

## **Appendix A**

### **List of Expert Panelists**



# Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers (SVF): The Influence of Fiber Length

## Panelists

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## **Appendix B**

### **Premeeting Comments, Alphabetized by Author (includes bios of panelists and the charge to the reviewers)**

Note: This appendix is a copy of the booklet of the premeeting comments that ERG distributed at the expert panel review meeting. The references for Dr. Lippmann's comments were inadvertently omitted from the booklets available at the meeting. Full citations for the references cited in Dr. Lippmann's premeeting comments are included in Appendix E (see pages E-4 through E-6).



**Division of Health Assessment & Consultation**

**Expert Panel on  
Health Effects of Asbestos and  
Synthetic Vitreous Fibers (SVF):  
The Influence of Fiber Length**

**Premeeting Comments**

New York, NY  
October 29-30, 2002

## **Notice**

This booklet includes the panelists' pre-meeting responses to the charge questions. It should be noted that the pre-meeting comments are preliminary in nature. The purpose of these comments is to stimulate meeting discussions. Some panelists' technical findings might change based on discussions during the meeting; therefore, pre-meeting comments should not necessarily be considered the panelists' final opinions.

Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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# Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length

## Charge to the Panel

The Agency for Toxic Substances and Disease Registry (ATSDR) is holding a panel discussion to review and discuss health effects associated with asbestos and synthetic (man-made) vitreous fibers (SVFs), especially those of less than 5 microns in length. ATSDR has invited a cross-section of scientific experts in the fields of toxicology, epidemiology, pulmonology/pathology, and medicine to participate in 12 days of discussions on a variety of topics, including depositional patterns of fibers in the lung and mechanisms of toxic action, the relationship of fiber size to toxicity, irritant effects of fibers, relationships between measured fiber levels and observed adverse health outcomes, and recommendations for future research. The meeting will have a public health focus, specifically related to evaluating environmental exposures and drawing public health conclusions associated with sites at which fibers and fibrous materials may be an issue. The agency will use input received during discussions to aid in developing scientifically defensible public health evaluations for human exposures to smaller-than-5-micron fibers.

### Background

ATSDR conducts public health assessments to evaluate possible public health implications of contaminants associated with hazardous waste sites and other environmental releases. A crucial part of this evaluation is the understanding of toxicological implications of exposure to substances that may be present. Recent events have highlighted a need to further explore the potential for health effects from exposure to biopersistent fibers, specifically asbestos and some SVFs. ATSDR is currently involved in several site assessments that address the potential for residential and community exposures to persistent fibers from past industrial operations (e.g., vermiculite processing plants across the country), hazardous waste sites, and dust generated from the World Trade Center (WTC) collapses in lower Manhattan. These sites are unique in that contaminant materials are/may be present in people's homes and communities. Additionally, there are potential concerns surrounding smaller length fibers which may have been generated by each of these past activities, especially in relation to the materials found in lower Manhattan.

Smaller fibers and non-fibrous particles may be generated as fibrous materials are processed, disposed of, or damaged, as in the case of the WTC collapses. In these situations, traditional fiber counting techniques may not quantify all of the materials present. Standard assessment methodology addresses fibers greater than 5 microns in length, based on the relative risk of longer fibers being greater than that of shorter fibers. Significant toxicology and occupational health research has focused on asbestos fibers and SVF greater than five microns in length, however, it seems that much less is known about the potential health effects of smaller fibers. ATSDR has identified a need to understand the potential for fibers less than 5 microns in length to contribute to adverse health effects.

*ATSDR is convening this panel to gain a greater understanding of asbestos and SVF toxicity, especially as it relates to fibers less than 5 microns in length.*

### Charge to Panel Members

The purpose of the panel is to discuss and summarize the best known science for each question. Consensus or specific advice on each question is not requested.

### ***Specific Charge Questions***

Discussions on the first day of the meeting will focus on answering questions that pertain to Topic #1, below. In asking these questions, ATSDR seeks a discerning review of the fate of inhaled asbestos and vitreous fibers less than 5 microns in length. The second day of the meeting will be devoted to critical assessment of the health effects that can be justifiably attributed to asbestos and vitreous fibers and to identifying critical data gaps and research needs that would further enlighten this subject (Topics #2 and #3).

#### **Topic #1: Physiological Fate of Asbestos and Vitreous Fibers less than 5 Microns in Length.**

Discuss/review current knowledge about the physiological fate of small fibers when they enter the body.

- What is the expected physiological depositional pattern for less-than-5-micron fibers in the lung?
- What is known about clearance/biopersistence of less-than-5-micron fibers once deposited in the lungs?
- What type(s) of migration are expected within the body for less-than-5-micron fibers?

#### **Topic #2: Health Effects of Asbestos and Vitreous Fibers less than 5 Microns in Length.**

Discuss/review health effects that may be due to less-than-5-micron asbestos and vitreous fibers present in air or settled dust.

- How robust are the animal and human cancer data for these fibers/particles? Do the data adequately address exposures where the majority of materials are less-than-5-microns in length?
- What is the state of the art understanding of the potential for SVFs to induce cancer in humans?
- Is there any direct evidence that less-than-5-micron fibers contribute to adverse health effects?
- Is there indirect evidence for less-than-5-micron fiber induced adverse health effects? Do the mechanisms of action of other materials (e.g., longer asbestos fibers, silicates, mineral dusts, amorphous silica) with potentially similar compositions aid in understanding small-fiber mechanisms of action?
- At what length does a material no longer exhibit fiber-like toxicity and can be considered particulate matter regardless of aspect ratio?
- Can any thresholds be defined for the mechanisms of action that may influence the toxicity of less-than-5-micron materials?
- Can an exposure threshold be developed for the irritant effects of SVFs for skin contact or eye irritation, based on either fiber loading or fiber content of handled materials? (What are fiberglass levels seen in housing and office areas where SVF insulation has been used, expressed as either fiber loading or fiber content of settled dust? Have irritant effects been associated with these levels?)

#### **Topic #3: Data Gaps.**

- What data gaps are evident when addressing the above questions?
- What research is needed to fill these data gaps?



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Dr. Case is a pathologist and epidemiologist at McGill University in Montreal, Canada. Following his residency in pathology at McGill University he obtained the Diploma in Occupational Hygiene at McGill, and worked as a post-doctoral fellow and instructor at the Mount Sinai School of Medicine, New York, from 1980-1983. While there, he performed some of the first studies on asbestos-mediated free radical release, with the help of the Young Investigator's Award of the American Lung Association. On his return to McGill he joined the Dust Disease Research Unit. The focus of this group was the epidemiological study of diseases related to mineral fiber exposure using lung-retained fiber in exposure assessment. In 1986, he received the National Health Scholarship of NHRDP (Canada) for his work in the field. In 1988, he moved to the University of Pittsburgh, where he succeeded Dr. Philip Enterline as Director of the U.S. EPA Center for Environmental Epidemiology, through their cooperative agreement with the University of Pittsburgh School of Public Health, where he was also associate professor of epidemiology. He returned to McGill in 1992 and continues research, teaching, and clinical work there in pathology, epidemiology, occupational health and in the McGill School of Environment. Dr. Case has participated in workshops, given lectures, and provided peer reviews and advice for many national and international agencies and professional societies on the subject of the exposure assessment and health effects of mineral fibers, including: EPA, CDC (through ATSDR and NIOSH), the U.S. Consumer Product Safety Commission (CPSC), the International Agency for Research on Cancer (IARC), the International Commission on Occupational Health (ICOH), the British Occupational Hygiene Society (BOHS), the American Thoracic Society (ATS), the Geological Society of America (GSA), and the Collegium Ramazzini. His research on asbestos and other mineral fiber and particle exposures and related diseases has been funded by American and Canadian public agencies including EPA, MRC (Canada) and NHRDP (Canada). Dr. Case has published over 100 papers on these subjects.

# HEALTH EFFECTS OF ASBESTOS AND SYNTHETIC VITREOUS FIBRES: The influence of fibre length.

Comments on the *Charge to the Panel*

For the meeting scheduled for New York City October 29/30, 2002-10-17

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First of all, there are some inaccuracies in the charge to the panel as stated. It is in fact not true that “much less is known” about the potential effects of “smaller” (sic) fibers. The effects of fiber length are *well known* for all of the asbestos-related diseases (except as noted below) and there is a general consensus that “*Fibre dose, dimension, and durability are **currently accepted** as important parameters...relevant to potential bioactivity*” (1); *emphasis added*). This statement refers to both diameter (in the sense of respirability) and length (in the sense of pathogenicity). What is unknown is the means by which these parameters, including fiber length, operate at a mechanistic level, although a great deal of work has in fact been done in animals.

In fact, although the choice of fibre length (usually 5  $\mu\text{m}$ ) by regulatory and other agencies such as NIOSH, OSHA, and WHO was originally practical, being based in part on the resolution of the light microscope, there has been for some time increasing consensus that *fibrogenicity and carcinogenicity are in fact not only related to, but proportional to, fiber length*, and many scientists believe that the use of a length of 5  $\mu\text{m}$  as a lower-limit cut-off is not overly liberal (that is, not “too long”) but overly conservative (that is, *too short*; what is lacking in research is not enough research on *short* fibres but on *long* fibres (where long refers to fibres longer than 10  $\mu\text{m}$ , 20  $\mu\text{m}$ , or even greater lengths). As some will know, EPA is currently considering a new risk assessment which will take this into consideration, at least for asbestos. There is a considerable paucity of such work on long fibres; see for example our own paper on intrapulmonary “long” (defined as  $> 18 \mu\text{m}$ ) fibres in relation to asbestos textile and mining work (2)), which is being used in current risk assessment revisions by EPA contractors (Berman W., personal communication). To the best of my knowledge this is the *only* published paper in the so-called “lung burden” (lung-retained fibre) literature which separately assesses long lung-retained fibres, whereas *the majority* of such papers assess “short” ( $< 5 \mu\text{m}$ ) fibres; sometimes by default by counting “all” fibres (which *overemphasizes the short fibres* and virtually eliminates evaluation of longer fibres due to the log-normal distribution of fiber lengths coupled with counting rules that “stop” after a given number of fibers or transmission electron microscopic “fields” are counted) and sometimes through categorizing this fibre interval (between the limits of resolution for length – usually around 0.2  $\mu\text{m}$  in most published work – and 5  $\mu\text{m}$ ).

**HEALTH EFFECTS OF ASBESTOS AND SYNTHETIC VITREOUS FIBRES:  
The influence of fibre length. (Bruce W. Case; McGill University; October 21, 2002) page 2**

Before a meaningful discussion of “short fibers” can in fact be held, a strict definition of terms must be performed. Most mineralogists and geologists and many health scientists do not regard structures having length “less than 5  $\mu\text{m}$ ” as fibers at all, regardless of their aspect ratio. Those who believe this would categorize such structures as particles of length less than 5  $\mu\text{m}$  and aspect ratio greater than a given ratio (usually 3:1 or 5:1, although here too, there is debate about what aspect ratio is acceptable in order to “define a fiber”). This is not a mere academic distinction, because the behaviour of these short particles in terms of deposition and lung retention is quite different than that of “true fibers”, and the biological effects are likely to (indeed have been demonstrated to) vary from those of “true fibers” as well.

In “short”... concentration on so-called “short” fibres (which are not fibres) is a scientific sense worse than a waste of time, it is a diversion from truly pathogenic fibres: fibres which are “long”. It remains of value to look at the question of fibre length *per se*, but not as defined in this Charge: The title is fine (“The Influence of Fibre Length”) as is part one of the principal charge (“ATSDR seeks a discerning review of the fate of inhaled asbestos and vitreous fibres...”) but the end of the latter sentence (“...less than five microns in length”) precludes useful discussion of the effects of fibre length on any of the parameters presented. Length cannot be arbitrarily divided in this way, although fibres “this short” are almost certainly not pathogenic except in their role as (not necessarily “fibrous” particles; see below) at sufficient bio-persistent dose. some of the underlying questions (especially the main heading of Topics # 1 and # 2, which in fact makes up most of the charge) appear to be limited to the influence of “short” (< 5  $\mu\text{m}$ ) length; a length range that is not in the range of fibres at all. The remainder of this discussion will focus on fibre length, not on “short fibres”, the term itself being an oxymoron.

The sole exception, in my view – and one worth some discussion – is the potential role of asbestos structures having aspect ratio greater than 3:1 and length less than 5  $\mu\text{m}$  in the pathogenesis of interstitial lung fibrosis (“asbestosis”). The publication of four articles (two from our laboratory and two from Dr. Churg’s) demonstrating an inverse relationship between fibre length and fibrosis in human lung-retained fibre studies is important in this regard (see below), although it may represent an epiphenomenon rather than a true picture of decreasing length as a factor in increasing fibrogenesis.

In the remainder of this document I will concentrate on studies of human exposure, often as demonstrated by tissue retained-fibre dose (so-called “fibre burden”) studies, as other members of the panel are likely to concentrate more on the animal and mechanistic data.

Brief comments on the printed charge “questions”:

Topic 1:

- What is the expected physiological depositional pattern for less-than-five  $\mu\text{m}$  fibres once deposited in the lung?

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This is well established in terms of the depositional mechanisms of impaction, settling, sedimentation, Brownian motion and (for fibres) interception. Particles larger than 2 mm. in *diameter* tend to be deposited directly on the respiratory mucosa (particularly in the nose, nasopharynx and bifurcation of bronchi) by inertial **impaction** of the particle on the wall as the air in which it is carried changes direction. Particles 0.5 mm. and less tend to remain suspended in gas and exhaled out of the lung (**Brownian motion**). Particles between 0.5 mm. and 2 mm. in diameter tend to be deposited on the mucosa of distal bronchi and membranous bronchioles through settling from the force of gravity (**sedimentation**; this is the most important deposition mechanism in humans). Fibre *length* is only important in the sense of interception, which is dependent on length; the longer the fibre the greater the degree of interception in a tube of fixed length.

- What is known about clearance/ biopersistence of less-than-5...etc.

