



CERTIFICATE OF ANALYSIS

Chain of Custody: 308006

Client: US Food & Drug Administration

Address: Office of Cosmetics & Colors

4300 River Road

College Park, MD 20740

Attention: John Gasper

Job Name: Task 3 - Analysis of Official Samples

Job Location: 4th Group - 15 Samples

Job Number: CLIN 1 - Task 3

PO Number: HHSF223201810337P

Date Submitted: 7/24/2019

Date Analyzed: 8/20/2019-9/18/2019

Report Date: 10/3/2019

Date Sampled: Not Provided

Person Submitting: Goran Periz

Revised: 10/11/2019 (Revision #2)

SUMMARY OF ANALYSIS

AMA Sample ID	Client Sample ID	TEM LOD Using ASTM D5756 Mass Calculation	TEM LOQ Using ASTM D5756 Mass Calculation	% Tremolite by TEM Using ASTM D5756 Mass Calculation	% Chrysotile by TEM Using ASTM D5756 Mass Calculation	% Total Tremolite & Chrysotile by TEM Using ASTM D5756 Mass Calculation	% Asbestos by PLM	% Organics	% Acid Soluable	% Other	Comments
308006-6	D-58	0.0000169%	0.00000675%	ND	ND	ND	ND	0.3%	6.7%	93.1%	Gravimetric Loss from PLM Prep: Organics = 0.3%; Acid Soluable = 7.1%; Other = 92.6%
308006-6A	D-58	0.0000133%	0.00001485%	ND	< 0.00001%	< 0.00001%	ND	0.2%	19.5%	80.2%	Gravimetric Loss from PLM Prep: Organics = 0.2%; Acid Soluable = 8.5%; Other = 91.3%
308006-6B	D-58	0.0000135%	0.00000540%	ND	0.00002%	0.00002%	ND	0.2%	11.2%	88.6%	Gravimetric Loss from PLM Prep: Organics = 0.3%; Acid Soluable = 5.5%; Other = 94.2%

LOD = Limit of Detection

LOQ = Limit of Quantification

ND = Not Detected

PLM = Polarized Light Microscopy

TEM = Transmission Electron Microscopy

Analytical Method(s): PLM by Modified NY ELAP 198.6
TEM by Modified NY ELAP 198.4/ASTM D5756

Analyst(s): PLM
TEM

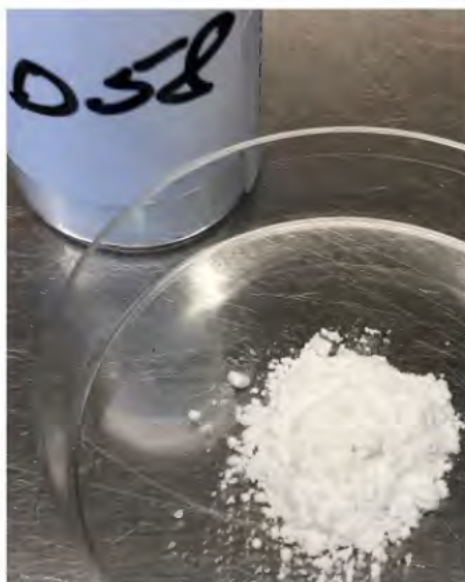
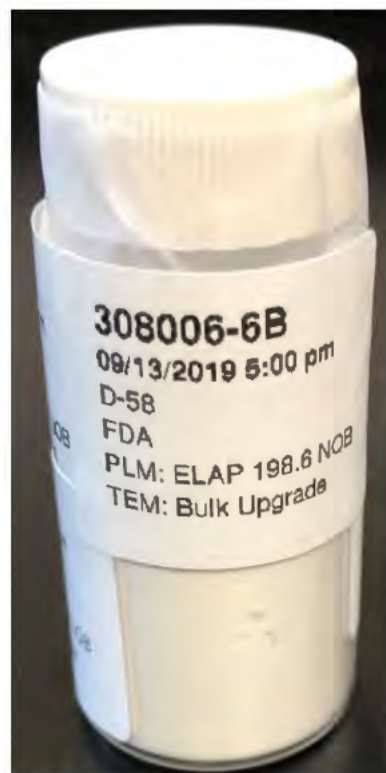
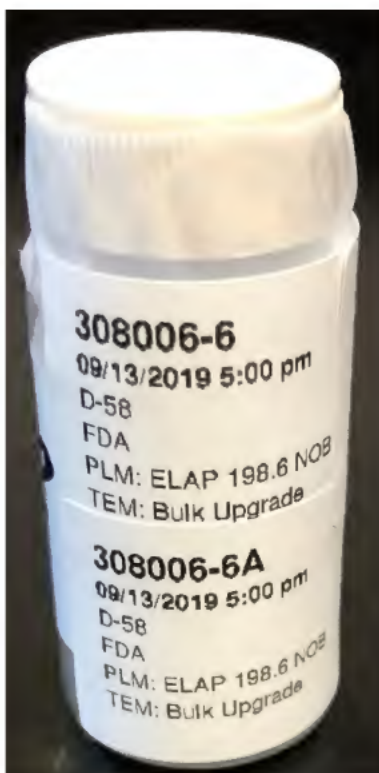
(b) (6)
(b) (6)

Technical Director: Andreas Saldivar

All results are to be considered preliminary and subject to change unless signed by the Technical Director or Deputy

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308006-6, 6A, 6B/D58



Sample Preparation

Samples were prepared for PLM and TEM bulk analysis by (b) (6) on August 13, 2019 through September 5, 2019.

Sample preparation consisted of the following steps:

- 1) Label and weigh two 8mL glass vials for each sample in the set – one vial for the PLM preparation and one vial for the TEM preparation.
- 2) Weigh out 0.1 to 0.8 grams of material and place in corresponding 8mL glass vial. Record weight.
- 3) Burn samples at 480° C for at least 12 hours.
- 4) Record Post-Ash Weight.
- 5) Treat ashed sample with concentrated hydrochloric acid.
- 6) Filter acid reduced material onto a pre-weighed 47mm 0.4um PolyCarbonate filter.
- 7) Place filter into drying oven for 30 minutes and then record Post-Acid Reduced weight.
- 8) Make four PLM slide preparations from the PLM residual ash for each sample in 1.550 dispersion oil. Make additional preparations in 1.605, 1.625, 1.680 and 1.700 dispersion oil as necessary for particle identification.
- 9) Weigh a portion of the residue from the TEM residual ash and place it into the corresponding pre-weighed 100ml jar.
- 10) Fill the 100ml jar with deionized water
- 11) Sonicate the jars for approximate 5-minutes.
- 12) Filter 0.2ml to 1ml of the solution onto a 47mm 0.22um MCE filter.
- 13) Dry the filter for 10 minutes then collapse, carbon coat, and place on a 3 TEM grids.

