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ASBESTOS BURDEN IN TWO CASES OF MESOTHELIOMA WHERE THE WORK HISTORY INCLUDED MANUFACTURING OF CIGARETTE FILTERS

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Asbestos has been used in many applications, but possibly one of the more unique was in the manufacturing of filters for cigarettes. The type of asbestos used in this application was crocidolite. Data from several resources indicate that crocidolite was one of the least utilized types of commercial asbestos in the United States. The present study provides quantitative tissue burden analysis data for two mesothelioma cases where the work histories included manufacturing of cigarette filters that contained crocidolite. The data include the number of asbestos bodies and uncoated fibers per gram of tissue, as well as the dimensions of these structures. The conclusion of the findings indicates that the individuals had an appreciable homogeneous exposure to crocidolite asbestos.

Asbestos has been referred to as a "magic mineral" due to its unique physical attributes that make it an excellent insulator and resistant to damage by heat and chemical reactions (Bowles, 1946). These attributes led to the use of asbestos in thousands of products (Hendry, 1965). Asbestos has long been recognized as posing a risk for producing disease in humans. Wagner (1972) in one of his earlier works noted that "inhalation" of asbestos dusts can lead to pulmonary fibrosis and carcinoma of the lung or to the development of diffuse mesothelioma of the pleura and peritoneum. Wagner and Pooley (1986) noted that 97% of the world's production of asbestos in 1976 was chrysotile. Amosite and crocidolite were the predominant other types of asbestos used in other commercial products in the United States. Production of anthophyllite for commercial uses was primarily localized in Finland (Wagner, 1972; De Vuyst et al., 1998), with only a very limited consumption in the United States (Dodson & Levin, 2001).

The use of either of the commercial amphiboles is usually based on properties that make them more functional in a given application than the more readily accessible chrysotile. These include applications where resistance to acids and alkalis (Wagner & Pooley, 1986) is needed or where high humidity

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would be encountered. Another unusual application of the crocidolite type of asbestos was in the manufacturing of filters for cigarettes (Knudson, 1956). The filters in which crocidolite was incorporated were reported to remove 40-60% by weight of the tars and nicotine. The original patent (Knudson, 1956) indicated the use of "Bolivian blue asbestos"—a type of crocidolite with a greater percentage of magnesium (Figure 1) than the form used in later production (Figure 2).

The limited data that exist as to disease produced from exposure to the crocidolite in such a filter consist of a report of a case with severe asbestosis in an individual with 9 mo of exposure while manufacturing the filters (Goff & Gaensler, 1972) and a review of a cohort of cigarette filter makers from a Massachusetts factory (Talcott et al., 1989). In the latter study, Talcott et al. (1989) reported that "extremely high morbidity and mortality in these workers were caused by the intensive exposure to crocidolite asbestos fibers." The potential release of crocidolite fibers from an asbestos-filtered cigarette was tested by Longo et al. (1995) using a simulated model.

Crocidolite is a fibrous silicate that contains an iron component. Iron has been shown to be an active participant in reactions producing chemicals that cause DNA damage (Jaurand, 1997). If longer fibers of crocidolite (>8 μm) are inhaled, the potential also exists for the formation of ferruginous bodies, which are the result of deposition by macrophages of iron-rich materials on the fibers. These structures have been isolated from animal tis-

