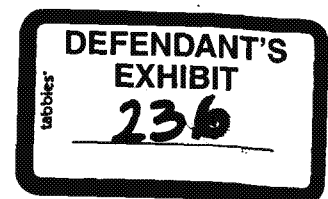


The biological sequence of events in the development of mesothelioma in rodents also appears to have a series of progressive steps (Boorman et al., 1990). In inhalation studies, the first event that is observed is fibrosis in the pleura immediately subjacent to the mesothelial lining. This is multifocal 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. Pseudovaculated 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 insight 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 μm diameter in rats and hamsters, monkeys ~ 15 μm , and humans ~ 21 μm 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. 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 μ m in length may not represent a significant carcinogenic risk. However, the same investigations, particularly the intraperitoneal studies also demonstrated that if the dose was high enough even so-called "innocuous" particulates, e.g. titanium dioxide, caused the induction of peritoneal

mesotheliomas, albeit at a lower incidence than long fibers. Additionally, the latter studies also demonstrated that if even long fibers, e.g. wollastonite and some SVFs, were not carcinogenic if they were not biopersistent in the peritoneal cavity. 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; Ilgen, 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 in vitro 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 Golyasny, 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 μ m 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 vitro studies could shed light on the dose response of both asbestos and SVFs. While these types of studies are primarily designed to capture and elucidate specific mechanisms of toxicity and carcinogenicity, there may be insights into dose response that could help in establishing thresholds of effect. One such study showed that short fiber amosite did not cause inflammation, while long amosite did. The only draw backs to and in vitro approach is that these techniques do not 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 lucigenin-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- α (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- κ B (NF- κ B) 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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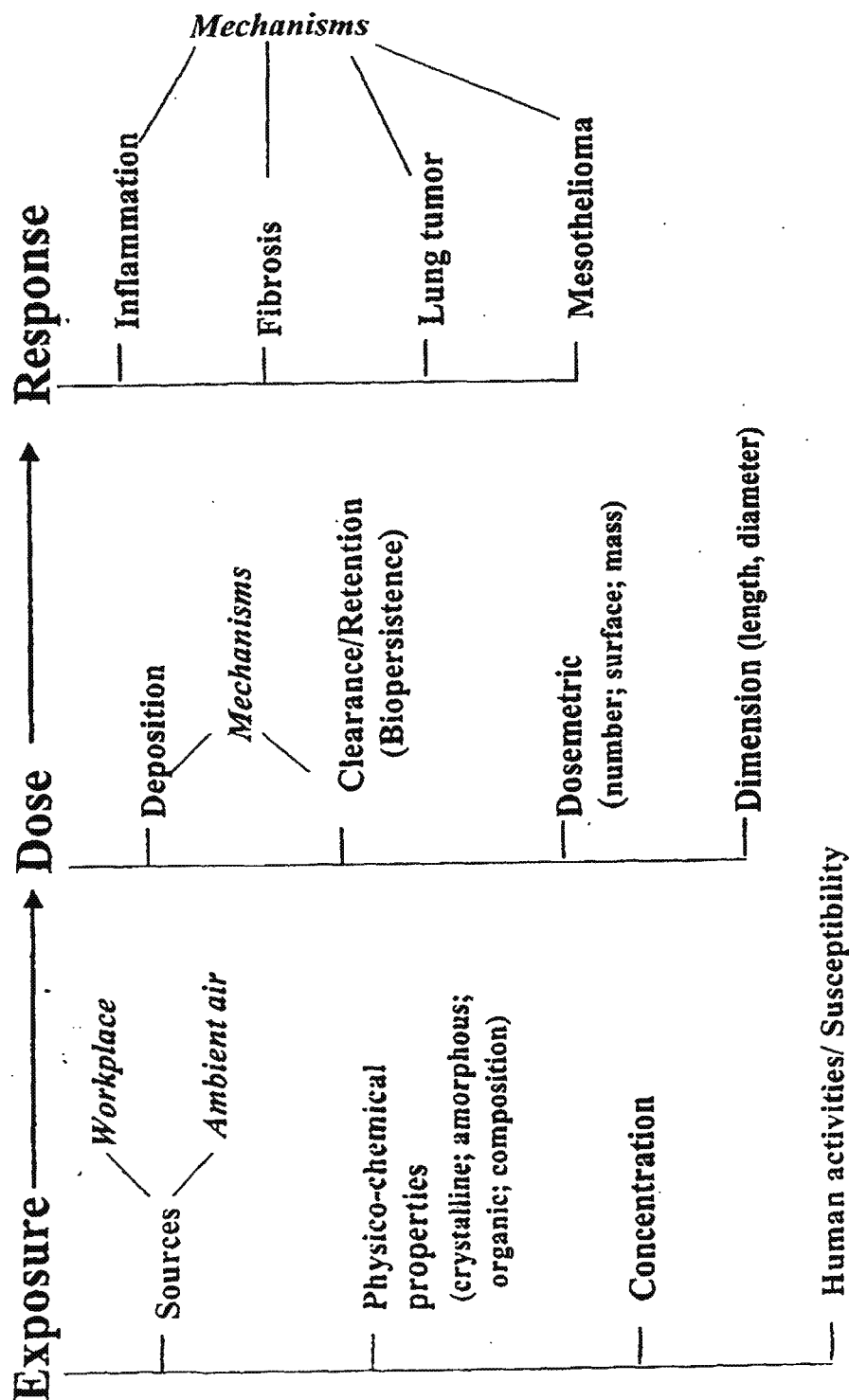
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Dr. Oberdörster's Post-Meeting Comments

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).

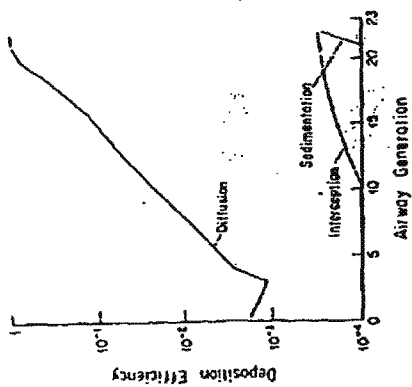
Airborne Fibers and Host Interactions



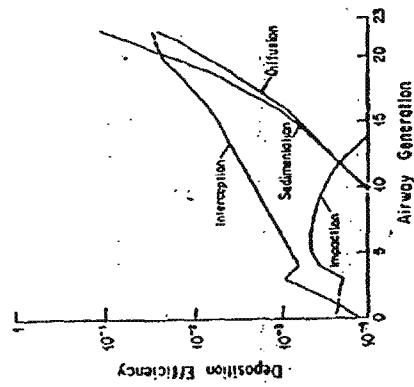
Main Deposition Mechanisms of Inhaled Fibers

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

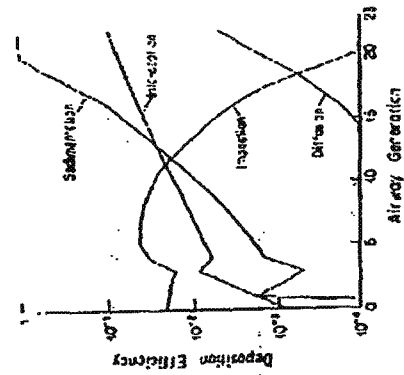
From: Asgharian and Yu, 1988



Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of 375 cm³/sec for $d_{pm} = 0.01 \mu m$ and Unit Density.

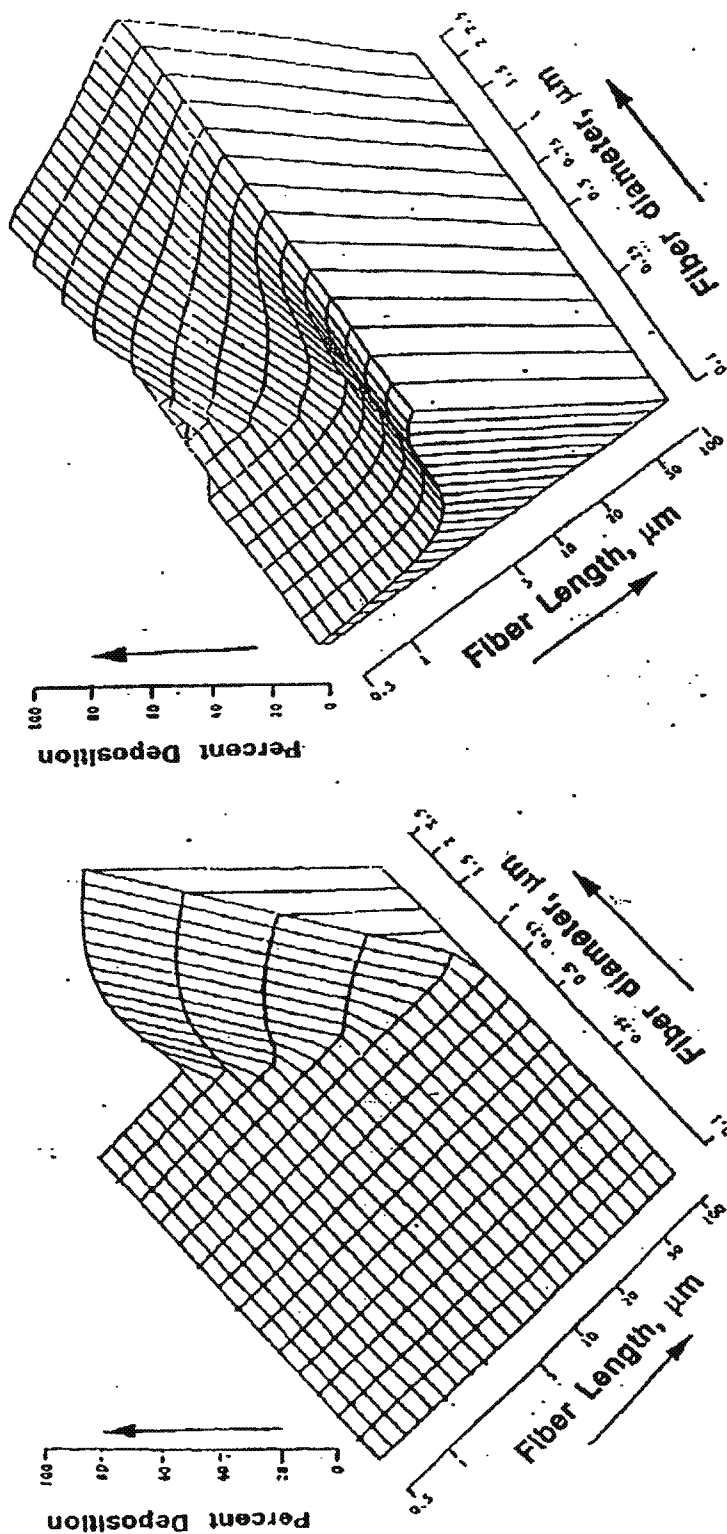


Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of 375 cm³/sec for $d_{pm} = 1 \mu m$ and Unit Density.



Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of 375 cm³/sec for $d_{pm} = 10 \mu m$ and Unit Density.

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



Nose-breathing

Mouth-breathing

Effective filtration of long fibers by nose

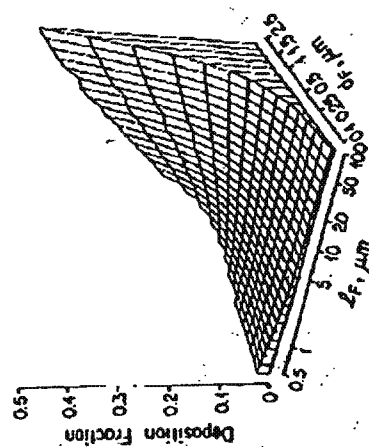


Fig. 3. Tracheobronchial deposition via trachea with no interstitial deposition at a lung volume of 10.78 cm^3 , a tidal volume of 1.68 cm^3 , a breathing frequency of $98 \text{ cycles min}^{-1}$ and $\rho = 3.37 \text{ g cm}^{-3}$.

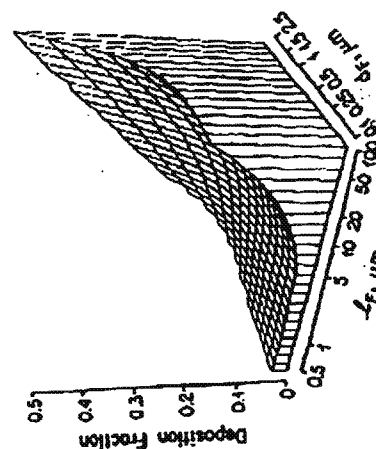
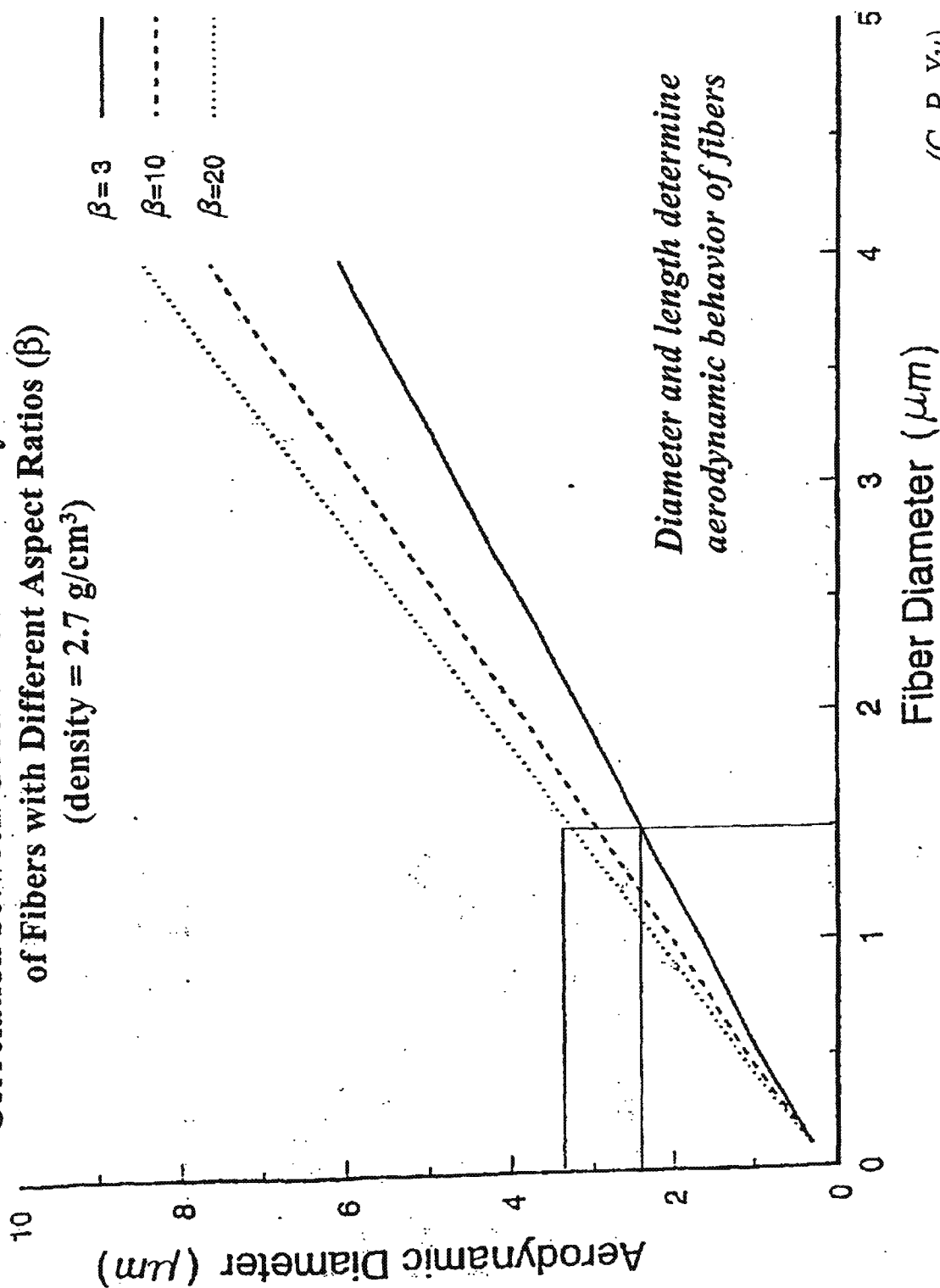


Fig. 4. Tracheobronchial deposition via trachea at a lung volume of 10.78 cm^3 , a tidal volume of 1.68 cm^3 , a breathing frequency of $98 \text{ cycles min}^{-1}$ and $\rho = 3.37 \text{ g cm}^{-3}$.

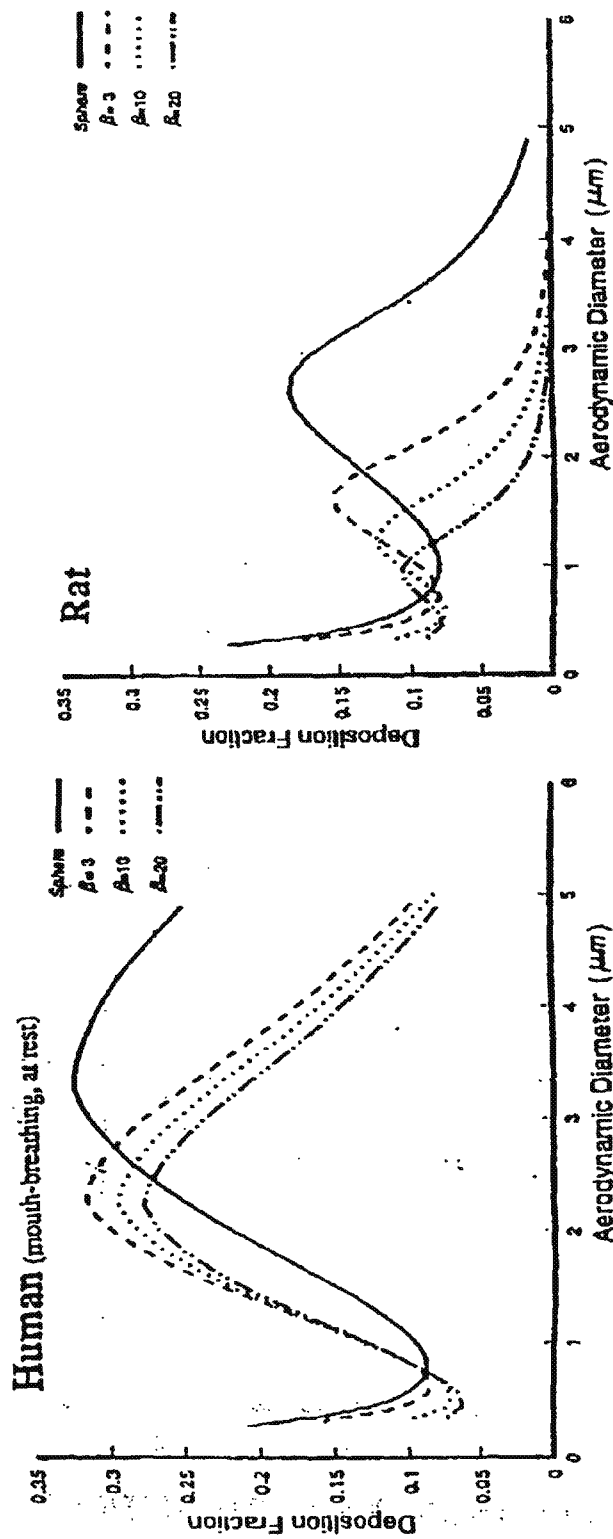
Correlation between Geometric and Aerodynamic Diameter of Fibers with Different Aspect Ratios (β)

(density = 2.7 g/cm³)



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)

Physiological Clearance Mechanisms of Deposited Fibers

- 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 Biopersistence 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!)

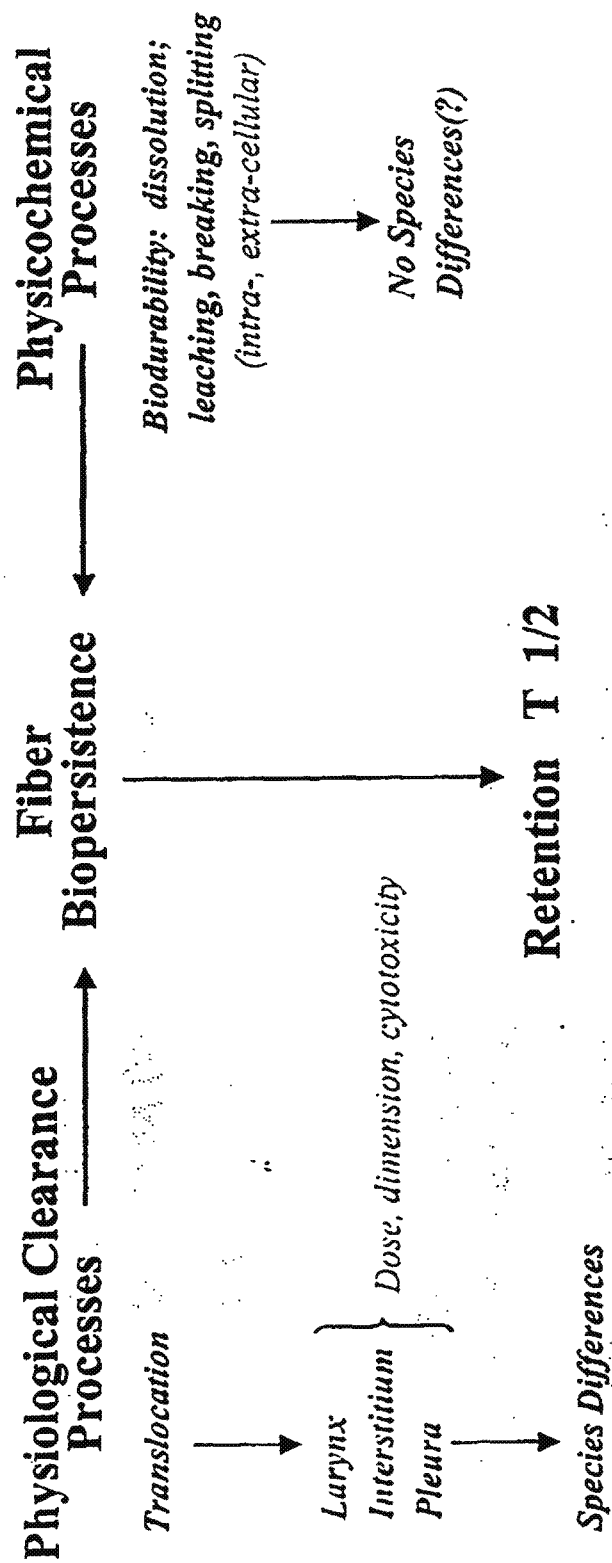
Size Dependent Lymphatic Clearance of Amosite Asbestos

Study: Intrabronchial instillation of amosite in dogs, followed by

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

	<u>Max. Diameter</u>	<u>Max. Length</u>
<i>Lymph node</i>	0.5 μm	16 μm
<i>Post-nodal lymph</i>	0.5 μm	9 μm

(Oberdörster et al., 1988)

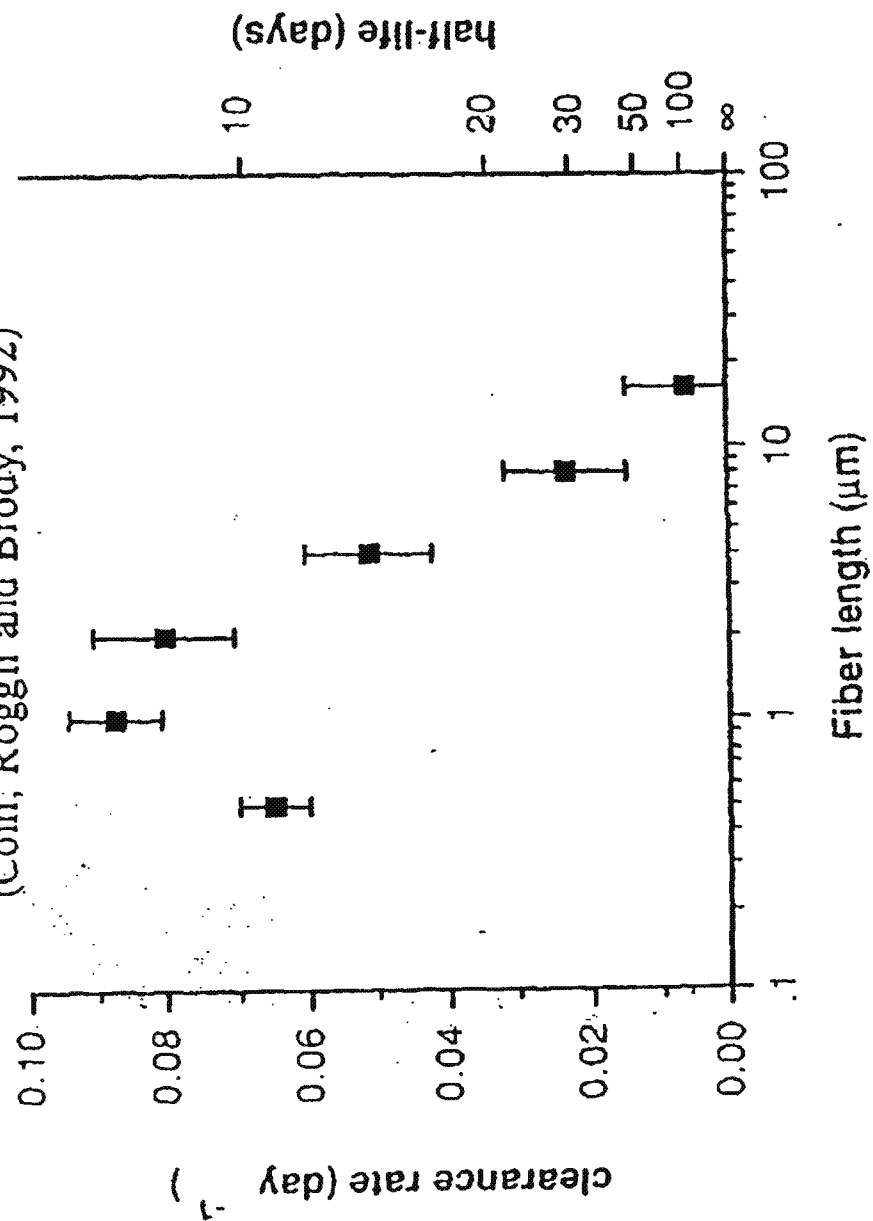


Biopersistence = Biodurability + Physiological Clearance

(Oberdörster, 1996)

Clearance of Chrysotile from Rat Lung

(Coin, Roggli and Brody, 1992)



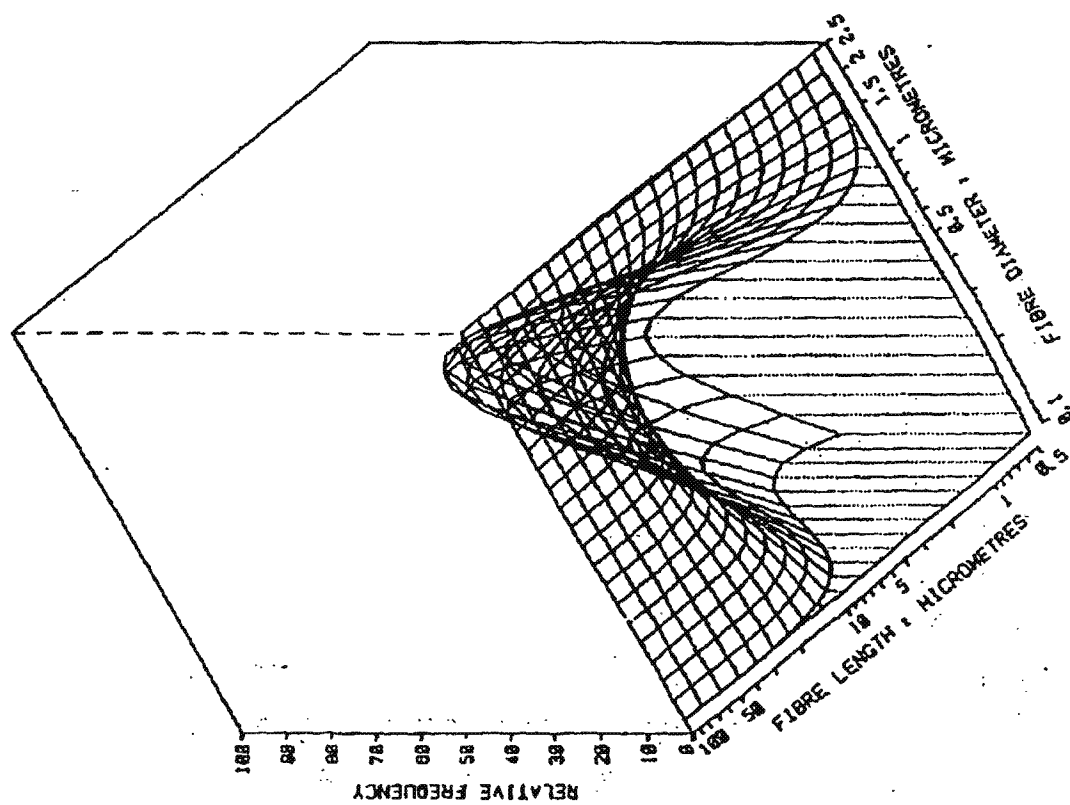


FIG. 8. Bivariate distribution of fibres in Paakkila bugging section.

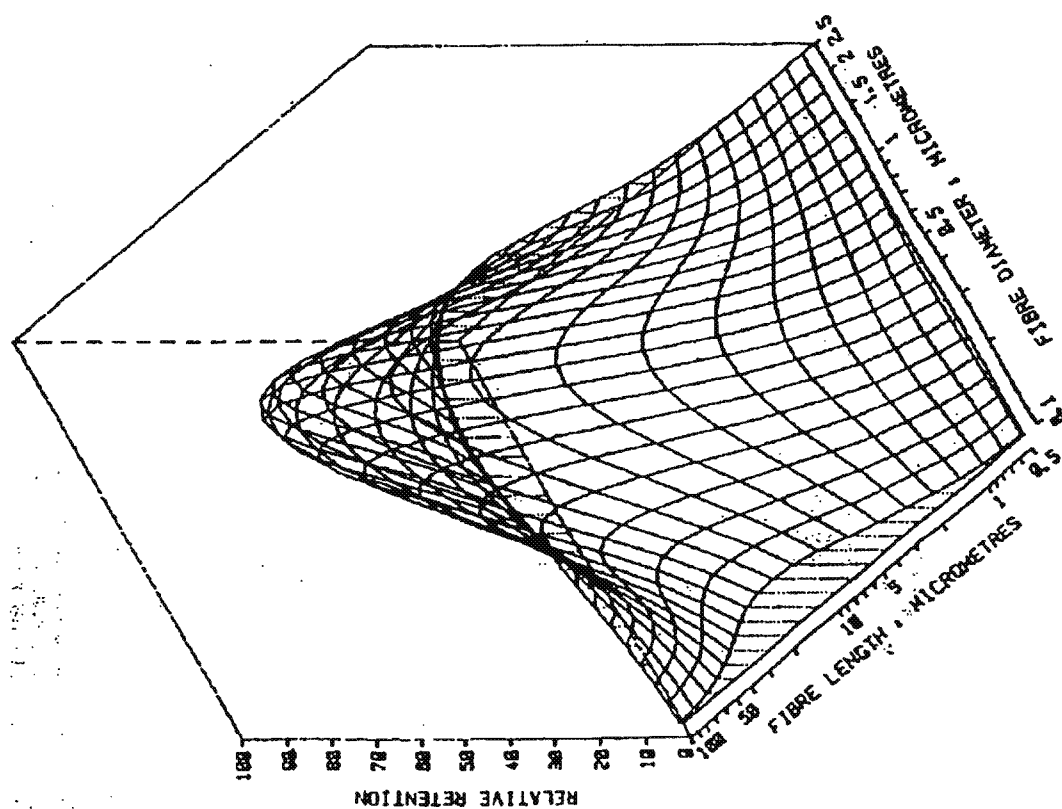
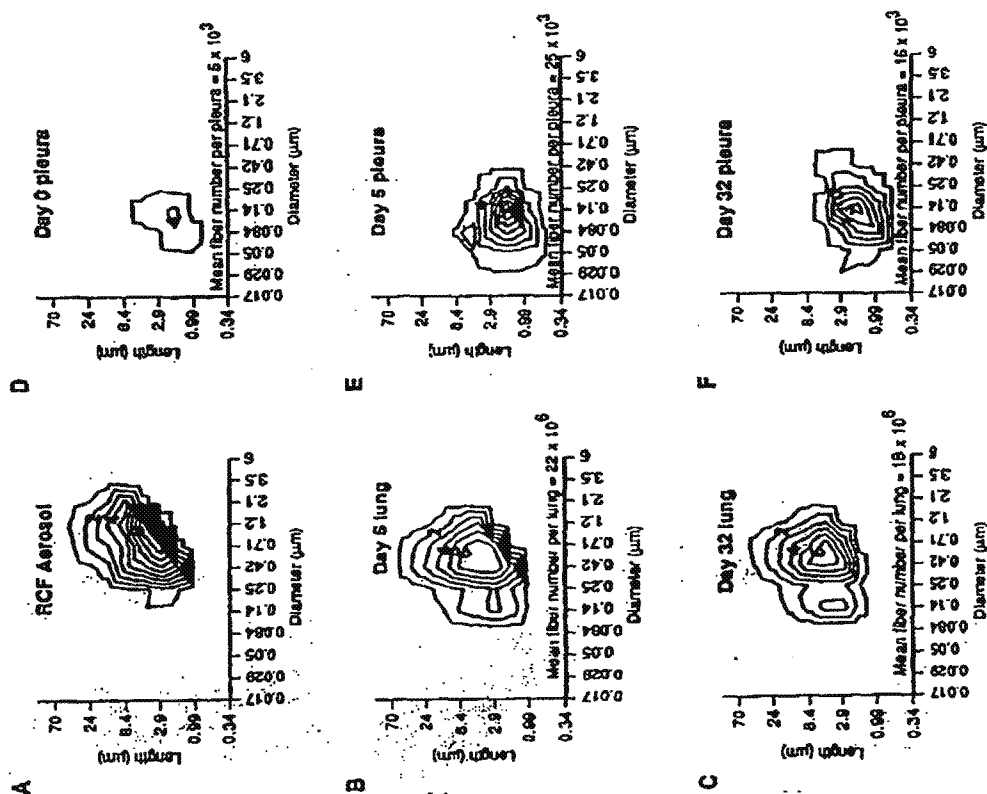


FIG. 10. Bivariate presentation of fibre retention.

FIBER DOSIMETRY FOLLOWING RCF-1 EXPOSURE



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

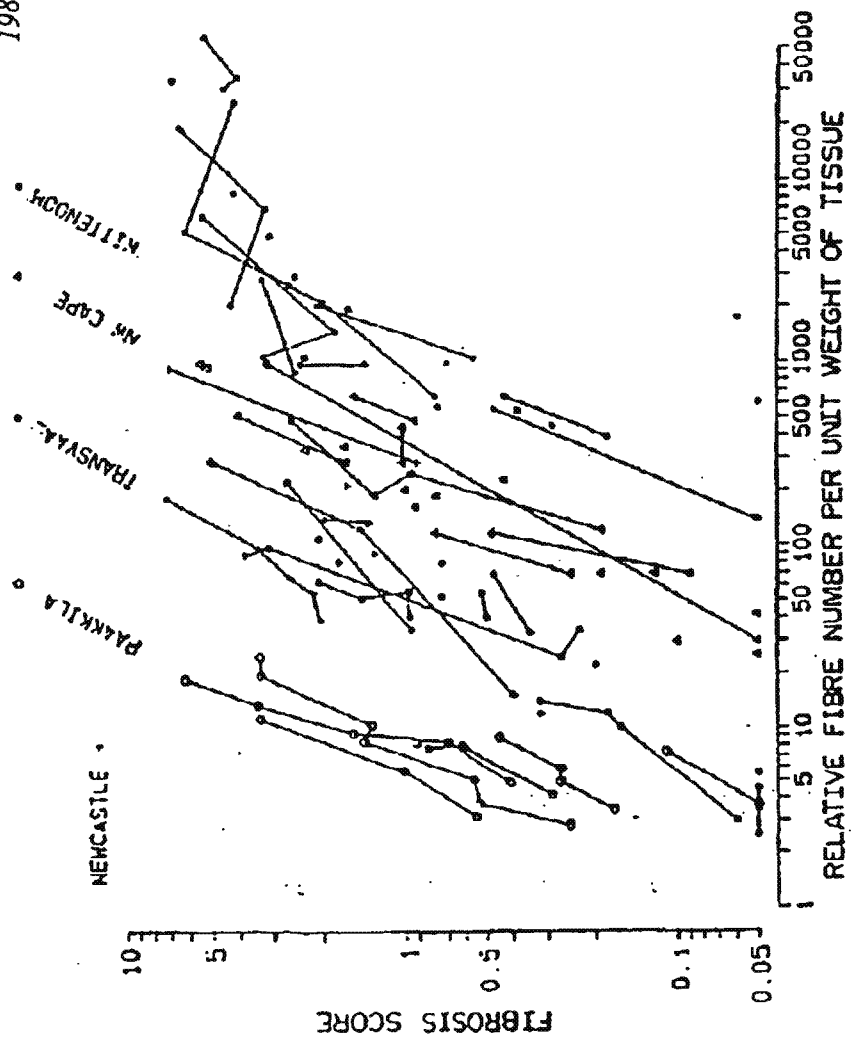


FIG. 5. Relationships when the parameter of fibre quantity is number.

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

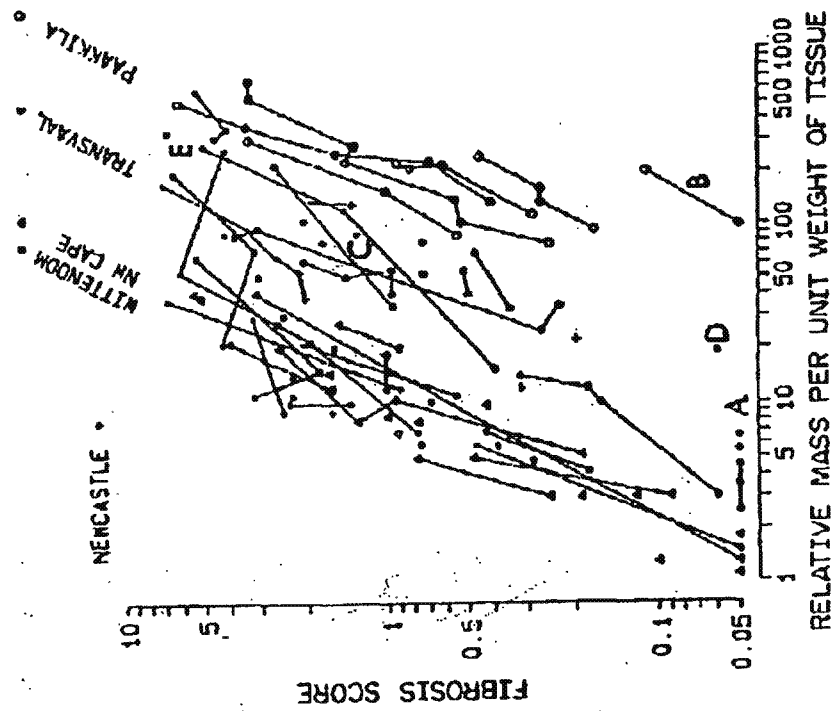


FIG. 3. Relationships when the parameter of fibre quantity is mass. Linked data points relate to tissue specimens from the same subject.

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

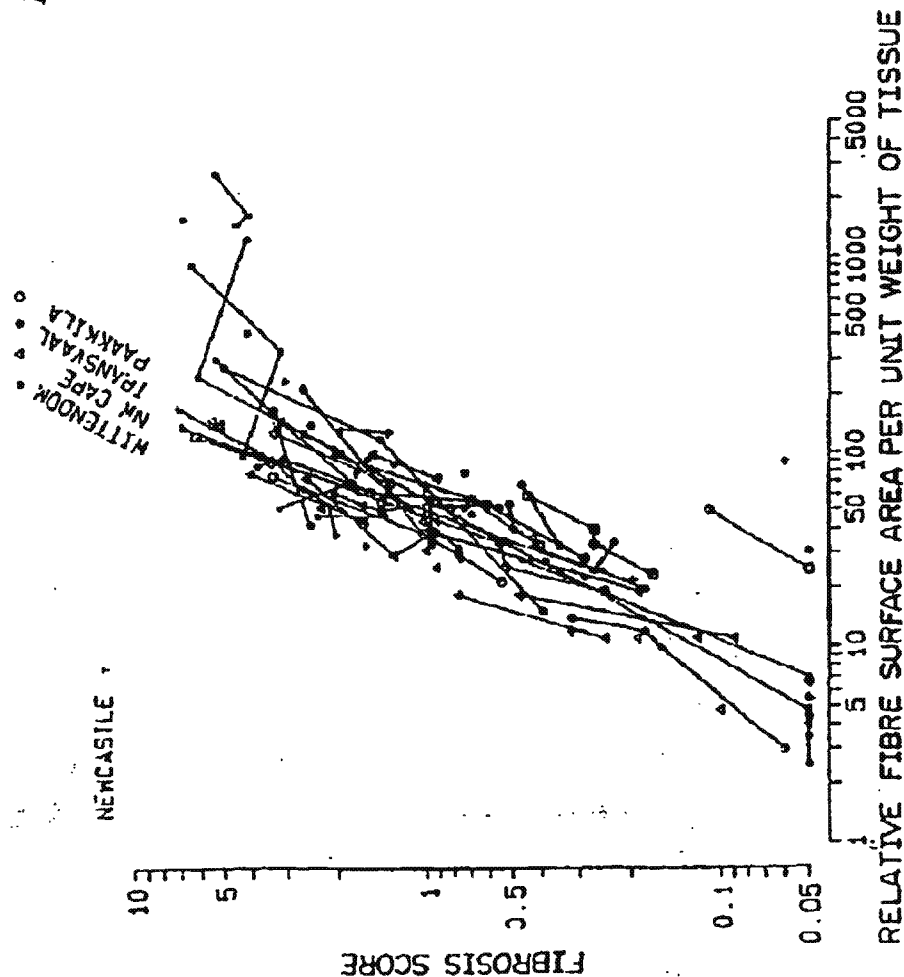
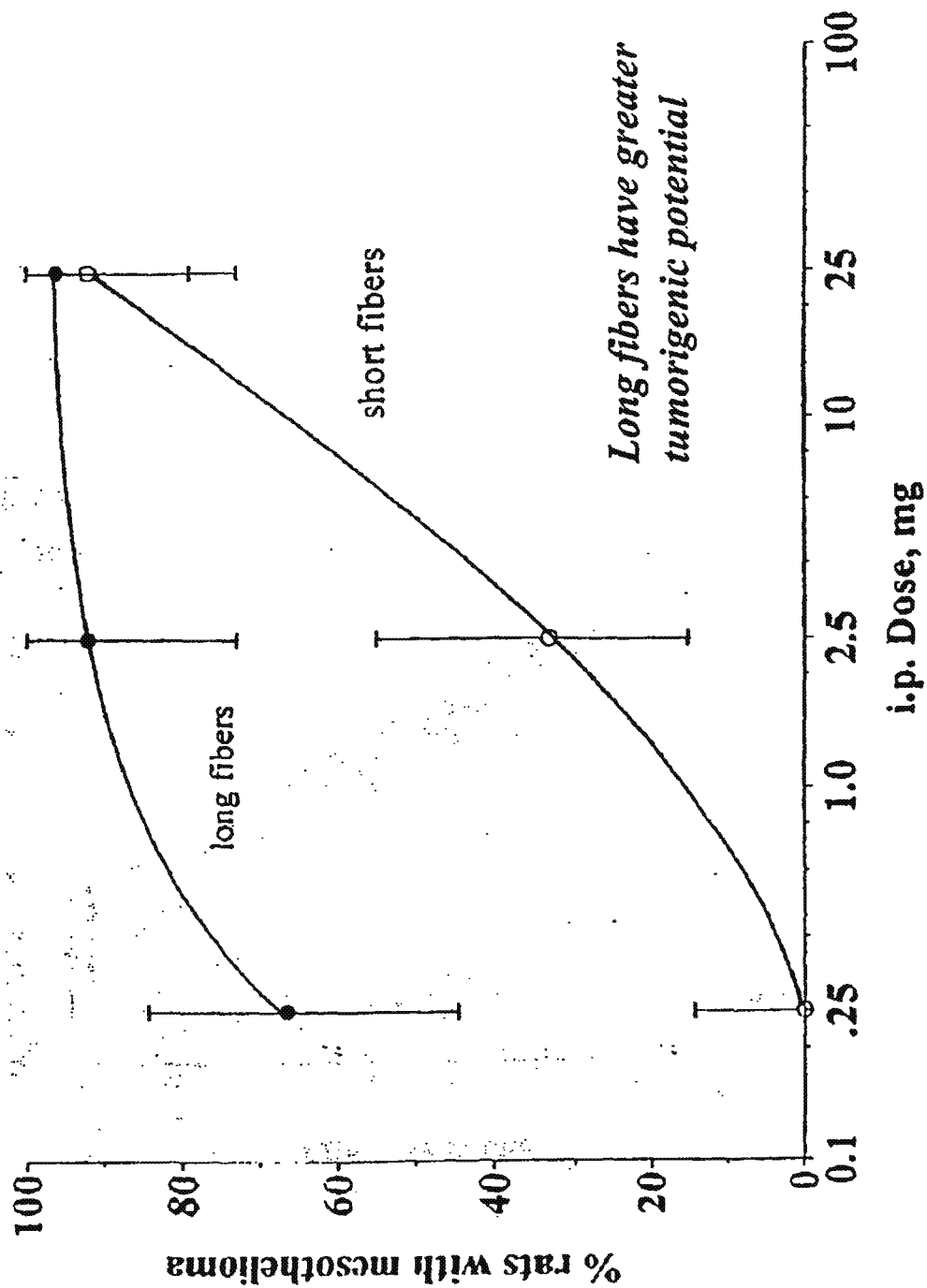


FIG. 4. Relationships when the parameter of fibre quantity is surface area.

Peritoneal mesothelioma After I.P. Injection of Long and Short Chrysotile (mean and 95% CI) (Davis and Jones, 1988)



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

Chrysotile or amosite (10 mg/m³) plus TiO₂ (10 mg/m³) or quartz (2 mg/m³),
1-year rat inhalation study plus 2-year observation period.

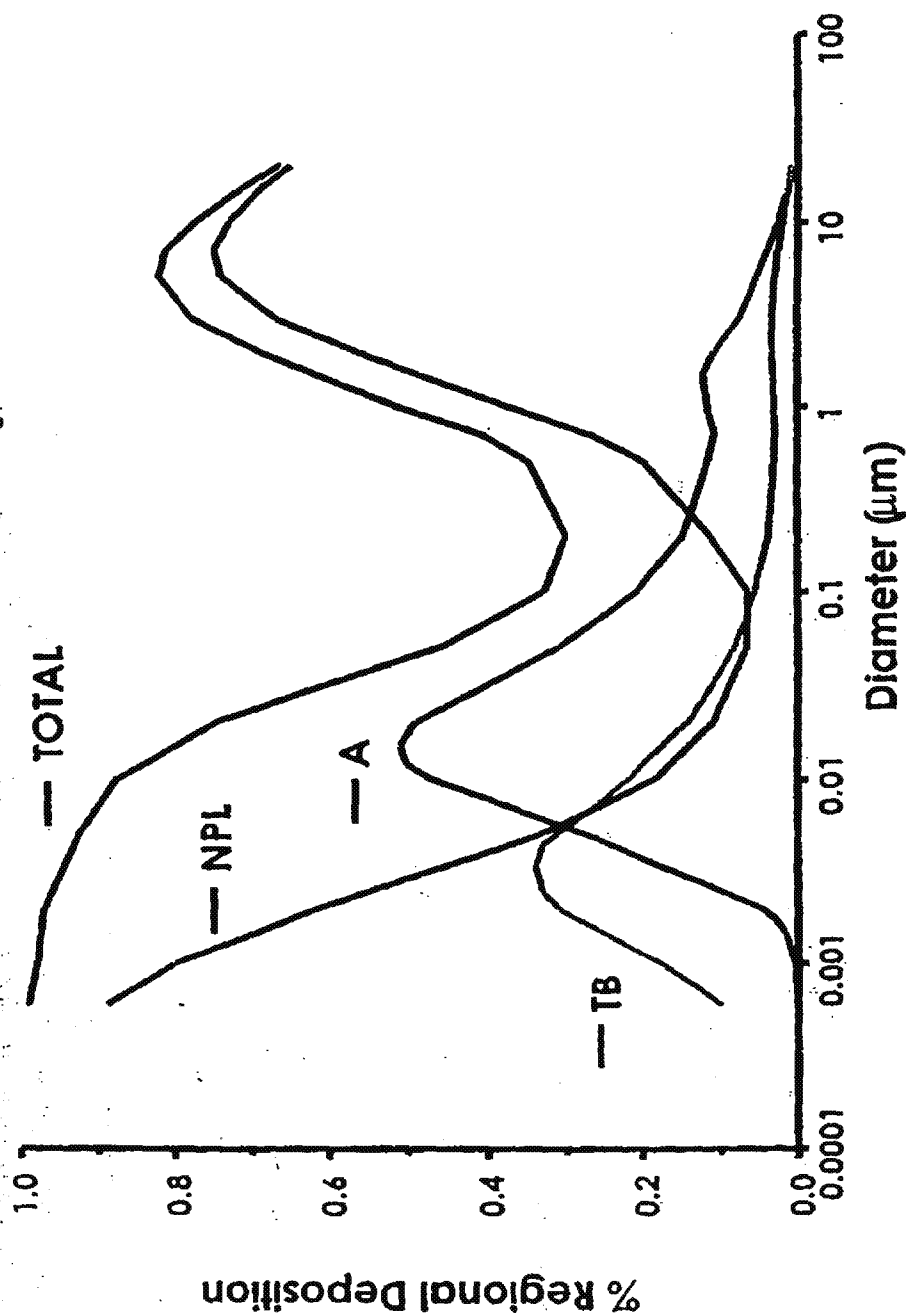
	Fiber retention	Pulm. fibrosis	Transport across visc. pleura	Lung tumors	Mesothelioma	Survival rate
Chrysotile + TiO ₂	↑	○	?	↑	↑ ^a	↑
Amosite + TiO ₂	○	○	↑	↑	↑	↑
Chrysotile + quartz	↓	↑	?	↑	↑ ^{a,b}	↓
Amosite + quartz	○	↑	↑	↑	↑ ^b	↓

○ no change; ↑ increased; ↓ decreased; (compared to asbestos alone)
(predicted lung burden of TiO₂ in "overload" range, ~10 mg/lung)

^a = no mesothelioma with chrysotile alone

^b = greater effect of added quartz than of added TiO₂

Fractional Deposition of Inhaled Particles in the Human Respiratory Tract (ICRP Model, 1994; Nose Breathing)



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

Classification

<u>Natural Fibers</u>		<u>Man-made Fibers</u>	
<u>Asbestos</u>	<u>Asbestosiform</u>	<u>Vitreous</u>	<u>Crystalline</u>
Serpentine	Fibrous clays	Glass wool	Alumina
chrysotile	polygorskite (attapulgite)	Glass filaments	Graphite
	sepiolite	Rockwool	Potassium titanate
		Slagwool	Silicon carbide
		Ceramic fibers	Sodium aluminum carbonate
			Synthetic zeolites
<u>Amphiboles</u>	<u>Other Fibrous Silicates</u>		
actinolite	wollastonite		
amosite	nematite (fibrous brucite)		
anthophyllite	talc		
crocidolite	zeolites: mordenite		
tremolite	erionite		

Mechanisms of Fiber Carcinogenesis

(Kane, 1996)

1. Fibers generate free radicals that damage DNA
 - direct generation of ROS and damage of DNA in cell free system
 - catalyze 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 intracellular 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 than 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 detoxification/re-toxification process is the mechanism distinguishing quartz and aluminosilicate 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- α , but have not been shown to induce fibrosis. And asbestos produces fibrosis in some systems without increasing TNF- α expression. Chrysotile contains little iron but is fibrogenic, albeit not as potent as amphibole. Churg et al. suggest a comparison of asbestos and silica-induced fibrosis data. Their paper compares the generation of ROS, RNS, and activation of NF- κ B and AP-1, and increased production of TNF- α 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

Asbestos 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 lucigenin-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 μm . 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 μm 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 serpentine 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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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.

