

FILE NAME: Colgate (COL)

DATE: 2014

DOC#: COL002

DOCUMENT DESCRIPTION: Journal Article - International Journal of Occupational and Environmental Health - Asbestos in Commercial Cosmetic Talcum Powder as a Cause of Mesothelioma in Women

Asbestos in commercial cosmetic talcum powder as a cause of mesothelioma in women

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Background: Cosmetic talcum powder products have been used for decades. The inhalation of talc may cause lung fibrosis in the form of granulomatous nodules called talcosis. Exposure to talc has also been suggested as a causative factor in the development of ovarian carcinomas, gynecological tumors, and mesothelioma.

Purpose: To investigate one historic brand of cosmetic talcum powder associated with mesothelioma in women.

Methods: Transmission electron microscope (TEM) formvar-coated grids were prepared with concentrations of one brand of talcum powder directly, on filters, from air collections on filters in glovebox and simulated bathroom exposures and human fiber burden analyses. The grids were analyzed on an analytic TEM using energy-dispersive spectrometer (EDS) and selected-area electron diffraction (SAED) to determine asbestos fiber number and type.

Results: This brand of talcum powder contained asbestos and the application of talcum powder released inhalable asbestos fibers. Lung and lymph node tissues removed at autopsy revealed pleural mesothelioma. Digestions of the tissues were found to contain anthophyllite and tremolite asbestos.

Discussion: Through many applications of this particular brand of talcum powder, the deceased inhaled asbestos fibers, which then accumulated in her lungs and likely caused or contributed to her mesothelioma as well as other women with the same scenario.

Keywords: Asbestos, Talcum powder, Chamber test, TEM, SEM, EDS, SAED, Mesothelioma

Introduction

Malignant mesothelioma occurs in both the peritoneum and in the lung pleura.¹ Mesothelioma cases have been attributed to direct occupational exposure, indirect exposure and secondary exposure.¹ A higher rate of “idiopathic” mesothelioma has been reported in women, as no link between asbestos exposure and patients has been identified.² Previous research suggests that ovarian cancer and peritoneal mesothelioma may be directly attributed to the use of talcum powder contaminated with asbestos or from exposure to partners occupationally exposed to asbestos.^{3–7} Using talcum powder in closed spaces may increase the likelihood of inhaling the powder laced with asbestos. Repeated applications increase the opportunities for inhalation and the asbestos could become concentrated in the peripheral airways and alveoli of the lungs of the talcum powder users. This has been supported by the presence of granulomas in the lungs of some talcum powder users.⁸

In 1976, Rohl and Langer tested 20 consumer products labeled as talc or talcum powder, including body powders, baby powders, facial talcums, and a pharmaceutical talc.⁶ Of the 20 products tested, 10 were found to contain tremolite and anthophyllite, principally asbestiform. The product with the highest asbestos content was the same product tested in this study. Both asbestiform anthophyllite and asbestiform tremolite were found in the Rohl and Langer tests. Given that asbestos has been determined as the primary cause of mesothelioma, it is important to note that cosmetic talc contained asbestos in the past.⁶ The contamination results from the mining process, since ore specimens taken directly from the mines have repeatedly been tested and shown to contain asbestos, most often anthophyllite and tremolite but also serpentine chrysotile asbestos.^{6,9,10}

In part from the review of corporate documents and the sworn testimony of those responsible for the sourcing of talc used in the products studied here, it was determined that three mines provided the raw material for use as talcum powder. The talc used by this cosmetic company that manufactured and

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distributed the talcum powder was from three distinct regions: the Willow Creek mine in Southwest Montana, the Regal mine near Murphy, North Carolina, and imported talc from the Val Chisone region of the Italian Piedmont.^{11–16} The specific geology of talc is an important indicator of whether a talc source may be contaminated with asbestos. These three mines all contained asbestos fibers; anthophyllite, and tremolite.^{11–18} The Val Chisone talc from Italy was studied by Pooley in 1972.¹⁸ Mine sample had intergrowths with serpentine-type, chrysotile asbestos along with tremolite and anthophyllite asbestos. The talc from Italy was named ‘American Ground Italian’ and designated as AGI 1615.^{19–21} This talc was diluted with a talc from another source to make it acceptable based on X-ray diffraction (XRD) protocols. However, it contained asbestiform tremolite and anthophyllite.²²

In this study, three laboratories analyzed a specific brand of talc from more than 50 containers of this cosmetic talcum powder product of different sizes and colors, produced over a 50-year time span to determine the presence of asbestos. The authors conducted independent product testing in unassociated laboratories in North Carolina, Georgia, and New York. A fourth laboratory, which also tested this product, will herein be referred to as Laboratory D. The lung and lymph node tissues from a woman who died from mesothelioma and testified to only using this specific brand of talcum powder were analyzed for the presence of asbestos and talc. This is the first report that explores the hypothesis that a specific brand of talcum powder coming from asbestos contaminated mines can find its way into the finished product that can be inhaled during use and cause or contribute to the development of mesothelioma

Materials and Methods

Laboratory A: product testing

In Laboratory A, over 50 containers of this particular brand of talcum powder were acquired from a variety of sources for bulk testing. Some of the containers were purchased online, while others were provided directly from the manufacturer. All of the containers were verified to be the correct brand and product.

Laboratory A tested talcum powder from each of the 50 samples using transmission electron microscope (TEM) methods. The procedure for testing by Lab A was as follows: 0.01 g of talcum powder was removed from its vial and suspended in 1 ml of distilled water with one to two drops of ethanol by brief sonication. From this suspension, 10 µl aliquots were removed and placed on a series of five formvar-coated nickel grids (100 grid openings each). In some cases, it was necessary to prepare additional sets of

five grids from the same 0.01 g sample of powder. The drops were allowed to dry in a covered Petri dish. The grids were then examined and analyzed with a Hitachi H-7000 STEM equipped with an Evex energy-dispersive spectrometer (EDS), for elemental composition and relative amounts of elements. The microscope was equipped with a tilt stage and a rotary specimen holder, which was employed with selected-area electron diffraction (SAED) analyses, as described below. Structures seen as fibers measuring at least five micrometers in length with aspect ratios of 5:1 or greater were analyzed to determine if they were regulated asbestos mineral fibers. We used EDS to chemically establish the presence of asbestos fibers and the crystalline structure was assessed using SAED. All 100 grid openings were observed and analyzed on each of the five grids for each product sample (at least 500 grid openings per sample analyzed).

Analyses were performed using a modification of the techniques described by Yamate *et al.*, and similarly adopted techniques used by the Environmental Protection Agency (EPA), American Society for Testing and Materials (ASTM), and International Organization for Standardization.^{23–26} All techniques required the use of a TEM equipped with an EDS system. Only in Yamate level III is the tilt and rotary stage optional to perform advanced SAED zone axis analysis. Yamate *et al.* stated that zone axis diffraction analysis is useful in differentiating between otherwise unidentifiable fibers.²³ In the Laboratory A analysis, zone axis analyses were not necessary as the identified amphiboles clearly demonstrated that they were asbestiform tremolite and anthophyllite confirmed by morphology, EDS chemistry, and characteristic 5.3 Å inter-row repeats on diffraction without tilting. Both asbestiform and non-asbestiform particles and fibers were present. However, in most cases this manuscript will refer to asbestiform fibers and state when they are tremolite, anthophyllite, or chrysotile type asbestos. A non-asbestos tremolite, anthophyllite will not be referred to as asbestos.