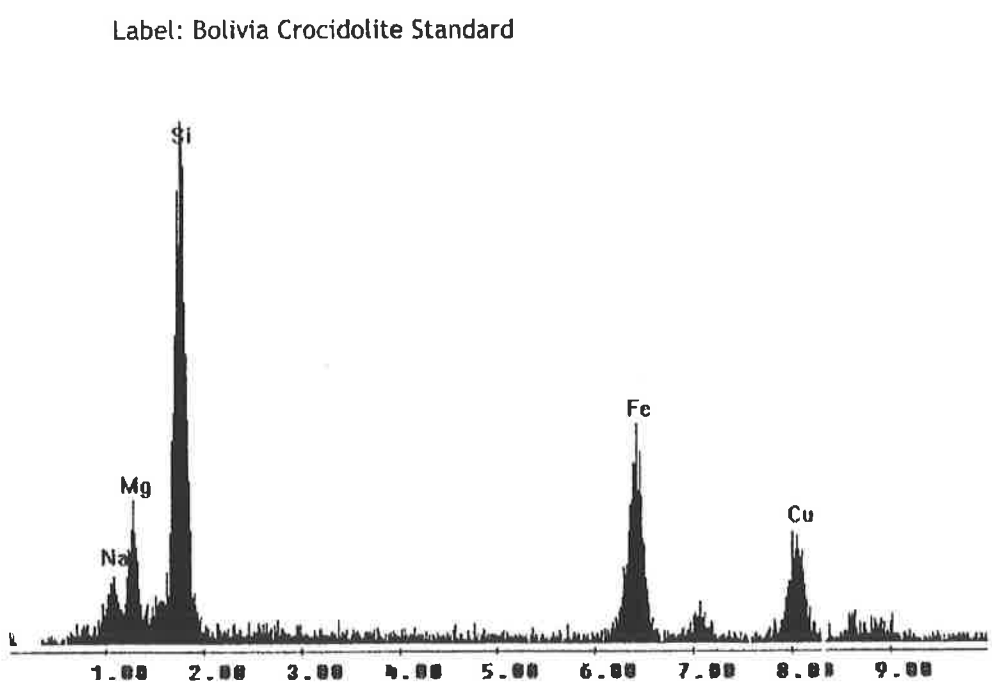

FIGURE 1. This x-ray energy-dispersive analysis is from the Bolivian type of crocidolite originally referenced in the filter patent. It contains an increased proportion of magnesium when compared to most samples of crocidolite.

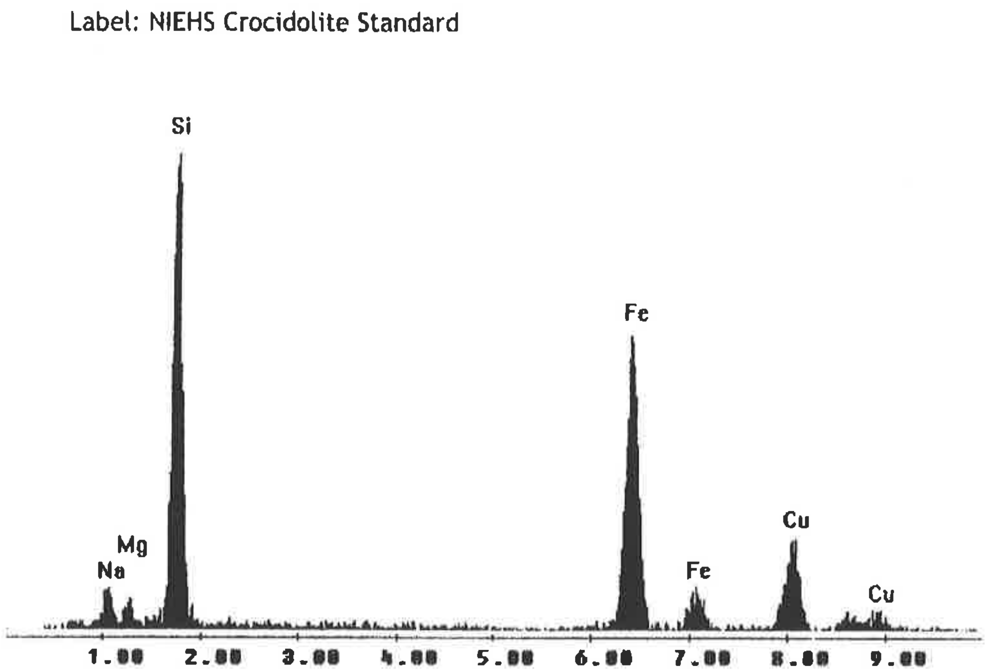

FIGURE 2. This spectrum of this NIEHS standard of crocidolite asbestos represents the more typical elemental ratios as found in most commercially used crocidolite. This spectrum is consistent with crocidolite fibers isolated from the exposed individuals.

sue (Ghio et al., 1997), studied in simulated stages of development (Fubini et al., 1997), and isolated and assessed for reactivity from human lung (Governa et al., 1999; Lund et al., 1994). In our own experience, the addition of these iron coatings on iron-rich amphiboles caused additional DNA strand brakes when compared to equal concentrations of uncoated fibers of the same type. Thus the presence of appreciable numbers of ferruginous bodies in these individuals poses additional risks for detrimental cellular reactions.

Both men studied in this report died from pleural mesothelioma and had a unique exposure to crocidolite asbestos in the workplace during the manufacturing of asbestos filters for cigarettes at the Louisville, KY, facility. The following study, to our knowledge, offers the first data regarding tissue burden of ferruginous bodies and uncoated fibers from lungs of individuals whose exposures were from such workplaces.

Case Reports—Case 1

The first man (case 1) in the study had a history of working in various vocations. As Becklake (1976) noted, workers whose primary job has no apparent association with asbestos may receive peripheral exposures without being aware exposures had occurred. This individual's occupational history indicated working on several jobs as a laborer. These included short periods of work for a window cleaning company, a flooring manufacturing company, a tree service company, and a seafood sales company. There were also work environments where his primary duties were defined as "sheet metal worker" or "welder." While some of this individual's jobs may have had the potential for exposures such as Becklake (1976) described, one assignment clearly involved direct exposure to asbestos.

