

A Technical Comparison of Evaluating Asbestos Concentration by Phase-Contrast Microscopy (PCM), Scanning Electron Microscopy (SEM), and Analytical Transmission Electron Microscopy (ATEM) as Illustrated From Data Generated From a Case Report

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As reported in the literature, there are more than 30 different standard methods available for the analysis of asbestos in a variety of situations. The methods include those for determining asbestos concentration in air, water, bulk building materials, surface dust, soil, and lung tissue (Millette, 2006; Dodson, 2006). Knowledge of the various methodologies is essential in determining which methodology is appropriate for any given situation. To better understand the use of various techniques in evaluating asbestos, we use an example of an individual who was a machinist in an auto supply/parts business. His work activity during much of his professional career included grinding, arcing, and drilling brake components. Asbestos has been identified as an important component of friction products, particularly brakes, and exposure to asbestos brake dust is of concern, particularly in workers where grinding, arcing, sanding, and drilling of brake components are recognized as releasing appreciable dust. Various methods can be used to evaluate asbestos in tissue and air. The case reported herein was of an individual who died from a pleural mesothelioma. Paraffin-embedded lung tissue was examined by a laboratory using scanning electron microscopy (SEM) and was reported to contain elevated asbestos body concentrations and five fibers, of which two were asbestos (one chrysotile and one tremolite). Tissue from the same paraffin block was analyzed by the laboratory of one of us (RFD) using analytical transmission electron microscopy (ATEM). While one might think the number of asbestos bodies and fibers would be similar using SEM and ATEM, this was not the case. Slightly elevated numbers of ferruginous asbestos bodies were detected in the digestate by light microscopy. Large numbers of uncoated chrysotile fibers were found by ATEM, but not by SEM. The majority of the chrysotile structures were fibrils whose detection required resolution levels attainable only at higher magnification by ATEM. The findings in this case clearly indicate that analysis of lung tissue digestates by ATEM at a higher magnification (15,000 \times) identifies significant numbers of asbestos fibers that are not identified by SEM at 1000 \times . These results further indicate that if causation of an asbestos-induced disease such as mesothelioma is based on asbestos concentration of lung tissue, erroneous conclusions can be made by analyzing tissue only by SEM. Thus, the methodologies that are available to analyze asbestos in lung tissue are extensively discussed here with respect to the type of procedure that should be utilized in various situations.

In 2003 Butnor et al. analyzed lung tissue from 10 individuals who had been diagnosed with mesothelioma whose only

stated exposure to asbestos was from brake dust. The collected samples of digestates were stated to have been evaluated by scanning electron microscopy (SEM) at 1000 \times and fibers equal to or greater than 5 μm in length with 3:1 aspect ratios were counted. The authors reported that “in every case with elevated asbestos fiber levels by SEM, excess commercial amphibole fibers were also detected. Elevated levels of chrysotile and non-commercial

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amphibole fibers were detected only in cases that also had increased commercial amphibole fibers.”

The report by Butnor et al. (2003) and a report by Langer and McCaughey (1982) are among the limited number of studies that have evaluated asbestos content of human lung tissue in individuals who suffered from mesothelioma and whose past exposure to asbestos was from asbestos-containing friction products. In evaluation of such lung samples, it must be recognized that shorter fibers are more rapidly cleared over time and that it can be difficult to detect short, thin fibers by scanning electron microscopy.

The purpose of this article is to compare lung tissue asbestos fiber digestion analysis using SEM at 1000 \times to analysis by analytical transmission electron microscopy (ATEM) at 15,000 \times . The results provided herein show that ATEM has a greater superiority in identifying short and thin fibers. This information can potentially be valuable in determining the past exposures an individual had to asbestos and can be important in determining all components of asbestos in lung tissue, rather than only a selected population based on an inherently biased count scheme.

CASE HISTORY

The individual whose tissue was submitted for analysis was diagnosed as having pleural mesothelioma. The individual at the time of death was a 62-yr-old male who had not been diagnosed with cancer during his life until the mesothelioma was diagnosed approximately 2 yr prior to his death in 2000. The deceased smoked only approximately one pack of cigarettes per day for approximately 2 yr in the early 1970s. He defined his work activities as being a “machinist” for over 40 yr. During this time he worked at several automotive supply/parts companies. He “cleaned blocks, bored blocks, turned cranks, turned brake shoes, drums, rotors.” The brake-related work activities included arcing brake shoes and drilling holes in brake linings. During the 40+ yr of his active work life, there were only short periods defined as “several months” and another for an apparent 2-yr period in which he worked in other professions. A review of his occupational activities did not identify other sources of asbestos exposure other than to friction products related to the automotive area. The individual stopped working once surgery was conducted and approximately 2 yr later succumbed to his disease.

METHODS AND MATERIALS

The case submitted to our laboratory for analysis consisted of 3 blocks (one of which had remaining lung parenchyma) and 17 slides. The primary information received consisted of a request for a tissue digestion procedure in order to determine the number of asbestos bodies and uncoated asbestos fibers in samples obtained from an individual who had been diagnosed as having a pleural mesothelioma.

