

US EPA ARCHIVE DOCUMENT

Mechanisms of Fiber-Induced Lung Disease

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Physiochemical Factors Affecting Fiber Toxicity

I. Durability – dissolution, breakage

II. Dimensions

Diameter – deposition

Length – retention

Biopersistence = durability + failed clearance

III. Surface Activity

- a. Generation of reactive species
- b. Surface area
- c. Role of length – frustrated phagocytosis in activation of reactive species production

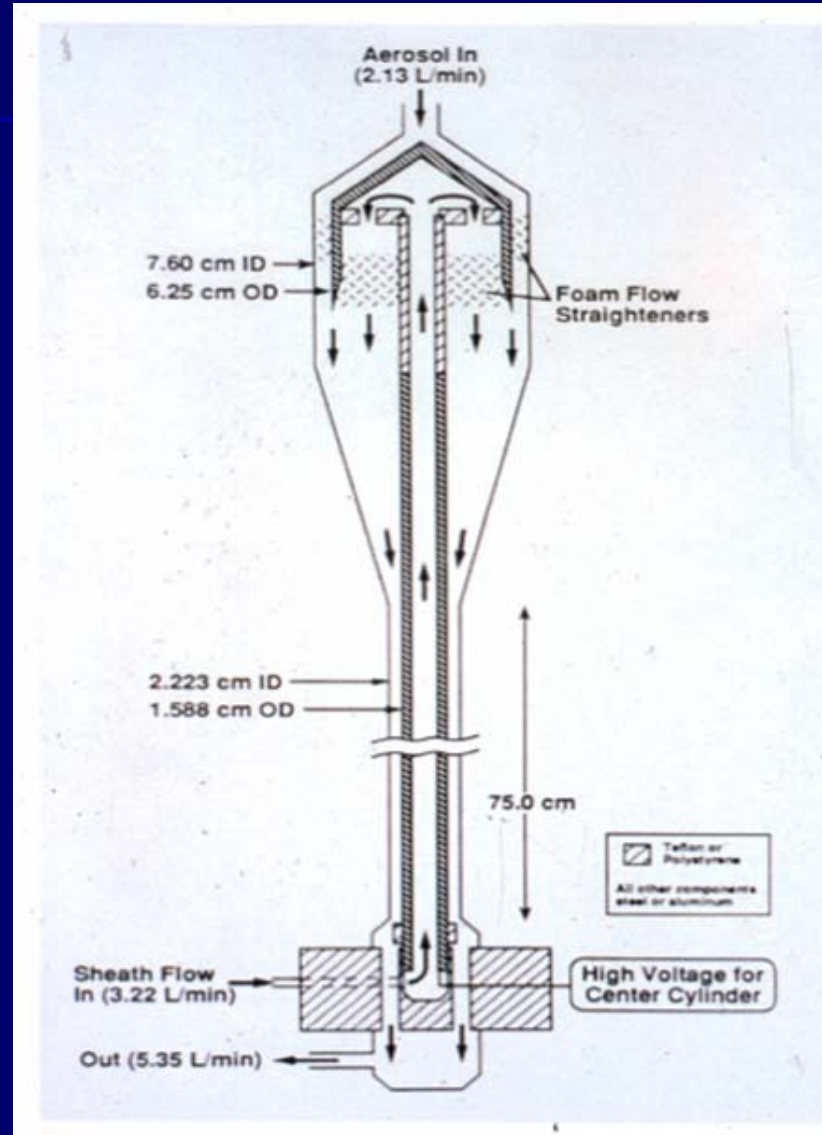
Problem

- Difficulty distinguishing between contributions of dimension vs. chemistry.

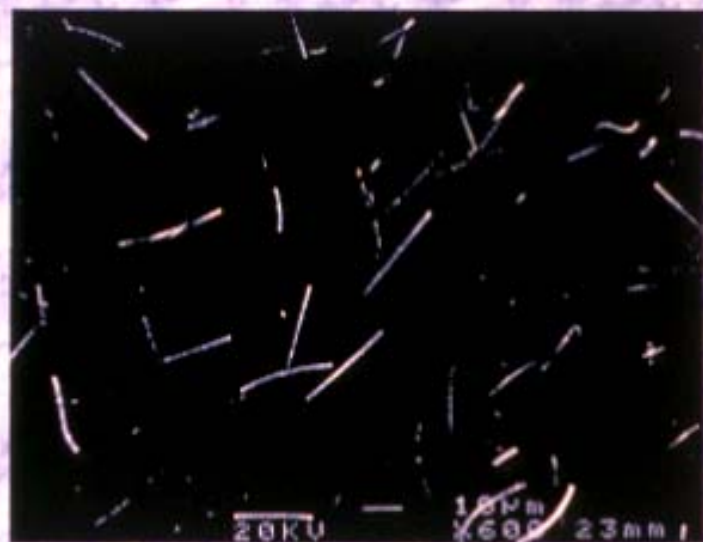
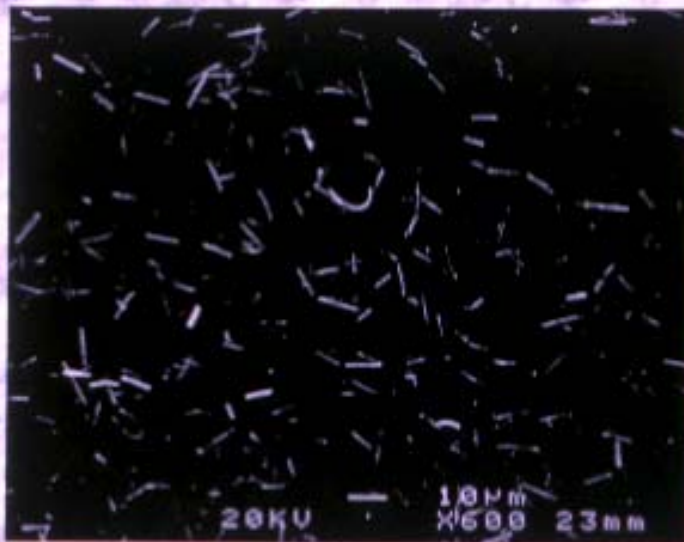
Approach

- Use of dielectrophoresis to obtain fiber samples of discrete lengths.

