Report on the Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length

Prepared for:

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NOTE

This report was prepared by Eastern Research Group, Inc. (ERG), an ATSDR contractor, as a general record of discussion for the expert panel meeting on "Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length." This report captures the main points of scheduled presentations, highlights discussions among the panelists, and documents the public comments provided at the meeting. This report does not contain a verbatim transcript of all issues discussed, and it does not embellish, interpret, or enlarge upon matters that were incomplete or unclear. ATSDR will use the information presented during the expert panel meeting to aid in developing scientifically sound public health evaluations for exposures to short fibers. Except as specifically noted, no statements in this report represent analyses by or positions of ATSDR or ERG.

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List of Abbreviations

ATSDR	Agency for Toxic Substances and Disease Registry
BAL	bronchoalveolar lavage
DPPC	dipalmitoyl phophatidyl choline
EPA	U.S. Environmental Protection Agency
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
IARC	International Agency for Research on Cancer
NIOSH	National Institute for Occupational Safety and Health
PCM	phase contrast microscopy
PMR	proportional mortality ratio
RADM	rear admiral
RCF	refractory ceramic fiber
ROS	reactive oxygen species
SMR	standardized mortality ratio
SVF	synthetic vitreous fibers
TNF-α	tumor necrosis factor-alpha
µm	micrometers
WTC	World Trade Center

Executive Summary

Seven expert panelists reviewed and discussed the state of the science on how fiber length relates to toxicity of asbestos and synthetic vitreous fibers (SVFs)—an issue relevant to the Agency for Toxic Substances and Disease Registry's (ATSDR's) ongoing work at several sites where fiber contamination is found in or near residential neighborhoods. The expert panelists included epidemiologists, pathologists, physicians, hygienists, pulmonologists, and toxicologists. During a 2-day meeting in October 2002 in New York City, the panelists thoroughly discussed the physiological fate of structures less than 5 micrometers (μm) in length having aspect ratios greater than 3:1, health effects of asbestos and SVFs of the same dimensions, and research needs.

The panelists' main findings and recommendations are listed below. The remainder of this report summarizes the discussions and observations that led to these findings, and reviews the panelists' comments on many topics not listed in this executive summary. This report provides insights and advice on how to interpret exposures to asbestos and SVFs less than 5 μm in length based on panelist discussions; however, the contents of this report should not be considered ATSDR policy.

- **Factors that influence toxicity.** Health effects from asbestos and SVFs ultimately are functions of fiber dose, fiber dimension (length and diameter), and fiber durability or persistence in the lung (as determined by the mineral type, the amorphous or crystalline structure, and the surface chemistry).

- **Fibers or particles?** Some panelists questioned why structures less than 5 μm long, regardless of their aspect ratio, were referred to as "fibers." This report refers to structures less than 5 μm long as "fibers," while acknowledging that some expert panelists have reservations about this terminology.

- **Deposition and retention of short fibers.** The lung depositional patterns of fibers less than 5 μm long have been well established and depend almost entirely on fiber width. For short fibers with diameters between 0.1 and 1.6 μm, total lung deposition in healthy people will be between 10% and 20% of what is inhaled, with most of that deposition occurring in the deep lung; the fibers that do not deposit will be exhaled. For short fibers

with diameters less than 0.1 μm, a greater proportion will deposit and there will be a somewhat greater proportion of deposition in the proximal airways.

The short fibers can be cleared from the lung by various mechanisms, depending on where the fibers deposit. Fibers depositing on the surface of conductive airways (i.e., the tracheobronchial region) are efficiently cleared by the mucociliary escalator, generally within 24 hours. Many of the short fibers that reach the gas exchange region of the lung are cleared by alveolar macrophages, and the rate of clearance by phagocytosis has been found to vary with fiber length and to differ across mammalian species. One panelist, for instance, cited *studies of mice and rats* suggesting that phagocytosis clears short fibers from the alveolar regions of the lung within a few weeks following exposure. On the other hand, another panelist noted that researchers have established that alveolar macrophage mediated clearance in *human* lungs takes considerably longer (retention half-times of 400 to 700 days). Overall, panelists noted that rodents clear short fibers from their lungs approximately 10 times faster than do humans. Deposition and retention patterns may differ in people with impaired capacities to clear foreign material from their lungs. The extent to which short fibers preferentially translocate from the gas exchange region to the pleura is not well known.

- **Cancer effects of short fibers.** Given findings from epidemiologic studies, laboratory animal studies, and *in vitro* genotoxicity studies, combined with the lung's ability to clear short fibers, the panelists agreed that there is a strong weight of evidence that asbestos and SVFs shorter than 5 μm are unlikely to cause cancer in humans.

- **Noncancer effects of short fibers.** The laboratory animal studies, epidemiologic studies, and *in vitro* studies generally suggest that asbestos and SVF pathogenicity increases with fiber length, but there are several notable exceptions. In laboratory animals, for example, short asbestos and SVFs at sufficiently high doses have been shown to cause inflammation, pulmonary interstitial fibrosis, and pleural reactions; however, the doses needed to cause these effects in humans may not be relevant to environmental exposures. In humans, four epidemiologic studies (Churg et al. 1989, 1990; Nayebzadeh et al. 2001; Case 2002b) involving highly exposed workers found that pulmonary interstitial fibrosis is correlated with the amount of *short fibers* in the lung at death; some researchers have hypothesized that this apparent association is explained by long fibers breaking down into shorter fibers between exposure and the time at which lung samples were collected. Finally, at least two *in vitro* studies (Ye et al. 1999, 2001) have found that short fibers are at least as active as, if not more active than, long fibers on a surface area or mass basis for multiple endpoints (e.g., tumor necrosis factor-alpha [TNF-α] production, activation of TNF-α gene promoter activity); however, the relevance of these *in vitro* findings to health effects *in vivo* is not known. Taken together, the findings from the laboratory animal, epidemiologic, and *in vitro* studies suggest that short fibers may be pathogenic for pulmonary fibrosis, and further research is needed to clarify this issue.

- **Research needs and recommendations.** Throughout the meeting, the panelists identified data gaps and made recommendations for filling them. Some recommendations addressed issues specific to sites (e.g., Libby, Montana; Lower Manhattan) with concerns about short fibers in residential communities. These recommendations are listed in Section 4.1. The panelists' recommendations for general research projects follow, in no particular order:

  - Encourage increased use of sampling human lung tissue or other biological indices, such as sputum collection, in known or suspected human exposure situations to improve both qualitative and quantitative exposure assessment.

  - Conduct a laboratory animal study to characterize the extent to which fibers of all lengths translocate into the pleura, and whether the translocation preferentially occurs for fibers of any dimension or type. Some panelists noted that translocation of fibers into the pleura does not necessarily imply causation of pleural disease, the mechanisms and site of action of these mechanisms being unknown (Kane et al. 1996). One panelist indicated that some studies (e.g., Gelzeichter et al. 1996; McConnell et al. 1999) have already examined this issue, to a certain extent, for refractory ceramic fibers; and a follow-up study has recently been completed, but not yet published, for amphibole fibers.

  - Develop and adopt standardized environmental and biologic sampling and analytical protocols to ensure that samples collected from different sites for different purposes can be compared.

  - Perform personal exposure sampling, or an equivalent, to quantify what exposures result when household surfaces are contaminated with asbestos or SVFs; analyze samples using conventional fiber counting methods (i.e., counting only fibers longer than 5 μm), but archive a subset of filter samples for further analysis.

  - Further investigate the possible association between short fibers and pulmonary interstitial fibrosis in humans and the impact of short fibers in regard to pleural changes, such as pleural plaques and diffuse pleural fibrosis.

  - Design and conduct an *in vitro* study to characterize the influence of fiber length on cell proliferation, DNA damage, and cytotoxicity endpoints that can then be confirmed in animal studies.

1.0 Introduction

The Agency for Toxic Substances and Disease Registry (ATSDR) invited seven expert panelists to a meeting to discuss the current understanding of health effects related to asbestos and synthetic vitreous fibers (SVF) less than 5 micrometers (μm) in length—an issue that is related to the agency's ongoing work at many sites. The expert panel review took place in a meeting open to the public on October 29–30, 2002, in New York City. Discussions at the meeting focused on three specific issues: the physiological fate of fibers less than 5 μm in length, health effects of fibers less than 5 μm in length, and data gaps.

This report summarizes the technical discussions among the expert panelists and documents comments provided by observers. The remainder of this introductory section reviews the background on ATSDR's concern about fibers less than 5 μm in length (Section 1.1), the scope of this expert panel review (Section 1.2), and the organization of this report (Section 1.3).

1.1 Background

ATSDR conducts public health assessments to evaluate the public health implications of exposure to contaminants from hazardous waste sites and other environmental releases. A crucial part of these evaluations is understanding the toxicologic implications of environmental exposures. Recent events have highlighted a need for ATSDR to explore the potential of exposure to biopersistent fibers—specifically asbestos and some SVF—to cause health effects. For instance, ATSDR is currently assessing the implications of residential and community exposures to fibers from past industrial operations (e.g., vermiculite processing plants across the country), contaminants at hazardous waste sites, and dust in Lower Manhattan generated from the collapse of the World Trade Center (WTC) buildings. These sites are distinct in that contaminants have been found, or are suspected of being present, in residents' homes. Moreover, ATSDR has received concerns specifically about the public health implications of exposure to shorter fibers, particularly for materials found in Lower Manhattan.

ATSDR has therefore identified a need to understand the potential of fibers less than 5 μm in length to contribute to adverse health effects. As one part of addressing this need, ATSDR convened an expert panel to discuss and review the current state of the science regarding the influence of fiber length on health effects of asbestos and SVF. ATSDR will use the panel's findings to help develop scientifically sound public health evaluations for human exposures to small fibers.

1.2 Scope of the Expert Panel Review

The expert panel review involved many activities before the meeting (see Section 1.2.1), at the meeting (see Section 1.2.2), and after the meeting (see Section 1.2.3). The following subsections describe what each of these tasks entailed.

1.2.1 Activities Prior to the Expert Panel Meeting

ATSDR selected seven experts in toxicology, epidemiology, pathology, pulmonology, hygiene, and medicine to serve as panelists for the meeting. Every panelist is either a senior scientist, physician, or researcher with extensive experience in the aforementioned fields, as demonstrated by peer-reviewed publications, awards, and service to relevant professional societies. ATSDR selected panelists with a broad range of affiliations (e.g., academia, consulting, other federal agencies) in hope that the expert panel would offer a balanced perspective on the meeting topics. Furthermore, during its search for expert panelists, ATSDR asked all candidates to disclose real or perceived conflicts of interest. Appendix A lists the names and affiliations of the seven expert panelists selected for this meeting, and Appendix B includes brief biographies that summarize the panelists' areas of expertise.

To focus the discussions at the meeting, ATSDR prepared written guidelines (commonly called a "charge") for the expert panelists. The charge included several questions that the expert panelists discussed during the meeting. These questions addressed the physiological fate of fibers less than

5 µm in length, the health effects associated with these fibers, and data gaps. A copy of the charge is included in Appendix B. Several weeks prior to the expert panel meeting, every panelist received a copy of the charge, logistical information for the meeting, a preliminary bibliography of publications on asbestos and SVF, and copies of six publications relevant to the meeting topics (Bourdes et al. 2000; Churg et al. 2000; Davis 1994; Kinnula 1999; Morgan 1995; Ohyama et al. 2001).

In the weeks after the panelists received these materials, the panelists were asked to prepare their initial responses to the charge questions. Booklets of the premeeting comments were distributed the expert panelists, and made available to observers who registered in advance to attend the expert panel review. These initial comments are included in this report, without modification, as Appendix B. It should be noted that the premeeting comments are preliminary in nature. Some panelists' technical findings may have changed after the premeeting comments were submitted.

1.2.2 Activities at the Expert Panel Review Meeting

The seven panelists and approximately 50 observers attended the expert panel meeting, which was held at the Jacob K. Javitz Federal Building in New York City, New York, on October 29–30, 2002. The meeting was open to the public, and the meeting dates and times were announced in the Federal Register. Appendix C lists the observers who confirmed their attendance at the meeting registration desk. The schedule of the expert panel meeting generally followed the agenda, presented here as Appendix D. The remainder of this section describes the introductory presentations given at the meeting.

- *Introductory remarks from ATSDR.* The meeting began with Rear Admiral (RADM) Robert Williams (Director of ATSDR's Division of Health Assessment and Consultation and Chief Engineer for the United States Public Health Service) explaining why ATSDR had convened the expert panel. He first reviewed ATSDR's site-specific experiences with asbestos contamination since 1980: assessing roughly 150 sites at which asbestos was a contaminant of concern, evaluating approximately 50 sites at which completed or potential exposure pathways were found for asbestos, and issuing public health advisories for sites

where the public might come into contact with elevated levels of asbestos-contaminated materials. RADM Williams indicated that the available environmental data for these previous evaluations were typically the percent of asbestos in a waste material, as quantified by measurement methods that count fibers longer than 5 μm. For most of these sites, detailed information on fiber size distributions is not available.

More recent work on sites with asbestos contamination, RADM Williams explained, has led to a greater need to understand the role of fiber length on asbestos toxicity. He reviewed ATSDR's activities at two sites with public health concerns regarding asbestos exposure. First, RADM Williams presented findings from medical testing that ATSDR conducted on residents of Libby, Montana, where vermiculite mining and exfoliation operations occurred for more than 50 years. ATSDR found that 18% of the residents tested (which included workers at the former mine and exfoliation plant) had pleural abnormalities, which were most prevalent among people who had lived in the area longest and who had completed exposure pathways for asbestos. RADM Williams described ATSDR's ongoing public health actions to address asbestos exposure issues in Libby. Second, RADM Williams described ATSDR's recent activities evaluating asbestos and SVF in dust generated during the WTC collapse. Activities included reviewing results of asbestos samples, conducting limited sampling in residential properties, evaluating whether buildings could be entered for occupational purposes, and assessing the need for maintaining the "exclusion zone" in Lower Manhattan.

RADM Williams indicated that ATSDR's experiences with the Libby, WTC, and other sites have raised unique challenges regarding asbestos and SVF. At these sites, for example, fibers are being found in homes, rather than at waste sites and in the environment; children are being exposed; and analytical methods are now quantifying amounts of shorter fibers (less than 5 μm) than were typically characterized previously. As one step in helping the agency respond to these challenges, RADM Williams indicated, ATSDR convened the expert panel to review the current state of the science on health effects of asbestos and SVF, focusing on the role of fiber length. RADM Williams explained that ATSDR often uses the expert panel forum to seek scientific input on priority issues the agency is evaluating. He noted that the panelists were invited to present their individual opinions and were not asked to reach consensus on any issue, and representatives from ATSDR were present strictly to observe the proceedings.

- *Introductory remarks from the meeting chair.* Dr. Morton Lippmann, the chair of the expert panel meeting, provided additional introductory remarks. After reviewing the charge to the panelists and the meeting agenda, Dr. Lippmann indicated that the goal of the expert panel meeting was to review health effects associated with asbestos and SVF, with a special emphasis on fibers shorter than 5 μm. He explained that the focus on fibers less than 5 μm emerged from conventions previously used to evaluate asbestos exposures. Specifically, risk assessment decisions related to asbestos, Dr. Lippmann noted, have typically been based on optical measurements of fibers longer than 5 μm, and one goal of

the expert panel was to evaluate the toxicity of the shorter fibers that are not counted by the optical analytical methods. Dr. Lippmann also emphasized that the expert panel's discussions should have a public health focus, such that ATSDR could apply the findings from the expert panel to sites where community members are concerned about exposure to asbestos and SVF.

To illustrate recent concerns about asbestos and SVF, Dr. Lippmann described ongoing research being conducted to evaluate contamination by WTC dust in Lower Manhattan. He indicated, for example, that his research group and colleagues have collected and analyzed numerous settled dust samples and ambient air samples following the WTC collapse and are evaluating health effects among approximately 300 firefighters and several thousand residents of Lower Manhattan. These dust samples reportedly were composed almost entirely of particles larger than 10 μm in aerodynamic diameter, with only 1% of fine particles less than 2.5 μm in aerodynamic diameter. Dr. Lippmann also noted that asbestos fibers detected in the dust samples were primarily small (less than 5 μm), because building materials were crushed by the force of the WTC collapse. He indicated that the purpose of the expert panel review was to help ATSDR interpret the public health significance of short fibers, like those detected in the WTC dust.

Following these opening presentations, Dr. Lippmann asked the panelists to introduce themselves by stating their names, affiliations, areas of expertise, and past research experience. For the remainder of the meeting, the panelists gave individual presentations and engaged in free-flowing discussions when answering the charge questions and addressing additional topics not specified

Are structures less than 5 μm in length fibers or particles?

The expert panel meeting was convened to address the health effects of fibers less than 5 μm, but some panelists questioned the appropriateness of the relevant terminology. One panelist, for instance, noted that many scientists would classify structures smaller than 5 μm as particles, regardless of the structures' aspect ratios (see Dr. Case's premeeting comments in Appendix B). During his introductory remarks, Dr. Lippmann reviewed these concerns and noted that mineralogists, geologists, health scientists, and individuals in other disciplines may use different definitions of fibers and these definitions may be based on size, aspect ratio, and other properties. Section 2.4 presents more detailed information on the panelists' opinions on the most appropriate terminology. This issue is raised here to inform readers that this entire report uses the term "fibers less than 5 μm," while acknowledging that some panelists had reservations about suggesting that structures less than 5 μm are fibers.

in the charge. Observers were given the opportunity to provide verbal comments throughout the expert panel meeting. Representatives from ATSDR were observers at the meeting and did not engage in or direct the panelists' discussions.

1.2.3 Activities Following the Expert Panel Meeting

The primary activity following the expert panel meeting was preparing this summary report. A technical writer who attended the meeting prepared a draft of this report. The expert panelists were asked to review and comment on the draft report, ensuring that its contents accurately reflect the tone and content of the discussions at the expert panel meeting. The draft report was revised based on the panelists' comments. The panelists were then given the opportunity to review the revised report; and the final expert panel review report (i.e., this report) was submitted to ATSDR. Some panelists submitted written comments after the meeting; these are included in this report, without modification, as Appendix E. ATSDR was not involved in the preparation of this report.

1.3 Report Organization

The structure of this report follows the order of the panelists' discussions during the meeting. For instance, Section 2 summarizes the discussions on the first agenda topic (physiological fate of asbestos and SVF less than 5 μm in length), Section 3 summarizes comments on the second topic (health effects of these fibers). Section 4 presents overall conclusions and recommendations. These report sections document comments raised both by the panelists and the observers. Finally, Section 6 provides references for all documents cited in the text.

The appendices to this report include extensive background information on the expert panel review. This information includes items made available to all meeting attendees, as well as items

generated since the expert panel meeting (e.g., a final list of attendees). The appendices contain the following information:

- List of the expert panelists (Appendix A).
- The panelists' premeeting comments, the charge to the reviewers, and brief bios of the expert panelists (Appendix B).
- List of registered observers of the expert panel meeting (Appendix C).
- Agenda for the expert panel meeting (Appendix D).
- Written comments that panelists submitted after the meeting (Appendix E).

2.0 Comments on Topic 1: Physiological Fate of Asbestos and SVF Fibers Less Than 5 Micrometers in Length

This section summarizes the panelists' discussions on the physiological fate of asbestos and SVF fibers less than 5 µm in length. Two panelists—Dr. Lippmann and Dr. Oberdörster—were designated discussion leaders for this part of the meeting, during which the panelists responded to the three specific charge questions regarding physiological fate of small fibers (Sections 2.1, 2.2, and 2.3) and addressed topics not identified in the charge (Section 2.4). Panelists also commented on the toxicity of asbestos and SVF fibers; these comments are summarized in Section 3. This section also summarizes observer comments made after the panelists completed their discussions (Section 2.5). Overall, this section presents a record of discussion of topics mentioned during the meeting, and it should not be viewed as a comprehensive literature review on the role of fiber length in the physiological fate of inhaled fibers. Dr. Lippmann's post-meeting comments (see Appendix E) also summarize these discussions.

Although the panelists focused their initial discussions on fiber length, several panelists stressed that length is not the only factor affecting fiber toxicity. These panelists noted that toxicity is rather a complex function of the fiber dose, dimensions, and durability, as has been widely documented in the scientific literature.

2.1 Depositional Pattern in the Lung

The first charge question asked the panelists: "What is the expected physiological depositional pattern for less-than-5-µm fibers in the lung?" When responding, the panelists provided relevant background information on lung physiology, reviewed what researchers have established for depositional patterns of particles, and then addressed what is currently known about depositional patterns for fibers:

- *Background on lung physiology.* Before addressing the specific charge questions on how fibers deposit in the lung, one panelist first reviewed fundamentals of lung physiology, which largely dictate fiber dosimetry. He explained how air flows through the respiratory system: inhaled air enters the body at the nose or mouth, passes through the larynx and trachea, and eventually enters the lung in airways that branch numerous times before reaching terminal bronchioles. These airways are all conductive, meaning that they move air to the deeper portions of the lung where gas exchange occurs. The air flow velocity decreases as air moves into the more distant bronchi, because the cross-sectional area of the branched bronchi is greater than that of the parent airways. After passing through the terminal bronchioles, inhaled air enters into respiratory bronchioles, then alveolar ducts, and eventually alveolar sacs, where most gas exchange occurs. Movement of air in the respiratory bronchioles and alveolar sacs is dominated by diffusion, rather than by convective forces.

  This panelist noted that clearance processes in the conductive airways differ from those in the airways distal to the terminal bronchioles. In the conductive airways, mucus is secreted onto the airways' surfaces, and ciliated cells on the bronchi and bronchioles gradually move the mucus up to the throat, where the mucus is swallowed. This mucus clearance mechanism efficiently removes particles that deposited on the conductive airways, typically within about 1 day following exposure. The clearance mechanisms for particles that deposit in the respiratory bronchioles, alveolar ducts, and alveolar sacs operate on a much longer time scale (see discussion on "phagocytosis" in Section 2.2).

- *Depositional patterns for particles.* One panelist then reviewed the state of the science of how inhaled particles tend to deposit in the respiratory tract. For both fibrous and non-fibrous particles, the deposition pattern is dictated largely by the particles' aerodynamic diameter. The aerodynamic diameter, another panelist noted, is equivalent to the geometric diameter of a unit density sphere that has the same terminal settling velocity in still air as the particle in question.

  The discussion leader then noted that researchers have long established that airborne particles with aerodynamic diameter larger than 10 μm typically do not pass the larynx, and the particles that enter the lungs deposit by one of three mechanisms—impaction, sedimentation, or diffusion (Brownian motion). The relative importance of these mechanisms is a function of the particle size. The largest particles that enter the lung, for example, have the most momentum, which causes them to have a greater tendency to deposit on airways by impaction as air flow changes direction at bronchial airway

branches. Smaller particles,[1] on the other hand, are less likely to deposit by impaction and therefore are typically carried by convective forces further into the lung.

Any particle that enters the respiratory bronchiole will likely deposit either by sedimentation or Brownian motion; impaction is relatively unimportant in regions where the air flow velocity is low. Sedimentation and diffusion tend to be the more dominant mechanisms in the small lung airways for particles, which diffuse in air much slower than gases. One panelist noted that sedimentation is the dominant deposition mechanism for particles with aerodynamic diameters greater than roughly 0.8 μm, while smaller particles are increasingly subjected to diffusional deposition in the airways. Particles depositing in the respiratory bronchioles, alveolar ducts, and alveolar sacs will remain in these regions of the lung until cleared by other mechanisms (see Sections 2.2 and 2.3).

- *Depositional patterns for fibers.* One panelist described depositional patterns of fibers, noting how their elongated shapes caused fibers to deposit differently in the lung than particles. The main difference between fiber and particle deposition is that fibers can be intercepted by airway surfaces, while particles generally cannot. For instance, as long fibers move through small airways, the end of a fiber might contact (and deposit on) an airway surface, even in cases when the fiber's center of mass is on a flow streamline in the center of the airway. Interception can therefore cause enhanced deposition of fibers, when compared to particles; and interception becomes an increasingly important deposition mechanism for longer fibers.

  This panelist indicated that many researchers have evaluated the depositional patterns of fibers in the lung. He cited the following studies as examples:

  - Studies using hollow airway cast models from human lungs have demonstrated that the extent of fiber interception varies with fiber length. Specifically, interception has been shown to be relatively unimportant for fibers less than 10 μm in length (Sussman et al. 1991). This panelist indicated that these shorter fibers will likely act like particles in the lung, because the one deposition mechanism unique to fibers is unimportant.

  - Other studies using these models have reported that fibers with aspect ratios greater than 10 behave aerodynamically like unit density spheres with diameters three times the fiber width (Stöber et al. 1970; Timbrell 1972). Interception accounts for the fact that longer fibers have proportionally greater deposition in the conductive airways than shorter fibers.

[1] Two panelists had different opinions on the particle sizes that should be cited in this sentence. One panelist indicated at the meeting that particles with aerodynamic diameters less than roughly 2 μm would be expected to be carried by convective forces further into the lung. Another panelist, when reviewing a draft of this report, recommended that the size cut-off for this sentence be 0.8 μm.

For fibers less than 5 μm in length, Dr. Lippmann indicated, the information available on particle deposition and longer fibers suggests that fiber diameter likely has the greatest influence on deposition patterns. He noted that fibers less than 5 μm in length will have diameters less than 1.66 μm, assuming the aspect ratios are at least 3:1. This panelist estimated that 10% to 20% of short fibers with diameters between 0.1 and 1.6 μm will deposit in the lungs of healthy people.

Another panelist reviewed findings from multiple publications to illustrate how the four mechanisms—impaction, sedimentation, diffusion, and interception—affect fiber deposition patterns. First, this panelist summarized results of a lung modeling study (Asgharian and Yu 1988), which predicted the relative importance of the four deposition mechanisms as a function of fiber diameter. For all fiber dimensions considered, diffusion (Brownian motion) accounted for an increased amount of deposition as air traveled further into the lung. Further, impaction, interception, and sedimentation were relatively unimportant for the thinnest fibers (those with diameters of 0.01 μm), yet accounted for most of the predicted deposition pattern for the larger fibers (those with diameter of 10 μm). Second, he reviewed the extent to which fibers are filtered from inhaled air in the nose versus the mouth, as predicted by mathematical models. The model predicted that, for all fiber dimensions considered, nose breathing is considerably more effective at filtering airborne fibers than is mouth breathing. In fact, appreciable filtration for mouth breathing was predicted only for fibers at least 1 μm in diameter. Overall, these comments highlight that researchers have already predicted how fiber dimension (both length and diameter) affect depositional patterns in the lung (see Dr. Oberdörster's premeeting comments in Appendix B for references to relevant peer-reviewed publications).

- *Role of laboratory animal studies in evaluating depositional patterns in humans.* The panelists acknowledged that laboratory animal studies have provided additional insights on how fibers deposit in the lung, but the panelists noted that inter-species differences in lung airway structure limit the utility of the animal data. One indicated, for instance, that laboratory animal studies have the advantage of being able to characterize lung fiber burdens at different time frames following highly controlled dosage conditions. On the other hand, he added, airway branching patterns in humans are nearly symmetrical, while rats (and most other mammals) have asymmetrical branching patterns. Such differences in branching patterns influence the cross-sectional air flow profiles, which in turn affect fiber deposition behavior. Consequently, lung deposition patterns in laboratory animals are expected to differ from those in humans.

  Another panelist showed how modeling results of lung deposition patterns support this expectation. Based on predictions of a mathematical lung dosimetry model developed by the International Commission on Radiological Protection, this panelist illustrated differences between rats and humans in estimated deposition fractions of fibers in alveolar regions. His figure indicated that the predicted deposition fraction in humans was greater

than that in rats for all fiber lengths considered, and this difference was most striking for longer fibers. Specifically, the model predicted that virtually no fibers with aerodynamic diameters of 3 μm and aspect ratios of 10:1 deposit in the alveolar region of rats, while more than 25% of these same fibers are predicted to deposit in the alveolar region of humans. Such predictions, this panelist noted, raise questions about whether rats are good models for humans in terms of fiber deposition in the lung.

2.2 Lung Clearance and Biopersistence

The second charge question asked the panelists: "What is known about clearance/biopersistence of less-than-5-μm fibers in the lung?" The panelists identified several mechanisms by which asbestos and SVF are removed from lung tissue. As Section 2.1 explains, fibers depositing on the conductive airways are cleared, typically within 1 day, by mucociliary transport; this clearance mechanism is not discussed further here. The panelists' comments focused primarily on phagocytosis and dissolution, but panelists considered several additional factors when discussing lung clearance. All of the panelists' comments are summarized below; Section 2.3 addresses clearance of fibers by migration to other tissues.

- *Phagocytosis.* Reviewing general lung clearance mechanisms, one panelist indicated that alveolar macrophages engulf and can eventually remove foreign materials (e.g., fibers, particles, bacteria) that reach the alveoli. Phagocytized material can then move to the ciliated airways, which would eventually clear the material up to the throat, or they can move into the pleura, lymphatics, or other tissues (see Section 2.3). Typical human macrophages have dimensions between 14 and 21 μm.[2] Consequently, alveolar macrophages can fully engulf fibers less than 5 μm long and remove them from the alveoli, but they are incapable of fully engulfing longer fibers. The extent of phagocytosis, therefore, clearly depends on fiber length, and may also depend on additional factors, such as surface properties of the inhaled fibers.

  The panelists noted that removal of asbestos and SVF from the alveoli by phagocytosis generally takes much longer than removal of these materials from the conductive airways by mucociliary transport—an observation that is supported by findings of lung clearance studies in rats (Coin et al. 1994). Specifically, the study reported how the half-life for lung

[2] Noting that rat alveolar macrophages have dimensions roughly between 10.5 and 13 μm, a panelist indicated that phagocytosis in rats is less effective than in humans at clearing fibers between 13 and 20 μm.

clearance in rats varied with the length of chrysotile asbestos fibers. For fibers approximately 20 µm long, the estimated half-life for clearance (by all mechanisms combined) was 100 days.[3] One panelist also presented data on time frames for lung clearance of fibers in humans, noting that the estimated half-life for alveolar macrophage clearance was estimated to be between 400 and 700 days; the panelist noted that these estimates apply to poorly soluble spherical particles of low cytotoxicity and to short fibers which can be engulfed by alveolar macrophages. He added that long fibers that cannot be phagocytized and that do not dissolve or break will not be cleared from the lung.

As one exception to the previous observations, one panelist noted that phagocytosis is not an effective clearance mechanism in "overload" conditions, or when high exposure doses overwhelm the lung's clearance mechanisms. The panelists questioned whether the environmental exposures that ATSDR typically evaluates would ever cause overload conditions, though they noted that overload conditions may be observed in some occupational settings or in unexpected accidental or emergency situations.

- *Dissolution.* Asbestos and SVF not only can be physically removed from the lung via phagocytosis, but can be chemically removed, or at least altered, by dissolution. A panelist indicated that the extent to which dissolution occurs depends largely on the fiber composition and the pH of the medium in which the fiber is located, and does not appear to depend on fiber length. Dissolution behavior can change when fibers are engulfed by macrophages, because pH varies considerably between the phagolysomes in the alveolar macrophages (pH = 4.5–5.0) and the extracellular fluid (pH = 7.4). Researchers already have documented the relative solubility of different fiber types (see Dr. Lockey's premeeting comments in Appendix B), which can be useful in characterizing the relative biopersistence of different fiber types.

- *Influence of fragmentation.* Asbestos and SVF fibers can fragment in the lung after being inhaled. Fragmentation is technically not a clearance process, because the fragmented fibers still remain in the lung. However, fragmentation can enhance clearance if the fragments formed are more easily cleared by phagocytosis than the original fiber. One panelist noted that glass and asbestos fibers fragment differently. Asbestos fibers, for example, tend to fragment longitudinally into thinner fibers of the same length. Therefore, an asbestos fiber that is too long to be engulfed by a macrophage tends to fragment into thinner fibers that are also too long to be engulfed by a macrophage. Glass fibers, on the other hand, tend to fragment transversely into shorter pieces that can more easily be cleared by phagocytosis.