To calculate the fiber concentrations per gram of talcum powder, we first determined the number of asbestos fibers on average per grid opening. This number was multiplied by 552. The product of that equation was multiplied by 100, and then divided by 0.01 to yield the fibers/gram talcum powder value. The constant, 552, is the number of grid opening areas on the entire grid. One hundred is the number of 10 µl drops in 1 ml that the talcum powder was dispersed and the 0.01 was the weight of the talcum powder dispersed. Quality control procedures, which included testing of blanks from water, working in a clean hood environment, and working with only one



Figure 1 Pouring of powder into hands in glovebox.

sample at a time ensured that no laboratory contamination of samples.

Laboratory B: asbestos releasability testing

To determine if the user could inhale asbestos during a talcum powder application, Laboratory B assessed asbestos releasability by air sample. Air samples were generated during simulation in a glove box, consistent with normal product use in a controlled environment. These three samples included the same samples tested by Laboratory A. Environmental and personal air samples were collected using standard airborne asbestos techniques, using high-volume air pumps for environmental (stationary) samples inside and outside of the controlled area, and low-volume air pumps for personal samples taken at a distance comparable to the breathing zone of the person simulating application. Standard TEM 385 mm² effective filter area 25 mm cassettes with 0.45 µm MCE filters were used on the flow-calibrated high (7–12 l/min) and low volume (1–4 l/min) air pumps (Figs. 1 and 2).

The resulting air samples were analyzed for airborne asbestos following the analytical procedures described in the U.S. Environmental Protection Agency Code of Federal Regulations 40 CFR part 763, subpart E, Appendix A — AHERA for direct preparation of MCE filters.²⁴ All final analyses by Laboratory B were conducted on a JEOL 2000FX TEM equipped with an energy-dispersive X-ray analyzer detector and SAED at magnifications up to $\times 50\,000$, using the fiber counting criteria specified by Yamate *et al.*'s protocols.²³

Laboratory C: product bulk testing and bathroom-sized chamber releasability

Bulk methods

Laboratory C examined nine samples under an Olympus SZ-40 stereomicroscope at magnifications from $\times 7$ to $\times 40$. Portions of the particulate found in the sample were mounted in Cargille refractive index liquids for analysis by polarized light microscopy (PLM) using an Olympus BH-2 PLM with a magnification range from $\times 100$ to $\times 1000$. The PLM analysis followed the procedures for bulk analysis of building materials described by the US EPA in 1993.²⁴ Characterization of the fibers was performed using a Philips EM420 100 kV TEM equipped with an Oxford INCA EDS x-ray analysis system and capable of SAED work involving tilting of amphibole fibers. Zone axis determinations were also conducted. We used TEM asbestos fiber counting criteria of fibers greater than 0.5 µm in length with at least a 5:1 aspect ratio as described in Asbestos Hazard Emergency Response Act (AHERA) and ASTM methods: D6281, D5755,

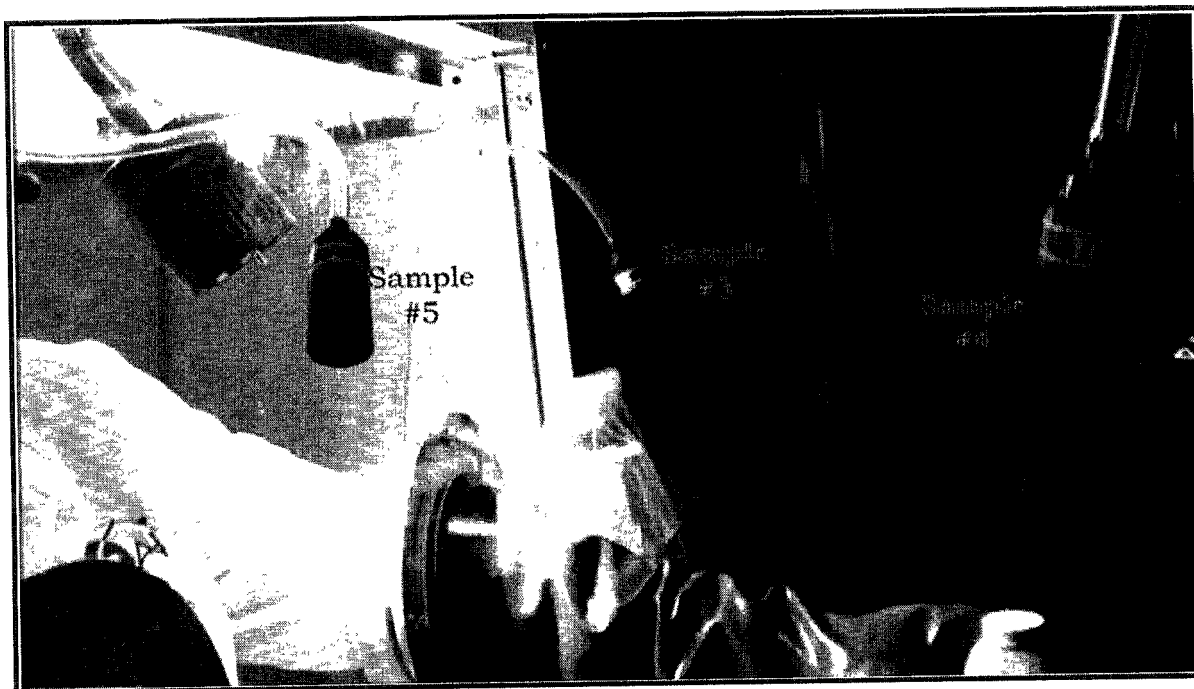


Figure 2 TEM cassettes in simulation area in glovebox.

D5756, and D648.^{24–28} Data were recorded using the ASTM D6281 format. XRD analysis was performed by an outside laboratory (DCM Science Laboratory, Inc., Wheat Ridge, CO, USA) scanning over a range of 3–45° 2 θ using 40 kV, 25 mA Cu K_{α} radiation. Mineral phases were identified with the aid of computer-assisted programs accessing a CD-ROM powder diffraction database.

Air testing

Tests to determine airborne levels of asbestos fibers resulting from application of this brand of talcum powder were performed in a testing chamber. The chamber was built to match the bathroom of the patient that used this brand of cosmetic talc. Her bathroom was measured at 7 feet, 9 inches high by 5 feet by 4 feet, 1 inch. All talc products used in these chamber tests had previously been tested in Laboratories A, B, or both.

Air test — shaker container

Using Personal Protective Equipment, a volunteer applied one of the bulk tested cosmetic talcum powders to his body using a shaker container. This particular talcum powder contained approximately 0.1% by weight and approximately 18 million anthophyllite asbestos fibers per gram. The container was weighed before and after the testing to determine the approximate weight of material applied. The talcum user wore a respirator and a bathing suit. The volunteer twisted the top of the container and shook material onto his hand. He applied the talc under his arm and around the shoulder and upper arm area. He then shook the talcum powder onto his other hand and applied it to the other underarm, shoulder and upper arm area. He shook out additional material and applied it to his neck and upper torso. He shook out and applied material two more times for a total of five applications. The total talcum application time was approximately 1 min and amounted to 0.37 g of the talcum powder. Two air samples were collected in the applicator's breathing zone at 0.5 l per minute (lpm) and two additional air samples were collected in the breathing zone at 1.0 lpm with commercial open-face air cassettes. The five-minute sampling time included the application time and a waiting period. The bystander in the test chamber had two air cassettes in his breathing zone for the five-minute period including application and the additional waiting time. The bystander wore a respirator and full protective clothing. These air samples were collected at rates of one and 2 lpm. No activities were conducted during the waiting period other than checking the pumps and cassettes. The air filters and two additional blank filters were analyzed by phase contrast microscopy (PCM) using National Institute for Occupational Safety and Health (NIOSH) Method 7400.²⁹ Two air

samples and two blanks were also analyzed by NIOSH Method 7402 via transmission electron microscopy to determine the percentage of asbestos fibers among the fibers counted by PCM.²⁹ An air sample collected from within the test chamber before the study was analyzed by a more sensitive TEM procedure following the EPA AHERA method.²⁴