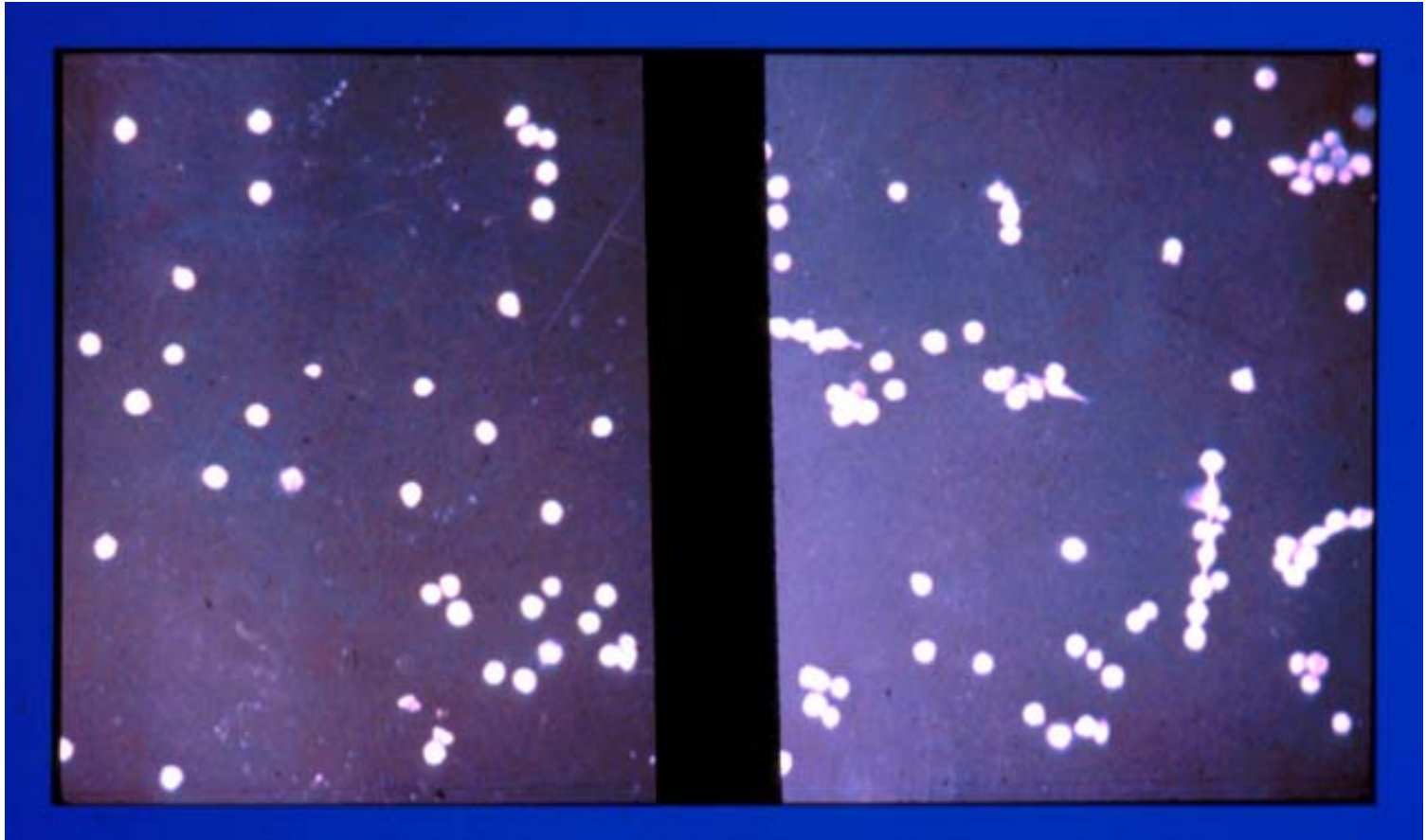
Dielectrophoresis System



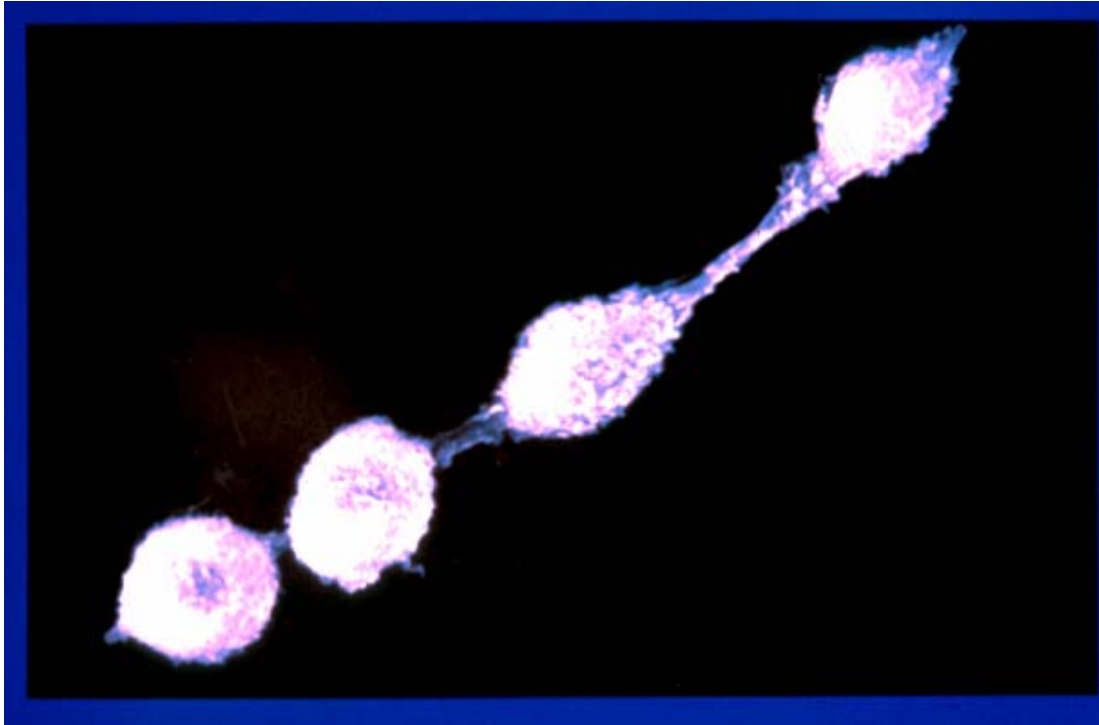
7 μm and 17 μm Glass Fiber Samples



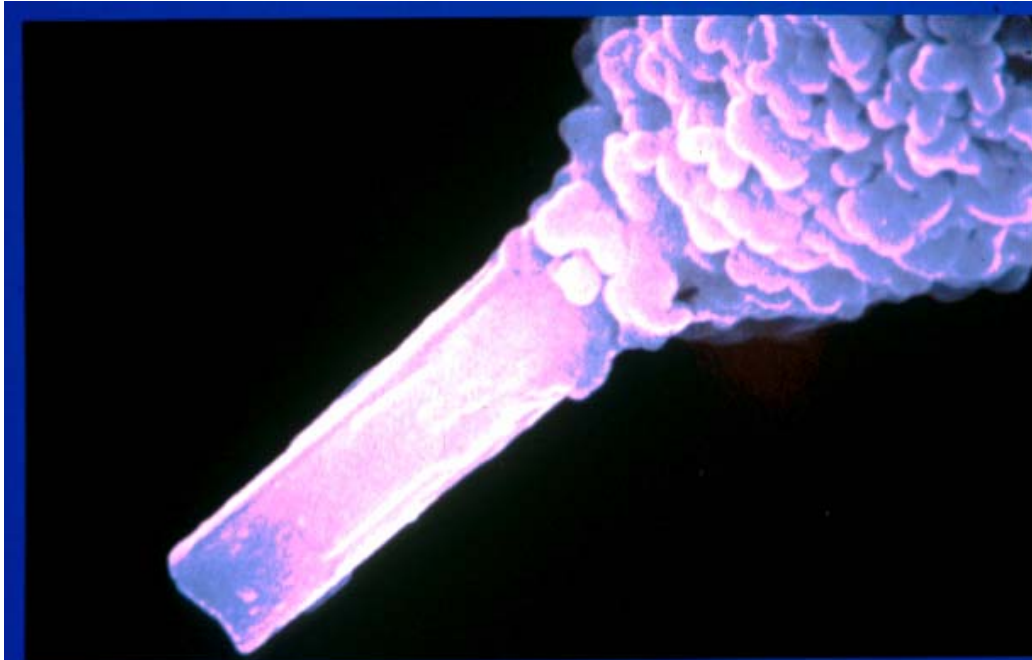
Fiber Uptake by Rat AM



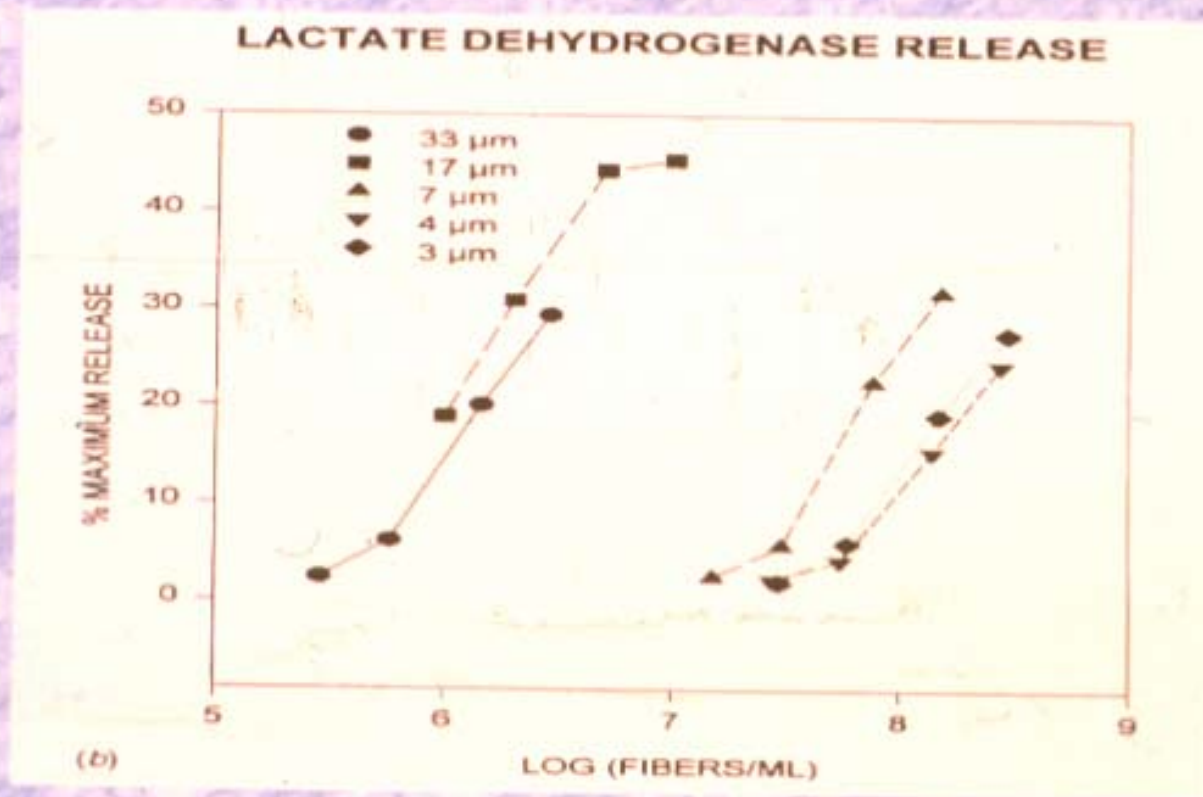
Frustrated Phagocytosis



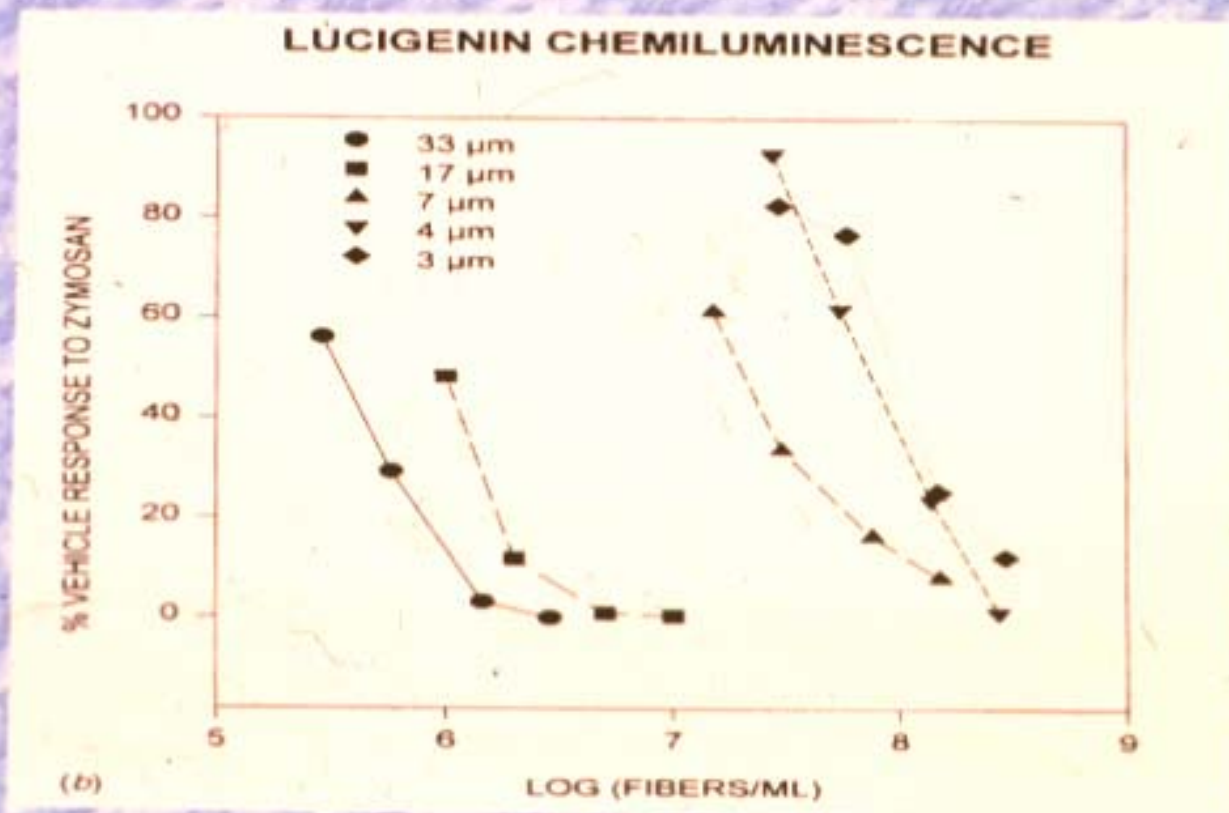
Activated AM and Frustrated Phagocytosis