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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 $<5 \mu\text{m}$ in length, deposition patterns and efficiencies will be determined almost entirely according to the fiber width, which for fibers $<5 \mu\text{m}$ long will be less than about 1.6 μm . For fiber widths between about 0.1 and 1.6 μm , 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 half-time of the fibrous or non-fibrous material in the lung. Physicochemical processes include dissolution, leaching, breaking and splitting, depending on the fibrous material, that can occur intra- as well as extra-cellularly, and differences in pH in both locations are of importance here. Clearance rates of fibers of different length categories have been determined from short- and long-term inhalation studies (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 phagolysosomes within alveolar macrophages as well as pH of 7.4 reflecting extra-cellular fluid. Short fibers that are ingested by macrophages will encounter the lower pH that overall could affect their biopersistence. In general, solubility tests identified the following rank order from lowest to greatest solubility of MMVF in comparison to asbestos fibers: crocidolite <amosite <RCF <special purpose glass fibers <rock wool <slag wool <conventional glass fibers (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 $\sim 0.1 \mu\text{m}$, which could be a significant fraction of fibers $< 5 \mu\text{m}$ in length, can penetrate through the respiratory epithelia and be transported through lymph channels to hilar and peripheral (mesothelial) lymph nodes and through blood to more distant body organs. 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 $1.5 \mu\text{m}$ (GSD ~ 2.0) and geometric mean diameter $0.09 \mu\text{m}$ (GSD ~ 1.5). For comparison parenchymal tissue GML = $5.0 \mu\text{m}$ (GSD ~ 2.3) and GMD $0.3 \mu\text{m}$ (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 $5 \mu\text{m}$ 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 Babbitt, 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 $<10 \mu\text{m}$ 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 $4.82 \pm 0.68 \text{ f/cc}$ (range 0.66–11.79) with $0.36 \pm 0.08 \text{ f/cc}$ (range 0.07–1.29) greater than $5 \mu\text{m}$ 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 Regarding Non-Cancer Effects of MMVF

There are no available morbidity studies of workers exposed to MMVF uniformly less than 5 μ m 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 μ m 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 μ m 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 $<5 \mu\text{m}$ in length have been associated with pleural or parenchymal disease in human. Any potential risk associated with fiber exposure $<5 \mu\text{m}$ 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 $<0.1 \mu\text{m}$ to $0.4 \mu\text{m}$ and lengths $<10 \mu\text{m}$ 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 $<5 \mu\text{m}$ 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 $<5 \mu\text{m}$ 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 $<5 \mu\text{m}$ in length but there has been some speculation that durable fibers $<10 \mu\text{m}$ in length and <0.1 to $0.4 \mu\text{m}$ in diameter may be associated with pleural plaques in relatively low concentration, in particular the amphibole tremolite.

For asbestos fibers $<5 \mu\text{m}$ 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 $5 \mu\text{m}$ 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 $>0.5 \mu\text{m}$ cannot reach the deep lung (alveolar region) which is the site of primary disease. Non-human primates would be an ideal animal model but are precluded because of their long life-span (would require at least 20-30 years to demonstrate a noncarcinogenic effect), availability (a cancer bioassay requires >200 animals/sex), and expense (such a study would cost $>\$20$ million).

Most chronic rodent inhalation bioassays of asbestos have been conducted in rats, have not shown significant strain differences and males and females are equally sensitive to its carcinogenic effects (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 insight 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 μm diameter in rats and hamsters, monkeys ~15 μm , and humans ~21 μm 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. 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 μ m in length may not represent a significant carcinogenic risk. However, the same investigations, particularly the intraperitoneal studies also demonstrated that if the dose was high enough even so-called "innocuous" particulates, e.g. titanium dioxide, caused the induction of peritoneal

mesotheliomas, albeit at a lower incidence than long fibers. Additionally, the latter studies also demonstrated that if even long fibers, e.g. wollastonite and some SVFs, were not carcinogenic if they were not biopersistent in the peritoneal cavity. 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 *in vitro* 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 Golyasny, 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 μ m 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 vitro studies could shed light on the dose response of both asbestos and SVFs. While these types of studies are primarily designed to capture and elucidate specific mechanisms of toxicity and carcinogenicity, there may be insights into dose response that could help in establishing thresholds of effect. One such study showed that short fiber amosite did not cause inflammation, while long amosite did. The only draw backs to and in vitro approach is that these techniques do not 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; Goodlick 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 lucigenin-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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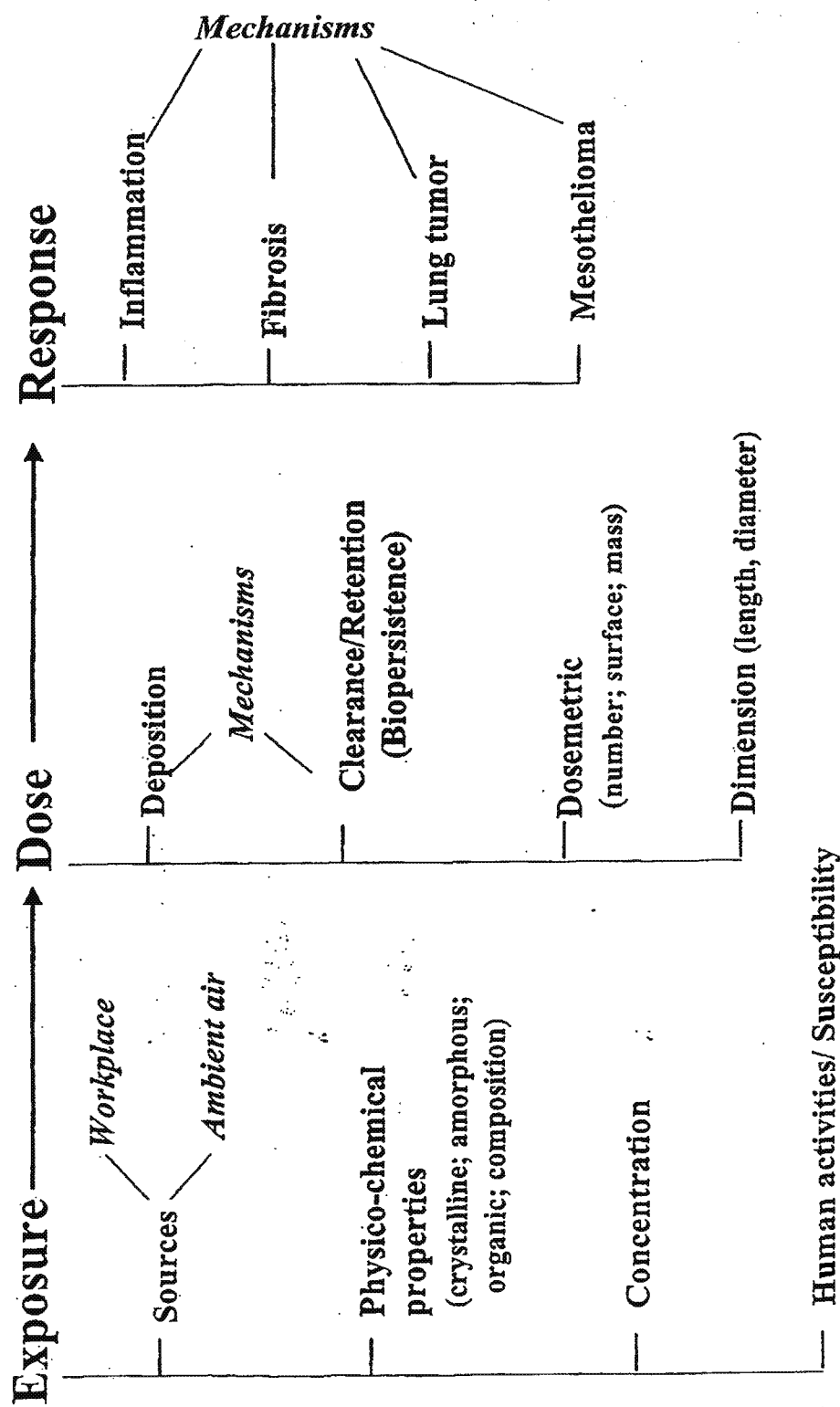
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Dr. Oberdörster's Post-Meeting Comments

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).

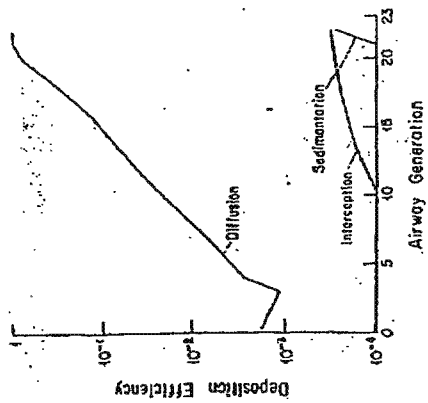
Airborne Fibers and Host Interactions



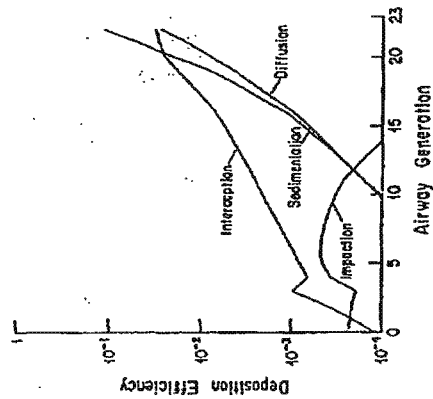
Main Deposition Mechanisms of Inhaled Fibers

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

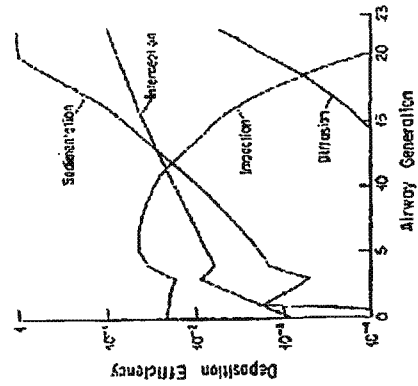
From: Asgharian and Yu, 1988



Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of $375 \text{ cm}^3/\text{sec}$ for $d_{gm} = 0.01 \mu\text{m}$ and Unit Density.

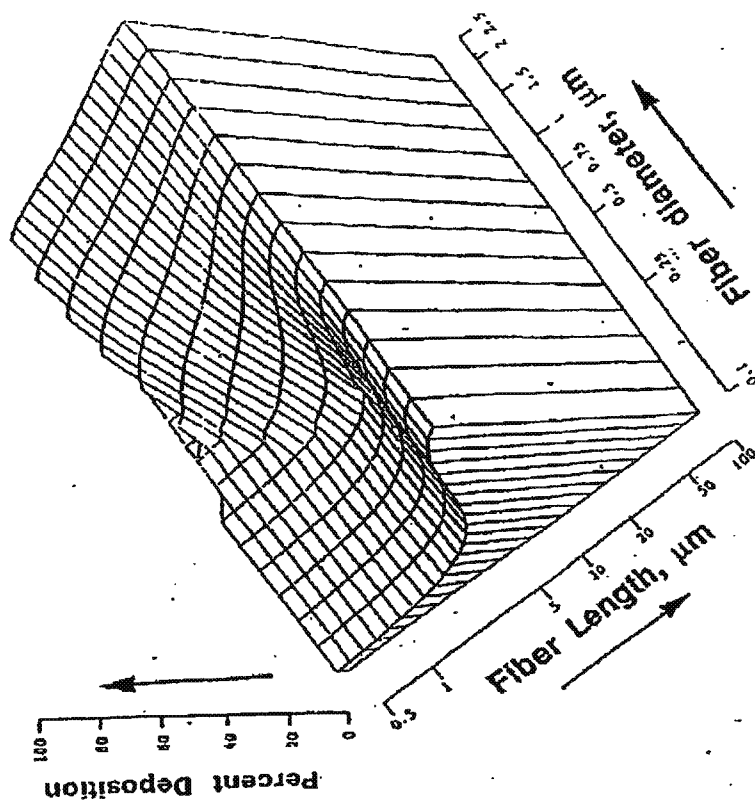


Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of $375 \text{ cm}^3/\text{sec}$ for $d_{gm} = 1 \mu\text{m}$ and Unit Density.

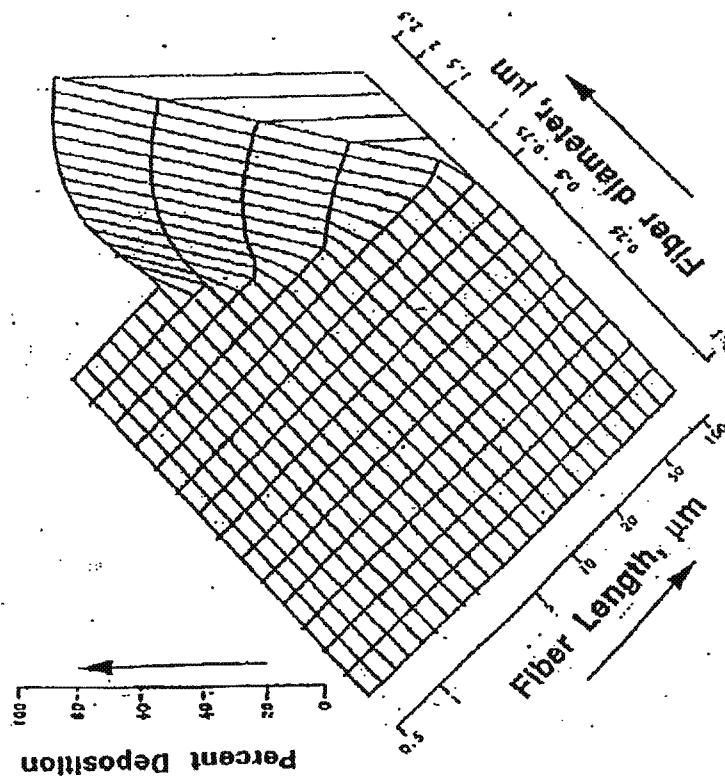


Deposition Efficiency of Fibers in Different Generations of the Weibel Lung Model at a Flow Rate of $375 \text{ cm}^3/\text{sec}$ for $d_{gm} = 10 \mu\text{m}$ and Unit Density.

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



Nose-breathing



Mouth-breathing

Effective filtration of long fibers by nose

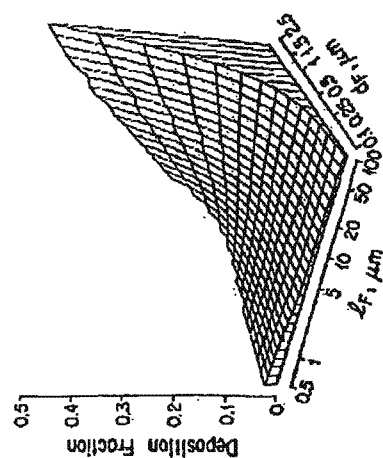


Fig. 3. Tracheobronchial deposition via trachea with no interceptional deposition at a lung volume of 1.68 cm^3 , a tidal volume of 1.68 cm^3 , a breathing frequency of $98 \text{ cycles min}^{-1}$ and $p = 3.37 \text{ g cm}^{-3}$.

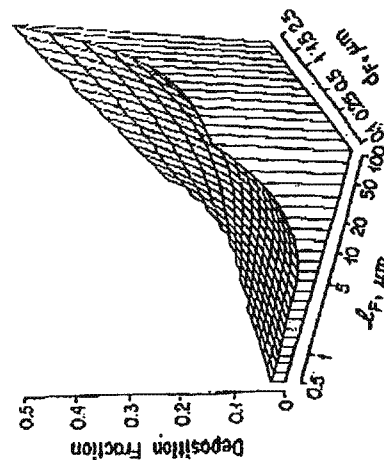
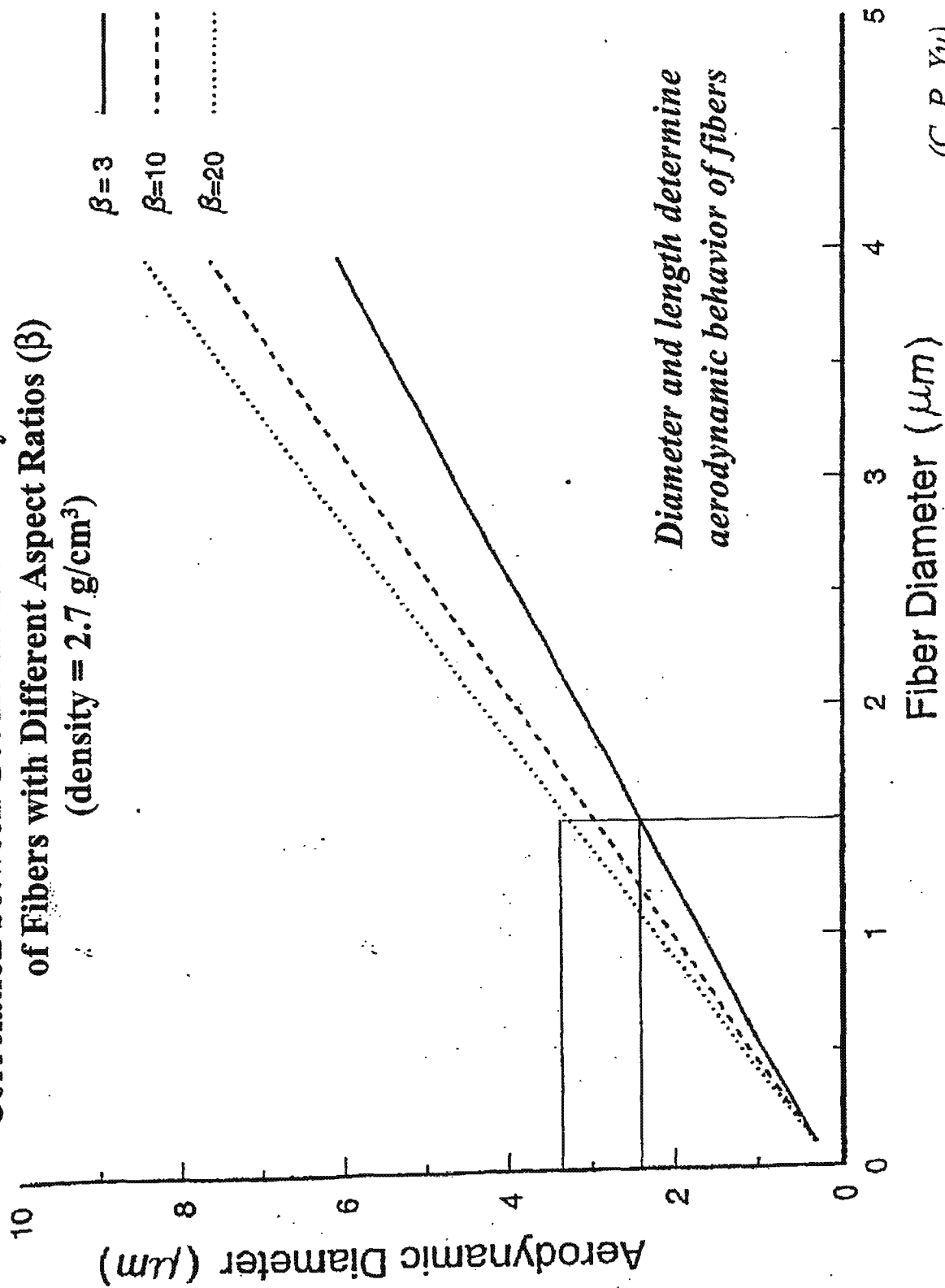


Fig. 4. Tracheobronchial deposition via trachea at a lung volume of 10.78 cm^3 , a tidal volume of 1.68 cm^3 , a breathing frequency of $98 \text{ cycles min}^{-1}$ and $p = 3.37 \text{ g cm}^{-3}$.

Correlation between Geometric and Aerodynamic Diameter of Fibers with Different Aspect Ratios (β)

(density = 2.7 g/cm³)

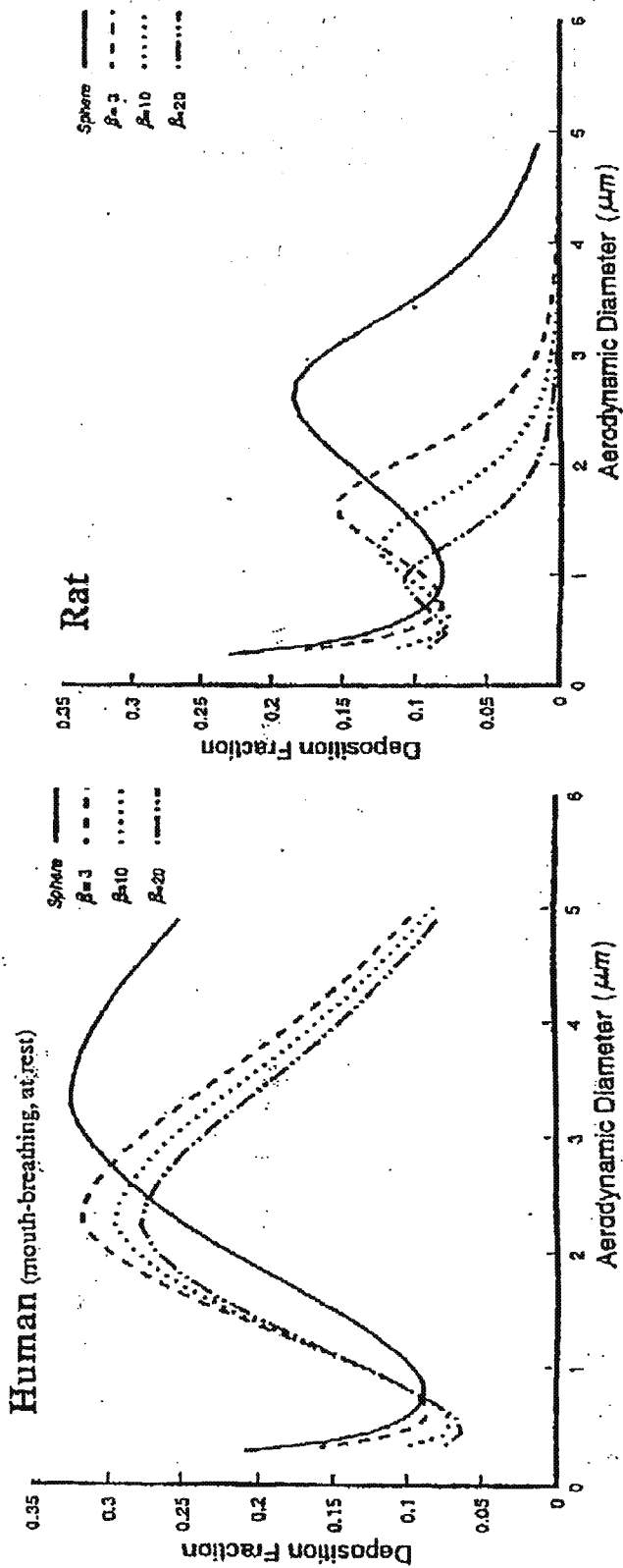


*Diameter and length determine
aerodynamic behavior of fibers*

(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)

Physiological Clearance Mechanisms of Deposited Fibers

- 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 Biopersistence 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

Human: 14 - 21 μm

Crapo et al., 1983; Lum et al., 1983, Stone et al., 1992;

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!)

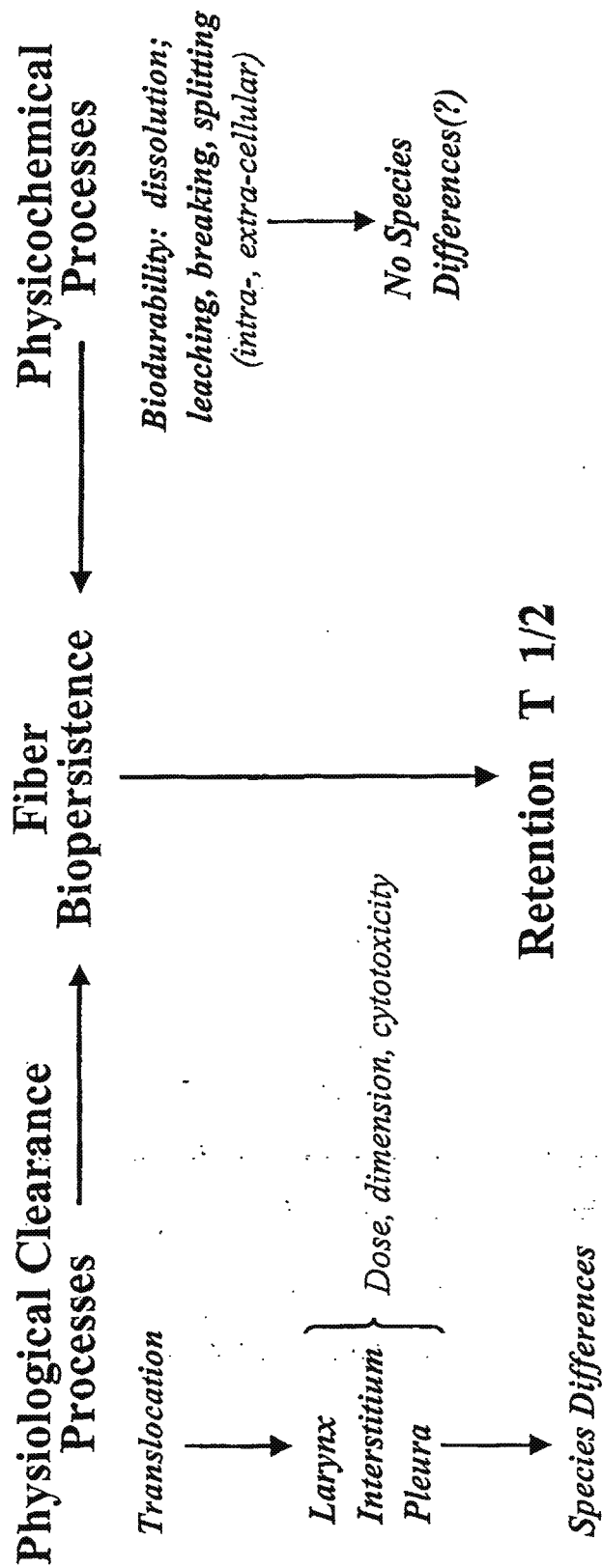
Size Dependent Lymphatic Clearance of Amosite Asbestos

Study: Intrabronchial instillation of amosite in dogs, followed by

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

	<u>Max. Diameter</u>	<u>Max. Length</u>
<i>Lymph node</i>	0.5 μm	16 μm
<i>Post-nodal lymph</i>	0.5 μm	9 μm

(Oberdörster et al., 1988)

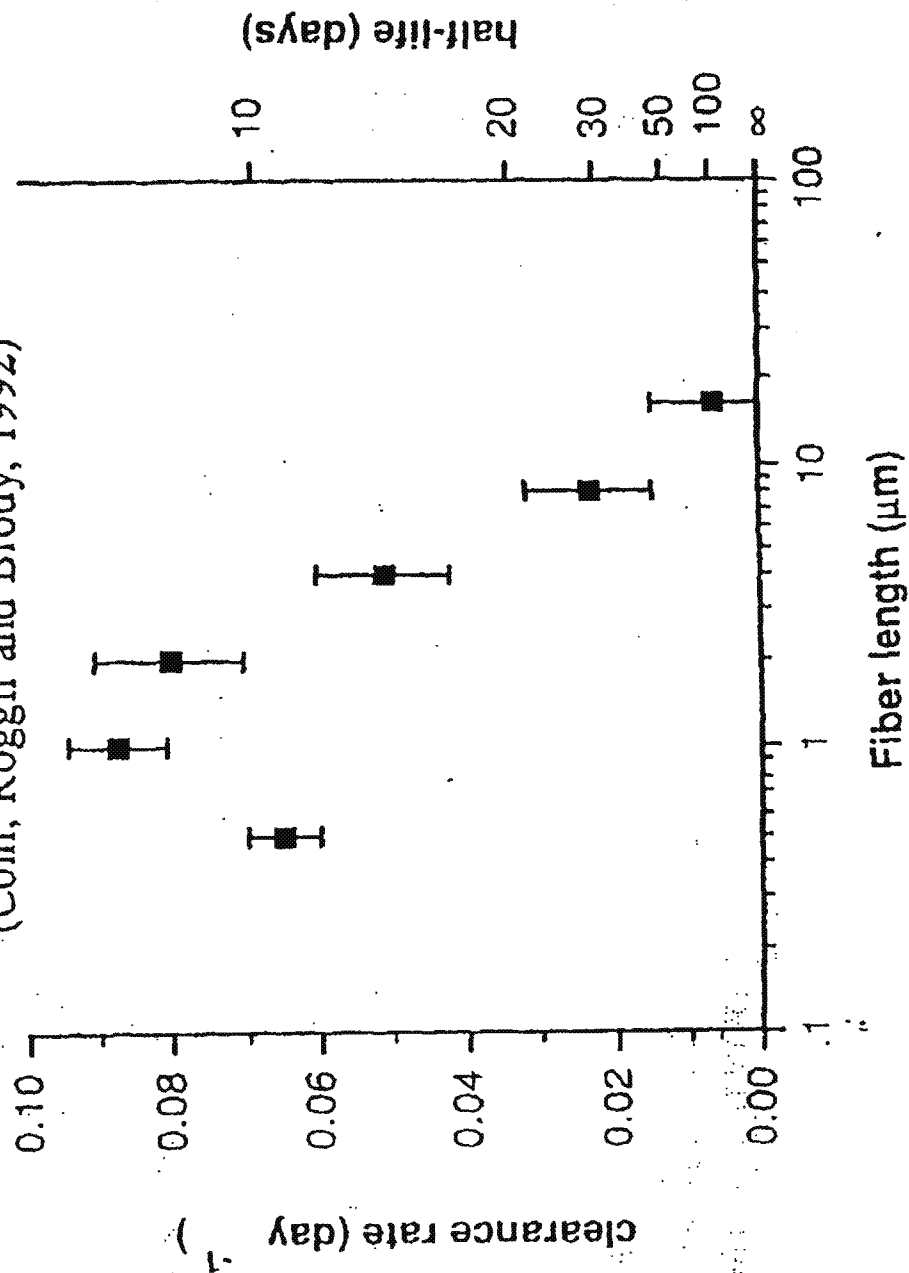


Biopersistence = Biodurability + Physiological Clearance

(Oberdörster, 1996)

Clearance of Chrysotile from Rat Lung

(Coin, Roggli and Brody, 1992)



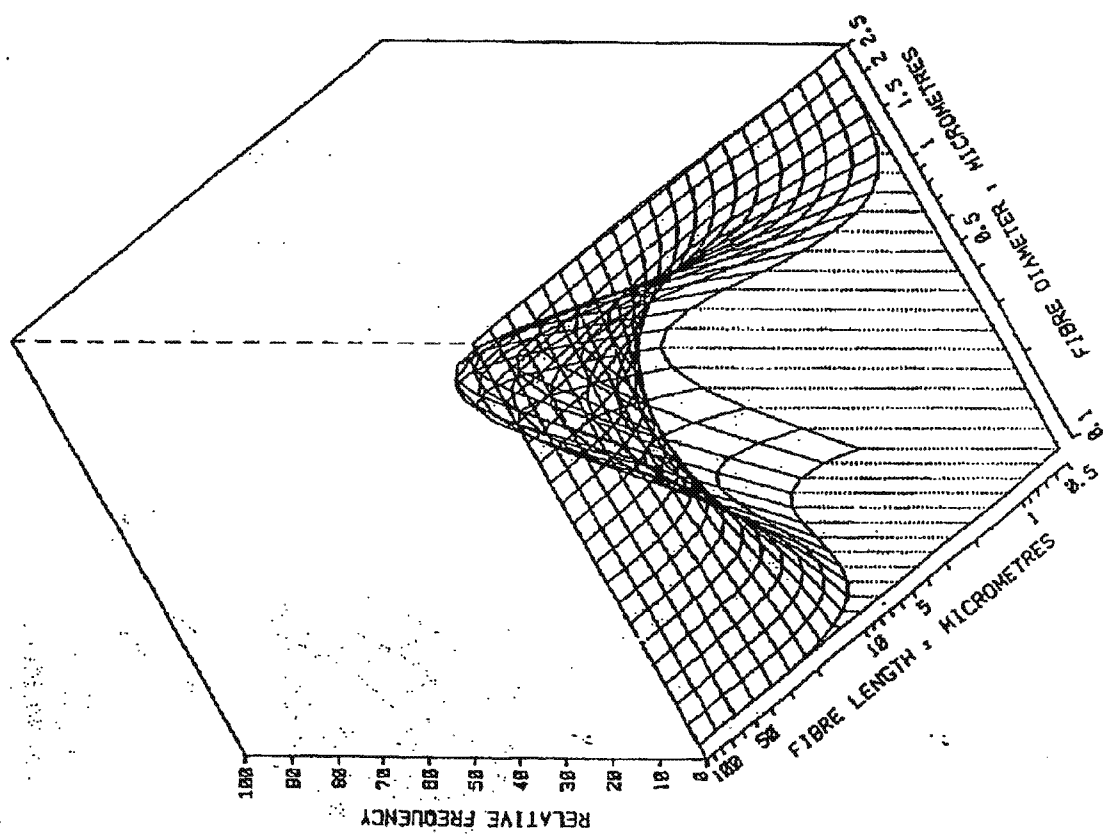


FIG. 8. Bivariate distribution of fibres in Paakkila bagging section.

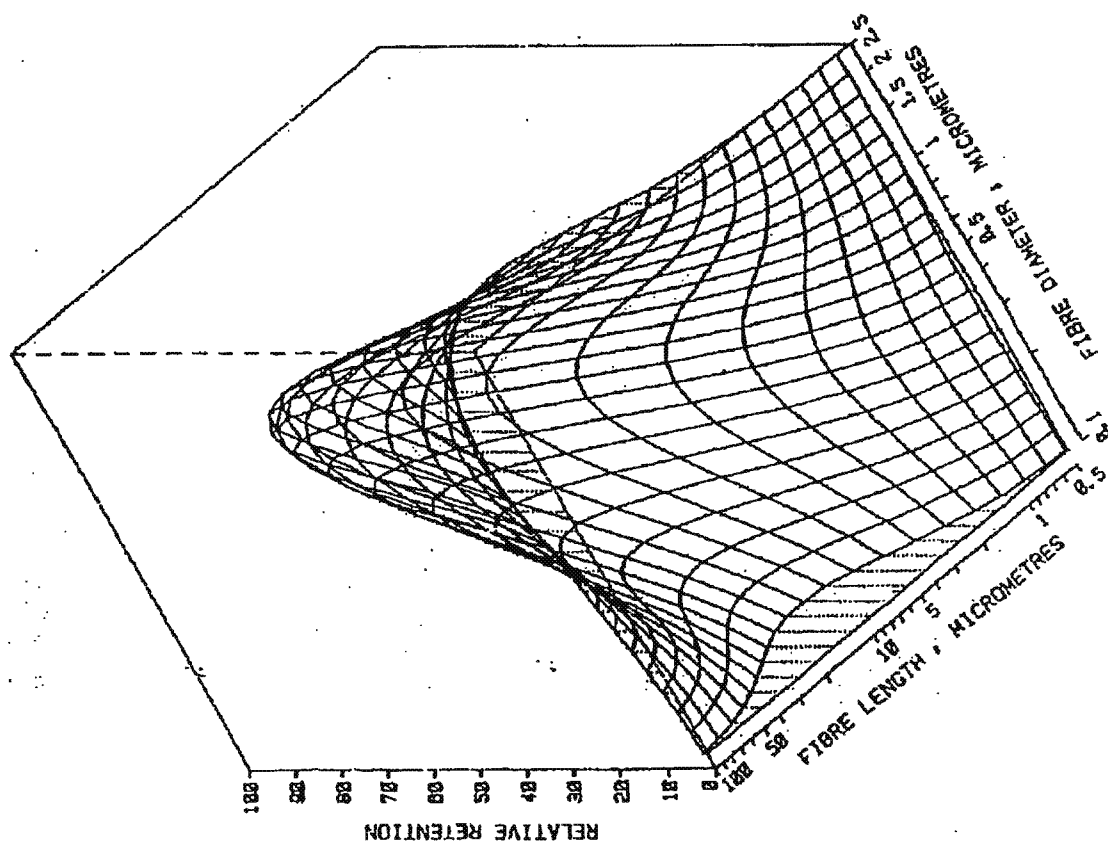


FIG. 10. Bivariate presentation of fibre retention.

FIBER DOSIMETRY FOLLOWING RCF-1 EXPOSURE

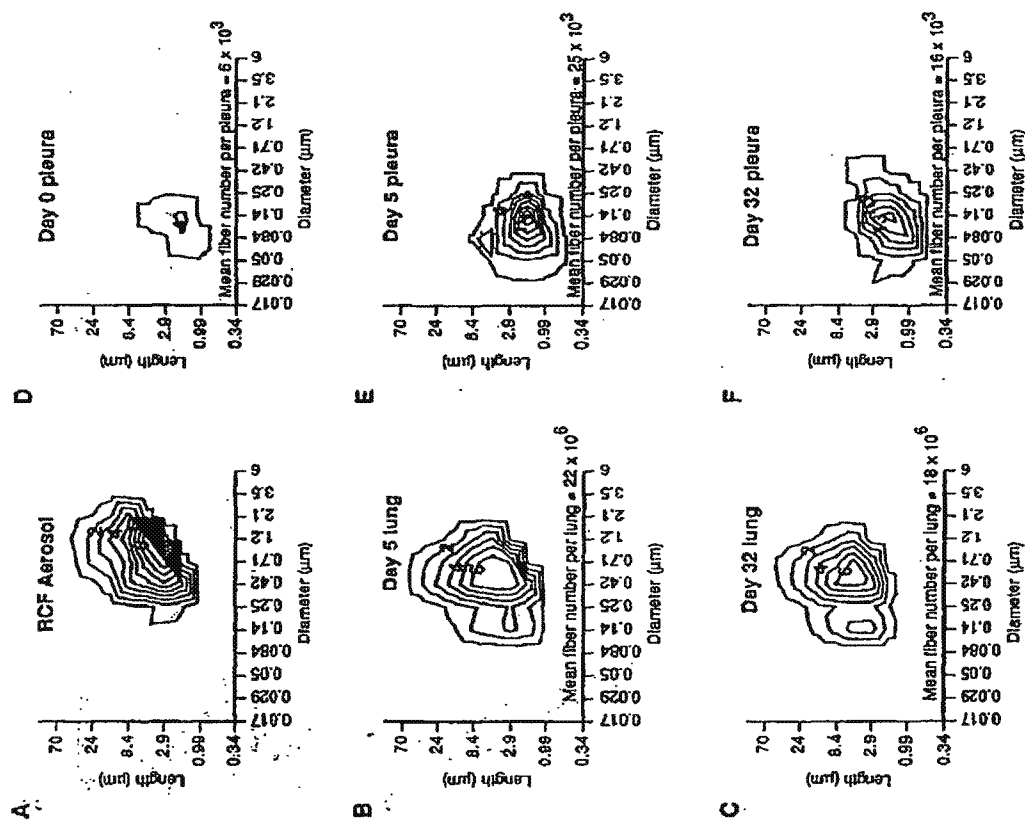


FIG. 2. Relative size distribution of fibers isolated from (A) aerosol, (B) Day 5 lung, (C) Day 32 lung, (D) Day 0 pleura, (E) Day 5 pleura, and (F) Day 32 pleura. Isobars were calculated based on histogram analysis of the aerosol cloud and fiber burden data (average of six rats). Median bin values are 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

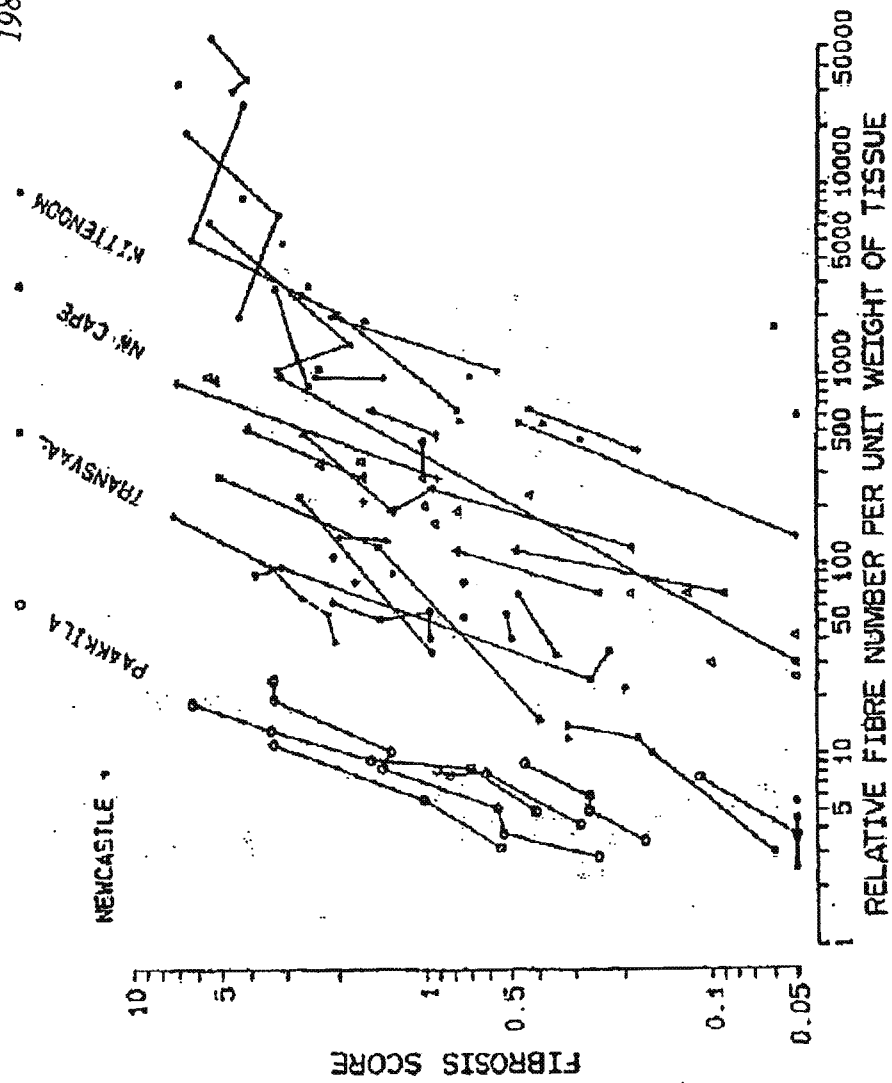


FIG. 5. Relationships when the parameter of fibre quantity is number.

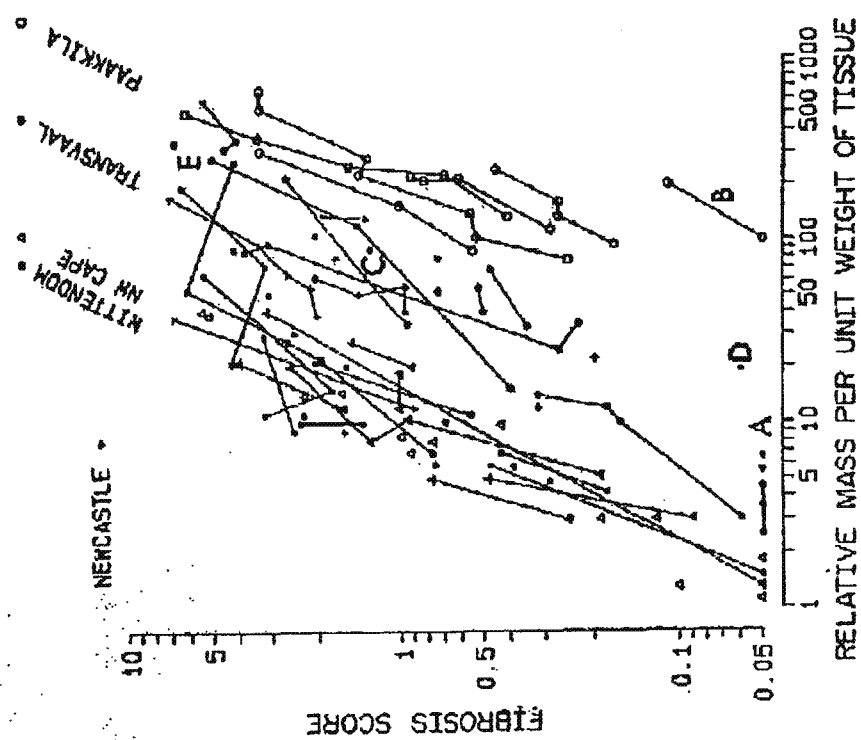


FIG. 3. Relationships when the parameter of fibre quantity is mass. Linked data points relate to tissue specimens from the same subject.

From: Timbrell et al.,
d Particles VI,

1988

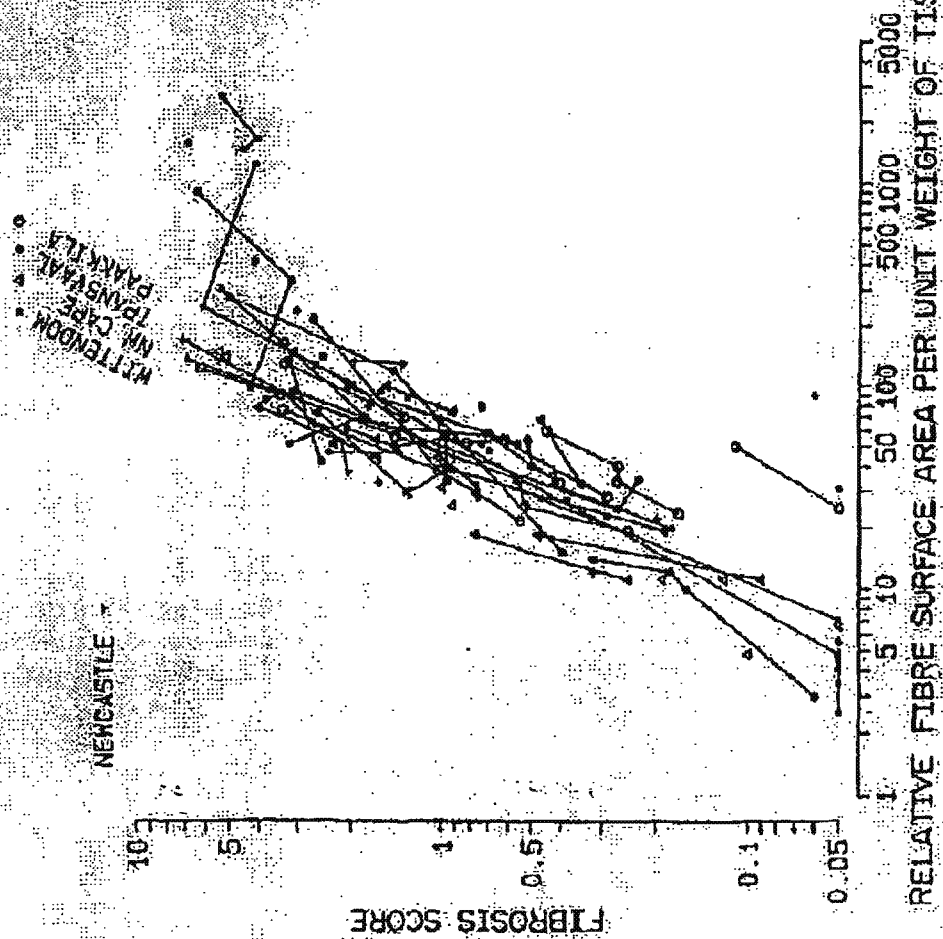
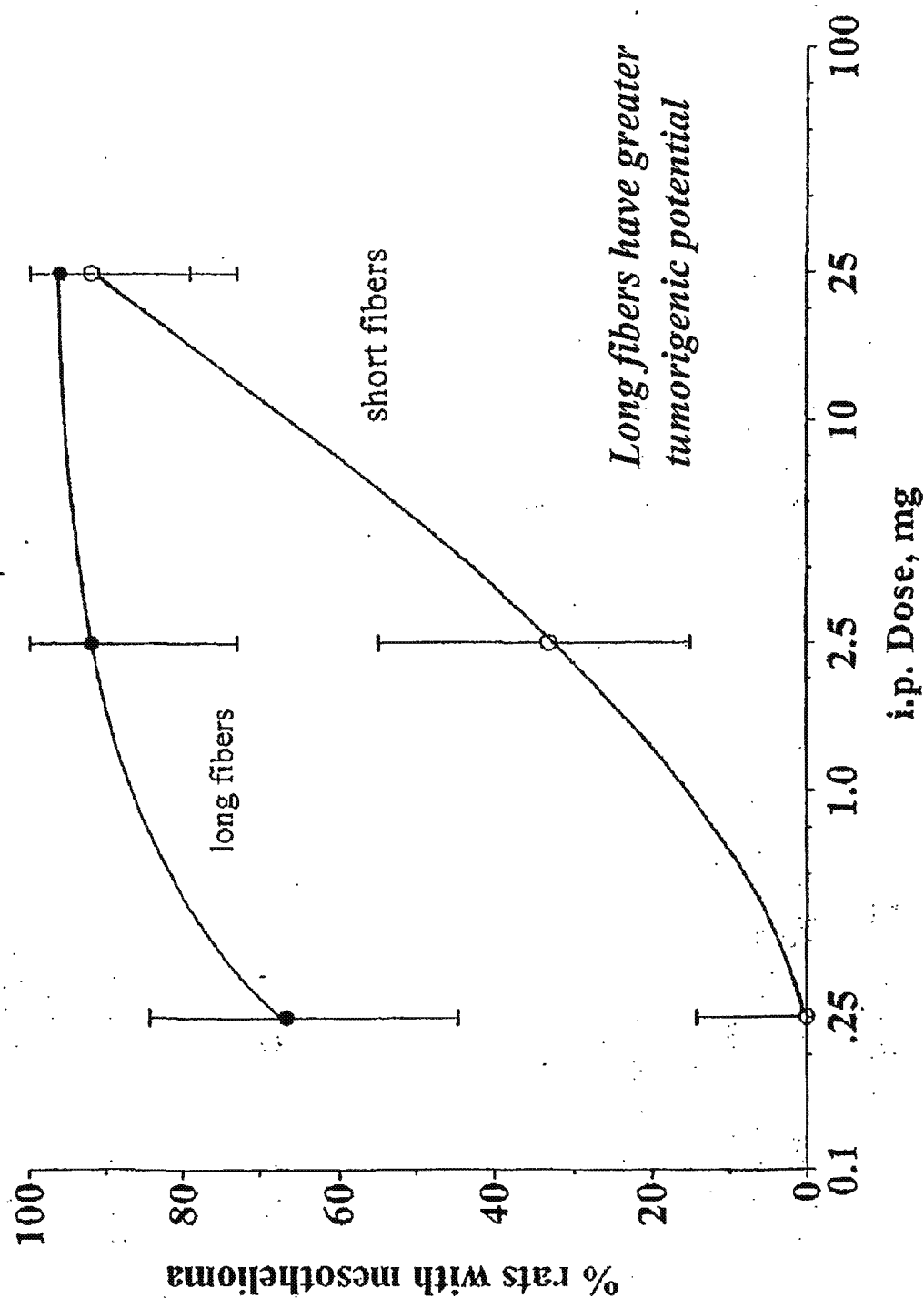


FIG. 4. Relationships when the parameter of fibre quantity is surface area.