Air testing puff applicator

In this test, a volunteer applied a different cosmetic talcum powder sample using a puff applicator. This particular talcum powder contained approximately 0.05% anthophyllite asbestos (approximately 70 million asbestos fibers per gram). The container was weighed before and after the testing to determine the approximate weight of material applied. The talcum user wore a respirator and a bathing suit. The talc user opened the puff container and applied the talcum powder as described above only this time with a powder puff. He then repeated the process for a total of six applications. The talcum application time was approximately 1 minute. Two air samples were collected in the applicator's breathing zone at 0.5 lpm for a sampling period of 4 minutes. One air sample was collected for a shorter period (3.3 minutes) that included the application period. Another air sample was to be collected after the application period but this sample was voided because the volunteer hit the air cassette and the cassette fell off the vacuum hose. The bystander in this test followed the same protocol as described above. Both air samples were collected at a rate of 0.5 lpm. No activities were conducted during the waiting period other than checking the pumps and cassettes. The air filters and two additional blank filters were analyzed by PCM using NIOSH Method 7400 as described above.²⁹ One air sample and two blanks were also analyzed by NIOSH Method 7402 via TEM to determine the percentage of asbestos fibers among the fibers counted by PCM.³⁰ An air sample collected from within was tested as described above by EPA AHERA method.²⁴

Human Tissue Analysis

TEM

Tissue samples from a woman with no other known exposure to asbestos other than her use of the product tested was supplied to Laboratory A. Human tissue analysis was performed according to the techniques described in Wu *et al.*²⁹ Lung and lymph node tissue was received fixed in formalin. Half of the tissue was removed from the lung and the lymph node tissue. Two grams of lung tissue were divided twice. The two halves of the lymph node weighed 0.16 g together. The two specimen types were separated throughout the study. The tissue from each was first digested in a 5% solution of potassium

hydroxide (KOH) for approximately hour at 60°C. The dissolved lung and lymph node material was then centrifuged in a high-speed centrifuge to separate the inorganic material from the dissolved organic tissue. The solute material containing the dissolved organic material and KOH was removed and distilled water was added. The inorganic material was re-suspended in the water by brief sonication. The material was re-centrifuged and the process of washing the inorganic material was performed five times. After the fifth wash, the distilled water was removed and replaced with 10 ml of fresh distilled water and the inorganic material was re-suspended by brief sonication. Ten microliter samples were removed from the suspension and placed on formvar-coated nickel grids on a metal mesh in a covered glass Petri dish to dry. Five grids were initially prepared and an additional set of five grids was prepared for each tissue type for a second analysis. The dried grids were observed with a transmission electron microscope. Four hundred grid openings on at least four grids were analyzed, and a fifth grid was used if grid openings were broken in the initial four examined grids. The fiber concentrations per gram wet weight lung or lymph node tissues were calculated from the number of fibers observed, the area analyzed, the aliquot ratio, and the total weight of the tissue sample digested.

Light microscopy

Tissue sections

Small lung tissue samples were put into 10% phosphate-buffered formalin and processed for embedding in paraffin. Five micrometer paraffin sections were cut, mounted on glass slides and stained with hemotoxylin, eosin, and an iron stain. The tissue was evaluated for the presence of altered morphology and/or ferruginous bodies; two characteristics often seen in lung tissues that are a byproduct of iron-rich protein deposits on asbestos fibers resulting from macrophage frustrated phagocytosis.

Digested lung and lymph node tissue

Two hundred and fifty microliters of digested lung and lymph node material suspension used for TEM analyses was placed in a cytocentrifuge and the slides were cover slipped and observed by phase contrast light microscopy. The entire area was counted for ferruginous bodies and calculated back to the weight of the tissue to determine the concentration of bodies per gram of wet weight tissue.

Scanning electron microscopy (SEM)

SEM samples were prepared by taking 250 µl of the suspended inorganic material used for the TEM and light microscopy analyses and placed on a 0.1 µm pore size Nucleopore filter mounted on a carbon planchette on an aluminum SEM stub. The material

was allowed to dry in a covered Petri dish. The stub was then coated with vaporized carbon and observed with a Hitachi S-4300 field emission scanning electron microscope equipped with an Evex EDS system. The entire filter sample surface was scanned for fibers and asbestos bodies.

Results

All three laboratories confirmed in multiple tests the presence of asbestiform anthophyllite and asbestiform tremolite in the talcum powder products, just as had been found and described by Rohl and Langer over three decades ago.⁶

Initial bulk analyses of 50 samples of this product in Laboratory A showed that all of the samples contained asbestos fibers. Eighty percent contained only anthophyllite asbestos, 8% only tremolite asbestos, 8% anthophyllite and tremolite asbestos and 4% anthophyllite, tremolite, and chrysotile asbestos. The range in asbestos concentrations of fibers >5 µm in length were calculated to be, at a minimum, between 1840 and 1 104 000 fibers per gram of talcum powder. More than 80% of the tested cans and plastic containers contained over 10 000 asbestos fibers/gram of talcum powder. Four of the containers had less than 5000 fibers per gram and six containers had more than 250 000 fibers per gram. However, it should be noted that there were many asbestos fibers that also had aspect ratios less than 8:1. These fibers were generally found to be shorter than 5 µm and were noted, but not counted in the original product testing or in the lung and lymph node tissue testing by Laboratory A. There were also a number of fibrous talc particles that were easily distinguishable from asbestos by morphology. If there was a question regarding their identity, both EDS and SAED were employed to recognize such fibers as talc. All the fibers that were actually counted in bulk and tissue preparations were 5 µm or greater in length, with aspect ratios for the most part greater than 10:1. The majority of asbestos structures counted demonstrated aspects ratios >15:1, with many >20:1. A minimum of four fibers was identified in each sample, making the concentration determinations of asbestos statistically significant and reproducible.

Laboratory C, using PLM, TEM, and XRD, tested nine samples of the specific brand of talcum powder described above. Generally, the PLM analysis showed that the samples contained both platy and fibrous talc, less than 1% by volume of the PLM visible amphibole fibers and some quartz. The majority of the PLM amphibole particles had low aspect ratios (length to width) but some were >10:1. By XRD, one of the talcum powder samples was found to contain 4% anthophyllite. No amphibole



Figure 3 Application of powder from shaker in bathroom-sized chamber.

minerals were detected in the other eight samples by XRD. The XRD detection limit was approximately 2% by weight. In TEM analysis, all nine samples were positive for amphibole asbestos (primarily anthophyllite), and were confirmed with zone-axis electron diffraction measurements. At least five asbestos fibers per sample were recorded in each sample, with concentrations ranging from 0.004 to 0.9% by weight and from 3 to 200 million asbestos fibers per gram of fibers greater than 0.5 μm in length with at least a 5:1 aspect ratio.

Air monitoring

Releasability of asbestos into the air from the products was assessed by glove box simulation testing by Laboratory B, and by full chamber testing by Laboratory C. In a manner consistent with methods used by the EPA, NIOSH or ASTM, study product body powders and dusting powders were applied hand to hand and hand to arm. Consistent with bulk testing results, anthophyllite and tremolite asbestos was repeatedly found in the air tests resulting from these simulations (Figs. 6–8).