- *Influence of co-exposure to other contaminants.* A panelist reviewed results from a mixed-dust exposure study in rats (Davis et al. 1991) to illustrate how co-exposures to other

[3] This half-life estimate likely understates the clearance half-life for amphibole fibers of the same length, one panelist noted, because more recent studies have shown that chrysotile fibers are cleared more readily from the lung than are amosite fibers of the same dimension.

contaminants affects fiber retention in the lung. In the study, groups of rats received different combinations of exposures: chrysotile asbestos and titanium dioxide, chrysotile asbestos and quartz, amosite asbestos and titanium dioxide, and amosite asbestos and quartz. Exposure concentrations for the chrysotile asbestos, amosite asbestos, and titanium dioxide were all 10 mg/m^3, while the exposure concentration for quartz was 2 mg/m^3. The animals were dosed for 1 year and lung tissues were analyzed for fiber retention after 2 years. The study found that co-exposure with titanium dioxide and quartz had no effect on lung retention of amosite fibers. For chrysotile fibers, on the other hand, co-exposure with titanium dioxide increased lung retention of the fibers (as compared to exposure to chrysotile alone) and co-exposure with quartz decreased lung retention of fibers. This panelist indicated that this study suggests that non-fibrous particles could affect fiber retention characteristics, though he acknowledged that the exposure concentrations used in the study are not relevant to typical environmental exposures.

- *Influence of physical structure: amorphous versus crystalline material.* The panelists briefly discussed how the physical structure of fibers (amorphous or crystalline) affects biopersistence and toxicity. One panelist noted that a laboratory animal study examined this issue by comparing lung samples from rats exposed for 3 months to amorphous silica to samples from rats exposed for 3 months to crystalline silica (Johnston et al. 2000). The study found that significant amounts of crystalline silica remained in the rat lungs 3 months after exposure ceased, while the lung-retained amorphous silica was near background levels. The panelist indicated that this trend suggests that the amorphous silica is more soluble than crystalline silica in the lung.

- *Populations that may have impaired capacity to clear fibers in the lung.* One panelist identified populations that may be susceptible to fiber-related health effects due to impaired capacity to clear fibers deposited in the lung. These populations included people with medical conditions (e.g., primary ciliary disorders, cystic fibrosis, asthma) that affect lung clearance mechanisms. Further, smokers with damaged cilia along the conductive airways may have impaired ability to clear fibers from the lung. Finally, some common pharmaceuticals are known to slow mucociliary transport (e.g., atropine), while others can enhance this transport (e.g., sympathomimetics).

- *Relevance of sputum samples.* When discussing lung clearance, the panelists discussed the utility of analyzing sputum samples to characterize the distribution of retained fibers. One panelist explained that, in at least one study, concentrations of asbestos in sputum, when compared to cumulative exposure estimates, were more predictive of radiological changes in the lungs of workers at vermiculite mines and mills (Sebastien et al. 1988). Though these and other findings suggest that sputum samples can provide useful insight into asbestos exposures, the panelists indicated that implementing a sputum sample study has a potential drawback. While smokers can produce voluntary sputum samples relatively easily, non-smokers often cannot. Induced sputum samples can be collected from non-smokers to characterize past exposure, and bronchoalveolar lavage has also been used for

this purpose. Both of these sampling techniques are invasive and require informed consent, and have a consistently better yield than simple sputum collection. More than 50 such studies conducted in North America, Europe, and Japan have already been published.

2.3 Migration of Fibers Deposited in the Lung

The third charge question asked the panelists: "What types of migration are expected within the body for less-than-5-μm fibers?" Both in their premeeting comments and during the expert panel review meeting, the panelists offered various perspectives on how fibers of different lengths migrate within the lung and from the lung to other organs. One panelist, for example, indicated that fibers with diameters less than 0.5 μm can penetrate through lung epithelia and be transported through lymph channels to lymph nodes, blood, and distant organs. However, most of the discussion focused on the extent to which small fibers translocate into the pleura. Three reviewers' perspectives on this matter follow:

First, one panelist indicated that several researchers have attempted to characterize the distribution of asbestos fibers in samples of human pleura. Although it has been reported that only short chrysotile fibers (average length <0.2 μm) translocate to the pleura, this panelist found these studies to be of questionable quality because they lacked matched controls or sampled tissue (such as tumors) other than the pleura. This panelist then reviewed two preliminary studies of fiber translocation, one in humans (Boutin et al. 1996) and the other in goats (Dumortier et al. 2002), which were based on more robust methods using controls. He noted that one study found that 22.5% of fibers detected in the pleura were longer than 5 μm and that the pleural samples had far greater amounts of amphibole asbestos fibers than chrysotile asbestos fibers (see Dr. Case's premeeting comments in Appendix B). The studies did not examine how fibers translocate to the pleura, though the findings suggest that lymphatic drainage paths may play an important role.[4]

[4] A panelist also noted that lymphatic transport has been demonstrated to occur in laboratory studies of dogs that were dosed with amosite asbestos by intrabronchial instillation (Oberdörster et al. 1988). Analyses of post-nodal lymph collected from the right lymph duct found fibers only of shorter dimensions: the maximum length of fiber detected was 9 μm, and the maximum diameter was 0.5 μm.

The authors of these studies hypothesized that the translocated fibers might contribute to formation of pleural plaques and mesothelioma.

Second, another panelist summarized the findings from a study of rats exposed via inhalation to kaolin-based refractory ceramic fibers with geometric mean length of 4.5 μm (Gelzeichter et al. 1996). The study reported that the fate of the fibers depended on fiber length: fibers in the pleural tissue 32 days[5] after exposure had a geometric mean length of 1.5 μm and geometric mean diameter of 0.09 μm, while fibers in the parenchymal tissue were much larger with geometric mean length of 5 μm and geometric mean diameter of 0.3 μm. Thus, the study indicates that very thin fibers smaller than 5 μm—fibers that would not be counted by conventional phase contrast microscopy (PCM) asbestos sampling methods—are capable of translocating to the pleural tissue (see Dr. Lockey's premeeting comments in Appendix B).

Third, a panelist reviewed findings of a rat inhalation study that investigated whether co-exposure to non-fibrous particles affects translocation of fibers to the pleura (Davis et al. 1991). The study found more amosite asbestos fibers translocated to the pleura in rats that were co-exposed to non-fibrous particles (quartz or titanium dioxide), as compared to rats that were exposed to amosite asbestos alone. The panelist noted, however, that the exposure doses of titanium dioxide (10 mg/m^3) might have overloaded the rat lungs and impaired alveolar macrophage clearance processes. If the observed fiber translocation to the pleura was caused by these overload conditions, the relevance of this study to environmental exposures is questionable.

The panelists noted that the extent to which fibers translocate to the pleura is not fully understood, but is likely an important consideration when evaluating pleural plaques, diffuse pleural thickening, and mesothelioma. For instance, if fibers must actually enter the pleura for these outcomes to occur (a hypothesis that has not been verified), then understanding fiber

[5] When reviewing a draft of this report, one panelist noted that 32 days is a relatively short period of time to examine translocation of fibers into the pleura. He indicated that it may take longer for long fibers to reach the pleura, especially if direct penetration is required for the long fibers to enter the pleura (as compared to lymphatic transport for shorter fibers).

translocation into the pleura is critical. If, on the other hand, fibers localized toward the lung periphery beneath the pleura can cause disease, perhaps through chemical mediators that cross into the pleural space, then translocation of fibers is less important. Therefore, without a more detailed understanding of the mechanisms of toxicity for pleural reactions and other outcomes, the significance of fiber translocation into the pleura is not fully known. The panelists revisited fiber translocation issues when discussing the role of fiber length, if any, in causing pleural abnormalities.

2.4 Open Discussion Among Panelists

After summarizing the panelists' responses to the three charge questions, the discussion leaders invited the panelists to provide comments on additional topics relevant to physiological fate of inhaled fibers. The panelists raised the following issues:

- *Terminology: fibers or particles?* One panelist had reservations about calling structures with lengths less than 5 µm fibers. He explained that mineralogists, geologists, and health scientists generally do not consider such structures to be fibers, regardless of the aspect ratio; such structures would instead be considered particles. This panelist noted that regulators have established a precedent for distinguishing between fibers and particles: the Occupational Safety and Health Administration, for instance, regulates structures smaller than 5 µm as particles not otherwise regulated, rather than as fibers. For these and other reasons (see Dr. Case's premeeting comments in Appendix B), this panelist had concerns about the terminology ATSDR used to characterize the structures with dimensions less than 5 µm. As noted previously, this report refers to structures less than 5 µm as fibers, and the concern about using this term has been documented.

- *Importance of the distribution of fiber lengths.* Noting that all mineral fiber exposures always involve inhalation of a wide distribution of fiber sizes, one panelist questioned the utility of focusing exclusively on fibers less than 5 µm in length. To illustrate this concern, he showed a graph depicting the fiber size distribution (in terms of length and diameter) in an ambient air sample collected at Libby. The graph showed that a clear majority of fibers

were less than 5 μm, as is often observed in occupational and environmental exposure situations.[6] The sample also included many fibers approximately 15 μm long, though in considerably smaller amounts than the short fibers. In such cases, the panelist cautioned about focusing exclusively on fibers smaller than 5 μm, even if they account for the overwhelming majority of the dose, because the smaller amount of longer fibers contribute more to overall toxicity.

To illustrate this issue further, the panelist presented data on the distribution of fiber lengths measured in surgical lung tissue samples from six miners and four cement plant workers who were exposed to asbestos fibers (primarily chrysotile) and non-asbestos fibers (Case et al. 2002a). The men were hospitalized with various lung diseases, which were mostly not related to their asbestos exposures. In these individuals, the majority (71%, by fiber count) of lung-retained chrysotile asbestos fibers were shorter than 5 μm, with lesser amounts (25%) of chrysotile asbestos fibers between 5 and 20 μm, and even lesser amounts (4%) of chrysotile asbestos fibers longer than 20 μm (Case et al. 2002a). A similar pattern was observed for the lung-retained non-asbestos fibers, with an even greater number of fibers shorter than 5 μm (85%) and none longer than 20 μm. Based on these results, this panelist reiterated that characterizing how toxicity varies with fiber length is critical, because retained doses can vary considerably between different fiber length intervals. The panelists revisited this topic when discussing whether a critical fiber length exists below which adverse health effects from environmental exposures would be unlikely (see Section 3.4).

- *Comments on fibers detected in Libby.* When evaluating the influence of fiber length on dosimetry, the panelists briefly discussed the significance of ambient air measurements in Libby, and asked Dr. Aubrey Miller (EPA) to summarize relevant data. Referring to trends among ambient air sampling data, Dr. Miller indicated that typically more than 60% of airborne fibers at the site are less than 5 μm long and therefore would not be counted by PCM testing for regulatory purposes. A panelist added that two asbestos amphibole minerals not currently regulated by the Occupational Safety and Health Administration (winchite and richterite) are included among the fibers in these samples. Dr. Miller noted that some Libby residents who were not occupationally exposed to asbestos and who had no household contacts with occupationally exposed individuals have developed pleural abnormalities, which raises questions about which fiber types are contributing to this disease. The panelists discussed this matter further when reviewing the current state of the science on human epidemiologic studies (see Section 3.1).

[6] During this discussion, one panelist cautioned about distinguishing environmental exposures from occupational exposures and instead encouraged scientists to focus on the exposure dose, regardless of whether it was experienced in an occupational or environmental setting. To illustrate this concern, he noted that some "environmental exposures," such as those experienced by Libby residents, might exceed "occupational" exposures in well-regulated work places.

- *Dose metric issues.* The panelists briefly discussed how the available dose metrics—mass, number, and surface area of fibers—correlate with toxicity. A panelist noted that one study (Timbrell et al. 1988) reported that surface area correlated best with pulmonary fibrosis scores and therefore might be the best dose metric for that endpoint. This panelist said this finding is consistent with toxicologic studies of non-fibrous particles, which also indicate that surface area correlates better with pulmonary fibrosis than do other dose metrics. Another panelist questioned whether surface area of retained fibers is an appropriate dose metric, noting that such a selection implies that short fibers (i.e., fibers less than 5 μm in length), if inhaled in substantial quantities, can be equally toxic as very long fibers. This issue was not resolved, but a panelist noted that surface area of fibers might be more predictive of certain endpoints (e.g., lung fibrosis) while other dose metrics may correlate better with carcinogenic endpoints.

- *Research needs.* While discussing the physiological fate of fibers in the lung, the panelists identified several research needs. One panelist, for example, suggested that a laboratory study comparing dosimetry of fibers less than 5 μm to that of non-fibrous particles less than 5 μm could provide insights into lung deposition and clearance of shorter fibers. Another panelist advocated research that characterizes dosimetry for a series of fiber length intervals, rather than focusing entirely on fibers shorter than a given threshold length (i.e., 5 μm), because people are ultimately exposed to airborne fibers of varying lengths. One panelist suggested that studies consider the relevance of susceptible populations, but other panelists indicated that research on susceptible populations should be conducted after key studies on healthy populations have been completed. The panelists discussed additional research needs later in the meeting (see Section 3.5).

2.5 Observer Comments and Ensuing Discussions

After the panelists finished addressing the first topic area, observers were invited to provide comments. The panelists were not required to respond to the observer comments. However, some comments led to further discussion among the panelists, as documented here. The observer comments are summarized in the order they were presented:

Comment 1: David Bernstein, consultant in toxicology

Dr. Bernstein presented findings from a chronic inhalation study that investigated the influence of fiber length and biopersistence on toxicity in rats. The study was conducted for the European Commission, but findings from the study have not been reported in the peer-reviewed literature and a written summary of the study was not provided to the expert panelists. Dr. Bernstein indicated that this study found that long fibers were more

biopersistent than short fibers. He further noted that exposure to fibers up to 20 μm long were found to be uncorrelated with toxic response, and only those fibers longer than 20 μm were correlated with toxicity. These findings were reportedly derived by comparing a toxic endpoint at 24 months following exposure to the distribution of fiber lengths retained in the rats' lungs. The toxic endpoint considered was collagen deposition at bronchoalveolar junctions—a precursor to pulmonary fibrosis. Dr. Bernstein claimed that the panelists can draw from this study's findings to make definitive statements on the toxicity of fibers shorter than 5 μm.

> **Panelists' Discussion:** When discussing this study, one panelist asked if preferential deposition of long fibers is expected to occur at the bronchial-alveolar junctions, and Dr. Bernstein said yes. This panelist noted that the apparent correlation between fiber size and toxicity might simply result from studying an endpoint where short fibers do not preferentially deposit. Another panelist encouraged Dr. Bernstein and his colleagues to publish these results.

Dr. Bernstein also presented data from an animal study on biopersistence of chrysotile fibers mined in Brazil. He explained that chrysotile fibers have a somewhat unique molecular structure, because more magnesium atoms are in the fiber surface; in amphibole fibers, on the other hand, these atoms are more concentrated internal to the fiber, away from the surface. Due to this unique structure, Dr. Bernstein argued, the chrysotile fibers are more readily dissolved in the lung. He reported that long chrysotile fibers (>20 μm) have a biopersistence half-life of only 1.3 days, while amphibole amosite fibers of similar length have a half-life of 466 days. He also showed a series of images depicting the fate of different length fibers in the lung as a function of days following exposure. Dr. Bernstein did not provide a reference for the data he presented.

> **Panelists' Discussion:** One panelist took exception to these studies, noting that his colleagues have published a study (Finkelstein and Dufrense 1999) indicating that chrysotile fibers longer than 10 μm have an estimated half-life of *8 years* in the lungs of Canadian miners. Further, he noted that a study of South Carolinian textile workers exposed to chrysotile fibers (Case et al. 2000) also supports a chrysotile half-life much longer than 1.3 days. That study found that the lung content of chrysotile fibers longer than 18 μm increased proportionally with the workers' cumulative exposure, suggesting that these longer fibers are more persistent in the lungs of occupationally exposed individuals than Dr. Bernstein's data imply.

Comment 2: Jay Turim, Sciences International, Inc.

Mr. Turim encouraged the panelists to consider the findings of two studies. First, he referred the panelists to a publication (Berman et al. 1995) that re-evaluated data from previous laboratory animal experiments in rats. This study reported that 99.7% of the potency for mesothelioma was due to asbestos fibers longer than 40 μm, with only 0.3%

of the potency attributed to fibers shorter than 40 μm. Mr. Turim suggested that the panelists consider these findings when commenting on the carcinogenicity of short fibers.

Second, Mr. Turim reviewed a recent study (Brown et al. 2000) in which two groups of rats inhaled formulations of different refractory ceramic fibers (RCF1 and RCF1a). The fiber formulations were reported as having approximately the same number of long fibers, but the RCF1 formulation contains much more non-fibrous particles than does the RCF1a formulation. In the study, the rats were exposed for 3 weeks (6 hours per day, 5 days per week), and were followed up for 1 year after exposure ceased. Mr. Turim noted that the lung retention of long fibers did not differ between the two exposure groups, even though the study authors reported that macrophage clearance processes were severely impaired in the rats exposed to RCF1, due to lung overload conditions. Mr. Turim also indicated that the study provides evidence that RCF (and SVFs, in general) behave differently from asbestos fibers in the lung, because the short RCF fibers were largely removed despite the impaired macrophage activity. Finally, because the study found more persistent inflammatory response, as gauged by bronchoalveolar lavage (BAL) analysis, in the rats dosed with the RCF1 mixture, Mr. Turim argued that the study shows that the presence of non-fibrous particles must be considered when evaluating the toxicity of SVFs.

> **Panelists' Discussion:** One panelist addressed this comment, noting that some aspects of the RCF study were not entirely clear to him. For instance, he did not think the publication adequately explained how lung clearance of short fibers could be similarly effective in the two groups, when macrophage activity was severely impaired only in the rats dosed with RCF1a. Further, he noted that the differences in toxicity between RCF1 and RCF1a were actually relatively minor, based on his interpretation of the BAL data and the histopathology results. Moreover, the panelist indicated that a follow-up study by the same group has found the non-fibrous FCF particles to be of high toxicity (Bellmann et al. 2002; Brown et al. 2002).

Comment 3: Jenna Orkin, 911 Environmental Action Concern

Ms. Orkin asked the panelists to comment on environmental contamination resulting from the WTC collapse, which blew contamination downwind toward downtown Brooklyn, where she lives. Concerned about ongoing exposure to WTC dust, Ms. Orkin indicated that she recently had a carpet sample from beneath a window in her house analyzed for fiber contamination using ultrasonication. She indicated that this analytical technique can detect about 100 times more asbestos fibers than can be found by ASTM MicroVac methods. Ms. Orkin noted that experts have reported that, for ASTM MicroVac samples, 1,000 structures per square centimeter is considered typical for rural homes and 10,000 structures per square centimeter typical for urban homes. However, she said that experts will not specify a safe level of structures measured by ultrasonication.

Ms. Orkin indicated that EMSL Analytical analyzed the carpet sample from her home and found "80,000 structures per square centimeter of asbestos." Seven chrysotile fibers were in the sample, including five long fibers. She indicated that the ultrasonication instrumentation eventually clogged, which she was told might mean that the contamination levels in the sample could not be measured because they were higher than the measurement sensitivity. Ms. Orkin asked if the panelists would comment on the data she presented, such as the exposure levels she and her family members might have experienced.

Panelists' Discussion: Three panelists and an EPA observer responded to the comment. One panelist noted that regulatory agencies have not established "safe limits" for measurements of asbestos fibers on fabrics. This panelist acknowledged that he was unfamiliar with the measurement method identified in the comment, but he did question why any sampling or analytical instrument would clog when analyzing a sample with only seven chrysotile fibers. Another panelist said the key issue for this scenario is characterizing the inhalation exposure, but he noted that no one has established how to estimate airborne exposure levels from asbestos levels in isolated carpet samples. Finally, noting that amphibole minerals make up 7% of the Earth's crust, a third reviewer suggested comparing the sampling results from the Brooklyn residence to measurements using identical methods in other locations that were not impacted by WTC dust.

Dr. Miller (EPA) indicated that EPA struggles with issues like those raised in the comment at many sites: What levels can be considered safe in homes? What fibers should one count when establishing these levels? When should regulatory agencies recommend abatement? He acknowledged that these decisions are beyond EPA's current regulatory guidelines.

Comment 4: Bertram Price, Price Associates, Inc.

Dr. Price's comment addressed asbestosis in Libby, Montana—a topic the panelists had questions about during their earlier discussions. Dr. Price indicated that ATSDR's recent study of Libby residents identified 12 cases of asbestosis: 11 among former mine workers, and 1 in a family member of a former mine worker. He said these findings illustrate the impact of dose on asbestosis, and he cautioned against attempting to distinguish environmental exposures from occupational exposures. Commenting on the influence of fiber length, Dr. Price noted that researchers have established a dose-response gradient between exposures to long asbestos fibers and asbestosis, though he acknowledged that the past studies used measurement techniques that did not count fibers shorter than 5 μm.

Panelists' Discussions: No panelists addressed this comment.

Comment 5: Suresh Moolgavkar, University of Washington

Dr. Moolgavkar's comments also addressed asbestos-related disease among residents in Libby, Montana. Dr. Moolgavkar noted that ATSDR has conducted two epidemiologic studies on Libby residents—the second was necessary after the agency realized that some death certificate data were inadvertently omitted from the initial report. He indicated that the second study reported that lung cancer mortality in Libby was higher than expected when compared to the state of Montana and the United States, while the first study found no excess. Regarding asbestosis, Dr. Moolgavkar summarized the available data on asbestosis cases (see Dr. Price's comment, above), and noted that asbestosis is linked to the most highly exposed individuals, regardless of whether their exposures were environmental or occupational.

Dr. Moolgavkar then commented on results from multiple mortality studies published on occupational cohorts of Libby mine workers (Amandus and Wheeler 1987; McDonald et al. 1986, 2002). He found no indication that asbestos from the Libby mines is more toxic than is predicted from cancer risk calculations using asbestos unit risk data from EPA's Integrated Risk Information System. Dr. Moolgavkar mentioned this to question the suggestion among the panelists that Libby asbestos is more toxic than asbestos from other sites (see Section 3). In fact, Dr. Moolgavkar noted, radiological examinations documented in the previous mortality studies found no evidence (based on prevalence of lung abnormalities) that Libby asbestos poses a greater health risk than asbestos from other sites.

> **Panelists' Discussions:** One panelist indicated that he agreed with the comment, in terms of lung cancer outcomes and lung parenchyma abnormalities, but he noted that mesothelioma cancer risks may in fact be uniquely higher at Libby. Specifically, the risk of developing mesothelioma among asbestos miners in Libby, as gauged by the proportional mortality ratio (PMR), is greater than that experienced by crocidolite asbestos miners in South Africa and Australia (see Section 3.1.1 for a more detailed summary of this argument).

Dr. Moolgavkar questioned this response, arguing that the PMR is not a good metric to use. He indicated that one would expect to see an elevated PMR if the Libby cohort had a strong "healthy worker effect."

> **Panelists' Discussions:** The panelist who addressed this issue agreed with this response, but noted that there is no evidence of a "healthy worker effect" among Libby miners, as demonstrated by the large number of accidental deaths in the cohort. This panelist defended use of the PMR for mesothelioma because it is a rare disease, and use of other cancer risk metrics (e.g., the standardized mortality ratio) might not be appropriate.

3.0 Comments on Topic 2: Health Effects of Asbestos and SVF Less Than 5 Micrometers in Length

This section summarizes the panelists' discussions on the role of fiber length in health effects from asbestos and SVF fibers. The meeting agenda (see Appendix D) lists the specific topics that the panelists addressed and identifies the discussion leaders for these topics. This section organizes the panelists' comments as follows: cancer effects (Section 3.1), noncancer effects (Section 3.2), mechanisms of toxicity (Section 3.3), general comments and interpretations (Section 3.4), and recommended research (Section 3.5). Section 3.6 summarizes observer comments made after the panelists completed their discussions. Some panelists submitted post-meeting comments to summarize their findings. These are included in Appendix E for the following topics: review of epidemiologic data (see Dr. Lockey's comments), review of laboratory animal studies (see Dr. McConnell's comments), and review of mechanistic studies (see Dr. Mossman's and Dr. Wallace's comments).

When evaluating health effects, panelists were asked to review findings from key studies that examined the role of fiber length on toxicity, whether *in vivo* or *in vitro*. Accordingly, this section should not be viewed as a literature review of all toxicity studies for asbestos and SVF; rather, it documents results from key studies that examined impacts of fiber length.

Although the panelists focused their initial discussions on fiber length, several panelists stressed that length is not the only factor affecting fiber toxicity. These panelists noted that toxicity is rather a complex function of the fiber dose, dimensions, and durability, as has been widely documented in the scientific literature.

3.1 Cancer Effects

This section summarizes the panelists' comments on the role of fiber length on cancer effects. The section is organized into three different types of studies: human cancer mortality studies (Section 3.1.1), studies of lung-retained fibers in humans (Section 3.1.2), and laboratory animal studies (Section 3.1.3). Within each section, comments are organized by type of fiber (asbestos or SVF) and type of cancer (lung cancer and mesothelioma).

3.1.1 Data from Cancer Mortality Studies

The panelists' comments on cancer mortality studies from occupational cohorts follow:

- *Asbestos.* One panelist indicated that no studies have evaluated cancer outcomes associated with fibers shorter than 5 µm, because no occupational cohort is exposed exclusively to such fibers. For insights into carcinogenicity of the short fibers, he reviewed findings reported for two occupational cohorts that were exposed predominantly (though not exclusively) to short asbestiform minerals:
  - The panelist first reviewed a study of workers at reserve mine deposits in Minnesota (Higgins et al. 1983). The workers at this site were exposed to cummingtonite-grunerite, a mineral related to amosite, and the vast majority of fibers were reportedly less than 10 µm in length. The study found no increase in overall mortality or mortality from respiratory cancers, but the panelist indicated that the average latency for the cohort was 14.7 years after initial exposure, with a maximum of 24.6 years, or a relatively short latency for development of cancer.
  - Second, this panelist reviewed studies of gold mine workers in South Dakota who also were exposed to cummingtonite-grunerite asbestiform material. He indicated that an initial study of this cohort (Gillam et al. 1976) found increased mortality from malignant respiratory disease among workers with at least 5 years of exposure. A follow-up study of this same cohort (McDonald et al. 1978), which considered workers who had worked for 21 years or longer, found no such increase, but did report increased risks of silicosis and tuberculosis. Average exposure concentrations for this site were 4.82 (±0.68) fibers per cubic centimeter, with 94% of airborne fibers being less than 5 µm in length.

This panelist indicated that these two studies were the closest approximation he could find to occupational cohorts exposed only to fibers shorter than 5 μm, and neither showed evidence of increased cancer mortality. He advocated follow-up studies of these cohorts in view of the passage of more than 20 years since the original publications to investigate carcinogenicity of short fibers more fully.

During this discussion, one panelist reviewed cancer mortality data published in two studies for an occupational cohort of Libby miners (McDonald et al. 1986, 2002). These studies considered 406 men who worked in the mine for at least 1 month prior to 1963; the more recent study, therefore, considers an average latency of period of more than 30 years from first exposure. This panelist noted that both studies reported elevated mortality rates for lung cancer, mesothelioma, and non-malignant respiratory disease (including asbestosis[7]). Of particular note, the panelist indicated that the more recent follow-up study (McDonald et al. 2002) suggests a PMR for mesothelioma of 6.7%—otherwise stated, 1 out of every 15 deaths identified in the follow-up study was from mesothelioma. He found this PMR significant because it is higher than those observed among most other cohorts studied, including crocidolite miners in South Africa and Australia.

- *SVF.* One panelist reviewed cancer mortality studies of occupational cohorts exposed to SVFs, including glass fibers, mineral wool, and RCFs. The panelist first noted that no studies have been conducted on occupational cohorts exposed exclusively to fibers less than 5 μm long, again because no cohorts appear to be exposed only to short fibers. However, he noted that cancer mortality at fiber glass and mineral wool production facilities has been extensively studied, both in the United States and Europe. He summarized these studies for different materials:

  - For fiber glass, the panelist noted that the studies did not find increases in respiratory cancer to be related to fiber glass exposures.

  - For rock wool and slag wool, the panelist indicated that studies of production facilities in the United States found no evidence of increased risk for respiratory cancer. At production facilities in Europe, on the other hand, initial studies have demonstrated increases in respiratory cancer, but no clear information to indicate that the increased cancer risk was specifically related to fiber exposure. A subsequent case-control study indicated no relationship between cumulative rock or slag wool exposure and lung cancer (Kjaerheim et al. 2002). The International Agency for Research on Cancer (IARC) authors did not conclude that the increased cancer was related to the exposures to rock or slag wool.

[7] One panelist, when reviewing a draft of this report, indicated that death certificate data typically use a single code for all non-malignant respiratory disease. He added that asbestosis probably accounts for a minority of these deaths when compared to chronic obstructive lung disease.

- For RCF, which are more durable fibers than the other SVFs, the panelist indicated that no cancer mortality data have been published for occupational cohorts exposed to RCF. This panelist noted that a recent study of a relatively small cohort of plant production workers has not demonstrated increased respiratory cancer risk for RCF, nor any identified mesothelioma, but he acknowledged that the study had limited statistical power for detecting an increased risk. Results from this study have been accepted for publication in a peer-reviewed publication (Lemasters et al. 2002).

The panelist who summarized these results also specifically noted that there is no indication of a relationship between exposure to SVFs and mesothelioma. Though a small number of mesotheliomas have been reported for workers at SVF manufacturing plants, these cases have since been explained by other factors (e.g., probable prior exposure to asbestos, incorrect diagnoses).

3.1.2 Data from Human Studies of Lung-Retained Fibers (Cancer)

Additional insights on the influence of fiber length on cancer outcomes was presented for studies that analyzed the amounts and sizes of fibers retained in the human lung. In these studies, lung-retained fiber is used to characterize exposure. The panelists identified limitations associated with such studies, most notably that the measurements of lung-retained fibers (typically at autopsy) are static and do not characterize when exposure occurred or temporal variations in exposure. Moreover, because lung-retained fibers can break or partially dissolve after exposure, it is possible that the length distribution of fibers observed after death is different from the length distribution of fibers in the original exposures. The panelists provided the following comments on available studies of lung-retained fibers:

- *General comments.* One panelist provided general comments on fiber accumulation and human disease. First, the panelist indicated that people are exposed to fibers of varying length, with shorter fibers generally accounting for the majority of exposure (by fiber count); a similar pattern—a majority of shorter fibers—is consistently observed in the lung-retention studies. Second, because asbestos fibers with widely varying lengths are detected in lung tissue samples from all populations, this panelist concluded that the human lung, under continuing exposure conditions, is not capable of completely clearing fibers of any length to background levels—a finding that is not replicated in inhalation

studies conducted in rats.[8] He demonstrated lung accumulation by displaying data from multiple studies (e.g., Sebastien et al. 1980; Case et al. 2000), which showed that all types of asbestos fibers (including long chrysotile fibers) accumulate in the lung with cumulative exposure.

- *Mesothelioma.* The panelists then commented on three case-control studies that examined the distribution of fiber lengths in people who died from mesothelioma (and most with matched controls). All three studies showed that risk of mesothelioma was considerably higher for individuals with larger amounts of long fibers retained in their lungs:

  - The first study (McDonald et al. 1989) examined lung tissues from 78 Canadian men and women who died of mesothelioma, as well as 78 lung tissues from age-, sex-, and hospital-matched controls. The lung samples were from pathologists' stock, without information on what parts of the lung the samples were collected from. Relative risk for developing mesothelioma was reported for different fiber types and lengths (<8 μm and >8 μm). The study found that the risk of mesothelioma was significantly related to concentrations of amphibole fibers longer than 8 μm and that fibers shorter than 8 μm accounted for none of the cancer risk.

  - The second study (Rogers et al. 1991) examined lung tissues from Australians who died of mesothelioma. Based on "the best fitting additive relative risk model," the study reported that mesothelioma risk was greatest for crocidolite asbestos fibers longer than 10 μm, followed by amosite asbestos fibers longer than 10 μm, and then by chrysotile fibers less than 10 μm. The authors suspected that the relative risk for chrysotile fibers less than 10 μm resulted from longer fibers breaking into shorter fibers.

  - The third study (Rödelsperger et al. 1999) evaluated lung tissue samples from 66 German individuals who died from mesothelioma and 66 matched controls. The study reported that "...a clear dose-response relationship up to an odds ratio of 99% has been demonstrated for the lung tissue concentration of total amphibole fibers longer than 5 μm." The study provided few details on the cancer risk associated with short fibers.