Lactate Dehydrogenase Release

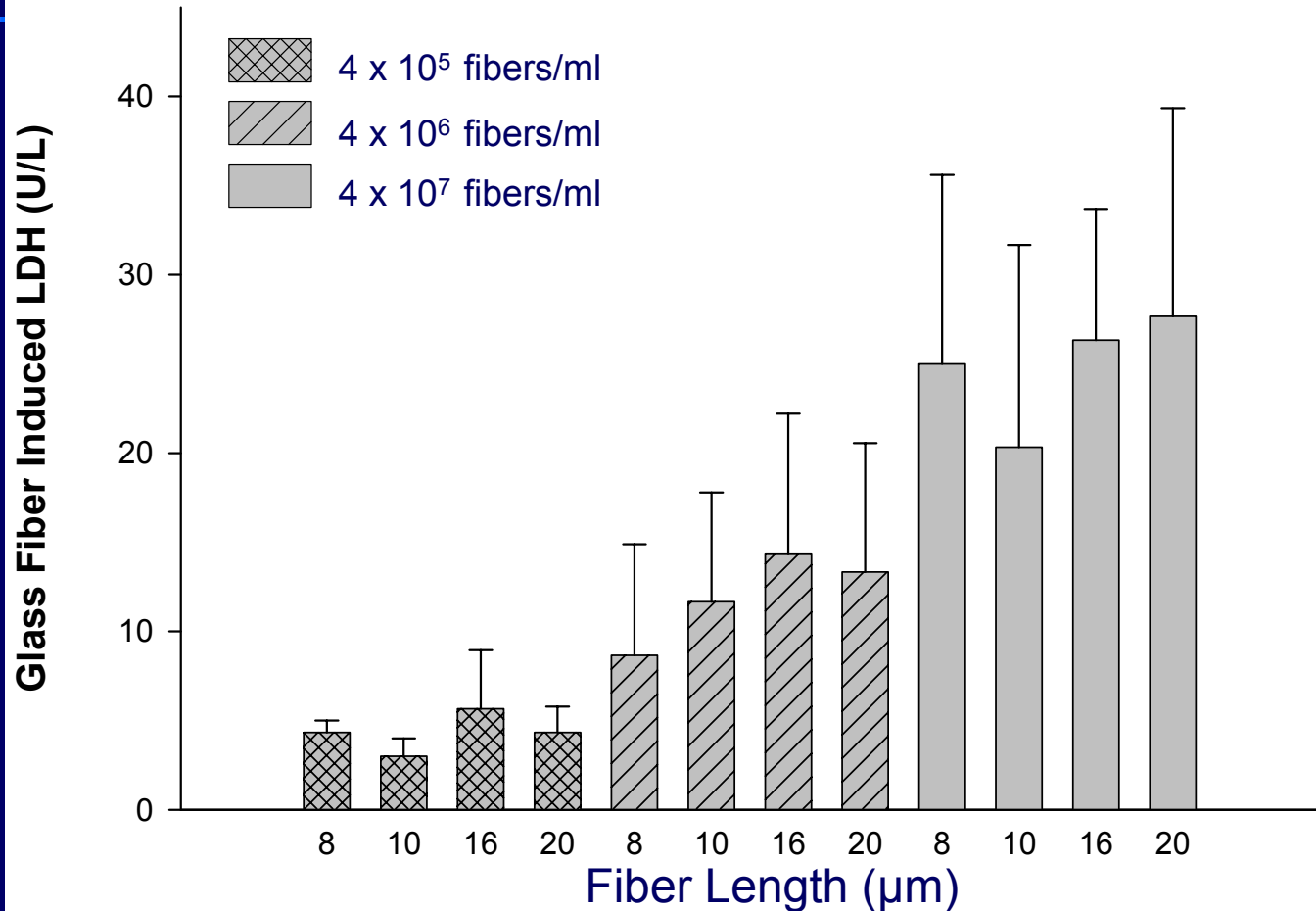


Lucigenin Chemiluminescence



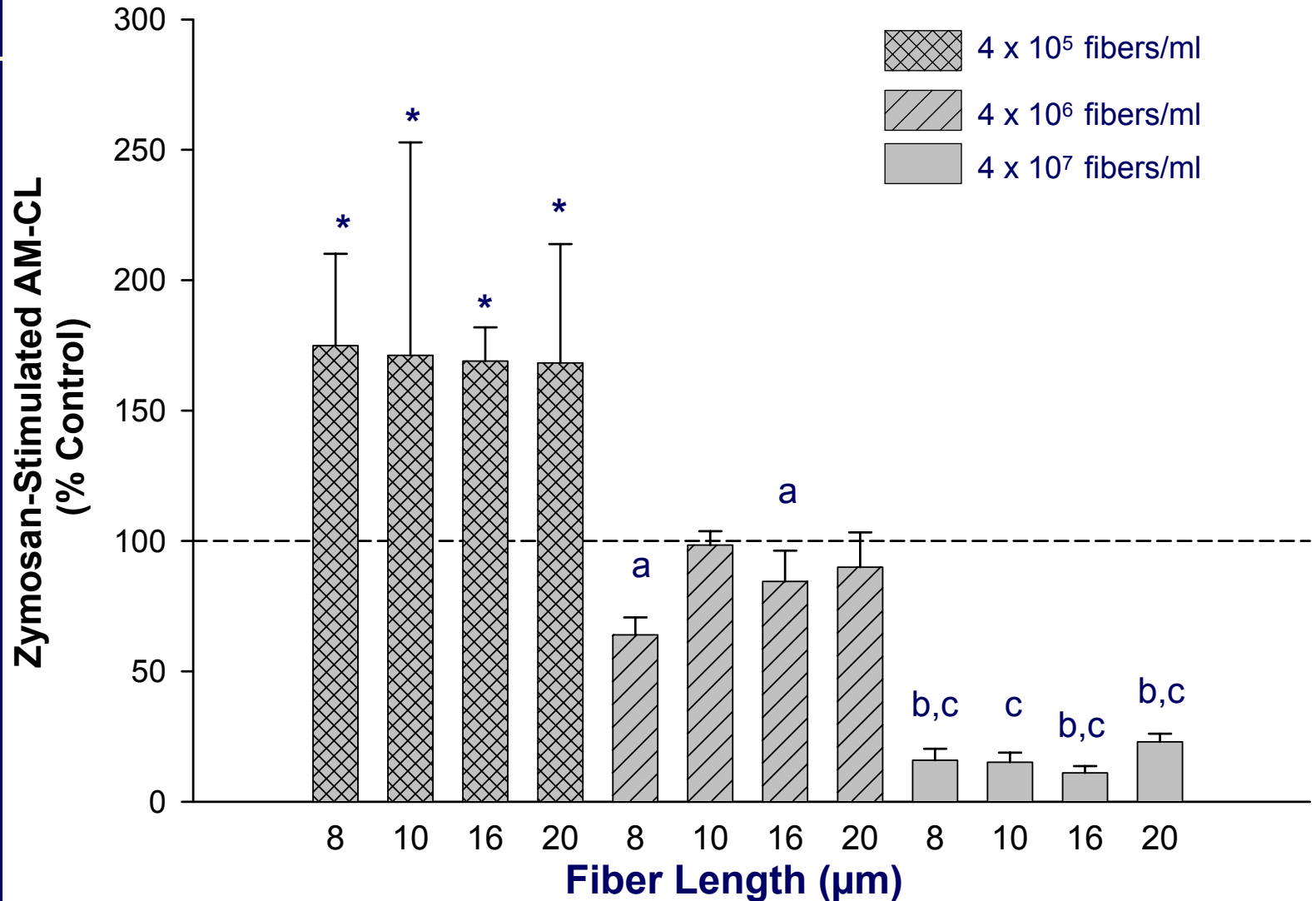
Human AM

Lactate Dehydrogenase Release



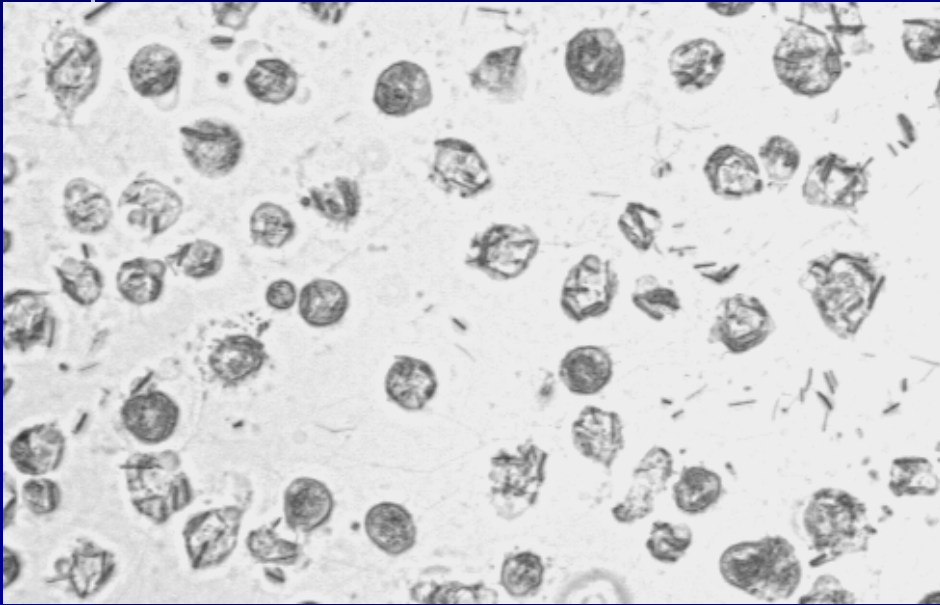