Shaker container test

The shaker application test used 0.37 g of talcum powder (Fig. 3). For the talc user, the average PCM fiber concentration in his breathing zone during application was 4.8 F/cc (3.1, 7.3, 3.9, and 4.9 F/cc). The asbestos to total fiber percentage as determined by TEM was 40%. Therefore, the asbestos concentration in the breathing zone of the talc user during application was 1.9 F/cc. For the bystander the PCM fiber concentration was 1.35 F/cc (0.9 and 1.8 F/cc) and the TEM derived percentage of asbestos was 35%, which results in a bystander asbestos concentration of 0.5 F/cc. No asbestos fibers were found in the sample collected in the chamber before the testing or in the blank filters.

Puff application

The puff application test used 6.25 g of talcum powder (Figs. 4 and 5). For the talc user, the average



Figure 4 Application with powder puff in bathroom-sized chamber.

PCM fiber concentration in his breathing zone during the 5-minute sampling period was 20 F/cc (23.6 and 16.5 F/cc). The asbestos to total fiber percentage as determined by TEM was 21%. Therefore, the asbestos concentrations in the breathing zone of the talcum powder user were 5 and 3.5 F/cc. The short term sample in the breathing zone of the applier had a PCM value of 60 F/cc. Using the TEM-derived percentage of asbestos of 10%, result for the short-term sample was an asbestos concentration of 13 F/cc. For the bystander, the PCM fiber concentration was 11.7 F/cc (13.7 and 9.7 F/cc). Using the minimum TEM-derived percentage of asbestos of 36% results in a bystander asbestos concentration of 4.9 and 3.5 F/cc. No asbestos fibers were found in the sample collected in the chamber before the testing or in the blank filters.

The tests performed independently by Laboratory C using a bathroom-sized room confirmed the findings for asbestos fiber release found by Laboratory B's glovebox testing. Samples showed that significant concentrations of anthophyllite, tremolite, and occasionally chrysotile asbestos were released in the simulated application of several iterations of the products. This confirmed not only



Figure 5 Application with a powder puff in bathroom-sized chamber.

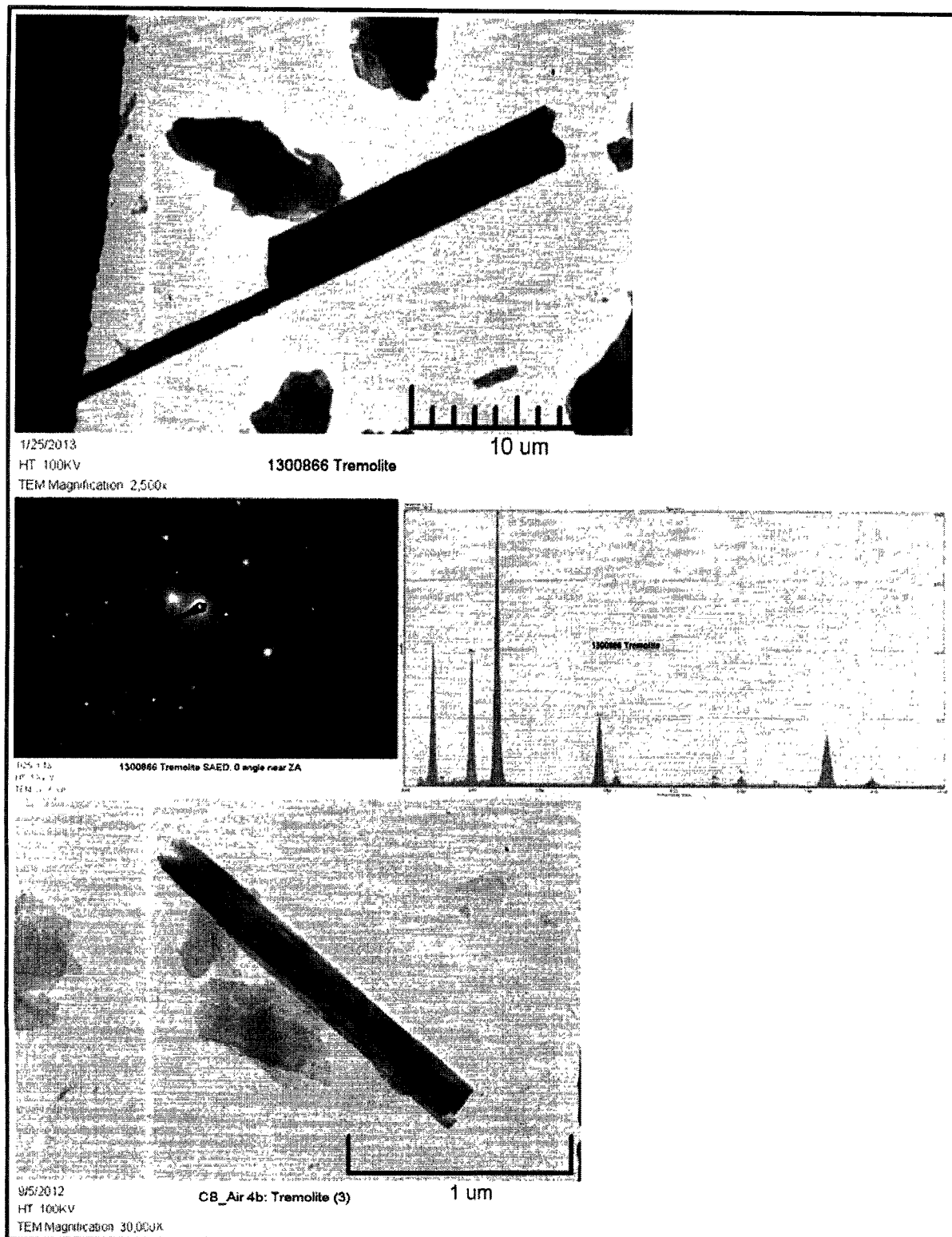


Figure 6 Tremolite asbestos from TEM analysis of releasability air testing of product (images, EDS, and SAED).

the presence of asbestos in the talcum powders, but also that the asbestos contained in the friable powders was easily aerosolized in a manner consistent with the products intended use; confirming the hypothesis that the cosmetic powders are capable agents of exposure to asbestos

Human tissue analysis

Electron microscopic analysis of the lung tissue revealed amphibole type asbestos fibers in a calculated concentration of 1380 and 4150 fibers per gram wet weight, respectively, with a limit of detection of 690 fibers per gram wet weight. All fibers counted