The data generated by our study were based on a “blind study.” The material was submitted from an attorney’s office and it was clarified once the report from our laboratory had been submitted that the case had been a medical/legal case that was

no longer active. The expert who utilized SEM for fiber identification had previously conducted tissue analysis, including ferruginous body and uncoated asbestos burden, at the request of the same client who referred the sample for our analysis. The fiber detection/analysis had been conducted using SEM at 1000 \times . The reason for submission to our facility was to evaluate asbestos burden by ATEM at a higher magnification from the remaining lung parenchymal sample in the same paraffin block. The goal was to determine what fiber burden would be found when the digestate of lung tissue was assessed by ATEM using a magnification of 15,000 \times and based on a count scheme that included analysis of all fibers $>0.5 \mu\text{m}$ long and thinner fibers that would require higher magnification for detection.

The remaining parenchymal lung tissue was removed from the paraffin block that had the original identification number as referred to in the earlier evaluation. The state of the block face indicated the tissue in the block had been re-embedded following the earlier sampling. The paraffin adjacent to the lung tissue was physically removed. The sample was then deparaffinized by passing the sample through six washes with xylene and six washes in ethanol. The total weight of the deparaffinized tissue block was 0.1441 g. The tissue sample was then subjected to a modified bleach digestion procedure (Williams et al., 1982), with the digestate collected on 0.2- μm polycarbonate filters. The entire surface of the filter was analyzed at 200–400 \times by light microscopy for ferruginous bodies prior to the filters being coated with a thin carbon film as part of the preparation for ATEM. Strips of the carbon-coated polycarbonate filters were mounted on 100-mesh copper grids and the filter matrix was dissolved using a modified Jaffe wick procedure using chloroform. This resulted in a carbon extraction replica that contained the entrapped fibers, ferruginous bodies, and other particulates. Scans were made of 30 random grid squares on 3 grids or all fibers in the last complete grid square in which 90 fibers had been analyzed (in this case, 20 complete grid squares) at 15,000 \times in a JEOL 1200EX transmission electron microscope. Fibers or cores of ferruginous bodies were analyzed as to crystalline characteristics by selected area diffraction (as is necessary for establishing an amphibole, serpentine, or other pattern, and particularly relevant in distinguishing fibrous talc from anthophyllite asbestos) and for elemental characterization with an IXRF Systems: Kevex pulse processor model 4461, which contained EDS 2000 software. Laboratory background evaluations were conducted for comparative assessment as quality controls of laboratory environment and the quality of the filters and solutions used in the process. The interest in comparing the findings by ATEM analysis of the remaining parenchymal lung tissue within the same paraffin block as previously evaluated by SEM prompted the request for the right to submit the findings for publication. Appropriate legal authorization was granted for this to be done.

RESULTS

Light microscopic evaluation of the ferruginous body burden in the previous study stated the tissue contained 1490 asbestos

bodies per gram of wet tissue (corrected for paraffin block). By SEM there were 10,900 uncoated fibers per gram of wet lung as determined at 1000 \times magnification. This value was based on analysis of "five consecutive uncoated fibers examined by EDXA." One fiber had elemental peaks and ratios consistent with tremolite asbestos and another had elemental ratios consistent with a chrysotile fiber. The other three fibers were talc fibers. Two structures defined as "coated fibers" were also seen. One was determined to have an amosite core and the other was stated to have an iron core.

Our evaluation of the digested lung tissue from the block resulted in the following findings: Light microscopic evaluation of the total surface of the polycarbonate filter prior to it being carbon coated for ATEM analysis resulted in identification of 7 small asbestos bodies, which were determined to be equivalent to 84 classical asbestos bodies per gram of deparaffinized wet tissue. The number of asbestos bodies per gram of digested lung tissue had been established as levels that differentiate exposures that occur in general populations from those in an occupational or paraoccupational setting. Several laboratories have determined that levels of asbestos bodies in samples from the general population contain 20 or less (often nondetectable levels) bodies per gram of wet tissue (Dodson, 2006).

Evaluation by ATEM of a total of 20 randomly selected grid squares on 3 grids revealed 92 uncoated fibers. These consisted of 1 silica fiber, 3 talc fibers, 4 amosite asbestos fibers, and 84 chrysotile asbestos fibers. The uncoated asbestos fibers were equivalent to 458,333 fibers per gram of deparaffinized wet weight of tissue. The detection limit was 5,208.33 fibers per gram of deparaffinized wet weight of tissue. There were 3 chrysotile-cored asbestos bodies found in the area scanned at 15,000 \times . The visible portion of two chrysotile cored asbestos bodies revealed they were formed on chrysotile structures consistent in size and unit structure with fibrils, while the third asbestos body was formed on an obvious bundle of chrysotile (Figure 1). The asbestos bodies found by ATEM were morphologically mature (beaded asbestos material along portions of the chrysotile core) but much shorter in length (average 10 μm) than those commonly formed on amphibole cores (which can often average 20 μm ; Dodson, 2006).

