The So-called Short-Fiber Controversy

Literature Review and Critical Analysis

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• Context.—Numerous articles in the scientific literature indicate that pathogenic fibers with respect to asbestos-related diseases are those that exceed 5 μm in length. Nonetheless, some authors have expressed concerns regarding pathogenicity of shorter fibers.

Objective.—To review the scientific evidence regarding pathogenicity (or lack thereof) of fibers less than or equal to 5 μm in length, with particular attention to publications indicating that such fibers might be hazardous.

Data Sources.—The scientific literature was reviewed for experimental animal studies and human studies that address the role of fiber size in causation of disease. Sources included original studies, as well as review articles related to the topic.

Conclusions.—Experimental animal studies involving inhalation of fibers have demonstrated that fibers greater than 5 μm in length are associated with both pulmonary fibrosis (ie, asbestosis) and malignancies (carcinoma of the lung and mesothelioma). There is no convincing evidence for a pathogenic effect for fibers that are 5 μm or less in length. Fiber analyses of human lung tissue samples provide further support for pathogenicity of long fibers, particularly the more biopersistent amphibole fibers. Similar observations have been reported for nonasbestos mineral fibers. Concerns expressed by some authors (eg, the greater abundance of short fibers) do not alter these conclusions. Similarly, in vitro studies demonstrating biological activity of short fibers do not override inhalational studies of whole animals or the epidemiological findings in humans.


Early on during studies of experimental animals exposed by inhalation to asbestos fibers, it was recognized that fiber length was a determining factor in pathogenicity. Vorwald et al1 concluded that fibrogenicity of chrysotile asbestos was related to fibers 20 μm or greater in length. The authors also observed that the latency interval for asbestosis was inversely related to the concentration of fibers inhaled by the animals. Subsequent investigations by Wagner et al2 demonstrated that all fiber types caused asbestosis in rats in a dose-dependent fashion and that the asbestos-related diseases are those that exceed 5 μm in length. Nonetheless, some authors have expressed concerns regarding pathogenicity of shorter fibers.

Mechanisms believed to be important in the fibrogenic effect of long fibers (ie, those >5 μm in length) include cytotoxicity, inflammatory cell recruitment, and tumor necrosis factor production. Long fibers, particularly the amphiboles, tend to accumulate in the lung and are sequestered for a long period of time.

EXPERIMENTAL ANIMAL STUDIES

Studies7–11 from the 1970s and 1980s showed that fiber length was an important determinant of fibrogenesis in inhalational models (Table 1). Lemaire et al7 reported that rats injected intratracheally with 5 mg of ultrashort chrysotile asbestos fibers, all of which were less than 8 μm in length, developed an alveolitis but no apparent fibrosis. In an inhalation model of rats exposed to chrysotile asbestos, Platek et al8 reported that a lung concentration of 23 million long fibers (>5 μm in length), 272 million short fibers (<5 μm in length), or a combination of the 2 was not sufficient to produce fibrosis 18 to 24 months after exposure. Similar results were reported with crocidolite asbestos fibers.7,8 Roggli et al14,15 showed that longer fibers are retained in the lung, whereas shorter fibers tend to be cleared. Furthermore, chrysotile demonstrated a progressive decrease in fiber diameter due to longitudinal fiber splitting, whereas crocidolite did not. Long amosite fibers have increased free radical and oxidizing potential and greater release of nitric oxide from lung epithelial cells compared with short fibers.16

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HUMAN STUDIES

McDonald et al. performed lung fiber analyses on 78 cases of mesothelioma and found that the risk of disease correlated with amphibole fibers (amosite, crocidolite, or tremolite) that were 8.0 μm or greater in length, with no additional information provided by fibers that were shorter than 8.0 μm (Table 2). Rödelspenger and colleagues similarly reported that the concentration of amphibole