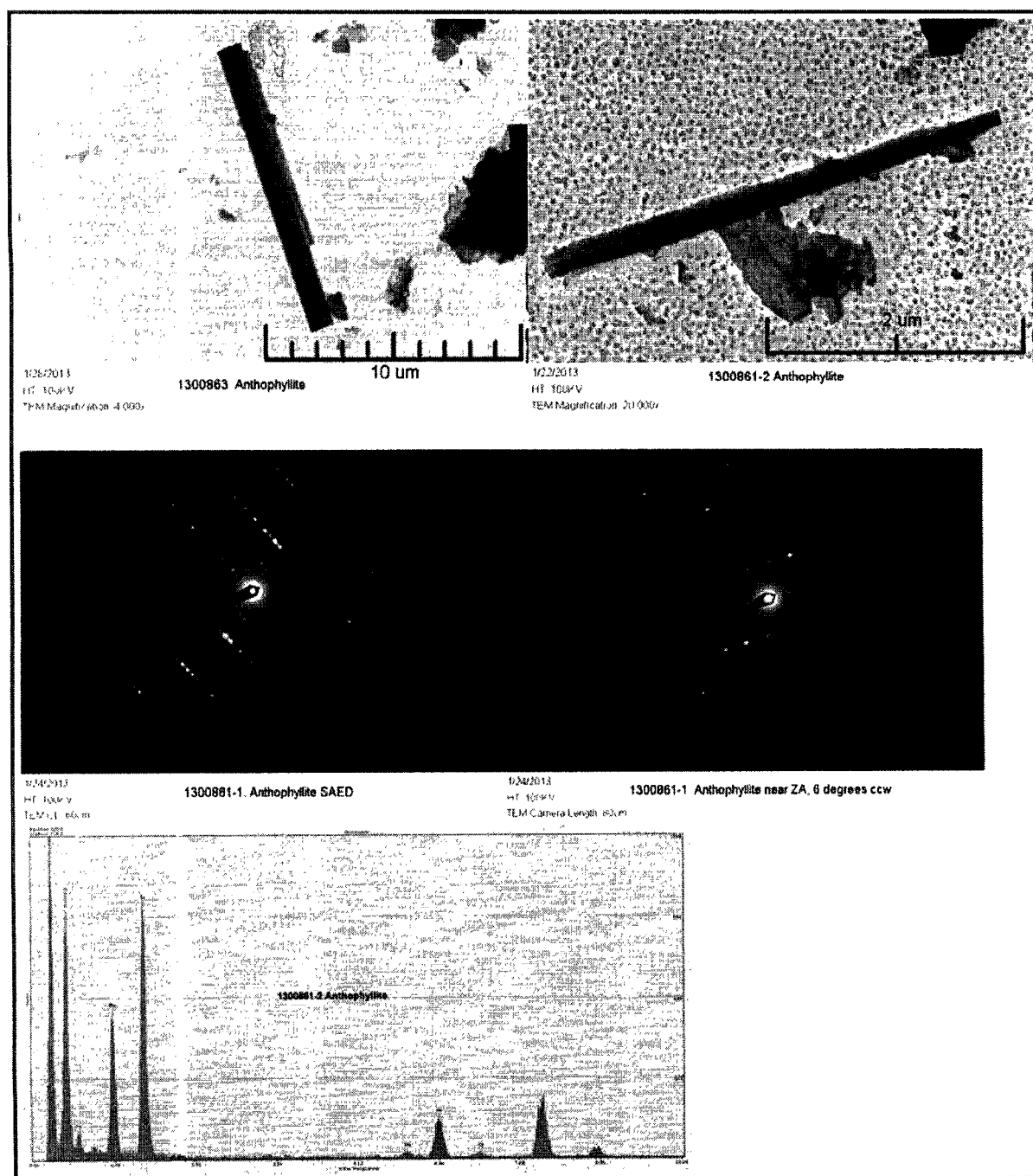


Figure 7 Anthophyllite asbestos from TEM analysis of releasability air testing of product (images, EDS, and SAED).

were 5 µm or greater in length and had aspect ratios of 20:1 or greater. The amphiboles were identified by EDS and SAED analysis as anthophyllite (Fig. 9) and tremolite (Fig. 10) asbestos. The asbestos fibers were seen in a ratio of 1:1 and 2:1, respectively (anthophyllite/tremolite). There were many anthophyllite and tremolite asbestos fibers less than 5 µm in length that were not counted. The majority of these smaller asbestos fibers were of the anthophyllite type. Light microscopic analysis of the cytocentrifuge preparation revealed a calculated concentration of 140 asbestos bodies per gram wet weight of lung

tissue by phase contrast light microscopy in both samples.

Electron microscopic analysis of the lymph node tissue revealed amphibole asbestos fibers in a calculated concentration of 12 738 fibers per gram wet weight, with a limit of detection of 2123 fibers per gram wet weight. All counted fibers were at least 5 µm in length with aspect ratios of 10:1 or greater. The amphiboles were identified by EDS and SAED analysis as anthophyllite and tremolite and they were seen in a ratio of 5:1 anthophyllite/tremolite. There were many anthophyllite and tremolite fibers less

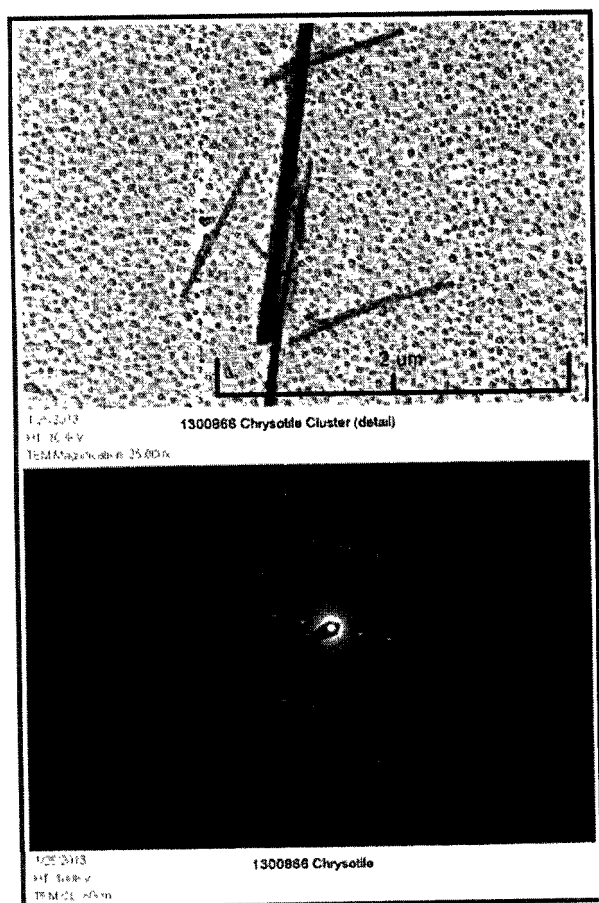


Figure 8 Chrysotile asbestos from TEM analysis of releasability air testing of product (image and SAED).

than 5 μm in length that were not counted. We also observed but did not count tremolite cleavage fragments. Light microscopic analysis of the cytocentrifuge preparation revealed a calculated concentration of 92 asbestos bodies per gram wet weight of lymph node tissue by phase contrast light microscopy (Fig. 11).

Histological sections of the tissue showed focal areas of mild parenchymal fibrosis and a more generalized pleural fibrosis. Although many ferruginous bodies were identified in the cytocentrifuge preparation, most were relatively small and not seen in the H&E-stained paraffin sections. These macrophages were clustered and contained a combination of fibrous and platy talc and small asbestos bodies.

In addition to the fibrous and platy talc described above, other inorganic materials were seen. Aluminum silicates and magnesium aluminum silicates in both fibrous and platy form were identified. We elected not to count these fragments. Their presence supports the hypothesis that the lung and lymph node samples match findings from the tested talcum powder.

The two analyses performed on the lung tissue were from two separate tissue digestions. The second was prepared with tissue not previously analyzed, but

saved from the original half of the tissue retained by Laboratory A. The results proved to be completely reproducible with no finding of any additional fiber types other than those reported above.

Confirmation of interlaboratory analyses

After several years of independent testing in separate laboratories, the authors became aware of one another's work through litigation. The finding that this historic brand of cosmetic talcum powder contained asbestos fibers with generally the same morphological and chemical assemblage was confirmed. A fourth laboratory (Laboratory D) tested many of the same samples, but did not report asbestos findings. Owing to the inconsistency with the other laboratories, re-examination of results from Laboratory D was warranted.