PLM Analysis

Analysis was performed in accordance with NY ELAP 198.6 protocols. The analysis was conducted using an Olympus BH-2 polarized light microscope (PLM) equipped with a dispersion staining objective. All four slide preparations for each aliquot were examined. 400-point count was performed for those samples on which asbestos was observed. If no asbestos was detected on any of the slides, the percentage of fibrous components was determined by visual estimation. The results of this analysis are detailed below in the *Discussion and Interpretation of Analytical Findings* section for each individual sample.

TEM Analysis

Analysis was performed in accordance with modified NY ELAP Method 198.4 protocols. The analysis was performed using a JEOL JEM-100CX II transmission electron microscope (TEM), equipped with a Thermo Fisher Quest Energy Dispersive X-Ray Analyzer (EDXA), at magnifications of 19,000x. Two grids for each aliquot were examined. Twenty (20) grid openings were examined per sample.

Modifications to the NY ELAP 198.4 Method were:

- 1) The residue was not placed in alcohol and prepared using the quick drop method. To obtain a more uniform preparation, the residue was placed in a jar and filled with 100ml of deionized water. The jar was sonicated, and a portion of the solution was filtered onto a 47mm 0.22um MCE filter.
- 2) The tremolite and chrysotile were not visually estimated. The length and width of the observed particles were measured, and the mass of each amphibole particle was calculated using the ASTM D5756 method.
- 3) All particles identified as tremolite were included with the counts/concentrations, regardless of size and aspect ratio.

The results of this analysis are detailed below in the *Discussion and Interpretation of Analytical Findings* section for each individual sample.

Calculations

ASTM D5756 Mass

$$M = \pi/4 L * W^2 * D * 10^{-12}$$

M = mass

L = length



W = width

D = density

Percent Calculation

$$\frac{EFA(mm^2) * 100ml * MA(g) * RW(g)}{VF(ml) * IW(g) * AA(mm^2) * RJ(g)}$$

The calculated value is then multiplied by 100 to convert it to percent.

EFA – Effective filter area

MA – Mass of asbestos

RW – Weight of residue

VF – Volume filtered

IW – Initial weight of the sample

AA – Area analyzed

RJ – Weight of residue placed into the jar

Limit of Detection and Quantification

We used the mass of a 0.5 x 0.04-micron tremolite or chrysotile fiber, depending on what was found in each sample, as the basis for our calculations. Limit of detection was defined as 1 fiber and limit of quantification was defined as 4 fibers.

Some aliquots of sample D58 contained very small amounts of asbestos that were either at or below our 4-fiber limit of quantification. For these samples we defined our limit of quantification as follows:

308006-6A: mass of the two observed chrysotile structures plus the mass of two chrysotile fibers measuring 0.5 x 0.04 microns

308006-6B: mass of 4 chrysotile fibers measuring 0.5 x 0.04-micron

Discussion and Interpretation of Analytical Findings:

308006-6, 6A, 6B Client Sample D-58

PLM

All three aliquots of sample D-58 were analyzed by (b) (6) on September 13, 2019. No asbestos or non-asbestos amphibole variants were detected the samples. The results were calculated using the equations detailed in the calculations section.

308006-6	NAD
308006-6A	NAD
308006-6B	NAD

TEM

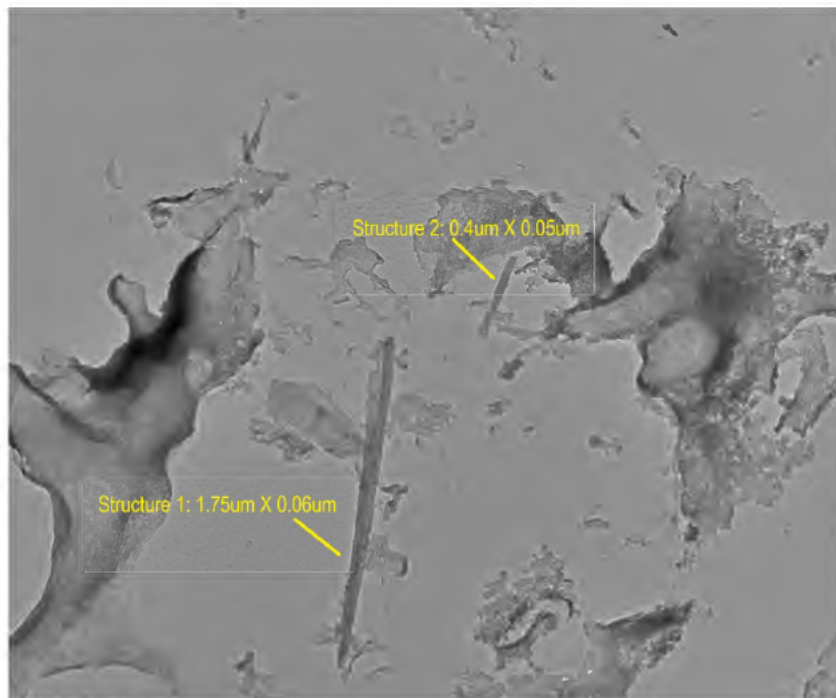
Sample 6 was analyzed by (b) (6) on September 3, 2019. Samples 6A and 6B were analyzed by (b) (6) on September 7, 2019. The primary particle observed was talc along with a few talc fibers, talc ribbons and mica particles. Two Chrysotile structures were detected on the aliquot for 6A and four chrysotile structures were detected on the aliquot for 6B. The results were calculated using the equations detailed in the calculations section.

308006-6	NAD
308006-6A	<0.00002%
308006-6B	0.00002%

Below are pictures, diffraction patterns, and chemistry from some of the observed particles. The unidentified peaks in chemistry spectra are copper, zinc, and carbon. Those peaks are from the TEM specimen holder and specimen grid.



Sample 308006-6A, Chrysotile Structures



308006 FDA_101.jpg
Chrysotile Structures
308006-6a
Cal: 0.001774 $\mu\text{m}/\text{pix}$
14:06 9/7/2019
TEM Mode: Imaging
Microscopist: CD
Camera: NANOSPRTS, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