Essentially, outside of *overload conditions* (which Dr. Oberdorster will be most familiar with), the particles tend to be removed from the lung by a variety of physiological mechanisms the most important of which are macrophage ingestion, dissolution (where chemically possible), and the muco-ciliary escalator. Absent abnormalities in phagocyte function these particles should be removed even if they are chemically resistant if (a) the dose is not too great to overwhelm these normal mechanisms and (b) the mechanisms themselves are intact. There are medical conditions which affect these mechanisms, however, so there are likely to be vulnerable populations (such as those with *primary ciliary disorders*; these tend to be genetic and very rare such as primary ciliary dyskinesia (incidence 1:20,000 to 1:60,000)). Of greater frequency is the lesser effect on muco-ciliary clearance in asthma. In addition environmental influences, including smoking and nitrogen dioxide (3), can affect these normal mechanisms through direct ciliary damage or disrupted function. Some common pharmaceuticals slow muco-ciliary transport (for example some general anaesthetics and atropine), while others accelerate it (for example theophyllines and sympathomimetics). Bronchial secretion is also an important contributor to clearance or impaired clearance, as can be seen most dramatically in cystic fibrosis. Overall, then, there are a number of possible factors which may interfere with particle clearance, but none have been associated with “fibre length” parameters with the possible exception of smoking (4).

- What type of migration are expected within the body for (“short fibres”)?

This *may be* an important subject, at least for the parietal pleura, *if* it is necessary for fibres to reach the pleura to cause lesions (plaques and mesothelioma). It remains possible that fibres still within the peripheral lung may be capable of contributing to the mechanisms of these diseases. Mechanisms remain speculative, but long amphibole fibres may tend to localize toward the lung periphery, and it remains possible (but unproven and indeed untested) that chemical mediators may cross the visceral pleura into the pleural space. Churg, among others, has observed that “accumulation of long fibers immediately under the upper lobe pleura may be important in the genesis of mesothelioma” (5). The idea that it is biologically *necessary* that fibres “reach the

parietal pleura” to cause mesothelioma is not scientifically tenable, although intellectually appealing.

Unfortunately, less is known about this than what has been published, due to the exceptionally poor quality of what *has* been published, with two exceptions from a group based in Brussels and Marseilles (6, 7). The few additional papers that have been published (in relation to human disease) have been for the most part based on static “fiber burdens” that purport to be in “the pleura” but which on careful reading are in fact in mesotheliomatous tissues and/ or pleural plaques; the false assumptions are then made that “short fibres” – usually very short chrysotile fibres, averaging less than 0.2  $\mu\text{m}$  *in length* – have “translocated” to the “pleura” from the lung. In fact the “pleura” was *not* studied, tumor and plaque which by definition could not contain fibres except via specimen contamination or incorporation, most likely from adjacent lung. Both Rogers et al. 1994 (8) and Case et al. 1994 (9) have also reported contamination by short *crocidolite* fibers of Nuclepore filter materials and in uncontrolled studies of this nature any material from air, fluids, and paraffin in the pathology laboratory from which the specimens originally were referred to specimen preparation materials are suspect.

All of the studies lacked matched controls, and in the American cases all were selected for litigation and subjected to a non-standard digestion technique. Examples of this are provided by all papers having Y. Suzuki as co-author on this topic (10-12) and some early papers by Sebastien (13, 14); the topic has been fully reviewed elsewhere (15).

It remains possible to *do* good studies of translocation of fibres, but for lung-to-pleura in humans at least only the two preliminary studies mentioned above have proved useful, and their results have been quite different: Boutin et al. and Dumortier et al. (6, 7) have found that “the distribution of asbestos fibers in the pleura was heterogeneous and that they might concentrate in...“black spots” of the parietal pleura”. Using thoracoscopy in living patients from “normal areas of the parietal pleura” rather than plaques and tumor, and using controls, they showed that “amphiboles outnumbered chrysotile in all samples” and that of all fibres 22.5% were in fact greater than or equal to 5 microns in length; a proportion at least as great as that usually seen in lung tissue. The means of translocation remains unknown, although these findings strongly suggest lymphatic drainage paths. The pathogenic significance also remains unknown, although the authors emphasized their hypothesis that these fibres might contribute to plaque and mesothelioma genesis.

## **Topic # 2: Health effects...**

### **■ How robust are the animal and human cancer data...do the data adequately address...less-than-5...**

As noted above, regrettably, this is the wrong question. The correct question would be, either “Do the data adequately address the effects of dimension” (the answer to which is a qualified yes) and “do the data adequately address **MORE THAN 5**

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*(substitute more-than-10, more-than-20...etc.)*. Here the answer is that the data clearly do not adequately assess the effects of long fibres, the fibres of greatest concern for disease, or more properly they do not address *the longest fibres*, as a result of *too much* concentration on published studies on fibres under 5 micrometres in length. This has been essentially “by default” in the lung-retained fibre field, since studies which take as their starting point “all fibres from 1 micrometre up”, or less, will by definition inadequately address the most pathogenic fibres, known to be the longest fibres.

- What is the state of the art understanding of the potential for SVFs to induce cancer in humans? Thanks to the excellent studies of the European Group, of Enterline, and most recently of Gary Marsh et al. at the University of Pittsburgh, it has now been established that SVFs *do not* induce cancer in humans.

Original concern arose when Saracci and the European group noted in a very large cohort of 13 plants an SMR for lung cancer of 192 (17 observed, 8.9 expected; 95% confidence interval 117-307). Although an initial dose-response relationship was believed present, subsequent study showed that the excess appeared in an anomalous group of workers; first, those limited to the “early technological phase” of the industry, but perhaps even more important workers having different lifestyles (and death-style; a high suicide rate), and in many cases short-term workers. No such excess was ever seen in the American workers except in some rock-wool plants, and lung-retained fibre studies demonstrated no excess of MVF *but an excess of amosite asbestos in the lungs of workers compared with controls dying in the same hospital* in one of the “higher lung cancer” areas. Enterline and colleagues demonstrated “no consistent evidence of a respiratory disease hazard related to exposure to man-made mineral fibers among the workers who produce these fibers” in the largest study of these workers (16); although lung cancer and non-malignant respiratory disease were increased in some rock- and slag-wool plants there was no evidence of a dose-response relationship. Complete follow-up of the study has recently been completed (17-24) and the lack of any relationship to disease confirmed, including for mesothelioma; of ten cases originally reported *most were not* mesothelioma and in any case almost all were asbestos-exposed. Overall, Marsh and colleagues noted “The excess in respiratory system cancer is largely a reflection of elevated lung cancer risks that we attributed mainly to confounding by smoking, to exposures outside the MMVF industry to agents such as asbestos, or to one or more of the several co-exposures present in many of the study plants (including asbestos)” (23).

- Is there any direct evidence that (short) “fibres” contribute to adverse health effects?

No, with the possible exception of asbestosis. For mesothelioma, at least three well-conducted case-control studies have now established that lung-retained fibre “risk” is entirely accounted for by *long amphibole lung-retained fibre content* and that any initial apparent effect of shorter fibres was a statistical artefact (15, 25-27). An initial observation by Rogers et al. of an increased risk associated with the intrapulmonary size

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category “shorter than 10  $\mu\text{m}$ ” (26) was later thought by the authors to lack biological plausibility, and if anything the “short” ( $< 10$ , not  $< 5$ ) fibres represented broken down “longer” fibres (8). Lung cancer, due to its overwhelming relationship to smoking, as proved difficult to investigate in this way. Animal studies and theoretical data are consistent with the overwhelming importance of fibres longer than 5 (indeed longer than 10, or longer than 20) micrometres (28-32). Recent observations that the half-life of chrysotile fibres longer than 10 micrometres may be more than eight years, at least in long-term chrysotile miners and millers (33), and that in mixed-exposure asbestos textile workers lung content of fibres longer than 18 micrometres was proportional to cumulative exposure (2) has shown that even for chrysotile, any effect appears to be related to long, not shorter, fibres.

■ At what length does a material no longer exhibit fiber-like toxicity and can be considered particulate matter...

This is one of the most important issues to be addressed; the most recent science from the point of view of risk assessment would imply that this figure is much *higher* than previously believed; certainly higher than 10 micrometres, much less 5. Modern risk assessments will take fiber length into account, possibly by the application of algorithms which assign increasing risk with increasing length. It is difficult to imagine setting one number below which a structure is a “particle” and not a “fibre”; it is much easier to say that structures less than five micrometres do not – ever – behave as fibres with respect to lung cancer and mesothelioma.

As alluded to several times above, there are a series of human studies which show an inverse relationship between fibre length and asbestosis severity. Studies by Churg and colleagues first of long term chrysotile asbestos miners and millers, in which “tremolite mean fiber length, aspect ratio, and surface area were, surprisingly, negatively correlated with fibrosis grade” (34). A similar study found a similar result for amosite in unselected litigation cases, mainly in shipyard workers and insulators, prompting the authors to conclude that “these observations again raise the possibility that short fibers may be more important than is commonly believed in the genesis of fibrosis in man” (35). However, there were some serious problems with these papers in that groups were small (no more than 21) and (as is always the case) fibre lengths were very strongly inter-correlated. Contradictory data initially came from our own laboratory (15), but more recent unpublished work with a much larger data base of workers selected across a more homogeneous base and divided into fiber length “strata” has once again demonstrated an apparent inverse association between interstitial fibrosis grade and (in this experiment) fiber length *interval*. Results, which are about to be published, will be discussed, as they and the previous human work contradict some animal work on fibrosis and fiber length. The latter however appears somewhat speculative with respect to postulated *mechanisms* of fibrosis. Is it reasonable to believe, for example, that “frustrated phagocytosis” results in much more chemical mediation than “simple” phagocytosis of much larger quantities of shorter fibre? If “short” fibres do have an effect which has not been adequately



explored – as fibres, rather than particles – then it surely the production of interstitial fibrosis.

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Dr. Lippmann is a Professor of Environmental Medicine at the New York University (NYU) School of Medicine. He holds a Ph.D. (NYU, 1967) in Environmental Health Science, an S.M. (Harvard University, 1955) in Industrial Hygiene, and a B.Ch.E. (The Cooper Union, 1954) in Chemical Engineering. At NYU, he directs a research program on Human Exposure and Health Effects, and the EPA-supported Particulate Matter Health Effects Research Center. He has been the recipient of numerous awards for his research and contributions in aerosol science and pulmonary physiology, human exposure assessment and dosimetry, chemical transformations in the atmosphere, population studies of exposure-response relationships in occupational and community cohorts, and factors affecting the toxicity of airborne fibers. Much of this research has been focused on specific chemical agents, notably ozone, sulfuric acid, and asbestos. Dr. Lippmann is a past President of the International Society of Exposure Analysis (1994-1995), past Chairman of: the ACGIH (1982-1983); the EPA Science Advisory Board's Executive Committee (2000-2001); EPA's Advisory Committee on Indoor Air Quality and Total Human Exposure (1987-1993); and EPA's Clean Air Scientific Advisory Committee (1983-1987). He has also chaired and been a member of numerous National Research Council committees, including committees on the airliner cabin environment and the health of passengers and crew, synthetic vitreous fibers, measurement and control of respirable dust in mines, indoor pollutants, toxicity data elements, and *in-vivo* toxicity testing of complex mixtures. His publications include 270 research and review papers in the scientific literature and two reference texts on environmental health science.

# RESPONSES TO ATSDR FIBERS PANEL CHARGE QUESTIONS

M. Lippmann

## *Specific Charge Questions*

### **Topic # 1. Physiological Fate of Asbestos and Vitreous Fibers less than 5 Microns in Length.**

Discuss/review current knowledge about the physiological fate of small fibers when they enter the body.

A. What is the expected physiological depositional pattern for less-than-5-micron fibers in the lung?

Fibers with aspect ratios  $>10$  behave aerodynamically like unit density spheres with diameters one-third their fiber width (Stöber *et al.*, 1970; Timbrell, 1972). The only exception, in terms of being influential in deposition in lung airways is for fibers longer than about  $10\ \mu\text{m}$ , where the mechanism of interception becomes influential (Sussman *et al.*, 1991). Thus, for fibers  $<5\ \mu\text{m}$  in length, deposition patterns and efficiencies will be determined almost entirely according to the fiber width, which for fibers  $<5\ \mu\text{m}$  long will be less than about  $1.6\ \mu\text{m}$ . For fiber widths between about 0.1 and  $1.6\ \mu\text{m}$ , total lung deposition in healthy people will be between 10 and 20%, with almost all of it in the deep lung. For fibers thinner than  $0.1\ \mu\text{m}$ , deposition will increase with decreasing width, and there will be a somewhat greater proportion of the deposition in the more proximal airways.

B. What is known about clearance/biopersistence of less-than-5-micron fibers once deposited in the lungs?

For these short fibers, which can be fully engulfed by lung cells and do not dissolve in airway fluids in less than a few weeks, their clearance will be similar to other mineral and vitreous particles. Those depositing in lung conductive airways will be largely removed to the G.I. tract by mucociliary clearance within about one day. Most of those depositing in the gas-exchange region will be phagocytized by alveolar macrophages and cleared to and through the

mucociliary escalator within a few weeks. Other particles may be engulfed by epithelial cells, primarily in the respiratory acinus, and retained for much longer periods, with gradual removal to lymph nodes.

C. What type(s) of migration are expected within the body for less-than-5-micron fibers?

Fibers with diameters less than  $\sim 0.1 \mu\text{m}$ , which could be a significant fraction of fibers  $< 5 \mu\text{m}$  in length, can penetrate through the respiratory epithelia and be transported through lymph channels to hilar and peripheral (mesothelial) lymph nodes and through blood to more distant body organs. However, quantitative aspects of these pathways have not been described.

## **Topic # 2. Health Effects of Asbestos and Vitreous Fibers less than 5 Microns in Length.**

Discuss/review health effects that may be due to less-than-5-micron asbestos and vitreous fibers present in air or settled dust.

A. How robust are the animal and human cancer data for these fibers/particles? Do the data adequately address exposures where the majority of materials are less-than-5-microns in length?

### Animal Toxicology Cancer Data:

The most definitive studies for short fibers ( $< 5 \mu\text{m}$  in length) was carried out by Davis *et al.* (1986, 1987) in Edinburgh using inhalation exposures and length classified amosite and chrysotile asbestos fibers. The short-fiber amosite (1.7%  $< 5 \mu\text{m}$  in length) produced no malignant cancers in 42 rats, whereas the long-fiber amosite (30%  $> 5 \mu\text{m}$  in length, 10%  $> 10 \mu\text{m}$ ), with the same diameter distribution, produced 8 cancers in 40 animals (Davis *et al.*, 1986). In the corresponding study using chrysotile (Davis *et al.*, 1987), the short-fiber material was less successfully depleted in long fibers. There were 330 f/mL longer than  $10 \mu\text{m}$  in the "short" chrysotile, versus 12 f/mL in the "short" amosite, and the "short" chrysotile produced seven

cancers (while the "long" chrysotile produced 22 cancers). These results suggest that the cancers produced by the "short" chrysotile preparation were, in fact, due to its contamination by longer chrysotile fibers. This conclusion is supported by the analysis of Lippmann (1994) using the fiber length distribution data for rat inhalation studies of unclassified fibers of amosite, brucite, chrysotile, crocidolite, erionite, and tremolite. He concluded that the tumor yield was better predicted by the concentration of fibers longer than either 10 or 20  $\mu\text{m}$  than by the concentration of fibers longer than 5  $\mu\text{m}$ .