- *Lung cancer.* A panelist indicated that no lung-retention studies in humans have attempted to examine relationships between the length distribution of retained asbestos fibers and lung cancer. He suspected that studies have not been conducted due to the high attributable risk from smoking. This panelist noted that many studies have reported the total concentration of asbestos fibers for lung cancer in lung samples, but none of these evaluated the role of fiber length.

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[8] When reviewing a draft of this report, another panelist indicated that animal studies have found that animals are also not capable of completely clearing fibers of all lengths to background levels.

3.1.3 Data from Laboratory Animal Studies (Cancer)

The panelists identified several laboratory animal studies that illustrate the influence of fiber length on carcinogenicity, and made general comments about the relevance of these studies to humans. Panelists specifically referred to the following three studies when discussing how fiber length has been shown to relate to lung cancer and mesothelioma in laboratory animals:

- In the first study (Davis et al. 1986), no malignant cancers were observed in 42 rats exposed via inhalation to a short-fiber amosite mixture, while eight malignant cancers were reported in the 40 rats exposed to the long-fiber amosite mixture (30% of fibers longer than 5 μm and 5% of fibers longer than 10 μm).

- In the second study (Davis and Jones 1988), seven malignant cancers were observed among rats exposed via intraperitoneal injection to a "short" chrysotile fiber mixture, while 22 malignant cancers were observed among those exposed to the "long" fiber mixture. Cancers in the former group, however, have since been attributed to contamination of the "short" fiber samples with longer chrysotile fibers (Lippmann 1994).

- In the third study (Wagner et al. 1985), rats exposed to mixtures of erionite fibers that were mostly shorter than 5 μm did not develop mesothelioma, while every rat exposed to the longer erionite fiber mixtures developed the disease. One panelist found certain aspects of this study surprising, such as the fact that all of the rats exposed to long fibers died within 15 months, even though mesothelioma typically is not lethal in rats, and that

General strengths and weaknesses of laboratory animal studies

The panelists provided several general comments on the utility of laboratory animal studies for understanding toxicity of asbestos and SVFs. Benefits of animal studies include the ability to (1) conduct highly controlled experiments using well-defined exposure levels and (2) evaluate health outcomes and lung-retention levels at many different time frames following exposure. Extensive lung tissue sampling and other highly invasive tests in humans, on the other hand, are only feasible at autopsy. However, panelists identified key factors that must be considered when interpreting laboratory animal studies. These factors include differences in life span, macrophage size, and airway branching patterns; relevancy of high dose and administration methods (e.g., peritoneal injection); and failure to address certain human exposure conditions (e.g., smoking). Overall, the panelists generally agreed that laboratory animal studies can provide useful insights into toxicity to humans, provided the studies are interpreted in the proper context regarding their relevancy to humans.

the histopathological slides showed very intense pleural reactions. The panelists revisited this study (see Section 3.4) when discussing how chemical composition and surface properties might affect toxicity.

One panelist synthesized the findings from these and other relevant laboratory animal studies. This panelist first noted that the rat is an adequate model for cancers in humans, because the rat has been shown to develop both mesothelioma and lung cancer, though he acknowledged that these cancers are not as aggressive in the rat as in humans.[9] He added that the laboratory animal studies have allowed researchers to observe the progression of disease for both lung cancer and mesothelioma. Regarding the administration method, this panelist indicated that the inhalation studies were more relevant to human exposures. He noted that fiber administration by intrapleural implantation and intraperitoneal injection does not represent human exposures for several reasons (e.g., extremely large doses are administered in very short time frames, alveolar macrophage and mucociliary transport clearance mechanisms are bypassed, and the fibers inserted into the pleura might not be capable of reaching these tissues following inhalation exposure).

Overall, this panelist believed that laboratory animal data using all administration routes have shown that short fibers of any type are less potent than long fibers, both for mesothelioma and cancer, but the relative potency has not been quantified.

3.2 Noncancer Effects

This section summarizes the panelists' comments on the role of fiber length on noncancer effects and is also organized according to the different types of studies: occupational studies (Section

[9] The panelists noted differences in asbestos-related cancers in rats and humans. One panelist said that lung cancer in rats tends to be bronchioalveolar, and develops in the distal lung, while lung cancer in humans largely tends to occur in proximal areas of the lung. He wondered if differences in fiber deposition patterns (due to differing airway sizes and branching patterns) might explain differences in where lung cancers develop in rats and humans. Another panelist cautioned against expecting that lung cancer would develop in the same parts of the lung in rats and humans, primarily because of the confounding factor of cigarette smoking in humans.

3.2.1), studies of lung-retained fibers in humans (Section 3.2.2), and laboratory animal studies (Section 3.2.3). Each section is further organized by noncancer endpoint. Although many different endpoints were discussed (e.g., irritation, nephrosis), the majority of discussions focused on pulmonary interstitial fibrosis and pleural abnormalities (e.g., pleural plaques, pleural thickening, and calcification).

3.2.1 Data from Occupational Studies

Overall, the discussion leader for this topic area indicated, there is limited evidence of noncancer toxicity being associated with fibers less than 5 μm in length, with two exceptions. First, he indicated that very high doses to short fibers, especially those that are durable in intracellular fluids, may have the propensity to cause interstitial fibrosis. Second, he noted that exposure to short, thin durable fibers may play a role in development of pleural plaques or diffuse pleural fibrosis if the dose is high enough. The following paragraphs review the discussion that led to these summary statements:

- *Asbestos.* One panelist noted that no epidemiologic studies have examined populations exposed only to short asbestos fibers, because actual exposures are inevitably to a broad distribution of fiber lengths. To address this issue, the panelists commented on data reported among Libby residents, particularly the prevalence of intense bilateral pleural fibrosis in community members—some of whom reportedly did not work in the local vermiculite mine or processing plant, and did not live with mine or mill workers. One panelist was particularly concerned about the role of short fibers, noting that a very large portion of fibers in the homes are too short or too thin to be counted by conventional PCM sampling methods. He added, however, that some researchers have speculated that short (<10 μm), thin (<0.4 μm), durable fibers, particularly tremolite asbestos, may preferentially deposit on the pleural surface and therefore be associated with pleural plaques. This panelist emphasized that the relevance of short, thin fibers and the risk for pleural abnormalities has only been speculated, and needs to be further investigated. The panelists also wondered if the intense pleural effects observed in the Libby cohort might be associated with the unique mineralogy of the Libby asbestiform fibers. Pleural plaques have been associated with environmental exposures in areas where tremolite fibers naturally occur (e.g., in certain regions of Greece, Cyprus, Turkey, Canada, the Czech Republic, Romania).

  Some discussion focused on the extent to which pleural effects are associated with occupational versus environmental exposures. Two panelists cautioned against attempting

to classify exposures in this manner, because some individual exposures were difficult to assess. For instance, some residents might not have worked at the mine or the mill or lived with mine or mill workers, but could have been highly exposed through routine contacts with these individuals in other settings. These panelists recommended that the discussion focus strictly on dose, regardless of the contributions from occupational and environmental exposures.

- *SVF.* One panelist indicated that the available epidemiologic studies provide no indication of increased mortality from nonmalignant respiratory disease among occupational cohorts exposed to SVF. He then summarized morbidity data for several cohorts. The summary focused on SVF production workers. Although SVF "end users" (e.g., insulators, pipe fitters, heating/ventilation workers) have also been evaluated, these studies are commonly confounded by potential asbestos exposures. Overall, this panelist concluded that the available occupational studies indicate limited overall toxicity associated with SVF exposure, with the exception of RCF exposure being associated with pleural plaques. This conclusion was based on the following observations:

  - For exposures to SVFs (all types), one panelist noted that multiple studies have found that SVF exposure among current or former smokers is associated with small additional decrements in forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1). He added that similar decrements in spirometric parameters are observed among other non-specific dust exposed industrial working populations, suggesting that the effect is not specific to SVFs.

    This panelist also reviewed studies, albeit limited ones, of skin irritation. These have reported irritation being related to mechanical effects of large diameter fibers (~5 μm in diameter), with increased irritation observed in hot, humid climates. Eye, upper respiratory, and lower respiratory irritation has also been reported in case studies among people accidentally exposed to high fiber concentrations, and these irritation effects are generally transient.

  - For fiber glass and mineral wool, this panelist noted that the available studies (e.g., Hughes et al. 1993), though limited in number, provide no indication of chest radiographic, interstitial, or pleural changes among production workers. The panelist added that studies have suggested an increased mortality risk from nonmalignant renal disease (e.g., nephritis, nephrosis) in occupational cohorts exposed to mineral wool, but not among those exposed to fiber glass; he questioned the biological plausibility of these outcomes.

  - For the more durable RCFs, the panelist indicated that occupational exposures have been associated with pleural changes, primarily pleural plaques. The pleural plaques were observed among approximately 3% of the production workers, but were found to be correlated with duration of RCF exposure, time since initial

exposure, and cumulative RCF exposure. The panelist added, however, that the available studies have not found RCF exposure to be associated with a statistically increased risk for pulmonary interstitial fibrosis.

- During this discussion, Dr. Ralph Zumwalde (NIOSH) informed the panel that, in the late 1970s, NIOSH studied the health implications among more than 2,000 miners who were exposed to an attapulgite clay that has fiber-like characteristics (Waxweiler et al. 1988). The clay "fibers" were less than 5 µm long, with diameters of approximately 0.1 µm. Dr. Zumwalde noted that this study, which he recalled found excess lung cancer among whites, might be useful in ATSDR's overall evaluation of short fibers. The increase in lung cancer deaths, however, was not associated with latency, duration of employment, or attapulgite exposure, and there was no increase in mortality from nonmalignant respiratory disease.

Overall, the relevance of short asbestos and SVFs to noncancer disease in humans was not entirely known. For the SVFs, only the durable RCF was found to be associated with pleural plaques; exposures to RCFs were not associated with pulmonary fibrosis, and exposures to fiber glass and mineral wools had no indication of chest radiographic, interstitial, or pleural changes. For asbestos fibers, no studies have examined the effects of exposures exclusively to short fibers. Given data collected in Libby, Montana, however, some panelists questioned whether short fibers might play a role in the observed cases of pleural plaques and diffuse pleural fibrosis; but others cautioned against inferring that the risk results from exposure to short fibers, given that the Libby samples contained significant numbers of long fibers as well.

3.2.2 Data from Human Studies of Lung-Retained Fibers (Noncancer)

Two panelists reviewed publications (case-control studies, a study recently submitted for publication, and a case report) that examine the influence of fiber length retained in the lung on the grade of pulmonary interstitial fibrosis, which is reported on a scale from 0 to 12. A summary of these studies, organized by fiber type, follows:

- *Findings for tremolite asbestos.* One panelist indicated that a study of tissues from chrysotile asbestos miners and millers reported an inverse relationship between fibrosis grade and length of tremolite fibers retained in the lung (Churg et al. 1989). In other

words, the most severe fibrosis was observed among those with smaller (on average) tremolite fibers in their lungs. Another study (Nayebzadeh et al. 2001) and a study recently submitted for publication (Case et al. 2002b) examined fibrosis grades for different length intervals of tremolite fibers: 0–5 μm, 5–10 μm, and 10–20 μm. Both studies found the highest average fibrosis grade occurred among those with the lowest tremolite fiber length interval, or for those with average tremolite fiber length less than 5 μm.

- *Findings for amosite asbestos.* One study (Churg et al. 1990) examined lung tissue samples from a small group (<20) of shipyard workers and insulators selected from litigation cases. This study also found an inverse relationship between fibrosis grade and length of retained asbestos fibers (amosite fibers, in this case).

- *Findings for total asbestos fibers.* One panelist summarized a study (Timbrell et al. 1988) that evaluated lung tissue samples at autopsy from workers exposed in different asbestos mines. Data were collected both for retained asbestos fibers and fibrosis score. The fibrosis scores were then correlated with lung-retained asbestos characterized by three different metrics: number of fibers, mass of fibers, and surface area of fibers. The correlation was best when the surface area of retained fibers was used as a dose metric. This panelist added that the surface area dose metric has correlated well with pulmonary inflammatory responses in other animal inhalation toxicity studies that examined inflammation, including fibrosis, following exposure to particulate contaminants that are poorly soluble with low chemical reactivity. The panelists referred to this study, which did not examine the role of fiber length, several times when discussing appropriate dose metrics.

- *Findings for aluminum oxide fibers.* One panelist reviewed data from a case report (Churg et al. 1993) on an individual with diffuse interstitial fibrosis who was occupationally exposed to aluminum oxide fibers. The lung-retained fibers in this case were predominantly 3–4 μm long and 0.01 μm in diameter. The panelist indicated that these findings raise questions about the significance of short, thin, durable fibers in the lung, though he acknowledged that conclusions should not be drawn from a single case report.

Several panelists commented on the trends among the aforementioned studies. Two panelists, for instance, noted that the trend of shorter fibers possibly being more toxic, at least in terms of interstitial fibrous, is counterintuitive. Two other panelists, on the other hand, noted that these findings suggest that, for interstitial fibrosis, the surface area of retained fibers may be more important than the fiber length, because larger amounts of short fibers would have considerably greater surface area than smaller amounts of long fibers. Finally, some panelists wondered if the apparent inverse relationship between fiber length and fibrosis score might be explained by long

fibers breaking down into shorter fibers between exposure and the time that lung samples were collected.

3.2.3 Data from Laboratory Animal Studies (Noncancer)

This section reviews the panelists' discussions on noncancer outcomes from asbestos and SVF exposure identified in laboratory animal studies. Before addressing this topic, one panelist summarized how the mammalian lung responds to exposures to inert materials, whether fibrous or particulate: once an inert material deposits in the lung beyond the conductive airways, it will either dissolve or be engulfed and cleared by alveolar macrophages; if the dose exceeds the lungs' capacity to clear the material, natural defense mechanisms may act, leading to fibrosis. Section 3.3 presents more details on the mechanisms involved in these steps. Specific comments on noncancer effects in laboratory animals, organized by endpoint, follow:

- *Inflammation, pulmonary interstitial fibrosis, and pleural reactions.* The panelists presented several observations when summarizing findings from laboratory animal studies on noncancer effects in the lung and pleura. First, two panelists noted that many laboratory animal studies have found pulmonary interstitial fibrosis following exposures to both fibrous material and non-fibrous particles. The sequence of events leading to the fibrosis was described (see Dr. McConnell's premeeting comments in Appendix B). When doses reach high enough levels, pleural reactions (e.g., localized acellular fibrotic changes) were observed, but one panelist questioned if the dose levels needed to elicit the pleural responses are relevant to environmental exposures in humans. Another panelist noted that the animal studies suggest that the pleural effects do not occur unless fibers are present in the pleura. When discussing interstitial fibrosis outcomes, one panelist said the long fibers appear to be more fibrogenic than the short fibers, though he stressed that short fibers alone are capable of generating fibrogenic responses if the dose is sufficiently high. The panelists' premeeting comments include specific references to studies that reported relative toxicity of short and long fibers for noncancer outcomes (see Dr. Mossman's premeeting comments in Appendix B).

  Reviewing specific studies, one panelist indicated that the intensity of noncancer responses in laboratory animals varies from one fiber type to the next. He noted, for example, that hamsters exposed to amosite asbestos had an increased incidence of pleural fibrosis, while hamsters exposed to comparable amounts of chrysotile asbestos did not; pulmonary fibrosis was evident, however, in both groups of hamsters. The panelist suspected that the different outcomes resulted from either the amosite fibers being more

durable (less soluble) in the lung or the amosite fibers being more likely to translocate to the pleura.

One issue that generated significant discussion was the extent to which interstitial fibrosis progresses in laboratory animals and the relevance of disease progression to humans. One panelist noted that, in every animal study he has conducted and reviewed to date, interstitial fibrosis is progressive only when asbestos exposure is ongoing. After asbestos exposure ceases, he noted, no overt signs of progressive fibrosis are apparent, although this has not been quantified in a definitive way. The inflammatory responses, microgranulomas, and bronchiolization also tend to decrease. This panelist added that fibrosis does not appear to progress and macrophage response tends to decrease when the exposure ceases, even though long asbestos fibers remain in the animals' lungs. He interpreted this trend as suggesting that short asbestos fibers in the original dose might play a role in stimulating an initial inflammatory response in the rats. Another panelist suggested that the lack of fibrosis progression, even in the presence of long fibers, might suggest that the retained fibers have been rendered inert (in comparison to the freshly inhaled fibers), possibly by being coated with biological fluids. In other words, he wondered if the freshly inhaled fibers are more likely to elicit cellular responses than fibers that have been in the lung for an extended period of time.

Though not questioning the comments on fibrosis progression in animals, two panelists emphasized that the trends discussed above are not observed in humans. Citing their experiences evaluating shipyard workers and chrysotile miners, these panelists noted that fibrosis and pleural changes have progressed in humans, even after asbestos exposures ceased. Reasons why fibrosis might progress differently in rats and humans were not discussed.

Commenting further on disease progression, one panelist indicated that certain noncancer effects and lung cancer appear to have consistent patterns in all animal studies he has reviewed, including studies of asbestos exposure and studies of exposure to non-fibrous particulate. Specifically, this panelist said he had not found any study in which a rodent had lung cancer, but did not have interstitial fibrosis; and he had never seen rodents with interstitial fibrosis in the absence of inflammation. These observations led the panelist to infer that lung cancer would not be expected to develop at doses that do not induce fibrosis or inflammation. He stressed that this inference is based solely on observations from previous animal studies and does not in any way suggest that fibrosis is a precursor to lung cancer—an issue that came up during the observer comments (see Section 3.6). He also added that this relative sensitivity of noncancer endpoints may not necessarily be observed in humans.

- *Irritation.* One panelist noted that laboratory animal studies have not studied the extent to which asbestos and SVF irritate the skin and eye. He added that histopathological studies of the nasal cavity, pharynx, larynx, trachea, and conductive airways have not identified

evidence of irritation, though he acknowledged that the histopathological techniques might not have detected certain responses (e.g., increased mucus production). He cautioned that these results do not necessarily suggest that humans will not experience fiber-induced irritation in the nasal cavity, larynx, and upper respiratory tract, because the rat studies did not consider populations with impaired mucociliary clearance, as might be observed in smokers. Finally, this panelist indicated that ingestion studies in rats and hamsters have shown no evidence of irritation in the gastrointestinal tract.

3.3 Mechanisms of Toxicity

This section reviews the panelists' comments on mechanisms of toxicity, primarily as presented by the two designated discussion leaders, Dr. Mossman and Dr. Wallace. After identifying several general advantages and disadvantages of *in vitro* studies, the discussion leaders reviewed current theories on mechanisms of toxicity for a wide range of fibers and analogous non-fibrous particles. This section reviews key points from those presentations. Emphasis is placed on what has been established or hypothesized regarding the relative toxicities of short and long fibers. For more detailed information on mechanisms of toxicity, refer to Dr. Mossman's and Dr. Wallace's post-meeting comments in Appendix E.

- *General comments on the utility of* in vitro *studies.* To initiate discussions, one panelist listed several strengths and limitations associated with *in vitro* toxicity studies. First, she indicated that *in vitro* studies, when compared to laboratory animal studies, offer a far more controlled setting for examining mechanisms of toxicity and dose-response behavior for specific cell types. She acknowledged, however, that interpreting trends among studies using widely varying doses and multiple cell types can be complicated. Moreover, the *in vitro* studies are all limited in duration, typically lasting a few days, due to the limited life spans of isolated cells in the *in vitro* environment. Consequently, the *in vitro* studies cannot characterize dissolution, macrophage clearance, and other processes that occur over longer time scales. Finally, this panelist noted that doses to *in vitro* samples cannot readily be extrapolated to human inhalation exposures.

- *Role of reactive oxygen species (ROS).* One panelist reviewed a widely accepted theory of how generation of ROS might explain asbestos-related toxicity. She indicated that alveolar macrophages, as they attempt to digest foreign fibers and particles, produce an "oxidative burst" and release ROS. (Other cell types that contact asbestos fibers also release ROS.)

These ROS can initiate sequences of events that have been shown *in vitro* to lead to outcomes such as genotoxicity, cytotoxicity, and cell proliferation.

This panelist highlighted two key observations regarding ROS. First, *in vitro* studies have shown that alveolar macrophages generate more ROS when attempting to digest longer fibers, while shorter fibers can be engulfed completely by macrophages (and other cell types) with no visible damage to the cells. Second, she noted that ROS can form highly reactive hydroxyl radicals via a reaction that is facilitated by the presence of iron. Therefore, long, iron-containing fibers, like several amphibole asbestos fibers, are capable of generating an intense "oxidative burst," which might explain their greater potency, when compared to fibers that do not contain iron. Finally, this panelist noted that researchers can prevent pulmonary fibrosis in animals by administering free radical scavengers or other substances that interfere with ROS formation and reactions—a finding that argues strongly for ROS having a causative role in inducing asbestos-related fibrosis.

Overall, this panelist noted that many aspects of the ROS theory help explain how fiber length and, to a lesser extent, mineral content relate to toxicity and why shorter fibers are substantially less toxic than longer ones. She presented results from several *in vitro* studies (e.g., Ohyama et al. 2001) that confirm that longer fibers generate a greater "oxidative burst" when they are not ingested by alveolar macrophages.

- *Effects on cell signaling events.* The panelist then described current research that has characterized effects of asbestos- and fiber-related cell signaling events. She explained that these events originate when fibers interact with cell surfaces, after which the cells activate transcription factors that mediate various outcomes which can be measured *in vitro*, such as cell proliferation, cell transformation, and cell death. She noted that cell proliferation is an important step in development in both malignant and nonmalignant disease.

  The panelist then described studies examining how selected signaling pathways are affected by asbestos and glass fibers of different lengths. She summarized studies that demonstrated activation of transcription factors and cell proliferation. First, the panelist reviewed a study (Ye et al. 1999) in which mouse macrophage cell lines were challenged with two formulations of fiber glass mixtures, one with average length of 6.5 μm, the other 16.7 μm. These challenges caused production of tumor necrosis factor-alpha (TNF-α), a cytokine involved in inflammation and fibrosis, which in turn caused activation of nuclear factor-κB. Gene promoter activation induced by the short fibers was found to be between one-third and one-half what was observed for the long fibers.

  Second, the panelist reviewed *in vitro* studies that examined specific aspects of cell proliferation. Although cell proliferation relates to cancer outcomes, she emphasized that development of mesothelioma and lung cancer is a multi-stage process with a long latency period, which cannot be captured in the short time frame of an *in vitro* study. The panelist identified many studies (see Appendix E) demonstrating that longer fibers are more apt to

cause cell proliferation than are short fibers, whether from tracheal explant studies (e.g., Sesko and Mossman 1989) or intratracheal models in rats (Adamson and Bowden 1990).

- *Studies of asbestos genotoxicity.* The panelist indicated that researchers have been studying the genotoxicity of asbestos, both *in vivo* and *in vitro*, for more than 20 years. These studies examined a wide range of endpoints (e.g., cell transformation, chromosomal aberrations, gene mutation) in various matrices. The panelist focused, however, on a series of studies conducted to examine the role of fiber length on cell transformation and cytogenetic effects (Hesterberg and Barrett 1984, 1985; Hesterberg et al. 1986). These studies demonstrated that long, thin fibers are most potent for both types of effects, and the shortest fibers examined (less than 1.7 μm long) had no indication of tumorigenic potential. These findings, she noted, indicate that longer fibers are again more toxic, with some suggestion that fibers below a certain length threshold may not be carcinogenic at all.

- *Observations regarding mechanisms of toxicity from non-fibrous particulates.* One panelist addressed mechanisms of action for non-fibrous particulates having compositions similar to those in asbestos and SVFs. First, he indicated that non-fibrous crystalline silica is strongly pathogenic for fibrotic lung disease, while two polymorphs of crystalline silica—quartz and cristobalite—have recently been classified as carcinogenic (IARC 1997). In contrast, amorphous silica (more akin to SVFs) has not been shown to cause lung cancer or mesothelioma in rodents (IARC 1987). Exposure to the crystalline silica polymorphs can directly damage cells, resulting in intracellular generation of reactive oxygen species and a cascade of events (e.g., synthesis and release of cytokines, cell proliferation, secretion of collagen into the extracellular space) similar to the those evoked by asbestos fiber. *In vitro* studies have shown that silanols (hydroxyl groups on the crystalline surface) are associated with the initial damage to cells: loss of surface silanols caused the crystalline silica to exhibit less damaging activity, and subsequent formation of silanols restored the silica's toxicity (Pandurangi et al. 1990). These and other studies (see Appendix E) suggest that surface chemistry plays a role in silica's toxicity.

  This panelist noted that an important but generally ignored component for physiologically representative *in vitro* bioassays is that particles and fibers depositing in the lung initially contact the aqueous "hypophase" lining on the terminal airway and airsac surfaces. The hypophase layer is rich with micellar dispersion of surfactant, composed largely of lipids and lipoproteins. Of particular note, the hypophase can be simulated *in vitro* with dipalmitoyl phophatidyl choline (DPPC) dispersed in physiological saline. Silica particles and other materials deposited in the lung have been shown to adsorb the surfactant, which extinguishes short-term cytotoxicity—another observation indicating that the toxicity of particles in the lung is affected by surface chemistry. This panelist noted that the alveolar hypophase contains more than enough surfactant to coat and neutralize the entire surfaces of respirable particles, even in most high dust exposures (Wallace et al. 1975).

Although the volume of surfactants in the alveolar hypophase is sufficient to coat respired particles completely (even in high dust exposures) and thus is theoretically capable of extinguishing the particles' toxicity, this panelist indicated that the toxicity can be restored when other cellular mechanisms remove the protective surfactant cover. Specifically, macrophages can engulf surfactant-coated particles, where they are subject to phagolysosomal enzymatic digestion which can remove the surfactant film and thus restore toxicity. Some study has shown that the surfactant film is more readily removed from crystalline silica than it is from kaolin, which suggests a mechanism by which quartz may be more toxic than kaolin; however, experimental study has not demonstrated that particle de-toxicification and re-toxicification explains the relative toxicities of these materials. Thus, surfactant coating of foreign particles deposited in the alveolar space again appears to play an important role in toxicity. The influence of surface chemistry has also been observed in quartz particles having alumino-silicate surface contamination; for such particles the surface contamination can delay for months or perhaps years the expression of fibrogenic activity.

Finally, this panelist noted that researchers might glean greater understanding of the main site of asbestos fibrogenic activity from theories reported for crystalline silica. He explained that a series of studies (e.g., Bowden et al. 1989) suggests that fibrosis results from a sequence of events following interactions between crystalline silica and interstitial cells, rather than interactions with alveolar macrophages. Specifically, it is hypothesized that interactions with the interstitial cells control the stimulation of exacerbated collagen synthesis by pulmonary fibroblasts; whereas, interactions with macrophages are hypothesized as being responsible only for an inflammatory response (not fibrosis) evoking neutrophil influx to the alveolus. This panelist suggested that further research on the mechanisms of fibrogenic toxicity for asbestos should consider interactions with interstitial cells, rather than focusing largely on responses initiated by interactions with alveolar macrophages.

- *Comparisons between fibrous minerals and crystalline silica particles.* This panelist noted that the available *in vitro* studies do not explain comprehensively how asbestos fibers and crystalline silica particles differ in inducing fibrosis. Although they identify several endpoints that asbestos and crystalline silica have in common, the studies cannot predict why asbestosis appears as a diffuse fibrosis, while silicosis appears in localized nodules.

  However, some research provides insights on differences between how long fibers, short fibers, and particles contribute to cytotoxicity. Specifically, an *in vitro* study (Liu 1994) examined whether surfactant coating inhibits the cytotoxicity of asbestos. (As the previous bulleted item indicates, similar studies found that surfactant coating virtually extinguished the short-term toxicity of crystalline silica particles.) In the study, Chinese hamster lung cells were tested for micronucleus induction after being challenged with surfactant-coated chrysotile asbestos. The study considered how induction differs between long fibers (average fiber length of 101 μm) and shorter fibers (average fiber length of 11.6 μm). It

found a slight, but not significant, decrement in cytotoxic endpoints for the long fibers and a considerable, statistically significant decrement for the shorter fibers. The findings suggest that surfactant coating is less effective at impairing toxicity for longer fibers.

Although the studies on crystalline silica underscored the role of surface chemistry in eliciting toxic responses, changes in the surface composition in chrysotile asbestos were found to have no significant effect on *in vitro* genotoxic activity (Keane et al. 1999). Specifically, fibers that had been mildly leached to remove near-surface magnesium atoms exhibited comparable genotoxicity to fibers that were not treated with the leaching solution.

Based on his review of these and other studies, one panelist suggested that more than one mechanism of toxicity may operate for asbestos and SVF, and the roles of the individual mechanisms might depend on fiber length. He explained that "frustrated phagocytosis" and its ensuing events clearly appear more relevant to long fibers (i.e., long fibers are much more likely to be only partially engulfed by alveolar macrophages), while a toxicity mechanism mediated by surface properties of phagocytized material (e.g., restoration of fiber toxicity in the intracellular matrix) would be more relevant to short fibers. In other words, part of the short fiber toxicity might be related to mechanisms involving surface chemistry, which were described in the previous bulleted item. This panelist added, however, that additional mechanisms could contribute to toxicity. As one example, he indicated that asbestos fibers penetrating the cell or cell nucleus may exercise modes of direct genetic or epigenetic damage. Whatever the mechanisms of direct fiber damage or stimulation of the cell surface, he noted, several components of the consequent intracellular response have been well defined. Appendix E provides additional detail on the responses that have been characterized, and the influence of fiber length on these responses.

- *Recent advances in fiber preparation methods.* One panelist noted that researchers at NIOSH have been developing a fiber size classifier (separator) that permits *in vitro* or perhaps limited *in vivo* experiments with sets of fibers of fairly well-defined length (Baron et al. 1994). The dielectrophoretic classifier reportedly can separate fibers from an airstream and produce about 1 mg/day of a given size interval. The panelist indicated that this preparation method recently was used to generate the following categories of fiber size intervals:

Cut	Fiber Length		Fiber Diameter	
	Average (µm)	Standard Deviation (µm)	Average (µm)	Standard Deviation (µm)
1	32.7	23.5	0.75	0.50
2	16.7	10.6	0.49	0.27
3	6.5	2.7	0.44	0.22
4	4.3	1.0	0.40	0.15
5	3.0	1.0	0.35	0.14

This panelist noted that the preparation technique may now allow researchers to investigate the influences of fiber length more rigorously. Some panelists noted that the distribution of fiber lengths in the first "cut" is quite broad, but other panelists indicated that the subsequent "cuts" were more narrowly distributed.

One panelist illustrated the utility of the fiber preparation technique by reviewing findings from a recent publication. In initial studies with these size-classified materials, NIOSH research compared fibers from "Cut 2" and "Cut 3" (see table above) for their induction of the cytokine cascade cellular responses (Ye et al. 1999). The longer fiber sample was more active when dose was measured as fibers per cell, but the shorter fiber sample was equally or more active when dose was characterized on a surface area or mass basis. One panel member noted that this was of interest in the context of the previously presented "counter-intuitive" histopathology reports (see Section 3.2.2) associating fibrosis with short fiber exposures.

3.4 General Comments and Interpretations

While discussing the influence of fiber length on asbestos and SVF toxicity, the panelists made several general comments and interpreted observations from the laboratory animal, human, and *in vitro* studies. This section summarizes these general comments and interpretations, while Section 4.1 reviews the panelists' individual summary statements provided at the end of the meeting.

- *Evaluating toxicity based on the "reasonable certainty of no harm."* When discussing asbestos and SVF toxicity, the panelists discussed the terminology they should use to characterize the hazard of fibers less than 5 μm long. One panelist recommended that the panelists consider whether the short fibers have a "reasonable certainty of no harm," drawing from the language promulgated in the Food Quality Protection Act. Given that dose-response data for humans or animals uniquely exposed to fibers less than 5 μm is largely not available, most panelists agreed that the terminology proposed was appropriate for their conclusions. They also noted that separate conclusions should be drawn for different endpoints, using a weight-of-evidence approach that draws from all types of data, including dosimetric, toxicologic, epidemiologic, and *in vitro* testing.

- *Do fibers shorter than a certain length have a "reasonable certainty of no harm"?* The panelists debated whether summary statements could be made regarding whether fibers of certain length intervals have a "reasonable certainty of no harm." Two panelists suggested that environmental exposures to fibers shorter than 5 μm would likely be free of

carcinogenic effects. Other panelists, however, felt uncomfortable making such judgments for noncancer effects (e.g., pleural abnormalities), especially considering the evidence summarized in Section 3.2. Refer to Section 4.1 for the panelists' individual summary statements regarding the influence of fiber length.