500 nm
HV=100kV
Direct Mag: 5800 x
AMA Analytical Services, Inc

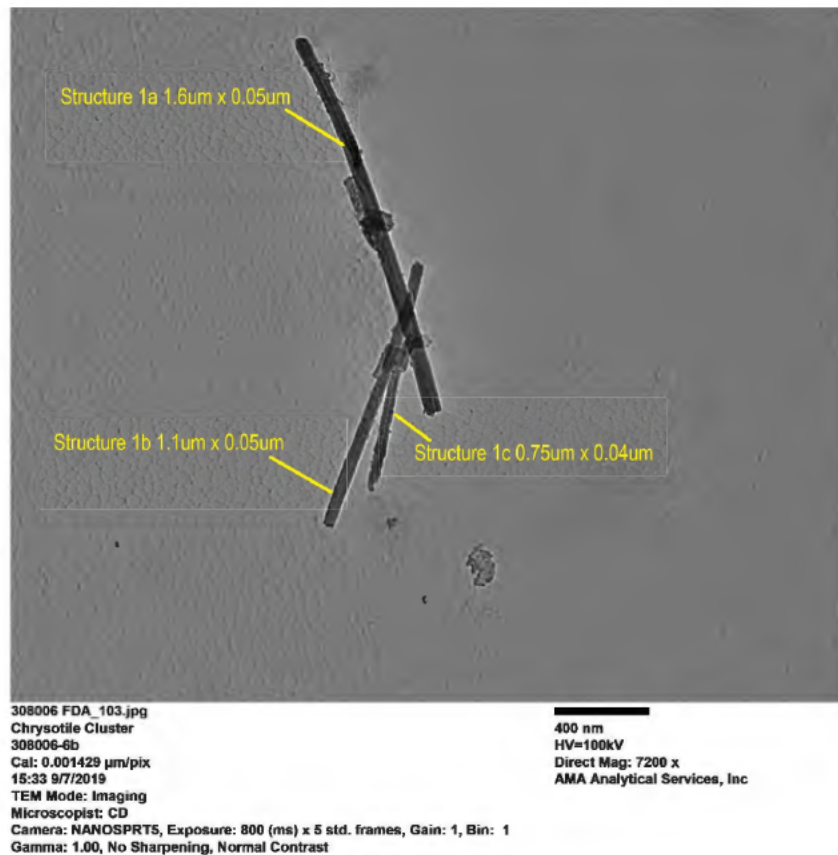
Diffraction Pattern from Chrysotile Structure 1 pictured above



308006 FDA_100.jpg
Chrysotile Dif
308006-6a
14:03 9/7/2019
TEM Mode: Diffraction
Microscopist: CD
Camera: NANOSPRTS, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

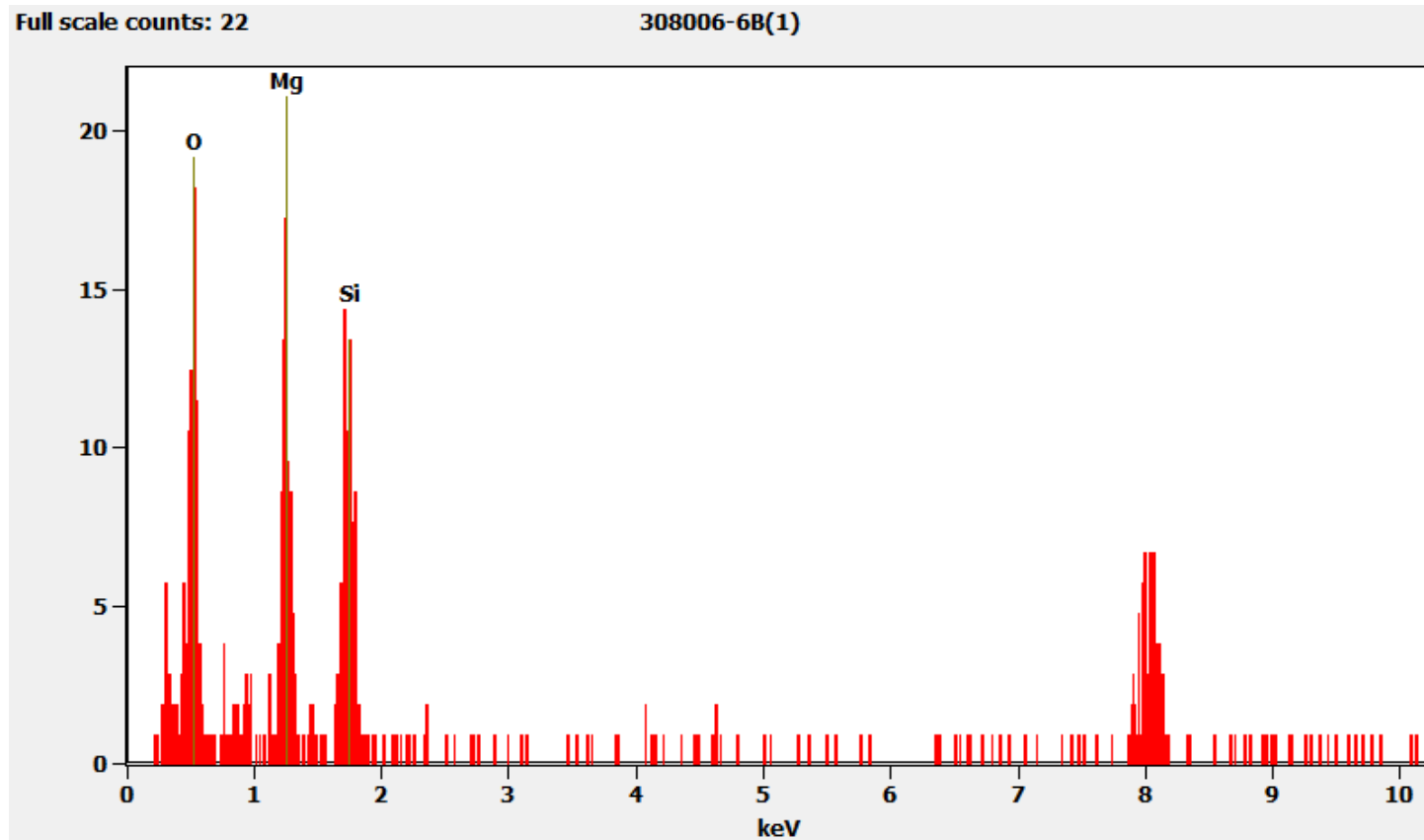
Sample 308006-6B, Chrysotile Structure 1



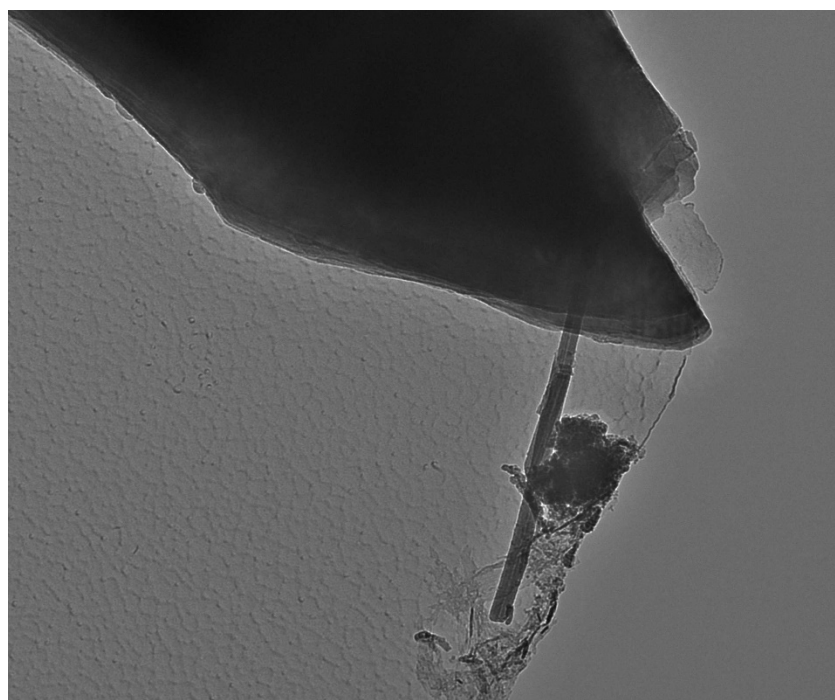
Diffraction Pattern from Chrysotile Structure pictured above