Peritoneal mesothelioma After I.P. Injection of Long and Short Chrysotile (mean and 95% CI) (Davis and Jones, 1988)



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

Chrysotile or amosite (10 mg/m³) plus TiO₂ (10 mg/m³) or quartz (2 mg/m³),
1-year rat inhalation study plus 2-year observation period

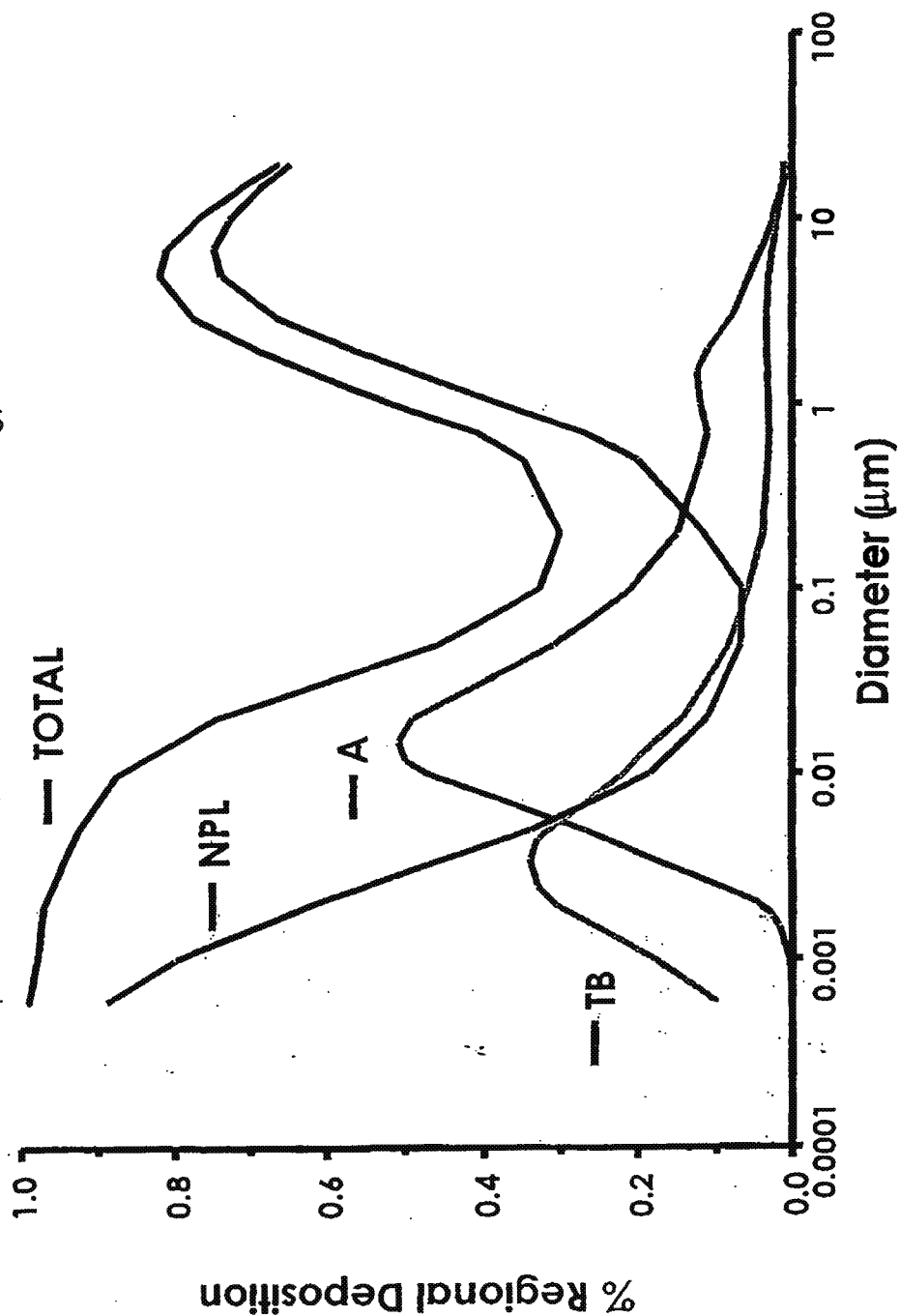
	Fiber retention	Pulm. fibrosis	Transport across visc. pleura	Lung tumors	Mesothelioma	Survival rate
Chrysotile + TiO ₂	↑	○	?	↑	↑ ^a	↑
Amosite + TiO ₂	○	○	↑	↑	↑	↑
Chrysotile + quartz	↓	↑	?	↑	↑ ^{a,b}	↓
Amosite + quartz	○	↑	↑	↑	↑ ^b	↓

○ no change; ↑ increased; ↓ decreased; (compared to asbestos alone)
(predicted lung burden of TiO₂ in "overload" range, ~10 mg/lung)

^a = no mesothelioma with chrysotile alone

^b = greater effect of added quartz than of added TiO₂

Fractional Deposition of Inhaled Particles in the Human Respiratory Tract (ICRP Model, 1994; Nose Breathing)



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

Classification

<u>Natural Fibers</u>		<u>Man-made Fibers</u>		
<u>Asbestos</u>	<u>Asbestiform</u>	<u>Vitreous</u>	<u>Crystalline</u>	<u>Organic</u>
<u>Serpentine</u>	<u>Fibrous clays</u>	Glass wool	Alumina	Para-aramic
chrysotile	polygorskite (antapulgite)	Glass filaments	Graphite	Cellulose
	sepiolite	Rockwool	Potassium titanate	Carbon
		Slagwool	Silicon carbide	
<u>Amphiboles</u>	<u>Other Fibrous Silicates</u>	Ceramic fibers	Sodium aluminum carbonate	
actinolite	wollastonite			Synthetic zeolites
amosite	nematolite (fibrous brucite)			
anthophyllite	talc			
crocidolite	zeolites: mordenite			
tremolite	erionite			

Mechanisms of Fiber Carcinogenesis

(Kane, 1996)

1. Fibers generate free radicals that damage DNA
 - direct generation of ROS and damage of DNA in cell free system
 - catalyze 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 intracellular 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 than 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 detoxification/re-toxification process is the mechanism distinguishing quartz and aluminosilicate 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 as 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

Asbestos 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 lucigenin-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 μm . 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 μm 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 serpentine 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 or 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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**Report on the Peer Consultation Workshop to Discuss a
Proposed Protocol to Assess Asbestos-Related Risk**

Prepared for:

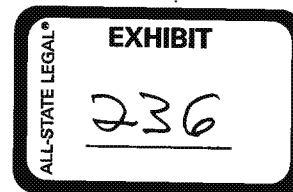
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FINAL REPORT
May 30, 2003



NOTE

This report was prepared by Eastern Research Group, Inc. (ERG), an EPA contractor, as a general record of discussion for the peer consultation workshop on a proposed protocol to assess asbestos-related risk. 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. EPA will use the information presented during the peer consultation workshop to determine whether the proposed risk assessment methodology can be used to support decisions at asbestos-contaminated sites. Except as specifically noted, no statements in this report represent analyses by or positions of EPA or ERG.

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LIST OF ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
EPA	U.S. Environmental Protection Agency
ERG	Eastern Research Group, Inc.
IARC	International Agency for Research on Cancer
IRIS	Integrated Risk Information System
NIOSH	National Institute for Occupational Safety and Health
PCM	phase contrast microscopy
SEM	scanning electron microscopy
SVF	synthetic vitreous fibers
TEM	transmission electron microscopy
μm	micrometers

EXECUTIVE SUMMARY

Eleven expert panelists participated in a peer consultation workshop to review a proposed protocol to assess asbestos-related risks. The protocol is documented in the report, "Technical Support Document for a Protocol to Assess Asbestos-Related Risk, Parts I and II" (Berman and Crump 1999, 2001). At the end of the 2½-day workshop, which was open to the public, the expert panelists drafted the following summary of their findings:

The peer consultation panel strongly endorsed the conceptual approach of developing an updated cancer risk assessment methodology that takes into account fiber type and fiber dimension. The opportunity is at hand to use substantial new information from epidemiology, experimental toxicology, and exposure characterization on what continues to be an extremely important societal issue—assessing the health risks associated with environmental and occupational exposures to asbestos. The panel recommended that EPA proceed in an expeditious manner to consider the panelists' conclusions and recommendations with a goal of having an updated asbestos risk assessment methodology. It is important that EPA devote sufficient resources so that this important task can be accomplished in a timely and scientifically sound manner. The panel urges that additional analyses underpinning the document, preparation of documentation, and further review be carried out in an open and transparent manner.

Prior to the workshop, the participants received draft copies of the "Methodology for Conducting Risk Assessments at Asbestos Superfund Sites Part 1: Protocol" and "Part 2: Technical Background Document." The panelists generally found that these documents did not provide a complete and transparent description of how the data were analyzed to support the conclusions presented. The incomplete documentation of methodology precluded the replication of the findings, in advance of the meeting, by several panelists. The methodology used was clarified by the comprehensive presentations that Drs. Berman and Crump made at the workshop. However, future drafts of these documents must

clearly describe the methodologies and include sufficient data, perhaps in appendices, such that the findings can be replicated.

The panelists made the following conclusions and recommendations:

- **Measurement methods.** Continuing advances have been made in the application of exposure measurement technology for asbestos fibers during the past two decades. These advances include the use of transmission electron microscopy (TEM) and allied techniques (e.g., energy dispersive x-ray detection, or EDS) as an alternative to phase contrast microscopy (PCM), thereby allowing the bivariate (i.e., length and width) characterization of fibers and fiber type. The proposed risk assessment methodology incorporates these advances in the development of an exposure index. The panel was in agreement that this aspect of the new risk assessment methodology represents a substantial advance over the existing methodology.
- **Integration of exposure and risk assessment models.** A key aspect of the proposed risk assessment methodology is a linking of specific exposure characterization methodology with exposure-response coefficients. It has been emphasized that any change in the exposure characterization metrics must be accompanied by changes in the exposure-response coefficients of the risk assessment models. This was emphasized in the report and the panelists endorsed this view.
- **Access to additional raw data sets.** The panelists strongly recommended that EPA make every attempt to acquire and analyze raw data sets from key human epidemiological studies. Where possible, it would also be desirable to obtain bivariate (i.e., length and diameter) fiber exposure information for these re-analyses. Several panelists believed that review of additional data sets offers substantial opportunity for improving the proposed risk assessment methodology. In the event that raw data cannot be obtained due to confidentiality reasons or other restrictions, the panelists suggested that the authors consider asking those who have access to the data to conduct the necessary statistical analyses and communicate their results directly to EPA for further consideration.
- **Fiber diameter.** The proposed risk assessment methodology uses a diameter cut-off of 0.5 micrometers (μm) for considering fibers. The report states that fibers 0.7 μm in diameter can reach the respiratory zone of the lung. A few panel members indicated that the fiber diameter cut-off could be as high as 1.5 μm during oral breathing. The 0.4 μm cut-off came from rat data, but larger diameters would be expected to be respirable in humans. There was general agreement that the diameter cut-off should be between 0.5 and 1.5 μm . This issue is deserving of further analysis.

- **Fiber length.** The Berman and Crump analyses made a significant contribution by obtaining and analyzing membrane filters from the animal inhalation studies in Edinburgh and conducting quality-assured bivariate length and distribution analyses by TEM—thereby greatly reducing the uncertainty of the exposure side of the exposure-response relationship for chronic fiber exposure in rats. Unfortunately, correspondingly detailed information on bivariate size distribution is not available for humans. This leads to the need to use the animal data, although one must always recognize the uncertainties associated with interspecies extrapolations such as anatomic characteristics and respirability between species. Future analyses may benefit from using other available laboratory animal data sets and human data sets.

The fiber length distributions for the human cohort exposures are much more uncertain. For the Wittenoom, Quebec, and South Carolina cohorts, there are limited fiber length distribution data based on TEM analysis from historic membrane filter samples, but only fiber categories longer than 5 μm and longer than 10 μm were counted. For all other cohorts, the measurements were limited to PCM fiber counts for all fibers greater than 5 μm in length in some, and particle counts (10x objective) on midget impinger samples in others. Both methods do not measure thin fibers, do not discriminate between asbestos and other mineral particles, and provide no information on the concentrations of fibers longer than 10, 20, or 40 μm , or inter-laboratory variations in optical resolution and counting rules. As one approach to addressing the varying uncertainty in assessing exposure in the different studies, Berman and Crump used the available information to make adjustments to the uncertainty ranges in the exposure-response coefficients. The workshop panel welcomed this initiative but suggested alternative approaches (see "Methods," below).

Some panelists felt that an Exposure Assessment Workshop, with participants having a broad range of expertise, could evaluate the uncertainties in historic occupational data sets' exposure measurements. They felt such a workshop could result in a more confident assessment of exposure-response relationships for populations exposed to a variety of amphiboles, chrysotile, and mixtures. With incorporation of other available knowledge on fiber type, process, smoking (if available), and the relative number of excess lung cancer and mesothelioma, it may well be possible to gain a much clearer understanding of the roles of these variables as causal factors for these asbestos-associated cancers. In addition, the workshop would prove valuable in further discussion of mineralogical, geological, and industrial hygiene issues with regard to application of the model to risk assessment in environmental sites of concern.

The Berman and Crump index assigns zero risk to fibers less than 5 μm in length. Fibers between 5 and 10 μm are assigned a risk that is one three-hundredth of the risk assigned to fibers longer than 10 μm . Panelists agreed that there is a considerably greater risk for lung cancer for fibers longer than 10 μm . However, the panel was uncertain as to an exact cut size for length and the magnitude of the relative potency. The panelists also agreed that the available

data suggest that the risk for fibers less than 5 μm in length is very low and could be zero. This specific issue was addressed by an expert panel convened by the Agency for Toxic Substances and Disease Registry (ATSDR) in October 2002. Some panelists suggested that, for mesothelioma, greater weight should perhaps be assigned to fibers in the 5 to 10 μm length range and to thinner fibers.

- **Fiber type.** For *mesothelioma*, the panelists supported the use of different relative carcinogenic potencies for different fiber types. The panelists unanimously agreed that the available epidemiology studies provide compelling evidence that the carcinogenic potency of amphibole fibers is two orders of magnitude greater than that for chrysotile fibers. There was some discussion about the precise ratio expressed due to questions about the availability of exposure data in existing studies (e.g., Wittenoom). There was recognition that time since first exposure is an important factor in determining risk for mesothelioma and some discussion is needed on the importance of duration and intensity of exposure.

For *lung cancer*, the panelists had differing opinions on the inferences that can be made on the relative potency of chrysotile and amphibole fibers. Some panelists supported the finding that amphibole fibers are 5 times or more potent for lung cancer than are chrysotile fibers. Other panelists did not think the statistical analyses in the draft methodology document supports this relative potency and wondered if additional review of the epidemiological data might identify factors other than fiber type (e.g., industry considered) that provide further insights on the matter. These other factors can then be considered when the risk assessment is applied.

- **Cleavage fragments.** The panel knew of little data to directly address the question as to whether cleavage fragments of equal durability and dimension as fibers would have similar or dissimilar potency for lung cancer. The general view is that data indicate that durability and dimension are critical to pulmonary pathogenesis. Therefore, it is prudent at this time to assume equivalent potency for cancer in the absence of other information to the contrary. Consideration of conducting a rat inhalation study using tremolite cleavage fragments was recommended to address this issue. For mesothelioma, it was viewed that thin fibers greater than 5 μm in length are more important. Cleavage fragments that do not meet these criteria would not contribute to risk of mesothelioma.

- **Other amphiboles.** The panel agreed with the report's conclusion that the potency of currently regulated and unregulated amphibole fibers should be considered equal based on the reasoning that similar durability and dimension would be expected to result in similar pathogenicity.

- **Methods.** The panelists extensively discussed the approach to conducting the meta-analysis of the large number of epidemiological studies. A number of the panelists urged that consideration be given to using more traditional approaches that would include development and application of specific criteria for inclusion of studies into the exposure-response analysis, examination of

heterogeneity and sources of the heterogeneity, and the use of sensitivity analysis to identify influential studies.

The panelists also urged, in the study-specific analysis, exploration of alternative exposure-response models other than the lung cancer and mesothelioma risk models EPA has been using since 1986. This would possibly include non-linear response models (e.g., log-linear models), examination of separate effects for concentration and duration, time since first exposure, time since cessation of exposure, possibly dropping the " α factor," and different methods for measurement error. The adequacy of different models should be examined using goodness of fit statistics across all studies. The possibility of internal analyses should be re-examined (i.e., it may be possible to obtain partial data, such as age-specific person years data, from authors). Exploration of non-linearity should also include shape of the curve in the low exposure area.

The panelists also urged alternative approaches to meta-analyses. In particular, panelists recommended meta-regression using original (untransformed) exposure-response coefficients, in which predictor variables include the estimated percentage of amphiboles, percentage of fiber greater than 10 μm , and categorical grouping of studies according to quality. Original exposure-response coefficient variances should be used in conjunction with random effects models in which residual inter-study variation is estimated. Analyses restricted to long latency and a predictor variable for industry type should be considered. A prior distribution for inter-study residual variance might also be considered. Meta-regression will allow simple inspection of likelihoods to consider the importance of different predictor variables. Sensitivity analyses should be conducted in which the inclusion or exclusion of specific studies or groups of studies is evaluated.

■ **Cigarette smoking.** Most panelists felt strongly that future analyses need to pay more attention to the effects of smoking on the lung cancer exposure-response model and extrapolations to risk. However, the current data sets have variable and limited information available on smoking. The panelists noted that smoking is the primary cause for lung cancer, but the lung cancer dose-response relationship for smoking is complex due to the effects of smoking duration, intensity, and cessation.

The impact of smoking has effects on both the estimation and the application of the model for projecting risk of lung cancer due to asbestos exposure. This may be an especially critical issue for low-exposure extrapolation. With respect to estimation, accepting the form of the proposed model, the effect of smoking may require different K_L values for smokers and non-smokers. The panelists recognized that there is limited epidemiologic data to address this issue, but recommend that it be investigated. With respect to applying the model to make risk projections for any future cohort, the background rate of lung cancer employed in the model needs to be carefully determined to capture the smoking behavior of the cohort.

- **Localized tremolite exposures.** During the course of public comments, the panel received input from several individuals who expressed concerns about environmental exposures to tremolite asbestos from localized geologic formations in California. The individuals suggested that inadequate attention had been given to characterization of the exposures to residents of these communities. While the panel was not in a position or charged with the evaluation of this issue, the panel did feel that this was a potentially serious matter deserving of attention by the appropriate public health authorities. Evaluation of these kinds of situations would benefit from the use of the improved risk assessment methodology being considered.

The remainder of this report summarizes the discussions and observations that led to these findings, reviews the panelists' comments on many topics not listed in this executive summary, and documents the observer comments provided at the workshop.

1. INTRODUCTION

This report summarizes a peer consultation by 11 expert panelists of a proposed protocol to assess asbestos-related risks. Contractors to the U.S. Environmental Protection Agency (EPA) developed the proposed protocol, which is documented in a report titled: "Technical Support Document for a Protocol to Assess Asbestos-Related Risk" (Berman and Crump 2001). The purpose of the peer consultation workshop was to provide EPA feedback on the scientific merit of the proposed protocol. The peer consultation workshop took place in a meeting open to the public on February 25-27, 2003, in San Francisco, California.

This report summarizes the technical discussions among the expert panelists and documents comments provided by observers. These discussions largely focused on three topic areas: interpretations of the epidemiology and toxicology literature, the proposed exposure index, and general questions about key assumptions and inferences in the protocol. The remainder of this introductory section presents background information on the protocol (Section 1.1), describes the scope of the peer consultation workshop (Section 1.2), and reviews the organization of this report (Section 1.3).

1.1 Background

EPA's current assessment of asbestos toxicity is based primarily on an asbestos review completed in 1986 (EPA 1986) and has not changed substantially since that time. The 1986 assessment considers six mineral forms of asbestos and all asbestos fiber sizes longer than 5 micrometers (μm) to be of equal carcinogenic potency. However, since 1986, asbestos measurement techniques and the understanding of how asbestos exposure contributes to disease have improved substantially. To incorporate the knowledge gained over the last 17 years into the agency's toxicity assessment for asbestos, EPA contracted with Aeolus, Inc., to develop a proposed methodology for conducting asbestos risk assessments. The proposed methodology distinguishes between fiber sizes and fiber types in estimating

potential health risks related to asbestos exposure. The methodology also proposes a new exposure index for estimating carcinogenic risk.

As a key step in determining the scientific merit of the proposed risk assessment methodology, EPA decided to obtain expert input on the draft report through a peer consultation workshop. The purpose of the workshop was to obtain feedback from subject-matter experts during the development stage of the proposed risk assessment methodology; the workshop was not an official peer review. Eastern Research Group, Inc. (ERG), organized and implemented the peer consultation workshop under a contract to EPA.

1.2 Scope of the Peer Consultation Workshop

The peer consultation involved many activities before the workshop (see Section 1.2.1), at the workshop (see Section 1.2.2), and after the workshop (see Section 1.2.3). The following subsections describe these activities.

1.2.1 Activities Prior to the Peer Consultation Workshop

This section describes the major activities ERG and the expert panelists conducted prior to the peer consultation workshop:

- **Select expert panelists.** ERG selected the expert panelists for the peer consultation workshop. ERG sought to compile a panel of experts with broad experience and expertise in the following disciplines: toxicology, epidemiology, biostatistics, asbestos sampling and analytical methods, EPA's human health risk assessment guidelines, and asbestos-related environmental and occupational health issues. Appendix A lists the expert panelists ERG selected, and Appendix B includes brief biographies that summarize the panelists' areas of expertise.

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. To ensure the peer consultation offered a balanced perspective, ERG intentionally selected expert panelists with a broad range of affiliations (e.g., academia, consulting, state and federal agencies). When searching for panelists, ERG asked all candidates to disclose real or perceived conflicts of interest.

- ***Prepare a charge to the expert panelists.*** ERG worked with EPA to prepare written guidelines (commonly called a "charge") for the peer consultation workshop. The charge includes 12 specific questions, organized into 4 topic areas. Discussions at the workshop largely addressed the technical issues raised in the charge, but the expert panelists were encouraged to discuss other relevant matters that were not specifically addressed in the charge questions. A copy of the charge is included in Appendix B.
- ***Distribute review documents and other relevant information.*** Several weeks prior to the peer consultation workshop, ERG sent every panelist copies of the charge and the proposed risk assessment methodology (Berman and Crump 2001). These items formed the basis of the technical discussions at the workshop. In addition, ERG distributed several additional publications on related topics (see Table 1, at the end of this section, for list of the publications). The supplemental publications were provided largely in response to panelists' requests for further background information on selected issues. The panelists also circulated publications amongst themselves on specific topics. Finally, one of the meeting chairs noted for the record that, upon arriving in San Francisco, he also received a memo and copies of many abstracts and other information from Cate Jenkins of EPA. The meeting chair offered to share these materials with other panelists during the workshop.
- ***Obtain and compile the panelists' premeeting comments.*** After receiving the workshop materials, the panelists were asked to prepare their initial responses to the charge questions. Booklets containing the premeeting comments were distributed to the expert panelists before the workshop and were made available to observers at the workshop. 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 Peer Consultation Workshop

The 11 expert panelists and approximately 75 observers attended the peer consultation workshop, which was held at the Westin St. Francis Hotel in San Francisco, California, on February 25-27, 2003. The workshop was open to the public, and the workshop dates and times were announced in the

Federal Register. Appendix C lists the observers who confirmed their attendance at the workshop registration desk. The workshop schedule generally followed the agenda, presented here as Appendix D.

The workshop began with introductory remarks from Ms. Jan Connery (ERG), the facilitator of the peer consultation. Ms. Connery welcomed the expert panelists and observers, stated the purpose of the workshop, identified the document being reviewed, and explained the procedure for observers to make comments. Mr. Richard Troast (EPA) then provided background information on the review document and EPA's ongoing efforts to assess asbestos toxicity (see Section 1.1). Mr. Troast identified the main differences between EPA's existing asbestos risk assessment methodology (EPA 1986) and the proposed methodology (Berman and Crump 2001). Mr. Troast noted that the expert panelists' feedback will ultimately help EPA complete its update of asbestos health risks for the Integrated Risk Information System (IRIS); he clarified that the final IRIS update will be subject to peer review or Science Advisory Board review before being implemented. Following these opening remarks, Dr. Wayne Berman and Dr. Kenny Crump—the authors of the proposed methodology—presented detailed information on the review document; Section 2 of this report summarizes their presentations.

After the background presentation, Dr. Roger McClellan and Dr. Leslie Stayner chaired the technical discussions that followed. For the remainder of the meeting, the panelists engaged in free-flowing discussions when answering the charge questions and addressing additional topics not specified in the charge. Observers were given the opportunity to provide verbal comments three different times during the workshop; these observer comments are documented in Appendix E. Representatives from EPA and the document authors provided clarifications on the proposed methodology periodically throughout the 2½-day workshop.

1.2.3 Activities Following the Peer Consultation Workshop

The primary activity following the peer consultation workshop was preparing this summary report. A technical writer from ERG who attended the meeting prepared a draft of this report, which ERG distributed to the 11 expert panelists and asked them to verify that the draft accurately reflects the tone and substance of the panelists' discussions at the workshop. After incorporating the panelists' suggested revisions to the draft report, ERG submitted the final report (i.e., this report) to EPA.

1.3 Report Organization

The structure of this report follows the order of the technical discussions during the meeting. Section 2 summarizes Dr. Berman and Crump's background presentations. Sections 3 through 6 are records of the panelists' discussions on the four main topic areas: interpretations of the epidemiology and toxicology literature (Section 3), the proposed exposure index (Section 4), general questions (Section 5), and conclusions and recommendations (Section 6). Finally, Section 7 provides references for all documents cited in the text.

The appendices to this report include background information on the peer consultation workshop. This information includes items that were on display at the workshop and items generated since the workshop (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 peer consultation workshop (Appendix C).
- Agenda for the peer consultation workshop (Appendix D).
- Observer comments provided at the peer consultation workshop (Appendix E).
- Observer post-meeting comments (Appendix F).

Table 1
References ERG Provided to the Expert Panelists

Berman, DW and Crump, K. 1999. Methodology for Conducting Risk Assessments at Asbestos Superfund Sites; Part 1: Protocol. Final Draft. Prepared for U.S. Environmental Protection Agency. February 15, 1999.
Berman, DW and Crump, K. 2001. Technical Support Document for a Protocol to Assess Asbestos-Related Risk. Final Draft. Prepared for U.S. Department of Transportation and U.S. Environmental Protection Agency. September 4, 2001.
Berman, DW, Crump, K., Chatfield, E., Davis, J. and A. Jones. 1995. The Sizes, Shapes, and Mineralogy of Asbestos Structures that Induce Lung Tumors or Mesothelioma in AF/HAN Rats Following Inhalation. Risk Analysis. 15:2,181-195.
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Committee on Nonoccupational Health Risks of Asbestiform Fibers. Breslow, L. Chairman. 1984. Asbestiform Fibers Nonoccupational Health Risks. Washington, DC: National Academy Press.
EPA 1986. Airborne Asbestos Health Assessment Update. U.S. Environmental Protection Agency. EPA 600/8-84-003F. 1986.
NIOSH Interdivisional Fiber Subcommittee Report. Prepared by the NIOSH Interdivisional Fiber Subcommittee. 1999.

2. BACKGROUND ON THE PROPOSED PROTOCOL TO ASSESS ASBESTOS-RELATED RISK

This section summarizes presentations given by the principal authors of the proposed risk assessment methodology. These presentations were given because several panelists asked ERG, prior to the peer consultation workshop, if the authors would provide detailed background information on how the methodology was developed. This section reviews the major presentation topics, but does not present the panelists' comments on the proposed protocol. Sections 3 through 6 document the expert panelists' technical feedback on the protocol.

■ **Motivation for developing the proposed protocol.** Dr. Berman identified several reasons for developing the updated protocol for assessing asbestos-related risks. These reasons include EPA's existing asbestos models being inconsistent with inferences from the scientific literature, the need for having uniformly-applied sampling and analytical procedures to measure asbestos characteristics most predictive of risk, and the belief that EPA's current asbestos risk assessment methodology may not be adequately protective in some circumstances. To improve upon the current methodology, the authors intended to develop a risk assessment model that adequately predicts cancer risk in all studied environments and can therefore be applied with much greater confidence to environments that have not been studied. Dr. Berman outlined the general approach taken to develop the proposed protocol, as summarized in the following bulleted items.

Dr. Berman provided background information on and definitions for asbestos, other fibrous structures, asbestos morphology, and cleavage fragments. He also described the capabilities and limitations of the analytical techniques that have been used to characterize asbestos exposures, such as midjet impingers, phase contrast microscopy (PCM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Dr. Berman explained how differences in these analytical techniques must be critically evaluated when comparing results reported in all epidemiological and other types of studies that examine asbestos exposure. Dr. Berman also stressed that it is not just differences in analytical techniques, but choice of specific methods for each analytical technique that affects results. Further information on these topics is included in Chapter 4 of the proposed protocol (Berman and Crump 2001).

■ **Re-analysis of human epidemiological data.** Dr. Crump described how the authors evaluated the human epidemiological data. He displayed a list of the studies that were considered, noting that he had access to raw, individual-level data for three occupational cohorts: chrysotile textile workers in South Carolina, United States; crocidolite miners in

Wittenoom, Australia; and chrysotile miners and millers in Quebec, Canada. All data sets with exposure data were considered in the analysis, and criteria were not established for selecting studies. Dr. Crump then presented findings for asbestos-related risks for lung cancer and mesothelioma.

For lung cancer, Dr. Crump first reviewed EPA's existing lung cancer model for asbestos exposure (see equation 6.1 in the proposed protocol), which relates the relative risk of lung cancer mortality linearly to cumulative asbestos exposure, with a 10-year lag time. Dr. Crump noted that the model predicts that relative risk for developing lung cancer remains constant after asbestos exposure ceases—an assumption he showed was reasonably consistent with findings from epidemiological studies. Dr. Crump also discussed how the model assesses interactions between exposures to cigarette smoke and to asbestos—an issue the panelists revisited several times later in the workshop (e.g., see Section 3.1.1 and the executive summary). Dr. Crump presented a series of tables and figures demonstrating the adequacy of multiple lung cancer models: first using EPA's existing lung cancer model, next using a modified version of the model that accounts for differences in the background rates of lung cancer, and finally using the proposed lung cancer model, which considers an exposure index that assigns greater carcinogenic potency to amphibole fibers and to longer fibers.

Similarly, Dr. Crump reviewed the performance of EPA's mesothelioma model for asbestos exposures (see equation 6.11 in the proposed protocol), which predicts that mesothelioma risks vary linearly with the average asbestos exposure and increase quadratically with time from onset of exposure. Dr. Crump presented several tables and graphs indicating how well EPA's existing model and the proposed protocol fit the human epidemiological data. He made several conclusions about the existing risk model, including that mesothelioma risk coefficients varied considerably across the cohorts and the risk coefficients were generally higher for cohorts exposed primarily to amphibole fibers, compared to those exposed primarily to chrysotile fibers. Dr. Crump also noted that the data did not support consideration of a sub-linear or threshold dose-response relationship. This latter point generated considerable discussion later in the workshop (e.g., see Section 4.3).

Dr. Crump then described the meta-analysis the authors conducted to evaluate the relative potency of amphibole and chrysotile fibers. First, he explained how the authors weighted the different studies in the meta-analysis, based on uncertainty factors assigned to the individual studies. Dr. Crump identified the four uncertainty factors and described generally how each factor was assigned. Sources of uncertainty included representativeness of air sampling data, the availability of conversion factors to express exposures in terms of PCM concentrations, and whether data on exposure duration were available. Dr. Crump then highlighted the main conclusions from the meta-analysis. For lung cancer, the meta-analysis suggested that amphibole fibers are approximately five times more potent than are chrysotile fibers, but the difference in potency was not statistically significant (i.e., the authors could not reject the hypothesis that

chrysotile fibers and amphibole fibers are equally potent). For mesothelioma, the meta-analysis suggested that chrysotile fibers are 0.002 times as potent as amphibole fibers, and the difference in potency was statistically significant.

- **Inferences drawn from the broader literature.** Dr. Berman described how the authors incorporated inferences from the broader scientific literature into the proposed protocol. He reviewed key findings on how various mechanisms are biologically related to how asbestos causes disease. These mechanisms included respiration, deposition, degradation, clearance, translocation, and tissue-specific biological responses. Chapter 7 of the review document provides detailed information on the relevance of these mechanisms, with emphasis on the influence of fiber type and fiber dimension.
- **Derivation of the exposure index.** Dr. Berman explained how the authors derived the exposure index, which is largely based on an earlier re-analysis (Berman et al. 1995) of six animal inhalation studies conducted by a single laboratory. That re-analysis found that lung tumor incidence is adequately predicted using an exposure index that assigns no carcinogenic potency to fibers shorter than 5 μm , relatively low carcinogenic potency to fibers with lengths between 5 and 40 μm and diameters less than 0.4 μm , and the greatest carcinogenic potency to fibers longer than 40 μm and thinner than 0.4 μm . However, these findings could not be applied directly to the human epidemiological data, because the epidemiological studies do not include exposure measurements that quantify the relative amounts of asbestos fibers shorter and longer than 40 μm .

Dr. Berman noted that the proposed protocol includes an *ad hoc* assumption that the fiber size weighting factors optimized from the laboratory animal studies can be applied to humans, but with a length cut-off of 10 μm in the exposure index, rather than a cut-off of 40 μm . Dr. Berman emphasized that this assumption was made to model the critical characteristics of asbestos in a manner that reasonably captures cancer risks observed across multiple epidemiological studies. He acknowledged that asbestos potency is likely a continuous function of fiber length, but the exposure measurements from the available animal and epidemiological studies do not support incorporating such a continuous function in the exposure-response model. The panelists commented on the proposed exposure index when discussing topic area 3 (see Section 4).

Dr. Berman also noted that the authors selected a conservative set of dose-response coefficients (see Table 6-30 of the review document), rather than using the optimized ones from the animal studies (see Table 6-29). However, the conservative and optimized dose-response coefficients were reasonably consistent: none of the conservative coefficients differed by more than a factor of 4 from the corresponding optimized ones.

- **Conclusions regarding proposed protocol.** Dr. Berman indicated that the proposed protocol is substantially more consistent with inferences documented in the scientific literature (i.e., that

long, thin structures contribute most to risk) than EPA's existing risk assessment methodology. Further, the proposed protocol provides a better fit to cancer risks observed in the human epidemiological studies than does EPA's existing model, and the proposed protocol appears to underestimate risks of lung cancer and mesothelioma less frequently and to a lesser degree than the existing approach. Finally, by recommending use of a standardized analytical method that links directly to the exposure index, the proposed protocol will help ensure that future risk assessments are conducted in a consistent fashion and their results can be readily compared from one study to the next.

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3. COMMENTS ON TOPIC AREA 1: INTERPRETATIONS OF THE EPIDEMIOLOGY AND TOXICOLOGY LITERATURE

This section summarizes the panelists' discussions on the interpretations of the epidemiology and toxicology literature. The meeting co-chairs—Dr. McClellan and Dr. Stayner—facilitated the discussions on this topic area, which focused first on lung cancer (see Section 3.1) and then on mesothelioma (see Section 3.2). This section presents a record of discussion of the topics mentioned during the workshop. Several panelists referred to their premeeting comments (see Appendix B) for additional suggestions for how the review of epidemiology and toxicology literature can be improved.

3.1 Lung Cancer

The panelists discussed at length whether the epidemiology and toxicology literature support the proposed protocol's finding for how lung cancer potency varies with fiber type and fiber length. This section summarizes these discussions, first on fiber type (Sections 3.1.1 and 3.1.2) and then on fiber length (Sections 3.1.3 and 3.1.4). General issues regarding the lung cancer evaluation are presented in Section 3.1.5.

3.1.1 Lung Cancer and Fiber Type: Inferences from the Epidemiology Literature

According to the proposed risk assessment methodology, amphibole fibers have a 5-fold greater lung cancer potency than do chrysotile fibers. The panelists had differing opinions on whether this finding is consistent with the epidemiology literature. On the one hand, some panelists indicated that the epidemiology literature is consistent with amphibole fibers being more potent for lung cancer, though the magnitude of this increase may not be known precisely. One panelist noted, for example, that multiple analyses (e.g., Hodgson and Darnton 2000, Berman and Crump 2001, and the statistical analyses a panelist presented during this discussion) all point to a consistent increased lung cancer potency for amphibole fibers compared to chrysotile fibers, albeit a small increase. On the other hand, other

panelists did not believe the epidemiology literature supports this conclusion, for reasons stated below. Finally, other panelists were not convinced that the epidemiology literature supports the higher lung cancer potency for amphibole fibers, but they believed the difference in potency seems likely based on evidence from the animal toxicology studies (see Section 3.1.3) and lung burden studies. A summary of the panelists' discussion on this topic follows:

- **Comments on specific publications.** Several panelists cited specific studies to support their positions on the relative lung cancer potency of chrysotile and amphibole fibers, but the panelists often had differing opinions on the inferences that should be drawn. The panelists mentioned the following specific studies:

Some panelists noted that a recent re-analysis of 17 cohorts (Hodgson and Danton 2000) indicates that the lung cancer potency for amphibole fibers is 10 to 50 times greater than that for chrysotile fibers. One panelist did not agree with this finding, due to the crude approach the article uses to characterize relative potency. Specifically, this panelist noted that carcinogenic potency was calculated by dividing the overall relative risk for a given cohort by the average exposure for the entire cohort, even for cohorts where the data support more sophisticated exposure-response modeling. He was particularly concerned about the authors' decision to omit the cohort of South Carolina textile workers from the meta-analysis. This decision was apparently based on the South Carolina cohort being an outlier, due to its much higher lung cancer potency when compared to other studies. The panelist noted, however, that the lung cancer risk for the South Carolina cohort is not unusually high when compared to other cohorts of textile workers. The panelist was concerned that omitting this study might have biased the article's finding regarding relative lung cancer potency. No other panelists discussed the review article.

One panelist cited a study of Quebec chrysotile miners and millers (Liddell et al. 1997, 1998) that reports that increased lung cancer risk was limited to the mining region with the highest level of tremolite asbestos, after correction for smoking and exposure. The article was distributed to the panelists on the first day of the workshop, but no panelists commented further on the study.

- One panelist noted that his review of multiple textile cohorts (Stayner, Dankovic, and Lemen 1996) found relatively small differences in lung cancer potency, even though some of the cohorts were exposed to asbestos mixtures containing different proportions of amphibole fibers.

One panelist indicated that further evidence on how fiber types relates to lung cancer potency can be gleaned from epidemiological studies that were not included in the meta-analysis due to inadequate exposure data for exposure-response modeling. Examples include a study of non-occupationally exposed women from two chrysotile asbestos mining regions (Camus et al. 1998) and a study of railroad workers employed by shops that processed different proportions of amphibole fibers (Ohlson et al. 1984). Both studies, she noted, provide evidence that amphibole fibers exhibit greater lung cancer potency. This panelist added that studies of auto mechanics have provided no convincing evidence of increased lung cancer due to chrysotile exposure, though she acknowledged that the absence of an effect might reflect the short fiber length in the friction brake products. One panelist cautioned about inferring too much from these studies regarding fiber type because they were not controlled for other factors, such as fiber length and level of exposure.

One panelist added that a recent study of a cohort of Chinese asbestos plant workers (Yano et al. 2001) should be considered in future updates to the proposed protocol; the workers in the cohort had increased risks for lung cancer and were reportedly exposed to "amphibole-free" chrysotile asbestos. However, another panelist cited a publication (Tossavainen et al. 2001) that indicates that asbestos from many Chinese chrysotile mines actually does contain varying amounts of amphibole fibers.

Several panelists noted that the proposed protocol's meta-analysis found a 5-fold difference in lung cancer potency between amphibole and chrysotile fibers. However, other panelists indicated that the reported difference was not statistically significant. Some panelists had additional reservations about the authors' meta-analysis, as summarized in the following bulleted items.

- **Comments on the meta-analysis approach.** Several panelists commented on alternate approaches the authors could have used to conduct their meta-analysis of the epidemiology studies. One panelist noted that the lung cancer potencies reported by the various studies exhibit considerable heterogeneity. In such cases, meta-regression is conventionally used to identify which factors account for the variability in the results (i.e., in the lung cancer potencies). This panelist suggested that the meta-analysis should have considered other factors in addition to fiber type and dimension; such other factors could include industry, follow-up time for the cohort, and estimated percentage of amphibole fibers in the exposures, to the extent that data on these other factors are available.

To demonstrate how more detailed investigation might reveal further insights, one panelist presented his own initial statistical analysis of the epidemiological studies. This analysis used a fixed effects model and a random effects model, both inverse weighted by the variance of the studies. His analysis examined how industry and fiber type contribute to the heterogeneity

observed among the cohorts and found that the industry of the cohort appears to be a stronger predictor than fiber type. The panelist explained that the purpose of displaying his statistical analysis was to highlight how other approaches to conducting meta-analysis can offer different insights on the epidemiological data. This panelist recommended that the authors conduct similar meta-regression analyses to investigate the importance of various variables on the lung cancer potency.

This panelist also demonstrated how a sensitivity analysis might yield additional information on influential studies. Using a fixed effects model, the panelist first showed how lung cancer potency factors (K_L) vary with exposure to chrysotile fibers, amphibole fibers, and mixed fiber types. When all epidemiological studies were considered in his analysis, the amphibole fibers were found to be three times more potent than the chrysotile fibers. When the cohort of chrysotile miners and millers from Quebec was omitted from this analysis, however, the amphibole fibers were found to be nearly two times less potent than the chrysotile fibers. Conversely, when the cohort of textile workers from South Carolina was omitted, the amphibole fibers were found to be more than ten times more potent than the chrysotile fibers. Given that the conclusions drawn about the relative potency of chrysotile and amphibole fibers appear to be highly sensitive to whether single studies are omitted from the analysis, this panelist was more skeptical about whether the increased potency of amphibole fibers is a robust finding. He recommended that the authors, when completing the proposed protocol, conduct similar sensitivity analyses to help reveal the factors or studies that appear to contribute most to lung cancer.

Another panelist agreed with this feedback, and provided further comments on the meta-analysis, noting that these analyses typically start with establishing criteria for study inclusion. After selecting studies to evaluate, she said, various statistical analyses can be used to test hypotheses and to understand the concordance and disparity among the individual studies. The panelist thought such an approach is needed to help understand the variability in potency factors observed across the multiple studies and to identify for further analysis the studies found to be most descriptive of exposure-response. To clarify the authors' approach, Dr. Berman indicated that the meta-analysis considered any published epidemiological study with sufficient quantitative exposure data that allowed for a reasonable estimate of the exposure-response relationship; uncertainty factors were then assigned to give greatest weight to the most robust studies. In response, additional panelists concurred with the original comment that meta-analyses conventionally begin with establishing explicit study inclusion criteria. These panelists clarified that they are not advocating removing a majority of studies currently considered in the proposed protocol, but rather being more judicious in selecting the studies to evaluate.

One panelist offered additional comments on the meta-analysis. He supported, for instance, the use of sensitivity analyses, and encouraged the authors to conduct additional analyses to identify influential studies, factors that contribute to risk, and the impact of different weighting factors. The panelist also noted that more sophisticated statistical methodologies (e.g., Bayesian

modeling, Markov Monte Carlo) can be used to generate distributions of outputs, rather than discrete values, which might offer greater understanding of the inferences that can be drawn from the epidemiological studies.

- **Disparate findings from the South Carolina and Québec cohorts.** Multiple panelists noted that the issue of the relative lung cancer potency of chrysotile and amphibole fibers depends largely on how one interprets the disparate findings from the cohort of textile workers in South Carolina and the cohort of chrysotile miners and millers in Québec. Two of these panelists indicated that the relative potency issue likely will not be resolved until the underlying reasons for the differences between these two studies are better understood. The other panelist viewed the difference in potency observed across industries (i.e., mining versus textile) as a more important matter than the difference between the two specific cohorts. When discussing these studies, two panelists indicated that the increased lung cancer risk for the South Carolina cohort might be attributed to exposure to amphibole fibers, which are known to be found in trace levels in commercial chrysotile.

- **Relevance of fiber durability.** One panelist noted that the issue of fiber durability often enters the debate on the relative lung cancer potency of chrysotile and amphibole fibers. Though he agreed that the animal toxicology data indicate that amphibole fibers are more persistent than chrysotile fibers, the panelist noted that trends among the human epidemiological data—particularly the fact that lung cancer risk does not appear to decrease with time since last exposure, even for chrysotile—suggest that the lower durability of the chrysotile fibers might not be important.

- **Influence of smoking.** The panelists had differing opinions on how the proposed protocol should address cigarette smoking. In terms of inferences drawn from the epidemiological literature, two panelists noted that very limited data are available on smoking, making quantitative analysis of its interactions with asbestos exposures difficult. Specifically, only one study includes detailed information on smoking, but that study found no difference in lung cancer potency between smokers and non-smokers. During this discussion, Dr. Berman explained that the proposed protocol assumes a multiplicative interaction between smoking and asbestos exposure, consistent with EPA's 1986 model. Dr. Berman noted that a multiplicative factor in the model, α , represents the background risk in the studied cohort relative to the risk in the comparison population, and both groups include smokers; he added that the influence of smoking is addressed implicitly in the model because it is a relative risk model in which the effect of asbestos is multiplied to the background risk that is present. A panelist clarified, however, that neither the potency factors nor α were derived based on observations of smoking prevalence in the epidemiological studies.

One panelist emphasized that the confounding effects of smoking greatly complicates the analysis of lung cancer potency. He noted that the relative lung cancer risk from asbestos exposure is

considerably lower than that for cigarette smoking. As a result, the panelist wondered how the meta-analysis can truly discern the relative potency of the asbestos fiber types from studies that present no information on cigarette smoking. This panelist provided an example to illustrate his concern: if a given cohort has between 5 and 10% more smokers than the typical population, this increased prevalence of smoking alone could totally confound relative risks attributed to asbestos. The panelist indicated that all future analyses of epidemiological data will suffer from similar limitations, so long as detailed information on smoking is not available.

- **General comments.** During this discussion, some panelists offered several general comments that apply to the entire proposed protocol. These comments included concerns about the transparency of the analyses, questions about data tables being inconsistent with text in the body of the report, and some panelists' inability to reproduce certain findings from the available data. These general comments are reflected in the executive summary of this report.

3.1.2 Lung Cancer and Fiber Type: Inferences from Animal Toxicology and Mechanistic Studies

The panelists offered varying insights on the inferences that can, or should, be drawn from animal toxicology studies and mechanistic studies regarding the relative lung cancer potency for chrysotile and amphibole fibers.

Citing various publications (e.g., Lippmann 1994), multiple panelists noted that the animal toxicology studies do not support the 5-fold difference in lung cancer potency between chrysotile and amphibole fibers. Two panelists added that the absence of different potencies might result from the animal studies being of too short duration (typically no longer than 2 years) for the greater dissolution of chrysotile fibers to be an important factor. Another panelist added that exposure levels in some animal studies are not relevant to human exposures; as an example, he noted that a recent rat inhalation study (Hesterberg et al. 1998) involved exposure levels at 11,000 fibers per cubic centimeter. These panelists indicated that the animal studies are generally more informative of how lung cancer potency varies with fiber length (see Section 3.1.4), and are less informative on how potency varies with fiber type.

The panelists noted that *in vitro* studies exhibit various findings, depending on the study design and endpoint assessed. One panelist, for instance, indicated that some *in vitro* studies suggest that chrysotile fibers are actually more potent than amphibole fibers. Other panelists added that many *in vitro* studies show crocidolite being considerably more toxic than chrysotile. These panelists cautioned against drawing firm conclusions from the *in vitro* studies, however, given that the study duration is far too short for any impact of dissolution to be observed. Finally, another panelist referred to the International Agency for Research on Cancer (IARC) consensus statement on fiber carcinogenesis for an overview of inferences that can be drawn from mechanistic studies: "Overall, the available evidence in favor of or against any of these mechanisms leading to the development of lung cancer and mesothelioma in either animals or humans is evaluated as weak" (IARC 1996).

Based on the previous comments, the panelists cautioned about attempting to draw inferences from the animal toxicology for several reasons. One panelist indicated that the animal studies have limited utility because lung cancer in humans results from a complex set of exposures, including cigarette smoke, and because rats, when compared to humans, develop different types of tumors at different sites. Another panelist reiterated that the duration of most animal studies precludes one from observing dissolution effects. Given these limitations, two panelists emphasized that conclusions should be based primarily on the epidemiological data, especially considering the volume of human data that are available. Though not disagreeing with this recommendation, one panelist noted that the exposure index—one of the major outcomes of the proposed protocol—is, in fact, based on observations from animal studies.

3.1.3 Lung Cancer and Fiber Dimension: Inferences from the Epidemiology Literature

The panelists made several observations regarding what can be inferred from the epidemiology literature on how lung cancer potency varies with fiber dimension, though they first noted that most published epidemiology studies do not include detailed data on the distribution of fiber dimensions to which cohorts were exposed. Overall, the panelists generally agreed that indirect evidence from the

epidemiological studies supports the proposed protocol's finding that longer fibers have greater carcinogenic potency for lung cancer. They added, however, that the epidemiology literature provides no evidence to support or refute the magnitude of the relative potencies used in the proposed protocol (i.e., fibers longer than 10 μm being 300 times more potent than those with lengths between 5 and 10 μm). The panelists made no comments about fiber diameter when discussing this matter. Specific discussion topics follow:

- **Observations from the epidemiology literature.** The panelists identified several studies that provide general insights on the role of fiber size in lung cancer. One panelist, for instance, noted that cohorts of textile workers, which were believed to be exposed to relatively longer asbestos fibers, exhibit higher lung cancer relative risks than do cohorts of miners or cement product workers. Another panelist indicated that studies of taconite miners from Minnesota (Cooper et al. 1988) and gold miners from South Dakota (McDonald et al. 1978) found no increased lung cancer risks among the cohorts, which were known to be exposed primarily to fibers shorter than 5 μm (see Dr. Case's premeeting comments for further information on these studies). This panelist added that the Minnesota Department of Health is currently updating the study on taconite miners and a publication is pending. Another panelist added that epidemiology studies of workers exposed to asbestos from friction brake products show no clear evidence of increased lung cancer. This panelist acknowledged that these epidemiology studies do not include exposure measurements, but other studies of this work environment have indicated that the asbestos fibers in friction brake products are predominantly short chrysotile fibers.
- **Relevance of fibrous structures shorter than 5 μm .** Some panelists noted that no epidemiology studies have examined the relative potency specifically of fibrous structures shorter than 5 μm ; thus no conclusions could be drawn from the epidemiology studies alone. While not disagreeing with this observation, one panelist reminded panelists that airborne particles and fibers have a broad distribution of fiber lengths, with a clear majority (75–90%) of fibrous structures being shorter than 5 μm . This panelist added that indirect inferences can be drawn from the epidemiology studies listed in the previous bulleted item. Another panelist noted that the fibrous structures shorter than 5 μm behave more like particles rather than fibers, at least in terms of lung deposition and clearance patterns. Finally, two panelists indicated that an ATSDR expert panel recently evaluated the issue of relative potency of fibers shorter than 5 μm ; however, the final report from that expert panel meeting was not available until after the peer consultation workshop. The final report has since been released, and a conclusion from that panel was that "there is a strong weight of evidence that asbestos and synthetic vitreous fibers shorter than 5 μm are unlikely to cause cancer in humans" (ERG 2003).

- **Statistical analyses in the proposed protocol.** As indirect evidence that longer fibers have greater carcinogenic potency, one panelist indicated that the exposure-response modeling by Drs. Berman and Crump showed an improved fit to the observed relative risk from epidemiology studies when using an exposure index that assigns greater weight to longer fibers and no risk to fibers shorter than 5 μm . Another panelist concurred, but added that the authors could have attempted to determine the specific weighting (i.e., between longer and shorter fibers) that would optimize the fit to the epidemiological studies.

3.1.4 Lung Cancer and Fiber Dimension: Inferences from Animal Toxicology and Mechanistic Studies

The panelists generally agreed that the animal toxicology studies and mechanistic studies indicate that fiber dimension—especially fiber length—plays an important role, both in terms of dosimetry and pathogenesis. However, panelists had differing opinions on the specific cut-offs that should be used for fiber diameters and lengths in the exposure-response modeling (though panelists generally concurred that fibers shorter than 5 μm should be assigned zero potency).

- **Fiber length.** Multiple panelists noted that the animal toxicology studies provide compelling evidence that lung cancer potency increases with fiber length. Another panelist agreed, but had reservations about assigning no potency to fibrous structures shorter than 5 μm , based on a recent study of refractory ceramic fibers (Bellman et al. 2001) that found that the incidence of inflammation and fibrosis appears to be related to the presence of small fibers in the lung. This panelist indicated that exposure to small fibers likely has some bearing on the oxidative stress state and inflammation in the lung, and he suspected that the exposure-response relationship for long fibers might depend on co-exposures or past exposures to shorter fibers. Based on these observations, the panelist was hesitant to exclude fibrous structures shorter than 5 μm from the proposed risk assessment methodology. On the other hand, another panelist added that animal toxicology studies have shown that fibrosis endpoints are strongly related to fiber length, with exposures to shorter fibers showing less evidence of fibrosis or lung damage. The panelists revisited the significance of fibers shorter than 5 μm when discussing the proposed exposure index (see Section 4).
- **Fiber diameter.** The panelists offered several comments on the role of fiber diameter in the proposed protocol. Noting that fibers with diameters up to 1.5 μm are capable of penetrating to sensitive portions of the lung during oral inhalation, one panelist indicated that this range of fiber diameters should not be excluded from future risk assessments. Other panelists shared the

concern of assigning no lung cancer potency to respirable fibers with diameters greater than 0.5 μm , especially considering that respirability patterns in laboratory animals differ from those in humans (i.e., thicker fibers are more likely to deposit in the human lung than they are in the rat lung).