The morphological characteristics of the uncoated chrysotile fibers indicate the value of assessing their presence by the ATEM. The longest uncoated chrysotile fiber was a 23- μm -long structure that morphologically could be defined as a bundle. Thirty-five percent of the uncoated chrysotile fibers were longer than 5 μm in length. The average length of the chrysotile fibers was 5.2 μm . However, of those longer than 5 μm only 3 were not in the form of fibrils (Figure 2). Only 7% of all lengths of chrysotile fibers were not fibrils (0.02–0.05 μm in diameter). The longest amosite fiber was 23 μm and all amosite fibers were longer than 5 μm . The amosite structures ranged from 0.4 to 1.5 μm (bundle) in diameter (see Table 1).



FIG. 1. The ferruginous body in this micrograph is formed on a bundle of chrysotile fibrils. Original magnification 20,000 \times .

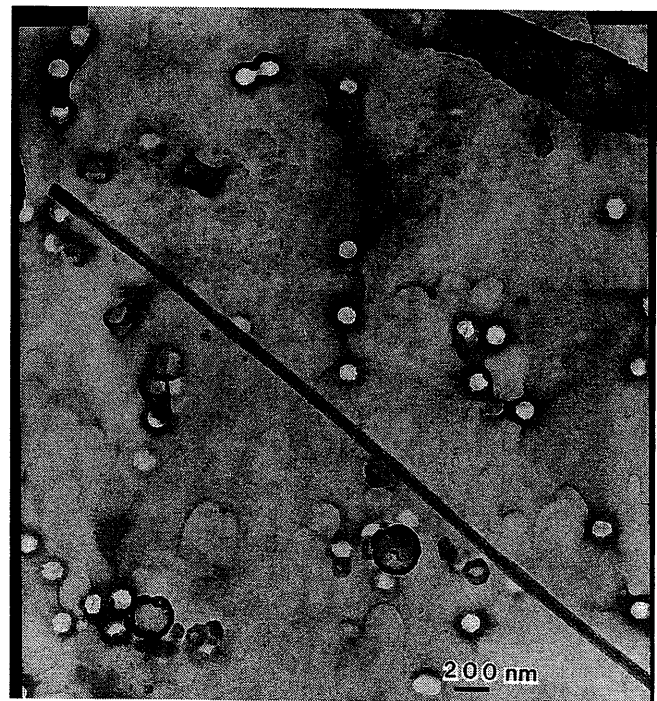


FIG. 2. The chrysotile fibril in this micrograph is typical of the thin form of uncoated chrysotile structure representative of the chrysotile burden found by ATEM. Original magnification 20,000 \times .

TABLE 1
Dimensions of asbestos structures

Asbestos	Length	Width	Asbestos	Length	Width
Chrysotile	5	0.05	Chrysotile	9	0.05
Chrysotile	13	0.05	Chrysotile	3.5	0.05
Chrysotile	3	0.05	Chrysotile	5	0.05
Chrysotile	23	0.3	Chrysotile	2	0.05
Chrysotile	2	0.05	Chrysotile	4	0.05
Chrysotile	3	0.05	Chrysotile	8	0.3
Chrysotile	6	0.05	Chrysotile	9	0.05
Chrysotile	9	0.05	Chrysotile	6	0.05
Chrysotile	13	0.05	Chrysotile	3	0.05
Chrysotile	1	0.05	Chrysotile	3	0.05
Chrysotile	3	0.05	Chrysotile	2	0.05
Chrysotile	3	0.05	Chrysotile	6	0.05
Chrysotile (FB)	14	0.3	Chrysotile	4	0.05
Chrysotile	6	0.1	Chrysotile	3	0.05
Chrysotile	4	0.05	Chrysotile	4	0.05
Chrysotile	6	0.05	Chrysotile	3	0.05
Chrysotile	5	0.05	Chrysotile	19	0.05
Chrysotile	5	0.05	Chrysotile	1.5	0.05
Chrysotile	13	0.05	Chrysotile	6	0.1
Chrysotile	4	0.05	Chrysotile	2	0.05
Chrysotile	7	0.05	Chrysotile	2.5	0.05
Chrysotile	8	0.05	Chrysotile	5	0.05
Chrysotile	4	0.05	Chrysotile	3	0.05
Chrysotile	3	0.05	Chrysotile	3	0.05
Chrysotile	8	0.05	Chrysotile	3	0.05
Chrysotile	4	0.05	Chrysotile (FB)	4	0.05
Chrysotile	4	0.05	Chrysotile	1	0.05
Chrysotile	1	0.05	Chrysotile (FB)	12	0.05
Chrysotile	4	0.05	Chrysotile	3	0.05
Chrysotile	18	0.05	Chrysotile	5	0.05
Chrysotile	2	0.05	Chrysotile	2.5	0.1
Amosite	19	1.5	Chrysotile	8	0.05
Chrysotile	1	0.05	Chrysotile	7	0.05
Amosite	23	0.5	Chrysotile	2.5	0.05
Chrysotile	5	0.05	Chrysotile	4	0.05
Chrysotile	2.5	0.1	Chrysotile	4	0.05
Amosite	6	0.6	Chrysotile	1	0.05
Chrysotile	2.5	0.05	Chrysotile	8	0.05
Chrysotile	3	0.01	Chrysotile	14	0.05
Chrysotile	1	0.05	Chrysotile	2	0.05
Chrysotile	3	0.05	Chrysotile	18	0.4
Chrysotile	6	0.05	Chrysotile	3	0.05
Chrysotile	12	0.05	Chrysotile	5	0.05
Chrysotile	2.5	0.5	Chrysotile	3	0.05
Chrysotile	6	0.05	Chrysotile	2.5	0.05
Chrysotile	8	0.05			

DISCUSSION

The purpose of this article is to use a case report, specifically a case report of a brake machinist who died from pleural mesothelioma, for evaluating methods of analyzing lung tissue to determine exposures to asbestos.