Chemistry from Chrysotile Structure pictured above



308006-6B, Chrysotile Structure 2



308006 FDA_105.jpg
Chrysotile Fiber
308006-6b
Cal: 0.001029 $\mu\text{m}/\text{pix}$
16:05 9/7/2019
TEM Mode: Imaging
Microscopist: CD
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

200 nm
HV=100kV
Direct Mag: 10000 x
AMA Analytical Services, Inc

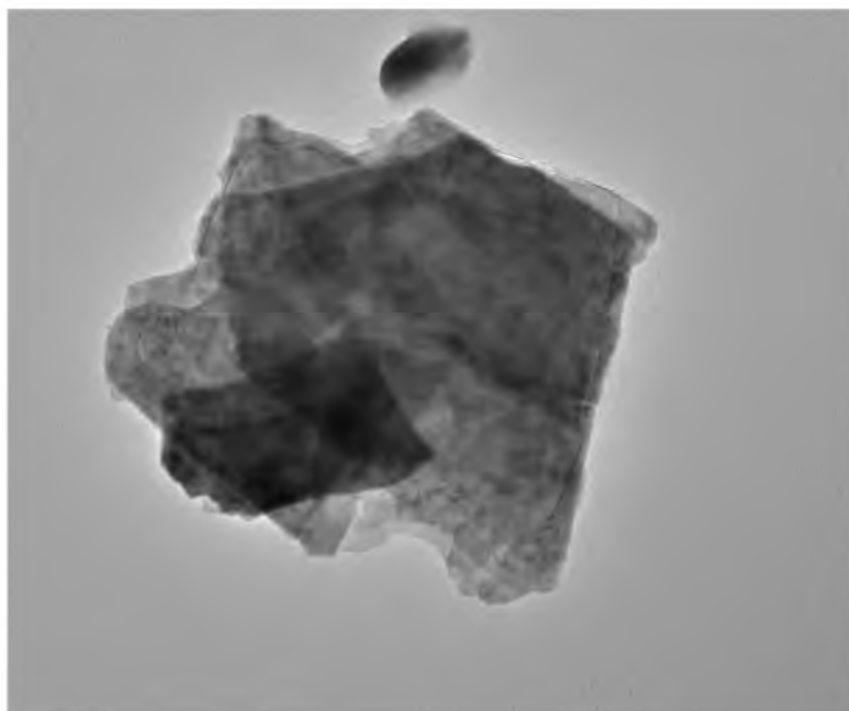
Diffraction Pattern from Chrysotile Structure pictured above



308006 FDA_104.jpg
Chrysotile Dif
308006-6b
16:03 9/7/2019
TEM Mode: Diffraction
Microscopist: CD
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 std. frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

308006-6, Talc Particle



308006 FDA_052.jpg
Talc Particle
Cal: 0.001774 $\mu\text{m}/\text{pix}$
17:18 9/3/2019
TEM Mode: Imaging
Microscopist: MG
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

500 nm
HV=100kV
Direct Mag: 5800 x
AMA Analytical Services, Inc

Hexagonal Diffraction Pattern from Talc Particle pictured above

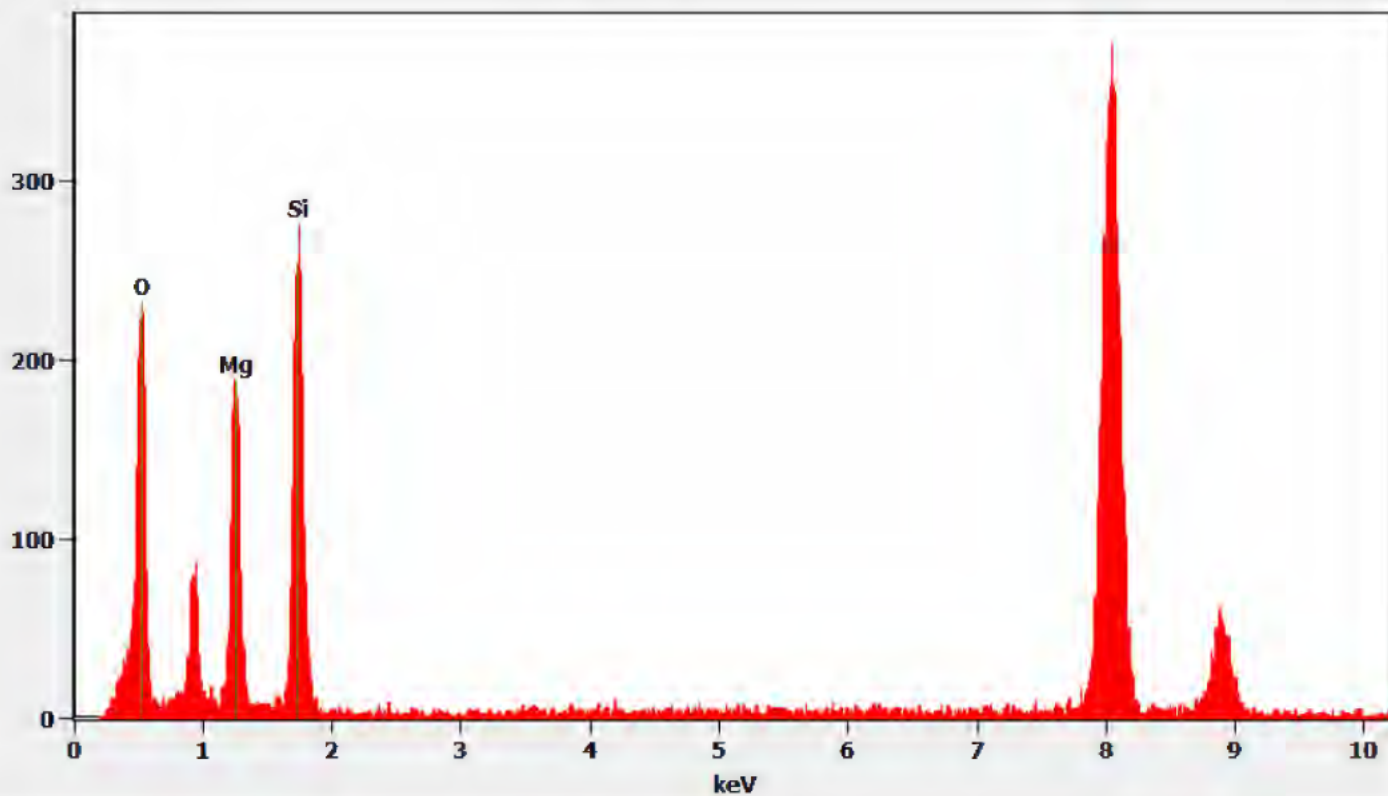


308006 FDA_053.jpg
Talc Particle
17:19 9/3/2019
TEM Mode: Diffraction
Microscopist: MG
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast
100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