It was discovered that the individual worked for a period of time extending from 1953 into 1954 for a company making asbestos-containing filters for cigarettes. This facility received "rods" of filters prepared at another facility. This individual's activity included working in the "plug room" where filter rods were cut. This work was reported under sworn testimony by the workers to be a very dusty process.

The individual was a 67-yr-old male who first sought medical attention in early August 1998, with his chief complaints being shortness of breath and right-side chest pain. A chest x-ray revealed a right pleural effusion. A computed tomography (CAT) scan indicated an interval increase in pleural thickening around the periphery of the inferior half of the right hemithorax. There were also peripheral linear densities noted at the base of each lung, which were reported as consistent with fibrotic changes related to asbestos exposure. These combined observations were reported as "worrisome" and suggested the pleural thickening could represent a mesothelioma. The person had smoked several brands of cigarettes, including the one with the asbestos-containing filters.

A right video-assisted thoracoscopy was performed on the right chest, and upon entry dense adhesions were noted between the parietal and visceral pleural surfaces. Pathological evaluation of biopsy material from the pleura and lung tissue revealed an infiltrating epidermal carcinoma. The histology and appearance of the tumor were considered diagnostic for malignant pleural mesothelioma. The pathologist further reported that the lung parenchyma showed variable interstitial thickening and fibrosis.

This individual died in September 1999 and a chest-only autopsy was performed. Light-microscopic sections of the lung indicated diffuse interstitial fibrosis with occasional asbestos bodies being seen in the lung tissue. The cause of death was reported as complications from the previously diagnosed mesothelioma of the pleura. There were also areas of pathologically defined asbestosis as well as effects of chronic pulmonary disease.

Case Report—Case 2

The second man (case 2) had worked in the filter manufacturing facility from 1951 until his retirement in 1983. He worked as a machine operator in the facility and as a mechanic where he put the filter on cigarettes and "rolled the plug." The individual presented in 1998 as a 67-year-old male complaining of exertional dyspnea and shortness of breath. His pulmonary function studies indicated mild restrictive lung disease. A computer-analyzed tomography (CAT) scan revealed pleural fluid accumulation at the back of the right lung. No mass was detected, and a sample of the pleural effusion was found to be negative for malignancies. Approximately 1 yr later he was found to have greater involvement of pleural effusion, which occupied a majority of the right pleural cavity. A video-assisted thoracoscopy and subsequently a pleurodesis were performed. The pathology confirmed a malignant mesothelioma. The patient succumbed to his illness in January 2001 at 70 yr of age. The pathology report indicated "diffuse, thick, white pleural plaques and pleural adhesions" in the right pleural cavity. Several small plaques were noted in the left pleural cavity. The right pleural surface was reported to be distorted secondarily to marked pleural adhesions. The left lower lobe contained a "white/yellow" nodule. Interstitial fibrosis was also present in the lungs. The individual's death was attributed to mesothelioma (pleural) with organizing broncheopneumonia as a contributing factor.

METHODS

Formalin-fixed tissues from the left and right lungs of both individuals were sampled by selectively using subpleural regions in which there was homogeneous parenchyma and excluded areas with larger airways. The tissue was prepared for analytical analysis for the determination of the presence and numbers of asbestos bodies and uncoated asbestos fibers. This procedure involved the use of a modified sodium hypochlorite digestion procedure (Williams et al., 1982). All solutions used in the analysis were prefiltered through 0.2-μm-pore polycarbonate filters. Both solutions and filter blanks were used for quality control assessments. The processes involved for either the light or electron microscopy preparations emphasized direct methods of preparation (Dodson, 1989). The aliquots from the digested material were collected on 0.22-μm mixed cellulose (Millipore) filters for examination by light microscopy to determine the numbers of ferruginous bodies. Another sample of the aliquot from the respective right or left digested lung was collected on a 0.2-μm polycarbonate (Nuclepore) filter for analysis by analytical transmission electron microscopy at 16,000× for determination of presence, sizes, and types of uncoated asbestos fibers. A lower magnification scan (1600×) was carried out in order to find ferruginous bodies and analyze the composition of their core material.

Nine sites collected from the left lung and two from the right lung of case 1 were digested. The digest pool from the right lung contained 0.3767 g dry tissue (2.5106 g wet). The pool from the left lung contained 0.5160 g dry tissue (3.3392 g wet).