Asbestos is a name given to a group of naturally occurring fibrous minerals. Their unique physical properties have resulted in their utilization in many industrial applications and their designation "silk of the mineral kingdom." (Bowles, 1946) One specific application of asbestos has been as a component of brakes/friction products. Blau (2001) stated, "history records the use of many kinds of material for brakes (friction materials). For example, wagon brakes used wood and leather. In fact, many current brake materials still contain organic-based materials, like polymers and plant fibers. In order to achieve the properties required of brakes, most brake materials are not composed of single elements or compounds, but rather are composites of many materials."

The DHHS (NIOSH) publication by Sheehy and colleagues (1989) reported, "asbestos is used for fiber reinforcement, flexibility, and heat resistance. Chrysotile is used almost exclusively and comprises from 40–50 percent of the brake lining." The potential exposures to asbestos while workers are doing brake repairs have been reported by Jacko and colleagues (1973). With regard to the projected amount of asbestos used in friction products over a designated period, they calculated an annual use for such application in 1973 of 118 million lb (of asbestos) (prior to grinding and drilling) in brake products and 4.5 million lb in clutch friction products. The work practices associated with replacing old brake components or working on such components historically involved the use of traumatic procedures. For example, Paustenbach and associates (2003) stated that "pending repairs, the wheels are elevated, removed, and then inspected. Loose dust is then cleared from the drums and the brake assemblies by vacuuming, wiping, brushing, using compressed air, or a combination of these methods." The processes associated with these job activities are similarly described by Lorimer and colleagues (1976). In addition, Lorimer et al. stated that "the new lining may require considerable manipulation to fit the brake shoe—beveling edges and punching holes in the material, for example. As an alternative, the brake shoe and lining may be replaced as a unit." As a further emphasis, the authors provide a photograph of a worker cleaning the brake drum with compressed air. A visible cloud of dust was shown being blown free from the assembly.

Rohl et al. (1976) evaluated the environment of garage mechanics during brake lining maintenance and repair work. They determined quantitative and qualitative features of free, unaltered chrysotile fibers found in the work zone. Their evaluation is important because of reported thermal influences that alter chrysotile asbestos to a nonasbestos entity: forsterite. They found that measurable concentrations of free chrysotile existed at least 75 ft from the work site and for at least 14 min after jet air blowing. They specifically stated that "unaltered chrysotile was

found in fiber and in fibril form, in air and brake drum dust samples." They concluded that "optically counting asbestos fibers may considerably underestimate the levels of total asbestos exposure."

Exposure to unused brakes that have not yet been subjected to the traumatic processing during instillation as described by Paustenbach (2003) and Lorimer et al. (1976) can release free chrysotile once the surface has been subjected to a gentle rinse (Atkinson et al., 2004). In a critique of the paper by Atkinson and colleagues (2004), Paustenbach and associates (2006) concluded that one would expect to find measurable asbestos fibers by ATEM on an unused brake component. The use of ATEM at higher magnification for detecting chrysotile asbestos in dust or tissue samples is more sensitive because chrysotile often separates into fibrils that cannot be seen by light optical microscopy nor easily seen at low magnification using ATEM or SEM (Dodson & Atkinson, 2006; Upton et al., 1991). Furthermore, chrysotile dust from brakes is often found as structures less than 5 μm long and/or as longer fibrils. Neither of these chrysotile structures derived from brakes would be counted in an optical microscopy counting scheme. As noted by Rohl et al. (1976) and in the study by Atkinson and colleagues (2006), the vast majority of chrysotile in brake dust is found less than 5 μm long or as longer fibrils. The majority of chrysotile fibers from brakes as shown in the work of Rohl (1976) and in the study by Atkinson and associates (2004) would not be counted as a "regulated fiber" (Dodson et al., 2003) in samples collected from air samples as used to determine the permissible exposure level (PEL)/time-weighted average (TWA) via a light microscopy count scheme. The fact that fiber release during any project involving chrysotile asbestos and monitored via light microscopy may indicate the PEL is not exceeded, when in reality there may be appreciable fibers not detected by light microscopy (Langer et al., 1991).