Human AM

Luminol Chemiluminescence

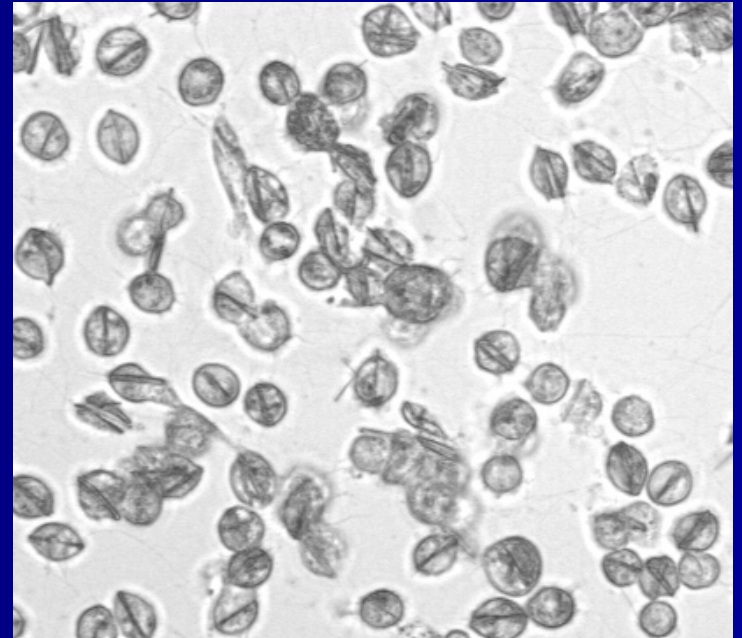


Fiber Uptake by Human AM

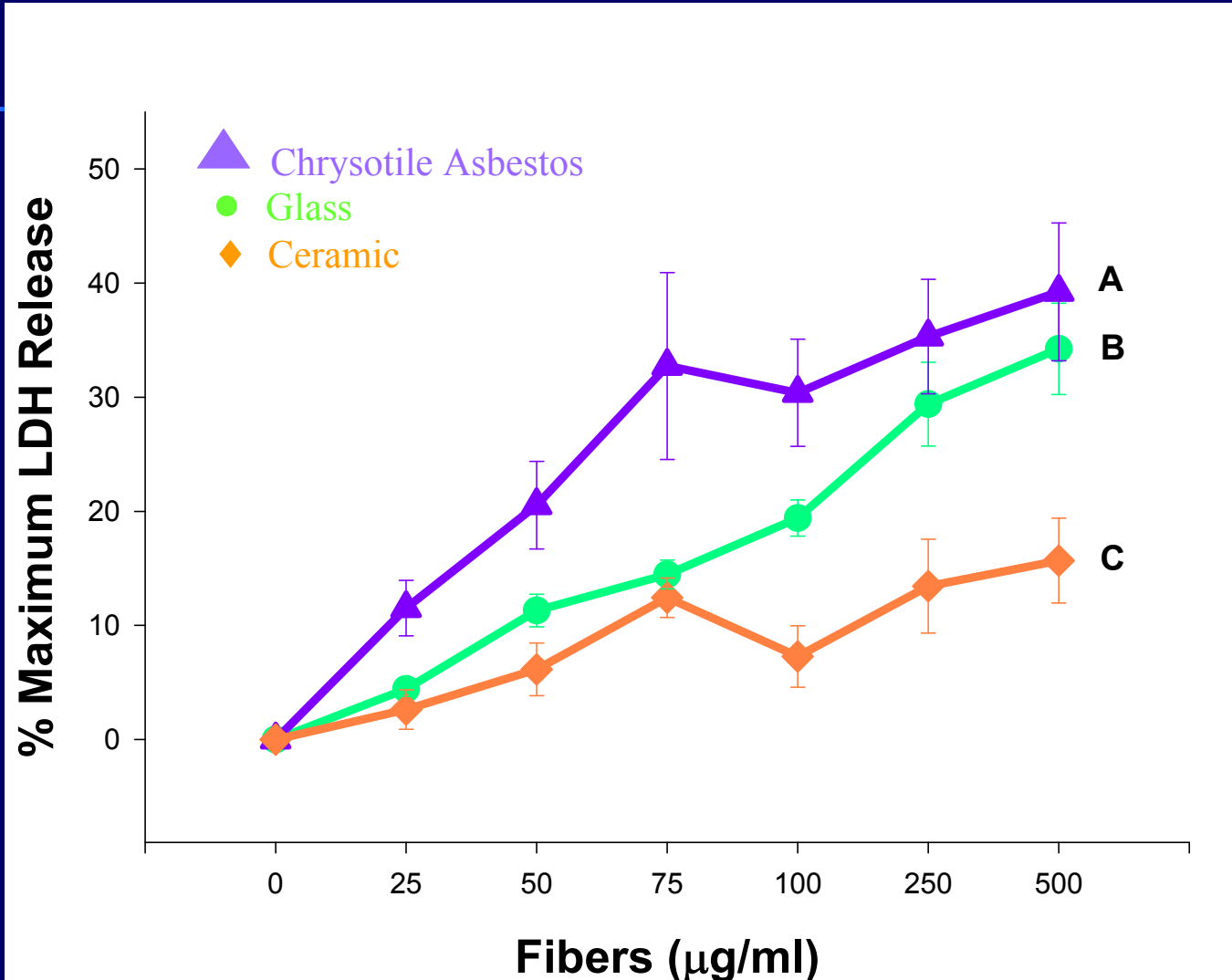
A.



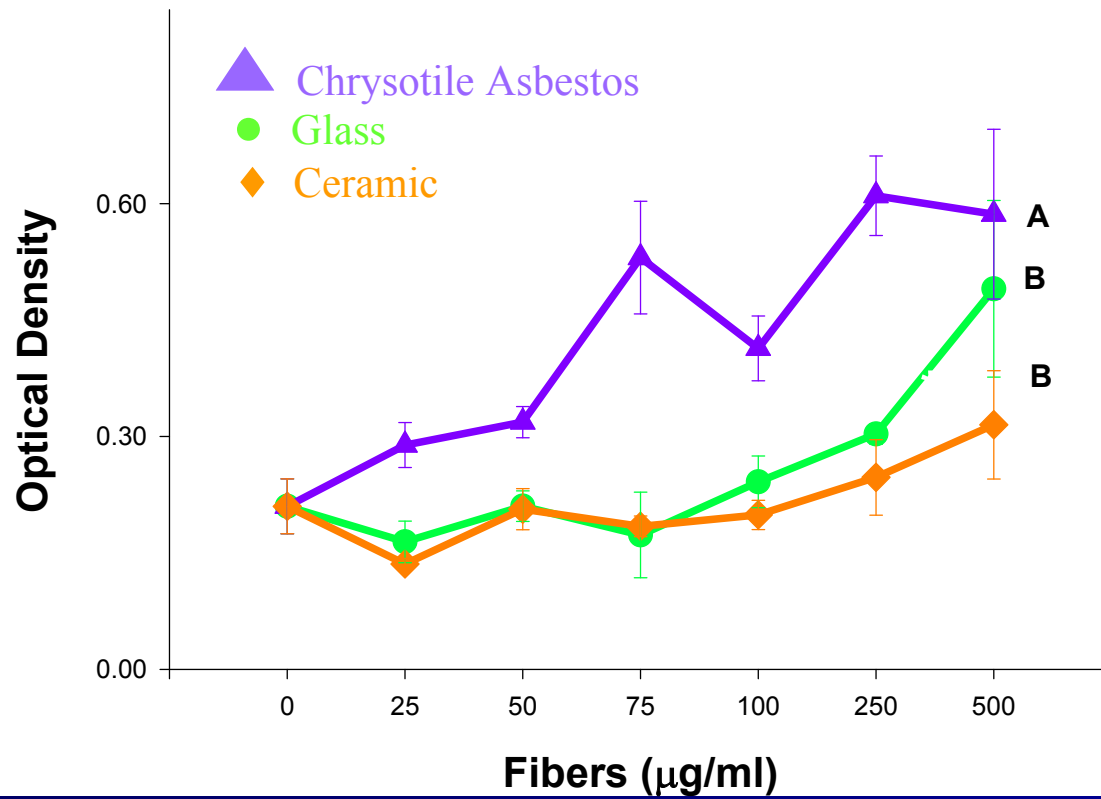
B.