- *Why arbitrarily establish critical fiber lengths?* Because humans are always exposed to fibers with a wide distribution of fiber lengths, one panelist wondered if ATSDR or environmental agencies could develop a universal algorithm that quantifies health risks associated with different types of fiber mixtures. For instance, an algorithm might include different relative toxicity factors for different fiber length intervals (e.g., 0–5 µm, 5–10 µm, 10–20 µm, and so on). Such data could then be applied to the distribution of fiber lengths measured in the environment to assess site-specific risks. This panelist acknowledged that the relative toxicity data do not appear to be available to support this approach, but he noted such a universal algorithm would be far less arbitrary than completely ruling out fibers having dimensions below a certain level. He added that such an algorithm can eventually account for other factors (e.g., biopersistence) that are also known to affect toxicity. In short, this panelist indicated that it is theoretically possible to express health risk as a function of fiber dose, dimension, and durability, though he noted that one would need additional research into dose-response and extensive inputs from biostatisticians to develop such an algorithm.

- *Other influences on toxicity.* While recognizing that the focus of the meeting was on how fiber lengths affect toxicity, the panelists noted that many additional factors determine the toxicity of a fiber mixture. Examples of other factors include dose, fiber composition (mineral type), physical state (amorphous or crystalline), surface area, and surface properties. The panelists cited several examples of why length alone might not adequately predict toxicity. First, the panelists noted that the cancers observed in the study of rats exposed to erionite (Wagner et al. 1985) could not be explained by fiber length alone; they suggested that the unique findings of this study might be best explained by unique surface chemistry or the mineral's relatively large internal surface area (2.5 m^2/gram). Second, the panelists noted that fiber durability likely explains why asbestos fibers and SVFs of the same length are not equally toxic. Due to these and other observations, a panelist noted, ATSDR might overlook other important factors that influence toxicity if it focuses exclusively on fiber length.

3.5 Research Needs

The panelists identified several research needs when discussing the influence of fiber length on health effects. In general, the panelists encouraged thorough planning of any future study, emphasized the need for having well characterized exposures, and advocated involving

researchers from multiple disciplines (e.g., epidemiologists, physicians, toxicologists, mineralogists). All research needs mentioned during this session of the meeting are documented here:

- Several panelists indicated that further study should be conducted among the residents of Libby, Montana, to understand the effect of fiber length on toxicity. One suggestion was, through the cooperation of the community and consent of residents, establishing a protocol to analyze lung and pleural tissue from community members who die, regardless of the cause of death. Another suggestion was to track the progression of the observed pleural disease.

- Given the health outcomes observed in Libby, one panelist encouraged focusing future research in laboratory animals on understanding fiber dose-response behavior for the visceral and parietal pleura. Such studies could use fibers from Libby to examine how doses to the pleura and progression of toxic responses vary with fiber length and between fibers and non-fibrous particles. Another panelist added that a well-constructed study can investigate multiple toxic endpoints.

- For added insights on toxicity of short fibers, possibly those from Libby or Lower Manhattan, the panelists suggested conducting an *in vitro* study using several cell types (e.g., rat pleural mesothelial cells, tracheal epithelial cells) to examine multiple endpoints that can be confirmed in animal models, such as cell proliferation and cytotoxicity.

- One panelist suggested that future study of environmental exposures could focus on residential development in areas with increased levels of naturally occurring tremolite asbestos (e.g., the Sierra foothills in California), but he added that a high level of cooperation from the local community would be essential to the success of any such study.

- To assess human health effects associated with exposures to short fibers, one panelist recommended follow-up study of two cohorts of miners, one in South Dakota and one in Minnesota, who were exposed predominantly to short cummingtonite-grunerite fibers. Further study would take into account a longer latency period and might reveal insights on the role of fiber length in toxicity.

- Several panelists encouraged NIOSH to continue to re-analyze personal exposure samples collected on membrane filters in the 1960s and 1970s from textile workers in Charleston, South Carolina. This suggestion followed an observer comment that informed the panel of NIOSH's planned work on this project.

3.6 Observer Comments and Ensuing Discussions

Observers were given two opportunities to provide comments on the second day of the meeting. The panelists were not required to respond to the observer comments. However, some comments led to further discussion among the panelists, as documented here. The observer comments are summarized in the order they were presented:

Comment 1: John Hadley, representing the North American Industrial Manufacturers

Mr. Hadley summarized selected IARC publications regarding the toxicity of SVFs. First, he noted that IARC has accounted for the influence of fiber length in one of its 1997 monographs (IARC 1997). Specifically, IARC classified palygorskite (attapulgite) fibers longer than 5 µm in "Group 2B," or "possibly carcinogenic to humans (limited human evidence; less than sufficient evidence in animals)." On the other hand, IARC classified palygorskite (attapulgite) fibers less than 5 µm in "Group 3," or "not classifiable." Mr. Hadley added that IARC researchers recently published an article on rock and slag wool production workers (Kjaerheim et al. 2002) indicating "no evidence of carcinogenic effect on the lung of rock and slag wool under exposure circumstances in the production industry during the last four to five decades."

Panelists' Discussions: No panelists addressed this comment.

Comment 2: David Bernstein, consultant in toxicology

Dr. Bernstein asked the panelists to provide more information on the lung-retention studies (e.g., how much of the lung was sampled, what parts of the lung were sampled, how representative are the samples of fiber loading in the entire lung).

Panelists' Discussions: One panelist summarized details of the lung-retention sampling performed in studies he authored, and he suggested that observers refer to the original publication for additional details. In one study, this panelist indicated, samples from the periphery and the central parenchyma were collected systematically from longitudinal sections of the entire lung. He noted that preferential sampling (e.g., diseased locations) did not occur, and he added that the study addressed concerns about sampling bias by collecting larger amounts of samples from a given lung.

Comment 3: Aubrey Miller, EPA

Dr. Miller asked the panelists to comment on research opportunities to examine why certain health outcomes (e.g., pleural abnormalities) are being observed in Libby, but have not been reported (and perhaps not examined) in other mining communities with generally similar doses as gauged by conventional fiber sampling methods (PCM). He wondered if research should be conducted in other mining communities to search for pleural abnormalities or if it should focus on understanding what makes the Libby experience unique.

Panelists' Discussion: One panelist indicated that extensive research has already been conducted to characterize mining communities in Quebec. He noted that the fibers have been well characterized and health effects thoroughly studied and identified key differences between these sites. For instance, there are far more asbestosis cases in Quebec miners, but the panelist noted that this might result simply from the larger size of the work force in Quebec. The proportional numbers of, and SMR for, lung cancers among workers are in fact twice as high among the vermiculite miners in Libby than among chrysotile miners and millers in Quebec. Additionally, there is more evidence of pleural disease in the Libby cohort.

Comment 4: Mark Maddaloni, EPA Region 2

Mr. Maddaloni asked the panelists to discuss residential cleanup issues associated with WTC dusts in Lower Manhattan, where fibers in dust samples are largely (80% to 90%) shorter than 5 μm and the asbestos fibers found are almost entirely chrysotile. He was specifically interested in dose-response data for short asbestos fibers and whether the panelists could establish a dose level for short fibers that constitute "a reasonable certainty of no harm."

Panelists' Discussion: Several panelists commented on this matter. One panelist, for instance, emphasized that focusing on fibers less than 5 μm is an arbitrary decision. He noted that residents are ultimately exposed to a complex mixture of fibers of many lengths. Further, this panelist indicated that virtually all dust and air samples contain large amounts (perhaps 80% to 90%) of short fibers, and the fact that WTC dust is composed largely of short fibers is not unusual. He indicated that, at most sites, concentrations of long fibers and concentrations of short fibers are correlated. Due to this correlation, this panelist argued, when measurements suggest that low levels of long fibers are present, one can have a "reasonable certainty of no harm" not only from the long fibers but also from the short fibers, because they are found in proportional amounts. Some panelists suggested that EPA consider using threshold limit values to evaluate the exposure levels.

Panelists expressed differing opinions on how to evaluate exposures. One panelist suggested that exposures to WTC have decreased considerably from the large amounts found immediately after September 11, 2001. One panelist, however, noted that the presence of fibers in household dusts presents an opportunity for ongoing exposure; he added that this exposure scenario differs from what has been evaluated in the literature among occupational cohorts of adults.

Comment 5: David Bernstein, consultant in toxicology

Dr. Bernstein commented on laboratory animal studies conducted for the European Commission. In these studies, rats were administered fibers both by inhalation and by interperitoneal injection. Though he agreed with the panelists' comments that inhalation administration is most relevant to human exposure, Dr. Bernstein cautioned against disregarding the data from interperitoneal injection studies, which have addressed the issue of fiber length. For example, he said recent data from the interperitoneal injection studies has shown that fiber length correlates better with cancer risk in rats than does the dose. Dr. Bernstein added that these studies found that the dose for short fibers had to be increased by orders of magnitude to elicit the same carcinogenic responses as observed for long fibers.

Panelists' Discussions: No panelists addressed this comment.

Comment 6: Joel Kupferman, New York Environmental Law Project

Mr. Kupferman urged the panelists, when discussing the WTC site, to not assume that exposures have ceased because much of the dust has settled. He noted that asbestos still remains throughout Lower Manhattan: in homes, in fire trucks, and in ventilation systems. He mentioned that dusts from some fire trucks have contained as much as 5% (by weight) asbestos. Mr. Kupferman asked the panelists to consider the fact that asbestos exposure is still occurring.

Panelists' Discussions: One panelist noted that the observer raised an important point. He added that researchers can investigate the exposure potential of these settled dusts through "comprehensive air sampling," during which time surfaces are disturbed to simulate actual work or home exposure situations. The panelists revisited this issue when making their final recommendations (see Section 4).

Comment 7: Ralph Zumwalde, NIOSH

Dr. Zumwalde suggested that, when recommending research needs, the panelists not only consider long-term projects that would help characterize dose-response, but also projects that might help ATSDR make prudent public health decisions in the short term. Regarding the short fibers, he asked the panelists to discuss research needs to characterize possible links between short fibers and inflammation and fibrosis (e.g., how do fibrosis grades in animals compare to those in humans? are rats an appropriate model for these endpoint?).

Panelists' Discussions: One panelist noted that several human studies have examined relationships between asbestos exposure (as gauged by lung-retained fibers) and fibrosis grade, but two panelists noted that comparable studies in which the length distribution of fibers was known have not been performed in animals.

Comment 8: Suresh Moolgavkar, University of Washington

Regarding the panelists' comments on progression of fibrosis, Dr. Moolgavkar cautioned the panelists about assuming that fibrosis is an intermediate endpoint for lung cancer, because these two endpoints result from very different pathogenic processes. Noting that toxicologists have long assumed linear dose-response relationships for cancer and threshold dose-response behavior for noncancer effects, he argued that low exposures levels might pose a risk (albeit small) for lung cancer and perhaps no risk for fibrosis.

Panelists' Discussions: One panelist agreed that fibrosis and lung cancer develop from different pathogenic processes. He explained that the animal studies he has conducted and reviewed involving fibrous and particulate materials all suggest that lung cancers are not observed in the absence of fibrosis. He emphasized that this does not mean that fibrosis is on a causal pathway for lung cancer, but rather demonstrates different dose-response behavior for the two outcomes, namely that fibrosis outcomes in animals appear to occur at lower doses than do cancer outcomes.

Comment 9: Jay Turim, Sciences International, Inc.

Mr. Turim asked the panelists to clarify comments made on disease progression.

Panelists' Discussions: One panelist responded, explaining that he has not observed overt progression of interstitial fibrosis *in animals* after asbestos exposures cease. He added that inflammatory response, microgranulomas, and bronchiolization tend to decrease after fiber exposures ceases, even for amosite. He said this has been observed both in rats and hamsters. This panelist acknowledged that these findings from laboratory animal studies may not be relevant to humans. Addressing this final point, two panelists indicated that progression of fibrosis has "absolutely" been observed *in humans* after cessation of exposure.

4.0 Conclusions and Recommendations

This section reviews the panelists' individual conclusions (Section 4.1) and summarizes remarks from the final observer comment period (Section 4.2).

4.1 Panelists' Final Statements

After addressing all agenda items, each panelist was asked to make a final statement with his or her individual conclusions and recommendations. These summary statements were used to draft the executive summary of this report. A review of the summary statements, in the order in which they were presented, follows:

- *Dr. Case's summary statement.* Dr. Case said there is a strong weight of evidence that asbestos and SVFs shorter than 5 μm do not cause cancer in humans and no further research is needed on this matter. For lung fibrosis or asbestosis, on the other hand, he noted that the role of fibers shorter than 5 μm is not as clear and might require further study. Dr. Case suggested designing a laboratory animal study to characterize the extent to which fibers translocate into the pleura, and to determine whether translocation preferentially occurs for any fiber dimensions or types.

  To prevent health effects from occurring in the future, Dr. Case noted that scientists need a better understanding of exposure levels; he advocated characterizing the fiber length distribution in exposure samples at sites with residential exposures. For the Libby site, Dr. Case indicated that further research is needed to understand the unusual pleural pathology among residents. He suggested conducting systematic further study of available data, including more blinded reading of x-rays and examination of pleural histopathology data, if they exist. Dr. Case also recommended cooperating with communities believed to have elevated asbestos exposure (e.g., Libby) to establish protocols to obtain human lung specimens after death; these protocols must ensure that blinded analysis of samples occurs and matched controls are selected.

- *Dr. Lockey's summary statement.* Dr. Lockey first said he concurred with the conclusions of Dr. Case. He then identified several research opportunities for Lower Manhattan and Libby—two sites where contamination with short fibers has been observed in residential communities.

Regarding the WTC site, Dr. Lockey recommended that health agencies characterize exposure in residential settings using proper industrial hygiene measuring techniques, such that the samples collected reflect personal exposures while individuals perform their normal activities of daily living. He suggested that samples be analyzed for asbestos fiber content using conventional analytical methods (i.e., those that count fibers longer than 5 μm) and that particulate samples be collected to characterize the amount of material shorter than 5 μm. Dr. Lockey indicated that health agencies could then compare the measured level of fibers longer than 5 μm to occupational exposure limits with appropriate adjustment factors to account for the fact that potential sensitive sub-populations, such as children, are being potentially exposed. To evaluate particulate levels, Dr. Lockey recommended, health agencies should compare the measured levels of particulates to current recommended occupational and environmental exposure levels and to sampling results from similar non-WTC urban areas to determine if elevated particulate exposures are occurring. He added that the available human and animal data suggest that "asbestos particulate" (i.e., asbestos fibers shorter than 5 μm) does not present a hazard for cancer or, in the case of the relative short term exposure from WTC, pulmonary asbestosis.

Regarding the Libby site, Dr. Lockey identified several data gaps and research needs. First, he again suggested that future exposure assessment work involve collecting air samples that best reflect personal exposure levels during typical activities of daily living, including personal air sampling for populations—such as children—that have potentially high exposures because of environmental activities. Dr. Lockey recommended that health agencies refer to the existing literature to determine the implications of exposures to fibers longer than 5 μm. Dr. Lockey was not sure how to evaluate risks of pleural abnormalities associated with exposures to short, thin, durable tremolite fibers, because these fibers typically have not been quantified in previously published scientific articles. He did recommend, however, that ATSDR study chest x-ray results from the Libby population to quantify the number of residents with pleural plaques and diffuse pleural fibrosis. This distinction, he noted, is important because the medical literature reports that diffuse pleural fibrosis can impair pulmonary function and can be a very progressive disease, while pleural plaques have, in themselves, more limited clinical significance. Dr. Lockey also recommended that ATSDR investigate whether correlations exist between the pulmonary function tests (e.g., spirometric results) and the types of pleural abnormalities observed. Finally, Dr. Lockey supported a recommendation made previously to initiate a protocol to conduct lung tissue analysis among residents.

- *Dr. McConnell's summary statement.* Dr. McConnell first said he supported most of the conclusions and recommendations identified by Dr. Case and Dr. Lockey. His main finding for the meeting was a review of the trends among the laboratory animal studies. Dr. McConnell indicated that these animal studies consistently demonstrate that fiber pathogenicity increases with fiber length. However, he noted that short fibers, if administered in high enough doses, can also produce disease.

Regarding future research directions in animal studies, Dr. McConnell encouraged health and environmental agencies to specify exactly what questions must be answered in order to address health issues at sites of concern. Once agencies indicate the fiber type, dose, fiber length distribution, and health endpoint of concern (e.g., pleural changes), then toxicologists can design and conduct animal studies to address these specific issues.

- *Dr. Lippmann's summary statement.* Dr. Lippmann supported the other panelists' recommendations for characterizing personal exposures at sites where asbestos and SVF contamination is in residents' homes. Sampling of indoor environments during simulated extreme activity was also recommended. Dr. Lippmann suggested that sampling from these sites continue to use the conventional fiber counting methods (i.e., counting those longer than 5 μm), but recommended that environmental and health agencies archive the sampling filters for further analysis in the future, should the need for examining shorter fibers become necessary. He added that fiber sampling and analytical protocols should be standardized and adopted to ensure that samples collected from different sites for different purposes can be compared.

  Dr. Lippmann encouraged further research into site-specific issues, such as pleural disease in Libby, but he also recommended that future laboratory animal studies quantify fiber dose-response behavior as a function of fiber composition and fiber dimension. He indicated that short-term research needs should be identified and met with appropriate screening studies or intermediate studies.

- *Dr. Mossman's summary statement.* Dr. Mossman agreed with other panelists' suggestions and recommendations. She supported initiating further human studies at sites such as Libby, but she added that additional studies of laboratory animals are needed to quantify how dose-response varies with fiber dimension and durability. Dr. Mossman also indicated that *in vitro* studies can (a) provide insights, within a short time frame, into important questions about relative toxicity of various materials (e.g., fibers of different lengths, fibers with different mineral content), (b) further examine theories of mechanisms of toxicity, and (c) direct future research in laboratory animals. She recommended that such studies challenge target cells with well-characterized fiber samples to study how fiber length relates to cell proliferation, DNA damage, and cytotoxicity. Dr. Mossman emphasized that such short-term studies should use appropriate positive and negative controls and should select endpoints that can be later confirmed in animal studies.

- *Dr. Oberdörster's summary statement.* Dr. Oberdörster concluded that most of the available data suggest that fibers less than 5 μm in length behave like non-fibrous particles; however, he noted that a few recent publications (e.g., Brown et al. 2000) have raised some questions about this. To determine more conclusively whether short fibers truly behave like particles and to assess how fiber dimension relates to toxicity, Dr. Oberdörster recommended conducting a simple intertracheal instillation study in rats with different fiber length categories using lung lavage, pleural lavage, and histopathology to

characterize toxic endpoints. For each asbestos and SVF material tested, he suggested evaluating dose-response for different size-selected fiber samples, as well as for an analogous non-fibrous material; if needed, this could be followed by a more expensive inhalation study with different well-defined fiber size categories. Second, Dr. Oberdörster recommended that future public health evaluations consider susceptible populations for asbestos and SVF exposure.

- *Dr. Wallace's summary statement.* Dr. Wallace recommended that future research take advantage of the emerging capability of generating samples of well-classified fibers, particularly those in the range of small fiber lengths. He believed this new capability can support very meaningful *in vitro* studies, such as those described in Dr. Mossman's summary statement, which can then lead into nasal-inhalation studies in rats. Recalling the experience of conducting *in vitro* studies for crystalline silica, Dr. Wallace urged very thorough planning of future *in vitro* studies of short asbestos and SVFs to ensure that the assays selected model the surface conditioning of deposited materials which occurs *in vivo*, especially for short fiber studies, to avoid false positive results. Dr. Wallace recommended that priority be placed on investigating the correlation between *short* asbestos fibers in the lung and pulmonary interstitial fibrosis (see Section 3.2.2), given the toxicologic findings of *in vitro* activities of short glass fibers (Ye et al. 1999) and the inverse correlations between fiber length and lung fibrosis score in some studies of human lung tissue (Churg et al. 1989, 1990; Nayebzadeh et al. 2001).

4.2 Observer Comments and Ensuing Discussions

Observers were given the opportunity to provide comments before the meeting adjourned. The panelists were not required to respond to the observer comments. However, some comments led to further discussion among the panelists, as documented here. The observer comments are summarized in the order they were presented:

Comment 1: Winona Rossel, Local 829 of industrial theatrical stage employees

Ms. Rossel commented that the role of industrial hygiene for the residences in Lower Manhattan is to get rid of the WTC dust. She urged removal of the dust because scientists truly do not know the health implications of the complex mixture of chemicals in the dust. Ms. Rossel said officials should take precautions when addressing this site and remediate and clean homes, rather than continue to study the dust samples. As an example of her concern, Ms. Rossel said, a local high school that had already been abated had to be cleaned further recently, when carpets were found to contain WTC dusts. She also

recommended that a registry be formed to track health effects among the community members.

Panelists' Discussions: The panelists discussed the concerns expressed by community members after all three comments in this section were presented. Refer to the summary following "Comment 3" for the panelists' remarks.

Comment 2: Katherine Ewes, resident of Lower Manhattan

Ms. Ewes, a resident of Lower Manhattan, informed the panel that asbestos, pulverized glass, and iron have been detected in samples from ventilation systems in residential buildings. Ms. Ewes said it would be helpful if the panelists would suggest research on these materials, particularly interactions between asbestos and iron.

Panelists' Discussions: The panelists discussed the concerns expressed by community members after all three comments in this section were presented. Refer to the summary following "Comment 3" for the panelists' remarks.

Comment 3: Kimberly Flynn, 911 Environmental Action

Ms. Flynn indicated that she is a member of 911 Environmental Action, a coalition of residents and community groups in Lower Manhattan. Ms. Flynn indicated that her group's priority is to stop all continuing exposures to WTC dusts. Ms. Flynn noted that the people who were exposed to dusts on September 11 should definitely be followed up on for health effects, but she emphasized that exposures in residential areas must stop. Ms. Flynn challenged use of occupational exposure limits to evaluate exposures to WTC dusts, because residents in the area are potentially exposed to WTC dusts 24 hours per day and some populations (e.g., housekeepers) might be receiving unusually high exposures. Ms. Flynn said she was pleased that the panelists advocated air sampling to characterize "real world" residential exposure scenarios, like children playing on carpets.

Ms. Flynn acknowledged that there are many uncertainties regarding the health effects associated with WTC dust, such as possible synergistic effects, but she was disappointed with how some agencies have responded to public concerns. She was particularly frustrated that agencies have acknowledged the complexities and uncertainties of the WTC dust issue, without taking precautionary measures to cease exposure or provide risk communication messages to the public. Ms. Flynn asked the panelists, in all of their thinking and research design, to be as protective as possible.

Panelists' Discussions: The panelists acknowledged the public concern about WTC dusts, and offered several insights in response. One panelist encouraged residents to participate in research projects that have already been funded, such as one being conducted by faculty at New York University. Another panelist made two comments. First, this panelist noted

that WTC dust has unique features (e.g., extreme alkalinity) that need to be considered in future site evaluations. Second, agreeing with the observers, he noted that eliminating exposures to WTC dusts is an important factor. Finally, a different panelist addressed a comment regarding exposures to short chrysotile fibers. He noted that the medical and scientific literature offer no evidence of exposure to short chrysotile fibers being of significant health concern, except in cases of prolonged exposures at extremely high doses; he added that the presence of long chrysotile fibers in residences would clearly be of greater concern. This panelist also acknowledged that other components of WTC dust (e.g., metals, polycyclic aromatic hydrocarbons) might be of health concern, but he indicated that the experts at this meeting were convened to discuss their knowledge of fiber toxicity.

During this discussion, a representative from ATSDR added that the agency has initiated a registry to track health effects that might be associated with the collapse of the World Trade Center buildings.

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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 premetting 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 1980B1983. 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 affects 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 μm) 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 μm 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 μm, 20 μm, 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 μm) 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 μm) 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 μm in most published work – and 5 μm).

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 μm" as fibers at all, regardless of their aspect ratio. Those who believe this would categorize such structures as particles of length less than 5 μm 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 μm) 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 μm 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 μm fibres once deposited in the lung?

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 μm *in length* – have "translocated" to the "pleura" from the lung. In fact the "pleura" was *not* studied, tumor and plaquew 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***

(substitute more-than-10, more-than-20…etc.). Here the answer is that the data clearly do not adequately access 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 be 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

category "shorter than 10 μm" (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 previous 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 interstitital 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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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 μm, where the mechanism of interception becomes influential (Sussman *et al*, 1991). Thus, for fibers <5 μm in length, deposition patterns and efficiencies will be determined almost entirely according to the fiber width, which for fibers <5 μm long will be less than about 1.6 μm. For fiber widths between about 0.1 and 1.6 mm, 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 μm, 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 ~0.1 μm, which could be a significant fraction of fibers <5 μm 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?

<u>Animal Toxicology Cancer Data:</u>

The most definitive studies for short fibers (<5 μm 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 μm in length) produced no malignant cancers in 42 rats, whereas the long-fiber amosite (30% >5 μm in length, 10% >10 μm), 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 μm 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 μm than by the concentration of fibers longer than 5 μm.

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 μm, 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 μm 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 μm and, for humans, probably closer to 10 μm.

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 (1990B1998). 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 phagolysomes 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 μm (GSD ~ 2.0 μm) and geometric mean diameter 0.09 μm (GSD ~1.5 μm). For comparison parenchymal tissue GML = 5.0 μm (GSD ~2.3) and GMD 0.3 μm (GSD ~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 μm in lengths <10 μm 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 μm and aspect ratio of 19.4 in patients with plaques versus 2.5 μm 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 (<5μm) 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]

Study authors	Experimental design	Fiber type	Critical dimensions	Pleural Effects
Data from the asbestos literature				
LeBouffant et al, 1973	Clinical analysis of human pleural tissue	Asbestos	L<2μm;D<0.03μm	Pleural plaques
Sebastien et al, 1979	Clinical analysis of human pleural tissue	Asbestos (chrysotile)	L=23μm;D=0.06μm	Pleural effusion, pleural fibrosis, mesothelioma
Stanton et al, 1981	Implantation of fibers in pleural cavity of rats	Asbestos $A10_2$ fibers	L>8μm;D<0.25μm L≥4μm;D≤1.5μm	Pleural sarcoma
Churg and DePaoli, 1988	Clinical analysis of human pleural tissue	tremolite chrysotile	L=2.4μm;D=0.15μm L=2.5μm;D=0.03μm	Pleural plaques
Lippmann, 1988	Review of scientific literature	Asbestos	L>5μm;D<0.1μm	Mesothelioma
Timbrell, 1989	Review of scientific literature	Asbestos	D<0.1μm	Mesothelioma
Dodson et al, 1990	Autopsy of lung and pleural tissue from former shipyard workers	Amphibole chrysotile	L=1.05μm;D=0.14μm L=0.85μm;D=0.06μm (Fewer than <10% of fibers in plaques had length >5μm)	Pleural plaques
Gibbs et al, 1991	Clinical analysis of human pleural tissue	Asbestos (all) Amosite	L=0.99μm;D=0.06μm L=1.23μm;D=0.17μm	Pleural fibrosis
Churg et al, 1993	Clinical analysis of fiber types, dimensions in human lung tissue	Asbestos (tremolite)	L=3.0μm;D=0.23μm	Mesothelioma, pleural plaques
Data from RCF literature				
Mast et al, 1995b	Animal study utilizing rats exposed to fibers by nose-only inhalation	RCF	L=5-10μm,10-20μm D≤0.5μm (retained)	Pleural fibrosis, lung neoplasm, mesothelioma
Gelzleichter et al, 1996	Animal study utilizing rats exposed to fibers by nose-only inhalation	RCF	L=1.5μm; D=0.09μm	Pleural inflammation

SUMMARY OF RECOMMENDATIONS ON ASBESTOS EXPOSURE INDICES

Disease	Relevant exposure index
Asbestosis	Surface area of fibers with:
	Length >2 μm; diameter >0.15μm
Mesothelioma	Number of fibers with:
	Length >5μm; diameter <0.1 μm
Lung cancer	Number of fibers with:
	Length >10μm; diameter >0.15μm

[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.15]

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 ≥ 5 can be classified as carcinogenic irrespective of their mineralogical composition if: diameter of fiber <1 μm or can split into such lengths of fibers >3 μm and durability *in vivo* longer than 3 years.) Schneider reported that from a transitional electron microscopy perspective only fibers longer than 3 μm 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 μm 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 um 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, not withstanding 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 phenomenons 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 a 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 um 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 um 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 carcinogenic 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 <u>vitro studies</u> 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 <u>in vitro</u> 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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Dr. Mossman has been studying the mechanisms of environmental lung disease for over 20 years and has generated over 200 publications. Her interest in the field began with graduate training in the lab of Andrew Sivak, Ph.D., at the NY University Institute of Environmental Medicine where she worked on the effects of phorbol esters in skin carcinogenesis. She completed her Ph.D. degree in the lab of John E. Craighead, M.D. in the Department of Pathology at the University of Vermont (UVM). She then pursued postdoctoral research on interactions of asbestos and cigarette smoke in lung tumors with Edward Bresnick, Ph.D., Department of Biochemistry, UVM. She is a past director of the Cell & Molecular Biology Program at UVM and is now a professor in the Dept. of Pathology and Director of the Environmental Pathology Program. Her current research, which focuses on cell signaling by asbestos, silica, and oxidant stress in cells of the respiratory tract, is funded by grants from the National Institute of Environmental Health Sciences and the National Heart, Lung and Blood Institute.

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/intrapleritoneal/ 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 Kuschner, 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 eriionite (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 tumorigencity. 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.

G. Oberdorster

ATSDR Panel Meeting

The term "fiber" should be defined first (WHO definition is different from the NIOSH definition). The selection of a 5 µm 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 µm 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 halftime 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 μm 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 µm for fibers recovered at the pleural site after RCF exposure, whereas the inhaled RCF fibers had a geometric median length of 4.5 µm with the longest fibers being longer than 100 µm. Very few fibers longer than 5 µm 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 [moe 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_2 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 ± TiO_2 or SiO_2; 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 µm) 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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Yu, C. P., Y. J. Ding, et al. (1995). "Deposition and clearance modeling of inhaled kaolin refractory ceramic fibers (RCF) in hamsters - comparison between species." Inhalation Toxicology **7**: 165-177.

Yu, C. P., L. Zhang, et al. (1995). "Deposition of refractory ceramic fibers (RCF) in the human respiratory tract and comparison with rodent studies." Aerosol Science and Technology.

From: Gelzleichter et al., 1996

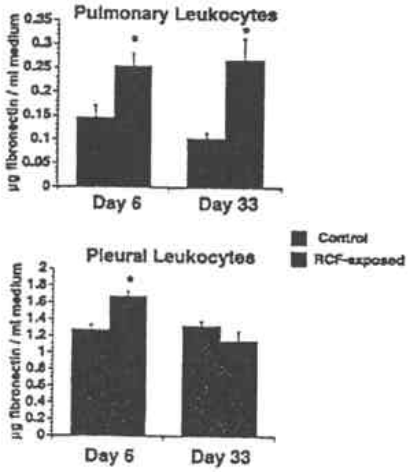
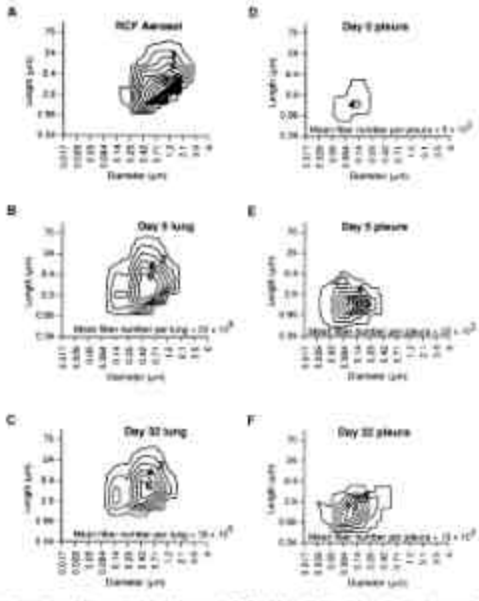


FIG. 3. Soluble fibronectin in conditioned medium following a 48-hr incubation with pulmonary leukocytes and pleural leukocytes. Lavaged leukocytes were plated in 24-well dishes, preincubated for 2 hr in RPMI media with 10% FBS, washed thoroughly with PBS, and incubated for 48 hr in complete medium.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

Gelzleichter et al., 1996



Asgharian and Yu, 1989

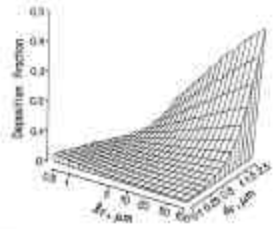


Fig. 3. Tracheobronchial deposition via trachea with no interoptional deposition at a lung volume of 10.78 cm³, a tidal volume of 3.08 cm³, a breathing frequency of 98 cycles min⁻¹ and $\rho = 3.57$ g cm⁻³.