Langer and colleagues (1991) stated that a "regulated fiber" by the OSHA standard indicated the 5 μm length limit was chosen for "practicality and theoretical considerations," with the full awareness that the "short fiber, <5 μm in length, was the predominant component in the air" and "it constituted a small component of the total dust assayed by light microscopy at 100 \times magnification." The governing document from OSHA (OSHA, 1994) that established the present PEL (0.1 fibers [f]/ cm^3 [cc] over an 8-h time-weighted period) stated that work with certain asbestos-containing materials might result in appreciable underestimation of the levels of undetected asbestos fibers released in the air during work practices when the air samples were evaluated by PCM. A clear example of such underestimation of total fiber content in the work environment where brake dust was being generated was shown by Rohl et al. (1976). The count by optical microscopy was reported to greatly underestimate the total levels of unaltered chrysotile in air samples when compared with the findings (with the inclusion of short and thin fibers) by ATEM. Sakai and colleagues (2006) concluded that "machine grinding and leveling of new brake-lining surfaces

represent potential sources of heavy asbestos exposure, unless enclosure and local ventilation are efficient."

There are reviews regarding the risk factors for the induction of asbestos-related diseases, including mesothelioma, from exposure to chrysotile in brake dust. Paustenbach and associates (2003) reviewed the "historical analysis of published data regarding exposure of brake mechanics to asbestos as a result of doing brake work." This review was "supported, in part, by funding from Daimler Chrysler, Ford Motor Company, and General Motors Corporation." The conclusions reached following the review of the references was often based on the premise that potential exposure could be extrapolated from evaluations of TWA/PEL in the workplace. TWA/PEL is based on light microscopy assessment of asbestos content in air samples and, as pointed out, much chrysotile generated from brake dust is not detectable via light microscopy. The points raised in the review that "airborne concentrations of asbestos for auto mechanics would have decreased substantially only over the past 20 years due to fewer asbestos brake linings on cars (conversion to non-asbestos linings), the conversion to disc brakes, and better techniques for controlling exposure to brake dust in repair shops" is encouraging.

Lemen (2004) reviewed the historical exposure to friction products and concluded "even the so called 'controlled' use of asbestos containing brakes poses a health risk to workers, users, and their families." Egilman and Billings (2005) provided a unique and critical perspective on influences made on the "debate" regarding the content of medical literature as related to exposure to asbestos dust from automobile brake work and the risk for developing asbestos-induced diseases. The amicus brief by Laura Welch (2007) provides additional information concerning the potency of chrysotile from brake dust in causing mesothelioma.

Langer and McCaughey (1982) and Butnor et al. (2003) evaluated asbestos content of human lung tissue in individuals who suffered from mesothelioma and whose past exposure was stated to have been from asbestos-containing friction products. In evaluation of such lung samples, it must be recognized that smaller particulates (in the case of fibers—smaller structures) are more readily cleared over time (Dodson, 2006). This concept is only partially true following multiple exposures. Pinkerton and colleagues (1984) studied responses of lung tissue in rats chronically exposed to aerosolized chrysotile for 7 h/day, 5 days/wk for 3 or 12 mo. Lung tissue was sampled for morphometric analysis by electron microscopy. Their conclusions regarding the status of chrysotile in the lung were as follows: "During exposure to asbestos fibers, macrophages and alveolar epithelial cells contain statistically significant amounts of asbestos and are associated with histological changes indicating marked epithelial injury. Increased amounts of fibers are also localized in the lung interstitium with continued exposure to asbestos and are associated with a progressive interstitial fibrotic reaction. Following cessation of exposure, macrophages and epithelial cells are cleared of fibers and resolve towards normal proportions. However, signifi-

cant clearance of fibers from the lung interstitium does not occur after cessation of exposure, and there is a continuing progress of fibrogenesis." Lung (Dodson, 1997) and extrapulmonary tissue (Dodson et al., 2000) has been shown to contain chrysotile asbestos at various time periods from the time of last occupational exposure. Recognition of the relevance of tissue burden left at the time the sample was taken reflects the materials left after being subjected to clearance, a process that favors short fibers. Additional reports involving the assessment of tissue burden of dust from friction products are specifically as to conclusions based on the influence of tissue preparation, instrumentation, and magnification, all of which may impact detection of smaller, longer, thinner chrysotile fibers. The size of chrysotile fibers puts them among the respirable population of fibers in workplace environments when brake repairs/replacements are being conducted. With respect to mesothelioma, Sebastien et al. (1980) stated that asbestos content in lung parenchyma may not accurately reflect the type and size of fibers that reach the pleura where most mesotheliomas develop.

The concentration of asbestos fibers in air samples determined by light microscopy, scanning electron microscopy, and analytical transmission electron microscopy is well described in the literature (Upton et al., 1991), although little information exists concerning asbestos fiber burden in lung tissue (particularly chrysotile fibers) as determined by SEM (asbestos analysis of fibers $>5 \mu\text{m}$ and achieved at lower magnification by SEM) when compared to the fiber burden determined by ATEM (when short fibers $>0.5 \mu\text{m}$ are included in a count scheme at high magnification).