The animal cancer data for injected fiber suspensions and the human epidemiologic data where fiber length distributions are available are consistent with carcinogenicity being attributable solely to fibers longer than 5  $\mu\text{m}$ , but are not by themselves definitive.

B. What is the state of the art understanding of the potential for SVFs to induce cancer in humans?

The state-of-the-art on this issue was summarized in a recent National Research Council report (NRC, 2000), as follows:

"In a review of the published epidemiologic literature with respect to respiratory system cancer, Lee *et al.* (1995) concluded that 'available data indicate that among those occupationally exposed, glass fibers do not appear to increase the risk of respiratory system cancer. Exposure to rock or slag wool may increase the risk of such cancers; however, the data do not convincingly prove that this association is causal.'

Recent studies, including case-control studies, make it clear that any lung-cancer SMRs based on national data must take into account the potential confounding effect of smoking. Evidence from the case-control studies demonstrates that there is no significant association between fiber exposure and lung cancer or nonmalignant respiratory disease in the MVF manufacturing environment. It is clear, for example, that of the Newark, Ohio, plant workers (who made up some 35% of the U.S. cohort) exposure to MVF, including respirable glass fibers, was not responsible for any increase in lung cancer risk (Chiazze *et al.*, 1993)."



- C. Is there any direct evidence that less-than-5-micron fibers contribute to adverse health effects?

In the absence of data that SVF fibers of all lengths cause respiratory disease, it is highly unlikely that the SVF fibers  $<5 \mu\text{m}$  in length cause any.

- D. Is there indirect evidence for less-than-5-micron fiber induced adverse health effects? Do the mechanisms of action of other materials (e.g., longer asbestos fibers, silicates, mineral dusts, amorphous silica) with potentially similar compositions aid in understanding small-fiber mechanisms of action?

There is no such evidence, and the greater rate of SVF fiber dissolution than of other materials cited in the question makes it very unlikely that short-fiber SVF causes health effects.

- E. At what length does a material no longer exhibit fiber-like toxicity and can be considered particulate matter regardless of aspect ratio?

The length limit is clearly not less than  $5 \mu\text{m}$  and, for humans, probably closer to  $10 \mu\text{m}$ .

- F. Can any thresholds be defined for the mechanisms of action that may influence the toxicity of less-than-5-micron materials?

Possibly, but if they exist, they would be much higher than any reasonably anticipated exposures in modern society.

- G. Can an exposure threshold be developed for the irritant effects of SVFs for skin contact or eye irritation, based on either fiber loading or fiber content of handled materials? (What are fiberglass levels seen in housing and office areas where SVF insulation has been used, expressed as either fiber loading or fiber content of settled dust? Have irritant effects been associated with these levels?)

Possibly, but not likely for airborne concentrations as measured in fibers/mL.

**Topic # 3: Data Gaps.**

A. What data gaps are evident when addressing the above questions?

The rat inhalation studies with size-classified amosite and chrysotile show how a well conceived study can help to resolve critical questions about lung cancer. More studies with better classified chrysotile, and with other well-classified asbestos and vitreous fibers of varying lengths and biopersistence properties, would be very informative.

A major data gap is the influence of fiber length on mesothelioma, which could be addressed in the hamster model.

B. What research is needed to fill these data gaps?

Inhalation studies in rats with size-classified fibers for lung cancer, and inhalation studies in hamsters with size-classified fibers for mesothelioma.

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Dr. Lockey has been associate director of the Department of Environmental Health, director of the Division of Occupational and Environmental Medicine, and a professor of environmental medicine at the University of Cincinnati College of Medicine since 1986. In addition, he is a consultant in employee health to the Children's Hospital Medical Center. Dr. Lockey completed his M.D. in 1972 (Temple University School of Medicine), and an additional M.S. in 1985 (University of Cincinnati College of Medicine). He was instrumental in developing the Center for Occupational Health (COH) at Holmes Hospital in the University of Cincinnati Medical Center while serving as director (1990-1998). Dr. Lockey continues to work in the COH through the Occupational Pulmonary Clinic where specialized services are provided in occupational medicine, occupational pulmonary services, disability management, and medical surveillance. In addition to his clinical activities, Dr. Lockey is a prolific researcher. The focus of his research has been on the health effects of exposure to man-made vitreous fibers (MMVF). He initiated, and currently directs, an industry-wide study of the health effects of refractory ceramic fibers. This study will continue until at least 2005 and has been the first to identify that exposure to certain types of MMVF is associated with scarring along the chest wall (pleural plaques). Dr. Lockey has developed a national, as well as international reputation, in regard to the health effects of MMVF and has written over 100 articles and book chapters on this topic.

**Topic #1: Physiological Fate of Asbestos and Vitreous Fibers less than 5 Microns in Length.**

What is the expected physiological depositional pattern for less-than-5-micron fibers in the lung?

What is known about clearance/biopersistence of less-than-5-micron fibers once deposited in the lungs?

Hazards associated with man-made vitreous fiber (MMVF) appears to be most strongly associated with the ability to persist within lung tissue. This is in part dependent upon chemical composition of the MMVF in that increased concentrations of stabilizers such as aluminum impact a greater degree of chemical durability. *In vitro* tests to measure fiber solubility should be performed to reflect an acid pH of 4.5 to 5.0 such as found in phagolysosomes within alveolar macrophages as well as pH of 7.4 reflecting extra-cellular fluid. Short fibers that are ingested by macrophages will encounter the lower pH that overall could affect their biopersistence. In general, solubility tests identified the following rank order from lowest to greatest solubility of MMVF in comparison to asbestos fibers: crocidolite <amosite <RCF <special purpose glass fibers <rock wool <slag wool <conventional glass fibers. [1]

In rodent exposure to mixed dust resulted in an increased transport of fibers across the visceral pleura and increase production of lung tumors and mesothelioma. [2]

Fibers may act as carcinogens or carriers of chemical carcinogens to the target organ. [2]

What type(s) of migration are expected within the body for less-than-5-micron fibers?

Gelzleichter et al, in 1996 exposed rats to nose only inhalation of kaolin-based refractory ceramic fiber. It was identified that fibers rapidly translocate to the pleural tissue with a difference between those in the pleural tissue and the parenchymal tissue. Within the pleural tissue the geometric mean length  $1.5 \mu\text{m}$  (GSD  $\sim 2.0$ ) and geometric mean diameter  $0.09 \mu\text{m}$  (GSD  $\sim 1.5$ ). For comparison parenchymal tissue GML =  $5.0 \mu\text{m}$  (GSD  $\sim 2.3$ ) and GMD  $0.3 \mu\text{m}$  (GSD  $\sim 1.9$ .) This would indicate the short thin fibers are capable of translocating to the pleural tissue. [3]

The efficiency of clearance by macrophages is greatest with fibers less than 5 microns in length and becomes less efficient with increasing length of fibers. Certain fibers may stimulate macrophages to move to the pleura rather than to be cleared from the lung which may be the case with crocidolite and erionite. [4]

**Topic #2: Health Effects of Asbestos and Vitreous Fibers less than 5 Microns in Length.**

*How robust are the animal and human cancer data for these fibers/particles? Do the data adequately address exposures where the majority of materials are less-than-5-microns in length?*

Conventional glass fiber has only a very small fraction that would be small enough from an aerodynamic diameter perspective to be able to penetrate into the lungs. Those conventional glass fibers that actually do penetrate into the lungs would rapidly break into shorter segments and rapidly dissolve. Therefore, the risk for lung cancer and mesothelioma from exposure to conventional glass fiber is extremely small unless there was an ongoing continuous exposure to high levels of long fibers. [5].

Probability of pleural sarcomas was best correlated with fibers 0.25 microns or less in diameter and >8 microns in length, but there was a high correlation with fibers up to 1.5 microns in diameter and length >4 microns. [6]

Further analysis of the Stanton hypothesis indicated that the type of mineral fiber was significant in relationship to predicted tumor incidence as was the number of index particles rather than log mean aspect ratio. [7]

*What is the state of the art understanding of the potential for SVFs to induce cancer in humans?*

Mortality studies of MMVF have not demonstrated a cancer risk regarding glass fiber and mineral wool production workers. There is no current published human mortality data available regarding refractory ceramic fibers.

*Is there any direct evidence that less-than-5-micron fibers contribute to adverse health effects?*

There is some indication that fibers with diameters of  $<0.1 - 0.4 \mu\text{m}$  in lengths  $<10 \mu\text{m}$  may have a greater propensity for inducing pleural plaques as reviewed in an article by Lentz et al.[8]

In regard to pleural plaques, Churg et al, identified that fiber size is significantly related to plaque formation with a geometric length of  $3.0 \mu\text{m}$  and aspect ratio of 19.4 in patients with plaques versus  $2.5 \mu\text{m}$  and aspect ratio 14.5 with no plaques. [9]

Some studies have suggested that short asbestos fiber may be carcinogenic when injected, but these results are difficult to interpret as the short fibers are only reported as a proportion of the total. With the ability of the fibers to cleave longitudinally as well as transversely, it is quite possible that the mean fiber length of the sample is reduced while actually increasing the number of long fibers per unit mass. A study of amosite fibers by an inhalational studies in rats with almost all fibers less than 5 microns in length was compared to normal amosite dust and the short fibers produced neither fibrosis nor neoplasm in comparison to the long fibers. [10,11]

Intrapleural inoculation studies and inhalation studies of short ( $<5 \mu\text{m}$ ) and long crocidolite and erionite fibers demonstrated tumors and fibrosis with long fiber exposure and tissue reaction only with the short fibers. [18]

Studies of asbestiform versus nonasbestiform tremolite by *in vivo* intrapleural injection demonstrated production of mesotheliomas with the asbestiform fiber exposure as well as markedly increased cytotoxicity.

[12]

**Studies of Exposures to Asbestos or RCF Which Include Fiber Dimensions and Pleural Effects.[8]**

<u>Study authors</u>	<u>Experimental design</u>	<u>Fiber type</u>	<u>Critical dimensions</u>	<u>Pleural Effects</u>
Data from the asbestos literature				
LeBouffant et al, 1973	Clinical analysis of human pleural tissue	Asbestos	$L < 2\mu m; D < 0.03\mu m$	Pleural plaques
Sebastien et al, 1979	Clinical analysis of human pleural tissue	Asbestos (chrysotile)	$L = 23\mu m; D = 0.06\mu m$	Pleural effusion, pleural fibrosis, mesothelioma
Stanton et al, 1981	Implantation of fibers in pleural cavity of rats	Asbestos	$L > 8\mu m; D < 0.25\mu m$	Pleural sarcoma
Churg and DePaoli, 1988	Clinical analysis of human pleural tissue	A10 <sub>2</sub> fibers tremolite chrysotile	$L \geq 4\mu m; D \leq 1.5\mu m$ $L = 2.4\mu m; D = 0.15\mu m$ $L = 2.5\mu m; D = 0.03\mu m$	Pleural plaques
Lippmann, 1988	Review of scientific literature	Asbestos	$L > 5\mu m; D < 0.1\mu m$	Mesothelioma
Timbrell, 1989	Review of scientific literature	Asbestos	$D < 0.1\mu m$	Mesothelioma
Dodson et al, 1990	Autopsy of lung and pleural tissue from former shipyard workers	Amphibole chrysotile	$L = 1.05\mu m; D = 0.14\mu m$ $L = 0.85\mu m; D = 0.06\mu m$ (Fewer than <10% of fibers in plaques had length $> 5\mu m$ )	Pleural plaques
Gibbs et al, 1991	Clinical analysis of human pleural tissue	Asbestos (all) Amosite	$L = 0.99\mu m; D = 0.06\mu m$ $L = 1.23\mu m; D = 0.17\mu m$	Pleural fibrosis
Churg et al, 1993	Clinical analysis of fiber types, dimensions in human lung tissue	Asbestos (tremolite)	$L = 3.0\mu m; D = 0.23\mu m$	Mesothelioma, pleural plaques
Data from RCF literature				
Mast et al, 1995b	Animal study utilizing rats exposed to fibers by nose-only inhalation	RCF	$L = 5-10\mu m, 10-20\mu m$ $D \leq 0.5\mu m$ (retained)	Pleural fibrosis, lung neoplasm, mesothelioma
Gelzleichter et al, 1996	Animal study utilizing rats exposed to fibers by nose-only inhalation	RCF	$L = 1.5\mu m; D = 0.09\mu m$	Pleural inflammation



SUMMARY OF RECOMMENDATIONS ON ASBESTOS EXPOSURE INDICES

<u>Disease</u>	<u>Relevant exposure index</u>
Asbestosis	Surface area of fibers with: Length $>2 \mu m$ ; diameter $>0.15 \mu m$
Mesothelioma	Number of fibers with: Length $>5 \mu m$ ; diameter $<0.1 \mu m$
Lung cancer	Number of fibers with: <u>Length <math>&gt;10 \mu m</math>; diameter <math>&gt;0.15 \mu m</math></u>

[Lippmann M. Asbestos exposure indices, *Environ Res* 46:86-106, 1988] [13]

Tremolite asbestos is an amphibole that can cleave resulting in short squatty cleavage fragments depending on crystalline plain or long thin asbestiform fibers with high aspect ratios. As tremolite can contaminate chrysotile deposits, it has been postulated that mesothelioma cases identified in chrysotile workers may in part be related to the tremolite amphibole content identified within the lungs. This has to be interpreted with caution in that even though tremolite only constitutes a few percent of the parent ore source, chrysotile tends to disappear from lung tissue over time and tremolite is much more durable and persist within lung tissue. If all fibers are counted in the lungs of Quebec chrysotile workers, the tremolite fibers are relatively short with low aspect ratio (geometric mean length 2 microns, geometric mean aspect ratio 8:1 to 10:1). If one were to count those fibers greater than 5 microns, the geometric mean aspect ratio of tremolite fibers is greater than 20:1. [14]