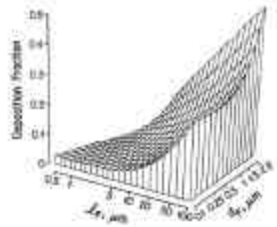
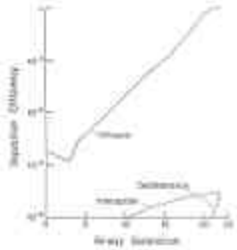
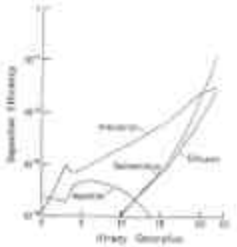
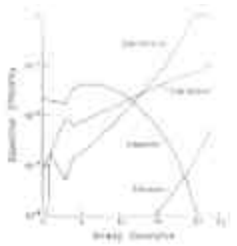


Fig. 4. Tracheobronchial deposition via trachea at a lung volume of 10.78 cm³, a tidal volume of 1.68 cm³, a breathing frequency of 98 cycles min⁻¹ and $\rho = 3.57$ g cm⁻³.

From: Asgharian and Yu, 1988







From: Timbrell, Inhaled Particles V, 1982

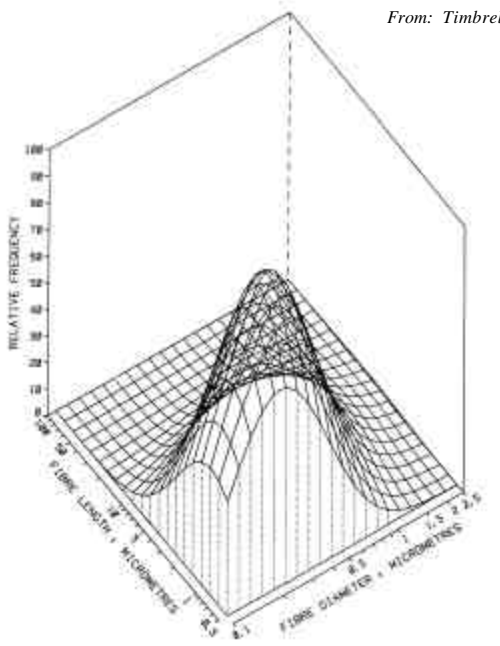


FIG. 8. Bivariate distribution of fibres in Paakkila bagging section

From: Timbrell, Inhaled Particles V, 1982

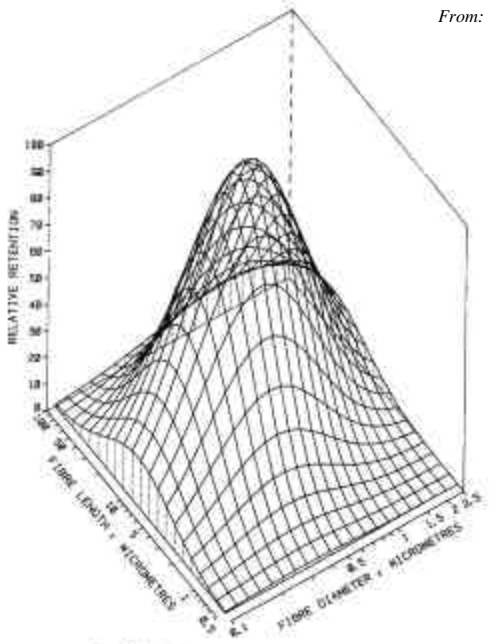


FIG. 10. Bivariate presentation of fibre retention

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

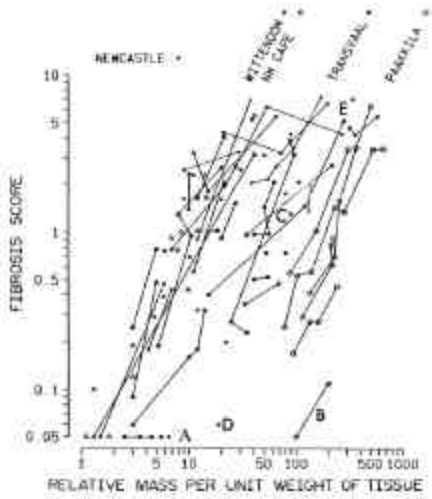


FIG. 3. Relationships when the parameter of fibre quantity is mass. Linked data points relate to tissue specimens from the same subject.

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

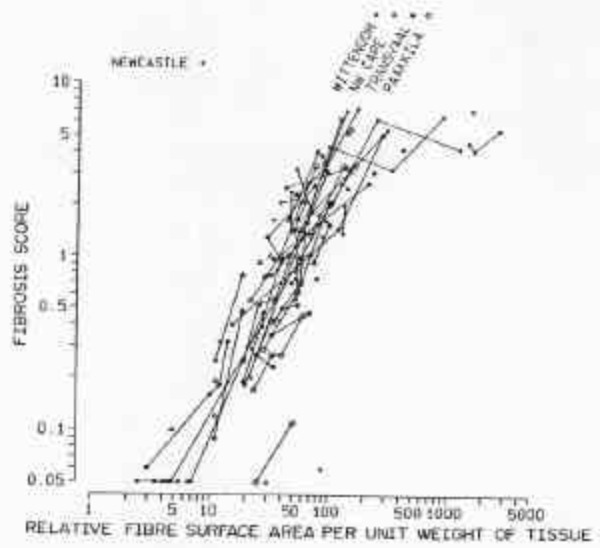


FIG. 4. Relationships when the parameter of fibre quantity is surface area.

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

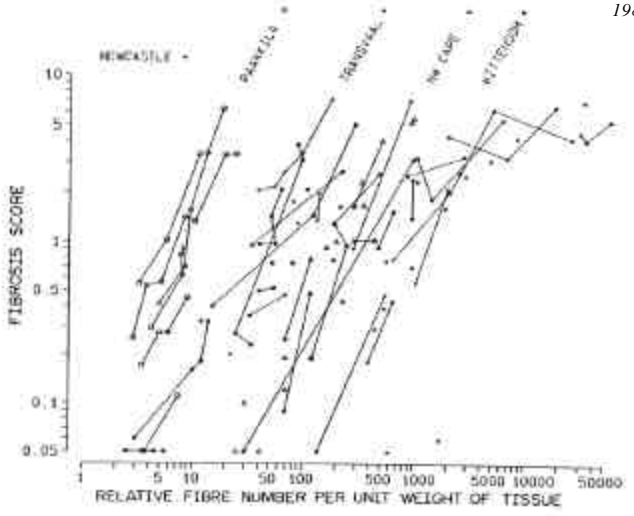


FIG. 5. Relationships when the parameter of fibre quantity is number.

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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 um (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 is 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 presenta 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 109:10331039,2001. This study of luciginen-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 um. 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 um :KT whisker, at 6 um, microglass at 3 um, TO whisker at 2 um, and SiC whisker at 6.4 um were only weakly active. Longer fiber activity correlated with length. This is consistent with the extensive literature indicating long, thin, durable fibers are tumorogenic.

Some other studies suggest and support a “frustrated” phaghocytosis mechanism. This includes some recent NIOSH research results: T Blake, et al. “Effect of fiber length on glass microfiber cytotoxicity” J Toxicol Environm 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 um. 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 Physiol276 (Lung Cell Mol Physiol 20):L426-L434, 1999. Glass fibers with lengths of 6.5 +/- 2.7 um and 16.7 +/- 10.6 um 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 a 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 povide 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 then. 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 know 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 is not 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 um mean with 65% > 10um) , and short length (11.6 um with 98% of fibers < 10 um) 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 preparetions : imtermediate 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" phagoctyosis-associated ROS induced damage, the latter dependent on the possible spread of fiber length values which permit or hinder phagocytosis with variations in cell siza.

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 pasivation 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.

Appendix C

List of Registered Observers of the Expert Panel Meeting

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

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Appendix D

Agenda for the Expert Panel Meeting

Agency for Toxic Substances & Disease Registry
Division of Health Assessment & Consultation

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

Agenda

Day #1, Tuesday, October 29, 2002 (afternoon only)

1:00 PM	Registration	
1:30 PM	Welcome and Introductory remarks	*Dr. Henry Falk, Assistant Administrator, ATSDR*
1:45 PM	Purpose of meeting	*RADM Robert C. Williams, Director, Division of Health Assessment and Consultation, ATSDR*
2:00 PM	Review of meeting charge and agenda	*Morton Lippmann, Ph.D., Panel Chair*
2:15 PM	Introduction of panelists	*Panelists*
2:25 PM	Meeting logistics	*ERG*

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

2:30 PM — What happens to small fibers when inhaled?
Discussion Leaders: *Dr. Lippmann and Dr. Oberdorster*

- *Depositional patterns in the lung*
- *Clearance*
- *Biopersistence*
- *Migration from the lung*
- *Fiber-like vs. particle-like activity patterns*
- *Asbestos vs. MMVF*

3:30 PM	Break
3:45 PM	Topic #1 (continued)
5:30 PM	Observer comments/questions
6:00 PM	Adjourn

Day #2, Wednesday, October 30, 2002

8:00 AM Review of Day #1 issues . . . . . *Morton Lippmann, Ph.D.*
Panel Chair

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

8:15 AM *What do human/epidemiological data tell us about small fibers?*
Discussion Leaders: Dr. Lockey and Dr. Case

- *Cancer effects*
- *Non-cancer effects*
- *Irritant effects*
- *Association between fiber length and fiber-like toxicity*
- *Thresholds of toxic action*
- *Asbestos vs. MMVF*

9:45 AM Break

10:00 AM *How do animal/experimental data augment our understanding of human health effects?*
Discussion Leaders: Dr. McConnell and Dr. Case

- *Cancer effects*
- *Non-cancer effects*
- *Irritant effects*
- *Association between fiber length and fiber-like toxicity*
- *Thresholds of toxic action*
- *Asbestos vs. MMVF*

12:00 PM Lunch

1:00 PM *What are the mechanisms of action of small fibers?*
Can the more robust knowledge base about other fiber or particle materials supplement this understanding?
Discussion Leaders: Dr. Mossman and Dr. Wallace

- *Immune system responses*
- *Cell signaling*
- *Role of reactive oxygen/nitrogen species*
- *Function of growth factors*
- *Other mechanisms*
- *Asbestos vs. MMVF*

3:30 PM Break

Topic #3: Data Gaps

3:45 PM Data gaps and research needs . . . . . *Panelists*

4:30 PM Observer comments/questions

5:00 PM Conclusions/recommendations . . . . . *Panelists*

5:30 PM Adjourn

Appendix E

Panelists' Comments Submitted After the Meeting

Note: The expert panelists were asked to provide premeeting comments and to participate in the discussions at the expert panel review meeting. In addition, several panelists chose to submit additional written comments after the expert panel review meeting. Some panelists submitted updated versions of their premeeting comments (see Appendix B), while others wrote summaries of the discussions they led at the expert panel review meeting. All post-meeting comments are presented here, regardless of their content. Panelists were not required to submit post-meeting comments.

This section presents the post-meeting comments exactly as they were submitted to ERG, with only minor changes to format and references. The expert panel was not asked to comment on the content of these post-meeting comments.

Contents:

Note: Dr. Case submitted post-meeting comments as a list of suggested revisions to an earlier draft of this report. Dr. Case's comments have been incorporated directly into the text of this report and are not replicated here.

Dr. Lippmann's Post-Meeting Comments

ATSDR Fiber Panel Review

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?

This is well established in terms of the depositional mechanisms of impaction, sedimentation, Brownian motion and (for fibers) interception. Fibers with aspect ratios >10 behave aerodynamically like unit density spheres with diameters three times 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 μm, where the mechanism of interception becomes influential (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). Thus, for fibers <5 μm in length, deposition patterns and efficiencies will be determined almost entirely according to the fiber width, which for fibers <5 μm long will be less than about 1.6 μm. For fiber widths between about 0.1 and 1.6 mm, 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 μm, deposition will increase with decreasing width, and there will be a somewhat greater proportion of the deposition in the more proximal airways. Particles that are not deposited remain suspended in the tidal air and are exhaled.

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.

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

vicinity of the bronchial-alveolar duct junctions, and retained for much longer periods, with gradual removal to lymph nodes.

The relatively rapid clearance of short fibers and compact particles from the lung has been demonstrated in a number of studies (reviewed in Health Effects Institute-Asbestos Research, 1991; Davis, 1994; Oberdörster et al., 1990; Morgan, 1995). Such particles 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 Lippmann, 1990) are more prone to dissolution and fragmentation than longer fibers and amphibole types of asbestos.

Absent abnormalities in phagocyte function of 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 mucociliary clearance in asthma. In addition environmental influences, including smoking and nitrogen dioxide (Case et al., 1982), can affect these normal mechanisms through direct ciliary damage or disrupted function. Some common pharmaceuticals slow mucociliary 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 that may interfere with particle clearance, but none have been associated with "fiber length" parameters with the possible exception of smoking (Takahashi et al., 1994).

The 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 μm are not. There are 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, which influences clearance, has to be considered. Inflammatory conditions in the lung (for example, smokers) also contribute to impairment of alveolar macrophage-mediated mechanical clearance and need to be considered.

Biopersistence is the sum of physiological clearance processes and physicochemical processes, which together account for the retention halftime 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 (Davis et al., 1986, 1987;

Wagner, 1990). Generally, short fibers are cleared rapidly if biosoluble (pH differs intracellularly vs. extracellularly), or at rates similar to nonfibrous particles. Breakage of long fibers will give input into short fiber category.

The hazards associated with man-made vitreous fiber (MMVF) appear 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 phagolysomes 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 (NRC, 2000).

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 (IARC# 140, 1996).

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

Fibers with diameters less than ~0.1 μm, which could be a significant fraction of fibers <5 μm 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. Gelzleichter et al. (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 μm (GSD ~ 2.0) and geometric mean diameter 0.09 μm (GSD ~1.5). For comparison parenchymal tissue GML = 5.0 μm (GSD ~2.3) and GMD 0.3 μm (GSD ~1.9.) This would indicate the short thin fibers are capable of translocating to the pleural tissue.

This may be an important subject, at least for the parietal pleura, *if* it is necessary for fibers to reach the pleura to cause lesions (plaques and mesothelioma). It remains possible that fibers still within the peripheral lung may be capable of contributing to the mechanisms of these diseases. Mechanisms remain speculative, but long amphibole fibers 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 and Wiggs (1987), among others, have observed that "accumulation of long fibers immediately under the upper lobe pleura may be important in the genesis of mesothelioma."

Two recent studies are informative (Boutin et al., 1996; Dumortier et al., 2002). They 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 fibers 22.5% were in fact greater than or equal to 5 μm 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 fibers might contribute to plaque and mesothelioma genesis.

Other 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 fibers" - usually very short chrysotile fibers, averaging less than 0.2 μm *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 fibers except via specimen contamination or incorporation, most likely from adjacent lung. Both Rogers et al. (1994) and Case et al. (1994) 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.

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Dr. Lockey's Post-Meeting Comments

What do human/epidemiological data tell us about small fibers?
Discussion Leaders: Dr. Lockey and Dr. Case

Cancer Effects

Short natural occurring fibers. A study by Higgins, et al. [1] in 1983 reviewed the mortality of workers employed at the Reserve Mining Company at Babbit, Minnesota. These workers were involved with mining taconite, which is a dense hard rock composed of silica, silicates and iron. Taconite mined in the eastern tip of the Mesabi range contained amphiboles in the cummingtonite-grunerite series. These fibers are short in length with reportedly the vast majority being <10 μm and are related to amosite asbestos. Of the 9,065 men employed by the company as of July 1, 1976, 5,751 had worked one year or more. The investigators established the vital status of 96% of those who worked for five years or longer and 75% of former workers who worked one to four years. The total respirable dust ranged from 0.02 mg/m^3 to 2.52 mg/m^3 and as high as 2.75 mg/m^3 with the modal range from 0.2 mg/m^3 to 0.6 mg/m^3. There were relatively few measurements of fibers and those that were available demonstrated concentrations usually low with a few at or above 0.5 fibers/ml in the crushing department. Reportedly none approached the OSHA threshold limit value which at that time was 2 fibers/ml. Results of the study indicated that there was no excess death in this population including those men with cumulative exposure of 1,000 to 3,000 total dust years or 500 to 1,000 silica dust years. The conclusions of the study indicated the death rates for all causes were significantly below expectations including selected respiratory disease and death from malignant disease was marginally below that expected for the State of Minnesota. There was no relationship between lifetime dust exposure and increased mortality, nor was there any indication that malignant neoplasm was increased after 15 to 20 years latency. The authors identified a weakness of the study in that the average latency of the cohort was 14.7 years with a maximum of 24.6 years, or a relatively short latency for development of cancer.

A study by McDonald, et al. [2] regarding the mortality from long-term exposure to cummingtonite-grunerite from a gold extraction process at the Homestake Mine, Lead, South Dakota was reviewed. Those workers who had worked 21 years or longer were traced and of 660 men who had died, the cause of death was ascertained for 657. Results of the study indicated pneumoconiosis, which was mainly silicosis along with tuberculosis, and heart disease were causes of excess death. There was a dust exposure relationship for both pneumoconiosis and respiratory tuberculosis, but reportedly no convincing increase in respiratory cancer. It was noted that more than 75% of the 660 men who had died started to work before 1925. The interval between first employment and death and the 76 fatalities from tuberculosis or pneumoconiosis ranged from 22 to 61 years with a median of 35 years. Average silica dust concentrations ranged from 11.0 to 24.6 mppcf before 1952. A study by Dement, et al. [3] reported that 80% to 90% of fibers in the mine had an amphibole diffraction pattern by transmission/scanning electron microscope equipped with an energy-dispersive X-ray spectrometer. The mean total fiber concentration was 4.82 $\pm$ 0.68 f/cc (range 0.66–11.79) with 0.36 $\pm$ 0.08 f/cc (range 0.07-1.29) greater than 5 μm in length. There was one potential mediastinal mesothelioma which could not be confirmed in the 17 respiratory malignancies (16.5 expected based on South Dakota rates).

The results of the study were in conflict with an earlier study by Gillam, et al of the same mine of 440 males who worked at least five years underground by 1960. Reportedly there were 10 deaths from neoplasm of the respiratory system between 1960 and 1973 where as 2.7 were expected based on the male population of South Dakota [4].

Conclusions

There is no data regarding human exposure to asbestos fiber uniformity less than 5 μm in length.

Studies of workers exposed to cummingtonite-grunerite, a type of amphibole related to amosite, demonstrated no consistent increase in overall mortality, mortality related to selected respiratory disease, or respiratory cancer. The vast majority of airborne fibers were reported to be less than 10 μm in length.

Studies of workers of a gold mine in Lead, South Dakota exposed to cummingtonite-grunerite initially demonstrated an increased mortality from malignant respiratory disease. A subsequent study did not confirm the initial finding but demonstrated an increase in silicosis and tuberculosis. Mean total fiber concentration was 4.82 f/cc with 0.36 f/cc greater than 5 μm in length.

Consideration should be given for performing a feasibility study regarding an updated mortality analysis of these two cohorts.

MMVF Mortality Studies. Mortality studies of glass fiber and mineral wool production workers have been ongoing in the U.S. most recently under the direction of Marsh, et al at the University of Pittsburgh, and within the European Union under the direction of the International Agency for Research on Cancer (IARC). The most recent follow up study by Marsh, et al. [5,6,7] of 10 U.S. glass fiber manufacturing plants demonstrate no excess mortality from all causes, all cancers combined, or non-malignant respiratory disease. For respiratory system cancer, there was an observed 6% excess that was statistically significant for the total cohort but not found in workers who had five or more years of employment. An association was seen with calendar time and time since first employment, but no relationship was found with duration of employment, or increase in exposure to respirable glass fiber. A case-control study of respiratory system cancer did not identify increased risk with duration of exposure, cumulative exposure, or time since first employment. An association with non-baseline levels of average intensity of exposure to respiratory fibers was not present when adjusted for smoking.

A previous case-control study of a glass fiber manufacturing facility included in the U.S. glass fiber study demonstrated that differences in local versus national smoking rates may have been a contributing factor in the excess respiratory cancer seen in that manufacturing facility. [8] The potential confounding impact of cigarette smoking in the U.S. glass fiber and rock/slag wool studies was further explored by Buchanich, et al. [9] and Marsh, et al. [10] and identified as the potential unaccounted for factor regarding the small excess respiratory system cancer not related to exposure indices.

Previous analysis of five rock and slag wool plants in the U.S. demonstrated increased lung

cancer mortality using U.S. but not local rates, and this was confined to short-term workers or those workers with less than five years duration of employment. There was no association with measures of respirable fiber exposure. [11] Within the U.S. a case-control study of 9 slag wool plants demonstrated an association with smoking but not MMVF exposure. [12]

Most recent analysis of the U.S. rock and slag wool workers as well as glass fiber production workers identified ten death certificates that mentioned the term mesothelioma. [13] Of the ten cases of mesothelioma, two on pathology review were definitely not felt to be mesotheliomas, one had a 50% chance of mesothelioma, and two others had less than 50% chance of mesothelioma. Medical records or pathology specimens were not available on the remaining five. Using a timeframe when specific malignant mesothelioma coding rubrics were available, the expected mesothelioma rate (local county comparison) was 2.19 versus 1 observed. Overall the authors felt there was no increased risk from the malignant mesothelioma in the U.S. MMVF cohort.

The IARC have followed the mortality of workers among 13 MMVF manufacturing facilities in Europe. [14] The most recent update demonstrated a significant increase in lung cancer mortality in rock and slag wool workers as well as glass wool workers, using national mortality rates which disappeared for the glass wool workers when using local adjustment factors to the national mortality rates. In addition, there was no association in the glass wool workers with time since initial employment or duration of employment, and with removal of glass wool workers with less than one-year employment no excess lung cancer was noted.

Within the rock and slag wool cohort there was an increase in lung cancer risk but the authors felt there was no clear information to indicate that the increased cancer risk was specifically related to fiber exposure. [14] A subsequent cohort study demonstrated similar results. [15] A case-control study nested in this latter cohort indicated no relationship between cumulative rock or slag wool exposure and lung cancer. [16,17]
Within the IARC study there were five cases of mesothelioma, two which occurred in workers with less than one-year employment and two in workers with most likely prior asbestos exposure. [14]

Preliminary results of a mortality study of U.S. RCF manufacturing workers demonstrate no significant increase in malignant or non-malignant respiratory mortality and no malignant mesothelioma. The power of the study was limited as the cohort was relatively young and small in number. [18]

Conclusions

There are no data regarding human exposure to MMVF uniformity less than 5 μm in length.

There is no persuasive evidence that exposure to glass fiber, rock wool, slag wool, or refractory ceramic fiber has been associated with increased lung cancer risks based on ongoing U.S. and European mortality studies.

There is no indication of an increased risk for mesothelioma.

Non-Cancer Effects

MMVF Morbidity and Mortality Studies. Non-malignant respiratory effects: Studies of five fiberglass and two mineral wool manufacturing facilities identified small opacities in 1.6 % of the population studied that were predominantly irregular in shape. [19] These workers were involved with working in facility manufacturing fibers over 3 μm in diameter and fibers averaging 1 μm to 3 μm in diameter. The overall rate of chest X-ray changes was no different in comparison to a non-MMVF exposed comparison group, and any relationship between exposure indices was seen at profusion level 1/0 but not 1/1. There was no increase in upper or lower respiratory tract symptoms. Similar results were seen in a study in Australia of glass and rock wool production workers with no findings of asthma, pulmonary fibrosis or pleural disease. [20] A similar study of rock wool workers also did not demonstrate increased respiratory symptoms or abnormalities with DLCO or DL/Va. A potential additive or synergistic effect, however, was seen regarding the FEV1/FVC ratio, fiber exposure, and those with greater than 40-pack year history of cigarette smoking. [21]

The IARC [22] study demonstrated no increased mortality from asthma, bronchitis or emphysema, which is similar to the most recent analysis of the glass fiber workers in the United States which did not identify increased mortality from non-malignant respiratory disease. [5] Of interest in the IARC study was the suggestion of an increased risk from non-malignant renal disease (SMR 0.97, 95% CI 0.36 to 2.11) in regard to duration of employment or employment at an early phase within the rock and slag wool industry. Within the U.S. mineral wool study a similar trend was noted ($p < .05$) with a SMR of 204 (observed 12) in regard to nephritis and nephrosis. [11] Similar type patterns have not been demonstrated in relationship to nephritis and nephrosis deaths in U.S. glass wool manufacturing facilities. [23]

There are very limited studies on end users of man-made vitreous fibers. One study identified increased prevalence of chest radiograph evidence of irregular opacities in workers using rotary spun fiberglass, but there was a question of airborne asbestos fibers within the plant site. [24,25] In insulators a decrease in FEV1 was identified in comparison to a non-exposed control group after adjusting for smoking habits and self-assessed former asbestos exposure. [26]

On-going morbidity studies of workers involved with refractory ceramic fiber (RCF) manufacturing have identified a relationship between pleural plaques and time from initial employment, duration of employment, and cumulative refractory ceramic fiber exposure. Pleural changes were seen 2.7% or 27 workers out of 1,008 of which 22 were pleural plaques. Of those with greater than 20 years latency from initial production job or 20 years duration in a production job, 16 workers or 8.0% and 5 workers or 8.1% had pleural changes, respectively. Interstitial changes were noted in 1.0% at profusion category $\geq$1/0, similar to other non-specified dust exposed worker populations and showed a non-significant elevated OR in regard to cumulative fiber exposure of 4.7 (95% CI, 0.97 to 23.5). In regard to cumulative fiber exposure, 5.4% (8 of 148) with greater than 45 to 135 fiber-month/cc exposure had pleural changes (OR 5.6, 95% CI, 1.5 – 28.1). For those with >135 fiber-months/cc exposure, 9.8% (6 of 61) had pleural changes (OR 6.0, CI 1.4 – 31.0). [27] European studies concurred that there was some evidence of a relationship between RCF latency and pleural changes including pleural plaques but not duration or intensity of RCF exposure, but it was difficult to separate the effects asbestos and RCF exposure and any relationship between RCF exposure and small opacities was at best ambiguous. [28]

Previous studies of the RCF workers demonstrated a relationship between 10 years of employment in production job tasks prior to 1987 and small decrements in FVC for current (165.4 ml) and past (155.5 ml) male smokers, but not never-smokers, and small decrements in FEV1 for current male smokers only (134.9 ml). For never-smoker women there was also a decrement in FVC (350.3 ml) per 10-years employment in production job tasks. [29] A longitudinal analysis in those male workers able to provide five tests or more did not demonstrate any further decrement of the FEV1 or FVC between initial and final tests. [30]

Conclusions Regading Non-Cancer Effects of MMVF

There are no available morbidity studies of workers exposed to MMVF uniformly less than 5 μm in length.

No increased mortality from non-malignant respiratory disease.

No indication of chest radiograph interstitial or pleural changes in regard to glass and mineral wool production workers but data is limited.

Refractory ceramic fiber (RCF) exposure appears to be associated with the occurrence of pleural plaques that most likely are related to increased exposure levels in the RCF manufacturing facilities prior to 1985.

Potential additive or synergistic effect with MMVF exposure and small decrement in FVC and/or FEV1 involving current or former smokers.

Within mineral wool cohort, question of potential increased mortality from non-malignant renal disease such as nephritis and nephrosis.

End user studies of MMVF users are limited and are confounded by potential previous asbestos exposure.

Irritant Effects

MMVF can cause skin irritation particularly in an area where clothing comes in close contact to the skin such as around the neck or forearms. Essentially this occurs in 5% of new workers involved with MMVF production. [31] Residential contamination of man-made vitreous fibers in high concentration can also cause irritation to the upper as well as lower respiratory tract. [32] Glass fibers with diameters greater than 5.3 μm have been reported to be more likely to cause skin irritation than the smaller diameter fibers, mainly due to mechanical irritation. [33,34] There has been documentation of eye irritation associated with MMVF as well as nasal and pharyngeal irritation with unusual MMVF dust exposure situations. [35,36]

Conclusions

Skin irritation appears to be related to the mechanical effects of fiber ~5 μm in diameter and appears to be worse in hot, humid weather.

Accidental exposure to increased concentrations of MMVF can result in upper and lower respiratory tract irritation as well as eye irritation.

Association Between Fiber Length and Fiber-like Toxicity

There have been no published studies that address whether asbestos fibers uniformly <5 μm in length have been associated with pleural or parenchymal disease in human. Any potential risk associated with fiber exposure <5 μm in length most likely would be related to an increased risk for pulmonary asbestosis, and most likely would occur at a substantially higher dose in comparison to exposures to asbestiform fibers (long fibers with high aspect ratios). [37]

There is some indication that fibers with diameters with <0.1 μm to 0.4 μm and lengths <10 μm may have a propensity for inducing pleural plaques. [38] Methodologies used to analyze pleural and/or parenchymal tissue for the presence of fibers and association of pleural changes differ markedly between investigators, however. Human studies of individual exposed to asbestos fibers are difficult to interpret in regard to toxicity solely related to fibers <5 μm in length because exposure situations almost uniformly contain a broad distribution of fiber diameters and length.

Preliminary results of residents of Libby, Montana that were exposed to asbestiform tremolite indicate a high propensity for pleural changes in comparison to interstitial changes. [39] There is some indication that exposure to tremolite fibers with relatively low aspect ratios in comparison to the asbestiform type tremolite may be capable of causing pleural plaques. [40] Pleural plaques can occur with minimal exposure to asbestos and can occur within a wide range of tissue burdens of asbestos fibers which overlap with control populations. [41]

Conclusions

Even though there are no human studies solely of MMVF <5 μm in length, the available morbidity and mortality studies of MMVF production workers indicate limited overall toxicity from MMVF exposure.

There are no human studies regarding exposures solely to asbestos fiber <5 μm in length but there has been some speculation that durable fibers <10 μm in length and <0.1 to 0.4 μm in diameter may be associated with pleural plaques in relatively low concentration, in particular the amphibole tremolite.

For asbestos fibers <5 μm in length, it would appear that very high doses may have the propensity to cause interstitial fibrosis, particularly if the fibers are durable within intracellular fluids.

Thresholds for Toxic Action

For asbestos and MMVF less than 5 μm in length, thresholds for toxic action in humans have not been established but most likely is substantially higher than the thresholds for long durable fibers with increased aspect ratios of respirable size.

Asbestos Versus MMVF

Based on animal and human studies, natural occurring asbestos fibers that are of respiratory size, long and thin with high aspect ratios, and durable within physiologic fluids represent the highest risk for malignant (lung cancer and mesothelioma) and non-malignant (interstitial fibers) respiratory disease. These abnormalities have not been demonstrated in MMVF manufacturing workers.

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Dr. McConnell's Post-Meeting Comments

How Do Animal/Experimental Data Augment Our Understanding of Human Health Effects

Background: There have been numerous studies of the effects of various types of asbestos (ATSDR, in press) and SVFs (ATSDR, in press) 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, disadvantages and limitations) for use for assessing potential health effects in humans (McConnell, 1995). 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 (McClellan et al., 1992). 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 (IARC, 1987) and SVFs (IARC, 2002) 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 (McConnell et al., 1995) or amosite asbestos (McConnell et al., 1999). 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 um 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 (ATSDR, in press). 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 (Wagner, et al., 1974). 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 (Boorman and Eustis, 1990). 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 (Pott et al., 1994). 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 (IARC, 1987; 2002).

Mesothelioma has also been found in rodent carcinogenic bioassays of asbestos and SVFs (IARC, 1987; 2002. 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 (Wagner et al., 1988). In contrast to inhalation, direct instillation into the pleural (Stanton et al., 1981) or peritoneal cavities (Pott et al., 1987) 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 (Boorman et al., 1990). 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 (McConnell, et al., 1999), 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 (Greim et al., 2001). However, there have been fiber studies where pulmonary fibrosis was observed without the development of fiber related neoplasms (McConnell, et al., 1994).