The asbestos body/uncoated asbestos fiber burden found in the lung tissue in this case is compared to that in a limited numbers of publications where lung tissue was evaluated from an individual (or individuals) with a defined past exposure to brake dust, and a comparison of our data with data obtained by SEM analysis of lung tissue. The evaluation by our laboratory was conducted as a blind study based on a request for services from a legal referral. Further clarification was made that the case had been a medicolegal case that was no longer active at the time the referral was made. To our knowledge, none of the data generated from our evaluation was used in any legal action and none of the authors was involved in this case during the time it was active in the legal process. We were informed that light and scanning electron microscopic analysis of digested lung tissue had been conducted at the request of the same law firm for determination of asbestos bodies and uncoated asbestos fibers in "one half" of the tissue sample prior to submitting the paraffin-embedded lung tissue to our facility. The digestate had been evaluated by light microscopy for ferruginous bodies and at $1000\times$ for asbestos fibers via SEM. The light microscopic analysis identified 1490 ferruginous bodies per gram of wet tissue. The number of uncoated fibers determined by SEM analysis was "10,900 uncoated fibers per gram wet lung." We were informed that "five consecutive uncoated fibers were examined by EDXA." The elemental ratio was interpreted as defining one fiber as consistent

with chrysotile, one fiber as tremolite, and three as talc fibers. Two "coated fibers" were examined by energy-dispersive x-ray analysis (EDXA) and the ratio of elements in the core material in one was characteristic of amosite and the other was characterized as iron.

The asbestos burden in lung tissue from individuals with mesothelioma has been studied by the same analytical procedure that was used in the ATEM assessment in this case. There is a wide variation in the total asbestos content in lung tissue from such individuals. This is reasonable since mesothelioma is recognized as a lower dose exposure disease when compared to some other asbestos-related diseases. When comparing the findings in this case with those of a 55-case study, the conversion of the deparaffinized wet burden per gram to dry weight burden would put this individual as the seventh highest asbestos burden (Dodson et al., 1997). A comparison of the burden in this case with data on lung burden from another study involving 54 cases of mesothelioma indicates that this individual would have had the fifth highest tissue burden (Dodson et al., 2005). Making the finding in this case of even more significant is the fact that chrysotile reportedly clears more readily from the lung than amphiboles (Dodson, 2006). In the occupationally exposed individuals comprising the 55-case study, 43% of cases did not have detectable levels of chrysotile. In the second study of 54 cases 50% were found not to have chrysotile within detection levels. Thus the finding of appreciable chrysotile fibers is unexpected in tissue analysis. As another comparative basis, the total asbestos burden determined for a group of 33 individuals considered as from the general population (Dodson et al., 1999) was 84,000 fibers of asbestos per gram of dry tissue.

Using a multiplier of 10 for converting the findings in this case from wet to a level per gram of dry tissue would indicate 4,583,330 fibers per gram dry weight of total asbestos fibers. In another study of tissue from 15 individuals from the general population, one lung sample had a single chrysotile fiber and the second had a single amphibole fiber. Thus 12 of the cases were not found to contain levels of asbestos within the detection limits used in the study (Dodson et al., 2001).

While this report is based on a single case and findings in a single block of tissue, the comparative findings based on light microscopy, and lower magnification assessment by ATEM and SEM (Upton et al., 1991), raise important issues concerning chrysotile asbestos content and strongly suggest the results of such an evaluation are determined in large part by the instrument and magnification used in an evaluation. This concept is relevant in interpretation of asbestos burden in areas that include environmental monitoring and in tissue evaluation when assessing the chrysotile burden in tissue from chrysotile-exposed individuals.

The work by Stanton and associates (Stanton et al., 1981) is often quoted as stating that long/thin fibers are most carcinogenically active. The model studied did not measure fibers recovered from the lung tissue but rather reactivity to a measured population of fibers in vitro before administering the material intrapleurally. Fiber dimensions and resultant responses in the

Stanton study are not to be confused by attempts to extrapolate the more carcinogenically active population of fibers for inducing mesothelioma based on fiber characteristics found in parenchymal lung tissue. The actual reference states that the "probability of pleural sarcoma correlated best with numbers of fibers that measured 0.25 μm or less in diameter or more than 8 μm in length. Relatively high correlations were also noted with fibers in other size categories having diameters up to 1.5 μm and lengths greater than 4 μm ." We evaluated the numbers of asbestos fibers counted in the case reported herein, which would be consistent with a "Stanton fiber," 0.25 μm or less in diameter or more than 8 μm in length; 17.8% of the chrysotile and none of the amosite fibers would qualify as a "Stanton fiber" by these criteria. Additional work has further shown the distinct increase in pathogenicity of long fibers when compared on a one-to-one basis with short fibers of the same type of asbestos. However, the fact remains that most fibers in human lung are less than 5 μm . Thus the impact of the total mass of short fibers to that of the minority longer fibers remains an open question. In this case the point of concern regarding short versus longer fiber, whatever that favorite length maybe, is of limited concern since the fibers that reach the pleura where the mesothelioma was located are overwhelmingly short fibers (<5 μm in length) (Sebastien et al., 1980; Dodson et al., 1990). The variation of risk for induction of tumors in the pleura with long versus short fibers is therefore of relevant concern when injections are given directly in the pleura to rats or other animals.