Table 1. Role of Fiber Dimensions in Experimental Animal Models

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Study Design</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asbestos</td>
<td></td>
<td></td>
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<tr>
<td>Vorwald et al.,1 1951</td>
<td>Chronic inhalation of chrysotile in various species</td>
<td>Fibrosis primarily related to long fibers</td>
</tr>
<tr>
<td>Davis et al.,1 1986</td>
<td>Chronic inhalation of long and short amosite fibers</td>
<td>Fibrosis and tumors only from long fibers</td>
</tr>
<tr>
<td>Davis and Jones,4 1988</td>
<td>Chronic inhalation of long and short chrysotile fibers</td>
<td>Considerably more fibrosis and tumors from long fibers</td>
</tr>
<tr>
<td>Platek et al.,13 1985</td>
<td>Chronic inhalation of short chrysotile in rats and monkeys</td>
<td>No observed fibrosis</td>
</tr>
<tr>
<td>Adamson and Bowden,7 1987</td>
<td>Mice instilled intratracheally with short crocidolite fibers</td>
<td>Minimal lung injury or fibrosis</td>
</tr>
<tr>
<td>Adamson and Bowden,8 1987</td>
<td>Mice instilled intratracheally with long crocidolite fibers</td>
<td>Severe fibrotic reaction resembling human asbestosis</td>
</tr>
<tr>
<td>Wright and Kuschner,9 1975</td>
<td>Guinea pigs instilled intratracheally with crocidolite fibers</td>
<td>Marked fibrosis with long fibers, macrophage reaction only with short fibers</td>
</tr>
<tr>
<td>Pott et al.,26 1987</td>
<td>Rats injected intraperitoneally with several types of asbestos</td>
<td>Length and durability of great significance for tumor potency</td>
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<tr>
<td>Other fibers</td>
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<tr>
<td>Wright and Kuschner,9 1975</td>
<td>Guinea pigs instilled intratracheally with synthetic fluoroamphibole and fibrous glass</td>
<td>Marked fibrosis with long fibers, macrophage reaction only with short fibers</td>
</tr>
<tr>
<td>Lee et al.,11 1981</td>
<td>Chronic inhalation of fibrous glass and titinate fibers in various species</td>
<td>Fibrosis and some tumors with fibers &gt;5 μm in length</td>
</tr>
<tr>
<td>Pott et al.,26 1987</td>
<td>Rats injected intraperitoneally with several fiber types</td>
<td>Length and durability of great significance for tumor potency</td>
</tr>
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Table 2. Role of Fiber Type and Dimensions in Human Studies

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Study Design</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>McDonald et al.,34 1989</td>
<td>Case-control study of mesothelioma patients</td>
<td>Mesothelioma risk associated with amphibole fibers ≥8.0 μm in length</td>
</tr>
<tr>
<td>Rödelspenger et al.,35 1999</td>
<td>Case-control study of mesothelioma patients</td>
<td>Mesothelioma risk associated with amphibole fibers ≥5.0 μm in length, with dose response</td>
</tr>
<tr>
<td>Rogers et al.,36 1991</td>
<td>Case-control study of mesothelioma patients</td>
<td>Strongest correlation with crocidolite fibers ≥10 μm in length</td>
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process include production of reactive oxygen and nitrogen species, cell signaling with cytokine and growth factor production, and matrix remodeling through matrix metalloproteinases and their inhibitors. Similarly, experimental studies have shown that long fibers correlate with the development of neoplasia. Davis et al. reported no cases of neoplasia among rats after long-term inhalation of an amosite preparation with extremely long fibers other than asbestos have also demonstrated a strong association between fiber length and carcinogenicity. In a series of pleural implantation studies, Stanton et al. showed that mesothelioma induction most closely correlated with the number of fibers that exceeded 8 μm in length and that were less than 0.25 μm in diameter. Using a model of intraperitoneal injection in rats, Pott et al. similarly concluded that the length and durability of fibers are of great significance for their carcinogenic potency. In vitro studies demonstrated a correlation between fiber length and transforming potency, enhanced incorporation of tritiated thymidine, biosynthesis of polyamines, and squamous metaplasia. Proposed mechanisms of carcinogenesis include cellular injury with DNA damage, leading to inactivation of tumor suppressor genes, activation of oncogenes, or mutagenesis. Reactive oxygen species generated directly by asbestos fibers or by inflammatory cells may also have a role. External environmental factors such as carcinogens in cigarette smoke or radiation may have a supplemental role in some malignancies.

Experimental animal investigations demonstrated that long crocidolite fibers were trapped on the peritoneal surface in the area of the diaphragmatic stomata, resulting in an intense inflammatory reaction. These stomata, which also exist in the parietal pleura, may be the site of accumulation of long amphibole fibers in the pleura. Earlier investigations in mice demonstrated that subcutaneous injection of asbestos fibers led to their concentration in milky spots in the parietal pleura. Animal investigations have also demonstrated long commercial amphibole fibers directly penetrating the visceral pleura and extending into the pleural space.
fibers exceeding 5 μm in length was associated with an increased odds ratio of mesothelioma and a clear dose-response relationship. In addition, Rogers et al.\textsuperscript{36} found that the strongest correlation of relative risk of mesothelioma was with the concentration of crocidolite fibers that were at least 10 μm in length.