The tissue samples in case 2 consisted of 10 sites from the left lung and 14 from the right lung. The digest pool from the right consisted of 0.7660 g dry weight (4.8088 g wet), while that of the left lung consisted of 0.7973 g dry tissue weight (4.9457 g wet).

RESULTS

Light Microscopy Findings: Case 1

For purposes of this report, a classical ferruginous body is defined by light microscopy as an elongated structure that has rust-colored surface material (often as a beaded coating) and a clear, thin core. These ferruginous bodies are morphologically consistent at the light microscopy level with those shown by analytical transmission electron microscopy analysis of core material as being asbestos bodies. Therefore, the two terms when used in this report are interchangeable.

The sample from the left lung contained 105,582 ferruginous bodies/g dry weight (16,315 ferruginous bodies/g wet weight). The limit of detection was 129 ferruginous bodies/g dry weight (20 ferruginous bodies/g wet).

The material analyzed from the right digest was found to have 185,057 ferruginous bodies/g dry weight (27,753 ferruginous bodies/g wet weight). The limit of detection for ferruginous bodies from the right side was 133 ferruginous bodies/g dry weight (20 ferruginous bodies/g wet).

Light Microscopy Findings: Case 2

The aliquot from the left lung from case two was found to have 8075 ferruginous bodies/g dry weight (1299 ferruginous bodies/g wet weight). The limit of detection for this sample was 124 ferruginous bodies/g dry weight (20 ferruginous bodies/g wet).

The sample from the right side contained 9906 ferruginous bodies/g dry weight (1577 ferruginous bodies/g wet weight). The limit of detection for ferruginous bodies for the right side was 125 ferruginous bodies/g dry weight (20 ferruginous bodies/g wet).

Transmission Electron Microscopy Findings—Case 1

The transmission electron microscopy scan of both cases revealed an appreciable and unique uncoated asbestos burden. The analysis of the first 100 fibers of the left lung revealed that 99 of the fibers were of the amphibole form of asbestos, with 98 being crocidolite with one being amosite. The remaining nonasbestos fiber was an aluminum silicate. The uncoated asbestos fiber concentration for the left lung was 71,127,051 fibers/g dry weight (10,990,840 fibers/g wet).

There were two ferruginous bodies found in the higher magnification scan (16,000×), and both were formed on crocidolite cores. An additional scan at lower magnification (1600×) yielded 12 additional ferruginous bodies also formed on crocidolite cores (Figure 3).

The analysis of the first 100 fibers from the preparation of the right lung indicated 96 were crocidolite asbestos (Figure 4), with the remaining fibers being aluminum silicates. One ferruginous body was found in the high-magnification scan (16,000×) and 10 were found in the 1600× scan. All were formed on crocidolite cores.