Lactate Dehydrogenase Release



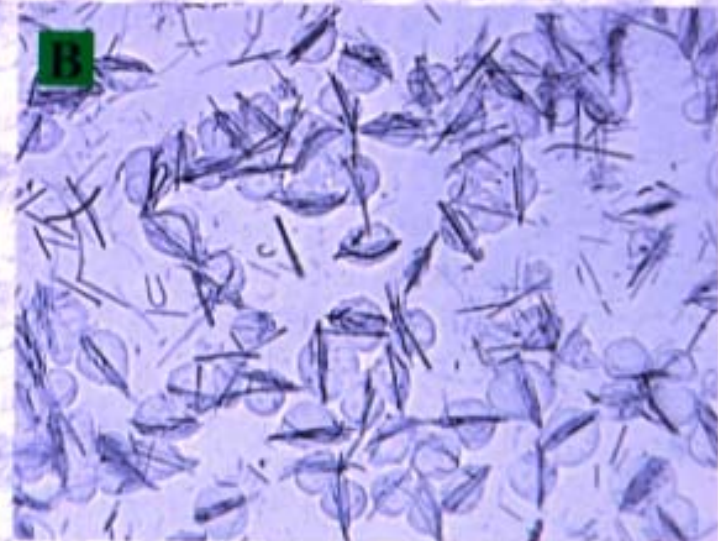
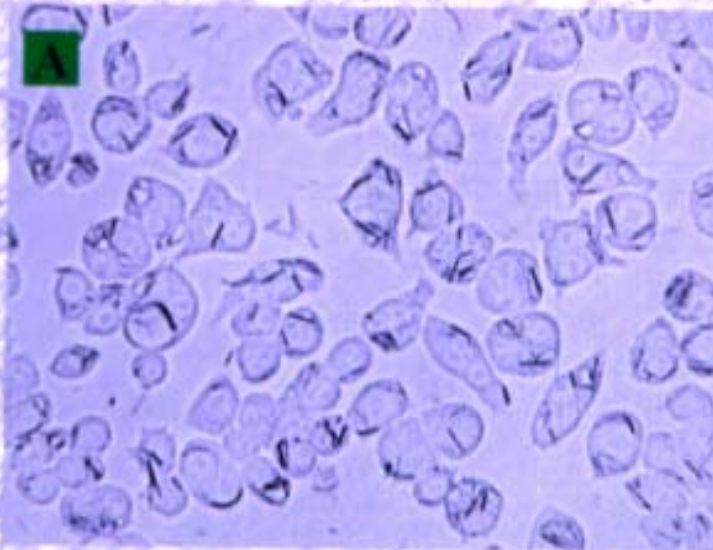
Apoptosis



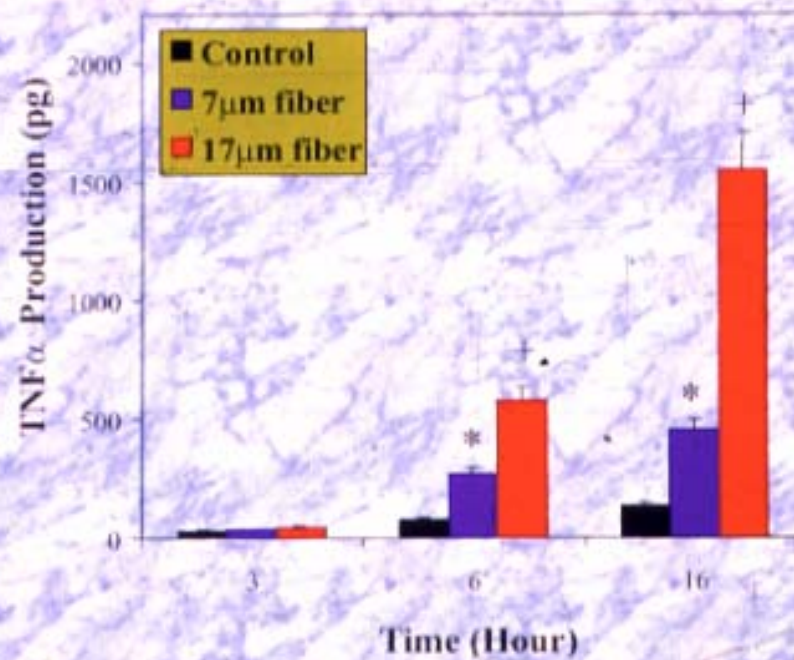
Conclusion I

- A. Dielectrophoresis is an effective method to separate fibers by length.
- B. With rat AM, fibers $\leq 7 \mu\text{m}$ in length are completely engulfed.
- C. With rat AM, fibers $\geq 17 \mu\text{m}$ in length are not completely engulfed, i.e., frustrated phagocytosis.
- D. With rat AM, glass fibers $\geq 17 \mu\text{m}$ in length are approximately 2 orders of magnitude more toxic than glass fibers $\leq 7 \mu\text{m}$ in length.
- E. With human AM, glass fibers $\leq 20 \mu\text{m}$ in length are completely engulfed, i.e., frustrated phagocytosis was not observed.
- F. With human AM, glass fibers 8-20 μm in length exhibited equal toxicity.
- G. With rat AM, chrysotile asbestos fibers were more toxic than glass or ceramic fibers of equal length

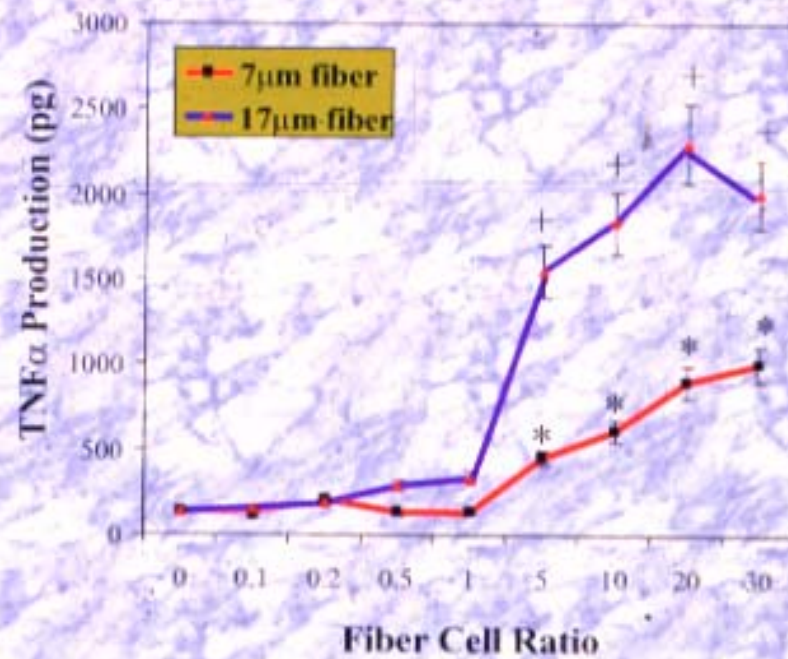
Morphology of RAW Cells Exposed to Glass Fibers



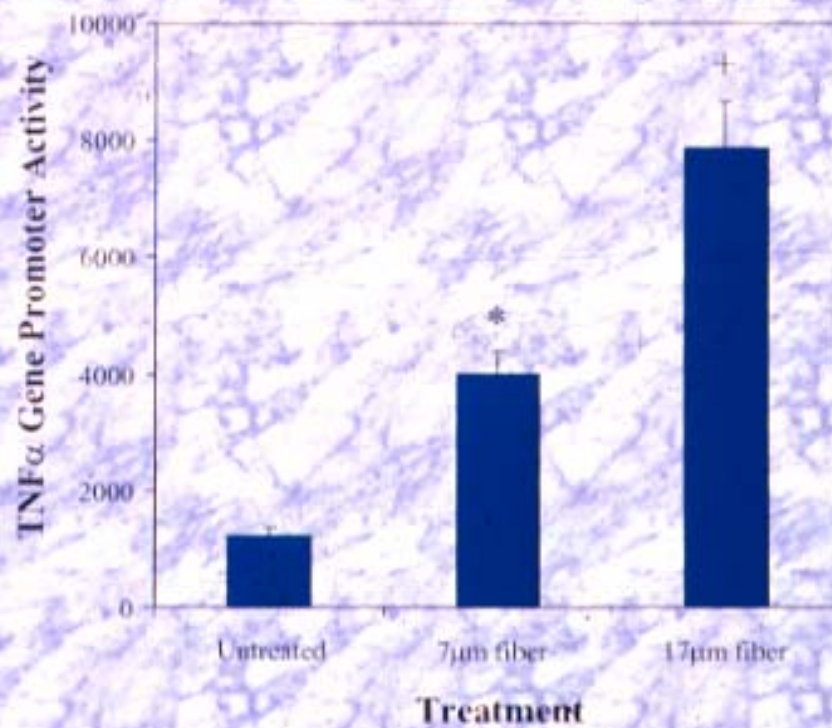
Time Course of TNF- α Production in RAW 264.7 Cells Exposed to Glass Fibers