Two of the three authors of this study went to the Laboratory D and were supplied with the prepared filters on TEM grids or SEM stubs previously analyzed by Laboratory D. They were also supplied with both TEM and SEM microscopes to re-analyze the specimens, along with data and locator sheets, allowing for the same grid openings and areas to be observed as in the initial analyses.

Reanalysis of subject product samples

One author re-analyzed the TEM preparations of 20 study products of talcum powder prepared by Laboratory D. Asbestos structures were found in the re-analysis, some of which were named in the original analysis as cleavage fragments, intergrowths, or fibrous talc rather than as asbestos. Although the author-reviewer agreed with many of the non-asbestos fibers identified, he concluded the original analyses were incomplete. Additional analyses by the author-reviewers showed some of the incompletely analyzed fibers to be asbestos. In other cases, asbestos found on re-analysis was located on areas of the filter where no fibers were recorded in the original bench sheets or reports. In some instances, the overall distribution of particulates on the preparations was inhomogeneous, in contrast with the method of choosing grid openings for the original analysis by skipping every other opening in a "checkerboard" fashion. Furthermore, the methods named on the analytical count sheets were not the same as the methods cited in the reports from Laboratory D.

Laboratory D reported no asbestos fibers in the 20 samples analyzed. In contrast, asbestos fibers were identified in all 20 of the same products in Laboratory A and in 16 of 20 products tested by Laboratory B. In the re-analysis of those same 20 samples originally analyzed by Laboratory D via TEM, eight were found to contain asbestiform anthophyllite, six asbestiform tremolite, and two were found to contain chrysotile fibers. These findings were significant because re-analysis was not a

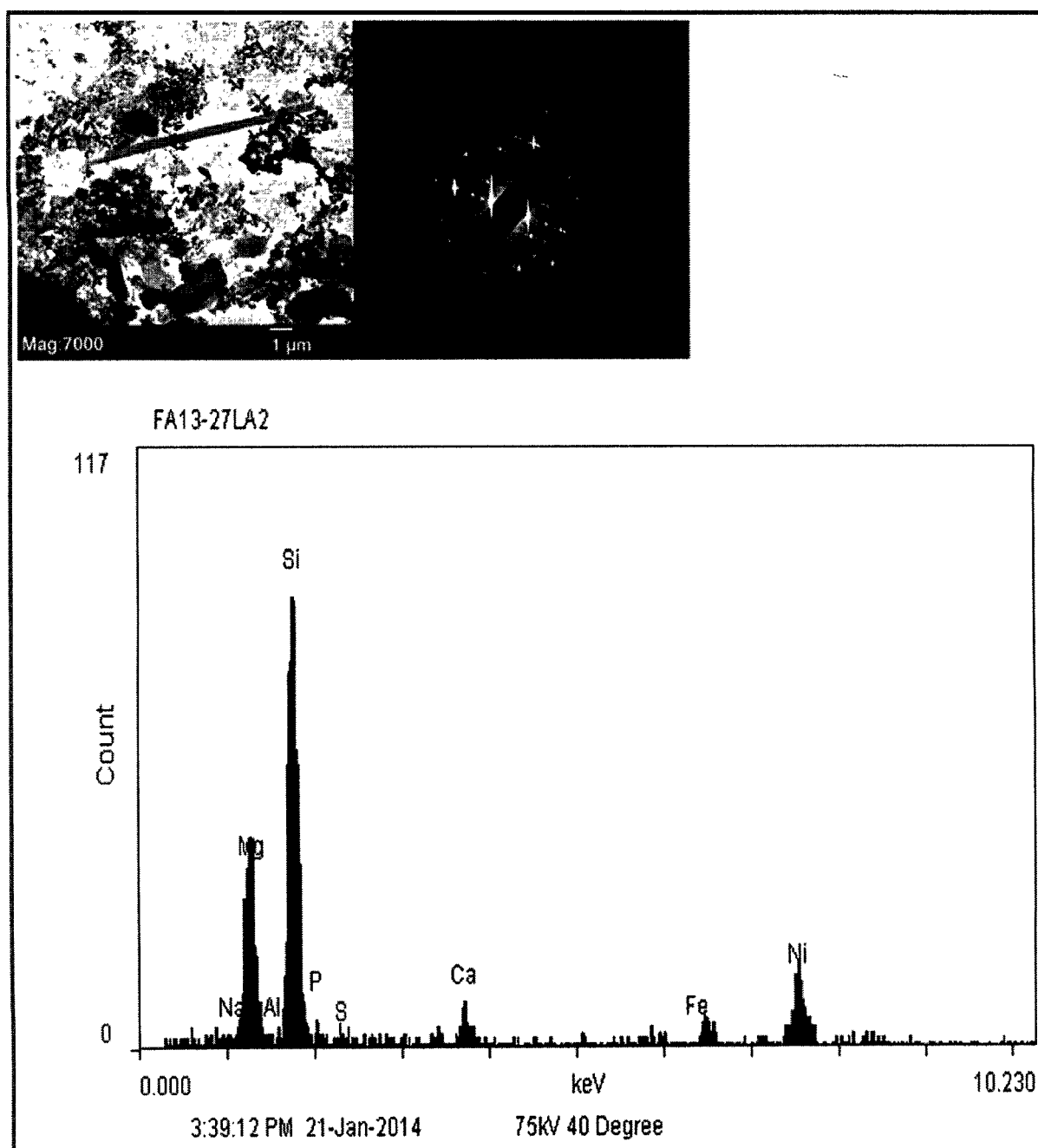


Figure 9 This asbestos fiber is a representative sample removed from the lung tissue of the patient exposed to cosmetic talcum powder. Anthophyllite asbestos fiber is observed and its SAED pattern is demonstrated beside it with the EDS spectra.

complete replication of the original analysis due to time constraints, damage, or unsuitable preparations. It was apparent that the technicians in Laboratory D missed fibers and misidentified asbestos fibers as non-asbestos.

Re-analysis of human tissue

Laboratory D also performed fiber burden analysis on human tissue with differing results than the study of the authors. Similar to the re-evaluation of bulk analyses, two author-reviewers analyzed the human tissue sample preparations of Laboratory D together and found significant differences in their analyses compared to the technicians who originally analyzed

the grids and stubs. We determined that the technicians misidentified anthophyllite asbestos fibers that had been coated with iron and protein (anthophyllite asbestos bodies) as either cleavage fragments or as amosite fibers (Fig. 12). Furthermore, it is the authors' consensus that there are no generally accepted criteria to classify individual fibers as cleavage fragments by TEM when the sample contains attributes of an asbestos fiber or countable structure. When Laboratory D technicians initially looked for asbestos bodies to determine the fiber core, they concluded that most were amosite. However, when the two author-reviewers examined