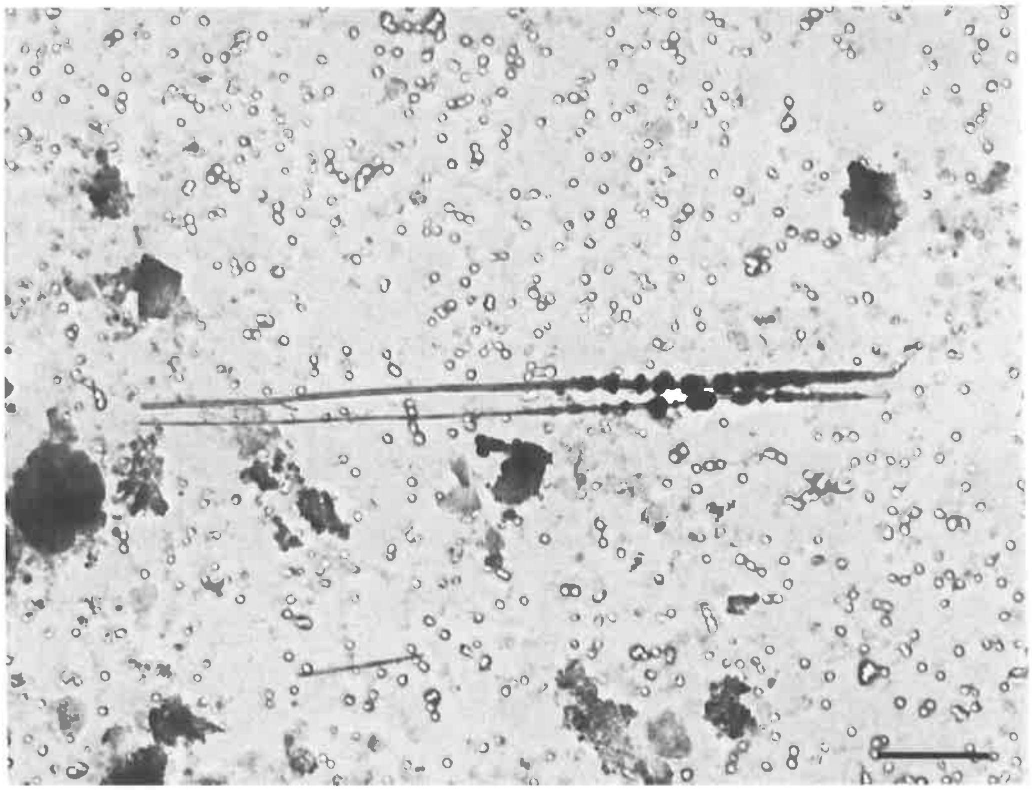

FIGURE 3. The two parallel ferruginous bodies seen in this figure are formed on crocidolite cores. There is also a shorter crocidolite fiber at the bottom of the print. Patient 1; 3300× original magnification.

The characteristics of the uncoated crocidolite fibers are shown in Table 1 and Figure 5. There were appreciable numbers of fibers greater than 5 μm in length in both sides, with the longest uncoated fiber found in the left side being 28 μm and in the right side being 65 μm. The overwhelming majority (91.8%) of the uncoated crocidolite fibers in both lungs were sufficiently thin (<0.25 μm) that detection by phase-contrast light microscopy would have been unlikely. The mean aspect ratio for the uncoated fibers from the left lung was 123, while that for the right lung was 172 (Table 1).

Transmission Electron Microscopy—Case 2

As with the first case, the uncoated asbestos burden was unique in composition. Of the 98 fibers analyzed from the left side, 83 were crocidolite asbestos. The remaining fibers analyzed were defined elementally as 12 aluminum silicates, 2 titanium fibers, and 1 talc fiber.

The asbestos fiber concentration in the left lung was 2,945,990 fibers/g dry (474,926 fibers/g wet). Two crocidolite-cored ferruginous bodies were found during the high-magnification scan (16,000×), while the 10 ferruginous bodies found by low magnification were also formed on crocidolite asbestos.

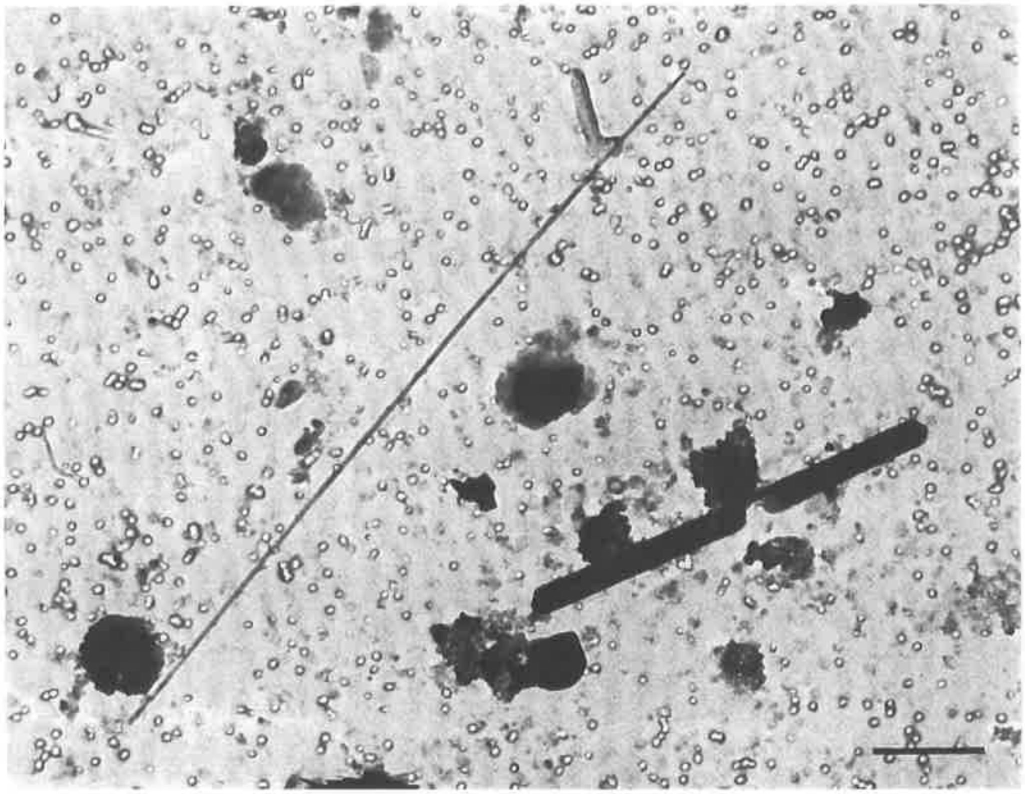

FIGURE 4. Even the long fibers of uncoated crocidolite found in the lungs were often very thin, as indicated in this figure. The replicas of the pores on the filters as seen in the background are 0.2 μm. Sample from lungs of patient 1; 3300× original magnification.