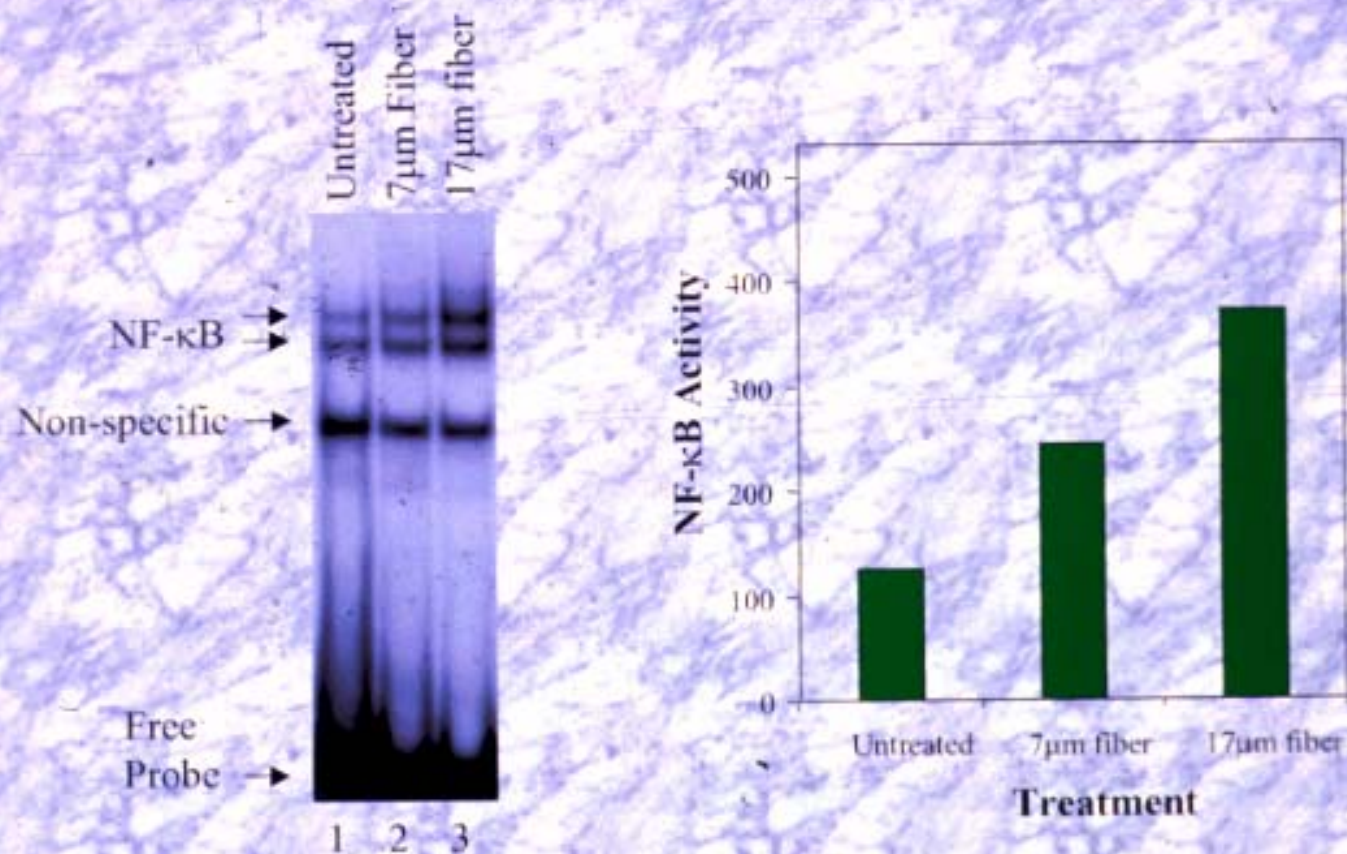
Dose-dependent Response of TNF- α Production with Glass Fibers



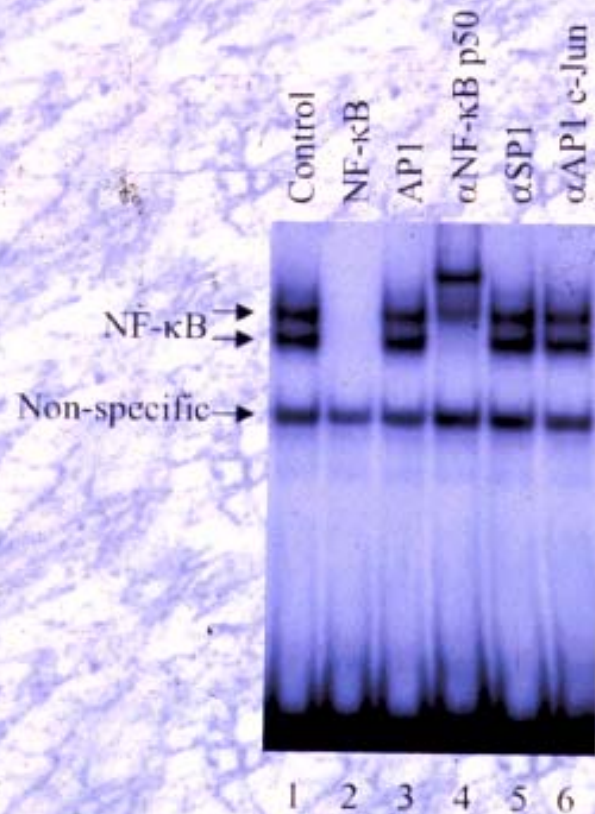
Activation of TNF- α Gene Promoter by the Glass Fibers



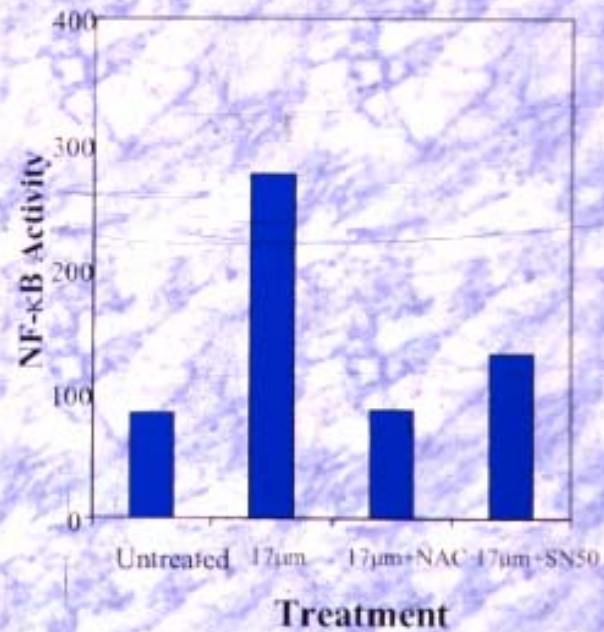
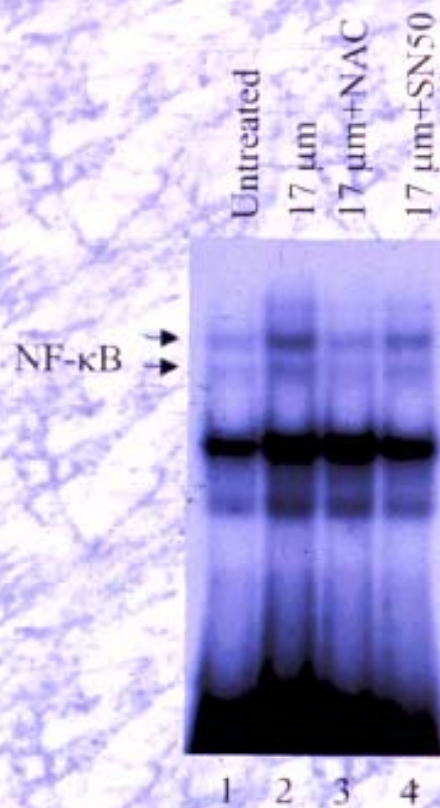
Activation of the DNA Binding Activity of NF- κ B by the Glass Fibers



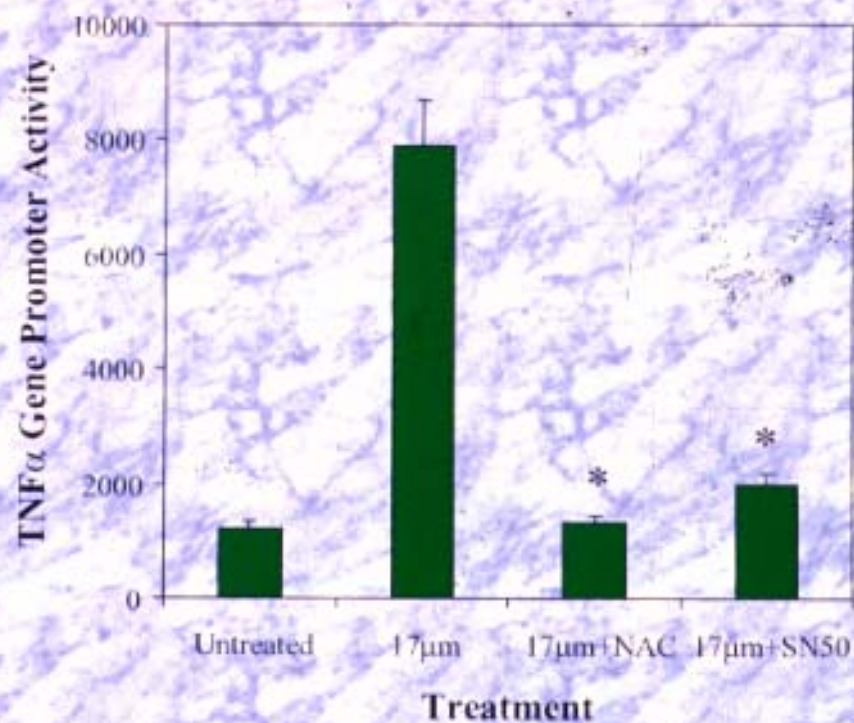
Characterization of NF- κ B Complexes by Oligonucleotide Competition and Antibody Supershift