The panelists also discussed a statement in the proposed protocol that "few fibers thicker than 0.7 μm appear to reach the deep lung." First, one panelist indicated that the proposed protocol includes outdated information on fiber deposition patterns; he recommended that the authors obtain more current insights from specific publications (e.g., Lippmann, 1994) and from the latest lung dosimetry model developed by the International Commission on Radiological Protection. Second, another panelist questioned the relevance of deposition in the deep lung because humans tend to develop bronchogenic carcinomas, while rats develop bronchoalveolar carcinomas. Another panelist cautioned against inferring that asbestos fibers must deposit on bronchial airways to cause lung cancer in humans, noting that significant accumulation of asbestos fibers does not occur in the airways where carcinomas develop in humans, due primarily to mucociliary clearance; this panelist suspected that deposition of fibers in the deep lung is likely related to lung cancer formation in humans, though the mechanisms of carcinogenesis are not fully understood.

3.1.5 Other Issues Related to Lung Cancer

The panelists discussed several additional issues related to the proposed protocol's evaluation of lung cancer potency. Most of the discussion focused on the utility of non-linear exposure-response modeling, but other topics were also addressed:

- **Consideration of non-linear exposure-response models.** The panelists had differing opinions on the extent to which the proposed protocol should consider non-linear exposure-response modeling. On the one hand, one panelist strongly recommended that EPA consider exploring the applicability of non-linear exposure-response models, given his concerns with linear low-exposure extrapolation. This panelist acknowledged that the revised linear model in the proposed protocol clearly provides an improved statistical fit to the epidemiological data when compared to EPA's 1986 lung cancer model, but he advocated more detailed exploration of non-linear cancer risk models, particularly to account for observations of cohorts with low exposures. This panelist was particularly concerned about the cancer risks that would be predicted for low exposures: because the slope in any linear lung cancer model will be determined largely by highly-exposed individuals, he questioned whether the slope derived from

high exposures truly applies to lowly-exposed individuals. To demonstrate his concern, this panelist indicated that the epidemiological studies consistently show that cohorts (or subsets of cohorts) with low exposure generally exhibit no increased lung cancer risk (standardized mortality ratios not statistically different from 1.0). To account for the possibility of a threshold or non-linearity in the exposure-response relationship, this panelist recommended that EPA investigate alternate exposure-response models, such as linear-linear models (i.e., models with two linear exposure-response regions having different slopes) or log-linear models.

Other panelists generally supported these comments. One panelist, for instance, noted that EPA's Draft Revised Guidelines for Carcinogen Risk Assessment indicates that exposure-response relationships should first be evaluated over the range of exposure observations, and then various approaches to extrapolate to exposure levels outside (i.e., below) this range should be investigated. Another panelist added that some studies finding no evidence of lung cancer risks among large cohorts with low exposures should factor into the decision of whether the lung cancer model should include thresholds; he cited a study of non-occupationally exposed women from chrysotile mining regions in Canada (Camus et al. 1998) to illustrate his concern. Other panelists noted that the utility of this study is limited, because exposures were not measured for individuals; further, a panelist clarified that approximately 5% of the individuals considered in this study were occupationally exposed. Finally, one panelist indicated that evidence from the epidemiology literature strongly suggests there are asbestos exposure levels below which lung cancer will not occur; this panelist added that he is unaware of any epidemiological study that has found evidence of lung cancer risk at exposure levels below 25 fiber-years. He recommended that the proposed protocol at least acknowledge the lowest exposure level at which lung cancer effects have been demonstrated.

On the other hand, some panelists were not convinced of the utility of conducting detailed analyses at low exposures and investigating possible thresholds. One panelist, for instance, indicated that a meaningful quantitative analysis of potential thresholds will not be possible, so long as the authors do not have access to raw data from additional epidemiological studies. Further, this panelist suspected that the protocol authors would find considerable heterogeneity among exposure-response slopes for low exposures, and he questioned what conclusions could be drawn by focusing exclusively on the low exposure region. Another panelist agreed, adding that the failure to find significantly increased cancer risks among lowly-exposed cohorts very likely results from poor statistical power and other uncertainties, and not necessarily from the presence of an actual exposure threshold for asbestos-related lung cancer. Finally, one panelist indicated that the National Institute for Occupational Safety and Health (NIOSH) previously examined a threshold model for the cohort of South Carolina textile workers, and that analysis revealed that the best fit of the exposure-response data was a threshold of zero (i.e., the best fit indicated that there was no threshold).

- **Consideration of cigarette smoking.** Several times during the workshop, the panelists debated the ability of the proposed risk assessment model to address interactions between cigarette smoking and asbestos exposure. One panelist recommended that the authors review a recent study that examined the role of cigarette smoking on lung cancer among chrysotile miners and millers in Quebec, Canada (Liddell and Armstrong 2002). Although the panelists generally agreed that smoking is an important consideration for developing and applying the model, some panelists were not convinced that the available data are sufficient to develop an exposure-response model that accurately portrays the interactive effects of asbestos exposure and smoking. The panelists further discussed this issue further later in the workshop.
- **Transparency of the proposed protocol.** Several panelists indicated that the review of epidemiological data in the proposed protocol is not presented in a transparent fashion. One panelist, for instance, sought more information on the uncertainty factors used in the meta-analysis, such as what ranges of factors were considered, what criteria were used to assign the factors, and a table of the factors that were eventually applied. This panelist also recommended that the proposed protocol identify the α -values that were determined for each epidemiological study and provide explanations for any cases when these values are unexpectedly large. Another panelist indicated that the proposed protocol should more clearly differentiate conclusions that are based on a meta-analysis of many epidemiological studies from conclusions that are based on a detailed review of just one or two studies.
- **The need to obtain additional raw data sets.** The panelists unanimously agreed that EPA should make every effort to try to obtain additional raw data sets for the epidemiology studies, such that the authors can further test how adequately the proposed risk assessment model predicts risk. The executive summary of this report presents the panelists' specific recommendation on this issue.

3.2 Mesothelioma

The following paragraphs document the panelists' responses to charge questions regarding inferences from the epidemiology and toxicology literature on how mesothelioma potency varies with fiber type (Sections 3.2.1 and 3.2.2) and fiber length (3.2.3 and 3.2.4).

3.2.1 Mesothelioma and Fiber Type: Inferences from the Epidemiology Literature

The expert panelists unanimously agreed that the epidemiology literature provides compelling evidence that amphibole fibers have far greater mesothelioma potency than do chrysotile fibers—a finding reported both in the review document (Berman and Crump 2001) and a recent re-analysis of 17 cohort studies (Hodgson and Darnton 2000) that reported at least a 500-fold difference in potency. Two panelists commented further that the epidemiology literature provides no scientific support for chrysotile exposures having a role in causation of mesothelioma—an observation that is generally consistent with the meta-analysis in the proposed protocol, which failed to reject the hypothesis that chrysotile fibers have zero potency for mesothelioma.

The most notable response to this charge question was the agreement among most panelists that amphibole fibers are at least 500 times more potent than chrysotile fibers for mesothelioma, as supported by two separate reviews of epidemiological studies. The panelists made additional comments on specific matters when responding to this question, as summarized below, but the key point in this discussion was the agreement that chrysotile is a far less important cause of mesothelioma than are amphiboles.

- **Relative roles of chrysotile and amphibole:** One panelist indicated that cohort studies with individual-level exposure-response data and the broader epidemiology literature both provide no evidence of increased mesothelioma risk due to chrysotile exposure. Further, this panelist noted that 33 of 41 mesothelioma cases previously identified as occurring among workers primarily exposed to chrysotile fibers (Stayner et al. 1996) were later reported as likely resulting from exposures to tremolite fibers found in the chrysotile mines (McDonald et al. 1997). This panelist noted that a recent finding of a small mesothelioma risk from chrysotile (Hodgson and Darnton 2000) results entirely on the assumption that the 33 mesothelioma cases mentioned above result entirely from chrysotile exposures. Based on these observations, this panelist indicated that the literature suggests that chrysotile exposures have limited, if any, role in causing mesothelioma. He nonetheless supported the relative potency attributed to chrysotile in the proposed protocol as a conservative measure in the overall risk assessment process.
- **Specific comments on the Connecticut friction products workers:** Another panelist commented on an epidemiological study of a cohort of workers employed at a friction products plant in Connecticut. The panelist noted that the original study (McDonald et al. 1984) did not identify any deaths from mesothelioma, but review of the state cancer registry (Teta et al. 1983)

revealed that three Connecticut residents who died of mesothelioma were employed by the same friction products company. One of these employees had amphibole exposures during the time he worked for a textile plant that was under the same parent company that owned and operated the friction products plant. The other two cases, the panelist noted, were females who indeed worked at the friction products plant. A pathology review found that one of these cases was a woman with probable pleural mesothelioma and 5 years of exposure; the other case was a peritoneal mesothelioma in a woman who also had asbestosis, and worked as a clerk for 30 years. This panelist noted that it was questionable to attribute the latter two mesothelioma diagnoses to the chrysotile exposures at the friction products plant, though she added that this possibility cannot be definitively ruled out. This panelist encouraged that future review of this epidemiological study should be revised given this new information.

■ **Comments on the proposed 500-fold difference in relative potency.** The panelists had several comments on the finding in the proposed risk assessment methodology that amphibole fibers are 500 times more potent for mesothelioma than are chrysotile fibers. Several panelists noted that this finding is consistent with that of a recent re-analysis of 17 epidemiological studies (Hodgson and Darnton 2000). Though not disagreeing that amphibole fibers are clearly more potent, one panelist was concerned that the risk coefficients (K_M) were largely derived from data sets with inadequate exposure-response information for mesothelioma, and assumptions had to be made to determine critical inputs to the mesothelioma model (e.g., average exposure, duration of exposure).

Other panelists commented on specific sections in the proposed protocol. One panelist, for example, recommended that the authors check the accuracy of data presented in Table 6-16 and Table 6-29 of the report, which are not reported consistently. Another panelist suggested that the authors better explain why separate risk coefficients for amphiboles and chrysotile were calculated for some cohorts (e.g., Hughes et al. 1987) but not for others (e.g., Berry and Newhouse 1983), even though the exposure information available for the studies appears to be comparable. Finally, one panelist recommended that the authors of the proposed protocol consider questions recently raised (Rogers and Major 2002) about the quality of the exposure data originally reported for the Wittenoom cohort (De Klerk et al. 1989) when evaluating exposure-response relationships for mesothelioma.

3.2.2 Mesothelioma and Fiber Type: Inferences from Animal Toxicology and Mechanistic Studies

The panelists discussed the inferences provided by animal toxicology data and mechanistic data regarding relative mesothelioma potency of different asbestos fiber types. Overall, two panelists

commented that the human epidemiological data clearly establish that exposures to amphibole asbestos fibers pose a greater mesothelioma risk than do exposures to chrysotile fibers. They added that the animal toxicology data are generally supportive of this finding, but the animal data suffer from some limitations. Two panelists, for instance, noted that the utility of animal toxicology studies is limited by the fact that rodents are rather insensitive to mesothelioma. These panelists added that the animal toxicology studies involving intra-tracheal instillation or peritoneal injection are not directly relevant to the inhalation exposures that occur in humans. These limitations notwithstanding, the panelists raised the following points when discussing the animal toxicology and mechanistic studies:

One panelist referred to one of his earlier publications (Lippmann, 1994) for further insights on the occurrence of mesothelioma in animal studies. At that time, this panelist noted, the animal inhalation studies found fewer than 10 cases of mesothelioma, and the number of cases appeared to be greatest among animals that were exposed to mixtures containing higher proportions of amphibole fibers. He found this consistent with the influence of fiber type observed in the human epidemiological data (see Section 3.2.1).

During this discussion, one panelist reviewed a publication (Suzuki and Yuen 2001) that was mentioned earlier in the workshop. The publication documents the amounts and types of asbestos fibers measured in samples of pleural plaques and tumor tissue collected for legal cases. These analyses reportedly found relatively large amounts of short, thin chrysotile fibers in the pleura, suggesting that these fibers should not be excluded from the group of fibers believed to induce mesothelioma. The panelist had several criticisms of the study. First, he indicated that the samples were analyzed using a non-standard technique, without any controls. Second, he questioned the major finding of fibers being detected in the pleura, because most of the samples analyzed were actually tumor tissue, in which he would not expect to find fibers. The panelist suspected that the chrysotile fibers reportedly found in the study likely result from specimen contamination—a bias that would have been more apparent had rigorous quality control procedures been followed. Finally, the panelist noted that a more rigorous study (Boutin et al. 1996) of

asbestos fibers in the parietal pleura found a mixture of fibers, including long amphibole fibers, among living patients with asbestos-related conditions. Based on these concerns, the panelist concluded that the publication of concern (Suzuki and Yuen 2001) is seriously flawed and its recommended should be excluded from EPA's analyses.

A specific issue raised regarding the analytical technique in the study (Suzuki and Yuen 2001) was that water was used during the digestion process. Noting that water may contain large amounts ($>30,000$ fibers/L) of small asbestos fibers, another panelist suspected that the fibers detected in the study might have resulted from contamination introduced during the digestion process. Because control samples were not analyzed, the panelist said the study offers no evidence that the fibers detected truly were in the original pleural plaques or tumor tissues. He added that studies of lung-retained asbestos fibers routinely detect primarily short, chrysotile fibers, and that the presence of the short fibers in the pleural tissue—even if the measurements from the study are valid—would not necessarily prove that short fibers cause mesothelioma.

3.2.3 Mesothelioma and Fiber Dimension: Inferences from the Epidemiology Literature

The panelists commented briefly on how the human epidemiological data characterize the role of fiber size on mesothelioma risk. Noting that exposure measurements in most every epidemiological study do not characterize fiber length distribution, one panelist indicated that these studies provide no direct evidence of how fiber length is related to mesothelioma. He added that the studies offer conflicting indirect evidence of the role of fiber length. Specifically, the higher mesothelioma risk coefficient among textile workers in South Carolina, when compared to that for the chrysotile miners and millers in Quebec, could be supportive of longer fibers being more potent, since exposures in South Carolina had a larger percentage of long fibers. However, a cohort of cement plant workers in New Orleans was found to have a higher mesothelioma risk coefficient than that of the South Carolina cohort, even though the South Carolina workers were exposed to higher percentages of long fibers. Finally, as indirect

evidence that carcinogenic potency increases with fiber length, this panelist noted that the mesothelioma risk model using the proposed exposure index, which is heavily weighted by long fibers, provided a considerably improved fit to the epidemiological data.

The panelists briefly revisited the inferences that can be drawn from studies of lung-retained fibers. One panelist again commented that results from a recent study (Suzuki and Yuen 2001) should be viewed with caution. He added that several other lung pathology studies (e.g., McDonald et al. 1989, Rogers et al. 1991, Rödelberger et al. 1999) have been conducted using more rigorous methods, such as using appropriate controls for age, sex, and hospital. These studies all showed that risk of mesothelioma was considerably higher for individuals with larger amounts of long fibers retained in their lungs.

One panelist indicated that results from a study of lung-retained fibers (Timbrell et al. 1988) suggest fiber diameter plays a role in mesothelioma risk: the study observed no mesothelioma cases among a population highly exposed to anthophyllite fibers, which tend to be thicker fibers. Citing his earlier review of mesothelioma cases (Lippmann 1988), the panelist also noted that crocidolite fibers are both thinner than and more potent than amosite fibers, which further supports the hypothesis that carcinogenic potency for asbestos decreases with increasing fiber diameter.

3.2.4. Mesothelioma and Fiber Dimension: Inferences from Animal Toxicology and Mechanistic Studies

The panelists made few observations on findings from animal toxicology studies regarding mesothelioma and fiber length. One panelist indicated that findings from the animal toxicology studies generally support the overall finding that mesothelioma risks are greatest for long, thin fibers. However, another panelist noted that his earlier review of mesothelioma risks (Lippmann 1988) hypothesized that the critical fibers for mesothelioma induction are those with lengths between 5 and 10 μm . This panelist added that fibers of this dimension are more likely to translocate to the pleura than are longer fibers, but

he acknowledged that it is unclear whether fibers must first translocate to the pleura in order to cause mesothelioma.

Some panelists indicated that fiber durability likely plays a role in inducing mesothelioma, based on the fact that mesothelioma is more easily induced in animals using administration methods (e.g., peritoneal injection) that remove the importance of dissolution.

3.3 Exposure Estimates in the Epidemiology Literature

The panelists raised numerous issues when responding to the third charge question: "To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?" Recognizing that the exposure estimates from the epidemiology studies are critical inputs to the exposure-response assessment, the panelists expressed concern about the exposure data: few studies provide detailed information on fiber size distribution; many studies report exposures using outdated sampling and analytical methodologies (e.g., midget impinger); individual-level data are not available for most studies; and many studies do not report detailed information on parameters (e.g., exposure levels, exposure duration) needed to evaluate exposure-response relationships, particularly for mesothelioma. Their specific concerns on these and other matters follow:

- **Concerns regarding exposure estimates in specific studies.** Some panelists expressed concern about the assumptions made to interpret the exposure data originally reported in the epidemiology studies. One panelist reviewed specific examples of these concerns:

The original study of workers at a Connecticut friction products plant (McDonald et al. 1984) reports exposures measured by midget impingers (in units of mmpcf), with no information on how to convert this to PCM measurements; and the original publication includes limited data on exposure duration.

The original study of workers at a New Jersey insulation factory (Seidman et al. 1986) did not report any exposure measurements from the factory studied; and data collected

from another plant with similar operations were used to characterize exposure-response for this cohort.

- ▶ The original study of workers at a Texas insulation factory (Levin et al. 1998) reported a range of exposure levels (15–91 fibers/mL), and the authors of the proposed protocol assigned an average exposure level (45 fibers/mL) to the entire cohort.
- ▶ The original study of U.S. insulation applicators (Selikoff and Seidman 1991) has no information on exposure. The proposed protocol assumes that all workers were exposed to 15 fibers/mL for 25 years, based on a separate review of exposures among insulation workers (Nicholson 1976).
- ▶ The original study of retirees from the U.S. Asbestos Products Company (Enterline et al. 1986) reported exposures based on midget impinger sampling, with no information on how to convert these exposures to PCM measurements.
- ▶ According to a recent letter to the editor (Rogers and Major 2002), the original study of the Wittenoom cohort (De Klerk et al. 1989) might have overestimated exposures, possibly by as much as a factor of 10.

The previous comments led to a discussion on whether certain studies should be excluded from the meta-analysis used in the proposed protocol (see next bulleted item). Prior to this discussion, one panelist expressed concern about being overly critical of the exposure estimates used for many of the studies listed above; he emphasized that all exposure estimates appear to be based on a critical review of the literature, and no estimates are completely arbitrary, as some of the panelists' comments implied.

- **Comments on using study inclusion criteria for the meta analysis.** Given the concerns about the quality of exposure data reported in some epidemiology studies, the panelists debated whether future revisions of the proposed protocol should exclude certain studies from the exposure-response analysis. The panelists were divided on this matter.

On the one hand, several panelists recommended that the authors develop and apply study inclusion criteria in the exposure-response evaluation, as is commonly done when conducting a meta-analysis. One panelist, for instance, recommended assessing exposure-response relationships for only those studies found to have adequate exposure data, and then using a sensitivity analysis to examine the effect of excluding studies with inadequate exposure data. These panelists clarified that they are not advocating disregarding the majority of studies; rather, they are suggesting simply that the authors of the proposed protocol use study inclusion criteria and sensitivity analyses to ensure that the conclusions are based on the best available exposure data.

On the other hand, several panelists supported the current approach of using as many studies as possible and accounting for the quality of the exposure measurements in the uncertainty factors. One panelist, for example, commended the authors for being as inclusive as possible when reviewing the studies; he supported the approach of recognizing the limitations of the available exposure data and accounting for these limitations in the uncertainty factors that were ultimately used to weight the studies in the meta-analysis. This panelist acknowledged that the exposure estimates in some of the epidemiological studies might be rough estimates, but he emphasized that the estimates are not worthless and should not be discarded. Other panelists concurred with these comments, and did not support applying overly restrictive study inclusion criteria.

- **Comments on the uncertainty factors assigned to each study.** The panelists made several comments on the uncertainty factors that the authors assigned to each study. Dr. Berman first explained the four uncertainty factors: the first factor (F1) characterizes the confidence in exposure estimates; the second factor (F2) represents the confidence in the conversion to PCM measurements from other exposure metrics (typically midget impinger analyses); the third factor (F3) characterizes the confidence the authors had on worker history data; and the fourth factor (F4) was a non-exposure related factor to account for other uncertainties (e.g., lack of information on confounders, incomplete or inaccurate mortality ascertainment). Dr. Berman described generally how the individual uncertainty factors were assigned and noted that each factor could range from 1 to 5.

The panelists' comments primarily focused on the transparency of how uncertainty factors were presented and incorporated into the meta-analysis. Multiple panelists, for instance, recommended that future revisions to the proposed protocol include a table that lists the uncertainty factors assigned to each study. Further, one panelist suggested that the revised protocol describe the assumptions inherent in the uncertainty factor weighting approach, such as explaining why some factors are assigned values over a broader range than others (e.g., why F1 values span a broader range than F4 values) and describing why the individual uncertainty factors have equal weights in generating the composite uncertainty factor. Another panelist agreed and added that the revised protocol should more explicitly describe how the uncertainty factors were combined into the composite factor and how this composite factor affects the weighting of studies in the meta-analysis. Expanding on this point, another panelist suggested that the final document more clearly explain that the final estimates of cancer risk coefficients (K_L^* and K_M^*) are actually weighted averages of the epidemiological studies, with the weights assigned to each study being a function of that study's uncertainty. This panelist also recommended that the revised document clearly state how, if at all, the fraction of amphibole fibers and the fraction of fibers longer than 10 μm are reflected in the uncertainty factors.

Some panelists debated the utility of alternate approaches that could be used to assign uncertainty factors. Two panelists noted that the approach used to assigning uncertainty factors is somewhat subjective; because different groups of analysts would likely assign different

uncertainty factors. To avoid the appearance of arbitrariness, these panelists suggested using alternate meta-analysis approaches that do not require using uncertainty factors. They noted, for example, that the authors could use a random effects model in which residual inter-study variation is estimated. Another suggestion was to conduct sensitivity analyses examining the effects of including or excluding studies, depending on the uncertainty factors assigned to them.

Another panelist disagreed with these comments and supported the analyses in the proposed protocol; this panelist indicated that the authors had no choice but to make judgments based on the information documented in the epidemiology literature. He suggested that EPA consider convening a separate expert panel to assign uncertainty factors, if panelists do not support those selected by Drs. Berman and Crump.

■ ***Assumptions made to convert exposure estimates from midget impinger sampling.***

Several panelists noted that the original publications for many epidemiology studies document exposure estimates based only on midget impinger sampling and do not include any information on how to convert these exposures to levels that would be measured by more modern methods (e.g., PCM, TEM). The panelists noted that the conversion factor (from mmpcf to fibers/mL) can vary considerably from one occupational setting to the next.

■ ***Interpretations of the study of South Carolina textile workers.***

The panelists had different opinions on interpretations of the study of South Carolina textile workers (Dement et al. 1994). One panelist, for instance, found this particular study to be an outlier among the other epidemiological studies, and he recommended that the authors exclude this study from the exposure-response analysis until the causes for the increased relative risks observed for this cohort are better understood. Another panelist suggested that the proposed protocol should classify the South Carolina cohort as being exposed to mixed asbestos fibers, rather than being exposed to chrysotile fibers. He indicated that some workers in the cohort were exposed to amosite and crocidolite, in addition to being exposed to chrysotile.

Other panelists, however, did not think the South Carolina study should be excluded from EPA's analysis. One panelist was troubled about criticisms of the exposure estimates for this cohort, given that this is one of few studies in which co-located samples were collected and analyzed using different methods, thus providing site-specific data for converting midget impinger

After reviewing a draft of this report, one panelist indicated that it is important to note that exposure data for the South Carolina cohort are available from more than just one reference (Dement et al. 1994). He suggested that EPA use data from studies conducted by McDonald in the 1980s of a parallel cohort in the same plant. However, he cautioned EPA against treating multiple studies of the same relatively small group of workers as separate studies, considering the large overlap of workers studied by the two groups of investigators. This panelist encouraged EPA to consider other data sources for this cohort, given that a recent re-analysis of epidemiological studies (Hodgeson and Darnton 2000) severely criticized the data source EPA uses (Dement et al. 1994), to the point of those data being dropped from the recent re-analysis altogether.

sampling results to PCM measurements. Another panelist challenged suggestions that the South Carolina study is an outlier; he indicated that the South Carolina study is one of the more rigorous epidemiology studies available for asbestos exposures, and he found no valid scientific reasons for discarding it. During this discussion, one panelist point out in response that the South Carolina study is indeed an outlier among the textile cohorts, with a slope which is higher than either of the two textile cohorts; this panelist did acknowledge that the lung cancer risk among the textile cohorts is greater than that among the mining cohorts. This panelist added that scientists need a better explanation for why the lung cancer risk among the South Carolina cohort is greater than that of other cohorts before the South Carolina study can achieve credibility, especially considering that exposures in South Carolina were supposedly to "pure" chrysotile.

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4. COMMENTS ON TOPIC AREA 2: THE PROPOSED EXPOSURE INDEX

This section summarizes the panelists' responses to the charge questions pertaining to the proposed exposure index. Section 4.1, 4.2, and 4.3 document the panelists' responses to charge questions 4, 5, and 6, respectively.

4.1 Responses to Charge Question 4

Charge question 4 asks: "The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk." The panelists discussed this matter earlier in the workshop (see Sections 3.1.3 and 3.1.4 for these comments), and provided additional insights on the matter. Overall, the panelists agreed that carcinogenic potency increases with fiber length, particularly for lung cancer. Most panelists supported assigning no potency to fibrous structures smaller than 5 μm . Some panelists agreed that the short fibrous structures are clearly less potent than long fibers, but they had reservations about assigning zero potency to the structures smaller than 5 μm ; these panelists acknowledged that the toxicity of the short fibrous structures might be adequately addressed by EPA's air quality standards for particulate matter. Specific comments on this charge question follow:

- **Reference to ATSDR's expert panel workshop on the role of fiber length.** Two panelists noted that ATSDR convened an expert panel in October 2002 to discuss the role of fiber length on toxicity, and much of that discussion specifically addressed fibrous structures smaller than 5 μm . A main conclusion of that panel was that there is "a strong weight of evidence that asbestos and synthetic vitreous fibers shorter than 5 μm are unlikely to cause cancer in humans" (ERG 2003). The panelists encouraged EPA to review the summary report prepared for that workshop, which was officially released on March 17, 2003, and is available on-line at: www.atsdr.cdc.gov/HAC/asbestospanel.
- **Evidence from epidemiological studies.** One panelist indicated that the epidemiological studies do not provide direct evidence of the role of fibrous structures shorter than 5 μm .

However, the panelist indicated that a growing body of evidence suggests that the cohorts predominantly exposed to shorter fibers (e.g., friction brake workers, gold miners, taconite miners) do not have statistically significant increased cancer risks. This panelist added that the mechanistic studies provide the strongest evidence for assigning no potency to fibrous structures (see next bulleted item). Another panelist agreed with these statements, and added that his interpretation of data compiled by the National Cancer Institute provide additional indirect evidence of short fibrous structures presenting little or no carcinogenic risk (see page 102 of the premeeting comments in Appendix B).

The panelists briefly revisited the findings from a recent publication (Suzuki and Yuen 2001) that reported finding relatively large amounts of short, thin chrysotile fibers in malignant mesothelioma tissue. Several panelists encouraged that these findings not be considered in the risk assessment methodology for reasons cited earlier in the workshop (see Section 3.2.2).

- **Evidence from mechanistic studies.** The panelists offered different interpretations of mechanistic studies. One panelist indicated that mechanistic studies have shown that shorter fibers are cleared more readily than long fibers from the alveolar region of the lung by phagocytosis, and therefore provide supporting evidence that short fibers play little or no role in carcinogenic risk. This panelist acknowledged that extremely high doses of particulate matter and other non-fibrous structures can generate biological responses (e.g., inflammation), but he doubted that such "overload" conditions would be relevant to the environmental exposures that the proposed protocol will be used to evaluate.

Another panelist agreed that long fibers are clearly more potent than short fibrous structures, but he questioned the conclusion that short fibrous structures have no impact on carcinogenic risk. This panelist noted that mechanistic studies have demonstrated that short fibrous structures and spherical particles, like silica, can elicit the same toxic responses (e.g., generate reactive species, stimulate proliferative factors) identified for asbestos fibers. This panelist added, referring to his premeeting comments, that exposure to short fibers could cause inflammation and generation of oxidative species that might increase the response to long fibers (see Bellman et al. 2001). Overall, this panelist acknowledged that long fibers are more persistent than short fibers in the lung and should be weighted more heavily in the exposure index, but he was hesitant to assign the short fibrous structures zero potency.

- **Implications on sampling and analytical methods.** One panelist commented on the practical implications, from a sampling perspective, of any changes to the exposure index. This panelist indicated that measuring all fibers (including structures shorter than 5 μm) in environmental samples would not only be expensive, but also would compromise the sensitivity of measuring the longer fibers that are most predictive of cancer risk. This panelist acknowledged that human exposure is predominantly to fibrous structures less than 5 μm , but he noted that the amounts of short fibrous structures retained by the lung tend to be very strongly

correlated with the amounts of long fibers retained by the lung. Due to this correlation, this panelist noted that measuring long fibers with sufficient accuracy would allow one to estimate amounts of short fibrous structures in a sample. This panelist added, however, that he sees no benefit of characterizing exposures to fibrous structures smaller than 5 μm , given the conclusion that such fibers do not cause cancer (ERG 2003).

4.2 Responses to Charge Question 5

Charge question 5 asks: "The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?" The panelists' responses to this question follow:

- **Consistency with epidemiological literature.** The panelists noted that the original epidemiology studies did not collect exposure information that provides direct evidence of the relative potency assigned to the two different fiber length categories: fibers longer than 10 μm , and fibers with lengths between 5 and 10 μm . During this discussion, one panelist recommended that EPA consider the results of a case-control study (Rogers et al. 1991) that suggests that mesothelioma risks are greater for individuals with larger amounts of the shorter fibers (i.e., between 5 and 10 μm) retained in their lungs. Another panelist was not convinced of the findings from this study, due to possible biases from selection of controls not matched for hospital of origin. This panelist encouraged EPA to refer to more rigorous lung-retained fiber studies (e.g., McDonald et al. 1989, Rödelberger et al. 1999) that have found that the majority of cancer risk for mesothelioma is attributed to exposures to longer fibers, even when measurements of short fibers are taken into account.
- **Questions about the fiber length-dependence used for mesothelioma.** Some panelists were not convinced that the relative potencies assigned to different fiber lengths were appropriate for mesothelioma. One panelist, for instance, noted that his previous review of the literature (Lippmann 1994) suggests that cancer risk for mesothelioma is most closely associated with exposure to fibers between 5 and 10 μm long. He indicated that this assessment is consistent with other human lung evaluations (e.g., Timbrell et al. 1988), which have reported that fibers retained by the lung tend to be longer than fibers that translocate to the pleura. This panelist added that the epidemiology literature clearly suggests that lung cancer and

mesothelioma have different risk factors, as the relative amounts of lung cancer and mesothelioma cases vary considerably from one cohort to the next. Based on these concerns, this panelist suggested that EPA consider developing separate fiber length weighting schemes for lung cancer and mesothelioma.

Another panelist indicated that the epidemiology studies provide indirect evidence that carcinogenic potency appears to increase with fiber length. Specifically, he noted that the studies consistently show that mesothelioma has a very long latency period—a trend that suggests that the most durable fibers (i.e., the longer fibers) are the most potent. The panelist added that the analyses in the proposed protocol provide further indirect evidence of mesothelioma risks increasing with fiber length: when the exposure index was used in the mesothelioma model, the proposed risk assessment methodology generated an improved fit to the epidemiological data.

During this discussion, a panelist cautioned about inferring that only those fibers that reach the pleura are capable of causing mesothelioma, because researchers have not determined the exact mechanisms by which mesothelioma is induced. Further, he cautioned about inferring too much from a single study (Timbrell et al. 1988), given that many additional studies are available on lung-retained fibers.

- **Questions about the relevance of animal toxicology data.** Some panelists expressed concern about basing the proposed weighting factors for different fiber lengths on observations from animal data. First, one panelist noted that the weighting factors were derived strictly based on lung cancers observed in laboratory animals, and he questioned whether one can assume that the weighting factors can be defensibly applied to mesothelioma. Second, other panelists noted that extrapolating the weighting factors from rodents to humans also involves uncertainty, due to inter-species differences in respiratory anatomy, macrophage sizes, and sites of lung cancers.
- **Suggested follow-up analyses.** Given the concerns about basing the proposed exposure index entirely on data from animal toxicology studies, two panelists recommended that EPA attempt to optimize the weighting factors applied to different fiber length categories using the available human epidemiological data. One panelist suggested that this optimization could be performed using the data compiled in Table 6-15 in the proposed protocol, which presents estimates of the fiber length distribution for different occupational cohorts. A panelist also suggested that EPA consider deriving separate weighting factors for lung cancer and mesothelioma, rather than assuming the same fiber length dependence for both outcomes.

4.3. Responses to Charge Question 6

Charge question 6 asks: "Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place." The panelists discussed several topics when addressing the question, because some panelists had different impressions of what the question was asking. Some panelists viewed the question as asking about the validity of low-dose linear extrapolations (see Section 3.1.5 for more information on this topic), and others viewed the question as asking about whether the proposed methodology is an improvement over EPA's current risk assessment model. A summary of the panelists' specific responses follows:

- ***Is the proposed exposure index an improvement to asbestos risk assessment?*** When answering this charge question, multiple panelists focused on whether the proposed exposure index is an improvement over EPA's 1986 asbestos risk models. These panelists agreed that the proposed approach is more consistent with the overall literature on health risks from asbestos, which show that cancer risks vary with fiber type and fiber dimension. Two panelists were hesitant to call the proposed approach an improvement for evaluating mesothelioma risks, because the fiber length weighting factors are based entirely on lung cancer data in animals. These panelists were particularly concerned that the proposed methodology might assign lower risks for mesothelioma in certain circumstances, because the fiber-length dependence in the methodology is not based on any toxicological or epidemiological studies of mesothelioma.
- ***Does the proposed risk assessment model support extrapolation from occupational exposures to environmental exposures?*** Some panelists commented on the applicability of the proposed risk assessment model to exposure doses below the ranges considered in the occupational studies. Referring to observer comments provided earlier in the workshop, two panelists indicated that some environmental exposures in areas with naturally-occurring asbestos do not appear to be considerably lower than those experienced by occupational cohorts. Another panelist agreed, and cautioned about distinguishing environmental exposures from occupational exposures; he instead encouraged EPA and the panelists to focus on the exposure magnitude, regardless of whether it was experienced in an occupational or environmental setting.

One panelist recommended that EPA investigate how cancer risks for lung cancer and mesothelioma vary between EPA's 1986 model and the proposed risk assessment methodology: for different distributions of fiber types and dimensions, does the proposed methodology predict higher or lower risks than the 1986 model? Dr. Berman indicated that the proposed methodology, when compared to EPA's 1986 model, generally predicts substantially higher risks for environments with longer, thinner fibers and environments with larger amounts of

Figure 1. The effect of the concentration of the *Agrobacterium* suspension on the transformation efficiency of *Agrobacterium* strains.

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5. COMMENTS ON TOPIC AREA 3: GENERAL QUESTIONS

This section summarizes the panelists' responses to charge questions 7-10 and 12. Responses to charge question 11 are included in Section 6, because this charge question sought the panelists' overall impressions of the proposed risk assessment methodology, rather than focusing on any one specific issue.

5.1 Responses to Charge Question 7

This charge question asks: "The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or 'bundles that are components of more complex structures'). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range." The panelists raised the following points when responding:

- **Terminology used in the charge question.** One panelist took strong exception to the wording in this question (see pages 30-33 in Appendix B) and strongly recommended that the panelists use correct terminology during their discussions. This panelist noted, for instance, that cleavage fragments are not equivalent to bundles, nor do cleavage fragments meet the regulatory definition of asbestos, as the charge question implies. He clarified that he defines cleavage fragments as non-asbestiform amphiboles that are derived from massive amphibole structures. This panelist was concerned that none of the panelists at the workshop has the mineralogical expertise needed to address issues pertaining to cleavage fragments. Another panelist echoed these concerns and agreed that this charge question raises complex issues.
- **Significance of cleavage fragments with respect to human health effects.** The previous concerns notwithstanding, several panelists commented on the role of cleavage fragments in the proposed risk assessment methodology. One panelist, for example, indicated that there is no reason to believe that cleavage fragments would behave any differently in the human lung than asbestiform fibers of the same dimensions and durability; he added that this conclusion was also reached by the American Thoracic Society Committee in 1990 (Weill et al. 1990). This panelist acknowledged, however, that expert mineralogists have differing opinions on the role of cleavage fragments. Several other panelists agreed that it is reasonable to assume that cleavage fragments and asbestos fibers of the same dimension and durability would elicit similar toxic responses.

- **Review of selected epidemiological and toxicological studies.** The panelists briefly discussed what information has been published on the toxicity of cleavage fragments. One panelist indicated that Appendix B in the proposed protocol (see pages B-3 through B-10) interprets results from an animal study (Davis et al. 1991) that evaluated exposures to six tremolite samples, including some that were primarily cleavage fragments. This panelist noted that the study provides evidence that cleavage fragments can cause mesothelioma in animals.

Another panelist, however, cautioned against inferring too much from this animal study for several reasons: the study was not peer reviewed; the fiber measurements in the study reportedly suffered from poor reproducibility; and the mesotheliomas observed in the study might have reflected use of intra-peritoneal injection model as the dose administration method. This panelist recommended that EPA conduct a more detailed review on the few studies that have examined the toxicity of cleavage fragments, possibly considering epidemiological studies of taconite miners from Minnesota (Higgins et al. 1983) and cummingtonite-grunerite miners from South Dakota (McDonald et al. 1978); he noted that a pending publication presents updated risks among the taconite miners.

- **Practical implications of measuring cleavage fragments in environmental samples.** One panelist added, and another agreed, that measuring cleavage fragments in environmental samples presents some challenges, because microscopists cannot consistently distinguish cleavage fragments from asbestiform fibers, even when using TEM.

5.2 Responses to Charge Question 8

Charge question 8 asks: "Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations." The panelists made the following general comments in response:

- **Review of evidence from toxicological and epidemiological studies.** The panelists identified few studies that address the toxicity of amphibole fibers other than actinolite, amosite, anthophyllite, crocidolite, and tremolite. One panelist indicated that animal toxicology studies have demonstrated that synthetic vitreous fibers with differing chemistry, but having similar durability and dimensions, generally exhibit similar potency for fibrosis, lung cancer, and mesothelioma. Another panelist added that lung cancer and mesothelioma exposure-response

relationships for a cohort of vermiculite miners from Libby, Montana, have been published for both asbestiform richterite and winchite.

- **Appropriateness of applying the model to non-asbestiform amphiboles.** Several panelists agreed that the proposed risk assessment methodology is relevant to amphibole fibers other than those listed in the federal regulations. The panelists noted that, in the absence of more detailed information on the matter, it is prudent to assume that fibers of similar dimension and durability will exhibit similar toxic effects. Two panelists expressed some hesitation on applying the proposed model to the non-asbestiform amphiboles: one panelist asked how confidently one can apply the cancer risk coefficients to amphibole fibers that have not been studied, and another panelist indicated he was not convinced that the model should be applied to the other amphiboles, let alone for the amphiboles that are listed in the federal regulations.

Given the amount of naturally occurring amphiboles in the Earth's crust, one panelist suggested that the proposed protocol clearly state that the non-asbestiform amphiboles being evaluated are only those with the same dimensional characteristics and biodegradability as the corresponding asbestiform amphiboles.

5.3 Responses to Charge Question 9

Charge question 9 asks: "The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?"

The panelists expressed varying opinions on this matter: some panelists saw no benefit of measuring fibrous structures shorter than 5 μm , based on responses to earlier charge questions (see Sections 3.1.3, 3.1.4, and 4.1); other panelists indicated that there is some utility to collecting information on shorter fibrous structures, particularly if the incremental analytical costs are not prohibitively expensive and if counting short fibers does not compromise accurate counts of longer fibers. The panelists raised the following specific issues when discussing measurement methods:

- **Support for using TEM in future sampling efforts.** The panelists unanimously supported the recommendation in the proposed protocol of using TEM, rather than PCM or some other method, to characterize exposures in future risk assessments. The panelists also emphasized that future measurement methodologies must focus on generating accurate counts of the most biologically active fibers, or fibers longer than 5 μm .
- **Practical implications of counting fibers shorter than 5 μm .** One panelist indicated that analyzing samples for fibrous structures shorter than 5 μm would compromise analysts' ability to accurately count the amounts of longer fibers that are of greater biological concern. Some panelists and an observer further discussed the costs associated with counting fibers in multiple length categories, including shorter than 5 μm . The panelists did not cite firm cost figures for these analyses. However, noting that environmental samples typically contain more than 90% short fibrous structures, one panelist suspected that counting the shorter structures would considerably increase the time a microscopist needs to analyze samples, and therefore also would considerably increase the cost of the analysis. A panelist indicated that the costs and benefits of counting fibers shorter than 5 μm might be more appropriately debated between microscopists and risk assessors, with inputs from industrial hygienists and mineralogists.
- **Relevance of fibers shorter than 5 μm for non-cancer endpoints.** One panelist noted that exposures to fibrous structures shorter than 5 μm can contribute to asbestosis in occupationally exposed individuals (Lippmann 1988), but he doubted that the exposure levels found to be associated with asbestosis would be experienced in non-occupational settings. Another panelist added that the role of shorter fibrous structures for other non-cancer endpoints is not known, such as the pleural abnormalities and active pleural fibrosis observed in Libby, Montana. No panelists were aware of any authoritative statements made on the role that short fibers play, if any, on these other non-cancer endpoints. During this discussion, one panelist indicated that the toxicity of fibrous structures shorter than 5 μm might be adequately addressed by EPA's particulate matter standards.

5.4 Responses to Charge Question 10

Charge question 10 asks: "The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber diameter appropriate?" Before the panelists responded to the question, Dr. Berman first clarified that the exposure index optimized from the animal studies (see Equation 7.12 in the proposed protocol)

assigns a far greater carcinogenic potency to fibers longer than 40 μm , with diameters less than 0.4 μm ; he noted that the proposed diameter cut-off (0.5 μm) was based on an *ad hoc* adjustment.

The panelists agreed that the proposed cut-off for fiber diameter (0.5 μm) would likely include most fibers of health concern; however, they also unanimously agreed that the exposure index should not exclude thicker fibers that are known to be respirable in humans. The main argument given for increasing the cut-off is that fibers with diameters as large as 1.5 μm (or with aerodynamic diameters as large as 4.5 μm) can penetrate to small lung airways in humans. Other panelists provided additional specific comments, generally supporting inclusion of thicker fibers in the proposed exposure index. One panelist, for example, advised against basing the fiber diameter cut-off strictly on observations from rat inhalation studies, due to inter-species differences in respirability. Further, noting that the proposed cut-off for fiber diameter would likely exclude some amosite fibers and a considerable portion of tremolite fibers with known carcinogenic potency, another panelist encouraged that the proposed exposure index include contributions from thicker fibers.

The panelists noted that consideration of fibers thicker than 0.5 μm was viewed as being most important for the lung cancer risk assessment model, as risks for mesothelioma appear to be more closely linked to exposures to long, thin fibers (see Section 3.2.3). Further, some panelists suspected that increasing the fiber diameter cut-off in the exposure index should be accompanied by changes to the exposure-response coefficients in the risk assessment models, but the panelists did not unanimously agree on this issue.

5.5 Responses to Charge Question 12

Charge question 12 asks: "Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options." The panelists briefly reviewed the strengths and weaknesses of the three options presented in the proposed protocol for assessing asbestos-related cancer risks. The panelists agreed that the first option—direct use of EPA's lung cancer and mesothelioma risk assessment models—allows for the greatest flexibility in evaluating site-specific exposure scenarios, particularly those with time-varying exposures. Dr. Crump indicated that he envisioned this option being coded into a computer program into which users enter their site-specific exposure information. Most panelists endorsed developing such a program. The panelists did not reject use of the second and third options, provided that EPA ensures that all three options generate equivalent risk estimates for the same exposure scenario.

The one issue discussed in greater detail was how sensitive predictions using the first option are to the mortality rates used in the evaluation. Noting that mortality rates as functions of age and sex differ from one location to the next, this panelist encouraged EPA to consider carefully whether nationwide mortality estimates would be programmed into the risk assessment model or whether risk assessors would have the option of entering site-specific mortality rates. The panelist also suggested that the authors of the risk assessment conduct sensitivity analyses to quantify how strongly the mortality data affect cancer risk estimates. These comments also raised questions about the fact that two populations with different underlying mortality rates could have different cancer risks, even though their asbestos exposure levels are equivalent.

6. COMMENTS ON TOPIC AREA 4: CONCLUSIONS AND RECOMMENDATIONS

This section reviews the panelists' individual conclusions and recommendations regarding the proposed protocol (Section 6.1), as well as how the panelists developed their overall conclusions and recommendations that appear in the executive summary of this report (Section 6.2).

6.1 Responses to Charge Question 11

Charge question 11 asks: "Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?" The panelists offered individual summary statements, which were not discussed or debated among the panel. Following is a summary of the panelists' individual summary statements in the order they were given:

- **Dr. Lippmann's summary statement.** Dr. Lippmann commended Drs. Berman and Crump on developing the proposed risk assessment protocol and supported use of a model that accounts for the factors (e.g., fiber type and dimension) that are most predictive of cancer risk. Dr. Lippmann supported the authors' attempt to make full use of the existing data and to interpret the results from the epidemiological studies. He strongly recommended that EPA make every effort to obtain individual-level data from additional epidemiological studies. Dr. Lippmann suggested that a follow-up workshop with experts in exposure assessment could help EPA evaluate the uncertainties in exposure measurements from historic occupational data sets. Dr. Lippmann supported an observer's suggestion to conduct an animal inhalation study using tremolite cleavage fragments to help resolve the issue of these fragments' carcinogenic potency. Overall, he encouraged that future work on the proposed protocol continue, through use of additional expert panels, to make more informed usage of the human exposure data.
- **Dr. Teta's summary statement.** Dr. Teta indicated that the proposed protocol is an impressive integration of the animal toxicology data and the human epidemiology data. She commended the authors for developing a scientific methodology that successfully reduces the variability in results across the epidemiological studies, suggesting that the studies might be more consistent than were previously thought. Dr. Teta recommended improvements to the meta-analysis of

epidemiological studies, such as establishing and applying criteria for use of human data in characterizing exposure-response relationships. Overall, Dr. Teta found no inconsistencies between the proposed protocol and the larger body of epidemiology literature, including studies of cohorts (e.g., gas mask workers, railroad workers, friction brake workers) that do not have well-defined exposure information. Though not disagreeing with the utility of other panelists' recommendations, such as re-analyzing data from additional epidemiological studies and convening additional expert panels, Dr. Teta encouraged EPA to move forward expeditiously with completing the proposed protocol and discouraged implementing additional steps that might delay the overall project.

- **Dr. Hoel's summary statement.** Dr. Hoel encouraged the use of more sophisticated modeling that incorporates data on exposure-response (including non-linear models), duration of exposure, cessation of exposure, and uncertainty in exposure. Dr. Hoel also strongly recommended that EPA attempt to obtain individual-level data from additional epidemiology studies, or at least obtain partial data sets. He encouraged Drs. Berman and Crump to use more sophisticated uncertainty analysis techniques, such as generating prior and posterior distributions of uncertainty. To ensure that the lung cancer model is not confounded by cigarette smoking, Dr. Hoel recommended that Drs. Berman and Crump more closely evaluate all available data on the interactions between asbestos exposure and cigarette smoking.

- **Dr. Steenland's summary statement.** Dr. Steenland indicated that the proposed protocol is a step forward in asbestos risk assessment; however, he had several recommendations for improving the analysis of epidemiological studies. For instance, Dr. Steenland suggested that the authors conduct meta-regression analyses using the original exposure-response coefficients, in which predictor variables include fiber size, fiber type, the estimated percentage of amphiboles, percentage of fiber greater than 10 μm , and categorical grouping of studies according to quality. He indicated that these factors can be examined using both fixed effects and random effects models. Dr. Steenland recommended that the proposed protocol explicitly state and defend the basis for choosing the 10 μm cut-off for fiber length in the exposure index. He suggested that EPA should consider using Bayesian techniques or other methods to determine which relative potencies assigned to different fiber length categories optimize the model's fit to the epidemiological data.

Focusing on specific topics, Dr. Steenland indicated that he disagrees with the approach of assigning amphibole fibers five times greater lung cancer potency than chrysotile fibers, especially considering that the statistical analysis in the proposed protocol could not reject the hypothesis that amphibole fibers and chrysotile fibers are equally potent. Further, he advocated suggestions of exploring the adequacy of other exposure-response models (e.g., non-linear models). Finally, Dr. Steenland suspected that cigarette smoking likely will not be a confounding factor in exposure-response analyses for two reasons. First, he noted that differences in smoking practices between working populations and general populations typically do not cause

substantial differences in standardized mortality ratios. Second, he indicated that it is highly unlikely that prevalence of smoking varies with workers' exposure levels. Dr. Steenland encouraged that EPA refer to a recent publication (Liddell and Armstrong 2002) for similar insights on interactions between asbestos exposure and cigarette smoking.

- **Dr. Crapo's summary statement.** Dr. Crapo complimented Drs. Berman and Crump on preparing the cancer risk assessment methodology, and he supported the general approach of expressing cancer risk as a function of asbestos fiber type and fiber dimension. Dr. Crapo indicated that the proposed protocol reaches several defensible conclusions, such as assigning greater mesothelioma potency to amphibole fibers and to longer fibers while assigning no risk to fibers less than 5 μm in length. However, he was concerned about some specific issues that are not yet adequately resolved. For instance, Dr. Crapo felt additional data are needed to rigorously define how mesothelioma potency varies with fiber length (i.e., fibers longer than 10 μm being 300 times more potent than fibers with lengths between 5 and 10 μm). Dr. Crapo recommended that EPA, when revising the proposed protocol, explore more sophisticated modeling techniques, including non-linear exposure-response models and consideration of threshold effects. He supported more detailed analyses of interactions between asbestos exposure and cigarette smoking, again through the use of non-linear models.
- **Dr. Sherman's summary statement.** Dr. Sherman first indicated that she concurred with several recommendations made by Drs. Hoel and Steenland. She focused her summary statements on the proposed exposure index, recommending that Drs. Berman and Crump use the epidemiology data to further investigate other formulations of an exposure index. Dr. Sherman recommended, for example, examining the goodness of fit of other formulations of the exposure index (e.g., assigning zero potency to all fibers shorter than 10 μm). Further, she recommended that the authors attempt to optimize the potency weighting factors in the exposure index to the epidemiological data. Finally, given that panelists expressed concern regarding how potency varies with fiber length for mesothelioma, Dr. Sherman suggested that Drs. Berman and Crump consider developing two different exposure indexes—one optimized for lung cancer, and the other for mesothelioma. Dr. Sherman added that she generally supported the lung cancer and mesothelioma exposure-response models, and questioned whether using more complicated models would necessarily lead to a better understanding of the data.
- **Dr. Castranova's summary statement.** Dr. Castranova concluded that the proposed protocol is a significant advance in asbestos risk assessment methodology. He strongly supported the recommendation that future measurements be performed using TEM, rather than PCM. Dr. Castranova also supported the approach of assigning equal carcinogenic potency to cleavage fragments and asbestos fibers of similar dimension—a finding, he noted, that could be tested in an animal inhalation study. Further, Dr. Castranova agreed that non-asbestiform amphiboles and asbestos amphiboles of the same dimension should be assigned equal carcinogenic potency. Dr. Castranova indicated that the epidemiology and toxicology literature clearly indicate that

mesothelioma potency varies with fiber type, but he was not convinced that this literature supports a difference in lung cancer potency between amphibole and chrysotile fibers.