Tremolite is the most commonly encountered amphibole fiber in lungs of urban dwellers in North America, and apparently they are short with low aspect ratios and actually shorter than those seen in the chrysotile miners. Lung burdens have been associated with pleural plaques particularly in individuals who would encounter dust from soil such as farmers. At these levels there is no evidence that chrysotile or tremolite produce an excess of lung cancer or mesothelioma. [14]

The ATS statement indicates that long high aspect tremolite fibers behave like other amphiboles with a high propensity for inducing mesothelioma, but the lower aspect tremolite fibers are capable most likely of causing pleural plaques in low concentrations but only are a risk factor for mesothelioma and asbestosis in high concentrations. This statement was made with caution because of the confounding factor of chrysotile versus tremolite and that the population had a high chrysotile exposure which was not reflected in lung tissue analysis because of the propensity of chrysotile to dissolve over time. [14]

*Is there indirect evidence for less-than-5-micron fiber induced adverse health effects? Do the mechanisms of action of other materials (e.g., longer asbestos fibers, silicates, mineral dusts, amorphous silica) with potentially similar compositions aid in understanding small-fiber mechanisms of action?*

An interesting case report regarding aluminum oxide fibers was reported by Gilks and Churg. Electron optical techniques identified 1.3 billion fiber particles of aluminum oxide per gram of dried lung tissue with a geometric mean aluminum fiber length of 1.0 micron and width 0.06 micron with an aspect ratio of 16. Ninety-eight percent of the fibers were shorter than 2.5 microns and no fibers were longer than 5 microns. There was also extremely high non-fibrous particulate content at 15 billion non-fibrous particles per gram of dry lung tissue. The authors raised the possibility of an association of the presence of the large number of fibers with diffuse interstitial fibrosis, and that a significant number of short fibers may be as dangerous as a smaller number of long fibers.<sup>15]</sup>

Recent results of the ATSDR medical testing program of residents in Libby, Montana indicated chest radiographic changes consistent with pleural plaques or diffuse fibrosis in 17.8% of those screened (994 of 5590) using PA and bilateral oblique chest radiographs. On PA views alone 780 or 14% had pleural changes and 49 or 0.9% had interstitial changes. Pleural changes were associated with increasing age at 5% for those individual 18 to 44, 22.2% in those individuals 45 to 64, and 37.8% in those individuals 65 years or older. There was also a gradient seen with years of residency in Libby, Montana as well as in regard to exposure pathway gradient. [Year 2000 Medical Testing of Individuals Potentially Exposed to Asbestiform Minerals Associated with Vermiculite in Libby, Montana. A Report to the Community, August 23, 2001. Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services, Atlanta, Georgia].

There is adequate documentation within the medical literature that local deposits of tremolite in various populations has been associated with the various types of asbestos related abnormalities and the threshold to which these do not occur has not been established. Plaques can occur with minimal exposures to asbestos

and can occur within a wide range of tissue burdens of asbestos fibers which overlap with control populations.[16]

At what length does a material no longer exhibit fiber-like toxicity and can be considered particulate matter regardless of aspect ratio?

Within this particular reference by Schneider, Dr. Potts from an article in 1987 is quoted as suggesting “All mineral fibers having an aspect ratio of  $\geq 5$  can be classified as carcinogenic irrespective of their mineralogical composition if: diameter of fiber  $< 1 \mu m$  or can split into such lengths of fibers  $> 3 \mu m$  and durability *in vivo* longer than 3 years.) Schneider reported that from a transitional electron microscopy perspective only fibers longer than  $3 \mu m$  need to be counted. It is interesting to note that for short tremolite fibers there was a relative low lung cancer risk and a higher relative mesothelioma risk, based on a study by Dement and Harris in 1979 involving talc mining and milling. Size distribution of man-made fibers and asbestos fibers is best described as bivariate log-normal size distribution. [17]

Can any thresholds be defined for the mechanisms of action that may influence the toxicity of less-than-5-micron materials?

Available data from asbestos fiber exposure is inadequate to establish thresholds other than for pulmonary asbestosis.

Can an exposure threshold be developed for the irritant effects of SVFs for skin contact or eye irritation, based on either fiber loading or fiber content of handled materials?

Glass fiber  $> 5 \mu m$  in diameter appear to be most irritating to the skin based on limited available data.

### **Topic #3: Data Gaps.**

What data gaps are evident when addressing the above questions?

From evaluating amphiboles such as tremolite, anthophyllite, and actinolite, the difficulty is differentiating the asbestiform from the non-asbestiform analogs that are chemically identical but have different crystalline planes resulting in cleavage fragments rather than long thin fibers. Presently the data in regard to these minerals both from an animal as well as a human epidemiology perspective is not sufficient to determine

whether the nonasbestiform varieties are as hazardous as are the asbestiform counterparts. [Reference 11, chapter 16: Bignon & Brochard]

What research is needed to fill these data gaps?

Animal studies on different types of tremolite indicate that the asbestos form of tremolite has a high propensity to produce both mesothelioma and carcinomas in experimental animals. Data regarding tremolite cleavage fragments compared to asbestos form tremolite are really inconclusive in that the fibers are usually composed of a mix containing both relatively and broad fibers as well as long fibers. ATS indicated that more definitive work is needed to determine whether cleavage fragments that are short and squatty are biologically different than the asbestiform type fragments.[14]

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He has also served as a panel member for IPCS Task Group Meeting on Synthetic Organic Fibers, London, England, September 1992; WHO European Programme for Occupational Health, "Validity of Methods for Assessing the Carcinogenicity of Man-Made Fibers", Copenhagen, Denmark, 1992; IPCS Task Group Meeting on Synthetic Organic Fibers, London, England, September 1992; WHO European Programme for Occupational Health, "Validity of Methods for Assessing the Carcinogenicity of Man-Made Fibers", Copenhagen, Denmark, 1992; National Research Council, Commission on Life Sciences, Subcommittee on "Review of the US Navy's Exposure Limits for Manufactured Vitreous Fibers", 1999-2000. He is also a member of Lovelace Respiratory Research Institute's "Research Programs Oversight Committee, 2002-present.



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To: Kate Schalk  
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Subj: Health Effects of Asbestos and Synthetic Vitreous Fibers (SVF): Influence of Fiber Length

Following are some comments regarding the subject "How do animal/experimental data augment our understanding of human health effects?" I have not included the reference citations at this point, but can do this in the future. Let me know if you want me to include Dr. Case's comments after you receive them.

**Background:** There have been numerous studies of the effects of various types of asbestos and SVFs in animals. Both fibrous and nonfibrous particulates have been used. Most studies have been conducted in rats and hamsters, but others, including nonhuman primates have been used. Routes of exposure have included inhalation (whole-body and nose-only), intratracheal instillation, intrapleural implantation/injection, intraperitoneal injection and ingestion. All of the routes of administration have their strengths and weaknesses (advantages and disadvantages) for use for assessing potential health effects in humans. However, the inhalation route appears to produce the most relevant data because it is the only route that duplicates all aspects of human fiber exposure and disease (inflammation, fibrosis, lung cancer and mesothelioma) resulting from the exposure. Also, the neoplastic changes typically occur late in the rodents' life, similar to what occurs in humans exposed to asbestos. Other routes of exposure are also useful for comparing the toxic potential of various types of fibers and understanding the mode of action and many of the mechanisms of fiber toxicity and carcinogenicity. Additionally, the oral route (ingestion) appears to be the most appropriate route of exposure for studying the potential hazard of ingested asbestos.

**Cancer Effects:** Rats and hamsters are the most frequently used species for assessing the potential carcinogenic effects as asbestos and SVFs and have been used with various routes of exposure. Of the two species, the rat appears to be the most appropriate one because it exhibits both lung cancer and mesothelioma in response to inhalation of known human carcinogenic fibers, e.g. asbestos. The hamster can be a useful model if one is only interested in the inflammatory, fibrogenic and mesotheliogenic effects of particulates. However, the hamster does not develop lung cancer after exposure to high levels of either chrysotile or amosite asbestos. Other species have been used but have significant limitations that preclude their general use for carcinogenic bioassays. For example, the mouse is not as useful as the rat or hamster because its terminal airways are smaller and therefore, particulates of a mean mass

aerodynamic diameter (MMAD) of greater than  $>0.5$   $\mu\text{m}$  cannot reach the deep lung (alveolar region) which is the site of primary disease. Non-human primates would be an ideal animal model but are precluded because of their long life-span (would require at least 20-30 years to demonstrate a noncarcinogenic effect), availability (a cancer bioassay requires  $>200$  animals/sex), and expense (such a study would cost  $>\$20$  million).

Most chronic rodent inhalation bioassays of asbestos have been conducted in rats, have not shown significant strain differences and males and females are equally sensitive to its carcinogenic effects. The only large series of studies of various types of asbestos showed that if there is a gender difference, males might be slightly more responsive. Therefore, either sex is appropriate with males slightly more preferable. Just as importantly, both sexes are probably not necessary. However, these same studies have shown that while life-time exposure to asbestos may not be necessary, it is important to observe the animals for most of their life-span (see below).

The types of cancer induced by asbestos and SVFs in rodents are comparable to those observed in humans, although the preponderance of a given type and its biologic behavior appears to be species specific. In inhalation studies in rats the preponderant form of lung cancer is bronchoalveolar in origin, arising from type II alveolar cells. They occur late in the animal's life, usually after 21 months of age. This is why lifetime studies may be necessary to fully exonerate a fiber from being considered carcinogenic. The tumors are slow growing and only occasionally are the cause of death. The biological sequence of growth is typically from bronchoalveolar hyperplasia to bronchoalveolar adenoma to bronchoalveolar carcinoma, although all aspects of the sequence of progression may not be found in a given lesion. Squamous cell metaplasia is not unusual and typically is found as part of the morphology of larger tumors. Squamous cell carcinoma may predominate in a small percentage of rodent tumors, but has rarely been observed to occur de novo. Squamous cell types may be more common with intratracheal instillation of the fibers. The malignant tumors are locally invasive and can metastasize but it is an unusual event for them to do so. When this occurs it is usually within the lung, but distant metastases have been observed. The presence of mitotic figures is in direct relation to the degree of malignant transformation. Tumors of the upper respiratory tract and airways have not been observed in response to inhalation exposure of asbestos or SVFs in rodents.

Mesothelioma has also been found in rodent carcinogenic bioassays of asbestos and SVFs. In inhalation studies in rats they are usually found at a lower incidence than lung cancer. Again, there does not appear to be a gender predisposition and the mesotheliomas in rodents typically occur late in life (after 21 months of age). They rarely are the cause of death. They grow by expansion, growing over the pleural surface. They typically do not invade the lung or other adjacent structures, although this has been observed. They usually present as multiple lesions on both sides of the lung and involve both the visceral and parietal pleura. Rarely, distant metastases have been observed. In inhalation studies, all of the major morphological types (tubulopapillary, sarcomatous and mixed) have been observed, although the tubulopapillary response is the predominate form. There is one exception to this and that is found in the inhalation study of erionite, where the sarcomatous type predominated, was highly invasive and the tumors were exceptionally lethal causing death in most of the rats by 15 months. In contrast

to inhalation, direct instillation into the pleural or peritoneal cavities results in a preponderance of sarcomatous neoplasms, and in fact, it may be difficult to find mesothelial cells in many of the tumors, particularly after peritoneal injection. However, even in these studies, the mesotheliomas seldom invade local tissues or metastasize to other areas of the body.

The biological sequence of events in the development of mesothelioma in rodents also appears to have a series of progressive steps. In inhalation studies, the first event that is observed is fibrosis in the pleura immediately subjacent to the mesothelial lining. This is multifocal in nature, possibly occurring more frequently in the interlobular pleura. In the few studies where the parietal pleura has been investigated, the initial change was found in the nonmuscular portion of the diaphragm and over the ribs (as compared to intercostal). The first indication of mesothelial change is found in these areas of pleural fibrosis. The mesothelial cells become cuboidal (as compared to a normal squamous morphology) and progress to focal hyperplasia of one to three cell layers thickness. The next step is the formation of papillary forms of growth and overgrowth of adjacent pleura. It is at this stage that mesothelioma is diagnosed. Pseudovacuolated tumor cells may be noted at this stage. Finally, the tumor evolves into the classical forms noted above. The course of events is somewhat different for instillation and injection studies. The initial response in the latter studies is inflammation, followed by a fibrogranulomatous reaction (assumed to be an attempt to wall off the fibers). A similar sequence of progression is assumed but results in a higher proportion of sarcomatous types of mesothelioma.

Pulmonary interstitial fibrosis (see below for description) is invariably found in studies where either asbestos or SVFs have caused either lung cancer or mesothelioma. However, there have been fiber studies where pulmonary fibrosis was observed without the development of fiber related neoplasms.

In vitro studies may not be of value for predicting the carcinogenic potential of a given type of fiber. There are several reasons for this. First, the fiber used is not subjected to physiological processes such as clearance and dissolution that are found in the lung. Also, the in vitro test systems use “fresh” fibers, so do not typically take into account pathology attenuating changes in fibers that occur over time in the lung. Finally, the in vitro “dose” may have no relevance to the lung fiber burden. However, notwithstanding this, in vitro methods are highly powerful tools for understanding fiber/cell interactions and mechanisms of toxicity (see Mossman for details).

**Non-cancer Effects:** Animal models have also demonstrated many of the same pathological responses that are found in humans exposed to particulates. The major noncancer endpoints that have been described in animals in experimental studies are phagocytosis, inflammation and pulmonary fibrosis. In regard to these endpoints, the rodent lung (and presumably other species) reacts to asbestos and SVFs as it would to any inhaled nonorganic foreign body that is not chemically toxic, e.g. beryllium. The lung can only react to such materials in a limited number of ways. In animals, if the particulate were deposited in the upper respiratory tract, one would assume that it would be possible for it to cause local irritation. However, this has not been observed in inhalation studies, even at high exposure levels. It is assumed that the resident time for such particles is brief, not allowing for a pathologic response. The mucous layer in these tissues is relatively thick compared to the size of the particulate and the methods of removal are quite efficient. The same is true for the major airways. In experimental animals the airways are

intact and have not been compromised by other toxicants as in humans, e.g. smoking. Therefore, particulates deposited on these surfaces are again efficiently removed via the mucociliary escalator and are either swallowed or expectorated. In either case, the resident time in the body is relatively brief.