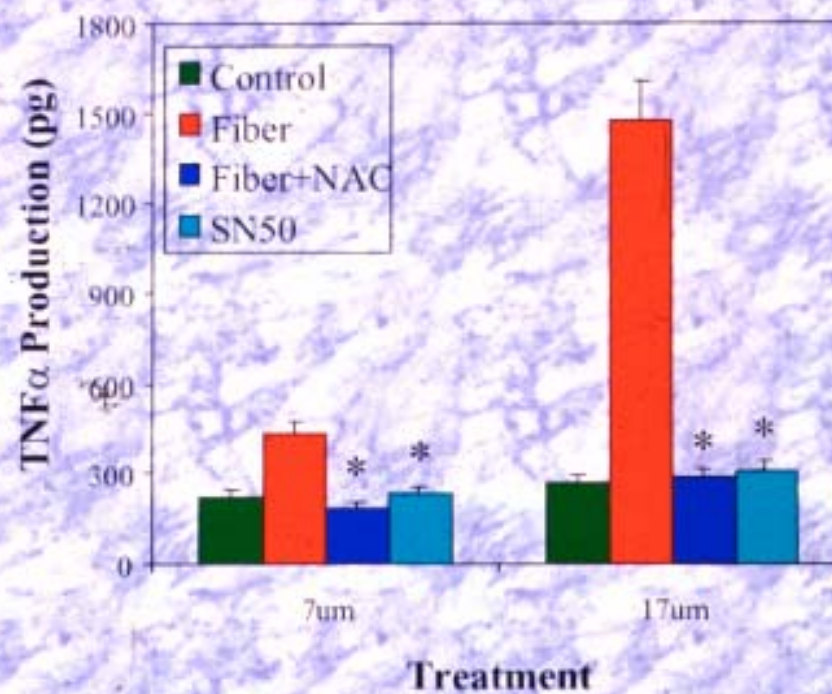
Suppression of NF- κ B Binding Activity by NAC or SN50



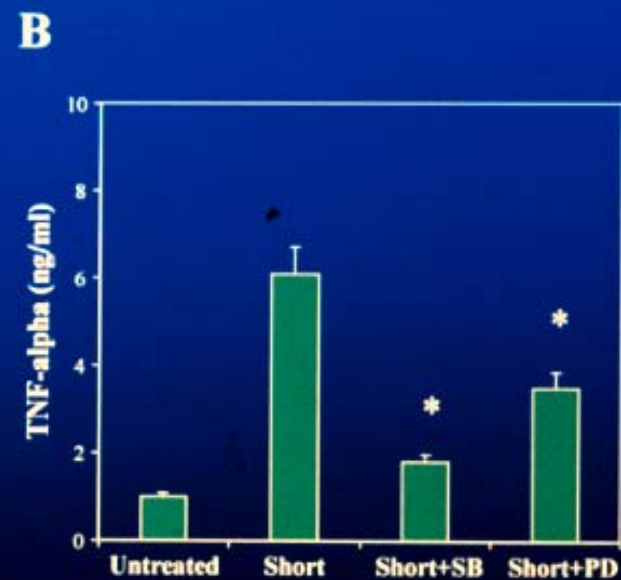
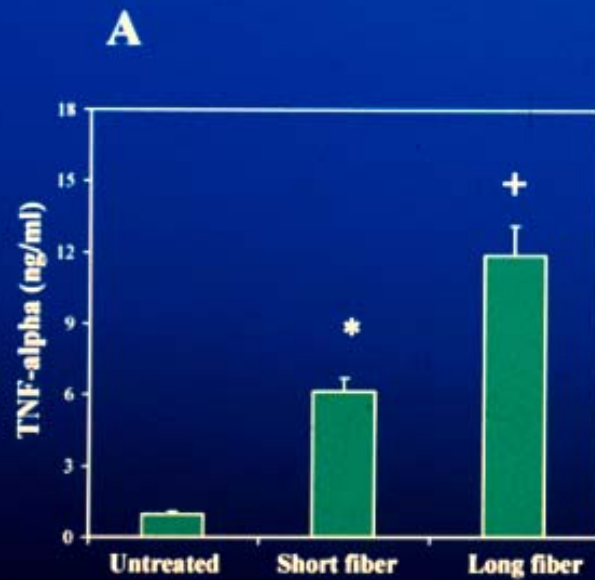
Inhibition of the TNF- α Gene Promoter by NAC or SN50



Inhibition of Fiber-induced TNF- α Production by NAC or SN50



Inhibition of TNF- α Production by MAP Kinase Inhibitors

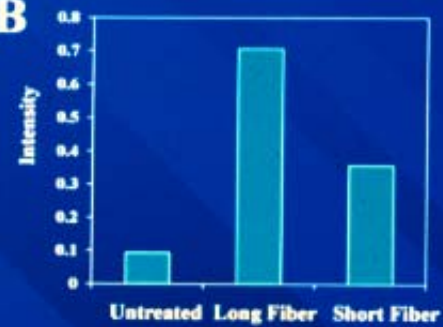


Phosphorylation of MAP Kinases p38 and ERK

A



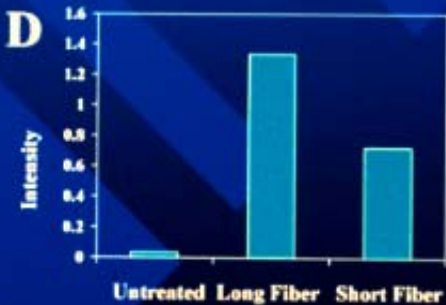
B



C

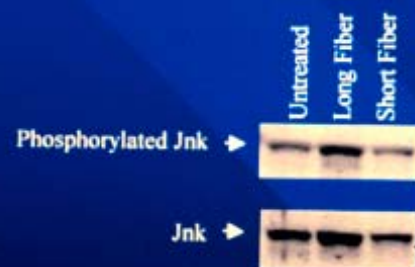


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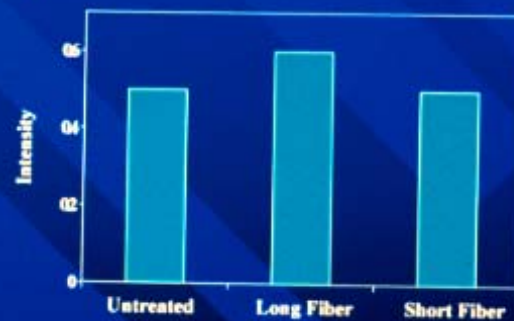