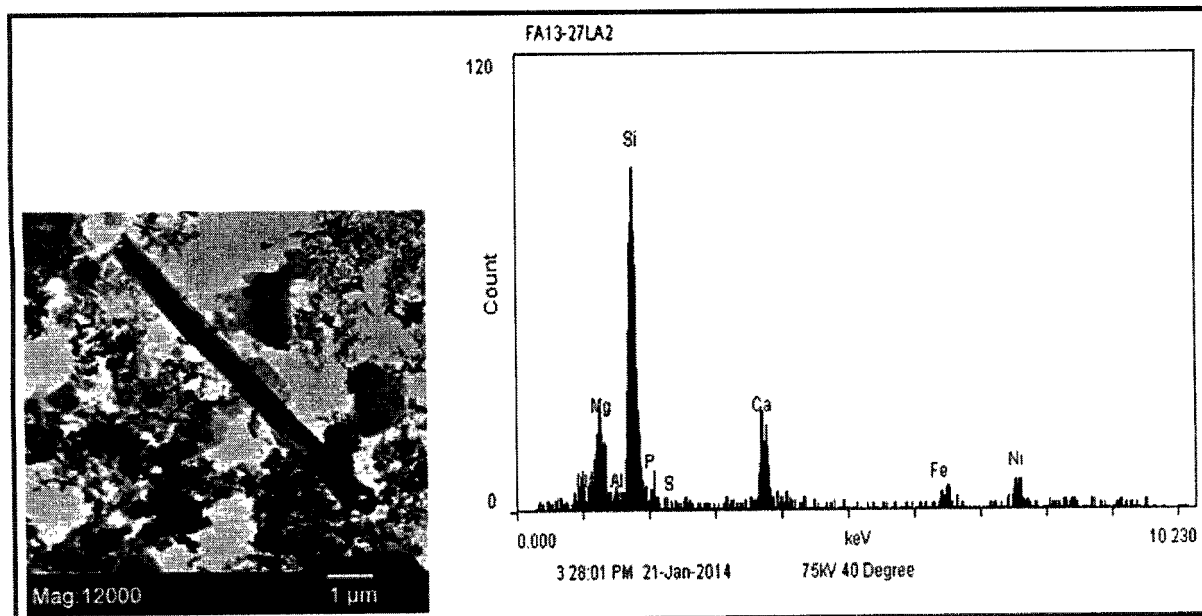


Figure 10 This asbestos fiber is a representative sample removed from the lung tissue of the patient exposed to cosmetic talcum powder. Tremolite asbestos fiber with its corresponding EDS spectra.

the same structures, it was clear that the cores were either anthophyllite or could not be determined because there was exposed fiber core. In previous studies of human tissue having anthophyllite and anthophyllite bodies (Fig. 11), it was common to find that the entire anthophyllite core, even if quite long, was completely coated.

Zone axis confirmation in bulk, tissue, and air
Laboratories A, B, and C confirmed original amphibole asbestos structures by zone axis diffraction. Laboratories A, B, C, and D re-analyzed archived preparations with the intent of confirming amphiboles by zone axis diffraction. In all four sets of re-analyzed preparations, anthophyllite and tremolite asbestos were consistently



Figure 11 These are asbestos bodies from the patients lung tissue taken by SEM. It is possible to see in the one to the left that the fiber is almost completely covered by the iron protein coating. This is compared to the one at the right which appears to have much more fiber exposed. However, upon EDS testing, it was determined that in both cases, these were anthophyllite fibers and they were both entirely coated, although much thicker in some areas as opposed to others.

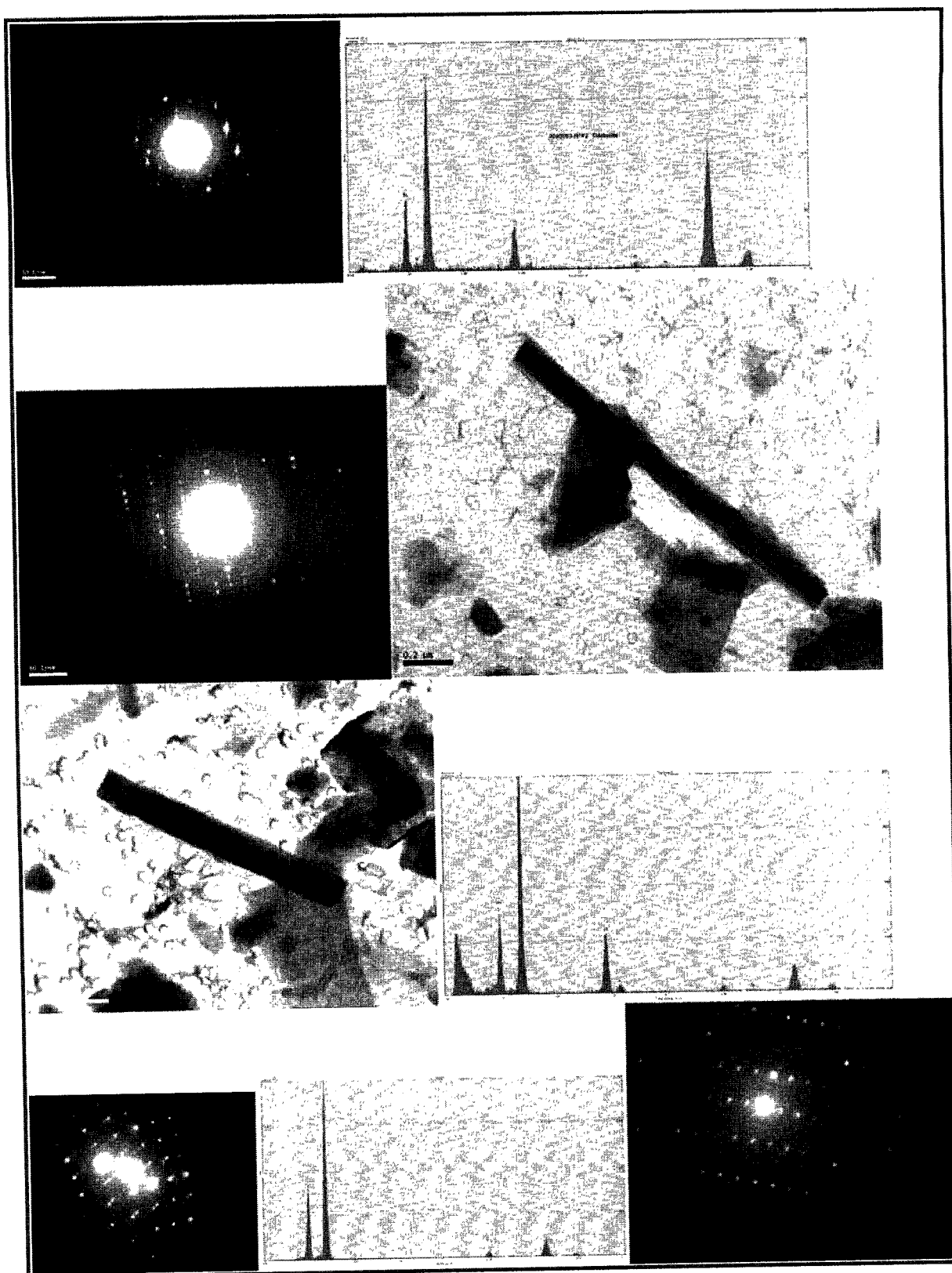


Figure 12 Tremolite and anthophyllite asbestos from re-analyses of 'Lab D' preparations (images, EDS, and SAED).

confirmed by zone axis diffraction pattern measurements. This included confirmation of asbestiform amphiboles, including anthophyllite and tremolite asbestos

from the original product testing, from the releasability air tests, and from TEM preparations of lung and lymph node tissues.

Discussion

Historically, many mesotheliomas, particularly abdominal mesotheliomas in women, have been labeled idiopathic due to a lack of an identifiable source for asbestos exposure. Further, there has been an increase in the number of idiopathic pleural and abdominal mesotheliomas in women using this specific brand of talcum powder. There have been a few studies that have examined talcum powder and its potential to cause ovarian tumors.³⁻⁵ The studies were inconclusive, but suggested that talc, asbestos, or both may cause these cancers through vaginal exposure.⁴ These studies attributed asbestos found within the women's lesions to result from contact with their partners. There was no consideration for the potential of the asbestos being a contaminant in the women's talcum powder.^{3,4} However, it has been reported that cosmetic talcum was contaminated with asbestos, and that asbestos was found in the mines from which the talc originated.^{6,9} Our findings indicate that historic talcum powder exposure is a causative factor in the development of mesotheliomas and possibly lung cancers in women.