For a particulate to cause pathology after inhalation, it must reach the alveolar region of the lung. Particulate size dictates whether this happens or not. If the particle reaches terminal bronchiole it causes a foreign body reaction which is dictated by dose, particle (fiber) size and to some extent physical chemistry. The lungs' initial response is an attempt to remove the offending substance. This is accomplished by resident macrophages. If the particle is of a size that the macrophage can engulf (phagocytize), it will be "captured" and removed from the lung either by translocation to the airways or draining lymphatics. As the dose (number of particulates) increases, more macrophages are recruited. However, if the dose is too large for the number of available macrophages to remove, an "overload" situation develops which results in other pathologic events. Such events have been documented in animals both by histopathology and physiological tests (see Oberdorster for details). If the fiber is too large to be phagocytized and removed, i.e. longer than the size of the macrophage (~13 um diameter in rats and hamsters; ~21 um diameter in humans), the fiber cannot be removed unless it is broken into shorter lengths or dissolves. Both of the latter two phenomena have been observed with several SVFs (see below).

If the dose overwhelms the physiological pulmonary defenses or the fiber is too large to be removed, the initial series of events in animals occur at the junction of the terminal bronchioles and proximal alveolar duct (this is where most of the fibers are initially deposited. In addition to stimulating the local macrophages, an influx of additional macrophages is recruited to the area. At this point, the local type II alveolar cells (in the proximal alveoli) undergo metaplasia to a cuboidal appearance and become hyperplastic. The resulting lesion has been termed "bronchiolization" because the change mimics the appearance of the terminal airways. Increased amounts of mucous production and sometimes inspissation of the material often accompany this. Coincident to the bronchiolization, microgranulomas are observed. These appear to form from a coalition of macrophages and fibroblasts. At this time the microgranulomas are restricted to the proximal portion of the alveolar duct, particularly along the alveolar duct ridge. With time and continued insult the process proceeds peripherally and becomes more apparent. If the offending fiber persists, collagen is laid down in the adjacent interstitium (presumably by direct invasion of the fiber into the epithelium and interstitium). At this time the lesion is referred to as interstitial fibrosis. In rodent studies, the fibrotic areas are initially focal and widely disseminated. But, if the insult persists or the dose is high enough, fibrosis becomes more widespread. Various schemes have been developed to describe these events and grade them as to their severity for comparative purposes. There is one notable difference between the qualitative appearance of the lesions produced by asbestos and SVFs in animals. Neutrophils are often a prominent part of the inflammatory reaction with asbestos, especially with amphiboles, while they are rarely found in studies of SVFs, even at doses that produce fibrosis. The inflammatory reaction can also be documented and quantified by conducting pulmonary lavage studies (see Oberdorster).

Stop studies (exposure is stopped and is followed by a nonexposed recovery period) have proved useful for determining the reversibility of the above lesions. Such studies have clearly shown

that the initial changes (macrophage response and bronchiolization) are totally reversible with most SVFs and to some degree with asbestos. Early fibrosis also is to some degree resolvable, at least with SVFs. Rodent studies have demonstrated that fibrosis, even with asbestos, is not progressive, once the exposure ceases.

While there is no exact correlate for pleural plaques in animals, localized acellular fibrotic changes reminiscent of this lesion have been observed, albeit on a much smaller scale. The qualitative changes in the pleura are somewhat different than in the lung. Macrophages and inflammatory cells are almost totally absent in the pleural response. Lavage studies have not been conducted with instillation or injection studies so it is not known if the same events occur with these routes of exposure. In addition, animal inhalation studies also suggest that fibers need to be present in the pleura for pathologic events to occur.

In vitro studies of mesothelial cells have been conducted using both human and animal cells. These have been primarily designed to study the mechanisms of carcinogenicity (see Mossman).

**Irritant Effects:** While there is evidence of dermal and ocular irritation of humans as a response to exposure to asbestos and SVFs, no such evidence has been observed in animals. Histopathological studies of the nasal cavity in rodents exposed via inhalation have not shown any evidence of pathology, although an increased mucous response could be missed with standard histopathology techniques. Similarly, ingestion studies in rats and hamsters of asbestos did not reveal any irritation of the alimentary tract.

We are unaware of in vitro studies on the irritant effects of either asbestos or SVFs.

**Association Between Fiber Length and Fiber-like Toxicity:** There are numerous animal studies that demonstrate the influence of fiber length and pathogenicity/carcinogenicity. The early studies using intrapleural implantation/instillation and intraperitoneal injection in rats clearly show a direct relationship between fiber size and carcinogenic activity. The longer the fiber, the more carcinogenic it was in these studies. These same studies provided the basis for the hypothesis that short fibers, i.e. shorter than 8  $\mu\text{m}$  in length may not represent a significant carcinogenic risk. However, the same investigations, particularly the intraperitoneal studies also demonstrated that if the dose was high enough even so-called “innocuous” particulates, e.g. titanium dioxide, caused the induction of peritoneal mesotheliomas, albeit at a lower incidence than long fibers. Additionally, the latter studies also demonstrated that if even long fibers, e.g. wollastonite and some SVFs, were not carcinogenic if they were not biopersistent in the peritoneal cavity. While few inhalation studies have been conducted to study the influence of the fiber length on the pathology of asbestos, there is one persuasive study of crocidolite asbestos in rats. In that study, short crocidolite (<2.0  $\mu\text{m}$  length) did not cause either pulmonary cancer or mesotheliomas in rats, even at relatively high exposure levels, while longer crocidolite was highly carcinogenic. Other circumstantial evidence for considering fiber length as being critical to the carcinogenic potential of fibers is provided by the observation that amorphous silica has been shown to be noncarcinogenic in several inhalation studies in rats, while some types of glass fibers of similar chemistry have shown to have carcinogenic activity. In fact, amorphous silica has been used as a “negative control” in rodent inhalation studies.

A final piece of evidence for the importance of fiber length for the carcinogenicity of asbestos and SVFs is found in the hilar lymph nodes that drain the lungs of animals exposed via inhalation to both asbestos and SVFs. These lymph nodes are literally filled with macrophages containing short fibers and fiber fragments with no evidence of pathology or neoplastic change in either the lymph nodes or adjacent tissues.

To summarize studies in animals of short fibers and nonfibrous particulates have shown that both are potentially carcinogenic if they are introduced into a confined cavity, e.g. pleural or peritoneal, at sufficiently high doses. But the same studies clearly show that the carcinogenic potential is definitely less than fibers of the same type that are longer. However, inhalation studies (although limited in number) suggest that short fibers have not caused cancer in animals. The other part of the equation that needs to be considered is the influence of pulmonary clearance and biopersistence on the carcinogenic potential of particulates. As noted above, even long fibers are not carcinogenic in animals unless they are biopersistent in the animal.

There are only a few in vitro studies that address this subject. In a study of Chinese hamster ovary cells (CHO) short amosite failed did not cause chromosomal aberrations while long fiber amosite did.

**Thresholds of Toxic Action:** There have been very few inhalation studies in animals of either asbestos or SVFs to assess a carcinogenic dose response. It needs to be remembered that to assess a carcinogenic dose response, one must have a multidose study that shows a carcinogenic response. Most asbestos and SVF studies were designed to address the carcinogenic potential of the fiber, not dose response. The only multi-dose inhalation study of asbestos used amosite in hamsters. In that study, there was a definite dose-related response with regard to both nonneoplastic (macrophage response, pulmonary fibrosis, etc.) and carcinogenic activity (mesotheliomas). Unfortunately, the potential lung cancer response could not be assessed because hamsters do not develop pulmonary tumors with particulates. There are a few inhalation studies of SVFs that address dose response. The only one that was positive for cancer involved refractory ceramic fibers in rats. In that study there was a clear dose response for both cancer and noncancer endpoints and a no-effect level. There are a few other multidose studies in rats using various types of SVFs, but since none showed carcinogenic activity, one can only evaluate the dose response for noncancer endpoints. Again, there was evidence in these studies of a dose-related change in the endpoints showing recognizable change. The “stop-studies” in many of these inhalation studies (both asbestos and SVFs) provide evidence for a dose response for noncancer endpoints. However, the number of animals evaluated in the “stop studies” is too small to address a cancer dose response. The only study in primates that addresses a potential threshold of action was with chrysotile asbestos. In this study, monkeys were exposed to chrysotile asbestos at an exposure level of 1 f/cc for two years. Ten months following the last exposure, lung biopsies were taken and evaluated for fiber burden and histopathology. There was no evidence of pathology although a few asbestos bodies were observed in the lung. The monkeys were then held unexposed for an additional 10 years at which time they were subjected to necropsy examination and the lungs for histopathology examination. Again, there was no evidence of pulmonary pathology and the number of asbestos bodies had decreased.

In summary, the totality of available data suggests that there is a dose-response for both neoplastic and nonneoplastic endpoints in animals and there is a no effect level for both asbestos and SVFs. One attempt at deciding if a given exposure in animals is potentially carcinogenic involves the use of noncancer endpoints. In this scheme it was assumed that a dose that caused pulmonary fibrosis could also represent an exposure that was potentially carcinogenic in animals. This was because no animal study has ever produced cancer in the absence of fibrosis. The next assumption was that since no inhalation study had ever shown fibrosis in the absence of inflammation, one could assume that an exposure that didn't result in inflammation would not reasonably be expected to be carcinogenic. The endpoint chosen for assessing inflammation was the presence of inflammatory cells over background in bronchoalveolar lavage (BAL) fluid after a 90-day inhalation exposure. Therefore, if one did not find an increase in inflammatory cells in BAL fluid, one could chose this exposure as a no-effect threshold.

It is reasonable to expect that in vitro studies could shed light on the dose response of both asbestos and SVFs. While these types of studies are primarily designed to capture and elucidate specific mechanisms of toxicity and carcinogenicity, there may be insights into dose response that could help in establishing thresholds of effect. One such study showed that short fiber amosite did not cause inflammation, while long amosite did. The only draw backs to and in vitro approach is that these techniques do no take lung clearance phenomena into consideration and fibers that are not biopersistent in the lung might not be differentiated from biopersistent ones because of the short time frame of the in vitro studies.

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Topic#1: Physiological Fate of Asbestos and Vitreous Fibers less than 5 Microns in Length:

Short fibers (<5 microns in length) may be less pathogenic because of their decreased deposition or penetration into the airways, and increased clearance by macrophages and other cell types (reviewed in Health Effects Institute-Asbestos Research, 1991). For example, fiber length governs fiber penetration into and along the airways, and as the length increases, there is more interception which can enhance deposition (Sussman et al., 1991) This also accounts for the fact that longer fibers have proportionately more deposition in the airways as opposed to peripheral alveoli. The fact that lung retention also increases more markedly with fibers greater than 10 microns is supported by theoretical calculations (Yu et al., 1990), analysis of lung dust content in humans (Timbrell, 1982; Churg and Wiggs, 1987; Pooley and Wagner, 1998) and studies using experimental animals (Morgan 1979, 1995). Aerodynamic diameter also is a feature of fibers governing their initial deposition, and it is unlikely that fibers with a diameter exceeding 3 microns reach the alveolar regions of the deep lung (Morgan, 1995). Since most commercial fibrous glass preparations exhibit fiber diameters of approximately 7.5 microns (equivalent to mean aerodynamic diameters of 22 microns), airborne fibers for the most part may not penetrate into the lung (Lippman, 1990). The increased clearance of short fibers from the lung has been demonstrated in a number of studies (reviewed in Health Effects Institute-Asbestos Research, 1991; Davis, 1994; Oberdorster et al., 1988; Morgan, 1995). These can be: 1) readily transported through tracheobronchial and other lymph nodes to more distal lymphatics, the pleura, or other organs, 2) cleared via the mucociliary escalator and alveolar macrophages, and 3) effectively phagocytized by a number of cell types in the lung including epithelial cells (Churg et al., 2000). Once within a phagolysosome or in general in lung fluids, shorter fibers of chrysotile asbestos (Hume and Rimstidt, 1992) or glass (reviewed in Lippman, 1990) are more prone to dissolution and fragmentation than longer fibers and amphibole types of asbestos.

Topic #2: Health Effects of Asbestos and Vitreous Fibers less than 5 Microns in Length:

Human Studies: Epidemiologic data indicate that there is no increased evidence of chronic neoplastic or nonneoplastic lung or pleural disease with occupational exposures to Man-Made Mineral Fibers (MMMF) (reviewed in Lippman, 1990; Health Effects Institute-Asbestos Research, 1991). Limited evidence suggests an increase of lung carcinomas among workers using rock or slag wool, but whether or not trace metals or other contaminants in the workplace setting play a contributing role is unclear. A difficulty in

assessing the role of fiber size in disease causation in man is that historical measurements of size dimensions of fibers in past workplace settings do not exist. Moreover, size dimensions of fibers in human lungs at autopsy may not reflect the actual sizes that individuals were exposed to in the 20 or 40 year periods prior to death.

Animal Studies: Data from a number of experiments overwhelmingly support the concept that the risks of lung cancer, mesothelioma, and fibrosis increase with increasing fiber length (reviewed in Churg et al., 2000; Lippmann, 1990; Mossman and Churg, 1998; Health Effects Institute-Asbestos Research, 1991). Short fibers in these studies have much less carcinogenic activity than long fibers. Chronic inhalation of short chrysotile fibers (less than 5 microns in length) for lifetime exposures (2 years) in rats or 28 months in baboons yielded no fibrosis nor pulmonary tumors despite the presence of asbestos bodies (Platek et al., 1985). Moreover, a lifetime inhalation study in Fischer 344 rats exposed to Jeffrey mine chrysotile fibers, UICC/B chrysotile fibers or short (< 5 microns) Coalinga mine fibers showed no fibrosis nor lung tumors with the short fiber preparation, although significant tumor induction and fibrosis were noted with both long fiber preparations (Ilgren and Chatfield, 1997, 1998a) The lack of pathogenesis of the Coalinga fibers was attributed to their increased lung clearance (Ilgren and Chatfield, 1998b).

Several experiments show that asbestos and erionite fibers less than 5 microns in length have less toxicity, inflammatory potential, and disease potential after inhalation or intratracheal/intraperitoneal/intrapleural injections (Davis et al., 1986; Donaldson et al., 1989; Wagner et al., 1985; Wagner et al., 1990). Injection studies using MMMF also reveal that they are carcinogenic or fibrogenic (Wright and Kushner, 1977) if they contain large numbers of long thin fibers, but carcinogenicity and pulmonary fibrosis in rodents is only achieved after inhalation of ceramic fibers (Davis et al., 1984; ) and Aramid fibers (Lee et al., 1988) as opposed to vitreous fibers (reviewed in Health Effects-Asbestos Research, 1991).