Seventy-four of 84 fibers analyzed from the right lung were crocidolite asbestos (Figure 6), with 1 anthophyllite asbestos fiber also found. The remaining nonasbestos fibers consisted of six aluminum silicates, one magnesium silicate (talc fiber), and two titanium fibers. Seven ferruginous bodies were found at the high-magnification scan (16,000×), and 10 were found at low magnification (1600×). All cores were confirmed to be crocidolite.

The characteristics of the uncoated crocidolite fibers from case two are shown in Table 1 and Figure 7. As with the findings in case 1, there was an appreciable population of fibers greater than 5 μm in length. Fibers as long as 50 μm were found on the left side and 61 μm on the right.

As with case 1, the findings in case 2 indicated that 97.5% of the diameters of the uncoated crocidolite fibers would have been at or below the detection limit of phase contrast light microscopy (≥0.25 μm). The mean aspect ratio of the left lung was 113 while that of the right was 136.

DISCUSSION

The utilization of chrysotile asbestos in commercial products has been recognized as accounting for over 95% of the total asbestos used. The remaining utilization of asbestos in commercial products was reported to

TABLE 1. Length and Width of Uncoated Crocidolite Fibers

	Average length	Average width	Average aspect ratio	Percent >5 μm length	Percent ≥8 μm length
Case 1					
Left lung	6.59 (4.53)	0.11 (0.08)	1.23 (56)	45.9	32.7
Right lung	9.40 (5.72)	0.12 (0.08)	172 (74)	59.4	38.5
Case 2					
Left lung	7.79 (5.42)	0.09 (0.07)	113 (73)	56.6	38.6
Right lung	9.84 (6.84)	0.09 (0.08)	136 (84)	60.8	43.2

Note. All dimensions are μm; numbers in parentheses are geometric means.

