ATLANTA
Corporate Headquarters
3945 Lakefield Court
Suwanee, GA 30024
(770) 866-3200 FAX (770) 866-3259

MAS Project 14-2732
Supplemental Talcum Powder Analysis
Ammens Containers

Prepared for: The Lanier Law Firm

Prepared By: William E. Longo, Ph.D., CEO
Materials Analytical Services, LLC

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PROJECT SUMMARY

This supplemental report corrects a few minor typos in the previous report and provides the results for the analysis of twelve Ammens powder containers. Eleven of the twelve Ammens containers were submitted to MAS by Darron Berquist on behalf of Lanier Law Firm. The final Ammens sample split was submitted to MAS by Lee Poye. The COC's for all twelve samples are found in section 2 of this notebook. All twelve Ammens powder containers were logged in accordingly and then placed in a secure laminar flow hood. The twelve Ammens powder sample containers were assigned the following MAS laboratory tracking numbers as shown below. Table 1 provides a sample description summary of the Ammens powders that were analyzed for asbestos.

Table 1
Ammens Powder Sample Container Descriptions

MAS Sample No.	Product	Amount of Powder in Container (oz)	Container Code	Source of Sample
M71482-001	(Bottled prior to 1958) Vintage Ammens Medicated Powder	5.5	4980	Purchased on eBay Submitted by Darron Berquist
M71483-001	1969 Medicated Ammens Powder	11.0	6G29	Purchased on eBay Submitted by Darron Berquist
M71484-001	1992 Ammens Medicated Powder	6.25	AJ2733-55-00 AJ4H3K	Purchased on eBay Submitted by Darron Berquist
M71484-002	1992 Ammens Medicated Powder	6.25	AJ0740-56-00 AJ4Y3K	Purchased on eBay Submitted by Darron Berquist
M71485-001	Ammens Medicated Powder	2.5	1230	Purchased on eBay Submitted by Darron Berquist
M71486-001	1981 Ammens Medicated Powder	6.25	L4A112	Purchased on eBay Submitted by Darron Berquist
M71487-001	1992 Ammens Medicated Powder	6.25	AJ2733-55-0 AJ4H4K	Purchased on eBay Submitted by Darron Berquist
M71487-002	1992 Ammens Medicated Powder	6.25	AJ2733-55-00 AJ4H3K	Purchased on eBay Submitted by Darron Berquist
M71501-001	Vintage Ammens Medicated Powder	4.5	3B35	Purchased on eBay Submitted by Darron Berquist

M71506-001	Vintage Ammens Medicated Powder Tin	0.5	23800	Purchased on eBay Submitted by Darron Berquist
M71507-001	Vintage Tin Can Bristol Myers Ammens Medicated Powder	2.5	4518	Purchased on eBay Submitted by Darron Berquist
M71520-001	Split sample of 1992 Ammens Medicated Powder	6.25	AI2733-55-00 AJ4J7K	Submitted by Lee Poye

OVERVIEW

This report provides the analytical results for the testing of twelve Ammens powder containers that MAS analyzed as requested by the Lanier Law Firm.

The talcum powder in the twelve Ammens Powder sample containers were analyzed for both chrysotile and amphibole asbestos using PLM and ATEM by the ISO-22262-1 and -2 methods, and by the NYELAP method.

For chrysotile, each sample was prepared by the Colorado School of Mines (CSMP) sample preparation method (with HLS). The samples were analyzed by PLM using refractive index liquids 1.550 & 1.560.[1,2]

For the detection of amphibole asbestos, the PLM sample preparation (with HLS) was utilized by the New York ELAP method and then analyzed by the ISO 22262-1 method with a refractive index liquid of 1.605. The ATEM sample preparation was utilized by the ISO 22262-2 method, then the filter was prepared and analyzed using the standard TEM methods.

Overview of Results

The CSMP Sample Preparation (with HLS) & Analyzed by the ISO 22262-1 PLM Method

The amount of chrysotile found in the twelve Ammens Powder samples had an average estimated volume weight concentration of 0.0003% to 0.004% (recovery weight corrected). The average amount of chrysotile bundles was 273,000 bundles per gram of talc (recovery weight corrected).