The importance of fiber length in pulmonary fibrosis has been shown in studies using asbestos by Vorwald et al. (1951), King et al. (1946), Scymczykiewicz and Wiecek (1960), and Klosterkotter (1968). Classical studies by the Stanton (Stanton and Wrench, 1972; Stanton et al., 1977; Stanton and Layard, 1978) and Pott laboratories (Pott and Friedrichs 1972; Pott, 1978) have indicated that the induction of mesothelioma by any asbestos or nonasbestos fiber is directly related to the presence of fibers > 8 microns

in length and diameters less than .25 microns. Although some studies have suggested that short fiber asbestos preparations may be carcinogenic after injection (Kolev, 1982; Le Bouffant et al., 1985), these preparations also contained a small percentage of long fibers, making results difficult to interpret.

Inhalation studies have more convincingly demonstrated the importance of fiber length in mesothelioma, lung cancers, and pulmonary fibrosis. In studies by Wagner using erionite (Wagner et al, 1985; Wagner 1990), an almost 100% rate of mesotheliomas was induced with long fiber material, which was reduced to zero when short fiber preparations were used. This is evidence of a threshold for short fibers in tumorigenicity. Studies by Davis et al., (1986) also show that short fiber (< 5 microns) preparations of amosite produced neither fibrosis nor lung tumors, and only a single mesothelioma after injection into rats as opposed to highly pathogenic long fibers. Results with chrysotile asbestos were similar (Davis and Jones, 1988), but the short-fiber chrysotile was contaminated with some longer fibers.

An intratracheal model in rats using long (> 2.5 microns) and short crocidolite asbestos has yielded some mechanistic information on the differential effects of long vs. short fibers (Adamson and Bowden, 1987a,b; 1990). These studies suggest that the increased fibrogenic response to long fibers may be due to selective increases in cell proliferation. In addition, both long and short asbestos fibers cause alveolar macrophages to secrete fibrogenic cytokines, but interstitial fibroblasts exposed to short asbestos fibers do not respond to these cytokines.

Mechanistic studies on cells in culture or tracheal explants have also supported the increased toxicity, mutagenicity, and proliferative potential of long vs. short fibers (Brown et al., 1986; Wright et al., 1986; Donaldson et al., 1986; Marsh and Mossman, 1988; Woodworth et al., 1983; Sesko and Mossman, 1989). These studies also show that nonfibrous, chemically similar analogs of both chrysotile and crocidolite asbestos are without effects on cell proliferation or cell survival.

Studies on cell transformation and cytogenetic effects in Syrian hamster embryo (SHE) fibroblasts also demonstrate that long thin fibers are most potent, regardless of composition (Hesterberg and Barrett, 1984, 1985; Hesterberg et al., 1986). After milling of fibers to reduce the length from 10 to 16 microns to less than 1.7 microns, morphologic transformation, an indication of tumorigenic potential, is completely inhibited (Hesterberg and Barrett, 1984). Thus, a threshold for fiber length in carcinogenesis may exist.

One theory advanced by these studies is that long fibers can penetrate the nuclear membrane during division of cells and interfere with the genetic apparatus.

The increased potential of long fibers in elicitation of toxicity (broadly defined as injury to cells), proliferation, inflammation, transformation, fibrosis and carcinogenesis may be related to their ability to generate reactive oxygen or nitrogen species (ROS/RNS) after frustrated or incomplete phagocytosis by cells (Hansen and Mossman, 1987; Goodglick and Kane, 1990; Kinnula, 1999; Ohyama et al., 2001). Studies show that even short fibers at massive concentrations may elicit ROS from elicited macrophages when clearance is impaired (Goodglick and Kane, 1990). Recent studies suggest that the release of oxidants from macrophages depends on fiber length as opposed to composition – there is a strong correlation between geometric mean length and the ability to induce an oxidative response in fiber samples > 6 microns in length (Ohyama et al., 2001).

### Topic #3: Data Gaps

The major data gap in demonstrating whether thresholds exist for the effects of short (or for that matter, long fibers) of any composition is the fact that standardized preparations of sized fibers are unavailable for experimental studies, especially inhalation studies which are expensive and require vast quantities of material. This has severely hampered experimental research. The information on airborne fiberglass levels and size dimensions in environmental settings in the US is another limitation in attempting to define risks.

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Dr. Oberdörster holds a D.V.M. (1964) and a Ph.D. in pharmacology (1966) from the University of Giessen, Germany. He is a professor of toxicology in environmental medicine and head of the Division of Respiratory Biology and Toxicology at the University of Rochester School of Medicine. Since 1999, he has also served as Director at the U.S. EPA-funded Particulate Matter Center on ultrafine particles in the Department of Environmental Medicine at University of Rochester. Dr. Oberdorster has served as chairperson or session chairperson at many national and international conferences related to aerosols, inhalation and pulmonary toxicology, and natural and man-made fibrous and non-fibrous particles. He has served as a peer reviewer for over 30 scientific journals. Dr. Oberdörster has been investigating the effects and toxicokinetics of occupational and environmental particles for more than 25 years, more recently focusing on ultrafine particles. His current research includes studies related to mechanisms of acute and chronic lung injury by inhaled particulate pollutants, including ultrafine particles (inflammatory responses, fibrosis); toxicological evaluation of air pollutants measured by the response of lavagable lung cells; mechanisms of pulmonary carcinogenesis of different inorganic compounds in rats and mice; inflammation, cell proliferation, and carcinogenesis of the lung; alveolar macrophage induced cytokines, chemotactic factors and growth factors; deposition and retention modeling of inhaled non-fibrous and fibrous particulate compounds (lung-dosimetry); species differences in pulmonary responses and extrapolation to man for risk assessment; pulmonary effects of air contaminants during space flights; preventive and therapeutic measures of polymer-fume induced lung injury; and relationships of age and disease for pulmonary responses of inhaled particles. He has published ~200 journal articles on these topics.

## ATSDR Panel Meeting

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The term “fiber” should be defined first (WHO definition is different from the NIOSH definition). The selection of a 5  $\mu\text{m}$  cut for a short fiber as a limit should also be discussed. I assume that both cancer and non-cancer endpoints are to be included in the discussion and it might be useful to list the different endpoints such as:

Cancer:

Lung tumor (bronchogenic); mesothelioma (pleura; abdominal)

Non-cancer:

Chronic inflammation (bronchial, alveolar), cell proliferation, interstitial fibrosis, pleural fibrosis, others.

### **Topic 1: Physiological fate of asbestos and vitreous fibers < 5 $\mu\text{m}$ in length**

#### Physiological deposition pattern for short fibers:

At present the references do not include publications by Yu *et al.*, on the deposition and clearance of fibrous and non-fibrous particles in humans and rodents. (A list of those publications is attached.) The nose is an efficient filter for long fibers, and less for shorter ones, depending on their aerodynamic properties as will be discussed at the meeting. The aspect ratio of the fibers is an important factor for their deposition, and several figures from Dr. Yu’s work are attached.

There are significant differences between humans and rats with respect to deposition efficiencies of long as well as short fibers; respirability is very different and the deposition fractions are significantly different as well between the two species (see attached figures). For very short fibers, their aerodynamic properties approach those of spherical particles. Material density also has to be considered.

### Clearance/Biopersistence of Short Fibers:

Biopersistence is the sum of physiological clearance processes and physicochemical processes which together account for the retention half-time of the fibrous or non-fibrous material in the lung. Physicochemical processes include dissolution, leaching, breaking and splitting, depending on the fibrous material, that can occur intra- as well as extra-cellularly, and differences in pH in both locations are of importance here. Clearance rates of fibers of different length categories have been determined from short and long term inhalation studies ( refs. to be provided). Generally, short fibers are cleared rapidly if biosoluble (pH differs intracellularly vs extracellularly), or at rates similar to non fibrous particles. Breakage of long fibers will give input into short fiber category.

Most important physiological clearance mechanism in alveolar region is clearance by alveolar macrophages (AM). Of importance is fiber length with respect to phagocytosis and removal by alveolar macrophages. Short fibers are easily phagocytized, fibers longer than 20  $\mu\text{m}$  are not. Species differences in AM size. Thus, clearance for long fibers is prolonged, as is that for short fibers when high lung burdens are reached (particle overload). Also, intrinsic toxicity of short fibers has to be considered which influences clearance. Inflammatory conditions in the lung (for example, smokers) also contribute to impairment of alveolar macrophage-mediated mechanical clearance and need to be considered.

### Types of migration:

Among physiological clearance processes are translocation along the mucociliary escalator from the conducting airways; translocation to interstitial sites, depending on fiber length and fiber load. Especially at higher lung burdens, short fibers are more likely to penetrate into the interstitium and translocate to pleural sites, lymph nodes, and short fibers can even enter the blood circulation.

Animal studies found preferentially translocation of short fibers to the pleural space in rodents when high lung burdens after inhalation exposure were achieved (Gelzleichter *et al.* 1996). They found a geometric median length of 1.5  $\mu\text{m}$  for fibers recovered at the pleural site after RCF exposure, whereas the inhaled RCF fibers had a geometric median length of 4.5  $\mu\text{m}$  with the longest fibers being longer than 100  $\mu\text{m}$ . Very few fibers longer than 5  $\mu\text{m}$  were found at the pleural site, whereas longer fibers were found in the pulmonary tissues but did not appear to migrate to the pleura.

Migration of the short fibers in the animal studies resulted in a pleural inflammatory response which was lower than in the lung and was also delayed compared to the response in pulmonary tissue.

In general, many studies with fibers have used fiber preparations with so-called “non-fibrous particles” which contain significant numbers of short fibers (if the WHO definition is followed). Such short fibers are of importance since they contribute to the overall lung burden and may actually amplify the effects of long fibers as has been shown in animal studies with mixed fibrous/non-fibrous particle exposures (both cancer and non-cancer endpoints).

In this context, inhalation studies by Bellmann *et al* (2001; 2002) using RCF with and without non fibrous particles are of interest: Reduction of the non fibrous particles restored impaired clearance of test particles, although other endpoints of toxicity (lung lavage data, histopathology) did not seem to be significantly different, and fiber clearance per se was not different between the 2 groups. (Bellmann, 2001). (Need to consider also total lung burden differences between the 2 groups!) Non fibrous particles of the same chemical composition induced high inflammatory responses in a subchronic inhalation study ( Bellmann *et al*, 2002,a,b: *Ann. Occup. Hyg.* 46, Suppl.1, 102-104; and 166-169, 2002)

## **Topic 2: Health effects of asbestos and vitreous fibers <5 microns in length.**

### Robustness of animal and human cancer data for short fibers:

Animal inhalation and i.p. injection studies consistently show that short fibers are clearly less tumorigenic than long fibers (*e.g.*, Davis *et al.* studies, example attached). An important factor in many studies is the existence of “a non-fibrous fraction” in fiber samples which contribute to both cancer and non-cancer effects of the fibers. Contamination of a fiber sample with other fibrous materials (for example, tremolite [more toxic] in chrysotile [less toxic]) is also important. With respect to cancer induction, the intrinsic toxicity of short fibers — like that of non-fibrous particles — is of high importance, both are readily phagocytized by alveolar macrophages and subjected to AM-mediated clearance unless they have significant cytotoxicity (*e.g.*, crystalline silica *vs.* TiO<sub>2</sub> for non-fibrous particles). Biopersistence is a most important factor as pointed out and discussed under Topic 1.

### Potential for SVF to induce cancer:

Biopersistence is a most important factor (see emphasis in new European regulations for testing of SVFs for biopersistence, in order for SVF's to be exonerated from a carcinogen label). Other important factors are exposure concentration (dose to the lung), length (long fibers most carcinogenic) and surface properties (crystalline *vs.* amorphous).

### Evidence of short fibers causing health effects:

Contribution of short fibers to effects caused by long fibers is probably similar to non-fibrous particles (mixed dust exposure studies). For rats a pathogenic mechanism due to lung overload from short fibers/non-fibrous particles becomes important for high doses, overload conditions in humans are not likely to be achieved (relevance of discussion on particle overload?). However, low doses

also result in AM activation. To be considered as well: compromised hosts (respiratory; cardiovascular); and pre-exposure history (e.g., development of tolerance).

Indirect evidence for short fibers to induce health effects:

Evidence exists from combination studies, *e.g.*, asbestos fibers  $\pm$  TiO<sub>2</sub> or SiO<sub>2</sub>; need to discuss effects of non-fibrous particles of different compositions, animal studies (usually very high doses) *vs.* human exposures.

Length of material to no longer induce fiber-like toxicity:

There is an no systematic study which would allow to define a specific fiber length to answer this question. There are a number of studies showing that short fibers (<5  $\mu$ m) are less biologically active than long fibers. The discussion here needs to focus also on what is “fiber-like toxicity”: A clear difference obviously exists when fibers are phagocytizable *vs.* non-phagocytizable, as well as their propensity to be translocated into and across epithelium. There are data for tangential *vs.* perpendicular uptake of fibers by AM, resulting in different responses (Okyama et al., 2001), but not conclusive. There is also the issue of nanofibers (*e.g.*, nanotubes). These fibers are so small that they very likely behave very differently with respect to interactions with cells, *e.g.*, translocation to interstitial and extrapulmonary tissues.

Thresholds for mechanisms of toxicity for short fibers:

Existence for threshold for pulmonary kinetics (accumulation and retention, can we extrapolate from non-fibrous studies? General threshold when exceeding physiological defense mechanisms (*e.g.*, clearance mechanism, antioxidant – anti-inflammatory defenses). LN accumulation as indicators of toxicity?



### **Topic 3: Data gaps.**

Data on toxic effects of fibers of one specific length only, without contamination with longer fibers (*in vitro*, i.t. instillation; inhalation) of materials of different compositions. Side by side comparison of different effects of different length fibers with non fibrous particles of the same material.

