibers (<5μm) cleared more rapidly than long fibers (>20μm)

Hesterberg, et al., 1998b

# COMPARISON OF BIOPERSISTENCE OF ASBESTOS FIBERS

## Fiber Half Times (Days)

| Fiber | <5µm | 5-20µ | >20µm |
|---|---|---|---|
| Amosite | NR* | NR | 418[1] |
| Crocidolite | 44 | (234)(142) | 817[1, 2] |
| Canadian Chrysotile | 107 | 29.4 | 16[3] |
| U.S. Chrysotile | NR | NR | <1[3] |
| Chrysotile (Cana-Brava Mine) | >2.4 | 2.4 | 1.3[4] |
| Tremolite | NR | NR | Very Long[3] |

*Clearance of fibers <5µm was faster than Clearance of fibers >20µm.

[1] Hesterberg, 1998
[2] Hesterberg, 1996
[3] Bernstein, 2003a, 2003b
[4] Bernstein 1999, 2000

# DIFFERENCES IN ASBESTOS FIBER BIOPERSISTENCE

| | |
|---|---|
| Chrysotile: | A serpentine composed of many fine fibers with Mg layer exposed |
| Amphiboles: | Rod like fibers where Mg is sandwiched in silicon layer |

## Typical chemical composition (percent)

| Compound | Chrysotile[1] | Tremolite[2] | Amosite[2] |
|---|---|---|---|
| $SiO_2$ | 40.6 | 55.10 | 49.70 |
| $Al_2O_3$ | 0.7 | 1.14 | 0.40 |
| $Fe_2O_3$ | 2.3 | 0.32 | 0.03 |
| FeO | 1.3 | 2.00 | 39.70 |
| MnO | -- | 0.10 | 0.22 |
| MgO | 39.8 | 25.65 | 6.44 |
| CaO | 0.6 | 11.45 | 1.04 |
| $K_2O$ | 0.2 | 0.29 | 0.63 |
| $Na_2O$ | -- | 0.14 | 0.09 |
| $H_2O^+$ | -- | 3.52 | 1.83 |
| $H_2$ | -- | 0.16 | 0.09 |
| $CO_2$ | 0.5 | 0.06 | 0.09 |
| Ignition loss | 14.0 | -- | -- |
| Total | 100 | 99.93 | 100.26 |

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

# DISSOLUTION

- In Vitro Dissolution - dissolution rates determined in neutral pH simulated living fluids using flow-through systems.

| | Dissolution Rate Constant (K) ($ng/cm^2/h$) |
|---|---|
| MMVF 10 | - 259 |
| MMVF 22 | - 23 |
| RCF-1 | - 8 |
| Crocidolite | - 0.3; 2* |
| Chrysotile | - Negligible |
| Amosite | - 8* |

*Estimated from In Vivo studies.

(Zoitis, et al., 1997;Law, et al., 1990; Eastes, et al., 2000)

# CONCLUSIONS

Chrysotile Asbestos Fibers L>20µm are less biopersistent than fibers L>20µm of Amosite, Crocidolite, or Tremolite.

Chrysotile <<<Amosite<Crocidolite<<Tremolite

Chrysotile fibers L>20µm appear to break up or dissolve in the lung.

There appear to be differences in the biopersistence of Chrysotile from different mines or locations.

# CONCLUSIONS

The biopersistence data for lung fibers supports the lower toxicity of Chrysotile fibers as opposed to amphibole fibers.

# SUMMARY

The EC short-term inhalation protocol appears to be a very useful study for comparing the potential toxicity of fibers, especially if it is coupled with histopathological characterization of pulmonary pathology.

- Use well-characterized fiber aerosols with comparable numbers of fibers >20μm.
- Should chronic inhalation fiber studies use levels that avoid "lung overload."

# REFERENCES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