In a study of black spots in the parietal pleura, Boutin et al.\textsuperscript{37} reported that these areas concentrated long amphibole fibers, which in some cases had levels exceeding those found in the lung tissue of the same individual. The fiber concentrations were at least an order of magnitude higher in the black spots compared with nearby parietal pleura. In a subsequent study, Mitchev et al.\textsuperscript{38} showed that the distribution of black spots at autopsy was primarily in the lower parietal pleura and on the diaphragm. This distribution correlates with the earliest lesions of malignant mesothelioma observed at thoracoscopy. In an analysis of pleural plaques, Dodson and colleagues\textsuperscript{39,40} noted that the only fibers found in pleural tissues that exceeded 10 μm in length were amphibole fibers.

In a meta-analysis of asbestos-related cancer risk, Berman and Crump\textsuperscript{41} concluded that there was no convincing evidence for carcinogenicity of fibers that were less than 10 μm in length. Indeed, the hypothesis that fibers less than 10 μm in length are nonpotent was not rejected by any of the fiber dimension metrics that were considered.

**CONTRARY OPINIONS**

Several authors have expressed concerns regarding the prevailing scientific opinion that short fibers (≤5 μm in length) have not been demonstrated to be pathogenic. In an analysis of pleural tissues and pleural tumors, Suzuki and colleagues\textsuperscript{42,43} reported primarily short chrysotile fibers in the pleura and claimed that these should not be ignored in terms of causation. In a review of the literature, Dodson et al.\textsuperscript{44} stated that one should not ignore short fibers present in tissues. Lemen\textsuperscript{45} opines that in vitro toxic effects of short fibers and their presence in the pleura imply that it is inappropriate to discount their role in asbestos-related diseases. Finally, in a review of chemical mechanisms of elongated mineral particle toxicities, Aust et al.\textsuperscript{46} call for a better definition of relative potencies of all sizes and shapes of respirable elongated mineral particles as a critical risk assessment need. In the following sections, I will examine each of these concerns and the relative merits of their respective arguments.

**SUZUKI PAPERS**

Suzuki and colleagues\textsuperscript{42,43} published 2 studies in which they claimed that the most abundant fiber type in the pleura was short chrysotile fibers and that the types and sizes of fibers present in the pleura differed from those in the lungs. In total, 64 cases of mesothelioma were examined in which both lung and pleural tissue from the same case were analyzed. Tissues were prepared by digestion using bleach or potassium hydroxide or using low-temperature plasma ashing of 25-μm-thick paraffin sections. It is unclear how many cases were examined by the quantitative digestion procedure versus the more unconventional and qualitative ashing of thick sections. A small number of control pleural tissues were examined.

There are numerous problems with the studies by Suzuki and colleagues. The vast majority of chrysotile fibers identified were less than 1 μm in length, which raises the issue of contamination or fragmentation during processing. Such short chrysotile fibers frequently are present in water, which means they are potentially present in formaldehyde used to fix the tissues, in bleach or potassium hydroxide solutions used to digest the tissues, or in paraffin used to embed the tissues.\textsuperscript{47,48} Even if one assumes that these short fibers actually derived from the tissue rather than these other sources, the authors seem to be arguing that these fibers must be doing something because there are so many of them. The fallacy of this argument is obvious when one considers that carbon particles are far more numerous in pleural tissues than short chrysotile fibers, so one could equally argue that carbon particulates are the cause of mesothelioma. The high levels of fibers in control tissues (up to $5 \times 10^6$ per gram of dry lung) also suggest contamination or procedural fragmentation (which would artifically increase fiber numbers).