■ **Dr. Price's summary statement.** Dr. Price found the proposed protocol to be an impressive compilation of the epidemiology and toxicology literature into a cancer risk assessment model that addresses most, but not all, risk factors debated since EPA's 1986 model. Dr. Price urged EPA to explore exposure-response models other than the models that involve linear, low-dose extrapolations, which he viewed as being inconsistent with the epidemiology literature. Dr. Price indicated that future revisions to the protocol should definitely consider non-linear models and threshold effects.

As an additional comment, Dr. Price emphasized that the two main elements of the protocol—the proposed exposure index and the exposure-response analysis—are closely inter-related and subsequent changes to the proposed exposure index could affect the robustness of the overall modeling effort. As an example of his concern, Dr. Price noted that increasing the fiber diameter cut-off in the exposure index from 0.5 μm to 1.5 μm could (according to an observer comment) lead to dramatic differences in the number of cleavage fragments counted in environment samples; however, he indicated that the animal studies used to derive the original exposure index did not include cleavage fragments. Such scenarios raise questions about using an exposure index derived from very specific exposure conditions in animal studies to evaluate human health risks associated with exposures of an entirely different character. Dr. Price encouraged further study of cleavage fragments, perhaps in an animal inhalation study, to resolve the role of cleavage fragments.

■ **Dr. Case's summary statement.** Dr. Case congratulated Drs. Berman and Crump for compiling what he viewed as a reasonable evaluation of the available toxicology and epidemiology literature, and he strongly supported the general approach of factoring fiber type and fiber dimension into cancer risk assessment. Dr. Case indicated that he agreed with the finding that amphibole fibers have slightly greater lung cancer potency than do chrysotile fibers, although he believed that fiber dose, fiber length, and especially smoking history and type of industry have greater importance in this regard. Dr. Case recognized that how one views the differences between the Quebec and South Carolina cohorts affects the conclusions drawn on this issue, and he encouraged EPA to classify the cohort of South Carolina textile workers as being exposed to mixed asbestos fibers, rather than being exposed to only chrysotile fibers.²

² When presenting the summary statements, one panelist (LS) indicated that NIOSH is re-analyzing filters that were collected in the 1960s from the South Carolina textile plant, and these re-analyses should indicate the distribution of fiber types in this cohort's exposures. Another panelist (BC) noted that these re-analyses will not characterize earlier exposures to amosite fibers, which are believed to have occurred primarily before 1950 (based on findings from studies of lung-retained fibers).

Dr. Case made several recommendations for further evaluating the existing epidemiological data and for collecting additional data. First, Dr. Case indicated that it is critically important for any lung cancer risk model to consider confounding effects of cigarette smoking, and he encouraged EPA to incorporate interactions with cigarette smoking into the lung cancer model to the greatest extent possible. Second, Dr. Case supported Dr. Lippmann's recommendation of convening an additional expert panel workshop to critically review inferences that should be drawn from the exposure measurements made in the epidemiological studies; such a panel, Dr. Case noted, would require inputs from experts in mineralogy, industrial hygiene, and measurement methodologies. Third, he supported comments recommending that EPA examine non-linear and threshold exposure-response models. Finally, Dr. Case agreed that conducting an animal inhalation study is probably the best way to examine whether tremolite cleavage fragments produce lung cancer, but did not advocate using rat inhalation studies to examine whether these fragments induce mesothelioma, because results from rat inhalation studies have been shown to be a poor model for mesothelioma in humans. He added, however, that it would quite probably be impossible to design an experiment in which rats were exposed only to "cleavage fragments" or "non-asbestiform fibers" with no asbestiform fibers present at all.

■ **Dr. Stayner's summary statement.** Dr. Stayner supported the general concept of incorporating fiber type and fiber dimension into cancer risk assessment, but he recommended that additional work be conducted before EPA accepts the proposed protocol as a new risk assessment paradigm. Dr. Stayner indicated that his confidence in the proposed protocol varies between the lung cancer and mesothelioma models.

For lung cancer, Dr. Stayner indicated that the available epidemiological data should be able to support a new risk assessment model, but he recommended that EPA consider the panelists' many recommendations for how the meta-analysis can be improved (e.g., using different statistical models, developing and applying minimal study inclusion criteria, conducting additional sensitivity analyses). Concurring with Dr. Steenland's summary statement, Dr. Stayner added that cigarette smoking is very unlikely to be a confounding factor in the lung cancer model and he questioned whether the available data would support a quantitative assessment of the interaction effects. While Dr. Stayner supported the recommendation for evaluating non-linear exposure-response models, he noted that the individual-level data needed to construct these models are not available for most epidemiological studies. Dr. Stayner added that obtaining raw data from additional occupational cohorts would provide the best opportunity for more detailed exploration of non-linear exposure-response relationships.

Dr. Stayner expressed greater concern about the foundation of the mesothelioma risk model. He indicated, for instance, that the relative potencies included in the proposed exposure index are based entirely on toxicology studies for lung cancer, and not on any epidemiology or toxicology studies specific to mesothelioma. Despite these concerns about the biological basis for the proposed mesothelioma model, Dr. Stayner noted that the proposed model does provide an

improved fit to the findings from the epidemiological studies. He recommended that EPA consider optimizing the relative potencies in the exposure index to the human data, especially if EPA can access raw data from additional occupational cohorts to evaluate how exposure-response varies with fiber size and fiber type.

- **Dr. McClellan's summary statement.** Dr. McClellan congratulated Drs. Berman and Crump for integrating the toxicological and epidemiological data into a reasonable evaluation of asbestos cancer risks. Overall, Dr. McClellan found the proposed protocol to be a substantial improvement over EPA's 1986 models and urged EPA to continue to move forward with completing the protocol based on the panelists' feedback. Though he found the presentation of information in the draft document to lack transparency on many important matters, Dr. McClellan indicated that the authors' presentations at the workshop addressed many of his concerns regarding the transparency of how the proposed model was developed. One suggested improvement to the protocol's transparency was to clearly describe what literature were reviewed and to specify what studies actually factored into the quantitative analyses.

Addressing specific topics, Dr. McClellan indicated that the analyses in the proposed protocol adequately characterize the general roles that fiber type and fiber dimension play in cancer risk. He supported suggestions for involving additional experts, perhaps in another expert panel review, to further review interpretations of the epidemiological studies. Further, Dr. McClellan agreed with other panelists' recommendation that EPA explore the utility of non-linear exposure-response models, consistent with the agency's proposed revised Cancer Risk Assessment Guidelines. If linear, low-dose extrapolation models are ultimately used, he suggested that EPA explicitly acknowledge the uncertainties associated with such an approach. Dr. McClellan indicated that obtaining raw data from additional epidemiological studies might be particularly helpful in the exposure-response modeling. Finally, Dr. McClellan emphasized that the exposure characterization in the proposed protocol is closely linked to the exposure-response assessment; thus, the authors must carefully consider how revisions to the exposure characterization affect the assumptions in the exposure-response assessment, and vice versa.

6.2 Development of Final Conclusions and Recommendations

After presenting their individual conclusions and recommendations, the panelists worked together to draft summary statements for the peer consultation workshop. Every panelist was asked to write a brief synopsis of a particular topic debated during the workshop. These draft statements were then displayed to the entire panel and observers, edited by the panelists, and then compiled into this document's

executive summary, which should be viewed as the expert panel's final conclusions and recommendations regarding the proposed protocol.

The expert panel has reviewed the proposed protocol and has concluded that the protocol is acceptable for use in the field. The panel has identified several areas for improvement, including the need for more detailed information on the protocol's objectives and the need for more detailed information on the protocol's procedures. The panel has also identified several areas for improvement, including the need for more detailed information on the protocol's objectives and the need for more detailed information on the protocol's procedures. The panel has also identified several areas for improvement, including the need for more detailed information on the protocol's objectives and the need for more detailed information on the protocol's procedures.

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Appendix A

List of Expert Panelists

Appendix B

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

Note: This appendix is a copy of the booklet of the premeeting comments that ERG distributed at the peer consultation workshop. One panelist (Dr. Bruce Case) submitted an edited form of his premeeting comments to ERG at the workshop. That edited version appears in this appendix.

Appendix C

List of Registered Observers of the Peer Consultation Workshop

Available upon request to the Bureau of the Census

Appendix C of the 1997 report on the peer consultation workshop is available upon request to the Bureau of the Census. The report is available in both print and electronic formats. The electronic format is available on the Bureau's website at <http://www.census.gov>. The print format is available upon request to the Bureau of the Census.

Appendix D

Agenda for the Peer Consultation Workshop

- Review of the project goals and objectives, and the role of the peer consultation workshop in the project.
- Presentation of the project findings and recommendations.
- Discussion of the project findings and recommendations.
- Presentation of the project findings and recommendations.

Appendix E

Observer Comments Provided at the Peer Consultation Workshop

Note: The peer consultation workshop included three observer comment periods, one on the first day of the workshop and two on the second day of the workshop. This appendix includes verbatim transcripts (to the extent that specific remarks were audible from recordings) of the observer comments, in the order the comments were given.

Appendix F

Observer Post-Meeting Comments

Appendix A

List of Expert Panelists



Workshop to Discuss a Proposed Protocol to Assess Asbestos-Related Risk

Westin St. Francis
San Francisco, CA
February 25-27, 2003

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**Workshop to Discuss a
Proposed Protocol to Assess
Asbestos-Related Risk**

Consultants' Premeeting Comments

February 2003

Notice

Premeeting comments were prepared by each consultant individually prior to the meeting. They are preliminary comments only, and are used to help consultants become familiar with the document and charge questions, develop the agenda, and identify key issues for discussion. During the meeting, consultants may expand on or change opinions expressed in their premeeting remarks and may introduce additional issues. For these reasons, premeeting comments should be regarded as preliminary and do not reflect the final conclusions and recommendations of individual consultants. These premeeting comments will be included as an appendix in the meeting summary report, along with other background materials.

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Workshop to Discuss A Proposed Protocol to Assess Asbestos-Related Risk

Charge to the Peer Consultants

The U.S. Environmental Protection Agency (EPA) is conducting a peer consultation workshop to solicit feedback from a panel of experts on issues related to the draft document, "Proposed Methodology for Conducting Cancer Risk Assessments for Asbestos" (Berman and Crump 2001). Eastern Research Group, Inc. (ERG), a contractor to EPA, is organizing the workshop. Discussions at the workshop will focus primarily on issues raised in this charge, which lists questions that EPA would like the peer consultants to discuss and answer. The charge questions are not intended to limit the peer consultants' discussions; they merely address issues that are important to EPA. Peer consultants are invited to raise and discuss additional relevant topics, as noted below. This charge provides background information, instructions to the peer consultants, and the charge questions.

Background

EPA's current assessment of asbestos toxicity is based primarily on an asbestos assessment completed in 1986 (EPA 1986), and EPA's assessment has not changed substantially since that time. The 1986 assessment considers all mineral forms of asbestos and all asbestos fiber sizes (i.e., all fibers longer than 5 micrometers) to be of equal carcinogenic potency. However, since 1986, there have been substantial improvements in asbestos measurement techniques and in our understanding of how asbestos exposure contributes to disease. To incorporate the knowledge gained over the last 17 years into the agency's toxicity assessment for asbestos, EPA has contracted with Aeolus, Inc. to develop a methodology for conducting risk assessments of asbestos. The proposed risk assessment methodology distinguishes between fiber sizes and fiber types in estimating potential health risks related to asbestos exposure. The proposed methodology and the charge issues (Berman and Crump 2001) are the subject of the peer consultation workshop.

A key step in the determination of whether the proposed risk assessment methodology can be used to support decisions at asbestos-contaminated sites is gaining feedback during this peer consultation workshop. During the two and one-half day workshop, EPA will seek feedback from the peer consultants on the technical issues outlined later in this charge. Time will be set aside each day to hear from observers. The Agency will consider feedback received at the workshop in making decisions as to the applicability of the updated risk assessment methodology.

Instructions to the Peer Consultants

ERG selected eleven scientists to serve as peer consultants for the workshop. The peer consultants have extensive expertise in related fields, such as inhalation toxicology, pulmonology, cancer risk assessment, and biostatistics. Before the workshop, each peer consultant will be asked to read the proposed methodology and technical support document for a protocol to assess asbestos-related risk (Berman and Crump 2001) and to prepare and submit pre-meeting comments, which are to be written responses to the charge questions listed in the next section. ERG will distribute a compilation of the pre-meeting comments to all peer consultants and will make copies of this compilation available at the peer consultation

workshop. At the workshop, the peer consultants will actively participate in discussions that will focus largely around the charge questions and they will help draft summary statements of their conclusions and recommendations. Following the workshop, a technical writer from ERG will prepare a draft summary report that documents the technical discussions at the workshop, including the observer comments. After the peer consultants review and comment on the draft summary report, ERG will submit a final summary report to EPA.

When preparing written comments, please write each question, followed by your comments (or state why you are not responding). Please include your name at the top of each page, but do not paginate. Please refer to the enclosed "Format Guidelines for Preparing Written Comments." Your written comments are due to ERG no later than February 14, 2003.

CHARGE QUESTIONS

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

1) For lung cancer.

A) Influence of *fiber type*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

B) Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than *fiber type* and *fiber length*. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

2) For mesothelioma:

A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

B) Influence of fiber length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

Topic Area 2: The proposed exposure index.

4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

5) The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

Topic Area 3: General questions.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or "bundles that are components of more complex structures"). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.
- 8) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.
- 9) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?
- 10) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber diameter appropriate?
- 11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?
- 12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

Topic Area 4: Development of Conclusions and Recommendations

At the end of the workshop, the peer consultants will be asked to draft conclusion statements identifying their most notable findings on the proposed methodology. As a prelude to developing these statements, the peer consultants are invited to provide any additional comments or concerns, both strengths and weaknesses, on topics not specifically addressed in the previous charge questions. After completing the discussions the peer consultants will prepare their conclusions, and they will also be asked to develop recommendations for how EPA can improve the methodology. Please note that, although recommendations for future research projects are welcomed, the focus of this workshop is on the proposed risk assessment methodology and related charge questions and issues.

References

Berman DW and Crump K. 2001. Technical Support Document for a Protocol to Assess Asbestos-Related Risk. Final Draft. Prepared for U.S. Department of Transportation and U.S. Environmental Protection Agency. September 4, 2001.

Berman DW and Crump K. 1999. Methodology for Conducting Risk Assessments at Asbestos Superfund Sites. Part 1: Protocol. Interim Version. February 15, 1999.

EPA 1986. Airborne Asbestos Health Assessment Update. U.S. Environmental Protection Agency. EPA 600/8-84-003F. 1986.

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

**WORKSHOP TO DISCUSS A PROPOSED PROTOCOL TO ASSESS ASBESTOS-RELATED
RISK: SAN FRANCISCO; FEBRUARY 25-27, 2003.**

COMMENTS ARRANGED BY CHARGE QUESTIONS

Topic Area 1: Interpretations of the epidemiology and toxicology literature

1) For Lung Cancer:

Note 1: Lung cancer risk conveyed by asbestos exposure is principally related to degree of asbestos exposure and subsequent retained asbestos dose; to smoking habit; to type of industry (in occupational exposure situations), and to fiber type, in approximately that order of priority.

Hence the following section is best addressed beginning with item (D), with some supplementation, rather than items (A) through (C), although many of these factors are inter-related.

Note 2: While it is not made clear what is meant by "mechanistic studies" in the questions below it is assumed that what is meant is *all* animal and toxicological studies, including both cell-free and in-vitro systems. In fact, in vitro and cell-free systems have not as yet proved successful in use in risk assessment and should not be considered (these are given too much attention in the documentation of the proposed model). This has been established by a fairly recent consensus statement by IARC in Scientific Publication 140; the Consensus Statement has been circulated to the panelists (IARC 1996). Briefly, although a total of five possible mechanisms for asbestos carcinogenesis were considered in some detail, "The exact mechanisms leading to the development of cancer after exposure to asbestos fibers are poorly understood...Overall, the available evidence in favour of or against any of these mechanisms leading to the development of lung cancer and mesothelioma in either animals or humans is evaluated as weak". However, with respect to the two parameters principally considered in the risk assessment model under consideration by the panel, the IARC panel accepted as fact that "*Fiber dose, dimensions and durability are currently accepted as important parameters*". (Emphasis in the original).

In the following sections therefore "mechanistic studies" which do not rely on whole animal exposures will not be commented upon and (in this observer's view) should not have any input into current risk assessment. In addition, for whole animal studies, only those based on inhalation (which is the model most useful for human risk extrapolation) will be commented upon unless otherwise noted. Finally, what is described by the proposal as "(human) pathology studies" but is actually a subset of such studies which includes (but is not limited to) lung-retained internal dose studies (sometimes called "lung burden studies") will be commented upon here where relevant, as such studies are most directly relevant to human exposure assessment and have been (in this panelist's view) afforded too little emphasis by the authors, partially because of a assumption that the sampling for such studies is virtually always "opportunistic". The authors also appear to ignore the possibility of human exposure indices such as broncho-alveolar lavage (BAL) and sputum asbestos body analysis in living subjects; both relatively simple techniques with BAL being quite reproducible (sputum production on the other hand is highly affected by smoking status unless an "induction" technique is used; it has nevertheless proved useful in some situations and in at least one situation is a better predictor of asbestos-related radiological abnormalities than is estimated exposure (Sébastien P, Armstrong, B., Case, B.W. 1988).

A) Influence of Fiber Type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g. chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response relationships for different *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

This is a much more difficult question for lung cancer than it is for mesothelioma, where there is a clear preponderance of the evidence for a very large effect of fiber type. This panelist

agrees with the authors of the proposal and with the recent analysis of Hodgson and Darnton (Hodgson JT and Darnton A 2000) of seventeen cohorts for which exposure data are available that (even having accounted for smoking, dose, and industry type) there is at least a tenfold increase in lung cancer asbestos-related risk for amphibole asbestos exposures over chrysotile asbestos exposures; it is difficult to differentiate however between amphibole fiber types, and also difficult to differentiate between "asbestiform" and "nonasbestiform" or "cleavage fragments of massive amphiboles" and "asbestiform" exposures *if the latter exposures are to structures having similar dimensions, regardless of their crystal structure*. An effective test of this is provided in the data on chrysotile miners, millers and factory workers of Liddell et al. (Liddell FD, McDonald AD and McDonald JC 1998), in which "it is now clear that for all practical purposes (lung cancer risk) was confined to (one mining area), probably due largely to fibrous tremolite and in dust conditions (averaging)... 7 mpcf or very roughly 24 fibers/ml".

The proposed risk coefficients in Tables 6-29 and 6-30 appear to be highly conservative with respect to what is known about the differential effects of fiber type for lung cancer risk, with only a five-fold difference. Since others have suggested that there is in fact a difference that is somewhere between ten and fifty-fold, this seems reasonable. Given the extreme importance of the other factors noted above and described in more detail in section DJ below (dose, smoking habit, and type of industrial setting (the latter perhaps being related to fiber length); a coefficient which is conservative for lung cancer risk and fiber type seems reasonable, as long as the other factors are taken into sufficient account by the risk model.

B] Influence of Fiber Length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response relationships for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see equation 7.13) that is weighted heavily by fibers longer than 10 micrometers (μm)?

This question was recently explored *in part* by an expert panel for ATSDR, the report of which is pending. Specifically, the latter panel was asked to assess charge questions which addressed any proven or putative risk for "short fibers", which were defined operationally as

those having length *less than 5 μm* (ATSDR 2002), for both cancer and non-cancer endpoints. The aim was not a consensus statement but to use the information presented during the expert panel meeting to aid in developing scientifically sound public health evaluations for exposures to "short fibers" defined as above. Because the final draft of this document has not been released it cannot be cited or quoted, but hopefully it will be made available to the current expert panel for EPA as it is directly relevant to issues of fiber length and risk. There is no reason to "reinvent the wheel" for the part of the current discussion which overlaps the previous panel's deliberations, although additional input from panel members not involved in the ATSDR-convened panel will be a valuable addition to this discussion. While the ATSDR-convened panel was not charged with looking at the converse proposition that *longer fibers* convey greater risk of the endpoints in question (including lung cancer), this is the other side of the same coin and was certainly discussed.

As acknowledged by the authors of the current proposal to EPA, there is little epidemiological data available which specifically assesses the role of fiber length on lung cancer risk. Most of the available epidemiological data on lung cancer risk for which any exposure assessment is available comes from occupational cohorts in which that exposure was assessed either by midget impinger counts (which historically counted all particles as million particles per cubic foot (MPCF); isometric particles as well as fibers). This method dealt with *all* particles visible by light microscopy and had a low resolution of approximately 1 μm diameter, with *no* information on particle length.

Some epidemiological studies have as indices of exposure to asbestos data derived from the membrane filter method through counting via phase contrast optical microscopy (PCOM). Results are expressed as fibers per cubic centimeter or milliliter (fibers/ml), but are always limited to fibers longer than 5 μm . As noted by the authors of the current proposal to EPA, a principal weakness of the membrane filter method and of PCOM counts is that they are not capable of determining whether the structures being counted are actually "asbestos" at all (although the authors do not appear to address the use of dispersion staining techniques in this regard. It is important that EPA receive competent mineralogical or industrial hygiene advice as to the suitability of dispersion staining techniques in association with PCOM/membrane filter counts to improve upon the identification of "asbestos", and individual types of asbestos fiber

using light microscopy alone, especially given the increased costs (and perhaps decreased sensitivity) of transmission electron microscopic techniques.

Some epidemiological studies combine both types of exposure index (MPCF and fibers/ml), using data-derived conversion factors from MPCF which vary from an approximate threefold to an approximate eightfold multiplication of the MPCF value in question to derive an analogous value in fibers/ml. The conversion factors appear to be to some degree study and workplace-specific; are by definition approximations; and should be used with caution; the most commonly used conversion factor is an approximate threefold multiplication of MPCF.

Since all situations in which there is exposure to asbestos fibers comprise a size distribution with respect to fiber length rather than a specific fiber length compartment, it is not surprising that epidemiological studies have not addressed this issue to a great extent. It should be emphasized however that all existing risk assessment models, including the 1986 EPA risk assessment, are largely derived on the exposure assessment side from measurements of, or approximations of measurements of, or conversions of other measurements to, exposures to fibers longer than 5 μm . In addition it is well known both from studies of size distributions of asbestos exposures and of asbestos retained dose that there is good correspondence of asbestos concentrations (even when broken down into individual fiber types) across fiber-length categories.

Some data does exist from epidemiological studies which may inform as to effects of fiber length on lung cancer incidence or mortality. There are two extremes for fiber length in epidemiological studies which have been examined with respect to the shape of distributions. Studies of workers in asbestos textile industries, in which there is some evidence that there is more skew of exposure fiber-length distributions to longer fibers; have generally shown a higher dose-response gradient for lung cancer risk (Knox JF, Holmes S, Doll R et al. 1968; Newhouse ML, Berry G, Wagner JC et al. 1972; Peto J, Doll R, Howard SV et al. 1977; Peto J 1980; McDonald AD, Fry JS, Woolley AJ et al. 1983; McDonald AD, Fry JS, Woolley AJ et al. 1983; Paci E, Buiatti E and Geddes M 1987; Sebastian P, McDonald JC, McDonald AD et al. 1989; Dement JM and Brown DP 1994; Dement JM, Brown DP and Okun A 1994; McDonald JC 1998;

Case BW, Duffresne A, McDonald AD et al. 2000; Hodgson JT and Darnton A 2000).

Conversely, studies of studies of gold mine workers in South Dakota and taconite miners in Minnesota suggested no lung cancer risk for a largely short ($< 5 \mu\text{m}$) fiber distribution. The South Dakota workers were exposed to cummingtonite-grunerite material with 94% of airborne fibers being less than 5 microns in length. Gilliam et al. (Gilliam JD, Dement JM, Lemen RA et al. 1976) found increased mortality from malignant respiratory disease among workers with at least 5 years of exposure. However, a follow-up study of this cohort which considered longer latency and the most highly exposed workers found no such increase at estimated average exposure concentrations of 4.83 fibers per cubic centimeter (McDonald JC, Gibbs GW, Liddell FD et al. 1978). A later study of 3,444 men employed for at least 3 months in Minnesota taconite mining operations (also believed to be exposed to a short-fiber distribution) during the years 1947 to 1958 (86,307 person-years of observation) found 41 deaths from respiratory cancer - an SMR of only 61 to 85 (for US white male rates or Minnesota rates respectively) (Cooper WC, Wong Q and Graebner R. 1988).

It seems reasonable to weight the exposure indices in question to assign greater risk for greater fiber length. It also seems unreasonable based on current knowledge to assign any weight at all to fibers of less than $5 \mu\text{m}$ in length. Finally, while it seems clear from what we know of mechanistic studies that tumor hazard is related to increasing length, a coefficient that assigns incrementally increasing weight to fibers in a continuous length distribution would be preferable to one that simply categorizes lengths. This however may be quite impractical for real-world assessments of hazard. Having said all this, the paucity of direct data on fiber length in the epidemiological studies makes it imperative to answer the question as posed - *is it appropriate to assess cancer risks using an exposure index (per equation 7.13) that is weighted heavily by fibers longer than 10 micrometers (μm)?* in the negative, *if one is referring to the supporting evidence from epidemiological studies alone.* Nevertheless, the very heavy weight put on the longest fibers for lung cancer risk in this equation does seem reasonable taking *all of* the available data into account. Strictly speaking, an equation which put greater weight on increasing length intervals would be better; and it must be remembered that the vanishingly small

coefficient for fibers between 5 and 10 μm in length will be modified by the fact that those fibers are far more numerous (and more likely to be disproportionately counted by any available technology, including transmission electron microscopy).

C] To what extent do animal studies (e.g. studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

These studies are clearly outlined in the protocol provided by the authors of this proposal. In general, inhalation studies support the role of fiber length (especially fiber length greater than 10 μm , or in some studies greater than 20 μm) in lung cancer risk and also support the assertion that there is no excess lung cancer risk in these models under 5 μm .

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology and toxicology literature for supporting these other properties into dose-response analyses?

Aspect ratio is simply the ratio of length to width and therefore should have no role in risk assessment independent from length and width. Surface properties, especially surface iron, may well be related to lung cancer risk through the mechanisms of lung cancer production (such as, for example, free radical generation and cell signaling mechanisms), but in my view are insufficiently developed or understood at this time to be useful for risk assessment, and are certainly not ready to be incorporated into dose-response analyses. This was also in essence the conclusion of the IARC panel, which was convened in order to determine *whether* mechanistic studies could contribute to risk assessment protocols and in the consensus statement concluded in effect that current evidence is "weak" (IARC 1996).

As noted above the principal factors driving risk for lung cancer related to asbestos exposure, in approximate order of priority, are not fiber factors *per se* but *asbestos dose*, *degree of smoking co-exposure*, *type of industry (in an industrial setting)*, and *fiber type*. With the exception of the latter, which was dealt with above, these are *not* necessarily directly related to fiber factors, and are *more important than fiber factors* (particularly fiber type and length) and

should to the degree possible be accounted for in any risk assessment model. They are in fact accounted for in one way or another in the proposed model.

Fiber dose (derived from *fiber exposure*) is so obviously related to risk that little further need be said here; in fact the charge questions *assume* the importance of this factor, while "jumping the gun" to assess the effect of other factors on "dose-response analysis". One cannot begin without a discussion of the influence of (externally measured) exposure, and subsequently of retained dose, *per se*. The authors of the proposal in fact do so in a number of ways, although their model itself is highly dependent on fiber factors *in addition to* dose. The question of linear extrapolation, the general use of the linear model (as opposed to other models), and the question of threshold, also arises in relation to the issue of total exposure and resultant total dose.

Individual smoking history is the second most important factor in risk after absolute exposure and absolute dose. The previous (1986) EPA model and the current model appear to assume through the derivation of the terms that risk for smoking and asbestos are multiplicative, with both assessments being heavily reliant on an early and flawed analysis of this relationship by Selikoff and Hammond ((Selikoff JJ, Hammond EC and Seidman H 1979). This is assumed through the use of a model which uses relative risk (to the underlying population) in which most of the absolute risk is due to smoking. This may overestimate lung cancer risk as the actual synergism between smoking and asbestos exposure is now generally thought to be less than multiplicative, although still more than additive (Liddell FD and Armstrong BG 2002).

Type of industry remains a powerful influence in risk. It is often assumed that fiber factors (perhaps especially fiber length) may be important in this regard, but this remains unproven and based largely on some assumptions about the large differences in the dose-response analyses between asbestos textile cohorts and asbestos mining cohorts, particularly those commonly associated with chrysotile. In fact, while there is no doubt that large differences in the slope of lung cancer risk exist between these industries, it remains unproven that these can be accounted for entirely by differences in fiber length, and recent thinking on this subject

suggests a more complex explanation (McDonald JC 1998; Case BW et al. 2000; Hodgson JT and Darnton A 2000) in which other factors (including but not limited to fiber type, and including other processing steps in industrial settings) play a role. For example, it is clear that

while exposure (externally measured) may show a greater proportion of longer fibers in the textile than in the mining setting, for any given fiber length interval the lung-retained concentration of fibers is greater in the mining situation — and it is the mining situation which shows lesser lung cancer risk. It does appear from close examination of the data however that the ratio of retained dose to exposure is higher in the textile situation for the longest fibers (unpublished analysis of data from (Case BW et al. 2000)).

It is hard to say how, if at all, this element which is a powerful one in industrial settings can be translated into risk assessments for environmental settings unless it is possible to determine for a given environmental setting (or site) which industrial cohort is most similar. For most superfund sites dealing with former mine sites, for example, mining cohorts (those with a lower slope of lung cancer risk) should clearly be those applied and the textile data is of little relevance. The model offered does not account for this possible discrepancy between sites.

Finally, as noted above, fiber type does play an apparent role in risk for lung cancer, with a ten to fifty-fold excess risk having been suggested by the best available analysis (Hodgson JT and Darnton A 2000) for commercial amphibole exposure as opposed to chrysotile, and for virtually all of the excess lung cancer risk in the chrysotile mining situation being explained by co-exposures to tremolite, at least in those with exceptionally heavy exposure (specifically greater than 300 million particle per cubic foot-years (MPCF-Y)). (Liddell ED et al. 1998).

2) For mesothelioma:

A) Influence of Fiber Type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g. chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response relationships for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

The epidemiology literature provides definitive evidence that carcinogenic potency varies from one fiber type to the next. Indeed, there is currently no real scientific support for the proposition that

chrysotile is a cause of malignant mesothelioma from available epidemiological studies. This is true across a wide range of industries and studies, including cohort studies of workers in a variety of asbestos industries, case-control studies of mesothelioma and occupation, and a variety of studies of non-occupational exposure to asbestiform amphiboles, including but not limited to tremolite asbestos and to "cleavage fragments" of massive tremolite amphibole having dimensions similar to those of asbestiform tremolite fibers. Even efforts to gather together all reported "cases" of mesothelioma related "mainly" to chrysotile exposure inevitably come up with small numbers of such cases limited mainly to chrysotile miners and millers also exposed to tremolite (e.g. (Stayner LT, Dankovic DA and Lemen RA 1996).

Mesothelioma is related to amphibole asbestos exposure in approximately 80% of cases in epidemiological and pathological studies (the attributable risk varies from about 60% (McDonald AD and McDonald JC 1980; Yeung P and Rogers A 2001), to about 88% (Spirtas R, Heineman EF, Bernstein L et al. 1994) depending on the population and time period covered. This is supported by studies which assess exposure to humans directly through lung-retained fiber content (what the authors of the proposal call "pathology studies"; for example (McDonald JC, Armstrong B, Case B et al. 1989; Rogers AJ, Leigh J, Berry G et al. 1991; Rodelsperger K, Weitowitz HJ, Bruckel B et al. 1999) or by occupational inquiry (McDonald AD and McDonald JC 1980; Teta MJ, Lewinsohn HC, Meigs JW et al. 1983; Spirtas R et al. 1994; Weitowitz HJ and Rodelsperger K 1994; Teschke K, Morgan MS, Checkoway H et al. 1997) (if properly conducted in a true analytical epidemiological study as opposed to a survey, "registry", or collection of "cases").

The percentage is higher in occupations with heavy amphibole asbestos exposure, and in relatives of those in some such occupations, or occasionally in areas of endemic exposure such as the neighborhood of some shipyards, factories, and mines. It is important to note in this regard that exposures thought to be "nonoccupational" may first of all simply have inadequate occupational history, and secondly may be truly "nonoccupational" but nonetheless be associated with exceptionally high dose. An example of both of the latter is offered by a recent case control study of pleural mesothelioma among women living in the neighborhood of chrysotile mines: of ten cases discovered, all had worked outside the home, five were known to have worked in the industry, nine had at lived with at least one and more frequently more than one asbestos worker, and all lived in the highest tremolite area (Case BW, CM, Richardson L, Parent M-É, Désy M, and Siemiatycki J 2002). In addition exposures were very high, estimated among cases on average at over 200 fiber/ml – years and never under 100 fiber-ml years. A

similar situation for environmental exposure to crocidolite has recently been reported from China (Luo S, Liu X, Mu S et al. 2003), where crocidolite was found in the surface soil in a rural county where the average number of mesothelioma cases was 6.6 per year in the 1984-95 period and 22 per year in the 1996-99 period, in a population of 68 000. The annual mortality rate for mesothelioma was reported as 85 per million, 178 per million, and 365 per million for three separate cohort studies; and there were no cases of mesothelioma in comparison groups where no crocidolite was known to exist in the environment. This provides an object lesson for parts of California in which asbestiform tremolite has been identified in the surface soil and there has been a large degree of recent and planned housing development.

Most exposed mesothelioma cases in other studies either worked with, or more rarely had relatives who worked with commercial amphiboles, whether crocidolite (Armstrong BK, de Klerk NH, Musk AW et al. 1988; de Klerk NH, Armstrong BK, Musk AW et al. 1989; de Klerk NH, Armstrong BK, Musk AW et al. 1989; de Klerk NH, Musk AW, Cookson WO et al. 1993; Hansen J, de Klerk NH, Eccles JL et al. 1993; Hansen J, de Klerk NH, Musk AW et al. 1998) and/or amosite (Sluis-Cremer GK 1991; Sluis-Cremer GK 1991; Sluis-Cremer GK, Liddell FD, Logan WP et al. 1992); although large quantities of non-commercial amphibole fiber (tremolite or other minerals in the tremolite-actinolite series) associated with chrysotile in mining occupations; mined as industrial "talc" (Abraham JL, Hull M., Case, B.W. 2002) or vermiculite (McDonald JC, McDonald AD, Armstrong B et al. 1986; Amandus HE and Wheeler R 1987; Wright RS, Abraham JL, Harber P et al. 2002) may also be causative. Relatives of such workers who are subject to domestic (household) exposure to mining or milling fibers brought home on clothes or shoes are also subject to mesothelioma risk.

Mesothelioma was first conclusively linked to "asbestos" by J.C. Wagner in South Africa in 1960 (Wagner JC, Sleggs CA and Marchand P 1960) in a large study of cases taken from the Cape crocidolite mines. Some pathologists including the late Dr. Wagner still believe that crocidolite is the most important or even the only causative fiber, but most now accept amosite as responsible for as many or more cases (at least in the United States), and lung-retained fiber surveys of cases by Churg and by Roggli et al. (Churg A and Green F 1990; Roggli VL, Pratt PC and Brody AR 1993; Churg A and Vedal S 1994) have established that the less potent amosite fiber is responsible for the largest percentage of cases in the United States, at least among plaintiffs in lawsuits from which their cases were mainly drawn.

A recent meta-analysis of 17 cohorts with established exposure histories has reconfirmed the over-arching importance of amphibole exposure in mesothelioma causation, including in cases of exposure to mixed fiber types, including situations where chrysotile is by far the most prevalent exposure (Hodgson JT and Darnton A 2000). These authors estimate the relative risks of fiber types for mesothelioma as crocidolite: amosite: chrysotile 500:100:1, even making the conservative assumption that the chrysotile-related fraction includes the mining cases.

Crocidolite, the form first shown to cause mesothelioma, remains the most potent cause, although use has been essentially banned in North America and Europe and the number of future cases has been overestimated according to the most recently available data. More North American workers (at least insulation workers and those in allied trades) have now been exposed to amosite, and therefore more cases are produced by it, even though, given equal exposures, the proportion of workers developing mesothelioma is higher among those exposed to crocidolite.

Studies of chrysotile miners and millers in Quebec (well-described by the proposal's authors, in general) show a mesothelioma death rate of approximately 0.4% (33/8009 or 1 in 240 deaths in recent years (Case BW; Churg, A., Dufresne, A. Sébastien, P. McDonald, A.D. and McDonald, J.C. 1997; McDonald AD; Case BW; Churg A et al. 1997). Lung tissue analytic study of miners from different locations show unequivocally that what was thought to be "chrysotile-related" mesothelioma occurs only in mining and milling situations where tremolite is present in sufficient quantity to produce high levels of long, thin, high aspect-ratio tremolite or tremolite-actinolite fiber in the lungs of workers.

In these studies the area in which mesothelioma risk was in greatest excess was that where the amphibole tremolite was (a) geologically likely to be present in highest concentration and (b) present in excess (compared to other chrysotile mines) in the lungs of miners and millers.

Commercial amphiboles, on the other hand, have long been known to cause mesothelioma, and at far lower dose. Wagner established the causal relationship between "asbestos" and mesothelioma in a crocidolite mining region, as noted above. Work by Hansen and colleagues have shown at the Wittenoom

¹ This applies to the 33 of 38 cases in this study who were miners and millers of chrysotile. (Another 5 cases worked in a factory producing asbestos products and used crocidolite asbestos. The total number of deaths given however also includes deaths among the small number of factory workers)

mine in Australia the causation of mesothelioma by crocidolite exposures as brief as one week and as small as 0.4 fiber-years (Hansen J et al. 1998). Similar work in South Africa has produced comparable results, both for crocidolite and for amosite, although the quantification of exposure is not as good as that observed in the Australian studies (Hodgson and Darnton 2000). Recent work from China suggests that in one rural province there the situation may be similar (Luo S et al. 2003).

Surveys of individual asbestos industries have confirmed that *within* those industries fiber type remains the key factor in mesothelioma production: effectively, wherever crocidolite or amosite have been used commercially some mesothelioma risk has been introduced. Acheson and others (Acheson ED, Gardner MJ, Pippard EC et al. 1982) looked at female respirator manufacturers: groups followed for 40 or more years. One group made "civilian" respirators containing chrysotile and showed no mesothelioma excess (and only one case, who had worked in the other plant as well). The other made "military" respirators (containing crocidolite) and had increased mesothelioma mortality. Similar results were observed for Canadian workers making military gas masks using crocidolite (McDonald AD and McDonald JC 1978).

A similar pattern was demonstrated for two asbestos cement plants in Louisiana by Hughes and Weill (Hughes JM, Weill H and Hammad YY 1987). Mesothelioma risk occurred in the plant in which crocidolite was used in one manufacturing process. Similarly, Gardner observed one case of mesothelioma in a cement plant using mainly chrysotile; but noted that the case was believed to be due to exposure elsewhere (Gardner MJ, Winter PD, Pannett B et al. 1986). A very recent study from Norway has again demonstrated the importance of a proportion of crocidolite in the cement manufacturing process in inducing mesothelioma risk (Ulvestad B, Kjaerheim K, Martinsen JI et al. 2002).

The manufacture of friction products is a particularly useful area in which to look at the distinctive differences in epidemiologic risk by fiber type. This is because for the most part these products were made with chrysotile asbestos, with only occasional "special contracts" in some plants having used crocidolite. Mesothelioma risk has been limited to those situations. This is true whether the studies have been of the plants in which friction materials were manufactured (for example (McDonald AD and Fry JS 1982; Newhouse ML, Berry G and Skidmore JW 1982; Berry G and Newhouse ML 1983; McDonald AD, Fry JS, Woolley AJ et al. 1984; Newhouse ML and Sullivan KR 1989; Berry G 1994)), or whether the studies were case-control studies of mesothelioma in which end-product users (including

identified groups of workers who worked with brake linings in garage settings) were included (McDonald AD and McDonald JC 1980; Teta MJ et al. 1983; Spirtas R et al. 1994; Woitowitz HJ and Rodelsperger K 1994; Teschke K et al. 1997). A recent meta-analysis has added statistical power to the latter analyses by combining them and again finding no mesothelioma risk for end-users of automotive friction products (Wong O 2001).

"Mechanistic" studies of mesothelioma add little of value to the question of fiber type. This is because of the large degree of interspecies difference as well as the technical difficulty of performing inhalation experiments with mesothelioma as an endpoint. One intriguing mechanistic point that has come to the fore with recent in vitro and cell-free work has been the question of the presence of iron and its effect on free radical generation and related effects. While this appears at first blush to be relevant due to the "structural" iron content of the commercial asbestiform amphiboles (crocidolite and amosite) (as well as the ferruginous "asbestos bodies"!), it does not explain the effects of some of the other amphiboles. Furthermore, chrysotile is not always "iron-free", as iron may be substituted in its structure or absorbed onto its surface.

The proposed risk co-efficients in Table 6-29 are quite consistent with the enhanced effect of amphibole fiber types on mesothelioma risk observed in epidemiological studies. The coefficients appear to be conservative in that they assign any mesothelioma risk at all to chrysotile asbestos for mesothelioma. It is interesting that although a different method was used than that of Hodgson and Darnton (2000), the "bottom line" in this model appears to be the same or even greater: an approximate five-hundred fold increase in risk for the amphiboles on a fiber-for-fiber basis.

B] Influence of Fiber Length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response relationships for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see equation 7.13) that is weighted heavily by fibers longer than 10 micrometers (μm)?

For the most part, epidemiology studies do not inform with respect to carcinogenic potency for mesothelioma for fiber length. This is because, as pointed out by the authors, the existing epidemiology

studies have not used methods for exposure assessment which are capable of assessing fiber length, other than (if PCOM is used with the membrane filter method or an approximation of or conversion to PCOM values used from MPCF) limiting exposures to those longer than 5 μm . Of "mechanistic" studies which inform as to fiber length, the classic studies remains those of Stanton (Stanton MF and Wrench C 1972; Stanton MF 1974; Stanton MF, Laynard M, Tegeris A et al. 1977; Stanton MF, Layard M, Tegeris A et al. 1981), although the method of "exposure" in those experiments was neither physiologic nor in any way related to actual human exposure. Nevertheless, no discussion of mesothelioma and fiber length can ignore the Stanton model, for which there has been additional support in many animal studies since. It must be realized however that the classic Stanton "carcinogenic" fiber dimension (fibers having length greater than 8 μm and diameter less than 0.25 μm) were not met by *all* carcinogenic fibers, and with specific respect to tremolite – a fiber for which two preparations produced a 100% tumor response in the model – Stanton specifically reported that his model *did not* fit the response, and that "...relatively high correlations (with tumor response) were also noted with fibers in other size categories having diameters up to 1.5 micrometer and lengths greater than 4 micrometer" (Stanton MF et al. 1981). On the other hand, there is no evidence that structures having the same chemistry and crystalline structure as "asbestos" but length less than 5 μm behave as fibers rather than in the same way as isometric particles, nor is there evidence that such particles convey any risk for malignant mesothelioma. There is also a great deal of animal data which suggests the converse, much of which is listed by the authors of the proposal.

C] To what extent do animal studies (e.g. studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Animal studies are of little value in assessing the carcinogenic potency of fiber type. Animal studies which assess mesothelioma risk using the exceptionally sensitive peritoneal injection model in rats are in my view of little value, and intra-tracheal instillation models are similarly flawed, in the latter case in part because of the difficulty of assessing either the size or the nature of the administered dose in terms of fiber number or morphology. Even animal inhalation models have proved disappointed in assessing the risk posed by different fiber types, principally because rats are rather insensitive in this model. A recent review (Muhle H and Pott F 2000) summarizes this well: "Inhalation experiments with rats need fiber exposure concentrations...about 1,000 times higher (than those of asbestos workers) to reach the same mesothelioma risk. Also, the striking difference between the low lung burden of

amphibole fibers of asbestos workers with mesothelioma and the more than 1,000 times higher lung burden of rats with a low mesothelioma risk demonstrates the low sensitivity of the inhalation test model for the carcinogenic potency even of crocidolite fibers." Fortunately the effect of fiber type for mesothelioma is established beyond question by the epidemiology studies, at least for the relative effects of chrysotile as compared to commercial amphiboles and tremolite asbestos.

To the degree that fiber length categories can be separated for the purposes of exposures in animals (there is no such thing as a perfect preparation in which there are "no long fibers" or "no short fibers") the animal studies do indicate increasing mesothelioma risk with increasing fiber length, although the fiber length varies somewhat from study to study. The fiber length most often mentioned above which a mesothelioma response was observed is 20 μm in more recent studies. These are well described in the proposal and will not be repeated here: key references include those with sized fiber preparations, with characterized length distributions, and with theoretical calculations (Davis JM, Addison J, Bolton RE et al. 1986; Davis JM and Jones AD 1988; Lippmann M 1990; McConnell EE, Axten C, Hesterberg TW et al. 1999; Miller BG, Searl A, Davis JM et al. 1999). While the same problems exist for studies using the intraperitoneal injection model in the rat with length as the independent variable as those for fiber type, one study (that was not peer-reviewed prior to publication of six naturally occurring tremolite preparations does suggest some effect (Davis JM, Addison J, McIntosh C et al. 1991). However the main purpose of this study was to test the relative effects of "asbestiform" versus "nonasbestiform" tremolite preparations (see below).

D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology and toxicology literature for supporting these other properties into dose-response analyses?

It is important here to distinguish between respirability and carcinogenicity, with respect to fiber diameter. As noted above, the original Stanton studies actually showed effects at diameters of less than 1.5 μm , not 0.5 μm . A cutoff of 0.5 μm is probably inappropriate, although it is quite true that almost all chrysotile and crocidolite fibers will be included. This is somewhat less true for amosite and is not acceptable at all for tremolite, which in many situations will almost reach an arithmetic average of 0.5 μm diameter. In general, as demonstrated by Berry (unpublished data on Witenoom and (Berry G 1999).

), who has shown that "the incidence of mesothelioma after exposure to asbestos is proportional to the intensity of exposure (fibers per milliliter of air) and the duration of exposure, and to the time that has elapsed since the exposure. The incidence increases with time since exposure to a power of between 3 and 4".

The latter variable – time since first exposure – is a very powerful component of mesothelioma risk in epidemiological studies across the board, if they are large enough and have long enough follow-up. No model which ignores this timing factor can be considered adequate for predicting risk. In addition Berry has recently demonstrated a large effect of elimination time on mesothelioma risk by applying this model to Wittenoom mesothelioma mortality data (Berry G, unpublished data presented at International Mesothelioma Interest Group meeting, Perth, Australia, December 2002).

I will take the opportunity here to separately and briefly discuss lung-retained fiber studies in human subjects for mesothelioma – a separate category of study which is called "pathology studies" by the authors which is capable of isolating effects of fiber type and length with the understanding that analyses are performed at an endpoint (either lung biopsy, pneumonectomy, or autopsy) which integrates lifetime dose and clearance at a single point in time. It is nonetheless useful, although to some degree dismissed by the authors of the model for a number of theoretical reasons, chief among them what the authors call "opportunistic" sample site selection and what the authors believe is poor repeatability of results. In fact, if such studies are well-controlled, sample selection is *not* opportunistic; in that samples from cases and controls, taken at the same time and in the same way by the same pathologists in the same hospitals, are very likely to be comparable. Similarly, there is little evidence other than a few studies based on very small numbers of samples that there is in fact significantly poor reliability in such measurements so long as they are compared within rather than across laboratories. Reliability is at least as good as that for TEM fiber measurements in air. In fact, in Quebec, this is the method used *routinely* to characterize exposure for workman's compensation purposes (when lung tissue sections are available). We have had the experience of hundreds of such analyses and our results do well in cross-disciplinary validation studies in comparison with semiquantitative job-based indices of asbestos exposure. We have not encountered difficulties with reliability; our published studies in fact are capable of distinguishing trends of fiber retention with age, with rural-urban gradient, and with distance lived from and time lived in mining areas for environmentally-exposed individuals (Case BW and Sebastien P 1987; Case BW, Sebastien P and McDonald JC 1987; Case BW and Sebastien P 1989; Case BW 1991; Case BW 1994;

Takahashi K, Case BW, Dufresne A et al. 1994). Here for example are results from our most recently analyzed mesothelioma case; note the consistency across samples.

Sample site	Asbestos body concentration (AB/ gram dry lung, PCOM at 320X, detection limit 40 AB/ gram dry lung)	Crocidolite fiber concentration* (and number)	Other asbestos fibers detected; detection limit 35 fibers/ mg dry lung
Right lower lobe #1	31,520 AB/ gram dry lung	722 fibers/ mg dry lung (N=22)	None detected
Right middle lobe	26,880 AB/ gram dry lung	620 fibers/ mg dry lung (N=18)	None detected
Right lower lobe #2	28,320 AB/ gram dry lung	790 fibers/ mg dry lung (N=23)	None detected
Right upper lobe	26,920 AB/ gram dry lung	550 fibers/ mg dry lung (N=16)	None detected

* Fibers (longer than 5 μ m, aspect ratio greater than 3:1) identified and counted by transmission electron microscopy at 13,500 X magnification and by energy dispersive x-ray spectrometry (EDS).

With specific reference to mesothelioma causation, several case-control studies using this type of exposure index have produced interpretable results which lend strong support to both the role of (amphibole) fiber type and of increasing fiber length. In one such example, McDonald et al. studied 78 case-control pairs of lung samples from mesothelioma victims and controls matched for age, sex, hospital, and time of acquisition of sample. (McDonald JC et al. 1989). There were "substantial differences...between cases and referents for amosite, crocidolite, and tremolite. Much less difference was noted for anthophyllite, talc, and chrysotile...Statistical analysis indicated that short fibers were not associated with increased risk for mesothelioma." It should be noted that no special care was taken to match sample sites for cases and controls or across cases or controls; this is in fact not necessary if cases and controls are matched by hospital and era as it is the routine practice of pathologists which determines sample site selection; in other words, while the authors of the proposal were justified in their belief that such studies might use "opportunistic" samples which are not from similar sites within the lung, the actual practice of pathologists taking lung samples from autopsy or from resected lung tissue is quite consistent in this regard, with most taking central parenchymal samples from fixed sites in a manner learned during any pathology residency.

- 3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

This question is unclear, and may not be the most relevant to ask. More important than the reliability of exposure estimates is their validity, which in general is excellent within the largest and best studies that have been generally used for risk assessment (combining them in meta-analyses has proved more difficult and controversial). Similarly, reliability is good across the largest individual studies but much poorer between studies, due to the differences in methodology employed. This is well discussed by the authors of the proposal and they suggest some additional work which might help to ameliorate this difficulty. However, the authors do appear to be unaware of some of the controversies extant about these estimates.² For example,

they make use the Witenoom data without referring to the controversy and debate between Australian researchers about these *(because of their exceptional importance these comments are attached to these comments as appendix A)*. The authors do note the exclusion of the Charleston (textile) data by Hodgson and Darnton but choose to include it; in my view this is not critical to their conclusions although the Charleston data is clearly an anomaly even within the studies of textile workers and should be viewed with extreme caution. It should only be used if balanced with use of the Quebec data on exposures in the mining and milling of chrysotile.

Finally, the set of Quebec data, as well, has attracted both positive and negative comment, the latter usually based on its exclusive use of midget impinger (MPCF) data. The latter are unavoidable (that was the data available); the data are extensive and internally consistent, and the authors of the current proposal do a good job of discussing this type of data.

For purposes of risk assessment my own opinion is that the operative question is "Can exposure be measured in such a way in sites which require evaluation that the exposure assessment is both valid and repeatable". Again, this is somewhat controversial. Rogers for example is on record as feeling that "A 'clear dose-response relationship' does not validate the actual exposure values used, but the decision about exposure values of course determines the slope, which influences the apparent potencies of different fiber types". My own view is that a clear dose-response relationship *does* validate the use of the exposure values; if the data are good enough to establish a dose-response relationship then they have internal validity. However,

² See the letter, reply, and editorial comment recently published as regards the Witenoom exposure data attached to these comments as Appendix A: (1) Rogers A and Major G. Letter to the Editor. Ann Occup Hyg (2002) 46: 127-128; (2) A.W. MUSK and N.H. DE KLERK. Reply. Ann Occup Hyg (2002) 46: 128-129; The Editors, Ann Occup Hyg: (3) Editorial Response. Ann Occup Hyg (2002) 46: 129.

it is quite true that in absolute terms *none* of the studies can give absolute confidence as to what the *actual exposure levels* were in these historical cohorts.