Phosphorylation of JNK after Glass Fiber Treatment

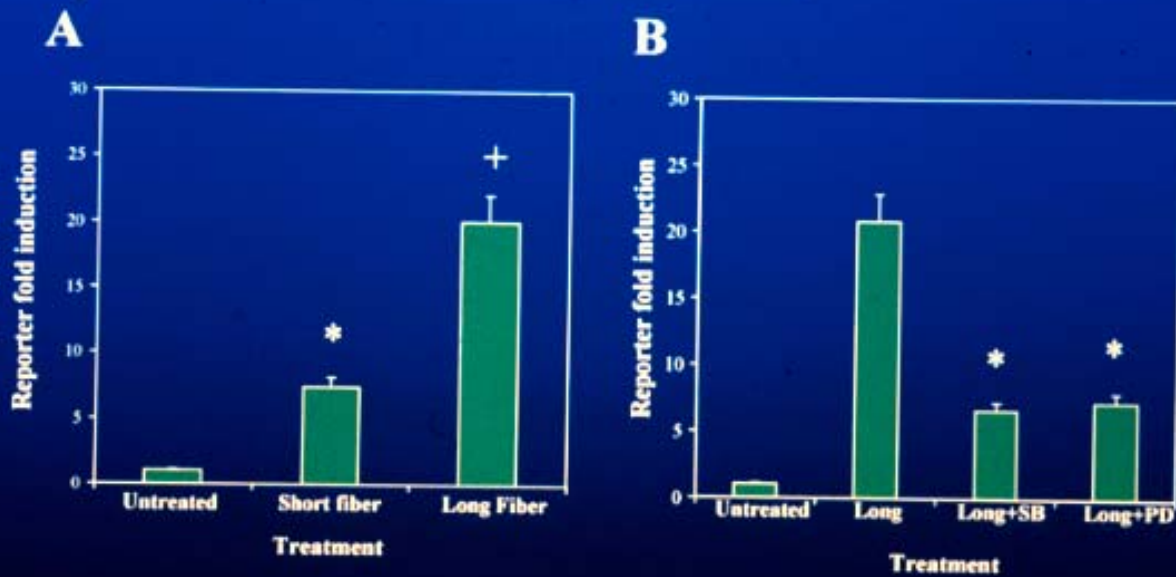
E



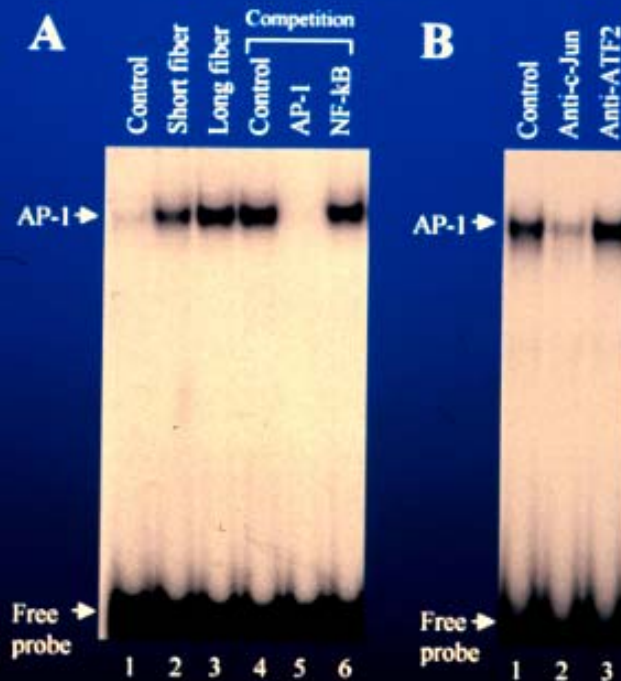
F



Inhibition of TNF- α Promoter Activity by MAP Kinase Inhibitors



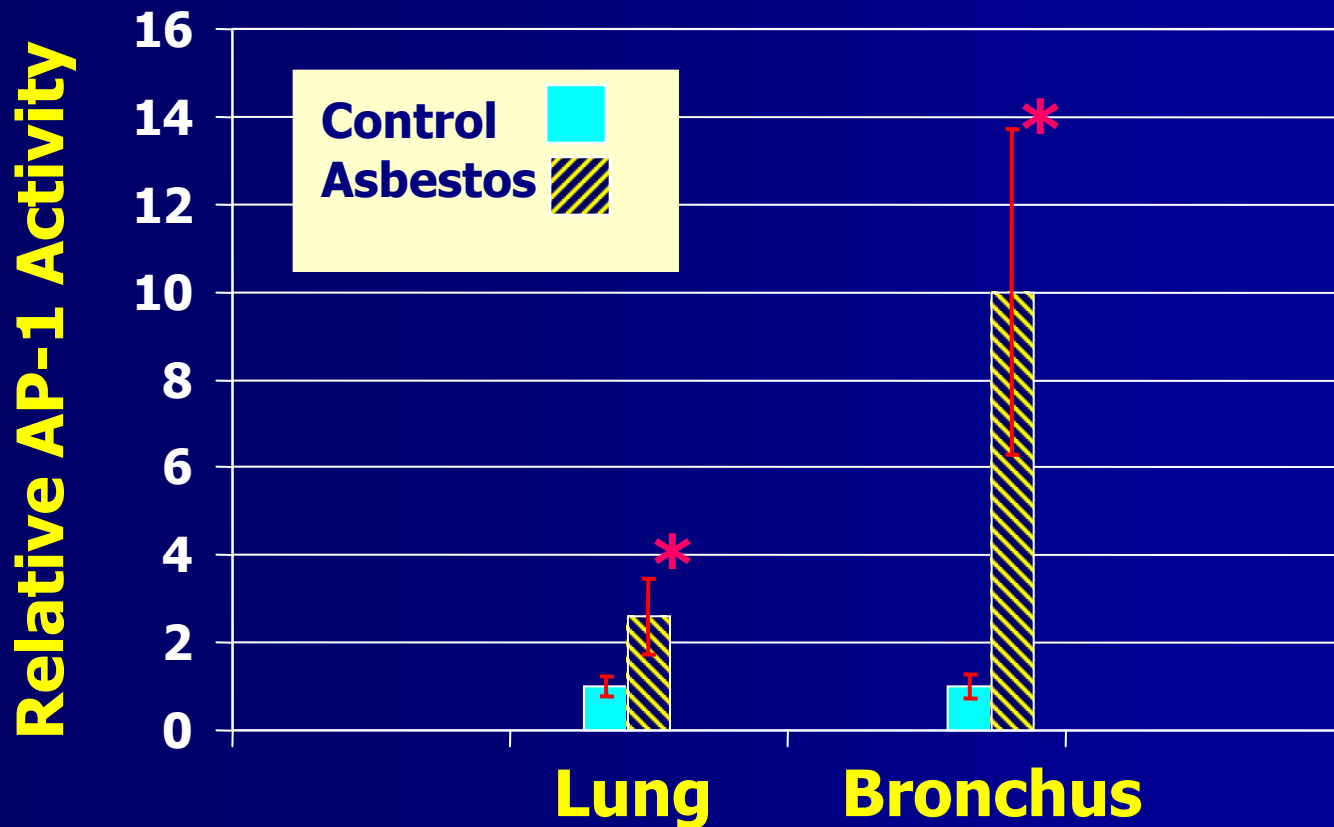
Activation of DNA Binding Activity of AP-1 in Rat Alveolar Macrophages by Glass Fibers



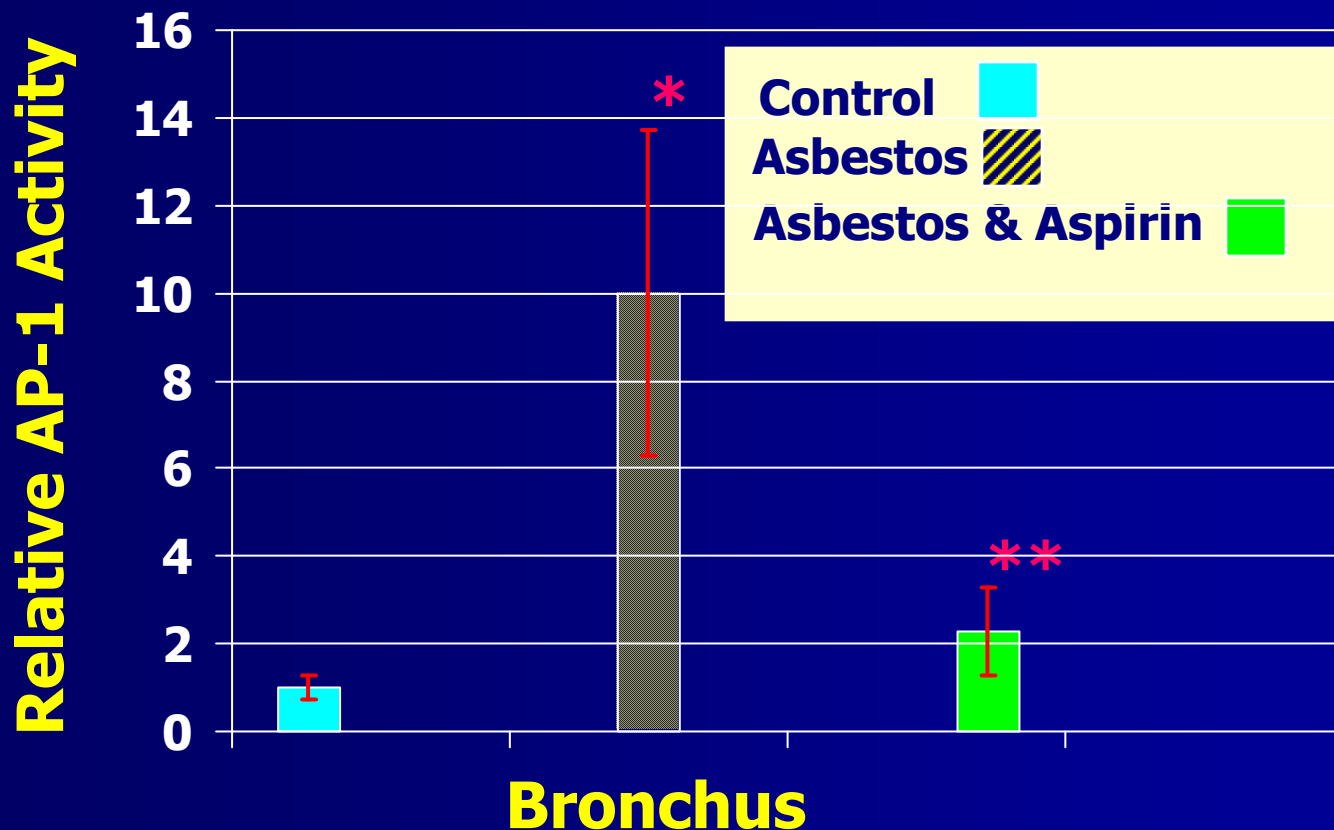
Conclusion II

- A. Exposure of macrophages to glass fibers results in:
 - NF κ B activation
 - phosphorylation of p38 and ERK
 - AP-1 activation
 - activation of the TNF- α gene promotor
 - TNF- α production
- B. Fiber-induced activation of the TNF- α gene promotor and TNF- α production is dependent on MAP kinase activity and NF κ B.
- C. Antioxidants inhibit fiber-induced activation of TNF- α gene promotion and TNF- α production.
- D. Long glass fibers (17 μ m) are more potent stimulants of signaling pathways and TNF- α production than short fibers (7 μ m), i.e., 2-3 fold more potent.
- E. The increased potency of long fibers in stimulating TNF- α production is explained by surface area.

Effect of IT Exposure to Crocidolite Asbestos on AP-1 Luciferase Reporter Transgenic Mice



Inhibition of Asbestos-induced AP-1 Activation by IP Aspirin Pretreatment



Conclusion III

- A. *In vivo* exposure to crocidolite asbestos induced AP-1 activation in mice.**
- B. Pretreatment with aspirin, an antioxidant, inhibited asbestos-induced AP-1 activation.**
- C. *In vitro* models appear to be useful in elucidating signaling pathways for fiber-induced cytokine production.**

Acknowledgements

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