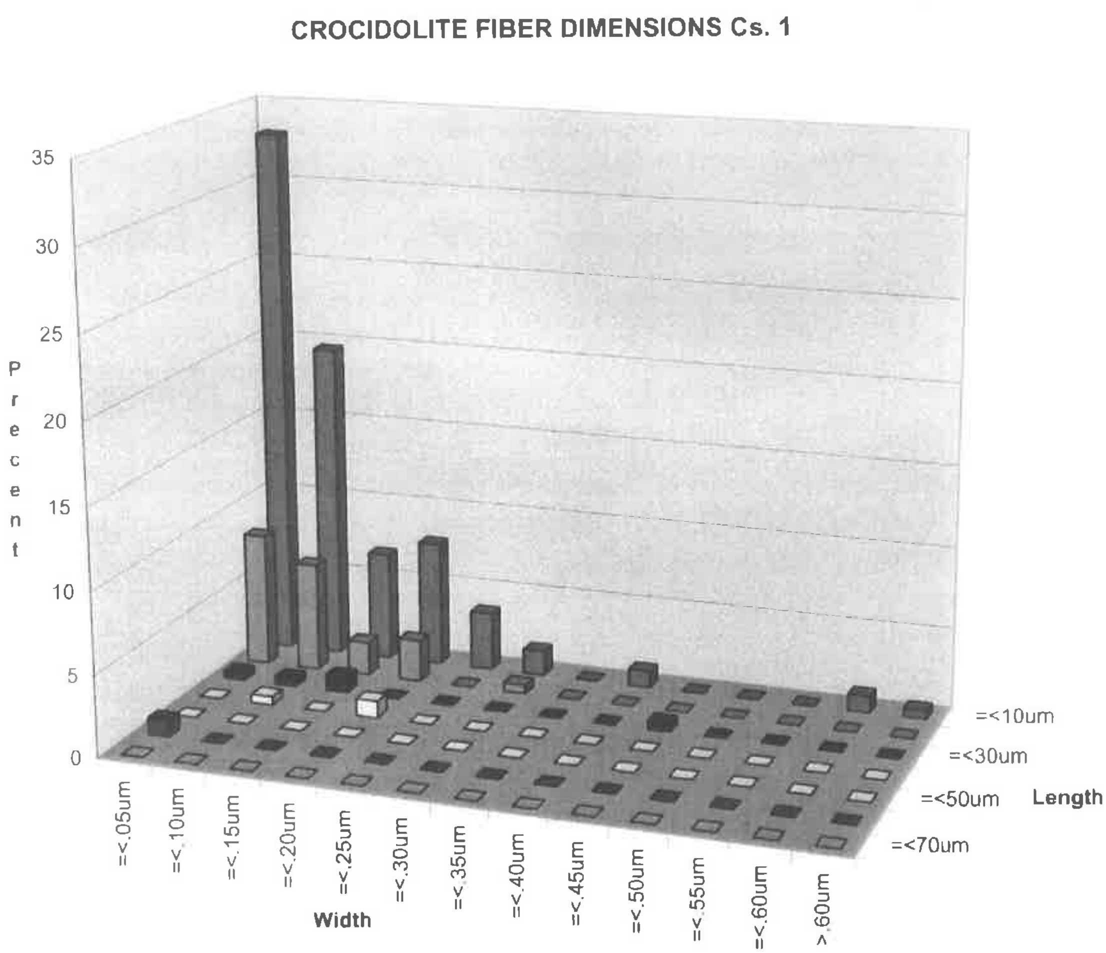

FIGURE 5. Combined left and right lung crocidolite fiber dimensions—case 1.

consist of 1–2% crocidolite, with the rest being amosite (Langer & Nolan, 1998).

The data from the lung tissue in the present cases reflect an almost homogeneous exposure to one of the less encountered commercial asbestos fibers—crocidolite. The uniqueness of finding such high levels of crocidolite contrasts with the finding of a study of 144 heavily exposed shipyard workers and insulators from the Pacific Northwest that reported that crocidolite fibers were "found in only a few cases, usually in quite small numbers, and have been excluded from all analysis" (Churg & Vedal, 1994). Therefore, based on the very high concentration of crocidolite-cored asbestos bodies and uncoated crocidolite fibers, it is evident that even with the relatively short duration of exposure (case 1) in the filter manufacturing facility, there was a high exposure.

A patient's risk for the occurrence of disease from any asbestos including exposure to crocidolite includes factors such as its clearance rate. This has been reported as lowest for the commercial amphibole fibers, including crocidolite (Du Toit, 1991). The high aspect ratio of crocidolite, among other features, has also resulted in it being labeled in one publication as the most fibrogenic asbestos in the lung (Murai et al., 1994). Further compounding the cancer risk for these patients were the actual physical attributes of the crocidolite used in the filter production as described in U.S. Patent 2,761,795. The patent indicates the importance of small diameter structures, which are "considerably longer than ¼ inch." Any degree of trauma to a bundle of these crocidolite fibers would immediately create a multitude of

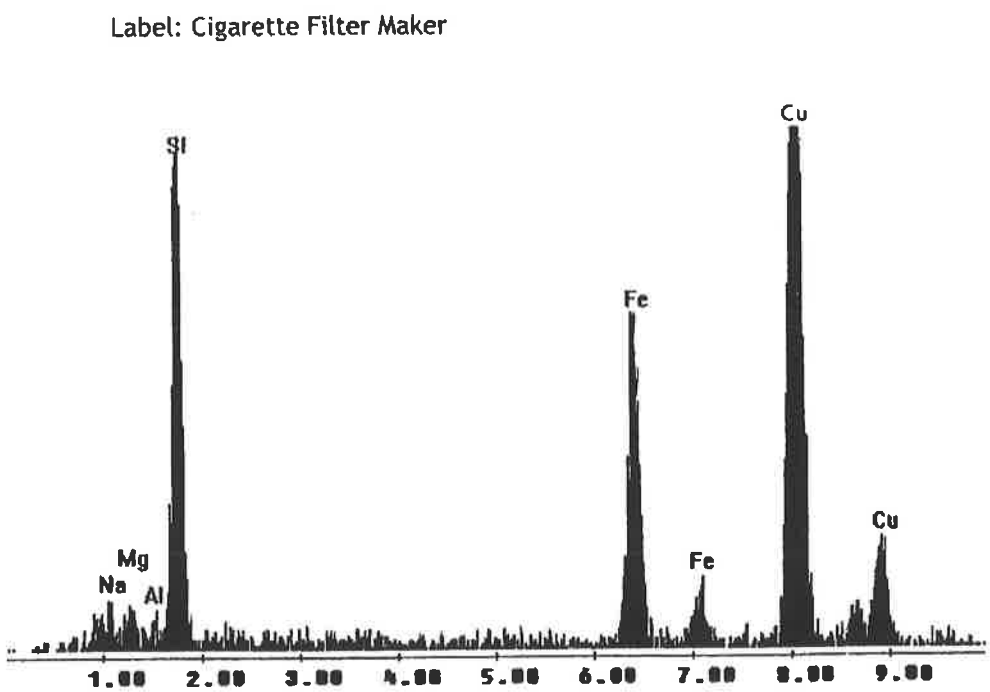

FIGURE 6. The x-ray energy-dispersive spectrum from an uncoated asbestos fiber from patient 2 is consistent with the NIESH standard for crocidolite shown in Figure 2.