Analytical sensitivity, however, is also especially important. Use of transmission electron microscopy, coupled with energy dispersive spectrometry of x-rays and in some instances, selected area electron diffraction should allow, at a minimum, the detection of fiber types and lengths in any such situation at a specified detection limit. At the Oakland conference of May 2001, Dr. Patrick Sebastian suggested that for environmental exposures the best use of TEM/EDS is the qualitative identification of the presence of individual fiber types rather than their full characterization in quantitative terms. The authors of the proposal appear to believe that "environmental" sites may be less homogeneous in their asbestos content, and perhaps more dilute in their asbestos content, than exposures in occupational settings. While this is no doubt true in sites where little is known about past use or exposures, it is certainly *not necessarily true* in superfund sites such as old mine sites.

The method of sampling is the key factor for evaluating environmental exposure (for example, Superfund) sites: for example, simple measurement of air samples in an undisturbed area which contains low concentrations of amphibole fibers will mislead the investigator of a site. One example is offered by a study of vermiculite insulation in the ceilings of a Canadian army base which unfortunately has been published only as the following abstract (and which is directly relevant to the exposure situations in Libby and to the question of exposures to amphibole from attic insulation) note the extreme effect of *conducting the air sampling during the demolition work*: concentrations of "asbestos" which were generally less than 0.1% by weight became tremolite levels by TEM of up to 172 fibers/ml
Cowan BW [1997]. Elevated Asbestos Exposures from a Building Demolition Which Contained Vermiculite Insulation. Proceedings of the American Industrial Hygiene Conference and Exposition (AIHCE 1997). Paper 65.

B.W. Cowan, Government of Manitoba, Brandon, MB, Canada
 Vermiculite is a silicate mineral which has been installed in many attics as a building insulation. An asbestos consultant collected bulk insulation samples from several locations scheduled for demolition on a Canadian Forces base. Asbestos concentrations ranging from less than 0.1% to

5-10% Actinolite and/or Tremolite were detected in this proactive survey. The majority of test results were quite low; generally less than 0.1% asbestos; however, the potential existed for asbestos fibers to become airborne during a routine demolition project. Air monitoring was conducted during the demolition work, which utilized no dust suppression, to determine representative worker exposures to airborne asbestos dust. Ten samples were analyzed by transmission electron microscopy (TEM) in accordance with NIOSH Method 7402 and concentrations ranged from 13 to 172 fibers per mL. The results of this study indicated elevated levels of airborne asbestos fibers were generated during the ceiling demolition and appropriate asbestos abatement procedures had to be initiated. These included the installation and operation of a negative pressure ventilation system and a decontamination facility, the wearing of adequate personal protective equipment, the prewetting of the asbestos contaminated material, the proper bagging of all asbestos waste, and regular on-site air monitoring to record the levels of airborne fiber concentrations.

TOPIC AREA 2: The proposed exposure index

- 4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

Such structures (they are not fibers and it stretching a point to call them "asbestos") present little or no carcinogenic risk. This was dealt with fully in the recent ATSDR workshop (ATSDR 2002) and will not be repeated here, although the panelists should if possible be provided with the current report from the ATSDR meeting in lower Manhattan in the fall of 2002, even though it has not yet been published. I sincerely hope we do not waste much time on this.

- 5) The proposed index is weighted heavily by fibers longer than 10 μm . Specifically, equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

It cannot be said to be "consistent with the epidemiology literature" since as the authors themselves point out that the epidemiology literature lacks such data (with the exception of lung-retained fiber studies or what are described by the authors as "pathology studies"). On the other hand it is quite consistent with the toxicology literature; and indeed an argument could be made that the critical length should be 20 μm rather than 10. The use of the more conservative 10 μm , although it will make many mineralogists unhappy (since they would not even regard such structures as "fibers"), is actually quite conservative in this regard.

Ultimately however the proof of the model (and of the index) is in its predictive ability; the authors do provide convincing evidence of this.

- 6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the workplace.

This will be exceptionally difficult since, as the authors note, virtually all of the published literature on workplace exposures (at least in the context of the epidemiological studies referred to) do not use similar methodology. In a broad qualitative sense the proposed exposure index will offer a better estimation of exposure than do the exposure measures offered in the historical workplace exposure measurements, since they include the biologically important descriptions of fiber type and length (with fiber type in particular being of proven importance in the epidemiology studies, although not from the historical measures of exposure but more from the qualitative descriptions of exposure offered in those studies; for example comparisons across similar industries having differences in fiber type which are not necessarily quantified).

Topic Area 3: General Questions.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or "bundles that are components of more complex structures"). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

This question must be withdrawn and reworded. It is badly mis-stated and contains errors of fact within its structure.

First, it is not clear whether the person or persons writing this question do not understand the distinction between "cleavage fragments" and "fiber bundles" (which are completely different animals), whether they are asking *only* about "cleavage fragments", or whether they are asking about *both* cleavage fragments and fiber bundles. Second, "cleavage fragments" are, by definition (as is clearly pointed out in the proposal), *not "asbestos"*, although this does NOT mean they are without effect. Third, the limitation of the question to *toxicological* significance ignores the published data on human exposure to "cleavage fragments" which is of greater importance than toxicological data (for example it has recently been estimated in a very detailed mineralogical study at the mine site that the nonasbestiform portion of the Quebec tremolite associated with one mine (the Jeffrey mine at Asbestos, Quebec) is 99% "nonasbestiform"). Fourth, the expert panel as constituted has no mineralogists or geologists, making any discussion of these points somewhat perilous. Thus this question, which is an exceptionally important one that has been addressed by the authors of the proposal, should be reworded. The question commented upon by this observer is reworded as the following: *I recommend that this or a consensus rewording, preferably with expert mineralogical input, be substituted BEFORE the meeting:*

7 REVISED) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments derived from massive amphibole structure (that is, nonasbestiform amphiboles). Please comment on whether cleavage fragments of this nature are as significant with respect to human health effects as fibers of the same size range, including reference to the toxicological and epidemiological literature.

Again, cleavage fragments are NOT "bundles that are components of more complex structures". The question of cleavage fragments of massive amphibole is a very important one, and the question of the assessment of complex bundles (which may or may not be composed of aggregates of asbestiform structures, nonasbestiform structures, or both) is an important but entirely separate issue. The phrase (or "bundles that are components of more complex structures") must be removed from this charge question before the meeting. If it is not the discussion of the exceptionally important issues surround

exposure assessment to cleavage fragments of massive amphibole, "nonasbestiform" amphibole, and so-called "transitional fibers" will be confused by this error. The following discussion responds to the restated charge question above (7 REVISED): it makes no mention of and does not apply to "bundles that are components of more complex structures".

This (inclusion of cleavage fragments of massive amphibole on the strict basis of structure dimension) is one of the greatest strengths of the proposed risk assessment approach, and may make up for the catastrophically inadequate approach taken by OSHA in their removal of such fibers from the asbestos standard in 1992. It may be recalled that the latter action was taken against the advice of NIOSH, of the scientific branch of OSHA itself (OSHA scientific staff, personal communication) and of the ATS Committee on the Health Effects of Tremolite (Weill HW AJ, Balnes J, Case BW, Chung AM, Hughes J, Schenker M and Sébastien P. 1990; Case BW 1991a; Case BW 1991b). The critical problems with excluding "cleavage fragments" and/or "nonasbestiform" amphiboles (of size and shape similar to analogous asbestiform amphiboles) from risk assessment were

- (a) That as a practical matter there was a debate as to whether there was a "bright line" separation between them;
- (b) That they often occur together, sometimes with only a small proportion of "asbestiform" structures;
- (c) That they are difficult to separate analytically (in fact they cannot be separated by the microscopist with certainty, not even with high-magnification transmission electron microscopy: on this point for example Patrick Sébastien has stated "To be able to tell whether fibers are asbestiform or not under the microscope is quite impossible. To me, the concept of "asbestiform" is not a microscopic one. Geologists may tell us whether a fiber is asbestiform, but certainly the microscopist cannot".

(Sébastien P, Discussion Part 14, Ann NY Acad Sci 643: page 505).

- (d) That most important, there is no convincing evidence that given similar dimensions and similar durability in the lung there is any reason to believe that "cleavage fragments" might be less toxic. Reproducing the ATS Committee document from page 1 on "Mineralogic Issues" and from the Conclusion:

Mineralogic Issues

As noted above, the focus on tremolite has raised the issue of the importance of cleavage fragments as opposed to asbestiform fibers. The fundamental issue is whether two fibrous particles of identical size and shape will have different biologic properties if the particles are pieces of mineral that have broken off a larger sample parallel to a crystal face (i.e., cleavage fragments) as opposed to particles that have originally grown in a fibrous habit (i.e., asbestiform fibers).

It became apparent, both from our review of the literature and from submissions made to this committee by experienced mineralogists, that the distinction between cleavage fragments and asbestiform fibers, although theoretically clear, is in practice extremely murky. Some mineralogists believe that these two types of particles are always distinct, whereas others believe that they shade off one into the other and that intermediate forms (byssolite) exist. Further, these same submissions were at odds with each other in identifying particular samples used in various experiments as asbestiform fibers or cleavage fragments. To complicate matters, it was also suggested to us that the important distinction is not that between cleavage fragments and asbestiform fibers but between nonasbestiform and asbestiform fibers.

Because of the lack of consensus among mineralogists, as well as the limited information about the minerals present in most published human and animal data (i.e., whether the particles used or observed really are fibers or cleavage fragments), we have to a great extent ignored the distinction and ended up treating most of the data as based on "fibers" of various sizes. The committee recognizes that this is not an ideal solution, and where stronger evidence of the cleavage fragment or asbestiform nature of a particular fiber exists, we have noted it. However, until there is reasonable mineralogic unanimity both on

(continues as "general definition and the classification of specific samples, and then animal experimentation with such classified materials, it appears to us impossible to draw general conclusions about biologic effects based on the distinction between cleavage fragments and asbestiform fibers"

and from the conclusion:

“ 3. The evidence for biologic effect distinctions based on mineralogic parameters, other than fiber dimension and fiber number, is currently inadequate.

4. At present, the prudent public health policy course is to regard appropriately sized tremolite “fibers,” in sufficient exposure dose (concentration and duration), as capable of producing the recognized asbestos-related diseases, and they should be regulated accordingly”

(Note: It is strongly recommended that panelists read the full statement in the American Review of Respiratory Medicine as referenced. Panelists should also be aware that the Environmental and Occupational Health Assembly of the American Thoracic Society has recently obtained funding to reconvene a new panel to update this statement, which is currently working on revisions and will meet in Seattle in May, 2003).

- 8) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations. The proposed cancer assessment approach is certainly relevant to all amphibole fibers which have been identified as capable of producing the recognized asbestos-related disease. In addition to the five designated types these include richterite, winchite, and possibly edenite in one location in Italy. Given the very large number of amphiboles (over 50) it seems likely that others may be found to have forms which may act in similar ways, but I am not aware of any at present. It should also be noted that “amphiboles” comprise a huge portion of the earth’s crust, and it would be totally impractical to try to regulate all forms of all amphiboles. In this regard the authors’ proposal is very useful in that it *limits* the nonasbestiform amphiboles assessed to those which have the same dimensional characteristics as the analogous asbestiform varieties.
- 9) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

This was answered above, with the exception of the point on “assessment of non-cancer endpoints”.

Again the panelists should be referred to the as yet unpublished ATSDR 2002 document which deals specifically with this issue; there is a general consensus that such short structures are not important in

non-cancer endpoints — specifically lung fibrosis — but there are (unlike the case for cancer endpoints) at least a few studies which contradict this.

- 10) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber *diameter* appropriate?

While the use of this cutoff would include most chrysotile and crocidolite fibers of concern, it would not count some amosite fibers and would not count a substantial portion of tremolite fibers of proven toxicity. Hence this cutoff is *not* appropriate; perhaps a weighted index could be applied similar to that for fiber length for thicker fibers, but ultimately it must be realized that no single technique for assessing exposure by electron microscopy in this regard will be equally applicable to all waste sites, and the hazard may be severely underestimated in some locations should such a liberal definition of diameter be adopted. In particular, it is not appropriate to exclude tremolite fibers under 1.5 μm in diameter from concern and from inclusion in assessments of sites where tremolite is the major mineral of concern.

- 11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

As a whole the proposed cancer assessment approach does appear to be a reasonable evaluation of the available health data, although it is mistaken in some details. These were described in previous sections. The emphasis on fiber type in risk assessment is long overdue; evidence for fiber length criteria in the approach is perhaps less solid, although certainly it is true that structures having length less than 5 μm need not be assessed, and indeed (through the fact of skewed length distributions) inclusion of this size category actually would provide risk assessment which may either overstate or understate health effects. Use of the greater-than-10 μm criterion as the most heavily weighted fraction, and the exact weight to attach to it (or to some other fraction), requires discussion by the panel.

- 12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

I will leave this to the actual panel/discussion.

Topic Area 4: ...the peer review consultants are invited to provide any additional comments or concerns, both strengths and weaknesses, on topics not specifically addressed in the previous charge/questions. ...the focus of this workshop is on the proposed risk assessment methodology and how it may be used to support decisions at asbestos-contaminated sites.

(signed)

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Monday, February 17, 2003

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CHARGE QUESTIONS

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

1) For lung cancer:

- A. Influence of *fiber type*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Response: A large body of data exist comparing rate constants for the in vitro dissolution of fibers of different chemical compositions. A good correlation exists between these in vitro dissolution data and biodegradability data collected in animal models. Furthermore, a correlation exists between durability values and the potency of fibers to cause fibrosis, lung cancer and mesothelioma in animal models. In vitro dissolution data indicate that chrysotile is less durable than amphibole fibers. However, in vitro toxicology data and animal studies do not consistently find chrysotile to be less bioactive (in vitro) or less fibrogenic or carcinogenic (in animal models) than amphibole fibers. The report proposes that the time frame of in vitro studies (hours-days) and animal studies (2 years) is too short for the dissolution of chrysotile to become a significant factor. In contrast, the 30 year time frame for asbestos-induced lung cancer is sufficiently long for chrysotile dissolution to influence the results. This is a reasonable argument, and it is supported by the modeling of the epidemiology data. The risk coefficients for lung cancer given in Table 6-29 and 6-30 suggest a 5 fold greater risk from amphibole exposure than from exposure to chrysotile. My view is that the epidemiological data support a greater risk coefficient for lung cancer with amphiboles than chrysotile. However, a 5 fold difference in risk is debatable considering the uncertainties inherent in the data used in this model.

- B. Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Response: In vitro mechanistic data generally support the hypothesis that long fiber are more bioactive than short fibers. Animal data for fibrous and lung cancer support this conclusion. Modeling of epidemiology also supports the hypothesis that long fibers are more potent in inducing lung cancer than short fibers.

Equation 7.13 heavily weighs the contribution of fibers $> 10 \mu\text{m}$ vs those between $5\text{--}10 \mu\text{m}$ in length by a factor of greater than 300:1. The equation dismisses particles $< 5 \mu\text{m}$ in length as having no influence of pulmonary response.

Mechanistic in vitro data on cell proliferation, generation of reactive species, and cytokine and growth factor production indicate that short particles are not without an effect. Indeed, although long fibers have been shown to activate transcription factors and increase cytokine production from cultured cells to a greater extent than short fibers, a relationship to surface area was noted (Ye et al. *Am J Physiol* 276: L426-L434, 1999; *J Biol Chem* 276: 5360-5367, 2001). Animal and epidemiological studies of asbestos toxicity indicate that short fibers are relatively less potent than long fibers. However, these were relatively pure exposures. In a mixed exposure condition, where exposure to short or non-fibrous particles is high, short particles may potentiate the pulmonary reaction to long fibers. The World Trade Center site is an example of an exposure to high levels of short particles along with fiber exposure. The potency factor of 300:1 for fibers longer than $10 \mu\text{m}$ seems high.

C. To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

Response: Animal studies do not support the 5:1 difference in lung cancer potency of amphiboles to chrysotile. The report's suggestion that a 2-year animal study is too short for dissolution of chrysotile to be an important factor has merit.

Animal studies support the hypothesis that lung fibers are more potent carcinogenesis than short fibers. However, animal studies do not support the hypothesis that short fibers or spherical particles are essentially inert.

D. Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Response: The major influence of fiber chemistry is expressed as differences in fiber durability. Surface properties, such as the ability of chrysotile vs amphiboles to generate reactive oxygen species, have not proven to greatly influence fiber carcinogenicity in animal models. Diameter and aspect ratio affect fiber deposition in the lung. However, the influence on carcinogenicity in animal models as independent of deposition has not been adequately evaluated.

2) For *mesothelioma*:

- A. Influence *fiber type*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Response: Intraperitoneal instillation data do not support a great difference between the potency of chrysotile and amphiboles to induce mesothelioma. In vitro mechanistic data do not support a great difference in potency by fiber type. However, animal data strongly indicate that chrysotile is less potent than amphiboles in producing mesothelioma. This is supported by epidemiological data. The relative risk coefficients of amphiboles vs chrysotile for mesothelioma in Table 6-29 and Table 6-30 are 500-600:1. Data support a large difference in risk.

- B. Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Response: Intraperitoneal instillation data support a strong dependence on fiber length. In vitro mechanistic data support a relationship between potency and fiber length, although the relationship is not all or none. Modeling epidemiologic data strongly support that long fibers are more potent than short fibers in inducing mesothelioma. Equation 7.13 indicates that fibers $>10 \mu\text{m}$ should be weighed 300:1 over fibers 5-10 μm in length for mesothelioma. The weighing for length and mesothelioma is mechanistically stronger than for lung cancer.

- C. To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Response: The difference in potency of chrysotile vs amphiboles and long vs short fibers to cause mesothelioma is supported by animal inhalation studies.

- D. Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Response: The major influence of fiber chemistry is expressed as differences in fiber durability. Surface properties, such as the ability of chrysotile vs amphiboles to generate reactive oxygen species, have not proven to greatly influence fiber carcinogenicity in animal models. Diameter and aspect ratio affect fiber deposition in the lung. However, the influence on carcinogenicity in animal models as independent of deposition has not been adequately evaluated.

- 3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

Response: The problem with these data have been adequately discussed in the report. Fiber characterization is not complete. Exposure levels for past exposures are often estimates. These uncertainties don't affect the conclusion that long fibers are more potent than short fibers or that amphiboles are more potent than chrysotile. However, they do make absolute quantitation of the potency differences difficult.

Topic Area 2: The proposed exposure index.

- 4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

Response: Cohorts for epidemiologic studies were chosen for the absence of major mixed dust exposure. Animal studies were controlled for fiber exposure alone. Therefore, the burden to particles less than 5 μm in length was minimized in the experimental designs. At such low burdens, long fiber toxicity would dominate. However, one could envision situations where high exposures to spherical particles or short fibers could occur. Mechanistic data is consistent with the hypothesis that such burden would elevate the oxidant/inflammatory set point and increase the response to long fibers. This point was discussed in 1B.

- 5) The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

Response: See response 1B and 4.

- 6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

Response: It seems possible that current and future exposures of environmental concern would be to mixed dusts rather than pure fibers. The proposed exposure index would dismiss what might be a high exposure to spherical particles or short fibers. The World Trade Center site is an example of such a mixed dust exposure. There are in vitro mechanistic data which would suggest that the responsiveness to long fibers might be enhanced if the system was under particle-induced oxidative stress and inflammation.

Topic Area 3: General questions.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or "bundles that are components of more complex structures"). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

Response: Assigning equivalent potency to individual fibers and cleavage fragments of equal dimension is a reasonable approach. Data from in vitro mechanistic studies do not indicate that cells can discern a difference between a single fiber or a bundle of equivalent dimensions.

- 8) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

Response: Response to fibers is governed by dose, durability and dimensions. No mechanistic or animal data exist which would suggest that two types of fibers which were similar in the three characteristics noted above would exhibit a different biological response in the lung. Therefore, long durable fibers not currently labeled as asbestos if inhaled at a similar dose would be expected to result in a similar degree of pathology.

- 9) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

Response: Use of TEM rather than PCM allows thin fibers to be counted. This is appropriate, since long thin fibers would be expected to be highly potent. In evaluating risk of fiber inhalation as part of a mixed dust exposure, it is possible that particles less than 5 μm in length could enhance the response to long fibers. The proposed assessment approach would ignore this possibility.

- 10) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner on 0.5 μm . Is this cut-off for fiber diameter appropriate?

Response: Since fibers up to 0.7 μm can be deposited in the respiratory zone of the lung, it seems more appropriate to raise the cut-off to this value.

- 11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

Response: The concepts that amphiboles are more potent than chrysotile and long fibers are more potent than short are reasonable. The debate is the weighing of these potencies. The approach used to model existing epidemiologic and animal data is reasonable. However, uncertainty of the weighing factors exists due to the uncertainty of exposure and size characterization in the individual studies used in the model.

- 12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

Response: All three options assume that the weighing factors for fiber dimension and fiber type are adopted. Given that assumption option 2 appears to be simple to apply to environmental conditions. Each option suffers from the uncertainty of the weighing factors and each option ignores the situation of a significant mixed particle exposure.

Topic Area 4: Development of Conclusions and Recommendations

At the end of the workshop, the peer consultants will be asked to draft conclusion statements identifying their most notable findings on the proposed methodology. As a prelude to developing these statements, the peer consultants are invited to provide any additional comments or concerns, both strengths and weaknesses, on topics not specifically addressed in the previous charge questions. After completing the discussions the peer consultants will prepare their conclusions, and they will also be asked to develop

recommendations for how EPA can improve the methodology. Please note that, although recommendations for future research projects are welcomed, the focus of this workshop is on the proposed risk assessment methodology and how it may be used to support decisions at asbestos-contaminated sites.

Response: The proposed methodology is founded strongly on the premise that lung cancer risk due to asbestos is independent of other exposures. This is not the case with smoking and asbestos exposure. Mechanisms for asbestos-induced cancer include oxidant damage, dysregulation of growth control, production of inflammatory cytokines and proliferation factors, down regulation of apoptosis, etc. Considering the current mechanistic understanding of fiber-induced cancer induction, it is not unreasonable to propose that lung burden to short fibers or spherical particles might change the oxidant stress and/or inflammatory set point of the lung and alter the responsiveness to long fibers.

agent agent.

B-52

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B-53

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Dr. Crapo is a professor of medicine at the University of Colorado Health Sciences Center, Director of the Ph.D. Program for Graduate Health Care Professionals, and Associate Dean of the School of Medicine, as well as serving as the Chairman of the Department of Medicine of the National Jewish Center. Previous to his appointments in Colorado, he was a professor of medicine and professor of pathology, and director of extrapolation modeling at Duke University. He is a member of the American Thoracic Society, American Federation of Clinical Research, Society of Toxicology, and the American Physiological Society, as well as a fellow of the American College of Physicians. He received his B.S. magna cum laude from Brigham Young University and his M.D. from the University of Rochester. His certified medical specialty is internal medicine, with a subspecialty in pulmonary disease. He is a member of the editorial boards for Inhalation Toxicology, Environmental Health Perspectives, and the American Journal of Physiology Lung, Cellular and Molecular Physiology. He has published several textbooks including Toxicology of the Lung, Extrapolation of Dosimetric Relationships for Inhaled Particles and Gases, and Textbook of Pulmonary Diseases. He has published numerous reviews, editorials, and book chapters including Aspects of respiratory tract structure and function important for dosimetry modeling: interspecies comparison, Fate and translocation of fibers, Models for Studying airways Response to Environmental Injury, Application of Morphometric Methods to Study Diffuse and Focal Injury in the Lung Caused by Toxic Agents, and Morphologic, Morphometric, and X-ray Microanalytical Studies on Lung Tissue of Rats Exposed to Chrysotile Asbestos in Inhalation Chambers. He has published many papers related to asbestos including Pulmonary macrophage accumulation and asbestos-induced lesions at sites of fiber deposition, Characterization of three types of chrysotile asbestos after aerolization, Airway Branching Patterns Influence Asbestos Fiber Location and The Extent of Tissue Injury in the Pulmonary Parenchyma.

Workshop to Discuss a Proposed Protocol to Assess Asbestos-Related Risk - Response to Charge Questions

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

1) For lung cancer:

- A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?
- I concur that the epidemiology literature and mechanistic studies now strongly suggest that the carcinogenic potency varies between fiber types. With respect to commercially used asbestos products, the research supports the carcinogenic potency as being: crocidolite > amosite >> chrysotile. The recent review done by Hodgson and Darton and the analysis prepared by Drs. Berman and Crump provide two approaches to assessing the relative potency of fiber types with similar outcomes. The epidemiologic data is now sufficient to support developing different risk coefficients for different fiber types. The coefficients shown on Table 6-29 are supported by the literature but are conservative. If the analysis done by Hodgson and Darton were used, it would result in larger differences in the risk coefficients than shown in Table 6-29.
- B) Influence of fiber length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

The epidemiology and mechanistic literature shows that the carcinogenic potential of asbestos is strongly correlated with fiber length. There is sufficient literature to support the development of dose-response relationships for different fiber lengths. The mechanistic literature shows that fibers less than 10-15 microns in length are cleared by macrophage action. The epidemiology literature supports the conclusion that the longer the fiber, the greater the carcinogenic potential, with fibers longer than 20 microns in length carrying most of the associated risk for carcinogenicity. Based on the additional studies generated over the past 15 years, it would now be appropriate to develop cancer risk estimates that are heavily weighted toward fibers longer than 10 or 20 microns.

- C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

Animal studies also suggest that carcinogenic potency varies with fiber type and fiber length, although the differences are often less than that suggested by human epidemiologic studies. This difference is likely due to the fact that animal studies are commonly done using extremely high doses (often given by injection or instillation) and shorter periods of observation (limited by the animal's life span). These differences have the effect of removing fiber durability and clearance as substantial factors in determining carcinogenic risk. Thus, differences in carcinogenic potency between fibers based on fiber durability are not adequately evaluated in animal studies. In spite of this, animal work in general supports the concept that carcinogenic potency varies with both fiber type and fiber length.

- D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How

adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Fiber diameter is an important element in carcinogenic potency probably by influencing the ability of the fiber to be inhaled into the deep lung. The recommendation by Berman and Crump that risk assessments should be focused on fibers with diameters of 0.5 microns or less is reasonable. Aspect ratio does not appear to be a factor in determining carcinogenic risk. Aspect ratio is a valuable function in characterizing fibers from other inhaled materials, but there is no evidence that aspect ratio is an important factor in predicting fiber toxicity. There is some evidence that other fiber characteristics, which include surface properties or chemical composition, may influence carcinogenic potential although this has not yet been sufficiently defined to be used in developing specific risk estimates. The current epidemiology and toxicology literature would support using fiber diameter as an important factor in determining carcinogenic potency, but would not support using aspect ratio as a factor, and is insufficient to develop specific risk estimates for other fiber properties.

One factor not adequately considered in the current document is the interrelationship between smoking and asbestos exposure in causation of lung cancer. Many historical studies were not appropriately controlled for smoking. Smoking is a higher risk factor for causation of cancer than is asbestos exposure and thus can easily confound risk estimates that focus only on asbestos exposure. The epidemiologic literature assessing the ability of asbestos exposure to contribute to cancer causation in the absence of smoking is weak. Another important issue in developing correct estimates for asbestos exposure contribution to lung cancer risk is whether or not asbestosis is required before cancer risk is elevated. There is a substantial body of literature suggesting that formation of asbestosis is required before asbestos exposure will increase lung cancer risk.

2) **For mesothelioma:**

A) **Influence of fiber type:** Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

My comments for mesothelioma are similar to those for lung cancer. The carcinogenic potency varies between fiber types. The epidemiologic literature shows a much larger difference in carcinogenic potential (based on fiber type) for mesothelioma than for lung cancer. The risk coefficients shown in Table 6-29 are conservative estimates based on current literature. A critical question not fully resolved by current literature is whether common human occupational exposures to chrysotile result in a low risk of mesothelioma or whether such chrysotile exposure carries no risk. It is possible that amphibole contaminants in chrysotile (commonly tremolite) create the low level risk of mesothelioma recorded in "chrysotile only cohorts." Most mesotheliomas found in chrysotile exposed cohorts are associated with the mining environment. MacDonald and colleagues have suggested that the mesothelioma risk in chrysotile mining cohorts is primarily associated with tremolite contamination. Based on current literature, I would concur that the conservative approach would be to use risk coefficients for mesothelioma such as those proposed in Table 6-29.

B) **Influence of fiber length:** Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

I concur with the assessment by Berman and Crump that longer fibers carry the primary risk for development of mesothelioma. It is appropriate to use exposure indices heavily weighted for fibers longer than 10 microns (or 20 microns). Fibers less than 5 microns in length have not been shown to carry significant potency.

- C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

Animal studies, in general, support that there are differences in carcinogenic potency with fiber type and fiber length. Depending on study design, those differences may be under-estimated or not present due to the dose and/or route of administration of the asbestos and due to the shorter duration of animal studies. The effects of fiber durability in determining carcinogenic potency are not adequately assessed in animal studies.

- D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Carcinogenic potency has clearly been shown to be a function of fiber diameter which influences the respirability of the fibers. There is no data associating aspect ratio with carcinogenic potency other than its ability to identify a fiber as opposed to a particle. Other factors such as surface property and chemical composition likely play an effect, but this is not adequately defined by current literature. For example, Faux et al. (2001) showed using rat pleural mesothelial cells that crocidolite had an impact on growth factor expression not seen with chrysotile and which was removed by milling the crocidolite. I would expect mechanistic studies to eventually define chemical or surface characteristics of fibers which could be included in risk estimates.

- 3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

Most of the asbestos epidemiology literature contains relatively crude estimates of exposure. Most estimates are qualitative and, at best, contain only intermittent assessments of exposure levels under specific work conditions. Even in those cases, exposure conditions are not generally well characterized as to fiber type, fiber length, or fiber diameter. The exposure estimates in the epidemiology literature are adequate for general conclusions but do not commonly allow rigorous comparison between studies. It would be advisable to recommend new criteria for assessing exposures which would include time-weighted exposure conditions and greater characterization of the fibers, including a more complete assessment of fiber length and fiber diameter distributions.

Topic Area 2: The proposed exposure index.

- 4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

The epidemiology and toxicology literature support the conclusion that fibers shorter than 5 microns in length do not significantly contribute to carcinogenic risk. There are sufficient epidemiologic studies at the present time to exclude fibers shorter than 5 microns in length from carcinogenic risk estimates for asbestos exposures.

- 5) The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

The fiber length coefficients used in Equation 6.7 and Equation 7.13 are consistent with the epidemiology and toxicology literature.

- 6) *Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.*

The proposed exposure index will be somewhat difficult to apply to historical exposures in the workplace because data regarding fiber length and width characteristics are not consistently available in that literature. However, many reasonable assumptions can be made based on known fiber characteristics from different products. Use of the proposed exposure index should enable a re-evaluation of historical workplace exposures and may help reconcile some of the unexplained risk differences between various workplace environments. Changing to a new exposure index will lead to problems in comparing to historical data, but this should not inhibit moving to a more correct exposure index. One problem not adequately considered in the current document is the relationship of smoking and asbestos exposure in lung cancer causation. When considering comparisons of current environment conditions to historic conditions, one must also recognize that there are major changes in the smoking characteristics of today's workers. This will confound interpretation of risk estimates related to asbestos exposures when comparisons are done to historical studies.

Topic Area 3: General questions.

- 7) *The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or bundles that are components of more complex structures). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.*

My interpretation of the existing literature is that cleavage fragments of asbestos are toxicologically significant only if the fragments remain of sufficient length (10-20 microns or longer). I am aware of no data showing that short cleavage fragments of asbestos show carcinogenic potential.

- 8) *Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.*

It is my general assumption that the proposed cancer assessment approach would be relevant to all amphibole fibers, however, there is rigorous data only on a limited number of amphibole types -- most of the data is focused on crocidolite, amosite and tremolite.

- 9) *The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μ m. Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μ m. To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?*

Based on current literature, I would not expect counting asbestos fibers shorter than 5 microns in length to significantly enhance one's ability to validate cancer risk assessment methodology or known cancer endpoints. It is, however, difficult to make firm statements about data one does not have. The critical question is the cost of including short fiber counts vs. the potential future value of the data. If costs were low, I would include such counts. The current data do not support including counts of short asbestos fibers at a high economic cost.

- 10) *The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μ m and thinner than 0.5 μ m. Is this cut-off for fiber diameter appropriate?*

James D. Crapo, M.D.

The use of counting methodology identifying only fibers longer than 5 microns in length and thinner than 0.5 microns in width is appropriate. This proposed change in methodology would be a substantial advance over the current use of a 3:1 aspect ratio.

- 11) *Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?*

The proposed cancer assessment approach is consistent with the epidemiology and toxicology literature for asbestos. My primary concerns relate to the absence of an adequate assessment of the confounding impact of smoking on lung cancer risk assessments and the absence of evaluating the role of asbestosis as a factor in determining lung cancer risk. The epidemiology literature shows that both of the above factors are major components in determining lung cancer risk in asbestos exposed cohorts. Neither of these factors have been clearly shown to have a linear correlation with asbestos exposure alone. Table 8-xxx on page 8-10 makes an attempt to assess smoking impact on both chrysotile and amphibole exposures. This assessment should be expanded and internal inconsistencies in the table resolved. Duration and intensity of smoking need to be more fully characterized. Looking at Table 8-xxx, why would a male nonsmoker who is not exposed to an amphibole have a 4 times higher lung cancer risk than a male nonsmoker not exposed to chrysotile?

- 12) *Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.*

All three proposed options have significant limitations. The use of a risk table is simpler for general use but is limited by factors such as the assumption of a constant exposure in both intensity and fiber characteristics. It is also limited by crude grouping with other characteristics such as smoking. Intensity and duration of smoking are also huge factors that modify the risk assessment. I would, in general, favor estimating risk using a unit risk factor if this approach were adequately developed and expanded, particularly if this unit factor could be accurately integrated with other major factors in cancer causation such as smoking, other exposures, and the formation of asbestosis.

Topic Area 4: Development of Conclusions and Recommendations

My initial recommendations are included in the previous comments.

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David Hoel

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Response to the Charge Questions

This is a very interesting and a very complex risk assessment. I am in support of what the authors and the agency are attempting to do. The question is whether relevant analysis and review can be accomplished in the short time period we have. I have restricted myself to the area of epidemiological data and the quantitative models being used with this data.

The general comments I have so far are as follows.

Models:

For both lung cancer and mesothelioma, two specific risk models are used. These models are applied to the fitting of grouped epidemiological data using Poisson regression. The models appear to describe the epidemiological data in a reasonable manner. I have several questions concerning the adequacy of these models and the impact they make on the final risk estimates.

For lung cancer, a simple relative risk model using cumulative exposure is used. For this type of data, one often sees the estimation of internal rates without the need of incorporating external lung cancer mortality rates. The authors are not clear as to why they prefer the use of external rates followed by an estimation of the alpha parameter, which allows an adjustment for the difference between the background lung cancer rates of the cohort and those of the general population.

Another issue is the choice of a linear relationship of cumulative exposure to risk as opposed to the separation into exposure rates and duration of exposure. For example, the lung cancer and cigarette smoking modeling of Peto and Doll find a linear quadratic effect of smoking rate with a 4th to 5th power of duration of smoking.

For mesothelioma, the model assumes that risk is proportional to cumulative exposure. Further, the effect is proportional to the 3rd power of time since first exposed, with a ten-year latency. Again, the question is whether this model is the appropriate one for dealing with the various cohorts that report mesothelioma.

It may be that the quantitative results of the overall analysis of the epidemiological data are fairly robust with respect to these two cancer risk models. If this is not the case, then it is important to understand the impact of the quantitative risk results on the choice of these two very specific cancer models.

Risk Estimates:

The optimized risk coefficients for pure fiber types are given in Tables 6-29. Table 6-30 gives conservative values. It would be more informative if simulations incorporating the estimated model uncertainties could be carried out and used in place of table 6-30.

Topic Area 1.

- 1) Lung Cancer: At this point in my review I believe that the epidemiological data is supporting the questions raised in A) & B). I have not reviewed the animal data so I have no answer for C). Based on the human data I do not believe we know beyond fiber type and length as in D). But I am still looking at this question.
- 2) Mesothelioma: Same answers as with Lung cancer.
- 3) Not my area of expertise.

Topic Area 2-4.

I hope to have answers for a number of these questions as we get closer to the meeting time.

In general, to appropriately answer many of the specific charge questions will necessarily require reviewing a large amount primary research papers.

CHARGE QUESTIONS

Topic Area 1:

Interpretations of the epidemiology and toxicology literature.

1)

For lung cancer:

A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Answer: A meta type analysis was used to group studies of similar fiber type from the epidemiological literature. These studies appeared to be fairly heterogeneous and the weighting factors were modified by incorporating ad hoc measures of study quality which resulted in increased confidence intervals. The resulting weighted potency estimates with confidence intervals by fiber type were not specifically given. However there did appear to be a difference in potency by fiber type (pure chrysotile v. amphiboles) although they may not necessarily be statistically different.

They linear RR model fits the South Carolina (Chrysotile) example very well while it was necessary to include an additional parameter (alpha) in order to fit the Wittenoom miner data (crocidolite) which continued to appear to be nonlinear. The reason for assuming that the spontaneous rate for lung cancer in this cohort being twice that expected is not clear other than the data is poorly fit

without the additional parameter. How well the linear model describes the data for other cohorts is not described with respect to residual patterns.

The risk estimates in Table 6-29 depend upon the concept that potency for a given asbestos type depends primarily length and diameter of the fibers. This is the result of animal inhalation studies (Davis et al.) which are assumed to directly apply to man. If this is correct the one can say that the Table 6-29 results are not inconsistent with the epidemiological data. The estimates can not apparently be derived solely from epidemiological findings.

B) Influence of *fiber length*. Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Answer: As stated above the epidemiology literature does not provide adequate information on fiber length and potency. Using the animal data to develop the exposure index 7.13 and applying it to the epidemiology data does not change greatly the forest plots given in Figures 6-3 and 6-4 with regard to heterogeneity.

C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Answer: It would be useful to have Appendix C available to answer this question. Based on the information from the animal studies it is clear that potency varies with fiber type and length.

D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than fiber type and fiber length*. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Answer: The epidemiology data does not provide adequate information on these measures with regard to cancer risk.

2)

For *mesothelioma*:

A) Influence of *fiber type*. Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Answer: The epidemiological data is somewhat limited for mesothelioma, however the available data shows a strong difference in potency by fiber type. Because of the limited data, potency is necessarily assumed to be linear in concentration. The model which assumes a third power of lagged duration since exposure is not specifically used. The exact method employed by the authors is as I understand a nonparametric description of time since exposure component. This is a reasonable approach which should be better than the parametric approach. The data is too limited to determine whether the parametric model is realistic. The coefficients in Table 6-29 are reasonable but because of limited data I have less confidence than for the lung cancer values.

B) Influence of *fiber length*. Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Answer: As with lung cancer the epidemiological data are insufficient to estimate potency based on fiber length.

C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Answer: The Davis data does show length and type differences but only a total of 13 tumors are available from the 18 experimental groups for the estimation of the differences. There are therefore large uncertainties which are not estimated and incorporated into the model. There seems to be the assumption that the fiber length and width effects for lung cancer are similar for mesothelioma.

D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Answer: The epidemiology data does not provide adequate information on these measures with regard to mesothelioma risk.

- 3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

Answer: I am not qualified to comment on the industrial hygiene aspect of the epidemiological studies. However, it seems that the comparisons between lung burden and air concentrations are reasonable given assumptions concerning retention of the fibers.

Topic Area 2:

The proposed exposure index.

- 4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

Answer: The animal data is clear that there is no cancer risk for exposures to fibers less than 5 μm . The epidemiological data provides no information on this issue due to the mixed fiber sizes in the occupational exposures. The epidemiological data is not inconsistent with this animal finding.

- 5) The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

Answer: The analysis of the animal inhalation data produces this finding. What is not given is the statistical uncertainty of this result. Also there may be physiological differences between rat and man that suggests that the species extrapolation may not be valid. This I simply do not know would like to see a discussion of the issue.

- 6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

Answer: Since the historical exposures are the basis for the risk models it should be reasonable to estimate risk from current environmental exposures. One issue I have is whether or not the simple linear assumptions are appropriate for relatively low current exposures. If not the environmental risks may be over estimated. There is simply no way of knowing this unless mechanistic data can provide an answer.

Topic Area 3:

General questions.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or "bundles that are components of more complex structures"). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

Answer: At this point I have no opinion on the concept of cleavage fragments.

- 8) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

Answer: I simply do not know enough about asbestos fibers to say whether an extrapolation beyond the five types is reasonable. I feel that the risk estimates using the types reported in the animal and epidemiology data are reasonable.

- 9) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

Answer: Obviously data on shorter fibers would be useful in future studies in order to confirm the currently proposed risk analysis.

- 10) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber diameter appropriate?

Answer: Based upon the limited animal and epidemiological data this seems reasonable to me. If this is not correct the contribution to risk for fibers outside this range would be very small at best.

- 11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

Answer: The only issue I have is whether effects are proportional to exposure. The alternative approach has been to consider the specific 2-stage model of Moolgavkar. I would also be interested in seeing an application of the more traditional Armitage-Doll multistage model as has been used recently with diesel exhaust and lung cancer.

- 12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment

options.

Answer: I prefer the second method since it is an estimate of the actual risk for a individual classified by gender and smoking status. It should be extend to cover scenarios of varying or terminated exposures and smoking status. The other approaches are crude general estimates of increased risk.

Topic Area 4:

Development of Conclusions and Recommendations

At the end of the workshop, the peer consultants will be asked to draft conclusion statements identifying their most notable findings on the proposed methodology. As a prelude to developing these statements, the peer consultants are invited to provide any additional comments or concerns, both strengths and weaknesses, on topics not specifically addressed in the previous charge questions. After completing the discussions the peer consultants will prepare their conclusions, and they will also be asked to develop recommendations for how EPA can improve the methodology. Please note that, although recommendations for future research projects are welcomed, the focus of this workshop is on the proposed risk assessment methodology and how it may be used to support decisions at asbestos-contaminated sites.

Answer: For both lung cancer and mesothelioma, two specific risk models are used. These models are applied to the fitting of grouped epidemiological data in a Poisson regression manner. The models appear to describe the epidemiological data in a reasonable manner. I have several questions concerning the adequacy of these models and the impact they make on the final risk estimates.

For lung cancer, a simple relative risk model using cumulative exposure is used. For this type of data, one often sees the estimation of internal rates without the need of incorporating external lung cancer mortality rates. The authors are not clear as to why they prefer the use of external rates followed by an estimation of the alpha parameter, which allows an adjustment for the difference between the background lung cancer rates of the cohort and those of the general population.

Another issue in the model is the choice of cumulative exposure as opposed to the separation into exposure rates and duration of exposure. For example, the lung cancer and cigarette smoking modeling of Peto and Doll find a relationship of a linear quadratic effect of smoking rate and a 4th to 5th power of duration of smoking.

For mesothelioma, the model assumes that risk is proportional to cumulative exposure. Further, the effect is proportional to the 3rd power of time since first exposed, with a ten-year latency. Again, the question is whether this model is the appropriate one for dealing with the various cohorts that report

mesothelioma.

It may be that the quantitative results of the overall analysis of the epidemiological data are fairly robust with respect to these two cancer risk models. If this is not the case, then it is important to understand the impact of the quantitative results on the choice of these two specific cancer models.

Finally, as I mentioned in response to question 11, I would be interested in the use of the multistage model as it describes degrees of initiation and promotion. Also the meta-analyses used appear to correctly use random effects model due to the heterogeneity of the studies. Publication bias was not considered i.e. funnel plots etc.

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Dr. Lippmann is a professor of environmental medicine at the New York University (NYU) School of Medicine. He holds a Ph.D. (NYU, 1967) in environmental health science, an S.M. (Harvard University, 1955) in industrial hygiene, and a B.Ch.E. (The Cooper Union, 1954) in chemical engineering. At NYU, he directs a research program on human exposure and health effects and the EPA-supported Particulate Matter Health Effects Research Center. He has been the recipient of numerous awards for his research and contributions in aerosol science and pulmonary physiology, human exposure assessment and dosimetry, chemical transformations in the atmosphere, population studies of exposure-response relationships in occupational and community cohorts, and factors affecting the toxicity of airborne fibers. Much of this research has been focused on specific chemical agents, notably ozone, sulfuric acid, and asbestos. Dr. Lippmann is a past president of the International Society of Exposure Analysis (1994-1995), past chairman of the ACGIH (1982-1983), of the EPA Science Advisory Board's Executive Committee (2000-2001), EPA's Advisory Committee on Indoor Air Quality and Total Human Exposure (1987-1993), and EPA's Clean Air Scientific Advisory Committee (1983-1987). He has also chaired and been a member of numerous National Research Council committees, including committees on synthetic vitreous fibers, measurement and control of respirable dust in mines, indoor pollutants, toxicity data elements, and in-vivo toxicity testing of complex mixtures. His publications include 260 research and review papers in the scientific literature and reference texts on environmental health science.

Responses to Charge Questions

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

1) For lung cancer:

A) Influence of *fiber type*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Response: The epidemiology literature, controlled animal inhalation exposure studies, and mechanistic studies cited by Berman and Crump are among the most appropriate for representing the differential potency of chrysotile and amphibole fibers for causing increased rates of lung cancer. The K_1 coefficients listed in Table 6-29 represent the best estimates currently available and are based on a reasonable interpretation of the available literature.

B) Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Response: The epidemiology literature and controlled animal exposure studies that have provided adequate data on fiber length and diameter distributions in the exposure atmospheres and/or delivered tissue dose clearly demonstrate that fiber length is a critical determinant of carcinogenic potency. The conclusion was firmly supported by the recent ATSDR Workshop Report: "Report on the Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length" (Draft Final of 12/23/02). The epidemiology literature supporting exposure-response analyses for different ranges of fiber lengths is still quite sparse, but a formulation that is weighted heavily for fibers longer than 10 μm is certainly justified. It may need further refinement in the future (e.g., giving greater weight to fibers longer than 20 μm) but the

proposed formulation is clearly superior to the pre-existing formulation that makes us distinction beyond length $> 5 \mu\text{m}$.

C] To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Response: The controlled exposure studies in animals (rats) by the John Davis group in Edinburgh and the Chris Wagner group in Penarth are among the most informative concerning the influence of fiber type (e.g., amosite, other amphiboles, chrysotile, and erionite) and fiber size (length and width), as summarized by Lippmann (1988, 1994), and the discussion Berman and Crumps document would have been strengthened by a more complete reference to the analyses cited in those papers.

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Response: Carcinogenic potency can be influenced by fiber diameter and surface properties, but aspect ratio, per se, has no biological significance. Fiber diameter can be influential in two different ways. One is that fiber diameter is closely related to aerodynamic diameter, which in turn largely determines deposition probabilities in the conductive airways and lung parenchyma. The mucociliary and macrophage mediated clearance pathways and residence times at deposition sites are determinants of toxic potential. The other way that fiber diameter affects carcinogenic potency is that very thin fibers appear to be able to penetrate through pores in the respiratory epithelium and thereby gain more ready access to interstitial lung cells and lymphatic drainage pathways.

Surface properties can affect dissolution rates and thereby biopersistence, the generation of reactive oxygen species, and the release of mediators from lung cells, and all of these factors may be important to carcinogenic potency for lung cancer.

Aspect ratio, i.e., the ratio of fiber length to fiber width, has no known biological significance in and of itself. Fiber lengths and widths themselves are the critical determinants of toxicity, as discussed above and in the Berman and Crump document. The information in the epidemiology and toxicology literature

provides quite adequate support for these conclusions in regard to exposure-response relationships for lung cancer.

2) For *mesothelioma*:

A) Influence of *fiber type*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Response: The epidemiology literature, controlled animal inhalation exposure studies, and mechanistic studies cited by Berman and Crump are among the most appropriate for representing the differential potency of various fiber types for causing mesothelioma. It is clear that, in terms of potency, erionite fibers > amphibole asbestos fibers > chrysotile fibers for given ranges of fiber diameter and fiber length. Table 6-29 provides coefficient estimates for mesothelioma (K_M) associated with amphiboles and chrysotile fibers that are based on an incomplete evaluation of the relevant literature, and need to be adjusted to reflect the influence of fiber length, as discussed below.

B) Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Response: The epidemiology literature and controlled animal inhalation exposure studies clearly indicate that fiber length is a critical determinant of potential to cause mesothelioma. As discussed by Lippmann (1988), short amphibole fibers (< 5 μm long) are essentially innocuous, in both studies in human lungs (Timbrell, 1983) and rats (Davis, 1986), and the critical fibers for mesothelioma induction are those between 5 and 10 μm in length. Fibers longer than 10 μm are not effectively translocated to the mesothelioma. Thus, for

mesothelioma, it is not appropriate for the exposure index to be heavily weighted for fibers longer than 10 μm .

CJ To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Response: Potency for mesothelioma induction clearly varies with both fiber length and fiber type. As noted above (in 2B), the critical fiber lengths are those between 5 and 10 μm , and, as noted (in 2A), fiber type is also a critical determinant, with erionite > amphibole > chrysotile. In fact, as noted by Lippmann (1994), the mesothelioma associated with exposure to commercial chrysotile are most likely due to the tremolite component of the commercial chrysotile.

DJ Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Response: As noted in 1D above, surface properties can affect dissolution rates and thereby biopersistence, the generation of reactive oxygen species, and the release of mediators from lung cells, and all of these factors may be important for carcinogenic potency. Accessible internal surfaces within fibers, such as that characteristic for erionite fibers, may account for the exceptional potency of erionite for producing mesothelioma in rats (Wagner et al., 1985) and humans (Baris et al., 1987).

As noted in 1D above, aspect ratio, per se, has no influence on carcinogenic potency.

3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

Response: The exposure estimates documented in the asbestos epidemiology literature clearly have weaknesses associated with: a) the different exposure indices measured (total dust count, PCM counts of fibers > 5 μm in length that could not detect very thin fibers and could not discriminate among fiber types, SEM, and TEM); b) the lack of information on fiber length and fiber diameter distributions in the PCM, SEM and TEM measurements; c) the relatively few long fibers seen in SEM and TEM measurements, resulting

in limited statistical validity for long-fiber counts. A significant contribution made in the Berman and Crump document was its ability to locate, access, analyze, and document better fiber distribution data from archived sampling filters collected during past epidemiology and controlled animal inhalation studies.

4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

Response: The recent ATSDR Workshop Report: "Report on the Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length" (Draft Final of 12/23/02) clearly indicates that fibers shorter than 5 μm present little or no carcinogenic risk.

5) The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

Response: The heavy weighting of fibers > 10 μm in length is quite appropriate for risk assessments for lung cancer, as documented in the literature review provided by Berman and Crump. On the other hand, as noted in my response to charge question 2B), such weighting is not appropriate for risk assessments for mesothelioma, where the risk is most closely associated with fibers between 5 and 10 μm in length.

6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

Response: The proposed exposure index is based largely on analyses of the past relationships between cancer incidence in asbestos exposed populations in the mines and mills in Quebec, a textile plant in South Carolina and crocidolite exposed workers at Wittenoom in Australia, and historic and retrospective analyses of the airborne fiber concentrations in those work environments. The extrapolation of that experience to the carcinogenic hazards associated with contemporary environmental exposures to people exposed to tremolite

fibers in Libby, Montana, various California communities around surface deposits of serpentine, people exposed to dust from the World Trade Center collapse in New York and New Jersey, and other places is reasonable and prudent insofar as the exposure concentrations in these communities are within about two orders of magnitude of those in the historic occupational cohorts.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or bundles that are components of more complex structures). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

Response: The toxic potential of inhaled mineral and vitreous fibers has been shown to depend most strongly on fiber length, fiber diameter, and biopersistence. There is very little evidence that amphibole asbestos cleavage fragments in the fiber diameter and fiber length range of concern are less hazardous than comparably sized asbestiform fibers. In fact, the only directly relevant comparison, i.e., the Davis et al. (1991) comparative study of six tremolite asbestos samples (three asbestiform fibers, and three cleavage fragment dusts), which was discussed in some detail in the Berman and Crump document (pp. B-3 through B-10), showed that the risks from the tremolite cleavage fragments, when appropriately adjusted according to their protocol structure formulation, had quite comparable potency to the asbestiform tremolite.

- 8) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

Response: Since all amphibole asbestos fibers can be expected to be biopersistent and be found in diameters and lengths that are associated with cancer causation, there is no good reason, based on biology, to limit regulations to the five specific types now regulated.

- 9) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on

shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

Response: Fibers shorter than 5 μm can contribute to asbestosis in occupationally exposed individuals (Lippmann, 1988). However, the asbestosis risk is not closely related to fiber number, but rather to fiber surface area. The counting of fibers < 5 μm in length would serve no purpose in cancer risk assessment, and asbestosis requires exposures to asbestos at concentrations far higher than any likely to be encountered in nonoccupational environments.

10) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber *diameter* appropriate?

Response: There is no good reason to exclude fibers between 0.5 μm and 1.5 μm in diameter ($\sim 5 \mu\text{m}$ in aerodynamic diameter) in a risk analysis for lung cancer. Such fibers can penetrate to small lung airways, and some asbestos minerals produce many fibers in this range of diameter (especially anthophyllite). On the other hand, there is little risk for mesothelioma for fibers thicker than 0.15 μm (Lippmann, 1988).

11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

Response: The Berman and Crump cancer assessment approach is quite reasonable for lung cancer risk assessment. However, as discussed in my responses to charge questions 2B and 5, it is not optimized for mesothelioma risk assessment.

12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

Response: The three different risk assessment options proposed by Berman and Crump are all usable, albeit with some variation in the convenience with which they can be applied. The easiest to use would be Option 2 (Risk Table), but as acknowledged by Berman and Crump, this could lead to errors for short-duration exposures.

For application of any of these options, reliance on Equation 7-13 may be appropriate for lung cancer risk estimation. However, it is almost certainly misleading for mesothelioma risk assessment, where its emphasis on fibers longer than 10 μm is not warranted.

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Some General Comments on the Berman and Crump Technical Support Document

This document needs a lot of editing for both technical content and organization. For example, there are numerous places where a statement in an earlier chapter relies on text in a later chapter. The text is overly encyclopedic and cites many papers whose relevance to the issues of concern in relation to the development of a better model for asbestos fiber risk assessment is not apparent. Also, there are indications of references to be supplied (see pp. 5.7, 5.8, and 5.10) as well as incomplete references in the reference list. Who is to do the needed work to make this document a better support for the recommendations offered? How much help for

this needed work is the responsibility of the Workshop's Peer Consultants? The merits of the basic formulations and recommendations of the Berman & Crump document should not be discarded because of the quite sloppy presentation in their document.

Some Specific Technical Comments on the Berman and Crump Document

- 1) Replace "dose-response" with "exposure-response" in all of the numerous places where the epidemiology and controlled animal inhalation exposure results are discussed.
- 2) Replace "asbestos-related risks" with "asbestos fiber-related risks". Nonfibrous asbestos dust exposures are a different issue.
- 3) The discussion of dust counts based on midjet impinger samples on p. 4.6 needs to be clarified for most potential readers of this document.
- 4) Their reliance on Raabe (1984) for a discussion on the quantitative aspects of particle deposition, and of Figure 7-1 from that paper to illustrate it, is inappropriate as an up-to-date and authoritative reference. A more appropriate reference is ICRP Publication 66: Human Respiratory Tract Model for Radiological Protection, Ann. ICRP, Vol. 24, Nos. 1-3, 1994.
- 5) The 4th, 5th, and 6th bullets in Section 7.1.4 are wholly or partially incorrect statements.
- 6) The first paragraph on p. 7.16 misspells "mucus" five times.
- 7) The last bullet on p. 7.18 indicates, incorrectly, that diffusional transport influences asbestos retention in the lung and other tissues.
- 8) There are various places where the authors have notes to themselves to reconsider or complete the text (see pp. 7.48, 7.65, and 7.103).

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Preliminary Comments for Workshop on "Proposed Protocol to Assess Asbestos-Related Risk"

A. General Comments

In addition to responding to the 12 specific charge questions formulated by the U.S. Environmental Protection Agency I believe it is appropriate to respond to a more general over-arching question. Specifically, "Has the Agency, and its Contractor, reviewed all of the relevant information on the carcinogenic risks of asbestos and interpreted, synthesized and integrated the information in a scientifically adequate manner for regulatory decision making"? In the following comments I will address the over-arching question I have posed.

1. The material provided by the Agency as background material for the Workshop does not reflect a comprehensive and thorough review of the literature. Neither does the material provide a high degree of confidence that all the relevant literature has been reviewed, interpreted, synthesized and integrated in a scientifically sound manner that lends confidence to the finished product meeting the high standards required for use in regulatory decision making. Three primary documents were provided to the Panel in sequential fashion; (a) a document labeled, "Final Draft - Technical Support Document for a Protocol to Assess Asbestos-Related Risk" prepared by D.W. Berman and K. Crump dated September 4, 2001, (b) a document labeled "Final - Methodology for Conducting Risk Assessments at Asbestos Superfund Sites, Part 1: Protocol, Interim Version" prepared by D. W. Berman and K. Crump dated February 15, 1999, and (c) a document, EPA/600/8-84/003F, June 1986, Airborne Asbestos Health Assessment Update prepared under the auspices of the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency.

Taken in aggregate these documents do not represent an up-to-date summary of the voluminous literature available on the health effects of asbestos and, specifically, the lung cancer and mesothelioma risks of exposure to asbestos. The base document, "Airborne Asbestos Health Assessment Update" was prepared by an EPA contractor, Dr. William J. Nicholson, nearly two decades ago and reviewed at that time by the Environmental Health Committee of EPA's Science Advisory Board. In the intervening years, numerous additional papers on the health effects of asbestos, including new epidemiological analyses and mechanistic studies on the carcinogenicity of asbestos, have been published. New and improved analytical methods for characterizing exposure to asbestos have also been

developed and adopted.

The two other documents noted above are also dated. Despite the intervals from September 4, 2001 (the Support Document) and February 15, 1999 (the Methodology) to present, the documents remain "works in progress" with incomplete references and omissions. It is a challenge to the reader to follow the logic being used to synthesize very complex data sets into relative simple algorithms to describe exposure-response relationships for lung cancer and mesothelioma induction by exposure to different types of asbestos fibers with varying dimensions.

The apparent haphazard and protracted approach to developing a scientifically sound approach to characterizing the risks of asbestos exposure is clearly not related to this being a "back burner" issue. During the last two decades, the issue of asbestos-related health effects has received substantial attention in the courts and resulted in the bankruptcy of some 60 companies.

To get the "asbestos-risk characterization" train on the track, so to speak, the Agency might consider using an approach that has served the Agency well in dealing with the criteria air pollutants. That approach is multi-phased. In the first phase, a criteria document is prepared periodically for each criteria pollutant by the Agency's National Center for Environmental Assessment, Office of Research and Development, with input from knowledgeable scientists both from within and outside the Agency. These encyclopedic documents describing all that is currently known about the pollutant are reviewed by the Clean Air Scientific Advisory Committee (CASAC), a part of the Agency's Science Advisory Board. CASAC notifies the Administrator by a "closure letter" when it has reached a consensus that the criteria document provides a scientifically adequate review of all the available information in the pollutant.

In a second phase, the Agency's Office of Air Quality Planning and Standards, Office of Air and Radiation Programs, prepares a Staff Position Paper, that draws exclusively on information in the criteria document, to critically assess the information specifically germane to assessing the risks of exposure to the pollutant in question. The Staff Position Paper is also reviewed by CASAC and when a consensus is reached by the Committee that the document provides a scientifically adequate basis for regulatory decision making, a "closure letter" is issued to the Administrator. The agency then proceeds to use the resulting information to set National Ambient Air Quality Standards and take other regulatory actions.

The process described above is transparent, open, and engages the scientific community, interested parties and the public. The process is not without controversy. However, the open and

participatory nature of the process results in controversy focusing on scientific issues. Legislative mandates for review of criteria pollutants every five years have rarely been met. Nonetheless, steady progress has been made in reviewing new information on a regular schedule.

Without question, the Agency would benefit from having an up-to-date comprehensive review of the current state of knowledge on the health effects of asbestos. The credibility and scientific and public acceptance of the review would be enhanced by obtaining input from a number of knowledgeable scientists in addition to Drs. Berman and Crump and having rigorous peer review by the Agency's Science Advisory Board.

A subsequent risk assessment prepared using information included within the health assessment document would have enhanced credibility if it were based on the input of a number of knowledgeable scientists. This statement is not intended to question the credibility and scientific credentials of Drs. Berman and Crump who are clearly two of the world's experts on the subject at hand. Despite their credentials, I submit that involvement of other scientists in a participatory and transparent manner would enhance the scientific credibility and acceptance of the final product.

As a third step, it would be appropriate for the Agency to provide a brief document detailing how the asbestos risk assessment will be used by the Agency in fulfilling its regulatory and enforcement agenda. The present documents leave these important matters open to speculation. This includes the scientific reviewers who do not know how the science, the associated uncertainties and the various assumptions will be used. For some applications a high degree of uncertainty and the use of many assumptions may be scientifically defensible. For other applications, this may not be the case.

2. Using only the three documents provided, it is difficult to assess if all the relevant information on asbestos-related health risks has been considered. Without question, the 1984/1986 Assessment is out of date. Thus, attention focuses on the two other documents. The manner of presentation in these documents is such that I am uncertain if other knowledgeable scientists could reproduce the calculations and quantitative results. The basic assumptions used in the various calculations are not always clearly spelled out. This leads to uncertainties as to the linkages between the various tables and related text as the document builds to summary conclusions (Tables 6-29 and 6-30).

3. The documents in numerous places acknowledge the substantial uncertainty in developing quantitative estimates of exposure-response coefficients for various types of asbestos (with

varied size characteristics) producing lung cancer and mesothelioma. Nonetheless, these uncertainties are rarely quantified and are absent from the summary conclusions (Tables 6-29 and 6-30).

4. Two key inter-related uncertainties that are not adequately addressed in the documents relate to (a) the shape of the exposure-response relationship over the range of observations from epidemiological studies of occupationally exposed populations, and (b) the basis of extrapolation from observations at high levels of generally prolonged occupational exposure to much lower levels of environmental exposure. These issues have been a focus of attention in EPA's revised cancer risk assessment guidelines. It is of interest that the Agency's proposed revised cancer risk assessment guidelines are not even referenced in either document.

The proposed revised cancer risk assessment guidelines emphasize the importance of using a two-step process. First, characterize exposure-response relationships over the range where observations can be made. Then in a second step, extrapolate to lower exposure levels. Neither step is adequately documented in the material at hand. Intuitively, one would anticipate considerable variation in extrapolated risk at environmental levels of exposure. To the extent it is possible the uncertainty should be quantified.

5. During the last two decades substantial progress has been made in understanding the mechanisms by which fibers may induce cancer. The present documents focus on advances in understanding the role of fiber dimensions as determinants of carcinogenic potency.

However, the documents do not adequately address a related issue, biopersistence, and especially the role of fiber solubility in biopersistence. Advances in this area have been extraordinary with regard to man-made fibers and have led industry to make revolutionary changes in commercial man-made fibers, i.e., increasing solubility and, thus, reducing their potential for causing human cancer. This body of science should be reviewed in the document because it may have applicability to some situations involving asbestos fibers. Specifically, provision should be made for changes in risk coefficients for asbestos fibers if it can be shown that the solubility of the fibers differs from the solubility of the asbestos fibers purported to induce cancer in the epidemiological studies used as the basis for the exposure-response models that have been advanced.

6. I am uncertain at this juncture if the proposed risk assessment approach is sufficiently

well developed and validated and the associated assumptions and uncertainties identified to warrant its use in the field for regulatory decision making. However, I do see substantial merit to the approach and urge the Agency to continue with development and validation of the approach on an accelerated basis. This accelerated process should include provision for broader scientific community participation in the development process and more peer-review than has occurred in the past.

B. Specific Comments

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

1) For lung cancer.

a) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

It is my professional opinion that the epidemiological literature and mechanistic studies provide strong evidence for the hypothesis that carcinogenic potency varies from one fiber type to another; crocidolite > amosite > chrysotile. There is also evidence that within a fiber type, differences in carcinogenic potency may also exist.

The proposed "optimized risk coefficients" in Table 6-29 may well be appropriate. However, the document in its present form does not clearly relate the origins of the "representative values" in Table 6-15 and their linkage to the "optimized risk coefficients" in Table 6-29 and the "recommended risk coefficients" in Table 2-1 of the protocol document. The Hodgson and Darnton (2000) analysis, cited in the document, provides different coefficients. The basis for the difference is not clear.

In future reports on this topic, it is important that additional attention be given to clarity of presentation including the origin of any values and associated assumptions and uncertainties. Whenever possible uncertainties should be quantified.

b) Influence of fiber length. Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure

index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)?
(Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

It is my professional opinion that the epidemiological literature and mechanistic studies clearly show a strong correlation between fiber length and carcinogenic potency for asbestos. If an integrated exposure index is developed and used, it is appropriate to give substantially greater weight to fibers greater than 10 μm in length as Berman and Crump have done.

c) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

The animal studies are clearly informative on the topics of fiber type and fiber length. This includes the early work of the Wagner group and the more recent work of the Davis group. This section of the report would be strengthened by more careful consideration of previous analyses including those of Lippmann (1988 and 1994).

d) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Fiber diameter is an important determinant of the carcinogenic potency of fibers. Fiber diameter is a major determinant of the aerodynamic diameter of fibers which strongly affects the deposition probability of fibers. In contrast, fiber length has only a small influence on aerodynamic diameter. The diameter of fibers influences the surface area of fibers which, along with surface chemistry, influences the dissolution rate of fibers and the interaction of fiber constituents with biological systems.

The aspect ratio is of importance in defining what is or is not characterized as a fiber. The definition of a fiber as an elongated particle with an aspect ratio of greater than 3 to 1 as typically used seems reasonable.

2) For mesothelioma:

a) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what

extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

The results of epidemiological investigations supported by mechanistic studies provide substantial support for a variation in mesothelioma induction potency associated with fiber type: erionite > tremolite/crocidolite > chrysotile. Because of difficulties in interpreting the various studies, it is not possible to rule out the hypothesis that pure chrysotile exposures are not associated with mesothelioma induction.

The proposed "optimized risk coefficients" for mesothelioma in Table 6-29 may be appropriate. However, the linkage to the individual studies from which they are derived is not always clear nor is the linkage to the "representative values" in Table 6-15 or the "recommended risk coefficients" in Table 2-1 of the protocol document.

In future reports, it is important that additional attention be given to clarity of presentation including the origin of all values, explicit statements as to assumptions used and statements of the underlying uncertainties. Whenever possible uncertainties should be quantified.

b) Influence of fiber length. Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

The epidemiological literature, supported by the results of controlled animal exposure studies, clearly indicate that fiber length is a major determinant of the potential for fibers to cause mesothelioma. In my professional opinion, fibers less than 5 μm in length are unlikely to induce mesotheliomas. The role of fibers 5 to 10 μm in length is less clear. Fibers 10 μm to 20 μm in length are most likely to induce mesothelioma. However, my statement as to the role of fiber lengths must be coupled with knowledge of the fiber type.

c) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

It is apparent that erionite and the amphiboles have the potential to induce mesothelioma. The available literature is not persuasive that pure chrysotile induces mesothelioma.

d] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

As the diameter of fibers decreases the relative surface area for a given mass of fibrous material increases. Thus, there is a greater opportunity for the surface of fibers to interact with the biological systems. Surface area will also influence the rate of dissolution of fibers. And, clearly, surface characteristics will influence the interactions between fibers and the biological system. Unfortunately, the specific surface properties of concern are not yet well understood.

As noted earlier, knowledge of exposure-response relationships extending from the high occupational exposure levels studied epidemiologically to environmental levels of exposure is lacking. The linear extrapolations from epidemiological studies of occupationally exposed populations to environmental levels of exposure have major uncertainties that have not been adequately stated in the Berman and Crump documents.

3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

The exposure estimates for asbestos reported in the occupational exposure asbestos epidemiology literature, are highly uncertain. In some cases, there is considerable uncertainty as to the fiber types to which the individuals were exposed. In other cases, major uncertainty exists as to the physical dimensions of the fibers because of the variety of evolving techniques used to characterize asbestos fibers. The extent to which other particulate matter or other toxicants were present is not always known. And for most studies there are only a relatively few exposure concentration measurements available for populations exposed for many years making estimates of cumulative exposure highly uncertain.

To the extent occupational exposures are under-estimated, the estimated risk coefficients will be too high, i.e., over-estimate the true potency. Conversely, if the occupational exposures have been over-estimated, the estimated risk coefficients will be under-estimates of true potency. For each study used to develop risk coefficients, the authors should provide a clear statement of their confidence in the exposure estimates and, if possible, provide a quantitative estimate of the uncertainty associated with the exposure estimates. These estimates of uncertainty for exposure should be carried over into estimates of uncertainty for the potency values.

4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

In my professional opinion, the asbestos fibers less than 5 μm in length do not pose a carcinogenic risk. Thus, it is appropriate to exclude them from the exposure-response index for asbestos-induced cancer.

5) The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

In my professional opinion, it is appropriate for the exposure-response index for lung cancer to be weighted toward the long fibers. The human literature is less certain with regard to mesothelioma induction.

6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

Embedded in this question are several issues. Historical exposure assessments are what have been reported. Whatever the technique and the reporting criteria used is what we have to work with, if it was phase contrast that is what we must work with. The second issue is the extent to which these measurements are truly reflective of the historical exposures of the population. The third issue following from the above is the degree of uncertainty in the derived estimates of exposure-response relationships. A fourth issue is whether these exposure-response relationships are valid for contemporary environmental exposures. A key consideration in this matter is the substantial extrapolation involved in going from historical occupational exposure levels to contemporary environmental levels including levels established for clean-up.

Topic Area 3: General questions.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or "bundles that are components of more complex structures"). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

In my professional opinion, the cleavage fragments have toxicological significance to the same extent as intact fibers of the same dimensions.

- 8) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

The proposed exposure-response index, if appropriately validated, would be appropriate for use in assessing the risks of asbestos fibers equivalent in type and size to those on which the index was based. Use of the index with other asbestos fiber types would involve an extrapolation of unknown uncertainty. It should also be emphasized that use of the index with asbestos or other fibers that have biopersistence characteristics different from those of the fibers used to develop the index would be inappropriate.

- 9) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoint)?

The document makes the point at several places that the output of the assessment of exposure must be matched to the exposure-response model being used. I strongly agree with this statement. Thus, if the Berman-Crump exposure-response indices are to be used, then it is appropriate to analyze samples by transmission electron microscopy and count only those fibers (particles with an aspect ratio of greater than 3 to 1) or bundles longer than 5 μm . This approach is justified if the only use of the exposure data is to match it to the Berman-Crump exposure-response indices.

However, it must be recognized that for many situations, exposures may be evaluated for multiple purposes. For example, the exposure estimates may be used as input to an epidemiological investigation of a specific population. In such a situation, it may be very useful to have a more comprehensive assessment of exposure. This might include enumeration of fibers by different increments of length, i.e., less than 5 μm , 5-10 μm , 10-20 μm , etc. Indeed, in some cases it may be

advantageous to have information collected in a manner that allows characterization of the variability of both fiber diameter and fiber length analyzed independently or in a linked manner along with electron diffraction analysis to provide information on chemical composition. The specific information must be matched to its intended use and also the cost of collecting the additional increments of information.

- 10) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber diameter appropriate?

This question addresses the inter-play between the definition of a fiber (an elongated particle with an aspect ratio [length to diameter] of 3 to 1), fiber length and diameter, and fiber aerodynamic diameter. Fibers with a length of 5.0 μm or longer and diameters up to about 1.5 μm could still meet the traditional definition of a fiber and have an aerodynamic diameter of about 5.0 μm . Such objects would still have a low probability of being inhaled and deposited in the pulmonary region. On this ground, there is no basis for excluding them from consideration. On the other hand, the proposed exposure-response index appears to have merit when only fibers under 0.5 μm in diameter are included.

- 11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

This issue is addressed in my general comments.

- 12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

It is appropriate for the document, and any subsequent versions, to provide multiple options for assessing cancer risks for different situations dependent on the information available. However, it would be appropriate for the document to more clearly define the circumstances under which it is appropriate to use each of the options. The "decision rules" for selection of options should be crafted to avoid providing the opportunity for a regulator to attempt to select an option to gain a particular pre-selected outcome.

Roger O. McClellan

I strongly favor retaining an approach that matches exposure-response risk coefficients to the particular type of asbestos fiber under consideration and calculation of risks separately for smokers and non-smokers.

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CHARGE QUESTIONS

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

I. For lung cancer.

A) Influence of *fiber* to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different *fiber types* ? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Answer: The epidemiology literature suggests that carcinogenic potency for lung cancer varies across fiber types, but the evidence is scattered throughout the literature and is not overwhelming. A unified analysis that incorporates all the lung cancer epidemiology data and that is focused specifically on the hypothesis - "potency for lung cancer varies with mineral type" is needed to answer this question. The Berman & Crump report (B&C) provides such an analysis. I am not aware of any other unified analysis of the lung cancer epidemiology data. The B&C analysis is innovative and, by necessity, employs various assumptions and interpretations of incomplete data. Before accepting the B&C conclusion, we need a better understanding of the B&C assumptions and data interpretations. A more detailed evaluation is needed of the B&C assumptions and applications of incomplete data than was possible at this time. (Note: Access to raw data used in B&C would be required for a detailed evaluation.) Since there is no competing unified analysis of the lung cancer epidemiology data that concludes otherwise, the "potency for lung cancer varies with mineral type" hypothesis should be accepted.

Concerning the risk coefficients for lung cancer in Table 6-29, the only way to determine if they are supported by the epidemiology literature is to conduct the type of unified analysis I mentioned above. Note that these risk coefficients are determined not only by the epidemiology data, but they depend also on the new proposed exposure index, the fiber size distribution adjustments to K_L , and the assumptions and data that were used to determine those entities. The B&C unified analysis, if

it survives more detailed peer review than was possible at this time, is itself a statement that the epidemiology literature supports the risk coefficients in Table 6-29.

However, the recorded values of the lung cancer risk coefficients in Table 6-29 are very likely incorrect because the B&C analysis relies on linear extrapolation of risk to low-exposure levels. An alternative unified analysis needs to be conducted that incorporates "threshold" models for lung cancer risk. Some researchers claim that epidemiology and clinical data suggest an exposure threshold for lung cancer of 25 f-yr/cc. Although there may be no asbestos exposure level where the risk of lung cancer is an absolute zero, the size of the potency coefficient for exposures below 25 f-yr/cc, or an appropriately determined alternative "threshold" exposure, is likely to be substantially less than the size of the potency coefficient for exposures greater than the "threshold."

The epidemiology literature supports the existence of this type of "threshold." Most, if not all the epidemiology data from studies used in B&C indicate "no statistically significant elevation of lung cancer cases" for exposure categories with exposures less than 15 to 20 f-y/cc. (As an example, my calculations applied to the Dement data analyzed in B&C (Table 6-2) indicate no statistically significant elevation of lung cancer risk below the exposure interval, [28-60] f-yr/cc. A simple linear-linear fit to these data picks a "threshold" exposure at 21.3 f-yr/cc.)

The "threshold" approach needs to be explored. However, simply fitting a standard exposure-risk equation to the full set of data from an epidemiology study will not necessarily solve the low-exposure problem adequately. Standard exposure-risk equations lack flexibility. The risk values at high exposures tend to pull the curve upward even at low exposure levels and it is likely that a statistical test will not be able to differentiate an s-shaped curve from a linear model due to the limited number of data points. Therefore, other approaches may be required, similar to the general approach in B&C that combines judgment based on information from animal studies, lung burden studies, and cellular studies with epidemiology data and statistical analysis. The "threshold" - low exposure linear extrapolation issue must be resolved before adopting new values for lung cancer potency.

B) Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is

information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Answer: Fiber length and fiber dimensions in general are extremely important factors in asbestos risk assessment. The epidemiology literature alone does not contain adequate data to determine the effects of different fiber lengths. Information concerning the lung cancer potencies of different fiber dimensions can be determined from animal studies and lung burden studies. An exposure index that weights long fibers more heavily than short fibers is justified for assessing lung cancer risk associated with asbestos exposure.

C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Answer: These studies indicate that carcinogenic potency varies with *fiber length*.

D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Answer: The epidemiology literature does not contain adequate data to determine the risk effects of fiber properties such as diameter, aspect ratio, and surface dimensions.

2) For *mesothelioma*:

A) Influence of *fiber type*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one *fiber type* to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different *fiber types*? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

Answer: The epidemiology literature clearly establishes the significance of *fiber type* for mesothelioma potency and supports different exposure-risk analyses for different *fiber types*.

Concerning the risk coefficients for mesothelioma in Table 6-29, the only way to determine if they are supported by the epidemiology literature is to conduct a unified analysis such as the B&C unified analysis. (For a more complete explanation, refer to my discussion above of a unified analysis for lung cancer). To answer this question, a more detailed peer review than was possible at this time would be required to test B&C assumptions and to dissect how supporting data were used (e.g., the calculation of fiber size adjustment factors for K_M). In addition, the "threshold" concept that I described above for lung cancer risk assessment also needs to be considered for mesothelioma risk assessment to avoid problems associated with low-exposure linear extrapolation.

B] Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Answer: Fiber length and fiber dimensions in general are extremely important factors in asbestos risk assessment. The epidemiology literature alone does not contain adequate data to determine the effects of different fiber lengths. Information concerning mesothelioma potencies of different fiber dimensions can be determined from animal studies and lung burden studies. An exposure index that weights long fibers more heavily than short fibers is justified for assessing mesothelioma risk associated with asbestos exposure.

C] To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

Answer: These studies indicate that carcinogenic potency varies with *fiber length*

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties

(e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Answer: The epidemiology literature does not contain adequate data to determine the risk effects of fiber properties such as diameter, aspect ratio, and surface dimensions.

- 3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

Answer: "Reliable" is a relative term. To answer this question, the word "reliable" has to be interpreted in a context. The context is "decision-making based on risk estimates derived, in part, from exposure estimates documented in the asbestos epidemiology literature." I am referring to decision-making applied to selecting exposure limits, managing or removing asbestos-containing materials in buildings, cleaning asbestos waste sites, or implementing product bans. The decision-making process must account for uncertainty in risk estimates, which is due, in part, to the uncertainty (i.e., the reliability or lack thereof) in the underlying exposure data used to develop the risk estimation method. B&C describe most and possibly all the well-known problems with exposure estimates in the epidemiology literature. We cannot claim to know the exact airborne fiber concentration or makeup of fiber types and sizes for any particular worker who is a subject in an epidemiology study. However, the collection of exposure estimates associated with the epidemiology studies, which have been developed from various and often disparate sources of information, appear to provide a relatively consistent characterization of exposure that is sufficient for developing a risk assessment method. Provided the uncertainty in risk estimates is treated with an appropriate degree of respect in decision-making, the exposure estimates documented in the asbestos epidemiology literature may be characterized as "reliable."

Topic Area 2: The proposed exposure index.

The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

Answer: The epidemiology literature alone does not contain adequate data to determine the effects of different fiber lengths. The toxicology literature supports the conclusion that long asbestos fibers are associated with greater carcinogenic risk than short asbestos fibers. The assertion that the 5 μ m limit is a "bright line" separating carcinogenic fibers from non-carcinogenic fibers is doubtful, but it is clear that fibers shorter than 5 μ m have diminishing potency.

An evaluation of the carcinogenic potential of short fibers also can be addressed from a completely different perspective, using a different set of epidemiology data -- cancer incidence data collected by the National Cancer Institute in its Surveillance, Epidemiology, and End Results (SEER) program. Briefly, the trend over time of mesothelioma incidence for US women does not reflect the pattern that would be expected if exposure to short fibers posed a significant carcinogenic risk. These data indicate that although exposures to short fibers may have increased over time, they have not resulted in an epidemic of asbestos-related cancer.

- 5) The proposed exposure index is weighed heavily by fibers longer than 10 μ m. Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μ m is more than 300 times greater than that of fibers with lengths between 5 and 10 μ m. How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

Answer: The epidemiology literature alone does not contain adequate data to determine the effects of different fiber lengths. The toxicology literature generally supports the conclusion that long asbestos fibers are associated with greater carcinogenic risk than short asbestos fibers. The proposed exposure index is consistent with the toxicology literature. There exist no data at this time other than those used in B&C to further confirm the use of the proposed classification of lengths and numerical weights.

- 6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

Answer: I believe the question should be - Can the proposed exposure index be used to evaluate cancer risk associated with current environmental exposures, given that the index was derived from animal data and the risk models were derived from data collected in occupational studies? The answer is "yes" with qualifications. "Yes" because the potency factors in the proposed risk models were adjusted to be applied with the proposed index. The qualifications are the uncertainties concerning the methodology and use of data to derive the adjustments as discussed in answers to earlier questions.

Topic Area 3: General questions.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or bundles that are components of more complex structures). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

Answer: From my reading of the scientific literature, it appears that cleavage fragments may be less potent for asbestos-related cancer than asbestos fibers. As a practical matter, it would be very difficult to evaluate the proportion of structures that were cleavage fragments in historical exposure measurements. Therefore it would be difficult to use a current exposure measurement that was adjusted for cleavage fragments in a risk assessment model.

- 8) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

Answer: The proposed cancer assessment approach is applicable only to the mixture of fibers that constituted exposure in the epidemiology studies. If it were subsequently confirmed that other mineral types were, in fact, included in the exposures (e.g., winchite and richterite at the Libby mine), the proposed cancer assessment approach, subject to adjustment of potency factors, could accommodate other amphiboles.

- 9) The review document recommends that asbestos samples be analyzed by transmission electron

microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

Answer: The evidence is reasonably strong that asbestos fibers with lengths less than 5 μm have minimal potency for asbestos-related cancers. Also, there is no history of an association between low level environmental exposures, which probably included a high percentage of short fibers, and asbestosis. There is no reliable evidence at this time that other non-cancer endpoints are associated with low level asbestos exposure or short fibers. Counting fibers shorter than 5 μm would increase the cost of measuring airborne asbestos. The cost would not be justified if the only use of the data were to validate the fiber length component of the cancer risk assessment. We need more information through a debate that has not yet been conducted to determine if the risk of specific non-cancer endpoints potentially associated with exposures to short fibers is sufficiently established to justify the extra cost of including short fibers in asbestos measurements.

- 10) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber *diameter* appropriate?

Answer: Determining the correct weights for fiber lengths is more important than fixing a specific diameter limit. The cut-off for fiber diameter should account for fiber respirability and clearance mechanisms. I do not have a recommendation at this time.

- 11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

Answer: As a whole the proposed cancer assessment approach is an impressive analysis of a wide-ranging collection of data to produce an asbestos cancer risk model that addresses almost all the significant risk issues that have been debated over the past 20 years. It is a reasonable evaluation of the available health effects data with one extremely important exception. It does not address the

"threshold" - "low exposure linear extrapolation" issue. The analysis, by virtue of this exception, is inconsistent with the epidemiology literature for asbestos.

- 12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

Answer: Each of the three proposed options is an approach with a counterpart that is currently available and has been available since 1988 when EPA last updated its Asbestos IRIS file. The simple equation reported in IRIS (counterpart of B&C Option 3) that determines asbestos cancer risk to be 0.23 risk units for each PCM fiber/cc measured in units of lifetime average daily exposure (LADE) is usually identified as "the EPA asbestos risk assessment." However, it is not the only option available to a risk assessor today. One may choose to utilize Tables 6-1 through 6-3 in the EPA Asbestos Health Assessment Update (1986) for separate risk estimates of lung cancer and mesothelioma by sex and smoking status (counterpart of B&C Option 2), or may use the underlying equations for lung cancer and mesothelioma with life-table data to estimate risks (counterpart of B&C Option 1). The changes in the currently available approaches that define the three proposed options are: (i) use of a new exposure index that explicitly accounts for fiber dimensions; and (ii) differential potencies based on mineral type that also are different for lung cancer and mesothelioma. These B&C innovations are significant, but more is needed.

Option 3 does not distinguish lung cancer from mesothelioma, and requires averaging over smoking status and sex. Option 3 may have some merit for "quick" asbestos risk comparisons, but is inflexible and subject to error. B&C does not explain how to implement Option 3. EPA should not rely on Option 3 for estimating risk.

Option 1 and Option 2 need to address "the low-exposure linear extrapolation" issue. EPA's risk assessment is intended to evaluate risk at low exposures. The currently available risk assessment methods lead to questionable risk estimates at low exposures. For example, using the current EPA unit risk equation published in IRIS, the incremental risk of asbestos-related cancer corresponding to a cumulative lifetime exposure of 0.30 f-y/cc is approximately 1 in 1000 (1×10^{-3}). However, epidemiology data suggest an exposure threshold that may be as large as 25 f-y/cc for lung cancer. Although there may be no exposure level where the risk of cancer is an absolute zero, it is highly

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unlikely that 0.30 f-y/cc lifetime exposure would lead to a 1×10^{-3} risk of cancer. Option 1 and Option 2 are likely to overstate risks at low exposures because they incorporate low exposure linear extrapolation risk assumptions.

Finally, Option 1 and Option 2 require estimates of mortality due to all other causes in order to produce risk estimates for lung cancer and mesothelioma. B&C provides mortality data that they use to create risk tables for Option 2. Since mortality patterns have been shifting (i.e., survival to older ages) and mesothelioma has a long latency period, the mortality data used in the model may be an important factor, especially for Option 2. (For example, using 1970 mortality data may lead to different risk estimates than mortality data from 2000.) The effect may be small, but it needs to be assessed before a particular set of data are locked-in to Option 2.

Topic Area 4: Development of Conclusions and Recommendations

At the end of the workshop, the peer consultants will be asked to draft conclusion statements identifying their most notable findings on the proposed methodology. As a prelude to developing these statements, the peer consultants are invited to provide any additional comments or concerns, both strengths and weaknesses, on topics not specifically addressed in the previous charge questions. After completing the discussions the peer consultants will prepare their conclusions, and they will also be asked to develop recommendations for how EPA can improve the methodology. Please note that, although recommendations for future research projects are welcomed, the focus of this workshop is on the proposed risk assessment methodology and how it may be used to support decisions at asbestos-contaminated sites.

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Dr. Sherman received her B.S. in mathematics from Pennsylvania State University, her M.A. in Biostatistics from the University of California-Berkeley, and her Ph.D. in statistics from the University of Waterloo. She is a Biostatistician with California Environmental Protection Agency, where she specializes in quantitative cancer risk assessment. She has served in various positions at NIEHS as a Biostatistician and has co-authored a number of papers with Dr. Christopher Portier including "Multistage stochastic models of the cancer process: a general theory for calculating tumor incidence," "The two-stage model of Carcinogenesis: overcoming the nonidentifiability dilemma," "The utility of the Kolmogorov backward equations in stochastic Carcinogenesis modeling," and "Numerically calculating the cumulative distribution function for the time to an observable tumor in multistage models of Carcinogenesis." She is the author of a book chapter entitled "the potential effects of chemical mixtures on the carcinogenic process with in the context of the mathematical multistage model," in Risk Assessment of Chemical Mixtures: Biological and Toxicological Issues, R. Yang (1994). She serves as a reviewer for several professional journals, including Biometrics, Environmental Health Perspectives, Journal of the American Statistical Association, and The Journal of Toxicology and Applied Pharmacology. She has presented numerous papers for government agencies and universities including "Assessing Cancer Risk from large Epidemiologic Cohorts: Tumor Incidence, Hazard Functions, and Identifiability," "Improving the mathematical modeling of Carcinogenesis via intermediate events and biomarker data." She is a member of the American Statistical Association, the International Biometrics Society, and the New York Academy of Sciences.

Prior to submitting these written comments, I would like to acknowledge the invaluable assistance and guidance of my colleagues at California EPA/OEHHA. Drs. John Budroe, Stan Dawson, and Melanie Marty have (probably) collectively spent more years working on the subject of asbestos than the number of years that I have been on this earth. Without their support, many of the questions that have been addressed would have been without comment. My only regret is that we did not have ample time to more thoroughly answer all of the questions within the charge.

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

1) For lung cancer:

A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

As has been documented throughout the asbestos literature and conditional on the relative risk model that has been used to describe asbestos induced lung cancer, carcinogenic potency varies for chrysotile and amphibole fiber types. What has not been reconciled are the results of Hodgson and Darnton (2000) with this report's conclusions regarding fiber type. Hodgson and Darnton (2000) determined differences in carcinogenic potencies for crocidolite, amosite and chrysotile. In this report, carcinogenic potency differences between the amphiboles are not reported nor is there a discussion to settle this disparity.

The overall values of KL of Table 6-29 appear to be in an appropriate relation to the adjusted individual values in Fig. 6-4 for pure chrysotile and pure amphibole and even for mixed fibers, suggesting agreement with the central tendencies in the epidemiological literature. This approach, however, does not give sufficient recognition to the high chrysotile

coefficients obtained in the South Carolina studies, a recognition that is needed for adequate health protection.

B] Influence of fiber length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (mm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

The adjustment of the risk coefficients for fiber length seems to be an appropriate concept applied to the animal studies. However, extrapolation to humans who have different airway geometry than rodents and may well respond differently to the same fiber dimension requires justification. The report needs some substantial basis for an extrapolation that will ultimately require a complex model to fit the human data.

C] To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

Hesterberg et al. (1998) exposed Fischer rats to chrysotile asbestos and several man-made fibers by nose-only inhalation for 6 h/day, 5 days/week for 2 years. The chrysotile asbestos inhalation concentration was 10,600-WHO fibers/ml (WHO fibers defined as being 5 μm in length and $> 3 \mu\text{m}$ in diameter and having a length/diameter ratio > 3). The geometric mean length and width of the dispersed fibers was 1.2 and 0.08 μm , respectively, suggesting that the non-WHO fiber concentration was higher than the WHO concentration. Additionally, no fibers were $> 20 \mu\text{m}$ in length, and very few were $> 10 \mu\text{m}$ in length. The geometric mean length and width of the lung burden of deposited chrysotile fibers after 104 weeks of exposure and 23 weeks of recovery were 1.6 μm and 0.07 μm , respectively.

Chrysotile asbestos caused significantly increased incidences of both lung cancer (12/69, 17.4%, adenomas and carcinomas combined), and pleural mesothelioma (1/69, 1.4%) compared to controls (lung cancer incidence 2/130, 1.5%; mesothelioma incidence 0/130). These results suggest that relatively short chrysotile asbestos fibers are capable of inducing both lung cancer and mesothelioma in rats. The authors stated that "fiber-induced lung toxicity is not always strictly dependent upon the numbers of long fibers retained in the lung", and "these data demonstrate that the toxic potential of chemically different fiber types cannot be predicted solely by the dimensions of the fibers retained in the lung. In the induction of fiber-induced pathogenesis, sheer numbers of fibers may thus be able to compensate for a lack of long fibers".

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

No comment.

2) For mesothelioma:

A] Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

As stated in Section 8.1.2, Hodgson and Darnton (2000) find substantially different carcinogenic potencies for amphiboles and chrysotile. Even though the report agrees with the conclusions of Hodgson and Darnton (2000) on this point, there have been references

in the literature over the years to differences in potency between crocidolite and all other asbestos minerals.

The overall values of KM appear to be substantially less than the individual values in Fig. 6-6 not only for pure chrysotile and pure amphiboles, but also for the mixtures. This appears to represent an irreconcilable difference between the optimal values given in Table 6-29 and the individual values for the epidemiology studies.

B] Influence of fiber length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (mm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Nolan et al. (1994) examined the lung contents of six workers who had been occupationally exposed to chrysotile asbestos. Five were lung cancer cases from Quebec, Canada. The sixth case was an American worker who had developed pleural mesothelioma. An analysis of two parenchymal lung tissue specimens from the pleural mesothelioma of the American worker demonstrated that the predominant fiber type was chrysotile. Chrysotile fiber length percentages in those parenchymal lung tissue specimens are described in Table 1. Fibers < 0.5 μm in length were not counted.

Table 1: Length distribution of chrysotile fibers from two parenchymal lung tissue specimens from an American pleural mesothelioma case (from Nolan et al., 1994)

Specimen	Percentage of fiber length		
	$\leq 4.99 \mu\text{m}$	$5 - 7.99 \mu\text{m}$	$\geq 8 \mu\text{m}$
A	96.7	2.5	0.8
B	98.8	1.2	0

The authors stated that "the fiber length distribution of the chrysotile recovered from the U.S. mesothelioma case was indistinguishable from that of chrysotile specimens known to produce mesotheliomas in rats". These data suggest that short fiber chrysotile may be capable of inducing mesothelioma in humans.

A study by Suzuki and Yuen (2001) characterized asbestos fibers in the lung and mesothelial tissues (mesotheliomatous tissue and hyaline plaque) taken from 151 human malignant mesothelioma cases. The most common asbestos types seen in the lung were a mixture of chrysotile with amphiboles followed by amphiboles alone and chrysotile alone. The majority of asbestos types seen in the mesothelial tissues were chrysotile alone, followed by chrysotile plus amphibole and amphibole alone. The majority of asbestos fibers detected in the lung and mesothelial tissues were shorter than $5 \mu\text{m}$ in length. Only 4% of the fibers found were $8 \mu\text{m}$ in length or greater. The authors stated that chrysotile asbestos can induce human malignant mesothelioma, since, in some of the mesothelioma cases, asbestos fibers detected in both the lung and mesothelial tissues, or lung tissue alone or mesothelial tissues alone were exclusively chrysotile fibers. Additionally, the authors concluded that "such short, thin asbestos fibers should not be excluded from those contributing to the induction of human malignant mesothelioma".

These studies suggest that short fiber chrysotile asbestos is capable of inducing both lung cancer and mesothelioma in rats, and may be capable of inducing mesothelioma in humans.

Similar to the case of lung cancer, the adjustment of the risk coefficients for fiber length suffers from inadequate data applied to the animal studies. Use of the same fiber-length adjustment obtained for lung cancer to mesothelioma, though not contraindicated, is not supported in the asbestos literature. Extrapolation to humans who have different airway geometry than rodents and may well respond differently to the same fiber dimension requires justification as well.

C] To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length?

See lung cancer section, part c.

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

No comment.

III. To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

The exposure estimates documented in the asbestos epidemiology literature suffer from many of the exposure uncertainties inherent in occupational epidemiological studies. Exposure uncertainties related to non-representative sampling, poor evaluation of job exposures, retrospective estimation of exposure levels, and the conversion of samples from counted particles (particle concentrations in million particles per cubic foot) to fiber concentrations (fibers per milliliter).

Topic Area 2: The proposed exposure index.

IV. The proposed exposure index does not include contributions from fibers shorter than 5 mm. Please comment on whether the epidemiology and toxicology literature

support the conclusion that asbestos fibers shorter than 5 mm present little or no carcinogenic risk.

The authors assume asbestos fibers shorter than 5 mm present little or no carcinogenic risk when insufficient information exists to validate this assumption. Potential counter-examples to this assumption would include Nolan et al. (1994) and Suzuki and Yuen (2001). Their conclusions were that short fiber chrysotile (< 5mm) may be capable of inducing mesothelioma in humans.

- 5) The proposed exposure index is weighed heavily by fibers longer than 10 mm. Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 mm is more than 300 times greater than that of fibers with lengths between 5 and 10 mm. How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?**

Equation 7.12 adequately fit tumor incidence data across 13 separate animal studies, but there is no justification for using the proposed exposure index to evaluate asbestos-related cancer risks for humans. Furthermore, as has been cited in earlier questions relating to fiber length, the induction of fiber-induced pathogenesis can be the result of the sheer numbers of fibers when there is a lack of long fibers.

- VI. Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.**

Given the lack of justification for the proposed exposure index in human studies, it is difficult to answer this question.

Topic Area 3: General questions.

- VII. The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or bundles that are components of more complex**

structures). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

Cannot comment.

- VIII. Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

In the absence of better information, it seems prudent to use the existing amphibole numbers, obtained from crocidolite or amosite studies or both, for other fibrous amphiboles. Based upon the study data of Armandus et al. (1987), tremolite has a potency between crocidolite and chrysotile for mesothelioma. For lung cancer, the potency of tremolite is more similar to chrysotile.

- IX. The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μ m. Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μ m. To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

Data on shorter fibers would certainly be useful to validate any cancer risk assessment methodology that is in current practice or development. Suzuki and Yuen (2001) characterized asbestos fibers in the lung and mesothelial tissues and noted that a majority of the asbestos fibers detected were shorter than 5 μ m in length. They concluded that short, thin asbestos fibers should not be excluded from those contributing to the induction of human mesothelioma.

The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μ m and thinner than 0.5 μ m. Is this cut-off for fiber diameter appropriate?

Cannot comment.

- XI. Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

The proposed assessment is predicated on the adjustment for fiber length, i.e. interim exposure index given by Equation 7.13, to be reasonable for humans. Without adequate justification, one cannot determine whether the approach outlined within the report is a

reasonable evaluation of the available health effects data. In addition, the proposed approach for developing coefficients has two serious problems: (i) For lung cancer, the potencies of the South Carolina textile studies for workers exposed to chrysotile are not adequately recognized. These should be included to afford greater health protection. (ii) For mesothelioma, the overall optimized coefficients are substantially below the trends of the coefficients for the individual studies.

X. Section 8.2 of the review document presents three options for assessing cancer risks from

asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

All three options could be used, depending on the application. Option 3, Estimating Risk from a Unit Risk Factor, has the advantage of being the most simple to apply and has been traditionally implemented. Option 3 would be particularly useful for inexpensive screening calculations.

Technical Comments:

Comment: Table 6-12 and Figure 6.3 display the likely ranges for the KL estimates. However, the uncertainty factors used to derive these "likely ranges" are not defined in a manner that allows one to replicate these analyses. A protocol that provides decision rules for assigning such factors is needed as well as the range for each factor. Thus, the text that describes the variation in the KL estimates could be misleading since the confidence intervals are effectively expanded.

Pg. 6.35: Among "pure" amphibole studies, the lowest and highest of the best-estimate KL values vary by a factor of approximately 20.... However, these two estimates are not statistically different (based on comparison of their confidence intervals).

Comment: The inference suggested above can be statistically tested via likelihood-ratio tests. By confining the slopes of the "pure" amphibole studies to be equal and then comparing the likelihood from this model to a model where the slopes may vary, one can objectively assert whether there is a statistically significant difference in the KL estimates.

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Dr. Stayner has been selected to co-chair the workshop. He recently served as a visiting scientist for the International Agency for Research on Cancer, where he worked with Dr. Jerry Rice on monographs for man-made mineral fibers and numerous other epidemiologic projects. He is the Chief of the Risk Evaluation Branch, which includes working on research on characterization of occupational health and safety risks and the development of better methods to characterize these risks. He was the 2000 recipient of the NIOSH Special Act Award for organizing a workshop on "Future Research for Improving risk Assessment Methods." He has lectured and served as an instructor on risk assessment and risk management for academia and professional societies, and international organizations. He has published numerous papers including "Exposure Response Analysis of Respiratory Disease Risk Associated with Occupation Exposure to Chrysotile Asbestos," "Silica, Asbestos, Man-Made Fibers, and Cancer," "Occupational Exposure to Chrysotile Asbestos and Cancer Risk: A review of the 'Amphibole Hypothesis,'" "Concordance of Rat and Human-based Risk Estimates for Particle Related Lung Cancer," "Exposure to Crystalline Silica, Silicosis and Lung Disease other than cancer in Diatomaceous Earth Industry workers: A Quantitative Risk Assessment." He has made numerous presentations at symposia, seminars, and workshops including "Using Epidemiologic Data for a Risk Assessment of Silica Exposure, 2001," "The Molecular Epidemiology of Asbestos and other Fibers, Harvard SPH, 1996, and "An Exposure-Response Analysis of Respiratory Disease risk Associated with Occupational Exposure to Chrysotile Asbestos."

1) For lung cancer:

A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

The epidemiologic literature does not provide compelling evidence that the carcinogenic potency differs by fiber type for lung cancer. This was the conclusion of a review that I authored about 5 years ago [Stayner et al. 1997], and I have not seen anything in this document or elsewhere that has changed my position. In fact, the epidemiologic literature provides in many cases evidence that chrysotile is just as potent as amphibole for inducing lung cancer. The studies of textile workers provide very similar estimates of potency (K_L), despite the fact that some of these studies involved pure chrysotile exposures [Dement et al. 1994], and others had mixed exposures [Peto 1985, and McDonald et al. 1983b]. In a study of cement workers, Hughes et al. [1987] observed an exposure-response for lung cancer that was nearly identical for workers exposed to chrysotile or to mixed fibers [In fact, $K_L = 0.4$ in both plants, see Table 6-16 of this report]. The "meta-analysis of K_L s presented in this report does not provide support for the hypothesis that chrysotile has lower potency for lung cancer than the amphiboles. In Table 6-21 the test for the hypothesis that the ratio of potencies (RPC) differs by fiber type was rejected ($p=0.42$ or $p=0.14$ depending on whether K was adjusted or not). Although this analysis resulted in a potency estimate for chrysotile that was either approximately 2 times (unadjusted K_L), or 5 times (adjusted K_L) lower than amphiboles these difference were not statistically significant, and therefore could be explained by chance.

In the end, I strongly suspect that decision of whether or not chrysotile is as potent as amphiboles for lung cancer is highly influenced by the disagreement between the Quebec miners and millers study, and the South Carolina textile study. It would be highly informative if a sensitivity analysis could be performed in which each of these studies as well as other studies were dropped from the analysis. I also suspect that differences in slopes may be more a function of industry type than fiber type, and it would interesting to see the analysis attempt to adjust for this factor.

While there are some mechanistic arguments that have been advanced to suggest that chrysotile may be less potent for lung cancer than amphiboles [e.g., Mossman et al. 1993], it is difficult to accept these arguments given that we do not presently know the mechanism and that these arguments appear to conflict with the empirical evidence from the epidemiologic literature discussed above (and toxicologic literature discussed below).

In summary, I do not believe that the choice of using separate lung cancer risk coefficients for chrysotile and amphiboles is well justified.

B) Influence of fiber length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

There is substantial evidence that fiber length is a critical factor in the carcinogenic potency for lung cancer. Unfortunately, this evidence is from toxicologic studies and there is little available information from epidemiologic studies. This is because the epidemiologic studies have not generally included characterizations of the fiber size distributions. There is some indirect evidence from epidemiologic studies. For example, Dement and Wallingford [1990] reported that the percentage of fibers greater than 10 μm was higher in the South Carolina textile facility than what had previously been reported in the Quebec mines and mills, or in asbestos cement manufacturing facilities. Thus differences in the fiber size distributions is a possible explanation for why a higher carcinogenic potency for lung cancer was observed in the South Carolina textile facility than in the Quebec mines and mills, and the cement manufacturing facilities.

Although there is limited epidemiologic evidence to support the need for an exposure index that gives greater weight to fibers longer than 10 μm , there is inadequate epidemiologic evidence to support the choice of the specific cutoff and exposure index that is proposed in this report [Equation 7.13].

There is also mechanistic data to support the increased carcinogenicity of long fibers. Davis and others have demonstrated that short-fiber preparations are cleared more rapidly from rat lungs than long-fiber preparations. As described in this report, a mechanistic hypothesis has been advanced that relates this difference in clearance to the inability of an alveolar macrophage to engulf fibers that are longer than the diameter of the macrophage. However, this mechanistic argument may imply a different choice of cutoffs for the exposure index than what is proposed in this report. The report lists 13.1 μm as the average diameter for a rat alveolar macrophage (AM), versus 21.2 μm for a human AM (p. 4.20). Therefore, based on this one might expect that model should use a fiber size cut-point in the model that would be around 13 μm for the rat model, and around 21 μm for the human model. Instead, the "optimum" rat model in the paper by Bernstein et al. 1995] uses 40 μm as a fiber size cut-point, and the "ad hoc" human model uses 10 μm as a fiber size cut-point.

Berman and Crump also cite studies suggesting that long fibers interfere with cellular division in a way that short fibers do not. There is evidence presented in some of these studies that specifically it is fibers longer than 15 μm that interfere with mitosis [Jensen CG and Watson M, Cell Biology International 23(12): 829-840, 1999, and Jensen CG et al., Carcinogenesis 17(9): 2013-2021, 1996] specifically refer to this as an effect of long fibers - either 15-80 μm long, in the 1999 paper, or 15-55 μm long, in the 1996 paper. Thus based on this mechanistic information one might suggest a cutoff of about 15 μm .

C] To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with fiber type and fiber length ?

There is little if any evidence from animal studies that carcinogenic potency for lung cancer by fiber type. The statistical analyses of the toxicologic data by Berman et al. [1995] failed to demonstrate any significant difference in carcinogenic potency for lung cancer by fiber type. It is suggested in this report [page 7-151] and elsewhere that this may be a reflection of the fact that animals have a much shorter lifespan than humans, and that given the long half-life of amphiboles the difference in potency might only be observable in humans. However, this arguments seems to conflict with the fact that the analysis by Berman et al. [1995] was able to detect a difference in potency by fiber type for mesothelioma.