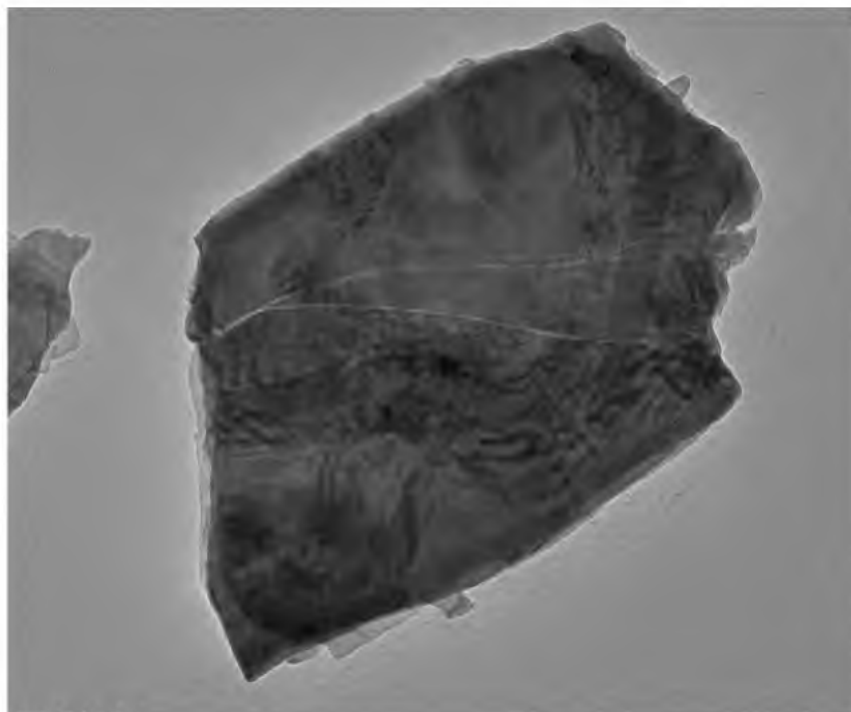
Chemistry from Talc Particle pictured above

Full scale counts: 377

308006-6(1)



306008-6, Mica Particle



308006 FDA_054.jpg

Mica Particle

Cal: 0.001429 $\mu\text{m}/\text{pix}$

17:21 9/3/2019

TEM Mode: Imaging

Microscopist: MG

Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1

Gamma: 1.00, No Sharpening, Normal Contrast

400 nm

HV=100kV

Direct Mag: 7200 x

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Diffraction Pattern from Mica Particle pictured above