Additional problems include improper controls and improper tissue for analysis. As indicated above, it would be useful to analyze samples of paraffin in each case to determine if short chrysotile fibers are present.\textsuperscript{48} Furthermore, it is improper to compare tumor tissue with normal pleura in terms of fiber concentration. The only proper comparison for tumors would be analysis of other pleural based tumors that encase the lung and are not asbestos related (epithelioid hemangioendothelioma of the pleura or some pseudomesotheliomatous carcinomas of the pleura). This is because blockage of parietal pleural stomata by a diffuse rind of tumor could impede the clearance of short chrysotile fibers and result in their accumulation in any type of tumor with this growth pattern. Indeed, tumor tissues are improper for analysis because they begin as a single cell and grow over time to form billions of cells that comprise the tumor rind. Whatever is present in tumor by necessity must have gotten there after the fact and cannot be considered a contributing factor to the disease. The finding of high concentrations of fibers in tumor raises the issue of contamination noted above. Therefore, because of issues regarding possible contamination, improper controls, unconventional analytical methodology, and false reasoning concerning causation, the Suzuki papers are not reliable for the proposition that short chrysotile fibers are causative of mesothelioma.

**DODSON AND LEMEN REVIEWS**

Dodson and colleagues\textsuperscript{44} reported a review of the literature regarding fiber dimensions and pathogenicity in which they argue that asbestos fibers of all lengths induce pathological responses and that it is difficult to exclude fibers of a particular dimension from a role in causing disease. The authors argue that short fibers cannot be ignored because they predominate in most samples, an argument that as shown above can equally be applied to nonfibrous particles in the lung and thus has no merit in terms of asbestos causation. The authors rely upon injection and instillation studies in the pleural or peritoneal cavities, while ignoring the more appropriate inhalational studies. For example, they quote a peritoneal injection study by Davis et al.\textsuperscript{39} but ignore inhalational investigations by Davis and Jones,\textsuperscript{4} as well as the reanalysis of the results by Davis and colleagues by Berman et al.\textsuperscript{3} Furthermore, the injection studies relied upon by Dodson and colleagues include investigations in which 3 of 25 animals injected with fibrous glass developed mesothelioma.\textsuperscript{49} Although such studies
may be of general interest, they carry little or no weight in terms of pathogenicity because there is no evidence that fibrous glass causes mesothelioma in humans. The authors also rely on the studies by Suzuki et al, which are as indicated above problematic. Finally, the authors cloud the issue of fiber length and pathogenicity by stating that other factors may also contribute to pathogenicity such as surface area, chemical composition, solubility, trace metal and organic content, and surface charge and reactivity. Such findings do not negate the observations that short fibers have no demonstrable pathogenicity in inhalational models.

Lemen reiterates that short fibers are the most numerous in the lungs and pleural samples, and therefore their role in the etiology of disease is implicated. This argument is fallacious for the reasons noted above. It should be noted that, regardless of fiber type, raw preparations of asbestos consist of 80% to 95% fibers 5 µm or less in length, so the predominance of short fibers is no great surprise. The fact is that in terms of clearance or phagocytosis the respiratory system has difficulties with long, thin fibers, so these become concentrated in the lungs and, as noted above, in certain hot spots in the parietal pleura. Lemen relies upon a 1979 article by Bignon et al noting that the percentage of short fibers in pleural samples is greater than that in lung tissue but ignores the 1994 text edited by Jaurand and Bignon in which every reference to fiber lengths indicates that it is the long biopersistent fibers that are pathogenic. Lemen also makes the mistake of relying upon in vitro and injection studies, while ignoring inhalational studies in animals whose defense mechanisms and reparative systems are intact.

It should be further noted that short chrysotile fibers are the most numerous fibers found in lung samples from the general population. If these fibers were of importance in causation of mesothelioma, then brake mechanics who are exposed to brake dust (in which the vast majority of residual fibers are <1 µm in length) should have very high rates of mesothelioma. However, numerous epidemiological investigations that have examined this question have found no evidence of an increased risk of mesothelioma among auto mechanics.

**AUST REVIEW**

Aust and colleagues reported a review of the literature regarding mechanisms of toxicity associated with elongated mineral particles. These authors make a number of references to short fibers and their potential for pathogenicity for the respiratory system. They comment that many mechanisms are applicable to both fibrous and nonfibrous particles and that potencies vary in proportion to effective surface area in terms of acute inflammatory response after intratracheal instillation. The authors provide an example with titanium dioxide. They note that grinding increases the ability of amosite and crocidolite to generate 5,5-dimethyl-1-pyrroline N-oxide adducts and to catalyze formation of 8-oxo-2′-deoxyguanosine, suggesting that this effect is due to greater surface area. They also discuss the role of iron catalysis of reactive oxygen species in tissue injury. Finally, the authors again claim that there are so many more short fibers than long fibers in the lung and in extrapulmonary sites that they cannot be ignored.