The mechanisms involved with inducing pathological events that may result in permanent changes in tissue involve more than simply a dimensional concept (Dodson et al., 2003). However, if vested interests could indeed eliminate concerns for disease from shorter fibers, the majority of chrysotile in any tissue would suddenly be of no concern for induction of disease since the majority is below 5 μm in length. This would thus make chrysotile defined as a safe or "good" form of asbestos. This is something that neither state, federal, nor international organizations have accepted (Welch, 2007).

SEM provides an appreciably greater amount of information regarding fiber type and burden in tissue than light microscopy. Since an earlier analysis by SEM was conducted with a count scheme including analysis of the population of fibers >5 μm , it seems appropriate to define which fibers would be identified under such a count scheme (excluding the factor of diameter) with the percent of chrysotile and amosite identified by our analysis that were >5 μm . In the best case scenario, 65% of the 84 chrysotile fibers would not qualify under such parameters, while all 4 amosite fibers would qualify for inclusion.

The individual in this report was not a brake mechanic but rather by his definition was a machinist. By his description his work included grinding, drilling, and arcing of brake components. There have been discussions regarding conversion of a portion of the chrysotile component of brakes by thermal/physical factors during wear. This includes either a "degradation" form of chrysotile or transformation to an olivine

structure (Langer, 2003). However, as Langer (2003) pointed out, "beveling and arcing of pads to fit vehicles, cleaning and refurbishing of brake surfaces especially large truck brakes [see Rohl et al., 1976], produce dust that has not been subjected to the same conditions as pad service. These practices require attention of the dust control engineer and industrial hygienist." Under such work conditions it would appear illogical that unaltered chrysotile asbestos would not be extensively freed into the environment, since a simple "gentle rinsing" of the surface of unused brake components resulted in a collection of large numbers of chrysotile structures (Atkinson et al., 2004).

A comparison of tissue burden in this case must be done with an appreciation that the majority of the chrysotile structures detected by ATEM analysis of freed fibers following the rinse procedure (Atkinson et al., 2004) and thus potentially releasable to the workplace air would not have been detected via light microscopy. Thus, such fibers/fibrils would not have been included in a phase-contrast (PC) light optical microscopy analysis of air quality in a count scheme used to assess "regulated fibers." The dimensions (length and especially width) of the chrysotile reported by Atkinson and colleagues (2004) raise the question of whether they would be detected at a low magnification evaluation by scanning electron microscopy (Middleton & Jackson, 1982; Middleton, 1982; Small, 1982; Small et al., 1983; Teichert, 1982; Upton et al., 1991). An earlier experience from our own laboratory found a variation between fiber burden of chrysotile when a comparison was made between lower and higher magnification assessment by ATEM from a preparation of the same lung parenchymal sample (Dodson et al., 1993). The lower magnification (5000 \times) of the digestate was conducted with parameters that included only fibers of size equal to or greater than 5 μm . Under such a count scheme one chrysotile fiber was noted. The average total asbestos burden for the 2 scans made at higher magnification (15,000 \times and 20,000 \times) that included fibers greater than 0.5 μm was 1.7×10^6 fibers/gram dry weight of tissue. The lower magnification scan excluded not only the majority of the fibers that were chrysotile (79% of total) but also amphiboles (21% of total) that were present.

The asbestos burden in pieces of lung tissue from the same block when evaluated by two laboratories resulted in consistent and variable findings. Both laboratories found the ferruginous body burden to be above that found in the laboratories as the level reflective of the upper limits of expected burden in samples from the general population (>20 ferruginous bodies/g wet tissue) (Dodson, 2006). The light microscopy assessment of isolated ferruginous bodies permits identification of their features in sufficient detail to permit a high degree of confidence that they are formed on asbestos cores. However, the definition as to the type of asbestos that constitutes the core material requires differentiation by electron microscopic analysis. Our observation of the isolated ferruginous bodies by light microscopy did, however, note that the structures were small when compared to those often found by light microscopy in samples from individuals occupationally exposed to asbestos. One ferruginous body

had been identified by SEM as formed on an amosite asbestos core. The three ferruginous bodies found by ATEM were determined to be formed on chrysotile cores, which in itself is an unusual observation since most asbestos bodies are formed on amphibole cores (Dodson, 2006). Chrysotile asbestos has been reported as the prevalent core of ferruginous bodies in certain unique exposure settings. These include in lavage samples from brake lining workers (Dumortier et al., 1990), within the lungs of chrysotile miners (Holden & Churg, 1986), and in lung tissue from a clutch refabricator (Levin et al., 1995).