#### **Research needed to fill gaps:**

Short-term studies, i.t. combination studies, long fibers alone; long combined with short fibers, different fiber length and non fibrous particles, dosed by different dosemetrics, *e.g.*, mass, number, surface area. Toxicokinetic studies (accumulation, retention)

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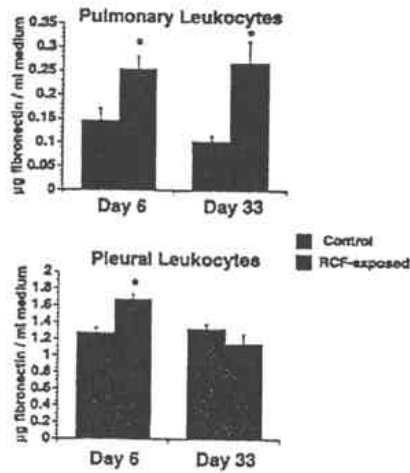


FIG. 3. Soluble fibronectin in conditioned medium following a 48-hr incubation with pulmonary leukocytes and pleural leukocytes. Lavaged leukocytes were plated in 24-well dishes, preincubated for 2 hr in RPMI media with 10% FBS, washed thoroughly with PBS, and incubated for 48 hr in complete medium. Asterisks denote that mean values were significantly different from control values ( $p < 0.05$ ).

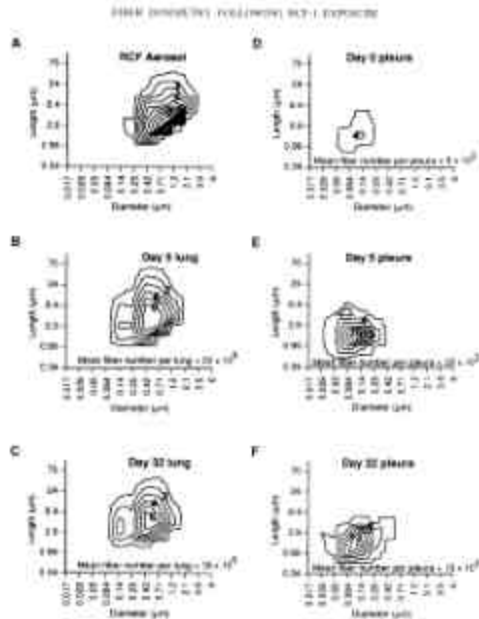


FIG. 2. Relative size distribution of bronchovascular (A-C aerosol), Day 9 (B), Day 33 (C) lung, and pleural (D-F) lesions. Relative size distribution based on stereologic analysis of the aerosol (A) and RCF lesion data coverage of the lung (B-E) and pleural (D-F) lesions. The relative size distribution of the lesions in the lung and pleural lesions were visualized at Day 9 and Day 33 with sizes added to the curves equal to 100 for Day 9.

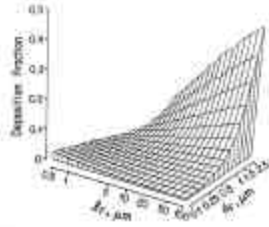


Fig. 3. Trichobronchial deposition via trachea with an intertracheal deposition at a lung volume of  $10.78 \text{ cm}^3$ , a tidal volume of  $3.05 \text{ cm}^3$ , a breathing frequency of  $56 \text{ cycles min}^{-1}$ , and  $\rho = 3.57 \text{ g cm}^{-3}$ .

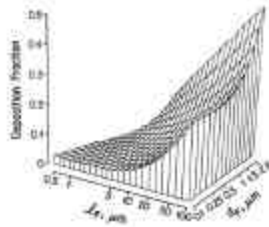
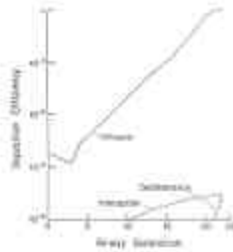
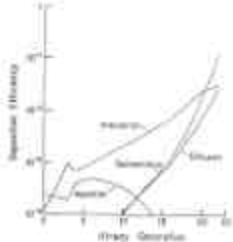


Fig. 4. Trichobronchial deposition via trachea at a lung volume of  $10.78 \text{ cm}^3$ , a tidal volume of  $1.68 \text{ cm}^3$ , a breathing frequency of  $66 \text{ cycles min}^{-1}$ , and  $\rho = 3.37 \text{ g cm}^{-3}$ .

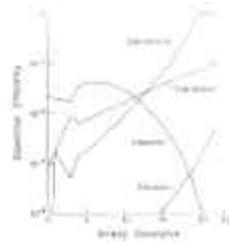


Deposition Efficiency of Fibres in Different Generations of the Naheki Lung Model at a Flow Rate of  $375 \text{ ml/min}$  and  $\rho_{\text{agg}} = 0.91 \mu\text{m}$  and Size Range:



Deposition Efficiency of Fibres in Different Generations of the Naheki Lung Model at a Flow Rate of  $375 \text{ ml/min}$  and  $\rho_{\text{agg}} = 1 \mu\text{m}$  and Size Range:

From: Asgharian and Yu, 1988



Deposition Efficiency of Fibres in Different Generations of the Naheki Lung Model at a Flow Rate of  $375 \text{ ml/min}$  and  $\rho_{\text{agg}} = 10 \mu\text{m}$  and Size Range:

From: Timbrell, Inhaled Particles V, 1982

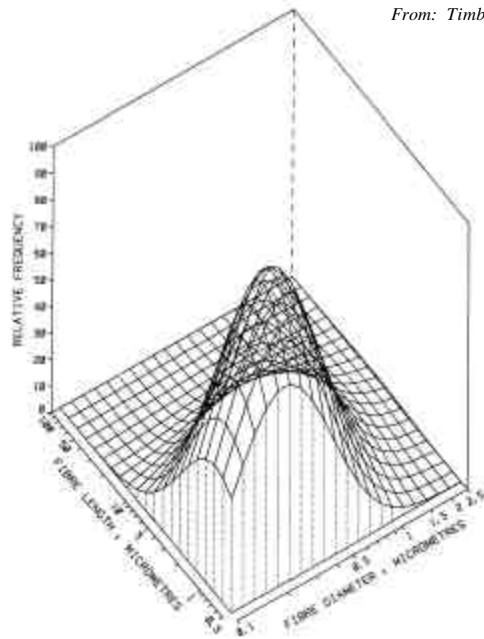


FIG. 8. Bivariate distribution of fibres in Paakkila bagging section.

From: Timbrell, Inhaled Particles V, 1982

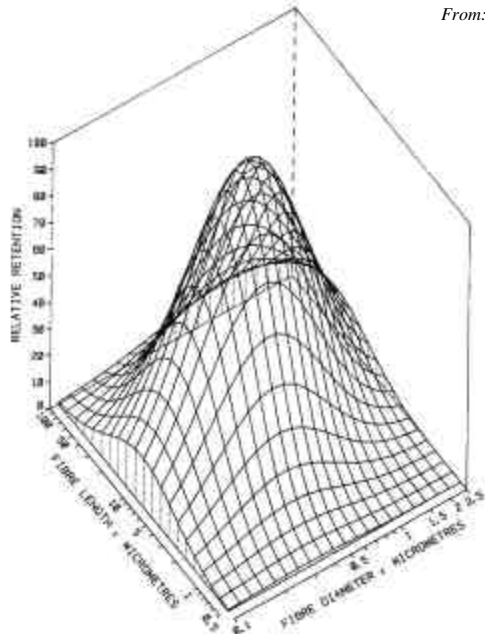


FIG. 10. Bivariate presentation of fibre retention.

From: Timbrell et al.,  
*Inhaled Particles VI*,  
1988

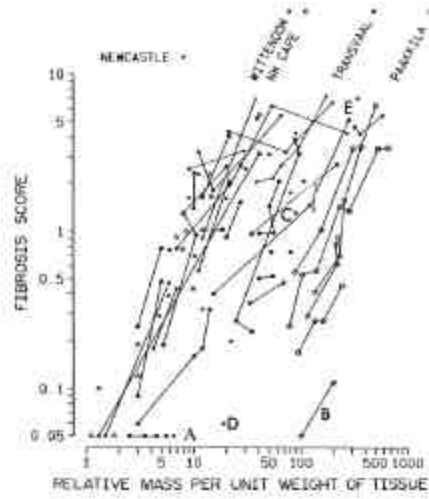


FIG. 3. Relationships when the parameter of fibre quantity is mass. Linked data points relate to tissue specimens from the same subject.

From: Timbrell et al.,  
*Inhaled Particles VI*,  
1988

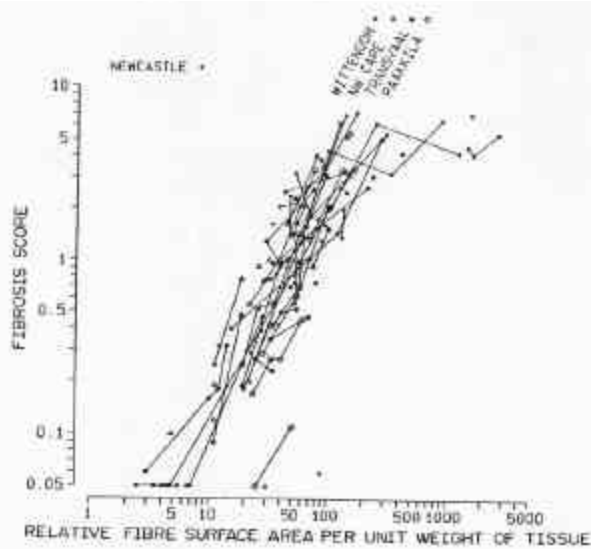


FIG. 4. Relationships when the parameter of fibre quantity is surface area.

From: Timbrell et al.,  
*Labeled Particles VI*,  
1988

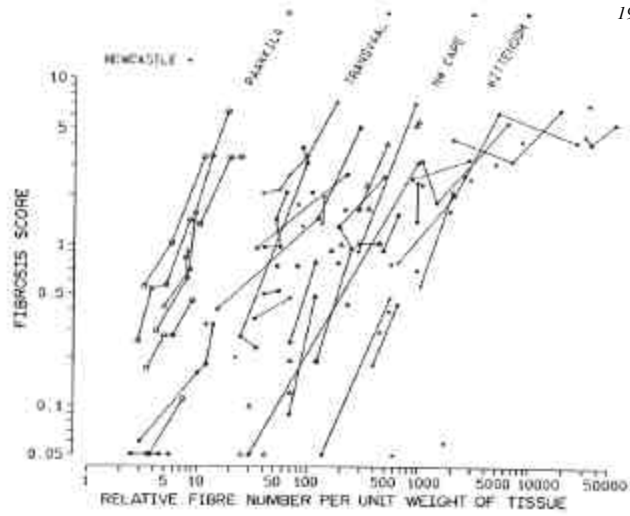


FIG. 5. Relationships when the parameter of fibre quantity is number.



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Dr. Wallace holds a Ph.D., M.S., and B.S. in physics from West Virginia University. He is currently serving as a molecular biophysics team leader and research physical scientist in the Exposure Assessment Branch, Health Effects Laboratory Division of NIOSH, in addition to acting as a National Research Council Postdoctoral Research Advisor. Dr. Wallace has been an adjunct professor in the Department of Chemical Engineering, Genetics and Developmental Biology Program, and a member of the graduate faculty, College of Engineering and Mineral Resources at West Virginia University since 1985. His current research at NIOSH centers around respirable particle surface properties and toxicity, explored through directing projects examining elemental surface composition of respirable dusts, surface chemistry characterization of particulate exposures, computational studies of biomolecular adsorption on mineral surfaces, and toxic respirable particle biological surface interactions. He has contributed articles to many books and journals including, papers related to the effect of chrysotile fiber surface composition on genotoxicity in vitro, particulate surface-phospholipid surfactant interactions affecting expressions of toxicity, in vitro genotoxic activities of diesel exhaust particulate soot and of quartz dust, intracellular surfactant removal from phagocytized minerals, the structure of silica surfaces in relation to cytotoxicity, and modulation of silica pathogenicity by surface processes.

## Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length

### Topic #2: Health Effects of Asbestos and Vitreous Fibers less than 5 micrometers in length

Question: Is there indirect evidence for less-than-5 micron fiber induced adverse health effects? Do the mechanisms of action of other materials (e.g., larger asbestos fibers, silicates, mineral dusts, amorphous silica) with potentially similar compositions aid in understanding small-fiber mechanisms of action?

#### Discussion:

There appears to be a significant difference in the pathogenic activity of respirable fibers with fiber length, with fibers below approximately 5  $\mu\text{m}$  (micrometers) in length being significantly less hazardous for cancer or pulmonary fibrosis.

This prompts the questions: What are the mechanisms of observed long fiber toxicity? Are compositionally similar non-fibrous dusts pathogenic? If so, what are the mechanisms of their toxicity? Do short fibers express either or both or combinations of those toxic mechanisms.

There is a profound literature on the topic of long fiber mechanisms of toxicity and fibrogenesis. The report by V Kinnula "Oxidant and antioxidant mechanisms of lung disease caused by asbestos fibers" *European Respiratory Journal* 14(3):706-716, 1999, reviews the possible roles of reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by asbestos fiber in cell-free and cellular and tissue systems. A primary step in response to asbestos fiber challenge of cells is agreed to be superoxide anion release in cells which have attempted to phagocytize fiber. This superoxide can further be dismutated to hydrogen peroxide, which can generate hydroxyl radical, catalyzed by iron via the Fenton reaction. That hydroxyl radical is extremely toxic and reactive, but therefore short-lived. There is some contention that fibers stimulate the release of ROS from inflammatory cells and not target cells. However, asbestos fiber can generate ROS spontaneously in cell-free systems.

Another pertinent review is by C Manning, V Vallyathan, and B Mossman: "Diseases caused by asbestos: mechanisms of injury and disease development" *International Immunopharmacology* 2:191-200, 2002. This explicates the central dogma that asbestos fibers activate transcription factors and early response genes involved in cell proliferation by generating ROS on iron-containing fiber surfaces, and that "frustrated" phagocytosis may be involved.

The paper by M Ohyama, T Otake, and K Morinaga presents a difficult argument against frustrated phagocytosis: "Effect of size of man-made and natural mineral fibers on chemiluminescent response in human monocyte-derived macrophages." *Environ Hlth Perspec* 109:1033-1039, 2001. This study of lucigenin-dependent chemiluminescence (CL) induced in vitro over a 2 h period found a strong correlation of response indicative of superoxide release with fiber length 6 to 20  $\mu\text{m}$ . All samples except wollastonite induced CL response in a dose-dependent manner. Superoxide release was non-specific for compositional type of fiber. The four fibers with lengths below 7  $\mu\text{m}$ : KT whisker, at 6  $\mu\text{m}$ , microglass at 3  $\mu\text{m}$ , TO whisker at 2  $\mu\text{m}$ , and SiC whisker at 6.4  $\mu\text{m}$  were only weakly active. Longer fiber activity correlated with length. This is consistent with the extensive literature indicating long, thin, durable fibers are tumorigenic.