308006 FDA_056.jpg

Mica Particle

17:22 9/3/2019

TEM Mode: Diffraction

Microscopist: MG

Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1

Gamma: 1.00, No Sharpening, Normal Contrast

100 $(1/\text{\AA})$

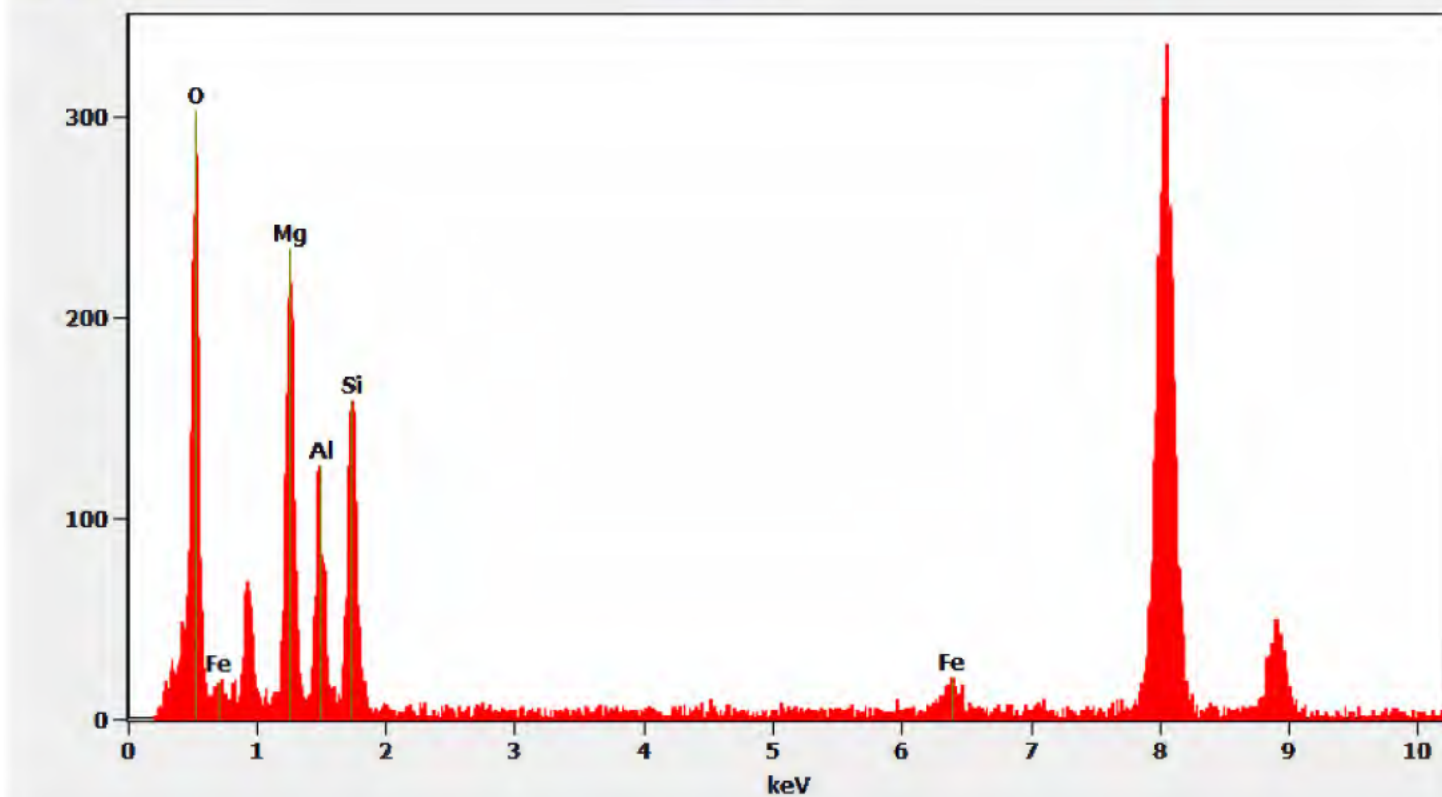
HV=100kV

Cam Len: 0.2200 m

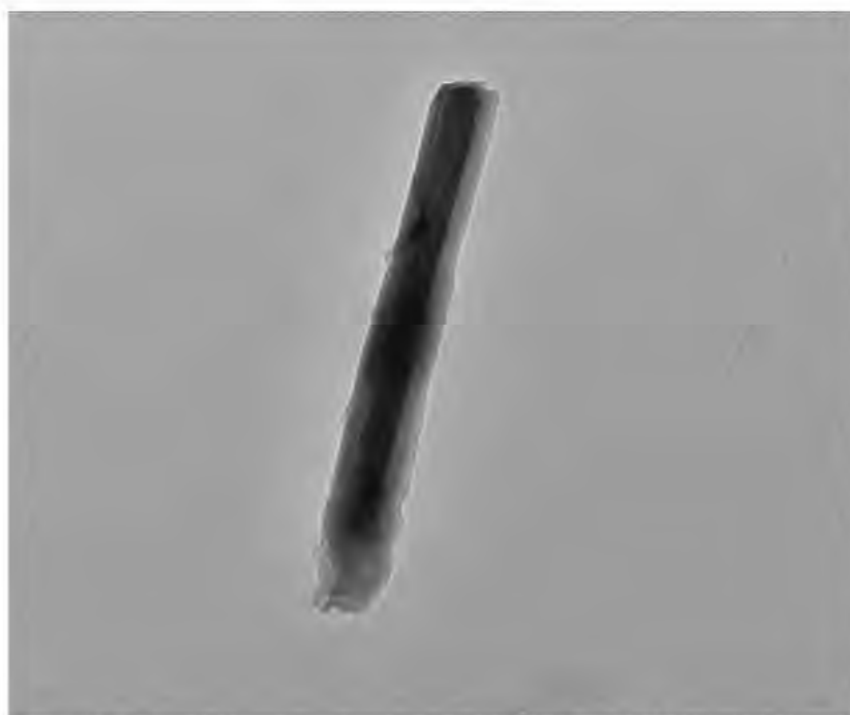
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Full scale counts: 337

308006-6(2)



308006-6, Talc Fiber



Camera: NANOSPRT5, Exposure: 800 (ms) x 5 d
Gamma: 1.00, No Sharpening, Normal Contrast

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Diffraction Pattern from Talc Fiber pictured above



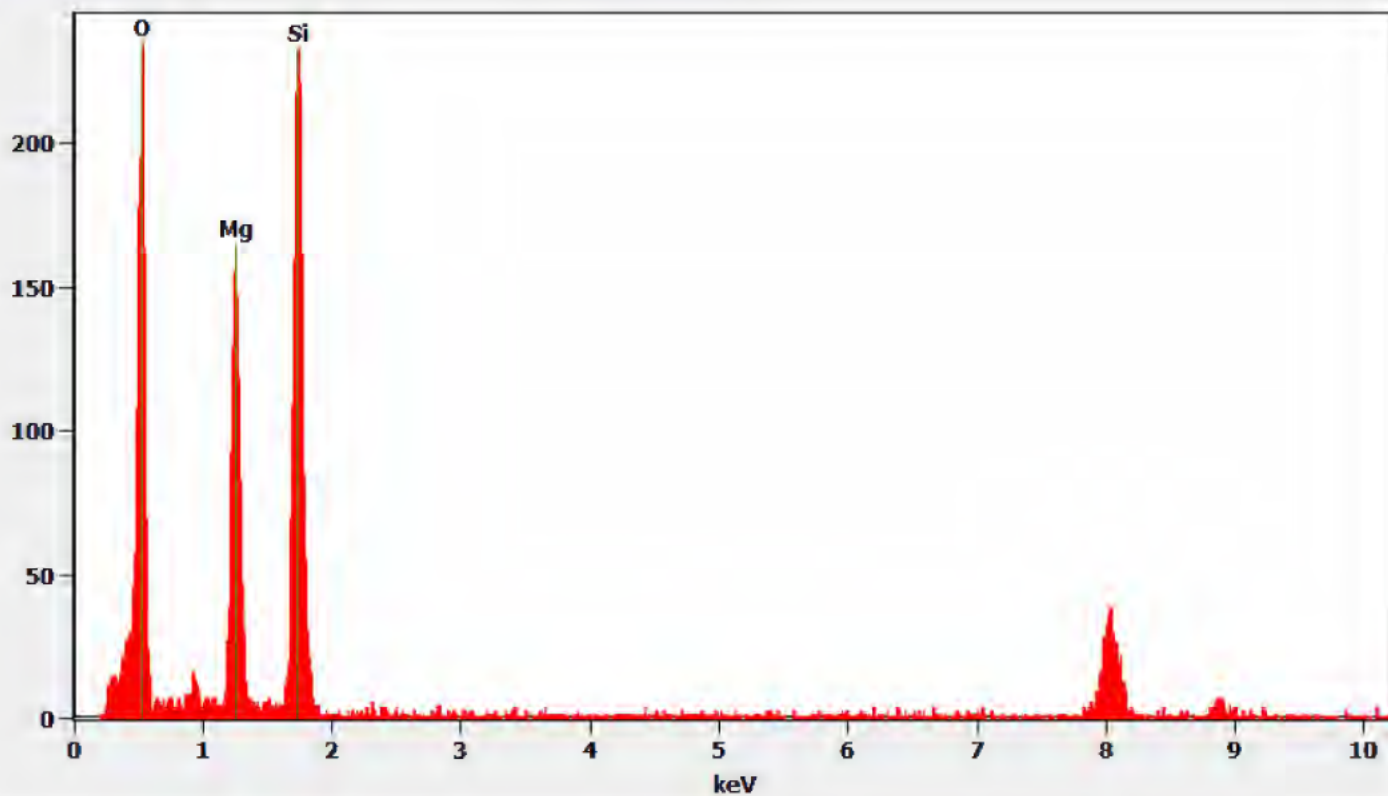
308006 FDA_058.jpg
Talc Fiber
17:28 9/3/2019
TEM Mode: Diffraction
Microscopist: MG
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

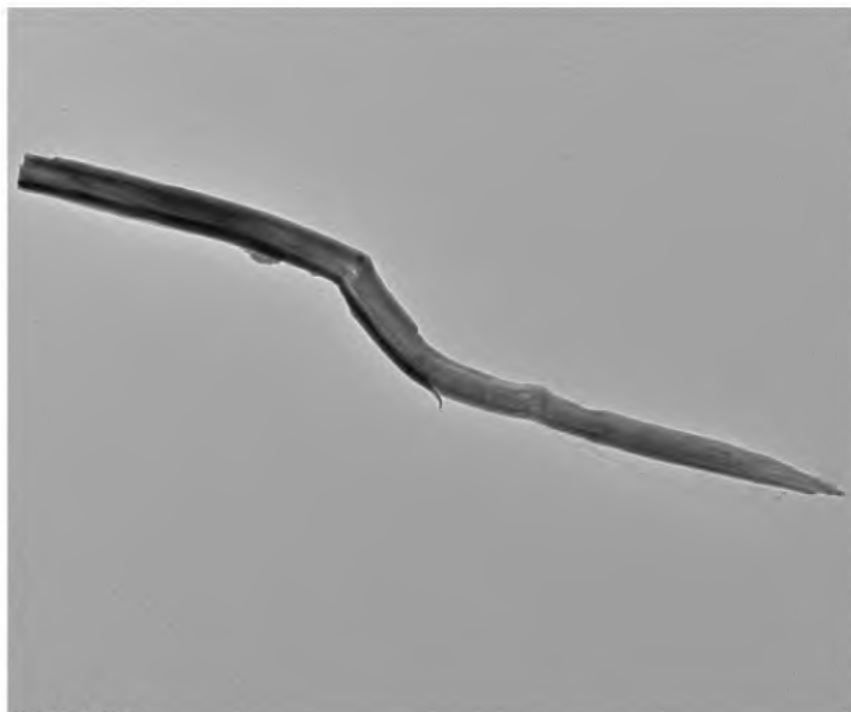
Chemistry from Talc Fiber pictured above