In vitro studies may not be of high value for predicting the carcinogenic potential of a given type of fiber, although they can give some incite into the difference between the carcinogenicity of long and short fibers. There are several reasons for why they may not as useful for predicting the carcinogenic activity of a given type. 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, not withstanding this, in vitro methods are highly powerful tools for understanding fiber/cell interactions and mechanisms of toxicity/carcinogenicity (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 (IARC, various volumes). 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 in experimental animals 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, monkeys ~15 um, and humans ~21 um diameter (Krombach et al., 1997)], the fiber cannot be removed unless it is broken into shorter lengths or dissolves (Maxim and McConnell, 2001). 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 - It should be noted that rodents do not have a respiratory bronchiole, as do humans). In addition to a 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 (McConnell et al., 1984: 2002). 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 using the results of pulmonary lavage studies (see Oberdorster).

Stop studies (where exposure is stopped and the animals are observed during 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. Microgranulomas become less apparent and early fibrosis is also, to some degree, resolvable, at least with SVFs. In rodents, studies have demonstrated that fibrosis, even with asbestos, is not particularly progressive, once the exposure ceases.

While there is no exact correlate for pleural plaques in animals, localized acellular fibrotic changes reminiscent of this lesion in humans 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 pleural instillation or peritoneal injection studies so it is not known if the same events occur with these routes of exposure of exposure. 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 (ATSDR, in press).

In vitro studies on the irritant effects of either asbestos or SVFs in animals have not been reported.

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 by using intrapleural implantation/instillation (Stanton et al., 1981) and intraperitoneal injection (Pott et al., 1976) 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 um 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. There have been a few inhalation studies have been conducted to study the influence of the fiber length on the pathology of asbestos, and all have been persuasive for showing that short fibers are not carcinogenic. This has been demonstrated for chrysotile (Davis and Jones, 1988; Ilgren, 1998; Wagner et al., 1980), amosite (Davis et al., 1987; 1986) and crocidolite (Davis et al., 1978; Wagner et al., 1984).

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 (IARC, 1987). 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 carcinogenic 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 with fibers of the same type that are longer. Inhalation studies have clearly shown 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 <u>in vitro</u> studies that address this subject but those that have clearly show a relationship between fiber length and genetic damage. For example, in a study of Chinese hamster ovary cells (CHO) short amosite failed did not cause chromosomal aberrations while long fiber amosite did (Donaldson and Golyasnya, 1995). See Mossman and others for other studies.

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 (McConnell et al., 1995). In that study, there was a definite dose-related response with regard to both nonneoplastic (macrophage response, pulmonary fibrosis, etc.) and carcinogenic activity (mesothelioma). 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 (Mast et al., 1995). 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 (Hesterberg et al., 1996). 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 (Patek et al., 1985). In this study, monkeys were exposed to chrysotile asbestos at an exposure level of 1 mg/m3 (0.8 f/cc >5 um length) for 18 months. 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 ~11 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 (not reported – personal observation).

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 <u>vitro studies</u> 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 <u>in vitro</u> 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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Dr. Mossman's Post-Meeting Comments

ATSDR Fibers Panel
Mechanisms of Short Fiber Toxicity

There appears to be a striking difference in the pathogenicity of respirable fibers directly related to fiber length, with fibers below approximately 5 microns in length being less hazardous for the development of cancers or pulmonary fibrosis. This prompts the questions: What are the observed mechanisms of long fiber toxicity? Does composition matter? Are short (<5 microns in length) fibers pathogenic? If so, what are the mechanisms of their toxicity?

One hypothesis is that long fiber effects are related to increased generation of oxidants; reviewed in Kinnula, 1999; Hansen and Mossman, 1987). It has been shown that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated by asbestos fibers spontaneously in cell-free systems, cells in culture, and lung tissue *in vivo.* A primary step in response to asbestos fiber challenge to a number of cell types is superoxide anion release from cells which have attempted to phagocytize long fibers whereas short fibers are encapsulated in phagolysosomes, often without visible damage to cells. Superoxide, however, can be further dismutated to hydrogen peroxide which can generate the reactive hydroxyl radical, catalyzed by iron vs. the Fenton reaction. Alternatively, superoxide can react with nitric oxide to form peroxynitrite that is associated with inflammation and lung injury. Asbestos stimulates the release of ROS and induces oxidants intracellularly in both inflammatory cell types (Hansen and Mossman, 1987; Goodglick and Kane, 1986; 1990) and target cells (Xu et al., 2002). Moreover, indirect evidence for oxidant stress by asbestos is indicated by elevations of antioxidant enzymes in cells in culture and lung tissue after inhalation of crocidolite asbestos (Janssen et al, 1992, 1994b). In human mesothelial cells, these increases were not observed with exposures to polystyrene beads, or riebeckite, a chemically similar nonfibrous analog of crocidolite (Janssen et al., 1994b). The role of oxidants by crocidolite asbestos in causation of inflammation and fibrosis has been confirmed in rodent inhalation studies (Mossman et al., 1990), and supports the central dogma that asbestos fibers activate transcription factors and early response genes involved in proliferation and inflammation by generating ROS via "frustrated phagocytosis" (reviewed in Manning et al., 2002).

Several papers show that "frustrated phagocytosis" and oxidant production occur selectively in response to long vs. short fibers of asbestos or glass. A study of luciginen-dependent chemiluminescence (CL) in human monocytes found a strong correlation between superoxide release and fiber lengths from 6 to 20 microns. All samples of fibers except wollastonite induced CL release in a dose-dependent manner. Superoxide release was non-specific for the compositional type of fiber, and fibers with lengths below 7 microns were only weakly active. In studies by Blake et al. (1998), CL induction after zymosan stimulation and LDH release, a measure of lytic cell death, were measured in Manville Code 100 (JM-100) fiber challenged rat alveolar macrophages. A novel feature of this study was the use of fibers carefully sized to average lengths of 33, 17, 7, 4, and 3 microns. The greatest toxicity was seen with the longer

fibers which had multiple macrophages attached along the surface, indicating that incomplete phagocytosis was associated with toxicity. These studies reinforce the many experiments in the literature showing that long fibers are more toxic than shorter fibers in a number of cell types, i.e., Goodglick and Kane, 1990.

Increased fiber length has also been linked to activation of transcription factors and cytokines. For example, Tumor Necrosis Factor-alpha (TNF) is a cytokine involved in inflammation and fibrosis. In a study by Ye et al. (1999), glass fibers with lengths of 6.5 +/- 2.7 microns and 16.7+/-10.6 microns were used to challenge a mouse macrophage cell line. Glass fibers stimulated TNF production and caused Nuclear Factor-kB (NF-kB) activation, a process involving ROS. Long fibers were more potent than short fibers which were effectively engulfed by macrophages. Short fiber-induced TNF and TNF gene promoter activation was on the order of one-third to one-half of long fibers. In another study (Cheng et al., 1999), crocidolite asbestos caused parallel increases in TNF production in macrophages in a dose-dependent manner, without cytotoxicity at the optimum stimulating condition. Titanium oxide dust was without effect. TNF production may also be linked to inflammation by asbestos, and it has been shown that injection of long vs. short amosite fibers intraperitoneally results in inflammation and macrophage activation related to the proportion of long fibers (Donaldson et al., 1989).

Another pathway leading to activation of protooncogenes (*fos/jun)* that comprise the Activator Protein-1 transcription factor is the Mitogen Activated Protein Kinase (MAPK) cascades, consisting of c-jun-N-terminal amino kinases (JNKs), Extracellular Signal Regulated Kinases (ERKs) and p38 kinases. In studies by Ye et al. (2001) using macrophages, long glass fibers were more potent than short fibers in activating MAPK which led to activation of c-Jun and the TNF promoter. Studies by Zanella et al. (1996) explored the stimulation of ERKs in mesothelial cells, and found increases with crocidolite and chrysotile asbestos , but not with the nonfibrous analogs, riebeckite or antigorite. Similarly, elevations in *c-fos* and *c-jun* expression were seen with asbestos fibers and erionite in mesothelial cells, but were not induced by a variety of particulates, MMVF-10 or RCF-1 fibers at comparable concentrations (Janssen et al., 1994a). Long fibers of crocidolite (> 60 microns) were selectively associated with phosphorylation of the Epidermal Growth Factor receptor in human mesothelial cells (Pache et al., 1997), an event not occurring with MMVF-10 or particles. In general, pathogenic dusts such as asbestos or silica, produce a variety of cytokines from cells and activate a number of transcription factors through ROS or RNS (Mossman and Churg, 1998; Churg et al., 2000).

Another ramification of transcription factor activation is cell proliferation. Mechanistic studies using target cells in culture or tracheal explants have shown that long fibers are more toxic and more apt to cause cell proliferation than short fibers (Brown et al., 1986; Wright et al., 1986; Marsh and Mossman, 1988; Sesko and Mossman, 1989; Woodworth et al., 1983). These events may be coupled, as compensatory hyperplasia may result from initial epithelial cell injury. In studies by Woodworth et al. (1983), epithelial proliferation and squamous metaplasia were observed with various types of fibers including glass and attapulgite, but not with nonfibrous analogs of asbestos, i.e. riebeckite and antigorite and other particles.

An intratracheal model in rats using long (>2.5 microns) and short crocidolite asbestos after intratracheal instillation 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.

Surfactant adsorption may be a mechanism whereby reactive particles or fibers are rendered inactive or nonpathogenic. To determine the effect of surfactant adsorption on chrysotile genotoxicity using an assay for micronucleus induction in Chinese hamster lung cells (V79) (Lu et al., 1994), two lengths of chrysotile fibers were used with and without pretreatment with DPPC, i.e. NIEHS intermediate (65%> 10 microns) and short (98% < 10 micron) fibers. The longer fibers 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 effect on the short fibers. One possibility is that the partial suppression of genotoxicity reflects suppression of a component of toxicity by surfactant on the mineral surface. Thus, short fiber genotoxicity, as reported here, may reflect a combination of mineral surface functional groups which direct membranolytic activity and can be modulated by interactions with components of the pulmonary surfactant system as well as phagocytosis-associated ROS.

In conclusion, studies summarized above show decreased or no effects of short fibers and nonfibrous analogs of asbestos in a number of bioassays. The effects of long glass and asbestos fibers may be comparable in some studies. However, the duration of these short-term assays may be too short to reflect important solubility changes occurring in lung over time.

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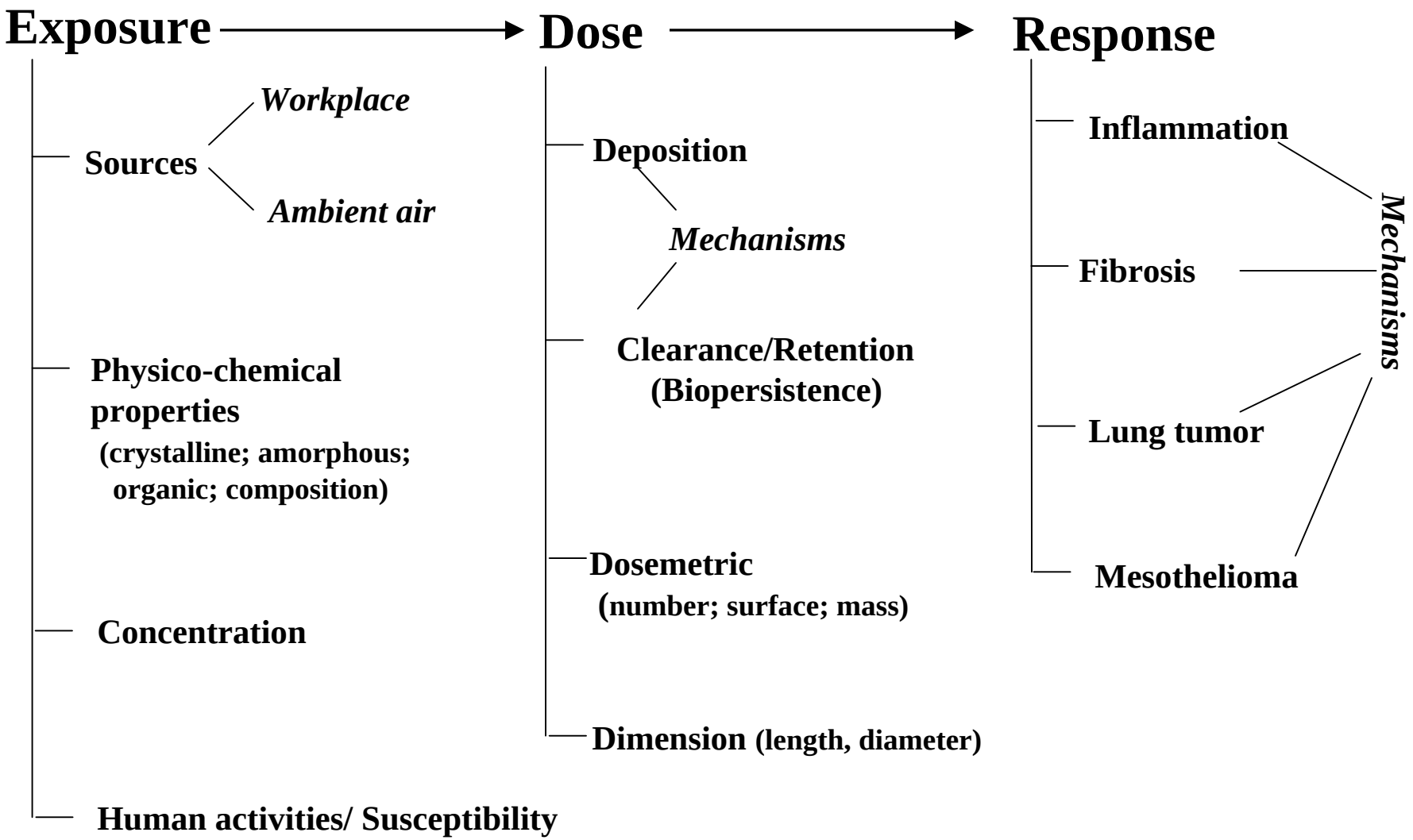
Dr. Oberdörster's Post-Meeting Comments

When responding to the charge questions in Topic Area 1 (physiological fate of asbestos and SVF fibers less than 5 micrometers in length), Dr. Oberdörster gave a brief presentation to the panel. He asked that a copy of the overheads from this presentation be included in this appendix of the report. A copy of the overheads Dr. Oberdörster prepared for the meeting follow, including some overheads that were not shown at the meeting due to time constraints.

Dr. Oberdörster also provided an additional comment not mentioned at the expert panel meeting. He noted that the panelists overlooked an important concept of short fiber toxicity which involves an increased retention in the lung of short fibers in people (e.g., smokers) who have disturbed alveolar macrophage mediated lung clearance. These people, he noted, can experience a marked increase in short fiber retention and thereby increase the potential for fiber toxicity significantly. Long fibers are reportedly not affected to the same degree as short fibers, as was described in a paper by Churg ("Effects of cigarette smoke on the clearance of short asbestos fibres from the lung and a comparison with the clearance of long asbestos fibres," International Journal of Experimental Pathology 73(3): 287-297, 1992).

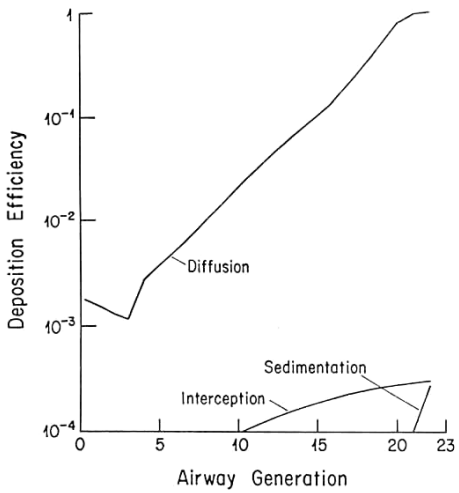
Airborne Fibers and Host Interactions



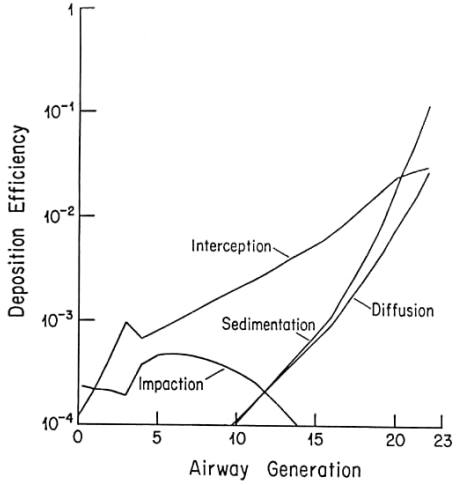
Main Deposition Mechanisms of Inhaled Fibers

- **Impaction** ***(abrupt directional changes)***
- **Sedimentation** ***(gravitational settling)***
- **Diffusion** ***(Brownian motion)***
- **Interception** ***(long fibers)***

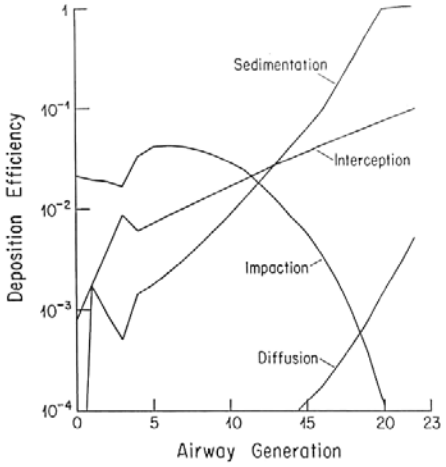
From: Asgharian and Yu, 1988



Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of 375 cm^3/sec for d_{em} = 0.01 μm and Unit Density.

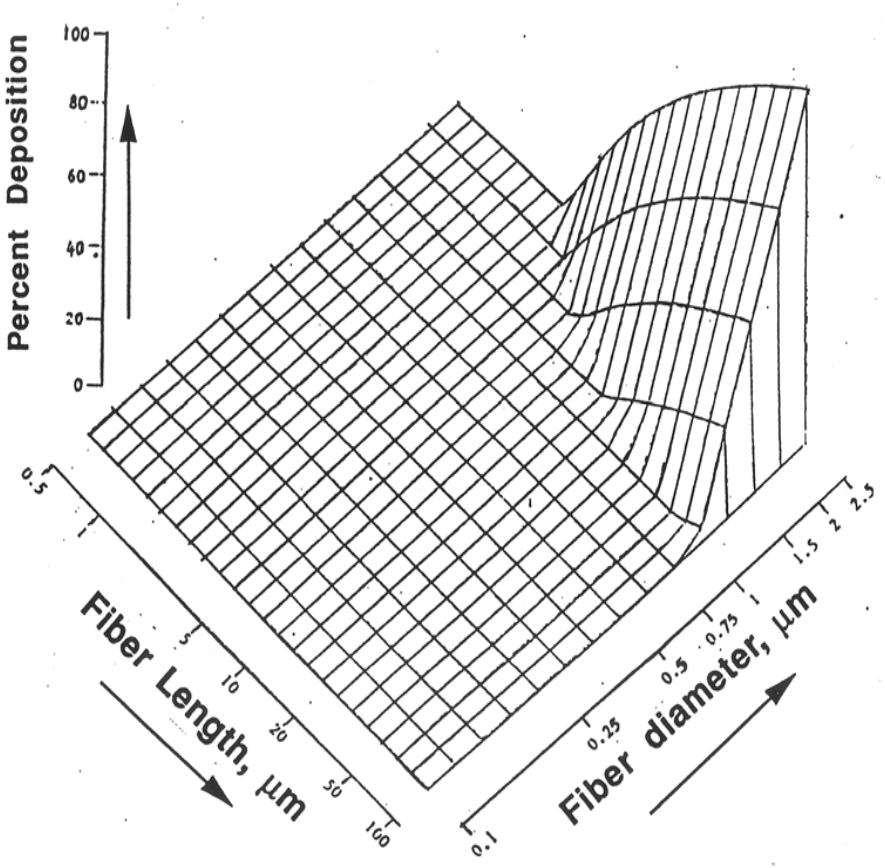


Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of 375 cm^3/sec for d_{em}= 1 μm and Unit Density.

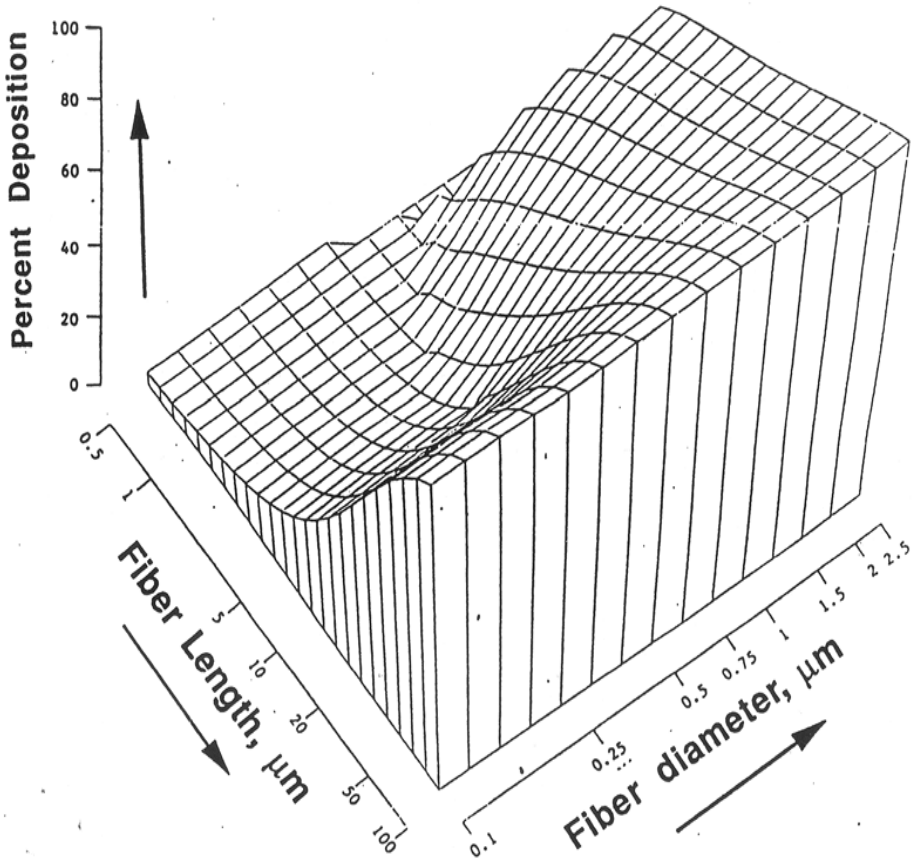


Deposition Efficiency of Fibrous in Different Generations of the Weibel Lung Model at a Flow Rate of 375 cm^3/sec for d_{em}=10 μm and Unit Density.

Predicted deposition of fibers in human extrathoracic airways
(after Yu, 1990)



Mouth-breathing



Nose-breathing

Effective filtration of long fibers by nose

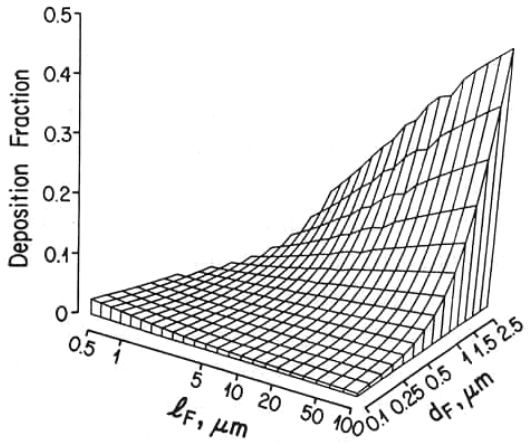


Fig. 3. Tracheobronchial deposition via trachea with no interceptional deposition at a lung volume of 10.78 cm^3, a tidal volume of 1.68 cm^3, a breathing frequency of 98 cycles min^{-1} and $\rho = 3.37\ g\,cm^{-3}$.

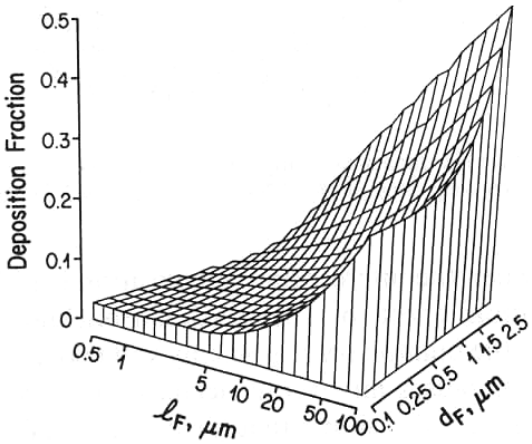
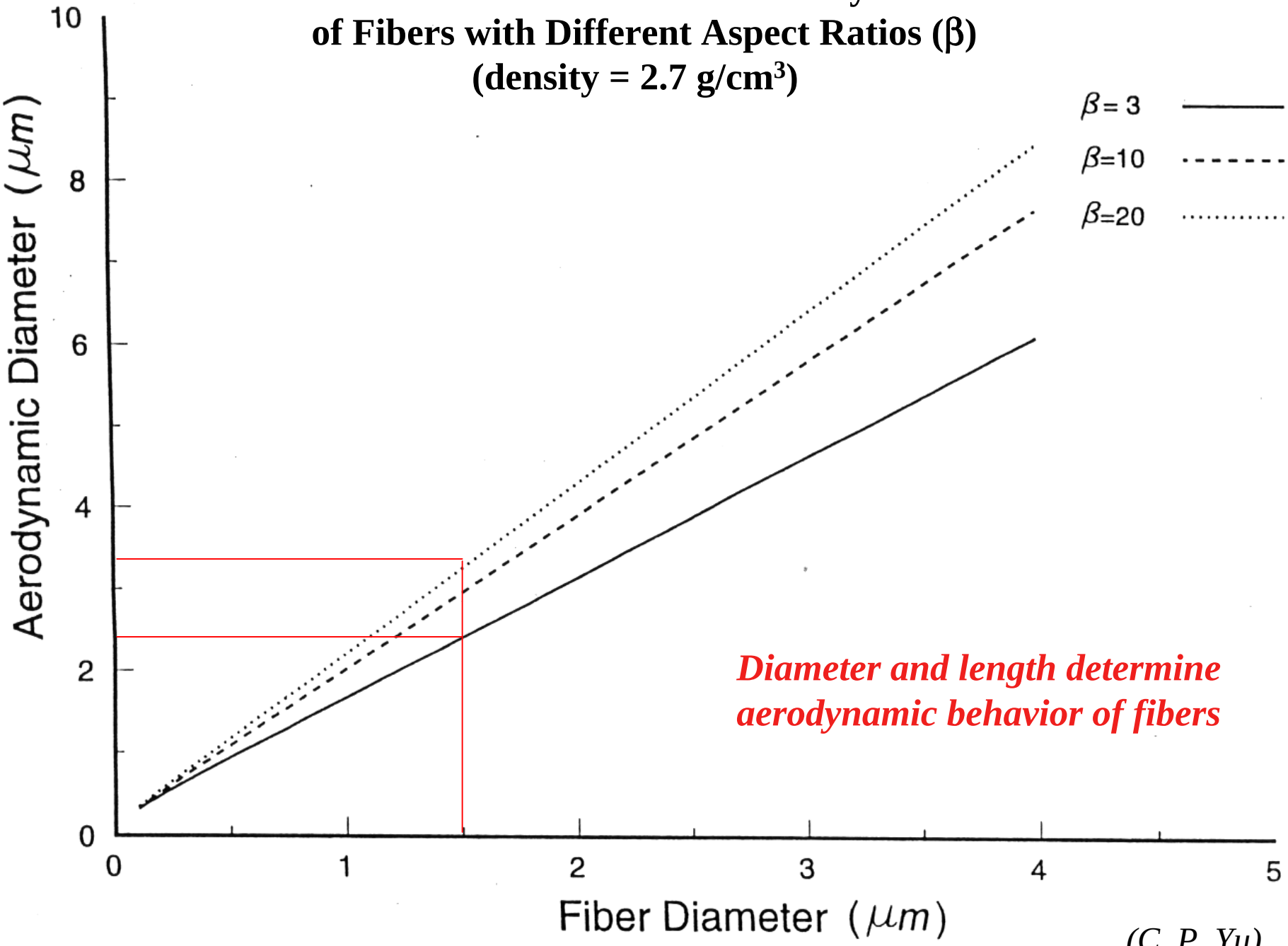


Fig. 4. Tracheobronchial deposition via trachea at a lung volume of 10.78 cm^3, a tidal volume of 1.68 cm^3, a breathing frequency of 98 cycles min^{-1} and $\rho = 3.37\ g\,cm^{-3}$.

Correlation between Geometric and Aerodynamic Diameter of Fibers with Different Aspect Ratios (β) (density = 2.7 g/cm^3)

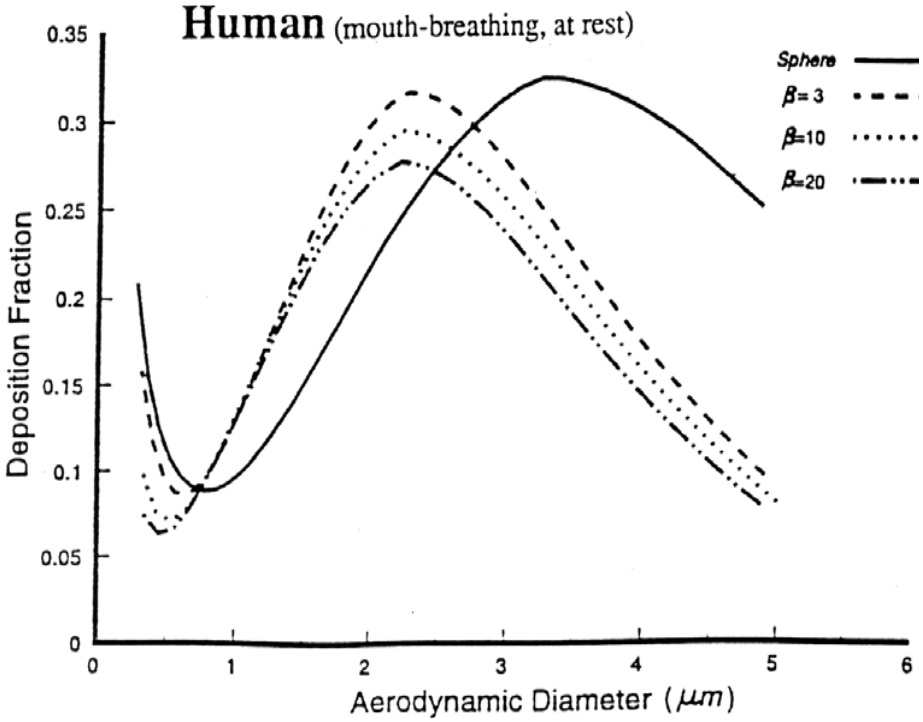


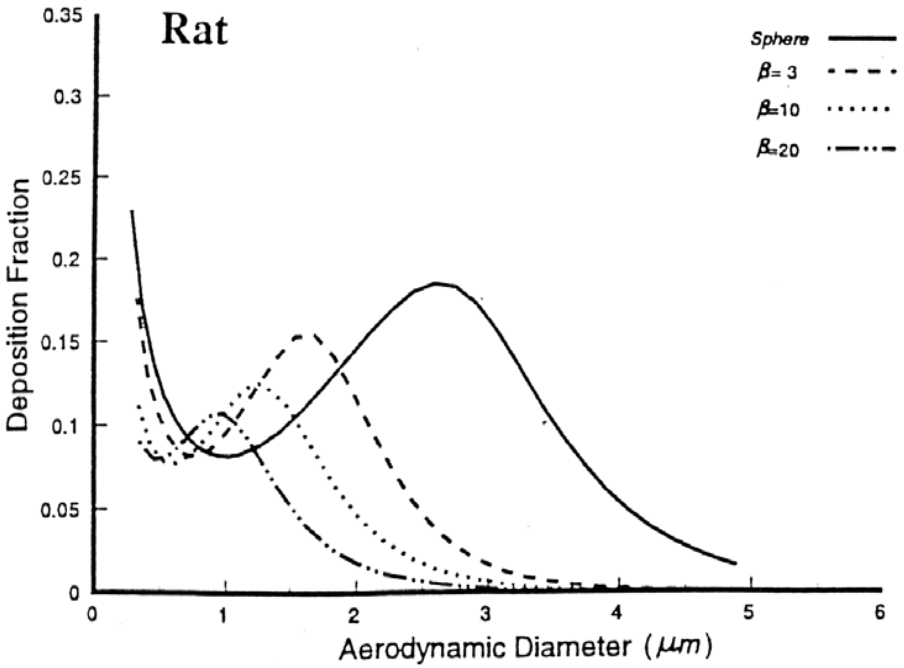
Diameter and length determine aerodynamic behavior of fibers

(C. P. Yu)

Respirability of Inhaled Fibers

Deposition in Alveolar Region





(C. P. Yu)

Long fibers are poorly respirable in rats

Retained Dose = Deposited Dose - Amount Cleared

(Retention = Deposition - Clearance)

<u>Physiological Clearance Mechanisms of Deposited Fibers</u>

— **mucociliary movement** *(nose; tracheobronchial region)*

— **alveolar macrophages*** *(size limitation)*

— **interstitial translocation** *(pleura)*

— **lymphatic clearance** *(size limitation)*

.....