Talc has been identified as a causative for mesotheliomas in New York talc miners.³¹ In recent years, more than 10 women developed mesothelioma and their only source of asbestos exposure was the use of one brand of talcum powder. This study demonstrates that the brand of talcum powder tested contained asbestos. Furthermore, we have traced the asbestos in the talc to the mines from which it originated, into the milled grades, into the product, and finally into the lung and lymph nodes of the users of those products, including one woman who developed mesothelioma.

Based on the testing and re-testing conducted by the authors, it is evident that this product line has been consistently contaminated with asbestos tainted talc derivatives. The amount of asbestos was variable based on the time of manufacture and the talc source. There have been numerous publications that have indicated that the talc in many talc deposits had asbestos contamination.³²⁻³⁵ The most common types of asbestos were tremolite and anthophyllite. These are the same asbestos fiber types found in the autopsied lungs and lymph nodes tested here for asbestos presence. In a few containers tested in this study, chrysotile was also found, consistent with the source ore geology.

Most, if not all, testing of cosmetic talc was performed using techniques designed for light microscopy, PLM, or by TEM criteria designed to test air and water samples. Testing determined if asbestos levels were above the EPA standards under AHERA or the Occupational Safety and Health Agency standards. These protocols are based on the parameters described

in the Yamate method.²³ There are significant limitations to these methods. PLM analysis misses small fine asbestos fibers or fibrils because the limits of the resolution are approximately 0.2–0.5 μm for different forms of light microscopy. Based on our findings, approximately 90% of the fibers identified fall into this category. Determining the number of TEM grid openings to be counted during the analysis requires stopping factors, or limits on the quantity of analysis to be performed. The Draft Yamate method (1984) gives the guidelines of "100 fibers or 10 grid openings, whichever is first."²³ This counting rule was instituted for cost limitation purposes. The Draft Yamate method describes that while this guideline of using 10 full-grid openings represents a judicious compromise between a reasonable experimental effort and a fairly low value of the detection limit, the analysis of additional TEM grid openings reduces the detection limit and improves the precision of the estimates. In the talc study described here, a very low level of detection was desired and therefore, in some cases, as many as 500 plus grid openings were analyzed to reduce the detection limit and improve sensitivity of the test. TEM testing has been adequate for evaluating building material asbestos abatement projects, local air sampling, and potential water contamination with asbestos.²³ However, these criteria are not acceptable for assessing asbestos fiber burden analyses in human tissues and for low asbestos content products that are used intermittently in small quantities over long periods of time, such as cosmetic talcum powder.³⁶ Talc related asbestos exposures can be heavy at times, above 4000 F/cc. The inhaled asbestos fibers are extremely variable in the causation of asbestos related tumors and fiber burdens found in the deceased woman were within the reported ranges for amphiboles to be causative factors in the development of such a tumor.³⁷

Therefore, it is imperative to analyze products such as talcum powder for small amounts of asbestos fibers. This requires that the limits of detection be lower than levels required in a typical Yamate analysis. The author-reviewers observed that the Laboratory D analyses were done using Yamate methodology and no more than 10–25 grid openings on bulk TEM grid preparations were observed.²⁴ Based on Laboratory D's protocols for testing, millions of fibers/gram of talc would have to present in order to find fibers. Lower concentrations in the ranges found by Laboratories A, B, and C demonstrated that fibers were detectable and present at levels sufficient to cause mesotheliomas.

Although long narrow asbestos fibers are highly carcinogenic, shorter, narrow fibers are also dangerous.³⁶⁻³⁸ It is now more common to find shorter narrow fibers in human tissue digestions than long narrow fibers, especially for chrysotile.³⁹ This

study provides evidence that low concentrations of asbestos in raw materials do not necessarily correlate to low health risk.^{38,39} Examples of recent studies of low asbestos content producing significant airborne concentrations in simulated activity include activity-based monitoring of asbestos as it naturally occurs in several sites, as conducted by the EPA and Agency for Toxic Substances and Disease Registry, and vermiculite-containing attic insulation studies.⁴⁰ These studies have repeatedly shown that substantial airborne concentrations could be derived from materials with only a fraction of a percent asbestos content.³⁶ This has been especially true when a product was in a friable state, or where the obvious use of material intimates aerosolization of fibers. Significant airborne concentration can be easily generated from such conditions when asbestos is a constituent.^{40–43}

The talc application studies were simulations of exposures to talc used by a deceased woman who had mesothelioma. The air volume in the testing space was 158 cubic feet. This is in the range of the chamber sizes used by talcum powder manufacturers in the 1970s in their studies of the quantity of talcum powder used in normal application. The space used by Russell was 171 cubic feet and the space used by Aylott was between 152 and 163 cubic feet. The amount of material used in the shaker test was 0.37 g. The amount used for the puff applicator test was 6.25 g.^{44,45} The shaker test was a light application and the puff a heavy application. However, the heavy application was within the ranges published by Russell of 8.84 ± 8.32 g and Aylott of 2.5 ± 12.5 g. The “talc times,” or the duration of talcum powder application, were approximately 55 seconds for the shaker test and approximately 57 seconds for the puff applicator test.^{44,45} These were within the ranges published by Russell of 83 ± 33 seconds and Aylott of 28–78 seconds for adult dusting.^{44,45} Laboratories A and B determined that the contaminated talcum powder released inhalable asbestos into the air.

Another issue in this study was the documentation and identification of cleavage fragments. The scientific community has not generally adopted cleavage fragment differentiation criteria.⁴⁶ It is unclear how to identify a cleavage fragment once the stone or material has been finely ground. Two criteria for distinguishing cleavage fragments from asbestos fibers have been proposed. The first is that the ends of cleavage fragments have oblique angles and second is that the aspect ratios are all less than 20:1. The ends criterion has not been validated with known asbestos/cleavage fragment standards and while an aspect ratio of 20:1 suggests that a fiber is likely to be an asbestos fiber, some fibers with aspect ratios below

20:1 are also asbestos. As the fiber aspect ratio increases, the percentage of asbestos fibers versus cleavage fragments also increases.⁴⁷ However, this criteria falls short when the fiber is extremely thin and is the smallest unit of diameter of a fiber. When these small fibers are removed and analyzed from human tissue, these criteria have to be discarded because enzymes with basic and acidic molecules within cells can leach elements from the surface, causing a breakdown of the fibers, especially when thin in diameter. van Orden *et al.* propose criteria to identify cleavage fragments by SEM.⁴⁶ The criteria are based on surface contours which identify a cleavage fragment.⁴⁶ However, this method has not been verified and is not generally accepted. There were no photographs of TEM or high-resolution high-magnification SEM provided by Laboratory D, which classified potential asbestos fibers as cleavage fragments

In conclusion, we found that a specific brand of talcum powder contained identifiable asbestos fibers with the potential to be released into the air and inhaled during normal personal talcum powder application. We also found that asbestos fibers consistent with those found in the same cosmetic talc product were present in the lungs and lymph node tissues of a woman who used this brand of talc powder and developed and died from mesothelioma.

Disclaimer Statements

Contributors All authors did studies relevant to the manuscript and all contributed and accepted all the writing.

Funding The work done was paid for by attorneys for litigation purposes. No funds were for writing of this manuscript.

Conflicts of interest Funding for all Labs was provided as part of litigation. No funds were for writing this article. Laboratories are available for defense or plaintiff litigation.

Ethics approval Ethical consent was not needed.

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