Full scale counts: 235

308006-6(3)



308006-6, Talc Ribbon



308006 FDA_059.jpg
Talc Ribbon
Cal: 0.001774 $\mu\text{m}/\text{pix}$
17:37 9/3/2019
TEM Mode: Imaging
Microscopist: MG
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

500 nm
HV=100kV
Direct Mag: 5800 x
AMA Analytical Services, Inc

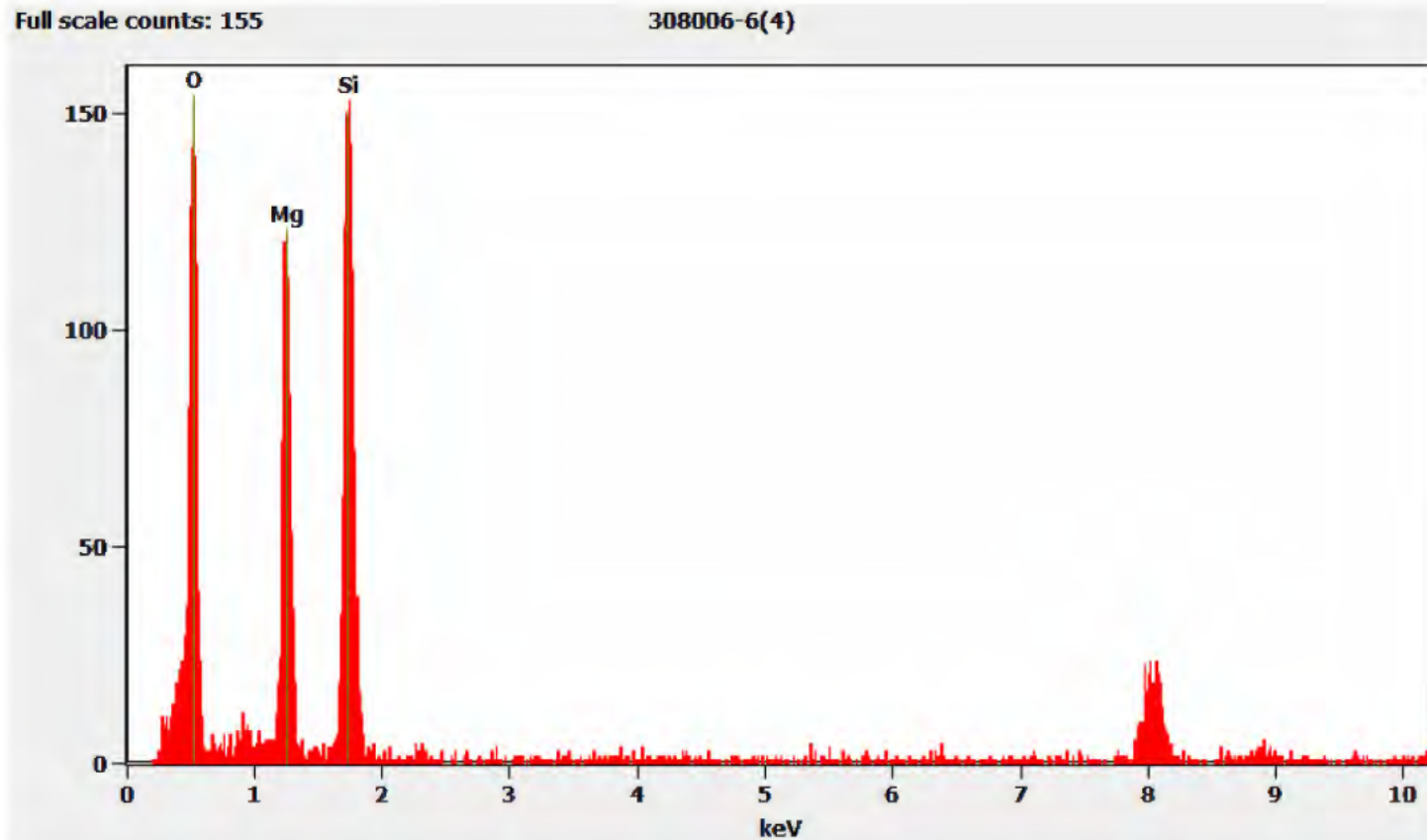
Diffraction Pattern from Talc Ribbon pictured above



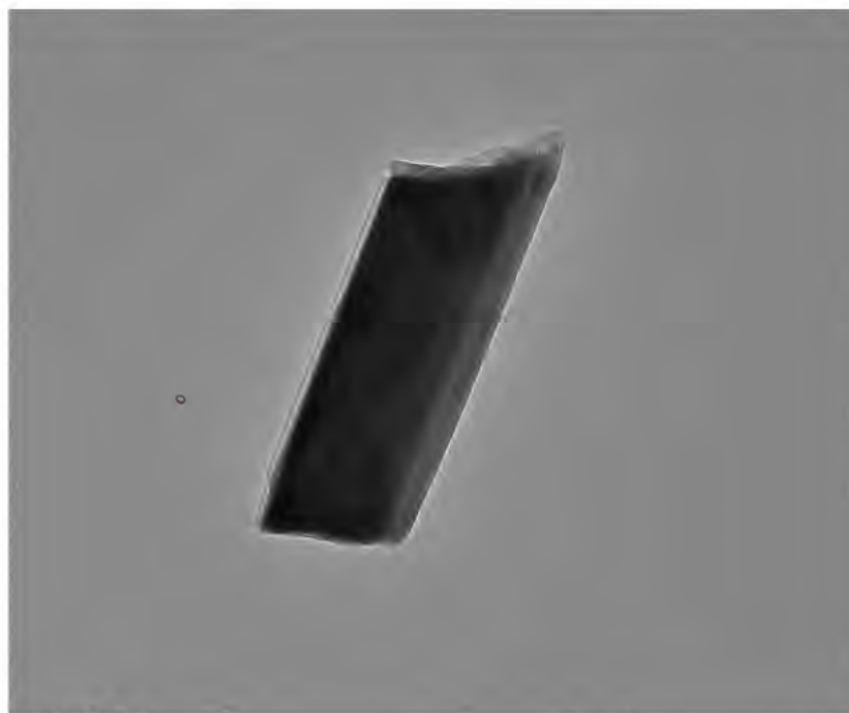
308006 FDA_060.jpg
Talc Ribbon
17:38 9/3/2019
TEM Mode: Diffraction
Microscopist: MG
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 $(1/\text{\AA})$
HV=100kV
Cam Len: 0.2200 m
AMA Analytical Services, Inc