Some other studies suggest and support a "frustrated" phagocytosis mechanism. This includes some recent NIOSH research results: T Blake, et al. "Effect of fiber length on glass microfiber cytotoxicity" *J Toxicol Environ Hlth* 54:243-259, 1998. CL induction after zymosan stimulation and LDH release were measured for Manville Code 100 (JM-100) fiber challenged rat AM in vitro in EMEM for 18h. A novel feature of this study was the use of fibers carefully sized to average lengths of 33, 17, 7, 4, and 3  $\mu\text{m}$ . The greatest toxicity was seen with the longer fibers. And multiple macrophages were seen attached along the length of the long fibers., suggesting "frustrated" or incomplete phagocytosis as a factor in increased toxicity with length.

This was also seen in J Ye et al. "Critical role of glass fiber length in TNF-alpha production and transcription factor activation in macrophages." *Am J Physiol* 276 (Lung Cell Mol Physiol 20):L426-L434, 1999. Glass fibers with lengths of 6.5 +/- 2.7  $\mu\text{m}$  and 16.7 +/- 10.6  $\mu\text{m}$  were used to challenge a mouse macrophage cell line in fetal calf serum (FCS)-containing culture

medium., for 3, 6, and 16 h. Glass fibers stimulated TNF-alpha production and caused NF-kB activation. Reactive oxygen species (ROS) were involved in the activation and production. Long fibers were more potent than short fibers. Short fibers but not long fibers were effectively engulfed by macrophages. However, short fiber induced TNF-a and TNF-a gene promoter activation was on the order of one-third to one-half that of the long fiber.

In a subsequent study by Ye et al. "Activation of mitogen-activated protein kinase p38 and extracellular signal-regulated kinase is involved in glass fiber-induced tumor necrosis factor-alpha production in macrophages" J Biological Chem 276:5360-5367,2001., it was found that the long fibers were more potent than short fibers in activating MAP kinases which activates transcription factor c-Jun which acts on the TNF-a gene promoter through the cyclic AMP response element and the AP-1 binding site.

In a study by Cheng et al. "Role of transcription factro NF-kB in asbestos-induced TNF-alpha response from Macrophages" Expt. And Mol Pathology 66:201-210, 1999, Crocidolite with a median fiber length of 11.5 um challenged lavaged rat AM in FBS-containing medium for 1 to 24 h.. Crocidolite caused parallel increases in TNF-a production and NF-kB activation.in a dose-dependent manner. Interestingly, at the optimun stimulating condition the asbestos did not cause a significant cytotoxic effect. A titanium oxide control dust had no stimulatory effect on TNF-a secretion.

One aspect of fiber production of toxic hydroxyl radical is that fibers long enough to be not fully phagocytized by a cell are involved in "frustrated" phagocytosis. One possible consequence is that the partially invaginated fiber stimulates the cell to release superoxide in a manner related to the respiratory burst upon normal phagocytosis, or that superoxide is produced by the cell in response to an autolytic effect of enzymes or other lysosomal or cytosolic agents released into the annular invagination of the fiber. The superoxide is then in close approximation with reactive iron species on the fiber surface in or extending beyond the partially invaginated fiber to create hydroxyl radical for strongly toxic effects at the cell or neighboring cells.

Mechanisms of toxicity for fibrous and non-fibrous materials are discussed by A Churg et al. In "Pathogenesis of fibrosis produced by asbestos and man-made mineral fibers: what makes a fiber fibrogenic?", Inhalation Toxicology 12(S3):15-26, 2000. The review highlights caveats to the general models of asbestos activity. Some fibers can evoke the responses from ROS

generation through the cascade to and including expression of TNF-alpha, but have not been shown to induce fibrosis. And asbestos produces fibrosis in some systems without increasing TNF-alpha expression. Chrysotile contains little iron but is fibrogenic, albeit not as potent as amphibole.

Churg et al. Suggest a comparison of asbestos and silica-induced fibrosis data. Table 3 of the paper compares the generation of ROS, RNS, and activation of NF-kB and AP-1, and increased production of TNF-alpha and other factors and find the dusts to be indistinguishable. In the face of this, asbestosis and silicosis differ in histopathological appearance: asbestosis is a diffuse fibrosis and silicosis is in localized nodules.. The conclusion is that the tabulated responses fail to explain comprehensively how asbestosis or silicosis develop.

Crystalline silica dust is a well established etiologic agent for pulmonary fibrosis, i.e., silicosis. However (a) the mechanism of the disease is still not fully known, and (2) the effect of silica in mixed dust exposures frequently is not proportional to the silica content of the dust, and (3) short-term in vitro investigations of toxicity fail to distinguish crystalline silica from some non-fibrogenic dusts.

Crystalline silica can directly cause membranolysis and induce the release of cytosolic and lysosomal enzymes from lavaged lung macrophages or other cell lines in vitro. Experiments with thermally treated crystalline quartz and cristobalite have shown the the membranolytic activity is associated with silica surface silanol hydroxyl (not hydroxyl radical) groups.

From a compositional standpoint, crystalline quartz is not a good non-fibrous analog of asbestos. Riebeckite is one such choice, and is not fibrogenic. A partially analogous set of non-fibrous minerals are layered alumino-silicate minerals, clays. Our research has been comparing in vitro cytotoxicities and physico-chemical surface properties of respirable quartz in comparison with the structurally-simplest clay, kaolinite. Those comparisons may provide some limited guidance in assessing the potential toxicities of short fiber asbestos which are distinct from fiber size and "frustrated" phagocytosis-associated mechanisms.

Respirable quartz is strongly fibrogenic, while respirable kaolin is not. Nevertheless, in vitro short term tests do not distinguish between them. On a surface area basis they are comparably active for membranolysis, lactate dehydrogenase (LDH) release, beta-glucuronidase release, beta-n-acetyl glucosaminidase release, and cytotoxicity as measured by trypan blue dye exclusion. It is important to note that these are short-term (one to a few hour) in vitro challenges in the absence of serum in the medium, or with the challenge managed such that serum is excluded from contact with the dusts during the challenge period. It appears that silica and silicate surfaces are comparably innately active for direct prompt cell membranolytic damage.

When a respired particle deposits in the terminal lung airways or the pulmonary alveoli, its first contact is not with the epithelium surface or with free macrophages on the lung surface, but with a thin hypophase environmental interface which is coated by and saturated with a dispersion of lung surfactants. This surfactant coating is known to function to reduce the surface tension of the air - aqueous layer interface. However, it appears to also function to suppress the otherwise prompt cytotoxicity of many non-fibrous mineral dusts, e.g., silica and silicates. Brief incubation of silica or clay dust in a dispersion of diacyl phosphatidyl choline (DPPC), the primary constituent of lung surfactant, in physiologic saline results in the immediate attenuation of dust cytotoxicity. That passivation is total if adequate surfactant-to-dust surface area is available. That is always the case for a normal lung under other than suffocating dust exposure conditions. That surfactant adsorption and passivation occurs for quartz dust as well as kaolin dust. So the question becomes not why both dusts are not strongly fibrogenic, but rather why either is active. Research indicates that multi-layers of DPPC surfactant will loosely adsorb to the particles, but a residual bilayer which cannot be water rinsed from the particle surface is fully prophylactic.

Surfactant coated dusts are phagocytized by lavaged rat macrophages in vitro and do not express otherwise prompt cytotoxicity, e.g., damage measurable in a one or two hour time after challenge. However, over a several day period there is a restoration of toxicity seen in the lavaged macrophage system or in vitro systems using several different cell lines. Radio-tracer studies show that the surfactant coating on the dusts is digested in parallel with the restoration of toxicity. Cell-free system studies show that phospholipase A2 hydrolyzes the particle bound DPPC surfactant. The lysolecithin product is partially water soluble. The restoration of membranolytic activity maps with the digestive removal of the adsorbed surfactant.

That is, adsorption of components of pulmonary surfactant promptly adsorb and passivate silica and silicate surfaces by prophylactically masking the membranolytic dust surface silanol hydroxyl groups. But following phagocytosis the particles are stripped of the protective coating by phagolysosomal enzymatic digestion. Research has indicated that the kinetics of the first half of the digestion process are rapid compared to the second half, and that toxicity restoration follows with removal of that second half of the surfactant. That is, the outer side of the adsorbed bilayer is readily digested and the surface-contacted layer more slowly. Using extracellular pH-neutral PLA2 in a cell-free system, the rate of digestive removal of DPPC is significantly greater for quartz-adsorbed in comparison to kaolin-adsorbed DPPC. However, for phagocytic cell in vitro systems, quartz and kaolin rates of surfactant loss and toxicity restoration are comparable.

Churg et al. Briefly discuss the principal site of asbestos activity, noting the alveolar macrophage is commonly regarded as the crucial effector cell. This is the background assumption also for most experiments on the cytotoxic and fibrosis-associated activity of crystalline silica dusts. However, Adamson, referenced by Churg et al in a different context, has published a suite of studies which make a case that it is interactions of silica particles with interstitial cells which control the stimulation of exacerbated collagen synthesis by pulmonary fibroblasts, and that the macrophage is responsible for only an inflammatory response evoking neutrophil influx to the alveolus but not tied to explicit fibrosis. Thus that model suggests that it may be the removal of surfactant under conditions of interstitial cell phagolysosomal or extracellular digestion which initiates cell response leading to fibroblast stimulation and fibrosis. While the mechanism of initial cell damage or stimulation may differ between silica or silicates and fibers, e.g., ROS from a “frustrated” phagocytosis mechanism for asbestos and surface silanol hydroxyl membranolysis by quartz or clay, a parallel analysis to Adamson’s silica study and findings should be considered for localization of the effective site of asbestos action for fibrosis.

We have briefly researched the question of the effect of surfactant adsorption on chrysotile in vitro genotoxicity, using an assay for micronucleus induction in cultured Chinese hamster lung cells (V79 cells). J Lu et al. “In vitro genotoxicity studies of chrysotile asbestos fibers dispersed in simulated pulmonary surfactant” *Mutation Res* 320: 253-259, 1994. Two lengths of chrysotile asbestos were used: NIEHS intermediate length (101  $\mu\text{m}$  mean with 65% > 10 $\mu\text{m}$ ) , and short length (11.6  $\mu\text{m}$  with 98% of fibers < 10  $\mu\text{m}$ ) chrysotile fibers. Fibers were pre-treated with DPPC and used to challenge V79 cells in FBS-supplemented medium for a total of 72 h. Four



types of fiber preparations : intermediate length +/- DPPC treatment, short length +/- DPPC, gave dose dependent micronucleus induction activity. The longer fiber samples were most active and DPPC treatment diminished the activity approximately 15%, the maximum activity of the short fiber sample was 70% of the activity of the non-treated intermediate; and the DPPC treated short fibers expressed about 45% of the activity of the untreated. That is, DPPC did not fully suppress the activity of the fibers, but had a much more pronounced partial passivation effect on the short fibers.. One possibility is that the partial suppression of the activity may reflect surfactant suppression of a component of toxicity due to mineral surface rather than “frustrated” phagocytosis mechanism.

We also attempted to see if a significant surface modification of chrysotile without a significant modification of fiber size would affect in vitro genotoxic activity. M Keane et al. “A study of the effect of chrysotile fiber surface composition on genotoxicity in vitro”. *J Tox & Environm Hlth*:57:529-541, 1999. NIEHS intermediate length chrysotile again was used in the V79 system, with parallel samples of the fiber which had been subjected to mild acid leaching to remove near-surface magnesium. Fiber modification was demonstrated and measured by X-ray photoelectron spectroscopy, scanning electron microscopy - X-ray spectroscopy, and zeta-potential measurement. No significant differences in genotoxic activity were found between the treated and untreated fibers.

Small fiber toxicity may be a combination of mineral surface functional group direct membranolytic activity as modulated by interactions with components of the pulmonary surfactant system, and some limited “frustrated” phagocytosis-associated ROS induced damage, the latter dependent on the possible spread of fiber length values which permit or hinder phagocytosis with variations in cell size.

In assaying for the first mechanism, the non-fiber mechanism, there is one additional caveat to be considered in experimental design. While the use of components of pulmonary surfactant or of surrogate surfactant, e.g., Survanta, may partially model a physiological prophylaxis in the lung, cell test systems may inadvertently introduce a non-representative prophylactic effect through the use of serum in the in vitro system media. Dr. Oberdorster and colleagues published an observation of such partial passivation of silica by lipoproteins in a serum medium seen in an in vitro test; and we have seen apparent similar phenomena in the passivation of kaolin in such a system.

This is a peripheral matter to the question posed, but of possible interest with regard to the overall question of health protection for anomalously high environmental or occupational exposures to respirable and potentially fibrogenic particles or fibers. Some of the provided literature and the body of medical experience indicate that pulmonary fibrosis resulting from asbestos or non-extreme silica dust exposures may be slow to progress, but detection can be subject to even greater delay: Early pulmonary fibrosis seen in tissue section histopathology frequently is not discernable on chest X-ray. Some of the new imaging modalities may provide an alternative to conventional radiological detection or grading of pulmonary fibrosis. Tritiated proline amino acid has been used for decades for autoradiographic analysis of collagen formation in lung necropsy sections of animal models of silicosis. Positron emission tomography using a positron-emitter labeled analog of the amino acid proline, which is used in great fractional quantity in collagen synthesis, may permit a relatively non-invasive method to detect and localize heightened collagen synthesis activity, i.e., localized fibrosis, in the lungs after exposure to asbestos or crystalline silica respirable dusts. Initial testing with a rabbit model of silicosis suggests the approach has the efficacy for detection of fibrosis as an early active metabolic event. Wallace et al. "Cis-4-[F-18]fluoro-L-proline PET imaging of pulmonary fibrosis in a rabbit model". J Nuc Med 43:413-420,2002. .Specificity of the method, e.g., for response to fibroblast collagen synthesis versus to a generally heightened metabolism associated with non-specific and transient inflammatory response by macrophages or neutrophils. The latter is seen with conventional fluoro-deoxy-glucose PET imaging. A non-invasive method for active fibrosis might also aid in surveillance of special populations for early indications of lung fibrosis or in evaluating medical management of advancing disease.