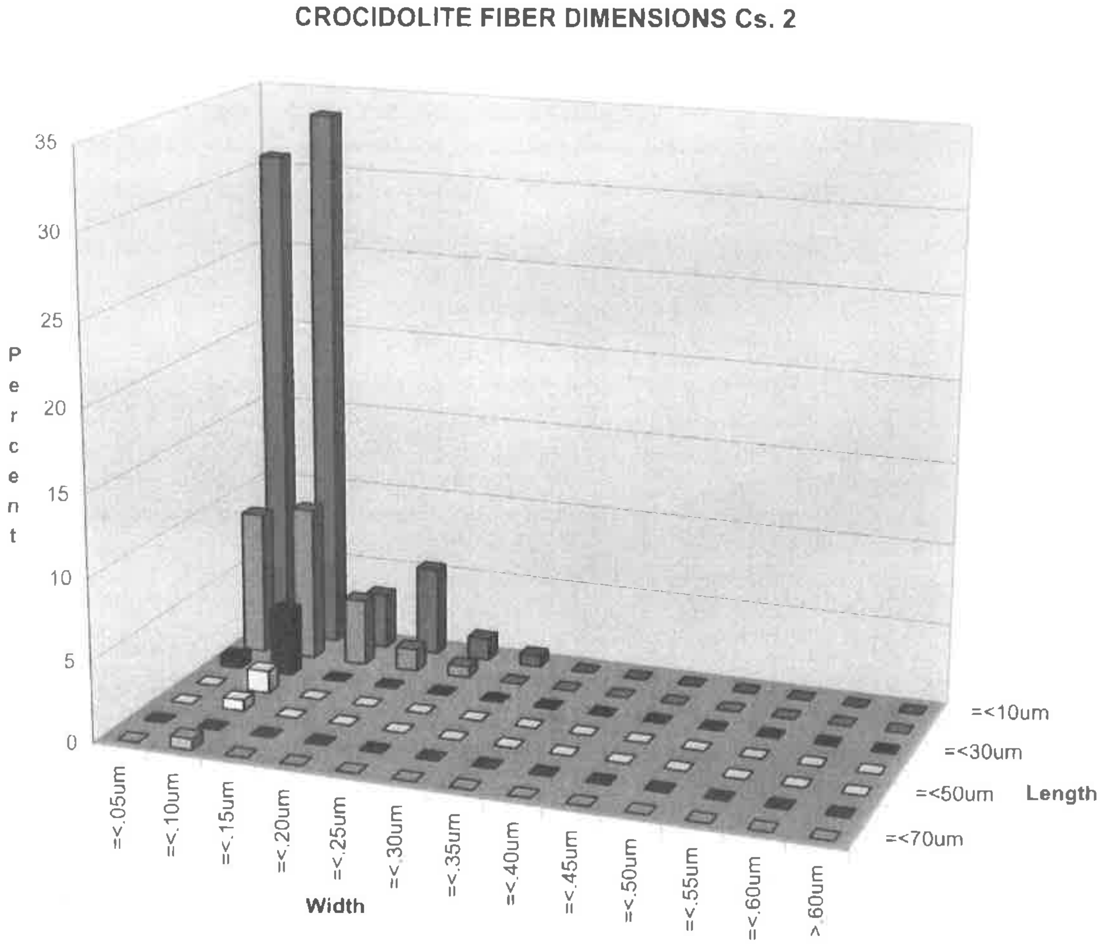

FIGURE 7. Combined left and right crocidolite fiber dimensions—case 2.

very respirable and potentially carcinogenically active fibers. Certainly the repetitious cutting process in the plug room resulted in very high-level exposures to such fibers, as reflected by the tissue burden that has remained in these individuals' lungs for the years since the date of last exposure. Shorter fibers are considered more readily cleared than longer fibers. Yet even with the impact of clearance over time from last exposure, fibers making up 39% (case 2, right lung) to 54% (case 1, left lung) of the total fiber burden were less than 5 μm in length. Thus, the remaining fibers at the time of death must reflect a fraction of the fibers that had earlier been in the tissue. The findings in tissue from these cases are consistent with the occupational exposure history of the individuals to crocidolite. The exposure setting is unique in that it represents one of the few "pure exposure environments" to one of the least utilized types of commercial asbestos—crocidolite.

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