Chemistry from Talc Ribbon pictured above



308006-6, Talc Fiber



308006 FDA_061.jpg
Talc Fiber
Cal: 0.001029 $\mu\text{m}/\text{pix}$
17:50 9/3/2019
TEM Mode: Imaging
Microscopist: MG
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

200 nm
HV=100kV
Direct Mag: 10000 x
AMA Analytical Services, Inc

Diffraction Pattern from Talc Fiber pictured above



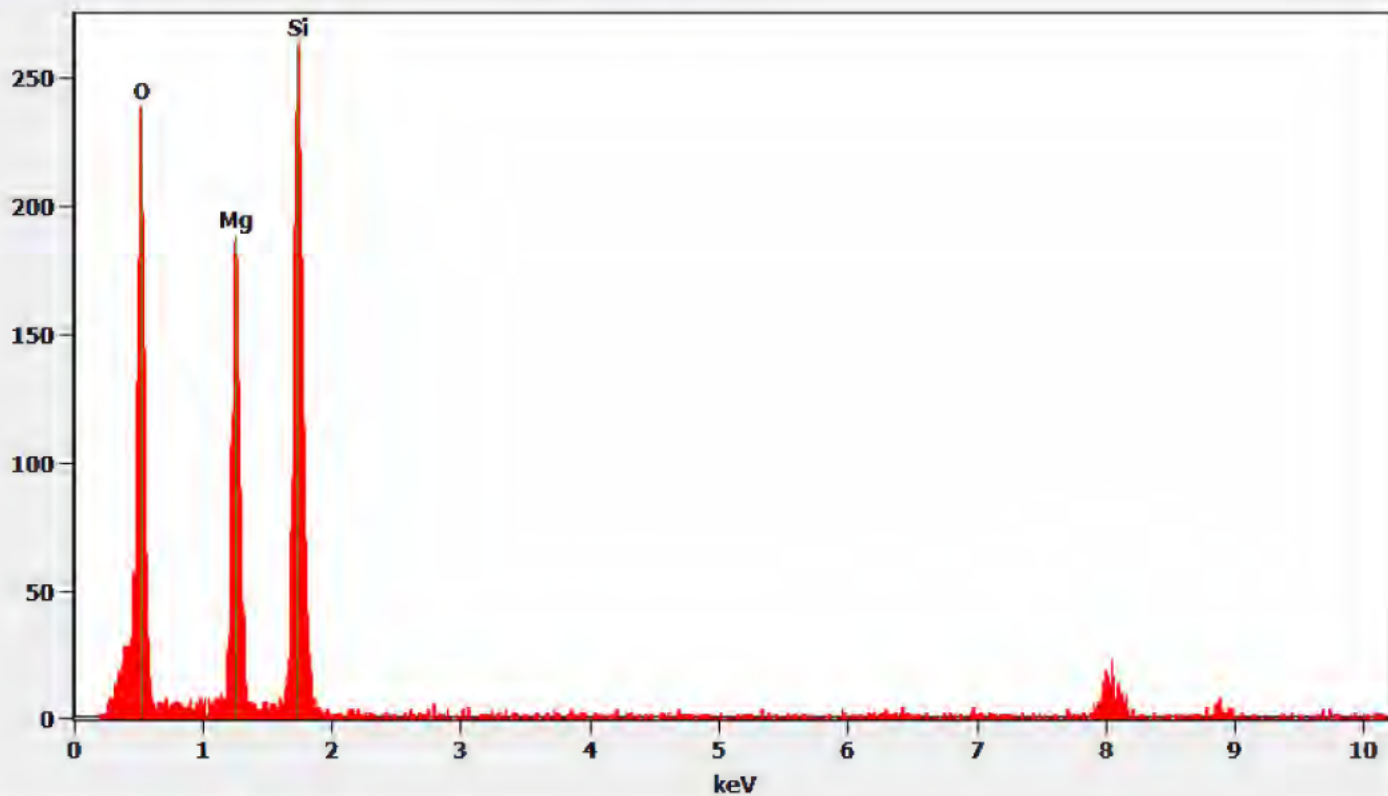
308006 FDA_062.jpg
Talc Fiber
17:51 9/3/2019
TEM Mode: Diffraction
Microscopist: MG
Camera: NANOSPRT5, Exposure: 800 (ms) x 5 drift frames, Gain: 1, Bin: 1
Gamma: 1.00, No Sharpening, Normal Contrast

100 (1/Å)
HV=100kV
Cam Len: 0.2200 m
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Chemistry from Talc Fiber pictured above

Full scale counts: 264

308006-6(5)



QC Discussion:

During preparation, three blank control samples and one reference control sample were prepared. These samples were prepared alongside the customer samples. The blank samples were prepared using Sigma-Aldrich Talc Powder, <10 micron, and was analyzed by (b) (6) on September 18, 2019. No asbestos was detected on the blank samples. The reference sample was made from the same Sigma-Aldrich talc powder spiked with 10% Chrysotile. The reference sample was analyzed by (b) (6) on September 18, 2019 and found to be within acceptable limits. Additionally, filter blanks were prepared with each batch of carbon coated filters. Filter blank number EB-54155 was associated with the carbon coating for samples 308006-6, 6A, 6B/D-58. No asbestos was detected on the filter blank sample.


Our laboratory information management system (LIMS) randomly selected samples 308006-2/D-54 and 308006-15/D-67 for additional replicate QC analysis. Separate preparations were made for PLM and TEM analysis. The replicate QC analysis was performed by (b) (6) on September 13, 2019, 2019 for PLM analysis and by (b) (6) on September 18, 2019 for TEM analysis. The QC results matched the original analysis.

Attachments:

The following items are attached to this case narrative for your reference:

- 1) Sample Log-In Sheet
- 2) Daily PLM Scope Calibration Log
- 3) Refractive Index Oil Calibration Log
- 4) Daily TEM Scope Calibration Log
- 5) QC Results Summary
- 6) Replicate & Duplicate QC Chart for (b) (6) for samples analyzed between 1/1/2019 and 9/18/2019
- 7) Replicate & Duplicate QC Chart for (b) (6) for samples analyzed between 1/1/2019 and 9/18/2019
- 8) Replicate & Duplicate QC Chart for (b) (6) for samples analyzed between 1/1/2018 and 9/18/2019
- 9) Raw Data Sheets
 - a. Gravimetric Data
 - b. Filtration Worksheets
 - c. PLM Analysis
 - d. TEM Analysis
 - e. QC Samples

I certify that all information contained in this report pertaining to laboratory events, procedures, and protocols is true and accurately describes the handling of this project by AMA Analytical Services, Inc. and its personnel.



Andreas Saldivar
Laboratory Director

10/11/2019
Date