The limited number of fibers analyzed by SEM included a fiber with an elemental ratio consistent with chrysotile in a count scheme that only included fibers >5 μm in length and reviewed at a magnification of 1000 \times . The contrast with the findings by ATEM is obvious in that there is a small population of amosite fibers in the tissue (4%), with the vast majority of the tissue asbestos burden being chrysotile (96%). If one considers that shorter/smaller inhaled dusts clear more readily from lung tissue (Dodson, 2006), then the representative portion of chrysotile left in the tissue at time the sample was collected logically must represent only a fraction of that having been in the lung from time of first exposure. The variation in sizes of chrysotile found in the lung tissue in the present study is consistent with the findings in analysis of asbestos composition from the lung of a mesothelioma patient whose exposure was as a brake repair worker (Langer & McCaughey, 1982). Langer and McCaughey (1982) reported that only 10% of the fibrils were longer than 10 μm and that fibrils less than 1 μm and others longer than 5 μm were present. Their publication (Langer & McCaughey, 1982) refers to the study by Rohl et al. (1976), which states, "besides this submicroscopic chrysotile fibre in brake drum housing, there are more significant sources of free, unaltered fibre in the beveling, refurbishing, and refitting of brake pads."

If one uses a phase-contrast light optical microscopy count scheme to assess tissue burden of chrysotile in this sample, then 97% of the chrysotile fibers would not be recognized as present. The size of chrysotile dust in this individual's lung tissue is consistent therefore with the dimension reported in the observation of chrysotile dust generated during brake lining maintenance and repair (Rohl et al., 1976, 1977) in that "most fibres (in the environmental samples) are too small to be seen by the optical microscopy." Rohl et al. (1976) further concluded the same from air samples as we have from tissue analysis, in that "the present technique of optically counting asbestos fibers may considerably underestimate the level of total asbestos exposures." The recognition of the discrepancies between the detection by light microscopy or lower magnification evaluation by electron microscopy of chrysotile in air and tissue samples must be appreciated based on what can be "seen" or counted using a particular count scheme, preparative procedures, selected instrument used in a count, the inherent resolution of that instrument at a given magnification, and most important what is counted and not counted as determined either by a selected count scheme or the

factors listed previously. As Langer et al. (1971) indicate, “optical microscopy delivers a select, biased population. First, we can only study what the microscope sees—and it only sees large fibers, those thicker than $0.5\ \mu\text{m}$ in diameter. Chrysotile, it has been noted, tends to split into finer fibrils in biological environments, and degradation to smaller particles occurs.” “Therefore selection of large asbestos bodies by means of light microscopy, resulting in a biased population of larger particles which were not altered in biological residence, stack the analytical deck” (Langer et al., 1971). This concept is just as valid a concern when data are presented that are based on an exclusionary count scheme in which only a selected population of larger fibers is analyzed or counted. The use of ATEM analysis is appreciably more time- and labor-intensive than PCM or SEM and thus is more costly. However, ATEM permits accurate determination as to the fiber types and, when used in at an appropriate magnification with a count scheme that includes short fibers, gives an accurate definition as to the actual composition of the asbestos fiber burden in tissue (Dodson & Atkinson, 2006). The majority (82.2%) of the chrysotile fibers from the lung sample are shorter than $8\ \mu\text{m}$ in length, which is consistent with findings in other studies when shorter fibers are included in a count scheme (Dodson et al., 2003). The percentage of short fibers ($<8\ \mu\text{m}$) become even more impressive in most studies when samples are evaluated from extrapulmonary sites (Dodson et al., 2003), including those where mesothelioma develop.

All asbestos-related diseases are dose-response related, which means that increased exposure to asbestos increases the risk of developing an asbestos-related disease. However, even in individuals who are exposed to high concentrations of asbestos (e.g., heat and frost insulators), only about 10% develop mesothelioma. There is no factual information on how much asbestos it takes to cause an asbestos-related disease such as mesothelioma in any given individual. One could argue that individual susceptibility (genetic susceptibility) is as important as asbestos concentration in determining who develops an asbestos-induced disease. With respect to cancer, the concentration of asbestos at the site where the tumor starts is thought to be the most important factor in determining causation. It is impossible to know how much asbestos it takes to produce an asbestos-induced disease. One would obviously think that with respect to mesothelioma, it was not the asbestos in the lung tissue that was responsible for causing the mesothelioma; rather, it would be the asbestos that was translocated to the pleura that caused the tumor. How much of the asbestos that was translocated to the pleura it took to cause the cellular changes that resulted in the development of mesothelioma is unknown and will probably remain unknown. As stated previously, the dominant fiber found in the pleura in most cases of pleural mesothelioma is chrysotile asbestos and the majority of chrysotile fibers are $<8\ \mu\text{m}$ long. Since all chrysotile fibers can potentially cause injury, there is no way to exclude chrysotile fibers of any specific length as having contributed to cause an asbestos-induced disease.

SUMMARY

As demonstrated by a case report of an individual whose only reported exposure to asbestos was from brake dust while working as a machinist, evaluation of lung tissue by ATEM at $15,000\times$ identified a significant elevated concentration of chrysotile asbestos that was not found by SEM at $1,000\times$.

This article described in great detail the issues that are encountered in accurately determining asbestos concentration in lung tissue and air.

Unless lung tissue and/or air is analyzed by ATEM at $15,000\times$ or greater magnification, a significant number of short fibers and long, thin fibers, most of which are chrysotile asbestos, will be missed. This lack of information could result in coming to conclusions that are not factually correct with respect to the type of asbestos found in lung tissue as reflective of past exposure and thus attribution for its potential contribution in causation of a given asbestos-related disease, including mesothelioma.

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