***In addition*:** **Clearance is determined by fiber specific physicochemical processes**

Together, these mechanisms define the <u>Biopersistence</u> of a Fiber

****normal AM-mediated clearance:*** $T_{1/2}$ ***rat ~70 days***
$T_{1/2}$ ***human ~400 - 700 days***

Pathogenicity and Fiber Length:

The Role of Alveolar Macrophage (AM) Size

Hypothesis: **Phagocytizable fibers** → **efficient clearance and prevention of target cell interaction**

Average AM Diameters:

Rat:	**10.5 – 13 µm**	*Crapo et al., 1983; Lum et al., 1983, Stone et al., 1992;*
Human:	**14 - 21 µm**	*Sebring and Lehnert, 1992; Krombach et al., 1997*

For cancer → **number of fibers longer than 20 µm**

For non-cancer → **all fibers (but: also impact for tumors!)**

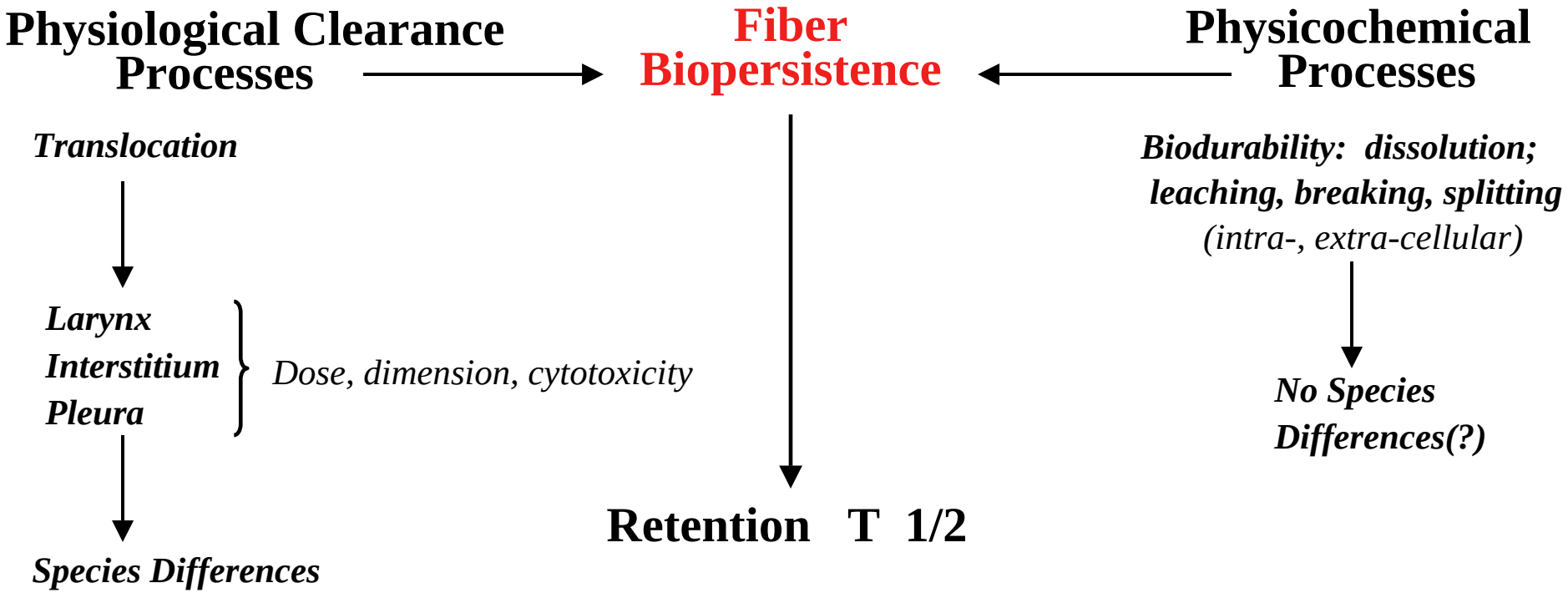
Size Dependent Lymphatic Clearance of Amosite Asbestos

Study: Intrabronchial instillation of amosite in dogs, followed by

Analysis of mediastinal lymph node and of lymph collected from Right Lymph Duct

	Max. Diameter	***Max. Length***
Lymph node	**0.5 µm**	**16 µm**
Post-nodal lymph	**0.5 µm**	**9 µm**

(Oberdörster et al., 1988)

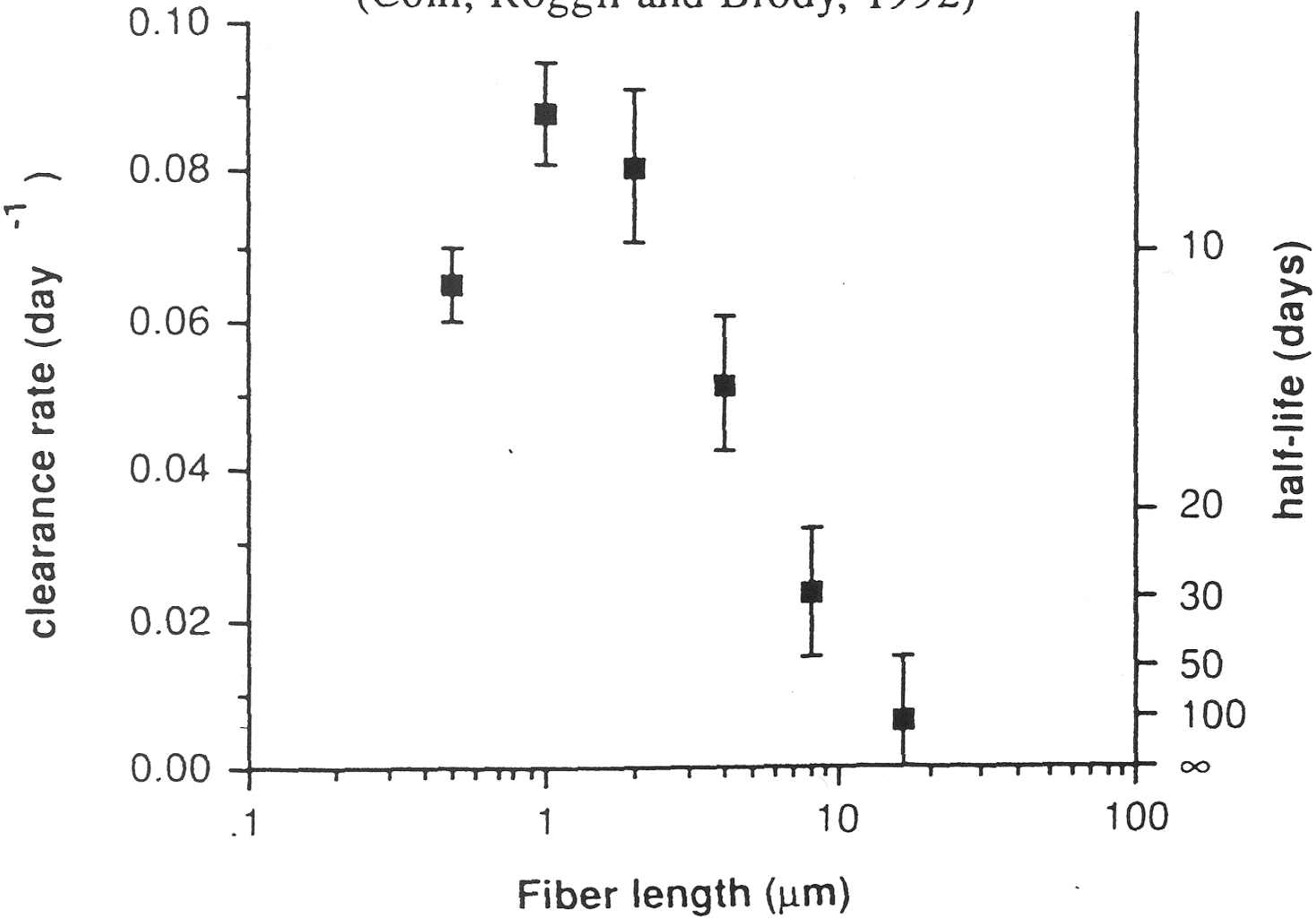


Biopersistence = Biodurability + Physiological Clearance

(Oberdörster, 1996)

Clearance of Chrysotile from Rat Lung

(Coin, Roggli and Brody, 1992)



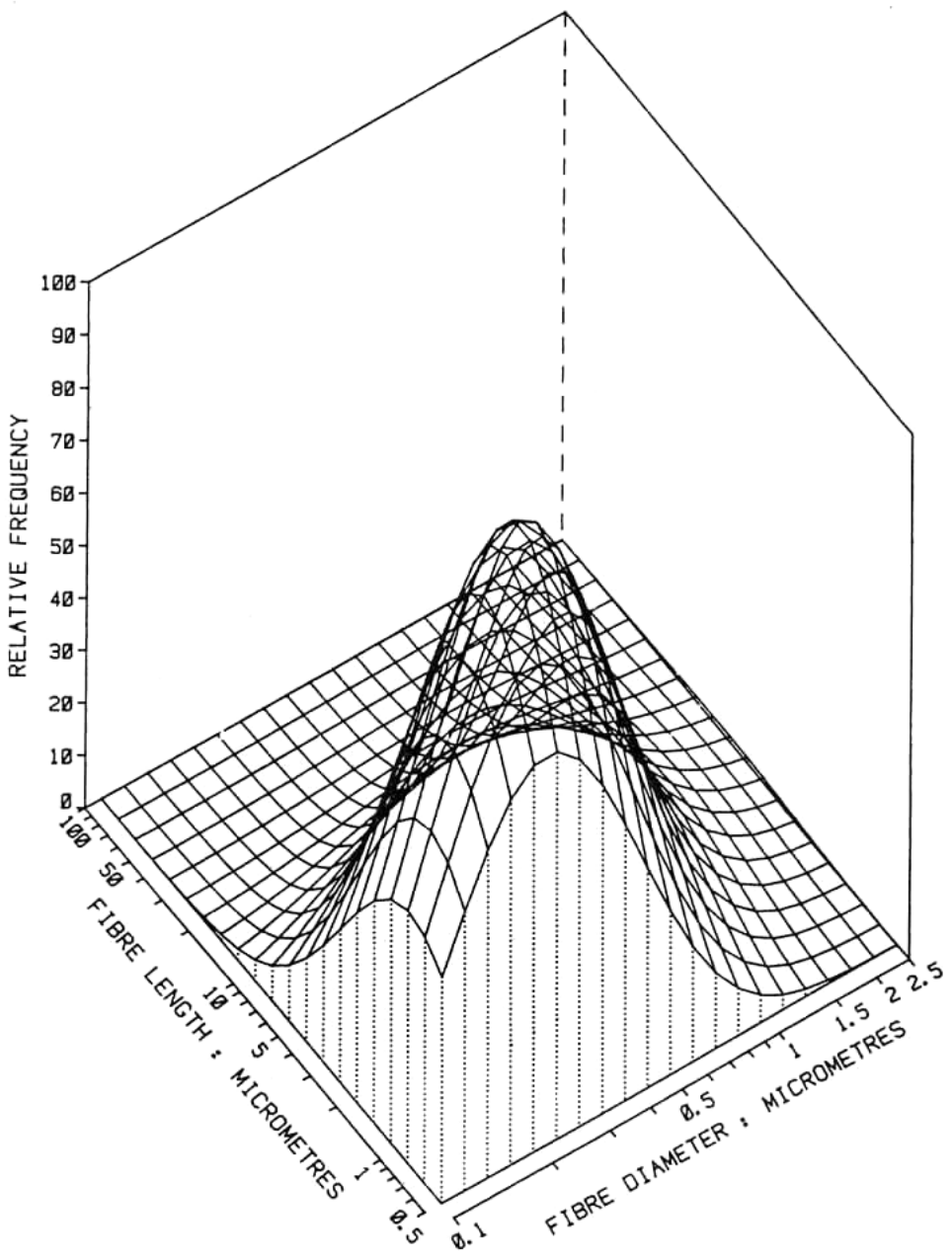


FIG. 8. Bivariate distribution of fibres in Paakkila bagging section.

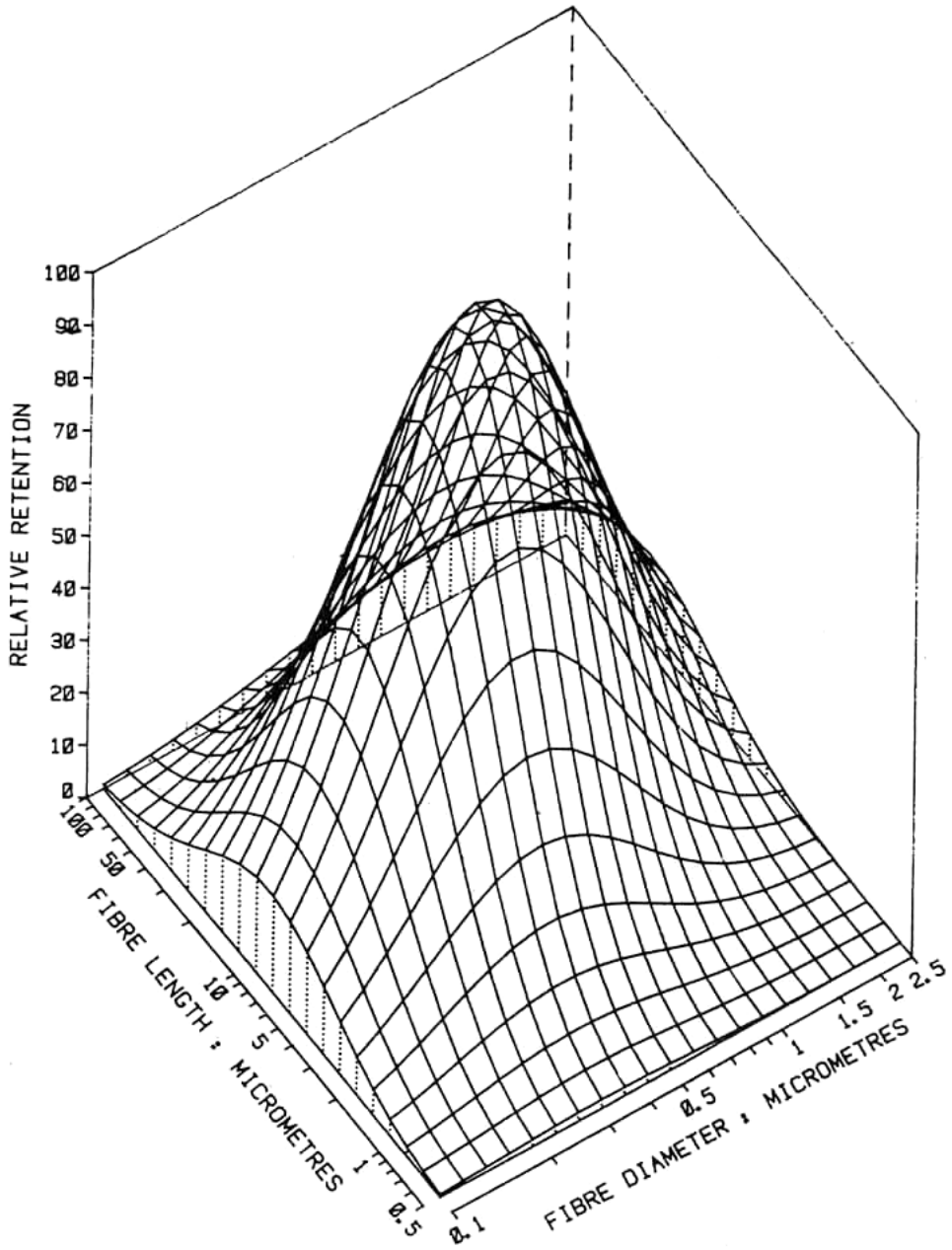


FIG. 10. Bivariate presentation of fibre retention.

Gelzleichter et al., 1996

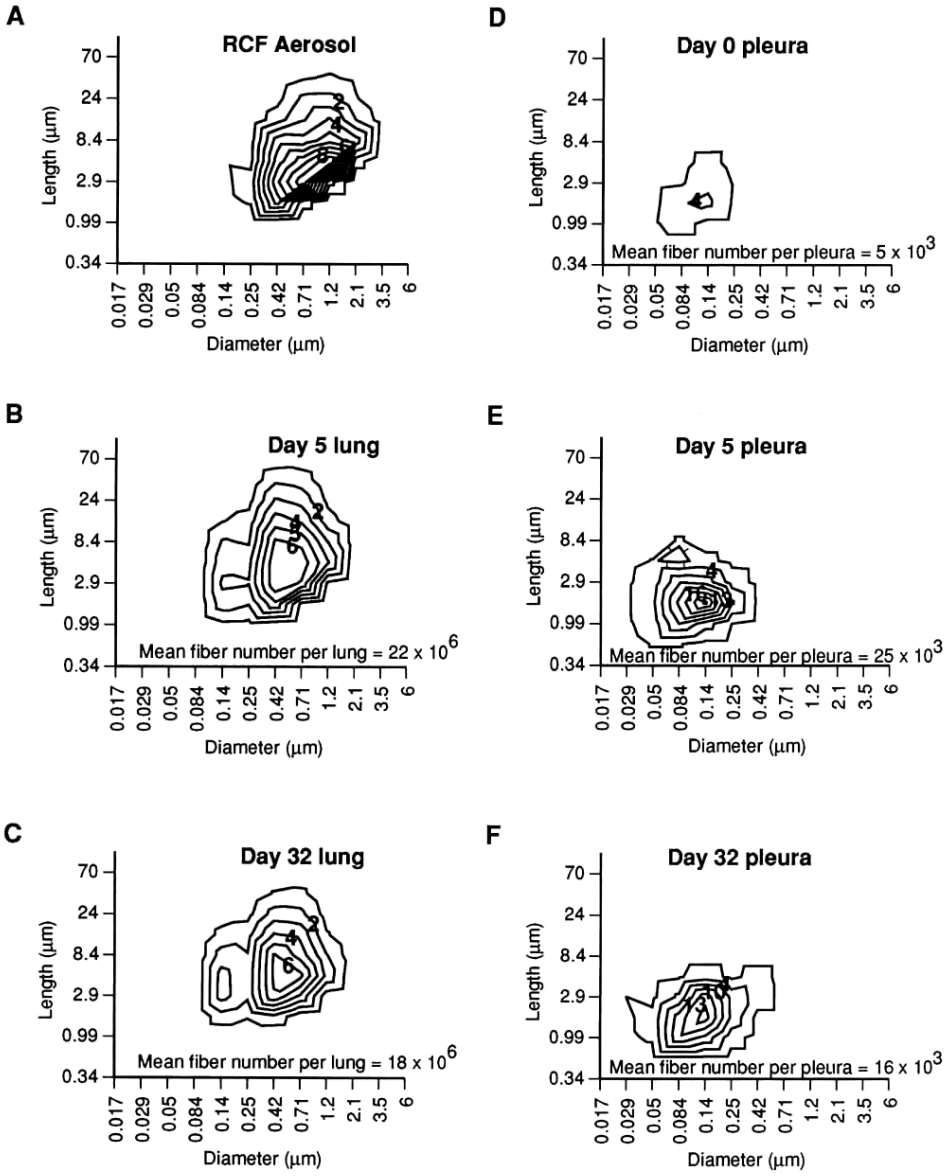


FIG. 2. Relative size distribution of fibers isolated from (A) aerosol, (B) Day 5 lung, (C) Day 32 lung, (D) Day 0 pleura, (E) Day 5 pleura, and (F) Day 32 pleura. Isobars were calculated based on histogram analysis of the aerosol cloud and fiber burden data (average of six rats). Median bin values are shown on the *x*- and *y*-axes. Pulmonary and pleural fiber burdens were normalized to Day 5 data with areas under the curve equal to 100 on Day 5.

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

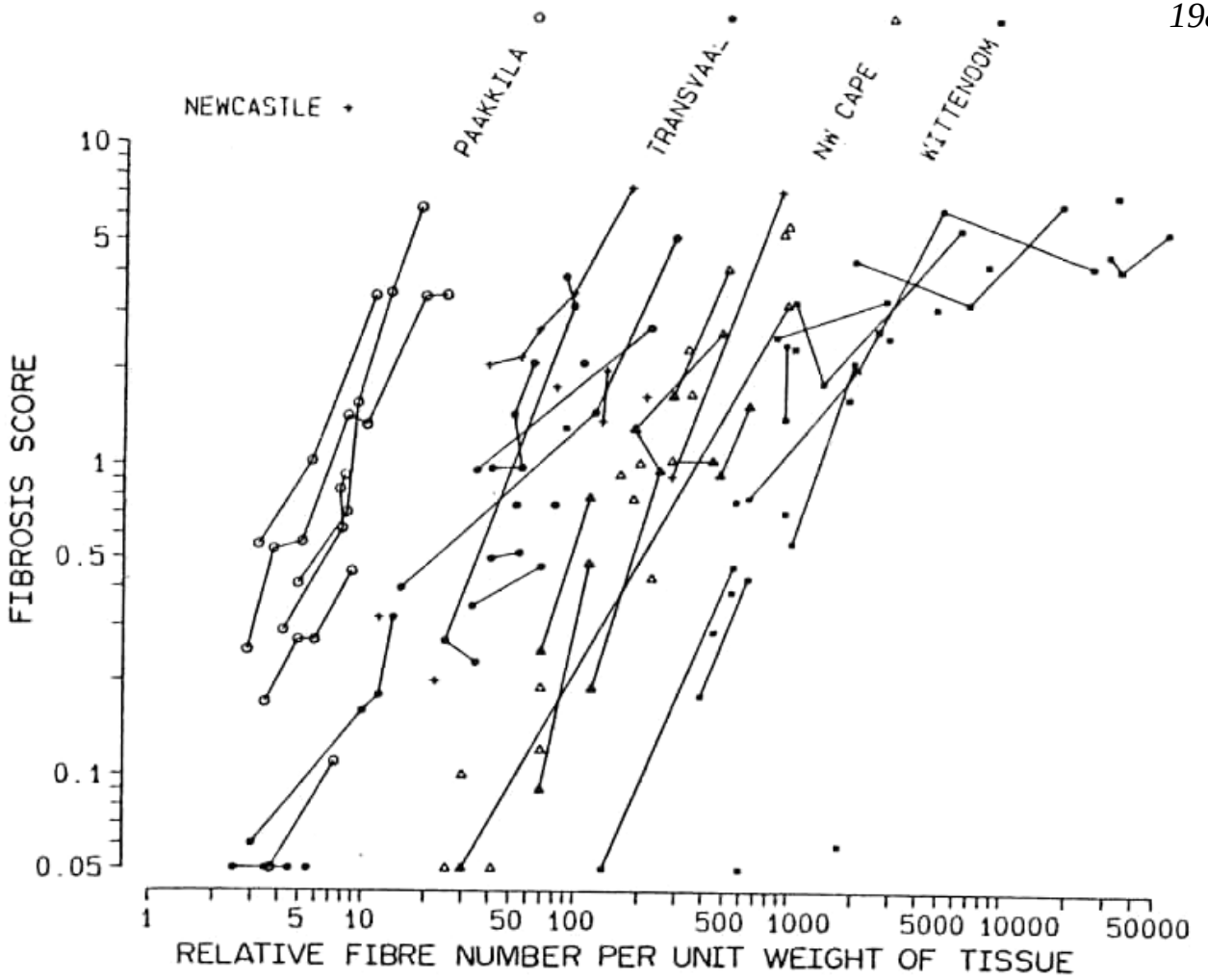


FIG. 5. Relationships when the parameter of fibre quantity is number.

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

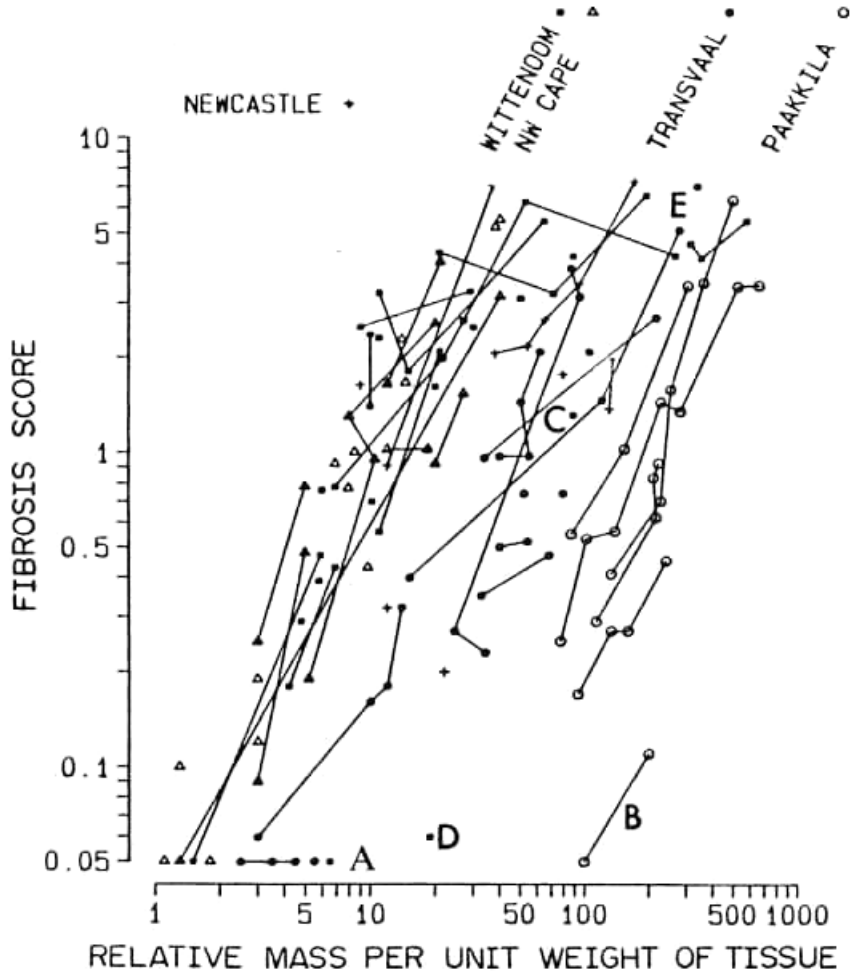


FIG. 3. Relationships when the parameter of fibre quantity is mass. Linked data points relate to tissue specimens from the same subject.

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

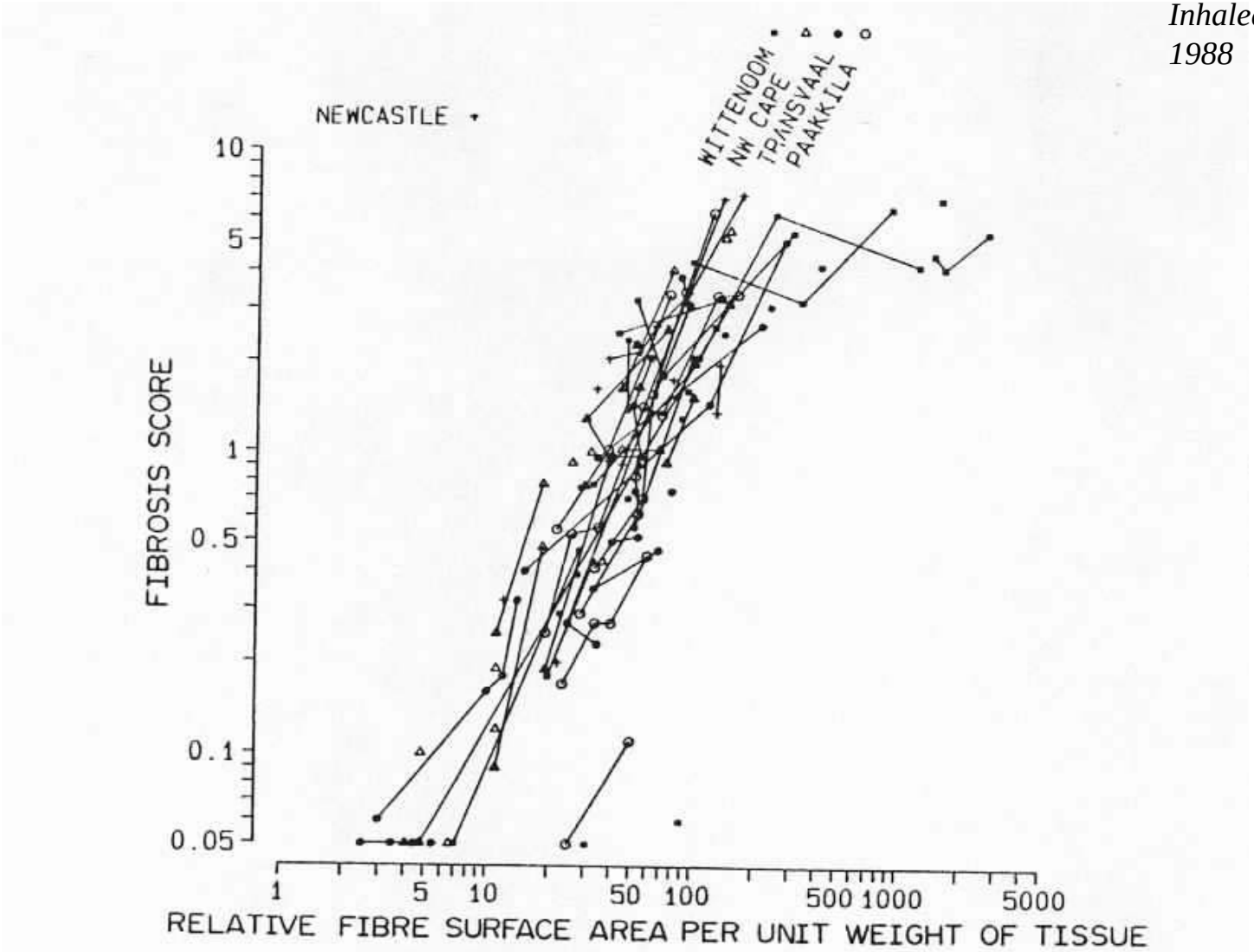
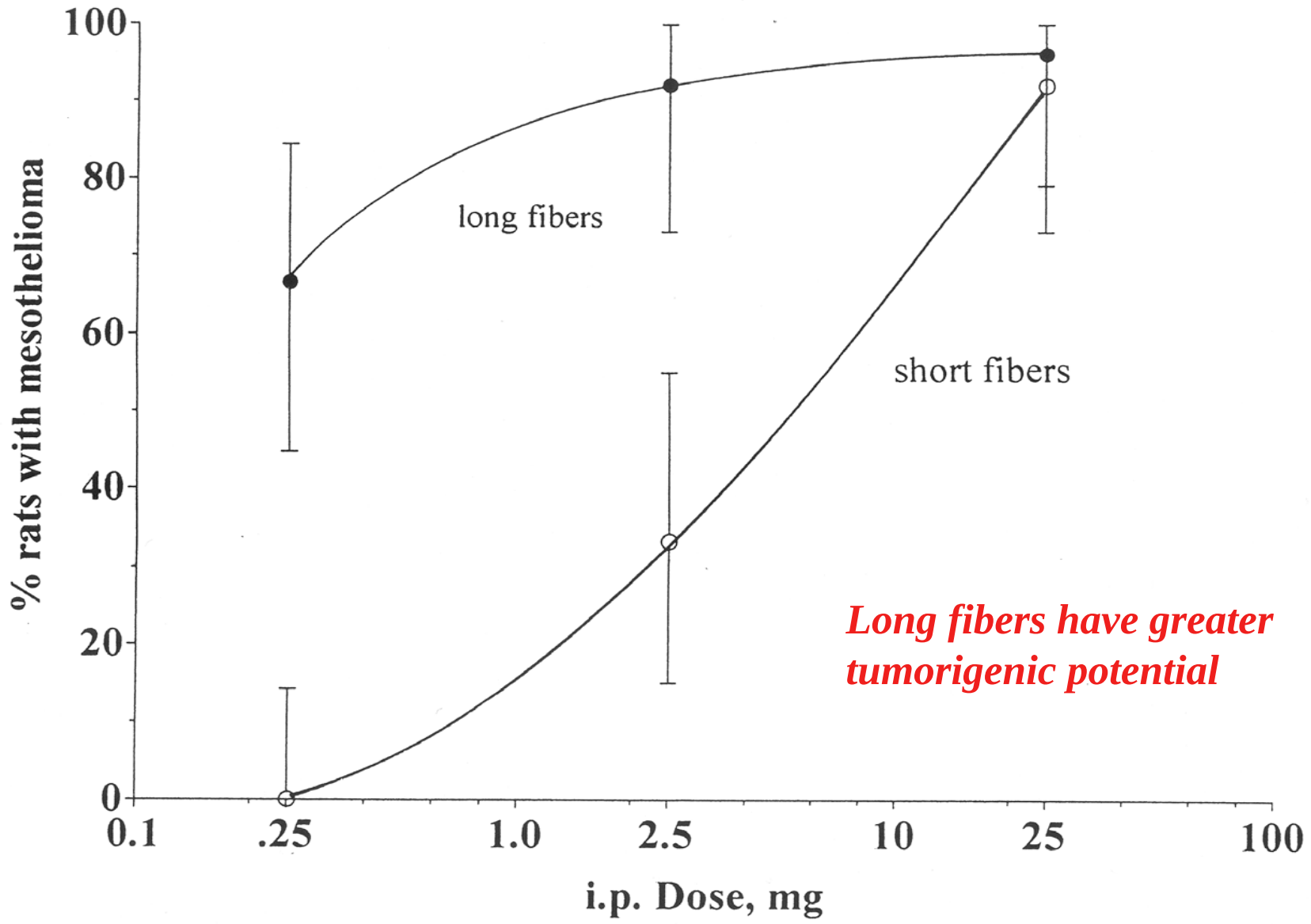


FIG. 4. Relationships when the parameter of fibre quantity is surface area.