Experimental studies using inhalational models in intact animals or epidemiological studies in humans can suggest that a particular agent may be dangerous in terms of disease causation, and in vitro studies may then suggest mechanisms by which cellular injury is likely to occur. However, the reverse is neither true nor logical: in vitro studies cannot be used to indicate disease causation when experimental animal models and human epidemiology do not support such causation. Obvious reasons for this include clearance mechanisms and reparative processes that are operative in the intact animal but bypassed in in vitro studies. Just to cite one example, as noted by Aust et al, titanium dioxide causes an acute inflammatory reaction after intratracheal instillation. However, there is no evidence that titanium dioxide causes significant tissue injury in humans. Similarly, the experimental animal models and human epidemiology do not support a pathogenic role for asbestos fibers 5 µm or less in length (see the discussion above). The fallacy of short fibers being so numerous that they must be doing something was dealt with in previous sections.

Aust et al also attempt to discount the study by Boutin and colleagues, noting that black spots in the parietal pleura do not correlate with the location of parietal pleural plaques and that miners in the Ruhr region of Germany do not have asbestos fibers in the black spots in their pleura. What the authors do not state is that the black spots do correlate very well with the earliest lesions of pleural mesothelioma (see the discussion above). It is unclear why there is a discrepancy between the location of plaques (posterolateral pleura along the direction of the ribs and diaphragm) and the earliest lesions of mesothelioma (diaphragm and lower portions of parietal pleura), but it may be related to mechanisms of the lung rubbing against the pleura overlying the ribs in association with protruding asbestos fibers. The Ruhr region of Germany is a coal-mining region, and there is no evidence that coal miners have an increased risk of mesothelioma. Indeed, the study by Boutin et al involved a natural experiment in a population exposed to significant amounts of soot (from coal-fired heat) and asbestos (the authors were studying individuals with mesothelioma). In this population, the black spots in the parietal pleura are simply acting as a guide to where the asbestos fibers are concentrated. In a population not exposed to significant amounts of asbestos (Ruhr coal miners), one would not expect to find asbestos in the black spots in the parietal pleura.

With respect to iron-catalyzed redox reactions, Aust et al suggest that iron deposited on asbestos bodies may be mobilized and thus participate in tissue injury. They are apparently unaware of our studies showing no toxic effects of asbestos bodies in vivo and the reduced availability of iron on the surface of asbestos bodies for redox reactions. The authors refer to an increased lung cancer rate among iron ore miners, but this is likely related to smoking and radon exposure in underground miners. They note that iron mobilization is not observed to any significant degree for chrysotile, tremolite, or erionite. However, any mineral particle or fiber can coordinate iron atoms on its surface, which are then available for redox reactions.

Aust et al conclude that a better definition of relative potencies of all sizes and shapes of respirable elongated mineral particles is a critical risk assessment need. However, exactly such an analysis was provided for the US Environmental Protection Agency by Berman and Crump, and their analysis showed no identifiable potency for fibers (referred to as respirable elongated mineral particles by Aust et al) that were less than 10 µm in length. The best model for understanding asbestos-related fibrogenicity and mesothelioma.
ificial carcinogenicity is the very slow clearance of long, thin biopersistent fibers, of which commercial amphibole asbestos (amosite and crocidolite), erionite, and refractory ceramic fibers are excellent examples, from respiratory compartments. The latter include the pulmonary interstitial and the stroma in the parietal pleura and peritoneum. Persistence of fibers in these locations for prolonged periods (decades typically) leads to activation of the inflammasome with smoldering long-term injury to the tissues, eventually resulting in fibrosis in the lung or mesothelioma in the pleura or peritoneum. Mechanisms by which these inflammasomes mediate tissue injury may include a variety of pathways, including cytokines, growth factors, or reactive oxygen and nitrogen species. However, there is no convincing scientific evidence that short fibers have a significant role in this process.

SUMMARY

In conclusion, studies in both experimental animal models and human tissue samples do not support a pathogenic role for fibers 5 μm or less in length. Claims concerning the numerical majority of such fibers in tissues carry no weight with respect to causation of disease, and in vitro studies of possible pathogenic mechanisms do not override the findings in inhalational animal models or human epidemiological studies. Further studies regarding mechanisms by which biopersistent mineral fibers produce constitutive activation of the inflammasome may enable more precise identification of fiber lengths beyond 5 μm that are responsible for disease, in addition to elucidating whether there is a difference in pathogenic length for fibrogenesis versus neoplasia pathways.

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