There is extensive evidence from toxicologic studies that fiber size is an important determinant of carcinogenic potency. The inhalation studies of Davis and co-workers [Davis et al. 1986; Davis and Jones, 1988] clearly demonstrated the effect of fiber size on potency. The total exposure concentration was held constant in these studies, and the preparations that contained a high proportion of long fibers were markedly more potent than the same fiber type with a short fiber length. The long amosite (with fibers up to 100 μm long) produced 11 lung tumors and 3 mesotheliomas in 40 rats; the short-fiber preparation (with fibers only up to 10 μm) produced no lung tumors and 1 mesothelioma in 43 rats. The long and short chrysotile preparations were not as different in lengths as the amosite, but Davis and Jones stated that the long-fiber preparation had over 80 times as many fibers $> 30 \mu\text{m}$ than the short-fiber preparation. In this case the long-fiber preparation produced approximately three times as many pulmonary tumors as the short-fiber preparation. Similar differences in pathogenicity were seen for mesotheliomas, as well, in intraperitoneal injection studies comparing long and short fibers. There are other studies in the tox literature that also suggest greater pathogenicity for long fibers, but the Davis et al. studies are sufficient to demonstrate the magnitude of the response differences that have been seen.

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

There is no epidemiologic data available to address this question.

Toxicologic evidence that carcinogenic potency for lung cancer is associated with fibers $> 0.15 \mu\text{m}$ in diameter was reviewed by Lippmann, Environ Res 46: 86-106, 1988. In addition, the analysis of Berman et al., Risk Anal 15: 181-195, 1995 suggests that fibers as large as $5 \mu\text{m}$ in diameter may be carcinogenic in the rat. In contrast, mesothelioma has primarily been associated with very thin fibers; Stanton et al., J. Nat'l Cancer Inst. 67: 965-975, 1981; reviewed by Lippmann, Environ Res 46: 86-106, 1988.

2) For mesothelioma:

A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

I believe that the hypothesis that the risk of mesothelioma varies by fiber type is pretty well established. The epidemiologic literature clearly demonstrates much higher incidence of mesothelioma among workers exposed to amphiboles than to chrysotile. For example the percentage of deaths in South African miners exposed to crocidolite is approximately 4.7% [Sluis-Cremer, 1992], and 2.4% among vermiculite miners [McDonald et al. 1986]; whereas, the percentage of deaths from mesothelioma among Quebec chrysotile miners and millers was only 0.4% [McDonald et al. 1993] and only 0.2% among South Carolina textile workers exposed to chrysotile [Dement et al. 1983]. In contrast to lung cancer, the meta-analysis performed in the report of the K_m s for mesothelioma indicated that the ratio of potencies for chrysotile and amphiboles was highly statistically different ($p < 0.001$) than 1 (See Table 6-21).

Although there are clear differences in mesothelioma risk by fiber type, the actual values of the risk coefficients presented in Table 6-29 have limited support from the epidemiologic literature.

Exposure-response information for estimating slopes (K_m s) was generally not available in these studies, and the K_m s could only be crudely estimated with assumptions about the average

exposures for most of these cohorts. True exposure-response relationships could only be determined from the studies by Liddell et al. [1997] and Dement et al. [1994], since these investigators made their data available to the authors of this report.

I am not aware of mechanistic data that indicates carcinogenic potency for mesothelioma varies by fiber type, although I must confess here that I am not totally up to date on my reading of this literature.

B] Influence of fiber length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

There is virtually no data available in the epidemiologic literature to evaluate how the carcinogenic potency for mesothelioma varies by fiber length. It is interesting to note, however, that the K_{ms} (0.013 for Asbestos, and 0.021 for Thedford) derived in this report from the analysis of raw data from the Quebec study of miners and millers [Liddell et al. 1997], was nearly an order of magnitude lower than the K_m (0.11) derived from analysis of the raw data from the South Carolina textile cohort. This may be consistent with the argument that the exposures in South Carolina had a larger percentage of long fibers, than in the Quebec mines and mills [Dement and Wallingford 1990], and thus with the hypothesis that longer fibers have higher carcinogenic potency for mesothelioma. On the other hand, the fact that the K_m for the cement plant study by Hughes et al. [1987] was higher than the South Carolina textile plant would seem to contradict this hypothesis, since Dement and Wallingford [1990] also suggested that the exposures at the South Carolina textile facility had a higher percentage of long fibers than the exposures at the cement factory.

There is no epidemiologic evidence to support the choice of the specific exposure index that is proposed in this report [Equation 7.13].

C] To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

The toxicological evidence to suggest that the carcinogenic potency for mesothelioma varies by fiber type is limited. There is considerable evidence from the toxicologic studies using fiber implantation that fiber length is an important determinant of carcinogenic potency for mesothelioma, but there is very limited data from inhalation studies. Part of the problem with answering this question is that very few mesotheliomas are produced in rodent studies where the route of exposure is via inhalation. The inhalation studies by Wagner et al. [1974] and the studies by Davis et al. produced small numbers of tumors, and overall provide little evidence [see review by Stayner et al. 1996]. The analysis by Berman et al. [1995] does suggest that chrysotile is approximately 3 times less potent than amphiboles. However, there are too few cases (n=13) to perform a direct evaluation of this question and it was evaluated by testing whether a direct constant of proportionality could be applied to the probability of lung cancer and mesothelioma. It was found that this proportionality constant was weakly significantly ($p=0.032$) for chrysotile and amphiboles. It should be noted that this analysis does not take into account differences in the fiber size distributions, and only indirectly the exposure level.

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) other than fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

There is no data from epidemiologic studies to evaluate these questions.

3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

The potential for exposure misclassification in many of the epidemiologic studies is extremely large, and possibly introduces far more uncertainty than the adjustments used by the authors of this report. Exposure intensity and even duration of exposure had to be estimated for many of the studies included in this analysis. For example, the study by Selikoff and Seidman of U.S. insulators did not include information on duration of exposure, and the US EPA (and this report) simply assumed that all workers in this study were exposed for 25 years, and to 15 f/ml in order to calculate a K_f .

There are also large uncertainties in the exposure estimates for studies that included analyses by cumulative or average asbestos exposure. One of the key issues is the conversion of measurements from impingers (mpcf) to the more modern methods based on PCM or TEM.

This may in fact be an explanation for the differences in potency between the South Carolina chrysotile textile cohort, and the Quebec chrysotile miners and millers study, which is a critical issue in this risk assessment. The South Carolina textile worker study included extensive side by side measurements for the conversion. It is noted in the report (page 5.3) that the conversion factors for the Quebec study came from studies at other facilities. It is also noted in this report (page 6.43) that in the mining environment there is a large potential for interference in using the impinger method and even the PCM method from non-asbestos dust and cleavage fragments. This suggests the possibility that fiber counts may have been over estimated in the Quebec study, which may explain in part the lower carcinogenic potency observed in this facility relative to the South Carolina cohort.

Topic Area 2: The proposed exposure index.

- 4) **The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.**

The epidemiologic literature does not have any information to contribute to this question. The toxicologic literature does, and it does strongly indicate that fibers shorter than 5 μm have little if any carcinogenic risk. For example, the multivariate analyses by Berman et al. (1995) indicated zero potency for fibers shorter than 5 μm . However, I think we need to be somewhat cautious here about overinterpreting these findings. It is still possible that short fibers (<5 μm) have a very low carcinogenic potency, and that the toxicologic studies did not have adequate statistical power to detect the level of risk associated with exposures to these fibers. One might expect short fibers to have similar carcinogenic potency as has been observed for other particles such as titanium dioxide or carbon black.

- 5) **The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?**

There is virtually no information in the epidemiologic literature to address this question. There is also very limited information from the toxicological literature with the exception of the paper by Berman et al. [1995], and even this paper does not clearly support the factor of 300, which appears to have been chosen somewhat arbitrarily (i.e., using an "ad hoc method"). Unfortunately, the Berman et al. paper did not present a model comparable to the proposed index. The closest model shown is the "intermediate" analysis, and in this analysis the potency of fibers that are 5 to 10 μm long is only approximately $1/4^{\text{th}}$ less than 10-20, $1/10^{\text{th}}$ less than 20-40, and 900 times less than $>40 \mu\text{m}$. Based on this model it would seem that assuming a factor of 300 in potency between fiber 5 and 10 μm , and $> 10 \mu\text{m}$ would only make sense if a very larger percentage of the fibers $> 10 \mu\text{m}$ were $> 40 \mu\text{m}$. It would be very informative if the analysis by Berman et al. could be repeated using the proposed exposure index.

One also must be concerned that this formula is based solely on the analysis of toxicological data. One might expect that humans might show a very different pattern in risk related to fiber size, given species difference in respiratory anatomy and the size of human and rat macrophages. The site of lung tumors is also different with rats developing tumors in the alveoli, and humans in the bronchus. Given these species differences, one would strongly suspect that the relationships between fiber size and carcinogenic potency could be species specific.

- 6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

At this time, it is not possible to use the exposure index to make meaningful comparisons between current environmental exposures and workplace because the workplace studies have not included the exposure measurements needed to use the index.

Topic Area 3: General questions.

- 7) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or "bundles that are components of more complex

structures"). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

I am unaware of any epidemiologic or toxicologic studies that have direct bearing on this question. It is interesting to note the concern raised in this report that studies of miners may have included counting of cleavage fragments, and that this might account for the very low lung cancer risk detected in these studies.

- 7) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

I am not convinced that the proposed methodology is even relevant for the amphiboles designated in federal regulation let alone for other fiber types.

- 8) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

I think its obvious that if we ever want to be able in the future to answer the question as to whether or not fibers < 5 μm are carcinogenic, than we will need to have studies in which these fibers are measured.

- 9) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber diameter appropriate?

In the "optimum" model in the paper by Berman et al., fibers longer than 40 μm , and > 5 μm in diameter showed a relatively high carcinogenic potency. This would suggest that 0.5 μm is not an appropriate cutoff, particularly when you have long fibers.

- 11) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

I believe what this report has done is clearly identify weaknesses in the current methodology using for measuring asbestos exposure, and for assessing risk. The choice of a 5 μ m cutoff with a 3 to 1 aspect ratio was clearly arbitrary, and not based on biologic principles as much as on the available sampling methodology. However, I am afraid the proposed methodology is also quite arbitrary, and lacks a solid scientific basis. I am most concerned that lower index for chrysotile and lung cancer risk is not supported by the analysis of the epidemiologic data. I am also concerned that while the proposed exposure metric may have more plausibility than the current one, that it still needs further evaluation before being adopted. In particular, I think that there is a real need to reanalyze some of the key epidemiologic studies using this index and other possible indices based on TEM analysis to determine what an appropriate index is.

- 12) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

Obviously the first option (using the dose-response model and a life table) is the most accurate, but the second (estimating risk from a life table) should be reasonably accurate under most circumstances (i.e., excess risks less than 1 per 100). As far as ease of use, of course the 3rd option is the easiest although the least reliable. All three methods may be useful for different audiences. EPA officials would probably want in most cases to use the first option since it is the most accurate. On the other hand, the unit risk option might be most appropriate for the lay public and this is what EPA might want to continue to use in its IRIS database.

Other Comments:

This document is in serious need of editing, and in many ways is a very rough draft. I have the following editorial and other minor comments to offer:

- 1) Page 4.21 – It seems that a bullet should be added stated that there are important species differences in the morphology of the lung and pleura, and these differences may have important implications for risk assessment.
- 2) Page 5.7, next to last paragraph – There is a missing reference (REF).

- 3) Page 6.17, last paragraph – it is stated that the animal data suggests that tumorigenicity is a function of in-vivo durability. However, this statement seems to be inconsistent with the fact that the animal studies have generally failed to demonstrate a difference carcinogenic potency between chrysotile and the amphiboles. The section 7.2.4 that is referred to is merely a discussion of differences in dissolution rates and does not provide any evidence to support this statement.
- 4) Chesson et al 1989, which is a critical reference cited several times in this document (e.g. page 6.46), is listed in the references as "Submitted for Publication 1989"?
- 5) In the meta-analyses of K_L and K_m I hope that they have not included both the McDonald and Dement analyses of the South Carolina cohort. This would obviously be a mistake.
- 6) Page 6.61 -The improvement in the range of K_L from when adjustments were made is not very impressive and seems to be due to improvements solely in the agreement between the South Carolina textile and Quebec miners studies. I am not sure that this can be interpreted as providing justification for the new index as the report does.
- 7) Page 6.65, formula 6-12 – the symbols for this formula need to be defined.
- 8) Page 6.69, last paragraph – It should be noted that the differences in mesothelioma risk was not statistically significant, which is clear in the presentation in the appendix.
- 9) Page 6.70 – It should be noted that the model does not fit locations 2 or 3&4 very well.
- 10) Page 6.90 – For the conservative risk coefficients why not use the largest value for chrysotile rather than only using the largest values for crocidolite and then scaling chrysotile?

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B-137

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EPA asbestos document (Berman and Krump), comments by Kyle Steenland, 2/10/03

Summary of approach

Exposure-response estimates based on a linear relative risk model for lung cancer, and an absolute risk model for mesothelioma, were re-calculated on summary data from the 15-20 epidemiologic studies in the literature with exposure-response data. For lung cancer, these analyses were based on SMRs comparing exposed populations to large non-exposed populations with background rates, and in the re-calculation an additional parameter was estimated as a multiplier of background rates. Using the results of these analyses, a meta-analysis of exposure-response coefficients was then conducted, for both lung cancer and mesothelioma. A correction was applied to exposure-response coefficients for lung cancer and mesothelioma based on fiber size (more weight on long thin fibers) and on fiber type (more weight on amphiboles vs chrysotile). The fiber size correction was taken a priori from animal data tempered by limitations in available exposure data, while the adjustment for fiber type was estimated from the data. The fiber type adjustment results in separate exposure-response estimates for chrysotile and amphiboles, a major difference from the current EPA approach. The fiber size correction has fewer implications.

The authors have done a thorough and competent job synthesizing a large body of literature and data, and have taken inventive approaches to old questions. Nonetheless, I have some questions and disagreements as outlined below. My comments are focused on epidemiologic issues, which is my area of expertise.

General comments

I. Style of the document.

It is actually quite difficult to de-cipher the text, the heart of what was done is contained in a few pages of a very long document. This document could be simplified. For example, pages 6.1-6.30 could be put in an appendix, as their results are essentially never used by the authors. I have put my comments on page 6.1-6.30 at the end of this document. Similarly, the animal data in section 7 seems to be summarized at the end on pages 7.148 through 7.158, and it is not clear to me that the preceding pages could not be reduced in size.

II. Meta-analysis of exposure response coefficients.

The meta-analysis differed from customary meta-analyses (DerSimonian and Laird, 1986) in which a random effects model is used (in the presence of heterogeneity) and an inverse-variance weighted average of study-specific exposure-coefficients is calculated (with the variance reflected in the confidence interval of each study-specific estimate), along with the addition of a variance component for between study heterogeneity. Here, in contrast, the between-study

variance component was estimated via a likelihood approach, a parameter for weighting fiber-type was also estimated via likelihood, and the individual study variance (confidence interval) was inflated in a way described in the Appendix.

This approach is not unreasonable, and is of importance in the estimation of the fiber-type weighting parameter from the data. One thing not clear, however, is the inflating of confidence intervals for each study as indicated in the Appendix. First, it should be made clear in the text that these inflated confidence intervals are used uniformly throughout the text, which I believe is the case – it would be better to consistently give them a different name altogether (eg, 'reasonable range' which is used sometimes). More importantly, Appendix A is not clear on how these inflated confidence are in fact calculated. They seem rather arbitrary. There appears to be an assumed value of 1.0 for up to 4 factors. This value would not appear to make sense as the formula in Appendix A has the logarithm of these factors, which would then be 0. I must be missing something here.

A different and more traditional approach, perhaps more transparent, would be estimate the weighting factor for fiber type from the data and then use it in a traditional meta-analysis, in which the traditional study-specific variances (CIs) are used, and an additional variance component (which would cover all the factors in Appendix A) was taken as the between study variance.

III. Why SMRs in the lung cancer re-analysis?

For lung cancer, why were internal analyses not considered, rather than SMRs? Use of SMRs leads to correction of background rates (estimation of 'alpha') for background rates, which in turn changes the estimated exposure-response coefficients. The raw data for the meta-analysis given in the Appendix uses a Poisson-SMR model in which background rates are incorporated. It is not clear why a Poisson model could not be used in internal analyses without recourse to national rates, which would get rid of the need to estimate the extra parameter alpha (background correction).

IV. Why these models (the usual EPA models for lung cancer and mesothelioma)?

Given that the authors have re-calculated exposure-response data for each study, why were models restricted largely to the usual EPA models? Why not the more common statistical models, such as the usual log-linear relative risk model for lung cancer?

For mesothelioma, the usual EPA model was originally based on animal data and mechanistic considerations. It is not a model used for any other disease, to my knowledge. The model, which is based on absolute risk rather than relative risk, is based on an average intensity

and time since first exposure, with no consideration of cumulative exposure. Cumulative exposure is the metric of interest in most occupational cancer studies. For example, it is the metric of interest used here for lung cancer (and lung cancer models do not include time since first exposure). This discrepancy between these two models could be mentioned.

If there is a reason to accept the EPA models a priori for historical reasons this should be stated. Of course, one reason is to simplify the task.

V. Throw out the outliers?

Another suggestion would be to throw out outliers, such as any plant where there is no exposure-response for lung cancer (this contradicts the great bulk of the evidence) and perhaps the Ontario plant for mesothelioma, where mesothelioma deaths were almost as numerous as lung cancer deaths.

Comments on adjustment for fiber size and fiber type.

The charge for peer reviewers primarily relate to these two questions.

The authors claim that 'by adjusting for fiber type and fiber size, the existing data base of studies can be reconciled adequately to reasonably support risk assessment'. The basis of this statement is not clear. Adjusting for fiber type and fiber size reduces somewhat the heterogeneity of the data; nonetheless, the heterogeneity remaining within the categories of amphibole and chrysotile studies remains very large, and any decision to accept a common risk coefficient across such heterogenous data is more a policy than a statistical decision (the authors do not do any tests for heterogeneity, referring rather to non-overlapping confidence limits, but tests are somewhat superfluous in the face of large and apparent heterogeneity.)

Adjustment for fiber type. Mesothelioma. It would appear that there is reasonable evidence that adjustment for fiber type is worthwhile for mesothelioma, in that the amphibole cohorts appear to have considerably higher risks than the chrysotile cohorts. However, there is great heterogeneity within the amphibole cohorts, making prediction within them quite difficult (the controversy over Whitenoom exposure estimates further increases uncertainty here, see below under 'minor points'). Furthermore, the chrysotile cohorts are also quite different, ie, between Quebec and S. Carolina/N. Orleans. The Carolina and New Orleans cohorts show high risk, and approach the lower bounds of some of the amphibole cohorts. Nonetheless, given the general increase in risk for all the amphibole cohorts vs the chrysotile cohorts, some adjustment for fiber type appears justified.

Animal data also apparently tends to support an increased risk of mesothelioma for amphiboles.

Adjustment for fiber type. Lung cancer. The evidence for adjustment for fiber type for lung cancer is more problematic, in that it relies almost exclusively on the low risk among Quebec chrysotile miners, or conversely on the high risk for Carolina textile workers and New Orleans cement workers exposed to chrysotile. The discrepancy of results these results for these 3 cohorts is unresolved, and yet upon it rests the conclusion that the lung cancer risk is substantially different between amphiboles and chrysotile. The evidence is weak, in my view, to make an adjustment for fiber type for lung cancer risk.

The animal data do not clearly indicate that lung cancer risk is higher for amphiboles vs chrysotile, further weakening the case for adjustment for fiber type for lung cancer. Theories about the short life of rats and the over-whelming of clearance mechanisms have been proposed to explain the lack of difference in rats by fiber type, but these explanations do not appear to have explained away the issue.

Adjustment for fiber size. The adjustment for fiber size on page 6.50 (a re-weighting of traditional exposure measures used in the current standards, which are based on fibers longer than 5 μm and with a 3:1 aspect ratio and greater than 0.25 μm diameter) is taken from the animal data in which long thin fibers ($>40 \mu\text{m}$) appear to be more carcinogenic, plus an ad hoc recognition that the epidemiologic studies permit exposure measures only based on a dichotomy of greater or less than 10 μm in length (so that a re-weighting using 40 μm cannot be done).

The data supporting the carcinogenicity of long and thin fibers appear reasonably uncontroversial. The application of this adjustment in fact does not change much the observed heterogeneity in the data. Another possible approach to adjusting for fiber size is to pick the weighting (currently 0.3% for $<10 \mu\text{m}$, 97.7% for $>10 \mu\text{m}$) such that heterogeneity in the data is maximally reduced. In practice so little weight is given to fibers $<10 \mu\text{m}$ that their weighting could simply be 0.

More minor points

a) Specific comments on pages 6.1-6.30.

While they are interesting exercises, it is not clear to me why the analyses of raw data for two specific cohorts was done, given the limited inferences which can be drawn about the universe of asbestos studies (a pooled analysis of all existing data, as opposed to a met-analysis, would be ideal but very time-consuming and possibly impractical). Tentative conclusions about the current EPA model are drawn based on these two studies, based on sparse data, which do not strike me as valid. In particular inferences about the pattern of rate ratios after employment termination strike me as unwarranted, as they are based on very sparse data. Time-related patterns of RRs, eg, stratified by time since termination, are affected by other variables involving the healthy worker survivor effect, independent of cumulative dose. Estimation of models using various assumptions about internal dose vs external dose are focused on patterns for time-since-termination. It might be more interesting to first evaluate dose-response by cumulative dose using estimates of internal dose vs external dose. But in any case, if only two cohorts can be studied using raw data, it does not seem worth the effort, as no generalizations can be extrapolated to other studies.

Similarly, it was not clear to me why the multi-stage model was included in the analysis of two cohorts. Again, while this was an interesting exercise, it could have been predicted that this model – which has not been adopted by epidemiologist conducting occupational studies – is complex and involves estimating a large number of parameters based on biological assumptions. It is difficult to interpret these parameters

b) There is a dispute about exposure levels in Whitenoom which is not reflected in this document, whereby exposure levels may have been overestimated by 4-10 times (see Hodgson and Darnton, 2000). This becomes relevant (see below), given the large weight of this study (large numbers of cases).

c) As a general comment, it would be worthwhile if the authors were to add the observed numbers of cancers to Table 6.12. These would enable an immediate appreciation of the strength of evidence provided by each study.

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Mary Jane Teta

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CHARGE QUESTIONS

Topic Area 1: Interpretations of the epidemiology and toxicology literature.

1) For lung cancer:

A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

There is compelling evidence from epidemiologic and mechanistic studies that carcinogenic potency varies by fiber type with chrysotile being a less potent lung carcinogen than the amphiboles. The formal analysis of the cohort studies included in the Berman/Crump report illustrates this fact, even after adjustment for fiber size. There are 16 cohort studies with quantitative data upon which to base these analyses and published studies with average TEM fiber size distributions relevant to all but two of them. This is adequate information. While uncertainties remain related to some of the assumptions and adjustments made in this methodology, the end result is much greater homogeneity among the studies within each fiber type and a clear distinction in risk between chrysotile and the amphiboles.

The analysis of relative risk (RR) with time, using raw data from Wittenoom (crocidolite) and SC (primarily chrysotile), is also informative with respect to potency variability. The RR remains constant after exposure for the Wittenoom miners but diminishes with time since last exposure for the textile workers. This is consistent with the findings of Finkelstein and Dufresne (1999) that chrysotile splits both longitudinally and transversely in the human lung and that lung burdens decrease substantially with cessation of exposure. The breakdown of chrysotile and its greater solubility has an inverse relationship with tumorigenicity. Although fiber size is the stronger influence on biopersistence, both rodent and human pathology studies indicate that in vivo durability (solubility) is a predictor of clearance and it is dependent on fiber mineralogy.

While unable to distinguish the effects of fiber size and type, the large body of asbestos epidemiologic studies, not included in this report because of inadequate exposure data for exposure-response modeling, is very consistent with the lesser potency of chrysotile. For example, Camus et al., (New England Journal of Medicine 1998; 338: 1565-71) reported no excess risk of lung cancer in a population of women with relatively high levels of nonoccupational asbestos exposure from two chrysotile asbestos mining regions. However, there are numerous examples of bystander and domestic amphibole exposures [e.g., shipyard, asbestos cement] linked with increased lung cancer rates. There is no clear evidence of increased lung cancer risk among vehicle mechanics despite potential exposure to chrysotile in brake dust and little or no risk associated with manufacture of (chrysotile) friction products. Similar fiber type risk differences have even been observed within the same cohort (e.g., Ohlson et al. 1984 Mortality among asbestos-exposed workers in a railroad workshop. Scand J Work Environ Health 10: 283-291) or industrial group (chrysotile v. amosite cement cohorts). The contrast in lung cancer risk among female gas mask workers follows a similar clear pattern of lower risk among those exposed to chrysotile than crocidolite. The exception of course is the textile manufacturing studies, where fiber size and the extent of mixed exposures to amphiboles confound the ability to address variability in risk due to mineralogy.

As Berman and Crump point out, the existing EPA model provides an adequate description of lung cancer mortality for the Wittenoon cohort but may not be adequate for the SC cohort. These findings, in addition to the differences observed in the optimized coefficients upon adjustment for fiber size (Table 6-29) and the consistent mechanistic data, provide compelling evidence that the dose-response curves for chrysotile and the amphiboles are too disparate to be represented by one curve or model.

The optimized coefficients for pure fiber types with a ratio of 5.3:1 (amphiboles to chrysotile) in Table 6-29 successfully reduce study potency variability (33% from a factor of 90 (based on 18 studies) to 60 (based on 16 studies)). Since the coefficients have been

adjusted for fiber type and size, a straightforward quantitative assessment of consistency with epidemiology studies may not be feasible. Furthermore, since the most informative epidemiology studies have been used to derive these values, they cannot be used as an independent test of consistency. This might have been possible, had some studies been excluded from the derivation of the coefficients. In this case, predictions based on the coefficients could have been compared to what was observed in the study cohorts. The disadvantage, of course, would be the reduction in the number of studies and the associated increased uncertainty in the optimization procedure. Would it be possible, however, to examine the observed number of lung cancer cases in each study against predicted, using the optimized coefficients to evaluate the goodness of fit?

B) Influence of *fiber length*: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with *fiber length*. How adequate is information in the epidemiology literature for supporting dose-response analyses for different *fiber lengths*? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Epidemiology and mechanistic studies provide convincing evidence that fiber size and shape (length and diameter) are important predictors of carcinogenic risk.

Mechanistically, fiber dimension is related to respirability, deposition, degradation, clearance and translocation and, therefore, is a major determinant of cumulative dose to the lung. Attempts to relate potency to asbestos air concentrations or worse, measures of dust containing asbestos, results in extreme variability in potencies, both among different fiber types and within the same fiber type.

The detailed review of experimental data from rodents and humans in the Berman/Crump report show that: short fibers ($<10 \mu\text{m}$) are cleared much more quickly than long ($>20 \mu\text{m}$) insoluble fibers, short fibers do not induce fibrosis, long fibers produce substantial inflammation, and deposition and translocation depend predominately on fiber size, while durability depends mostly on fiber type. Even longer fibers may be cleared efficiently if

they are soluble. Studies were examined that included varying fiber lengths within the same fiber type to enable distinctions to be made, free of confounding. Both the epidemiology and mechanistic data support using a weighted exposure index that recognizes the predominant influence of fibers longer than 10um.

The analysis of the lung cancer crude potencies (not adjusted for fiber size) based on the Quebec miners and SC textile worker studies, together with a re-analysis of the lung pathology results of workers from these two locations are very informative. Higher measured concentrations in Quebec do not translate to higher lung cancer potency; in fact the epidemiology studies confirm higher lung cancer risks in the textile workers. The Berman/Crump analyses show that airborne concentration ratios between the two work settings are not predictors of lung burden (relative ratios of fiber types [chrysotile or tremolite]). More importantly, size distribution comparisons indicate that textile dust in SC may have been highly enriched with long tremolite fibers (>20 um). The published size distributions in these environments (Gibbs and Hwang, 1975, 1980) and knowledge of the raw fiber purchased by the textile plant further support these findings.

The absence of a clear increase in lung cancer in epidemiology studies of auto and brake mechanics, after adjustment for smoking, may be explained in part by the potential exposure to short fiber chrysotile, in contrast with the long fiber types in textile settings needed to facilitate weaving of the fibers.

Table 6-15 shows a clear correlation between fiber length and lung cancer potency coefficients. Wittenoom, however, seems to be an exception, with a predominance of shorter fibers and one of the higher potencies. Potencies adjusted for fiber size (by using the new exposure metric) are presented in Table 6-15. All coefficients increase by factors of 2 to 7, with the exception of Wittenoom, whose increase is less than 2, dropping it down to 9th most potent (of 20 cohorts). The Wittenoom results merit some discussion and clarification.

M. Jane Teta, Dr.PH

The Berman/Crump approach to adjusting the lung cancer potencies for fiber size (length and width) is very effective in reconciling the variability in the unadjusted estimates.

This body of epidemiological data is adequate for supporting these dose-response analyses. The approach of using relevant TEM size distributions from the published literature to implement the size adjustment is both rational and innovative. The uncertainty values assigned, however, need to be more clearly explained and justified. The range of uncertainty values should be described for each consideration and the criteria used clarified.

C) To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

As an epidemiologist, I will defer to the toxicologists on this issue.

D) Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

Fiber diameter is a more important characteristic with respect to respirability (0.02-2um) than fiber length. In addition, experimental studies clearly show that long, thin fibers have the greatest potency. Few fibers thicker than 0.7 um appear to reach the deep lung. Berman and Crump make a persuasive case that maximum diameter is more important than aspect ratio as a criteria for an exposure metric. I do not think epidemiology informs this issue.

2) For mesothelioma:

A) Influence of fiber type: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies from one fiber type to the next (e.g., chrysotile versus amphibole fibers). How adequate is the information in the epidemiology literature for supporting dose-response analyses for different fiber types? Specifically, to what extent do you think the proposed risk coefficients in Table 6-29 are supported by the epidemiology literature?

The evidence from epidemiologic and mechanistic studies that carcinogenic potency varies by fiber type with chrysotile being less potent than the amphiboles is even more dramatic for mesothelioma than for lung cancer. The formal analysis of the cohort studies included in the Berman/Crump report illustrates this fact, even after adjustment for fiber size. There are 12 cohort studies with quantitative data upon which to base these analyses and published studies with average TEM fiber size distributions relevant to all but one of them. This is adequate information. (See 1A for comments regarding the mechanistic evidence.) While uncertainties remain related to some of the assumptions and adjustments included in this methodology, the end result is much greater homogeneity among the studies within each fiber type and a clear distinction in mesothelioma risk between chrysotile and the amphiboles (about 600 times more potent).

The percentages of deaths from mesothelioma in amphibole-exposed cohorts greatly exceed what is seen among workers primarily exposed to chrysotile. Paraoccupational and bystander mesothelioma excesses examined in formal epidemiology studies have been associated with amphibole exposures. In a recent publication, Case et al. identified six mesothelioma cases among women in the Quebec chrysotile mining region. All resided in Thetford, where the mines have a higher tremolite concentration and none in Asbestos. The ubiquitous uses of crocidolite in Australia have led to the highest rates of mesothelioma in the world. The clear increased rate of lung cancer in the SC textile cohort contrasts with the few observed suspect deaths from mesothelioma. The cases of mesothelioma identified in the Quebec miners and millers study track with the crocidolite exposure at location 2 and the higher tremolite content at Thetford. In a mostly chrysotile friction products plant, the 11 cases of mesothelioma were attributable to uses of crocidolite at the plant or other employment (e.g., Berry and Newhouse, 1983). The contrast in

lung cancer risk among female gas mask workers follows a similar clear pattern of lower risk among those exposed to chrysotile than crocidolite.

In addition, the current EPA model, which applies potency derived from cohorts heavily exposed to amphiboles, has been shown to overpredict mesothelioma in a chrysotile-exposed population (Carnus et al., 2002).

The CT friction product plant studied by McDonald et al. (1984) with no reported deaths due to mesothelioma based on death certificates is worthy of comment, since it has had special treatment in this report. There were 3 deaths due to mesothelioma that were employed at this location, identified from the CT Tumor Registry as part of a case/control study (Teta et al., 1983; Letters to the editor, JOM 1986 28: 808-809). One man worked at an asbestos textile plant, 1921-32, which was the parent company to the friction products plant studied by McDonald. His hire date preceeded the start of her cohort, 1938, and involved amphibole exposure. There were two women whose cause of death on their death certificates did not list mesothelioma. One was classified as probably pleural mesothelioma and the other as a confirmed case of peritoneal mesothelioma during a pathological review. There were issues related to possible domestic exposures and possible involvement of other jobs, so it is not known whether these cases are attributable to exposure at the friction products plant. Peritoneal mesothelioma is virtually unheard of due to chrysotile exposure. The Berman/Crump report included in their analyses suspected mesothelioma cases from the SC textile cohort and cases in other studies for which there was possible involvement of other employment. This seems reasonable, given the under-reporting of cases in the past on death certificates. In this same spirit, perhaps the two women might be included in the analysis of the McDonald cohort.

The treatment, in general, of uncertain mesothelioma cases should be evaluated for consistency in computing Km values. For example, in Hughes, 1987, Kms were derived for chrysotile and amphibole cohorts separately. However, no Kms were calculated for Berry and Newhouse (1983), although the study had 0 cases exposed to chrysotile and 8-11 for crocidolite. Neither study permitted CIs to be computed directly.

The optimized coefficients for pure fiber types with a ratio of 600:1 (amphiboles to chrysotile) in Table 6-29 successfully reduce study potency variability, a remarkable reconciliation from a factor of 1000 to about 26. Since the coefficients have been adjusted for fiber size, a straightforward quantitative assessment of consistency with epidemiology studies may not be feasible. Furthermore, since the most informative epidemiology studies have been used to derive these values, they cannot be used as an independent test of consistency. This might have been possible, had some studies been excluded from the derivation of the coefficients. In this case, predictions based on the coefficients could have been compared to what was observed in the study cohorts. The disadvantage, of course, would be the reduction in the number of studies and the associated increased uncertainty in the optimization procedure. Would it be possible, however, to examine the number of observed mesothelioma cases in each study against predicted, using the optimized coefficients to evaluate the goodness of fit?

B) Influence of fiber length: Please comment on the extent to which the epidemiology literature and mechanistic studies suggest that carcinogenic potency varies with fiber length. How adequate is information in the epidemiology literature for supporting dose-response analyses for different fiber lengths? In general, is it appropriate to assess cancer risks using an exposure index (see Equation 7.13) that is weighed heavily by fibers longer than 10 micrometers (μm)? (Note: Topic area 2 includes more detailed questions on the proposed exposure index.)

Epidemiology and mechanistic studies provide convincing evidence that fiber size and shape (length and diameter) are important predictors of carcinogenic risk. Mechanistically, fiber dimension is related to respirability, deposition, degradation, clearance and translocation and, therefore, is a major determinant of cumulative dose to the lung. Attempts to relate potency to asbestos air concentrations or worse, measures of dust containing asbestos, results in extreme variability in potencies, both among different fiber types and within the same fiber type.

As with lung cancer, it is evident from Table 6-15 that the unadjusted mesothelioma potencies for the 12 cohort studies correlate with fiber size, with the exception of Wittenoom, which ranks #2 in potency but #10 in total fibers $> 10\mu\text{m}$. The most dramatic changes after adjustment are in the insulating manufacturing and insulator cohorts, whose potencies dramatically increased, due to the highest proportion of long fibers. The smallest change occurred for Wittenoom, consistent

with a distribution favoring short fibers (crocidolite). (Note: the KL values for Wittenoom and SC in Table 6-16 don't seem to agree to the values in Tables 6-1 and 6-2.)

The Berman/Crump approach to adjusting the mesothelioma potencies for fiber size (length and width) is very effective in reconciling the variability in the unadjusted estimates. This body of epidemiological data is adequate for supporting these dose-response analyses. The approach of using relevant TEM size distributions from the published literature to implement the size adjustment is both rational and innovative. The uncertainty values assigned, however, need to be more clearly explained and justified. The range of uncertainty values should be described for each consideration and the criteria used clarified. The authors might consider a section describing each decision in the process that was made to account for uncertainty, in both the use of TEM values from other studies and the F1-F4 factors related to exposure and other uncertainties.

C] To what extent do animal studies (e.g., studies by Davis and other researchers) suggest that carcinogenic potency varies with *fiber type* and *fiber length*?

As an epidemiologist, I will defer to the toxicologists on this issue.

D] Please comment on the extent to which carcinogenic potency is a function of fiber properties (e.g., diameter, aspect ratio, surface properties) *other than* fiber type and fiber length. How adequate is information in the epidemiology or toxicology literature for supporting these other properties into dose-response analyses?

I have nothing to add beyond my brief comment in 1 D.

3) To what extent are the exposure estimates documented in the asbestos epidemiology literature reliable?

Obviously, some studies have more complete and more accurate exposure estimates and the amount of error and imprecision is a function of time, with estimates of historical exposures being the most difficult to reconstruct. In the past they are more likely, however, to be worst case, with representative sampling becoming routine around the mid to late 1970s, if the chemical industry pattern in the U.S. holds. The authors used uncertainty factors (F1-F3) to account for uncertainties in exposure estimates for each of the studies: F1 for uncertain recreation

of past exposures, F2 for conversion and F3 for use of a crude estimate of average exposure. F4 was used for other types of uncertainties.

While these issues capture the key exposure factors, the choice of F values ("at least 1.5 and is at least 2 or more in most cases") is inadequately explained and appears arbitrary. Furthermore, the formula for the overall uncertainty factor (A.6), the square root of the exponent of the sum of the logs squared of the factors, needs more justification than the reasonable one that it accounts for errors in different directions. In addition, the factors are only applied to the confidence intervals (CI), as if the only influence on the coefficients would be related to random variability or precision. It is unclear how the CIs impact the final coefficients in Tables 6-29 and 6-30.

I would disagree with the use of F3 as an uncertainty factor and argue that use of an overall cohort average exposure is too uncertain and studies with this severe a limitation should not be used for exposure-response. It appears this occurs for only one study, Selikoff and Seidman, 1991. The limitation of this study is even greater because the average exposure concentration did not even come directly from the study itself. (A case study by Nicholson, 1976, is cited in the 1986 EPA Health Assessment). Furthermore, there were no data on duration of exposure, requiring another outside average to be used. This was a very important study with respect to identification of asbestos hazards, but the available information is not adequate for use in exposure-response analyses. Another study I would reconsider including in the exposure-response is Lacquet et al., 1980. The follow up is much too short and the exposure information inadequate.

Topic Area 2: The proposed exposure index.

- 4) The proposed exposure index does not include contributions from fibers shorter than 5 μm . Please comment on whether the epidemiology and toxicology literature support the conclusion that asbestos fibers shorter than 5 μm present little or no carcinogenic risk.

There is adequate evidence that fibers shorter than 5 μm present little or no risk. Experimental data confirm that clearance of fibers is dependent on fiber length and that short fibers do not produce fibrosis. Mechanistic and other experimental studies show fibers < 5 μm clear readily

and the small proportion that do not clear are sequestered in alveolar macrophages. Fibers shorter than 10-20 μm are almost completely handled by macrophages. The strongest evidence comes from Berman's re-analysis of the toxicological studies of Davis et al. using TEM based fiber length distribution data. None of the epidemiology studies have TEM data and there is no way to identify workers in the studies that are exposed only to fibers $< 5 \mu\text{m}$. Therefore, epidemiology cannot specifically address this issue. There is corroborative epidemiology, however, in studies of vehicle and brake mechanics, who have potential exposure to short fiber asbestos (chrysotile) and have not been found to be at increased risk of mesothelioma in numerous studies examining this issue.

- 5) The proposed exposure index is weighed heavily by fibers longer than 10 μm . Specifically, Equation 7.13 suggests that the carcinogenic potency of fibers longer than 10 μm is more than 300 times greater than that of fibers with lengths between 5 and 10 μm . How consistent is this difference in carcinogenic potency with the epidemiology and toxicology literature?

This difference is based on the re-analysis of the Davis et al. studies, using TEM based fiber distributions. Other experimental evidence is corroborative indicating that fiber lengths shorter than 10 to 20 μm are readily handled by macrophages. In addition, the optimum exposure index indicates little impact of exposures as high as 40 μm in length.

As indicated in #4 above, epidemiology cannot offer specific guidance on this issue.

- 6) Please explain whether the proposed exposure index will allow meaningful comparisons between current environmental exposures to asbestos and historical exposures to asbestos that occurred in the work place.

Current environmental exposures to asbestos can be fully characterized by fiber type and size using TEM. Berman and Crump have characterized the exposures of workers in the cohort studies used for exposure-response by these same characteristics. Factors have been introduced, however, to address uncertainty in the exposure data from the studies and in the derivation of fiber size distributions from these studies for purposes of potency calculations and their CIs. Therefore, if I understand the question correctly, direct comparisons of exposure would not be a meaningful

exercise. However, use of the models with the new potency estimates can be effectively employed to estimate lifetime risk associated with the particular environmental circumstance.

Topic Area 3: General questions.

- 1) The proposed risk assessment approach assigns carcinogenic potency to individual fibers and to cleavage fragments (or Bundles that are components of more complex structures@). Please comment on whether cleavage fragments of asbestos are as toxicologically significant as fibers of the same size range.

This issue is beyond my area of expertise.

- 2) Please comment on whether the proposed cancer assessment approach is relevant to all amphibole fibers or only to the five types of amphibole fibers (actinolite, amosite, anthophyllite, crocidolite, tremolite) designated in federal regulations.

I am not familiar with any other amphibole fibers and doubt that there is any epidemiology to inform the issue.

- 3) The review document recommends that asbestos samples be analyzed by transmission electron microscopy (TEM) and count only those fibers (or bundles) longer than 5 μm . Such counting practices will provide no information on the amount of asbestos fibers shorter than 5 μm . To what extent would data on shorter fibers in samples be useful for future evaluations (e.g., validation of the cancer risk assessment methodology, assessment of non-cancer endpoints)?

Since fibers shorter than 5 μm do not contribute to fibrosis, there would be no value in collecting these data for non-cancer endpoints. To validate the risk assessment methodology, more research would be needed. The only circumstance I could think of that would be of interest is where there is an exposure scenario (e.g., work environment, environmental source) where exposures are limited to $< 5 \mu\text{m}$ and there is an ability to identify increased risk, if it existed for lung cancer and mesothelioma. It would be extremely difficult, however, to design a valid study with reasonable precision. Power issues and confounding exposures would likely be insurmountable.

- 4) The proposed risk assessment methodology suggests that exposure estimates should be based only on fibers longer than 5 μm and thinner than 0.5 μm . Is this cut-off for fiber diameter appropriate?

With the exception of mouth breathing, few fibers $> 0.7 \mu\text{m}$ in diameter reach the deep lung.

And not all those that do will adhere to the lung surface. Experimental data also indicate that it is the fibers thinner than 0.7 μm and longer than a minimum of 10 μm that likely contribute to disease. Timbrell (1982) reported complete clearance of short ($< 4 \mu\text{m}$) fibers with diameter less

than 0.6um. (Berman and Crump make another argument supporting 0.5 um as the cut-off related to diffusional diameter. This discussion is outside the scope of my expertise.) Reliance on the re-analyses of the Davis et al. toxicology studies using TEM based exposure results in an adequate fit of the data with a cut-off of 0.3 um diameter for structures between 1 and 40 um in length. Adequate fit was also seen with a 0.4 um cut-off. In light of this evidence 0.5 um seems appropriate.

- 5) Discuss whether the proposed cancer assessment approach, as a whole, is a reasonable evaluation of the available health effects data. What aspects of the proposed cancer assessment approach, if any, are inconsistent with the epidemiology or toxicology literature for asbestos?

This is a very impressive piece of work that considers all the evidence and integrates it effectively. The methodological approach is supported by a solid foundation of scientific evidence and data. Uncertainties in the human data are considered and factored into the method. The experimental data is carefully scrutinized and reasonably evaluated in a balanced fashion.

The variability in the results of the epidemiology studies are well described, but no standards are provided to judge the acceptability of the studies for risk assessment.

I found the epidemiology data to be accepted at face value as evidenced by the placement of study summaries in the Appendix. The information from these studies is fundamental to the methodology. Justification for the uncertainty factors needs to be clearer (see response to question #3). What criteria were used for acceptability of the studies for inclusion (see prior discussion of Selikoff and Seidman and Lacquet et al.)? I support the use of human data and the methodology proposed but question whether study quality and the suitability of each study for exposure-response was measured against any standard. I am not convinced that introducing the uncertainty factor, F4, for example, solves all the limitations unrelated to exposure, such as a sizeable proportion of subjects lost to follow up. Would it be more appropriate to exclude the study?

I see no substantive inconsistencies with the existing epidemiology and toxicology literature. The extreme variability in estimates of risk from the epidemiology studies has troubled scientists for

some time. It is well accepted in the scientific community that asbestos fiber size and type are key determinants of risk. More specifically, it is well recognized that the long, thin fibers are the most potent and that amphiboles have greater potency than chrysotile. This scientific understanding and a practical approach to applying it has been captured in the Berman/Crump proposed methodology. The result is a vast improvement in reconciling the differences in the epidemiology studies.

This is an innovative piece of work that vastly improves the risk assessment methodology for asbestos and makes excellent use of all the currently available scientific information.

- 6) Section 8.2 of the review document presents three options for assessing cancer risks from asbestos exposure. Please comment on the technical merit of the proposed risk assessment options.

I support options #1 and #2 as appropriate approaches to assessing cancer risks for asbestos exposure. They are both technically correct with option #1 being more flexible (e.g., can handle time-varying exposure), being the more general case; but option #2 being easier to implement (only need estimates of long-term exposure). Choosing between these options depends on the circumstances of the population of interest. To make a judgment about lifetime risk to an urban U.S. population with a relatively constant exposure, option #2 would be the easiest to use and would provide the same result, had option #1 been employed. If a demolition project is under consideration in which exposure concentrations might vary by task and workers would have various fixed durations of employment, then option #2 would not be suitable, but the more general case, option #1 would be.

I would not recommend option #3, some sort of combined unit risk, because it defeats the purpose of taking into account how potencies vary by fiber size and type and introduces an additional weighting procedure. While single unit risk estimates have the advantage of simplicity, the disadvantages in this case outweigh the advantage, particularly with the ease of use of the risk table.

Topic Area 4: Development of Conclusions and Recommendations

At the end of the workshop, the peer consultants will be asked to draft conclusion statements identifying their most notable findings on the proposed methodology. As a prelude to developing

these statements, the peer consultants are invited to provide any additional comments or concerns, both strengths and weaknesses, on topics not specifically addressed in the previous charge questions. After completing the discussions the peer consultants will prepare their conclusions, and they will also be asked to develop recommendations for how EPA can improve the methodology. Please note that, although recommendations for future research projects are welcomed, the focus of this workshop is on the proposed risk assessment methodology and how it may be used to support decisions at asbestos-contaminated sites.

Additional comments:

- It is noted in 6.65 that background cases of mesothelioma are rare in the general population. Is 2 per million, the estimate for the U.S. small enough to not impact the model?
- Why are 90% CI preferred in this document?
- Discussion of two-stage model may be better placed in the Appendix, since it didn't turn out to be useful
- Very long section on factors governing cellular and tissue response (i.e., mechanism of carcinogenicity) should be substantially shortened (with more detail in Appendix), since it resulted mostly in hypotheses that were not integral to methodology.
- Clarify how CIs are incorporated into final coefficients, if in fact they are. If not, how do the uncertainty factors make a difference?
- Would incorporation of a maximum latency period into the EPA model improve its performance?
- There is little discussion of peritoneal mesothelioma -- would potency estimates be any different for this endpoint?
- P. 5.6 notes one needs incidence of meso. by "age at first exposure" to implement EPA model. Is this correct or should it be "time since first exposure"?
- P. 6.65, description of equation 6-12. "assuming that exposure remains constant" may be incorrect.
- P. 8.7 notes consistency with Stayner yet he concludes that with respect to lung cancer, epidemiology doesn't support lower potency for chrysotile?
- Is there any mechanistic data to understand why chrysotile is closer in potency to amphiboles for lung cancer but so much less potent than amphiboles for mesothelioma?

References

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United States
Environmental Protection Agency
Office of Solid Waste and Emergency Response

Workshop to Discuss a Proposed Protocol to Assess Asbestos-Related Risk

Westin St. Francis
San Francisco, CA
February 25-27, 2003

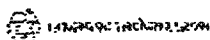
Agenda

Workshop Co-Chairs: *Roger McClellan, Toxicology & Human Health Risk Analysis*
Leslie Stayner, National Institute for Occupational Safety and Health

Facilitator: *Jan Connery, Eastern Research Group, Inc.*

TUESDAY, FEBRUARY 25, 2003

- 8:00AM Registration/Check-In
- 8:30AM Welcome and Announcements..... Jan Connery
- 8:35AM Opening Remarks Richard Troast
U.S. Environmental Protection Agency (U.S. EPA)
Office of Solid Waste and Emergency Response (OSWER)
Office of Emergency and Remedial Response (OERR)
- 8:45AM Peer Consultant Introductions and
Conflict-of-Interest Disclosure..... Facilitated by Jan Connery
- 9:00AM Goals, Purpose, and Ground Rules Jan Connery
- 9:10AM Background on the Proposed Protocol to
Assess Asbestos-Related Risk Wayne Berman,
Aeolus, Inc.
and Kenny Crump,
ENVIRON
- 10:10AM BREAK
- 10:30AM Charge to the Peer Consultants



TUESDAY, FEBRUARY 25, 2003 (continued)

10:40AM Topic Area 1: Interpretations of the Epidemiology and Toxicology Literature
Discussions facilitated by Roger McClellan (Animal Toxicology and Mechanistic studies)
and Leslie Stayner (Epidemiology)

LUNG CANCER

- (1) Fiber Type**
 - a. Epidemiology**
 - (b) Animal Toxicology and Mechanistic Studies**

12:00 Noon LUNCH (on own)

1:00PM Observer Comment Period Facilitated by Jan Connery

2:00PM Topic Area 1: Interpretations of the Epidemiology and Toxicology Literature

LUNG CANCER (continued)

- II. Fiber Dimensions and Surface Properties (length and other considerations)**
 - a. Epidemiology**
 - b. Animal Toxicology and Mechanistic Studies**

3:30PM BREAK

3:45PM Topic Area 1: Interpretations of the Epidemiology and Toxicology Literature

MESOTHELIOMA

- II. Fiber Type**
 - a. Epidemiology**
 - b. Animal Toxicology and Mechanistic Studies**

- II. Fiber Dimensions and Surface Properties (length and other considerations)**
 - a. Epidemiology**
 - b. Animal Toxicology and Mechanistic Studies**

5:30PM ADJOURN

WEDNESDAY, FEBRUARY 26, 2003

8:00AM Observer Comment Period

9:00AM Review of Day One Discussions and Day Two Charge to Peer Consultants

WEDNESDAY, FEBRUARY 26, 2003 (continued)

9:10AM **Topic Area 1: Interpretations of the Epidemiology and Toxicology Literature**
EXPOSURE ESTIMATES *Facilitated by Leslie Stayner*

9:40AM **Topic Area 2: The Proposed Exposure Index**
Question #4 *Facilitated by Roger McClellan*

10:10AM **BREAK**

10:30AM **Topic Area 2: The Proposed Exposure Index**
Question #5 *Facilitated by Leslie Stayner*

11:15AM **Topic Area 2: The Proposed Exposure Index**
Question #6 *Facilitated by Roger McClellan*

12:00Noon **LUNCH**

1:00PM **Topic Area 3: General Questions**
Question 7 *Roger McClellan*
Question 8 *Leslie Stayner*
Questions 9 *Roger McClellan*
Question 10 *Leslie Stayner*
Question 12 *Leslie Stayner*
Questions 11 *Roger McClellan*

3:15PM **BREAK**

3:30PM **Discussion of Other Key Issues** *Facilitated by
Roger McClellan and Leslie Stayner*

4:50PM **Wrap Up of Day Two Discussions and Review of Goals for Thursday**

5:00PM **ADJOURN**

THURSDAY, FEBRUARY 27, 2003

8:00AM **Topic Area 4: Development of Conclusions and Recommendations**

10:15AM **BREAK**

10:30AM **Topic Area 4: Development of Conclusions and Recommendations**

11:35AM **Closing Remarks**

11:45AM **ADJOURN**