Peritoneal mesothelioma After I.P. Injection of Long and Short Chrysotile

(mean and 95% CI) (Davis and Jones, 1988)



Long fibers have greater tumorigenic potential

Mixed Dust Exposures in Rats *(Davis et al., 1991)*

Chrysotile or amosite (10 mg/m^3) plus TiO_2 (10 mg/m^3) or quartz (2 mg/m^3), 1-year rat inhalation study plus 2-year observation period

	Fiber retention	Pulm. fibrosis	Transport across visc. pleura	Lung tumors	Mesothelioma	Survival rate
Chrysotile + TiO_2	↑	○	?	↑	↑[a]	↑
Amosite + TiO_2	○	○	↑	↑	↑	↑
Chrysotile + quartz	↓	↑	?	↑	↑[a,b]	↓
Amosite + quartz	○	↑	↑	↑	↑[b]	↓

○ *no change;* ↑ *increased;* ↓ *decreased; (compared to asbestos alone)*

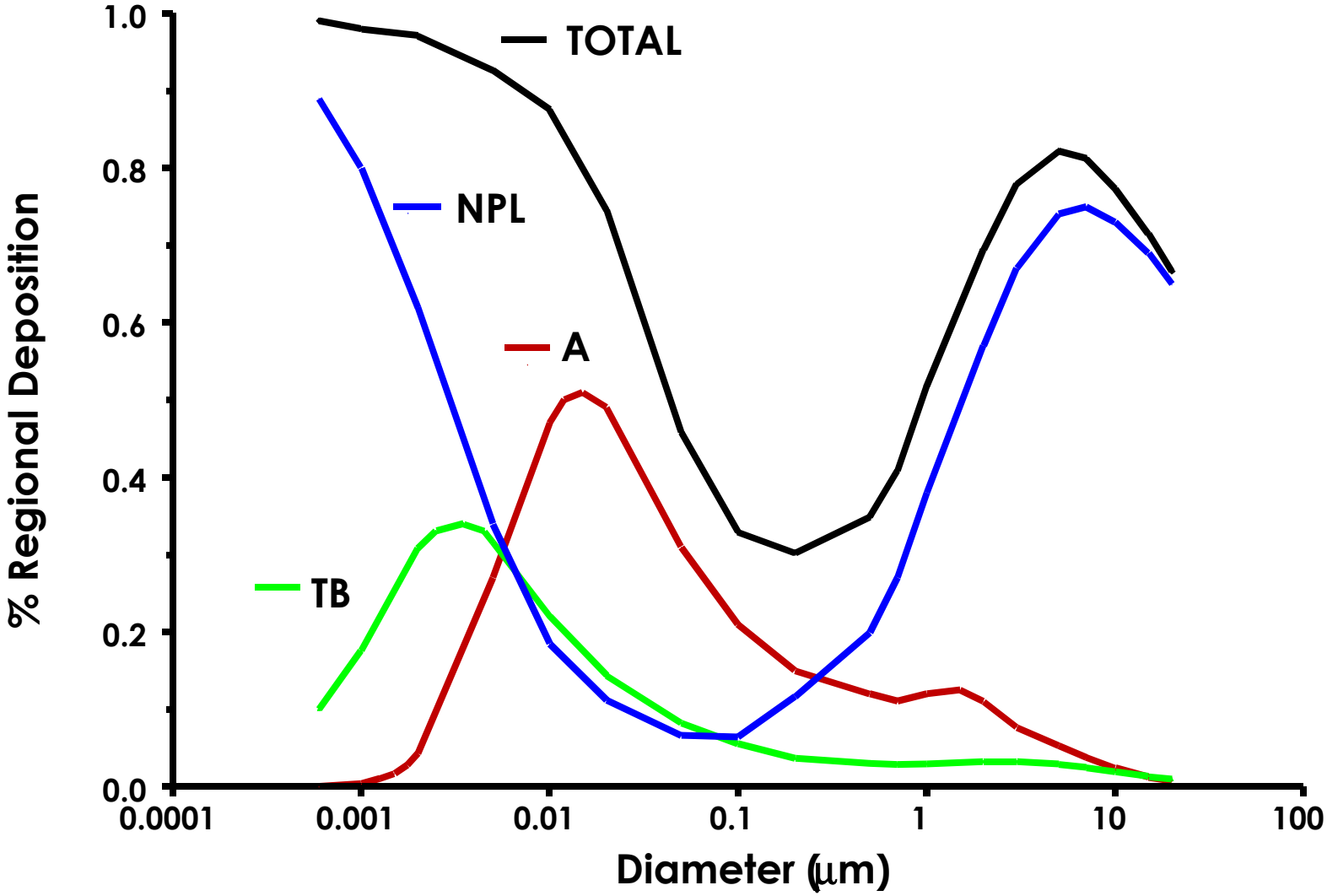
*(predicted lung burden of TiO_2 in "**overload**" range, ~10 mg/lung)*

[a] = *no mesothelioma with chrysotile alone*

[b] = *greater effect of added quartz than of added TiO_2*

Fractional Deposition of Inhaled Particles in the Human Respiratory Tract

(ICRP Model, 1994; Nose Breathing)



A = Alveolar; TB = Tracheobronchial; NPL = Nasal, Pharyngeal, Laryngeal

Classification

Natural Fibers

Asbestos	*Asbestiform*
Serpentine	Fibrous clays
chrysotile	polygorskite (attapulgite)
	sepiolite
Amphiboles	Other Fibrous Silicates
actinolite	wollastonite
amosite	nemalite (fibrous brucite)
anthophyllite	talc
crocidolite	zeolites: mordenite
tremolite	erionite

Man-made Fibers

Vitreous	*Crystalline*	*Organic*
Glass wool	Alumina	Para-aramic
Glass filaments	Graphite	Cellulose
Rockwool	Potassium titanate	Carbon
Slagwool	Silicon carbide	
Ceramic fibers	Sodium aluminum carbonate	
	Synthetic zeolites	

Mechanisms of Fiber Carcinogenesis

(Kane, 1996)

1. **<u>Fibers generate free radicals that damage DNA</u>**
   - *direct generation of ROS and damage of DNA in cell free system*
   - *catalyse oxidation of PAH to free radical*
   - *activate phagocytes to release ROS (esp. long fibers)*

2. **Fibers interfere physically with mitosis**
   - *in vitro studies (high doses!), phagocytosis by target cells, interference of long fibers with mitotic spindle and chromosome migration*

3. **Fibers stimulate proliferation of target cells**
   - *compensatory cell proliferation*
   - *stimulation of intracellular signal transduction pathways*
   - *direct mitogenesis (proto-oncogene expression)*
   - *induction of growth factors and growth factor receptor expression*

4. **Fibers provoke chronic inflammation and release of ROS, cytokines, and growth factors**
   - *tissue injury, cell proliferation, inflammatory cells (neutrophils)*
   - *link between sustained inflammation, fibrosis, cancer?*

5. **Fibers act as co-carcinogens or carriers of chemical carcinogens to target cells**
   - *synergism between cigarette smoke and asbestos exposure*
   - *interaction between fibrosis and non-fibrous dusts*

Dr. Wallace's Post-Meeting Comments

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

I participated in the Agency for Toxic Substances and Disease Registry (ATSDR) expert panel on "Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length", held in New York City on October 29-30, 2002. I limited my comments to one of the topics which ATSDR requested that the panel consider: "Topic #2: Health Effects of Asbestos and Vitreous Fibers less than 5 micrometers in length." My research background has involved some studies of the surface properties and associated toxicities of respirable silica and silicate particulate dusts, which may have some indirect relevance to one of the questions asked of the panel under Topic #2, specifically: "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?"

Dr. Ralph Zumwalde of NIOSH, who has an extensive background in the epidemiology of fiber-associated diseases, attended the proceedings as an observer and contributed information and recommendations concerning the availability of data and the analyses of epidemiology studies of occupational exposures to fibers.

In this review and revision of my comments on the panel, I also comment on the question: "Is there indirect evidence for less-than-5 micron fiber induced adverse health effects?" because of its association with the question of mechanism and because of some reports of inverse correlations of fiber length with fibrosis seen in asbestos workers' lungs.

As discussed in the following review, my evaluation of information presented and commentary made by and to the panel is that there is a need for focused and short-term research on short fiber hazard; and that there are new opportunities for the design of that research.

Question: 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?

A. Some lessons from non-fibrous particulate studies

1. Non-fibrous crystalline silica is cytotoxic, fibrogenic and carcinogenic.

Respirable crystalline silica particles, which are non-fibrous by any definition, are strongly pathogenic for fibrotic lung disease, and IARC, the US EPA, and others have recently evaluated quartz and cristobalite, two crystalline silica polymorphs, to be carcinogenic (1).

Exposure to these crystalline silica dusts can directly damage cells. Research suggests that consequent to this damage, there can be intrarcellular generation of reactive oxygen species and a cascade of events similar to the those evoked by asbestos fiber (2,3). As depicted by Dr. Mossman and others, that sequence may lead to the synthesis and release of TNF-alpha or other cytokines which stimulate near-by fibroblasts to proliferate and to up-regulate their synthesis and secretion of procollagen into the extracellular space of the pulmonary interstitium. There the procollagen matures into one or several forms of collagen fibers causing simple or progressive lung fibrosis.

The initial damage by quartz dust and by cristobalite dust to cells in vitro has been shown to be associated with the presence of silanols, hydroxyl groups on the crystalline silica surface. Bolasitis et al. (4,5) showed that calcining, e.g., heating, quartz resulted in the loss of surface silanols and a parallel loss of direct membranolytic cell damaging activity. As the dust aged in normal humidity air, the silanols re-formed on the surface over a period of days, and toxicity was restored parallel to that restoration. Saffiotti et al. (6) observed similar behavior with cristobalite. In some circumstances, e.g., sand-blasting occupational exposures, highly reactive free radical species are formed on the freshly broken crystalline silica surface; these exhibit heightened toxicity to cells in vitro in the absence of materials which can react to quench that activity, and may provide a additional mechanism of heightened toxicity (7).

2. Mineral-specific fibrogenicity: Short-term in vitro bioassays for mineral particles do not work

Some silicate dusts are cytotoxic in vitro but are not strongly pathogenic in vivo. Clays, layered alumino-silicates, are not associated with strong fibrogenic activity in human workplace exposures or in animal model exposure studies (8). In particular, respirable-sized kaolin clay dust, perhaps the structurally simplest alumino-silicate clay, is comparable to respirable-sized quartz dust for in vitro cytotoxicity (9) as measured by short term assays of cell damage, e.g., membranolysis, cytosolic or lysosomal enzyme release, or dye-exclusion measures of cell viability. Therefore, direct short-term in vitro cellular assays do not distinguish the distinct in vivo fibrogenic potentials of quartz versus kaolin clay dusts. Because of this, the general prevalence of clays in many mixed dust exposures prevents the use of short-term in vitro cytotoxicity systems to predict dust hazard.

3. The first events in particle or fiber interaction with the deep lung surface:

An important but generally ignored component for physiologically-representative in vitro bioassays:

a. The environmental interface of the deep lung is surfactant-coated

Particles or fibers depositing in the deep lung respiratory bronchioles or pulmonary alveoli will first contact the aqueous “hypophase” lining on the terminal airway and airsac surfaces. This thin layer is coated at the air-liquid interface with surfactant which acts to reduce the surface tension and physically stabilize the airspaces (10). The hypophase layer is also rich with micellar

dispersion of surfactant. The surfactant is comprised principally of lipids and lipoproteins. The major constituents are phospholipids: diacyl phosphatidylcholines. Dipalmitoyl phosphatidyl choline (DPPC) dispersed in physiological saline provides perhaps the simplest model of lung surfactant, representing the major surfactant constituent and generally reproducing the surface tension-lowering characteristics of full lung surfactant.

b. toxic particles adsorb surfactant and are promptly neutralized

Both quartz and kaolin clay dust particles promptly adsorb DPPC surfactant from dispersion in physiological saline; this immediately coats the particle surfaces and prophylactically extinguishes their short-term cytotoxicity (12). The amounts of surfactant in the alveolar hypophase compared to the surface areas of respirable mineral dusts and their adsorption isotherms for DPPC suggest that there is adequate surfactant in the lung to coat and neutralize depositing particles even in most high dust exposures (13).

c. Restoration of particle toxicity and a possible basis for mineral-specific fibrogenicity

Subsequent to the suppression by pulmonary surfactant of otherwise prompt cytotoxic activity, the surfactant-coated particles can be phagocytized by macrophages and subjected to phagolysosomal enzymatic digestion (14). Cell-free experiments have correlated the digestive removal of DPPC from quartz and kaolin particle surfaces by phospholipase A2 enzyme with the restoration of membranolytic activity. In cell-free tests using pH -neutral acting phospholipase A2 and in limited in vitro/in vivo tests, quartz is stripped of surfactant significantly more rapidly that kaolin(15). Cellular in vitro studies have found that macrophage-like cells in vitro digest quartz- and kaolin-adsorbed DPPC at comparable rates over a period of about 7 to 10 days with initial partial restoration starting at 3 to 5 days (16). It has not been demonstrated that this de-toxification/re-toxification process is the mechanism distinguishing quartz and alumino-silicate expression of toxicity in vivo.

4. Site of particulate-induced fibrogenic activity

Churg et al. (17) 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(18). 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 findings might be considered in researching the site of asbestos action for fibrosis.

5. Possible interferences in short-term bioassays

Oberdörster and others (19) have found that the conventional protocol for extended-term in vitro cellular assays may inadvertently cause a non-physiologic surface conditioning of mineral particles which significantly affects assay results. The use of fetal bovine serum can confer a prophylaxis on silica and perhaps on kaolin (20), probably due to the mineral surface adsorption of lipo-proteins from the FBS. That may not represent a physiological situation in the intact lung in vivo and may interfere with attempts to model the condition of particle surfaces upon deposition in the lung and resultant effects on their expression of toxicity in vivo. For purposes of in vitro investigation of fiber or particle toxicity, this interference might be circumvented, e.g., by excluding serum from the medium during a short-term period for particle or fiber challenge.

6. Environmental conditioning of particle surfaces can affect their in vivo pathogenic activity

Even animal model in vivo tests can fail to be predictive in the case of a cytotoxic and fibrogenic mineral in mixed composition dusts, e.g., quartz particles in workplace dusts: conventional mineralogical and cytotoxicity assays may not correlate with short-or intermediate-term in vivo fibrogenic response. Alumino-silicate surface contamination of quartz particle surfaces can delay for months or perhaps years the expression of fibrogenic activity. Aluminosilicate or other mineral occlusion of the underlying host particle can alter the expression of toxicity in vivo during the bio-persistence of the surface contamination. This has been seen worldwide in anomalies in the fibrogenicity of coal mine dust exposures (21). This was clearly demonstrated by LeBouffant et al. (22) by in vitro and in vivo studies of the fibrogenicity of silica in coal mine dusts and in natural lightly contaminated sands. More recently, new spectroscopic surface analysis methods have demonstrated natural clay occlusion of quartz dusts from some workplace where epidemiology studies had detailed anomalies in disease risk correlation with conventional measures of dust exposure (23).

B. Fibrous mineral and crystalline silica particle differences and similarities

1. Mechanisms of toxicity for fibrous and non-fibrous materials

a. Conventional assays do not clarify the bases of asbestos or silica particle toxicity

Churg et al. (17) review highlights and caveats to the general models of asbestos activity. Some fibers can evoke the responses from ROS generation through the cascade to increased 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 a potent as amphibole. Churg et al. suggest a comparison of asbestos and silica-induced fibrosis data. Their 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. Their conclusion

is that the tabulated responses fail to explain comprehensively how asbestosis or silicosis develop.

b. Surfactant does not fully suppress all asbestos fiber in vitro cytotoxicity

Asbsetos fiber as well as particulate silicate can adsorb the DPPC and components of pulmonary surfactant (24). We have briefly researched the effect of surfactant adsorption on chrysotile in vitro genotoxicity, using an assay for micronucleus induction in cultured Chinese hamster lung cells (V79 cells) (25): in our test of two chrysotile asbestos fiber samples, pre-treatment with DPPC in physiological saline surrogate lung surfactant did not fully suppress a short-term toxic activity to cells in vitro. NIEHS intermediate length chrysotile asbestos fiber (average 101 micrometer length, 65% > 10 micron) and NIEHS short chrysotile asbestos fiber (average 11.6 micron, 98% < 10 micron) were tested for micronucleus induction in V79 macrophage-derived cells for 72 hour challenge +/- DPPC surfactant pre-treatment of the fibers. For the longer fiber sample, DPPC did not significantly affect the activity, a numerical reduction of about 20% in the activity was observed but was not statistically significant. However, DPPC treatment reduced the shorter-length fiber sample activity significantly, to about half that of the untreated shorter fiber sample. Similar effects were seen for multi-nuclei induction and for dye-exclusion viability measure for cell toxicity. No activity was seen for either sample in a sister chromatid exchange assay.

c. A surface modification which did not affect long asbestos fiber toxicity in vitro

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 (26). The NIEHS intermediate length chrysotile asbestos fiber used above was mildly acid leached to remove near-surface magnesium, but to retain fiber length. The treatment resulted in a 20% reduction in fiber length in each of three length categories: <3 micron, 3-10 micron, > 10 micron. Spectroscopic surface analysis and zeta-potential measurements showed significant reduction in surface-associated magnesium and in its influence on surface chemistry. However there was no significant change in measured activity for micronucleus induction between the treated and non-treated fibers.

d. Is there more than one mechanism of fiber cytotoxicity? Do short fibers also act as particles?

One interpretation of these two experiments is this: at least two mechanisms are involved in the initial damage or interaction of fibrous particles with the lung: a component which is at least transiently suppressed by surfactant conditioning which significantly contributes to shorter fiber activity, and a component which is not suppressed by surfactant conditioning, and which is not affected by one significant modification of surface composition and chemistry, and which is the principal mechanism for longer fibers. That is, a model which suggests itself is the combination of the frequently discussed “frustrated phagocytosis” mechanism for longer fibers, e.g., those which are too long to be fully phagocytized and internalized by the cell target, and a surface

property-mediated toxicity mechanism for internalized particles or short fibers, i.e., fibers which are internalized and subjected to conventional phagolysosomal processes.

One possible consequence of "frustrated phagocytosis" of longer fibers 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. The paper by M Ohyama et al. (27) provided to the panel presents a difficult argument against frustrated phagocytosis: The study used luciginen-dependent chemiluminescence (CL) induced in vitro over a short (2 hour) period, and found a strong correlation of response indicative of superoxide release with fiber length 6 to 20 um. 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 um were only weakly active. Longer fiber activity correlated with length.

Research on the surfactant suppression and subsequent lysosomal enzymatic restoration of mineral particle cytotoxicity within a cell, suggests that short fibers which are fully taken into the cell in a phagosome may express, in part, a cytotoxicity within the cell after removal of adsorbed prophylactic surfactant. That is, some part of short fiber toxicity may be related to the mineral surface-specific mechanism of non-fibrous particulate toxicity.

Those do not exhaust the possible mechanisms for long or short fiber damage to cells. Asbestos fiber penetrating the cell or cell nucleus may exercise modes of direct genetic or epigenetic damage. In our above study of surfactant effects on chrysotile genotoxicity in vitro, a limited investigation using immunofluorescent kinetochore staining indicated that both clastogenic and aneuploidogenic effects were associated in similar proportion with the observed micronucleus induction. That is, fibers may directly or indirectly interact with the spindle mechanism involved in chromosomal separation during cell division. During mitosis, the nuclear membrane disintegrates, possibly providing intracellular fibers access to the genetic material or kinetochores and spindle apparatus.

2. Intracellular response to fiber challenge

a. Long fiber challenge

Whatever the mechanisms of direct fiber damage or stimulation of the cell surface, some components of the consequent intracellular response have been well-defined. Mossman and others have detailed the cascade of events following fiber challenge to pulmonary macrophages or perhaps to other cells. A recent review (3) explicates the central dogma that damage to or stimulation of the cell by fibers is followed by an increase in intracellular reactive oxygen species which trigger a cascade of transcription factor activation leading to the up-regulated production

and release of TNF-alpha or other cytokines. This also was recently the subject of a NIOSH study by Cheng et al. (28) in which 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-alpha production and NF-kB activation.in a dose-dependent manner. A titanium oxide control dust had no stimulatory effect on TNF-a secretion. The report by V Kinnula which was provided to the panel (29) 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 "long" fiber challenge of cells is agreed to be superoxide anion release in cells which have attempted to phagocytize fibers. 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. This fiber-prompted production and release of TNF-alpha can stimulate nearby pulmonary fibroblasts to proliferate and increase pro-collagen synthesis, which is released extra-cellularly to mature into collagen scarring.

b. Intracellular response to challenge by well-classified shorter fibers and particles

Dr. Baron of NIOSH has been developing a fiber size classifier (separator) which can permit in vitro or perhaps limited in vivo experiments with sets of fibers of fairly well-defined length (30). A dielectrophoretic classifier can separate fibers from an airstream producing about 1 mg/day of a size cut. These classes of JM-100 glass fibers were recently produced for in vitro toxicology study:

cut 1: Length = 32.7 micrometer +/- 23.5 SD; Width = 0.75 micrometer +/- 0.50 micrometer
cut 2: L = 16.7 u +/- 10.6 u; W = 0.49 u +/- 0.27 u
cut 3: L = 6.5 u +/- 2.7 u; W = 0.44 u +/- 0.22 u
cut 4: L = 4.3 u +/- 1.0 u; W = 0.40 +/- 0.15 u
cut 5: L = 3.0 u +/- 1.0 u; W = 0.35 u +/- 0.14 u

In recent NIOSH studies by Dr. Castranova and colleagues, these samples were used in a comparison of "long" and "short" fiber cytotoxicity and of induction of the cytokine cascade in vitro: Blake et al. (31) used 18 hour challenge of rat alveolar macrophages in vitro and the lactate dehydrogenase (LDH) release assay, the 17 micrometer sample expressed about 2 X the activity of the shorter samples (and also 2X the activity of the 33 micrometer longer sample) on a mass basis. However, all samples were active well above control levels. The 7 micrometer fiber set had about 8 X more fibers per gram than the 17 micrometer set, or about 3 X the linear surface area. Thus, the 17 micrometer long fibers were on the order of 6 or 7 X more cytotoxic than the shorter 7 micrometer fibers on a linear surface basis. Similar effects were seen with an assay using chemiluminescent response to zymosan challenge. And multiple macrophages were seen attached along the length of the long fibers, suggesting "frustrated" or incomplete phagocytosis was occurring for longer fibers.

J Ye et al.(32) challenged a mouse macrophage cell line with the 7 and with the 17 um glass fiber cuts, for 3, 6, and 16 h. Glass fibers stimulated TNF-alpha production, activation of TNF-alpha gene promoter activity, and activation of DNA binding activity of nuclear factor (NF)-kB. Reactive oxygen species (ROS) were involved in the activation and production. Dose was set at 5 fibers per cell; by that metric the longer fibers were more potent than short fibers by a factor of about 3. However, on a basis of length of fiber exposed to the cell or surface area, the activities were about equal for the long and short fibers. As seen in photomicrographs, short fibers but not long fibers were effectively engulfed by macrophages. In a subsequent study by Ye et al. (33) it was found that the long fibers were more potent than short fibers at the same dose of 5 fibers/cell in activating MAP kinases which activate transcription factor c-Jun which acts on the TNF-a gene promoter through the cyclic AMP response element and the AP-1 binding site. Again, the activities were comparable for long and short fibers on the basis of exposed fiber length or surface area.

Question: Is there indirect evidence for less-than-5 micron fiber induced adverse health effects?

A. Human studies

1. Churg et al. (34) found the grade of interstitial fibrosis asbestosis in the lungs of a group of chrysotile miners and millers to be directly proportional to tremolite or chrysotile fiber concentrations, but inversely proportional to mean fiber length and length-related parameters. Churg et al., (35) graded fibrosis in the lungs of some shipyard and insulation workers, finding fibrosis grade to be strongly positively correlated with amosite concentration and negatively correlated with mean fiber size parameters including fiber length; they suggested "...these observations again raise the possibility that short fibers may be more important than is commonly believed in the genesis of fibrosis in man." In a study of chrysotile miners and millers, Churg, et al., found pleural plaques were strongly associated with mean tremolite fiber aspect ratio, but no differences in mean fiber size, including length, were seen for any other disease studied (mesothelioma, airway fibrosis, asbestosis, or carcinoma) (36). One member brought to the panel's attention a recent publication (37) analyzing fibers in lung tissue from two groups of former chrysotile miners and millers: the study concluded that "...fiber dimension does not seem to be a factor that accounts for the difference in incidence of respiratory disease between the two groups". It has been generally speculated that shorter fibers in lung tissue may be the residue of fibers which were longer when deposited, and disease initiation was due to the originally long fibers, which were subjected to subsequent in vivo dissolution or degradation into the observed short fibers (17). This appears to be one plausible explanation of the inverse correlations reported between fibrosis and fiber length in human lungs. But this does not limit the research opportunity or imperative, provided by the seemingly anomalous or "counter-intuitive" results, to address possible short fiber-associated disease mechanisms.

2. A possible "short fiber" exposure cohort. Dr. Zumwalde of NIOSH suggested to the panel that a past NIOSH study of 2,302 workers at an attapulgite mining and milling facility (38) may have

involved exposures, in part, to short mineral fibers. A significant deficit of mortality (SMR = 43, 90% CI 23-76) from nonmalignant respiratory disease (NMRD) was observed for the cohort; but a statistically significant excess of mortality from lung cancer was observed among whites (SMR = 193, 90% CI 121-293), but a deficit occurred among nonwhites (SMR = 53, 90% CI 21-112). This may present an opportunity for review and re-analysis and a source for collection of materials for study. NIOSH also is re-analyzing archived materials available from a past study of asbestos workers in South Carolina.

Question: Are short fibers pathogenic? What should we do?

1. Review of in vitro toxicology

For non-fibrous particles:
- Non-fibrous mineral particles can be cytotoxic, fibrogenic, and carcinogenic.
- That pathogenicity is mineral-specific.
- Surface characteristics may delay expression of that pathogenic activity in vivo.
- That pathogenicity is not necessarily reflected in short-term in vitro cytotoxicity assays.
- The first interaction of particles depositing in the deep lung, namely, adsorption of the lung lining surfactant, strongly affects mineral particle prompt toxicity.
- The bio-persistence of that surfactant prophylaxis may be a critical factor in the timing and severity of mineral particle expression of toxicity.
- After expression of the primary toxic event in particle challenge to cells, the intracellular response may be much similar to the cascade induced by asbestos or fiber challenge: leading to the induction of pathogenic, e.g., fibrogenic activity by nearby cells.

For fibrous particles:
- Many studies have found an association of pulmonary fibrosis, cancer, and mesothelioma with occupational exposures to long fibers, e.g., fibers with length greater than the dimensions of the target cells.
- Long fibers clearly are cytotoxic in vitro.
- Long fiber cytotoxicity and the initiation of pathogenic processes are generally considered to be resultant from a "frustrated phagocytosis" mechanism.
- Some studies of fiber burden and disease in tissue from asbestos workers have shown an inverse correlation of disease with fiber length.
- Those disease-correlated shorter fibers appear in some of the cases to be mineral specific, e.g., associated with contaminant amphibole more than with seprentine asbestos.
- Limited in vitro study data suggest that shorter fibers may have a component of cytotoxicity which is surface associated, perhaps independent of a "frustrated phagocytosis" mechanism involved in long fiber toxicity.
- In short term in vitro assays, well-controlled for fiber length, shorter glass fibers can cause intracellular events comparable on a fiber or surface basis to those associated with longer fiber challenge and with asbestos fiber challenge.

2. Interpretability of short-term in vitro assays

The ability to interpret many in vitro experiments on particle of fiber toxicity is complicated by the lack of modeling of initial conditioning of particles in the lung and the time course of expression of toxicity in vivo. Surfactant adsorption in the lung can dramatically alter the short-term in vitro toxicities of mineral particles, and may determine if toxicity is expressed in times long or short compared to clearance.

This surfactant effect and associated delay in toxicity expression does not appear to be a factor for long fiber asbestos expression of in vitro toxicity. Whether this is a factor for short fibers is unknown. That is, if short fibers have a component of toxicity independent of a "frustrated phagocytosis" mechanism but dependent on a surface-property mechanism then such conditioning and time delays in expression of toxicity could be critical in the design of experiments for the detection and analysis of short fiber toxicity by in vitro or short-term in vivo assay.

3. Research opportunities

a. The ability to collect milligram quantities of well-classified (sized) small fibers presents the opportunity to do carefully size-controlled in vitro studies and possibly some (more limited) in vivo studies, e.g., by nose-only inhalation or tracheal instillation.

b. Epidemiology study results suggest types of fibers which should be compared and contrasted in such experiments, e.g., short tremolite vs. short chrysotile.

c. A review of epidemiological studies of attapulgite or other short fiber exposures may provide an identification of other short fiber asbestos and non-asbestos materials for toxicological study for which human disease epidemiology information is available for comparison.

d. Preceding the initiation of new toxicology studies, a review of past in vitro studies might identify the controls for surface conditioning of the test fibers: were effects of lung conditioning modeled, or were non-physiologic effects of medium adsorbates possible in past studies?

e. So-designed in vitro toxicology studies of classified short fibrous materials which have known positive or negative correlations with pathology could be attempted to determine if there is a short fiber toxicity with a reasonable potential to initiate disease in vivo, and if that potential is dependent on fiber mineral type or surface property or morphology.

f. A similar review of short-term in vivo studies might help the design of methods of challenge and time course for tests of materials selected from the in vitro study results.

g. Results of the in vitro and in vivo studies would suggest if useful application could be made to dusts of current concern, e.g., Libby vermiculite or World Trade Center disaster-associated dusts.

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