The NYELAP Sample Preparation (with HLS) Analyzed by the ISO 22262-1 PLM Method

For the PLM analysis showed that one of the twelve Ammens powder samples (M71482-001) was reported positive for tremolite asbestos at a concentration range of 0.001 of 0.002 wt.%.

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[1] Colorado School of Mines Research Institute February 26, 1973 Report Re: Mineralogical Examination of Five Talc Samples to W.H. Ashton from W.P. Reid and W.T. Caneer.

[2] Colorado School of Mines Research institute April 2, 1973 Report re: Mineralogical Examination of four Samples for Tremolite and Chrysotile from W.P. Reid to W.H. Ashton.

ISO 22262-1&2 Sample Preparation Method with (HLS) Analyzed by ATEM for Amphibole Asbestos

Of the twelve Ammens Powder samples, three were positive for tremolite asbestos and are as follows: M71482-001, M71506-001 & M71507-001 with a tremolite asbestos concentration at a range of 36,600 to 56,100 fibers/bundles per gram of Ammens talcum powder.

MATERIALS & METHODS

Ammens Powder Sample Containers

After the Ammens Powder sample containers were logged in at MAS, the containers were transferred to the cosmetic talc archive room where all twelve samples were photographed in the received condition and inspected for damage or tampering. The MAS chain-of-custody documents can be found in Section 2 of this report, and photographs of each container can be found in Section 16 of this report.

Muffle Furnace

For this procedure, approximately 1 gram from each of the twelve talcum powder samples were removed from their containers (Sartorius Research Balance) and placed in twelve separate glass scintillation vials. Each scintillation vial was then placed in a Fisher Scientific Iso-temp muffle furnace Model #620 at 480°C for a minimum of 12 hours to remove any organic material. Typically, the muffle furnace samples are run overnight.

CSMP Sample Preparation Method (with HLS) and ISO PLM Analysis (Chrysotile Asbestos)

CSMP Sample Preparation

Approximately 200 milligrams from each of the twelve muffled talcum powder samples were transferred into separate 15 ml centrifuge tubes (VWR 10026-076). Through the use of DI water, approximately 5 ml of adjusted Lithium heteropolytungstates (HL) solution, GeoLiquids, Inc., Cat. No. LST010 (stated density of 2.85 g/cc), was diluted to a new density of 2.72 g/cc, as determined by a VWR Hydrometer, Model Number 34620-1109.

The newly diluted HL was added to each of the VWR centrifugation tubes containing the talcum powder samples and then shaken vigorously for 10 to 20 seconds. Each VWR centrifugation tube was then placed in an Ohaus Frontier 5000 series centrifuge set at 2000 RPM for 24 hours at room temperature without breaking. After removing the tubes from the centrifuge, the talc/heavy liquid (light fraction) was pipetted off the top of each centrifuge tube. The pellet along with the DI water was then filtered onto a new 0.45um 47mm PC filter and allowed to dry under a drying lamp for 20 to 30 minutes. This washing step was repeated two more times for the sample.

After drying, each of the final MCE filter/talc samples (heavy fraction or pellet) were provided to the PLM analyst. All the 47 mm MCE filters were weighed before HLS recovery process, then after

the filtration and drying of the heavy fraction or pellet.

PLM – New York ELAP Method (with HLS Sample Preparation) for Amphibole Asbestos

Approximately 200 milligrams from each of the twelve muffled talcum powder samples were transferred into separate 15 ml centrifuge tubes (VWR 10026-076). Through the use of DI water, approximately 5 ml of adjusted HL (Lithium heteropolytungstates solution, GeoLiquids, Inc., Cat. No. LST010 (stated density of 2.85 g/cc), was diluted to a new density of 2.78 g/cc, as determined by a VWR Hydrometer, Model Number 34620-1109.

The newly diluted HL was added to each of the VWR centrifugation tubes containing the talcum powder samples and then shaken vigorously for 10 to 20 seconds. Each VWR centrifugation tube was then placed in an Ohaus Frontier 5000 series centrifuge set at 2000 RPM for 24 hours at room temperature without breaking. After removing the tubes from the centrifuge, the talc/heavy liquid (light fraction) was pipetted off the top of each centrifuge tubes. The pellet along with the DI water was then filtered onto a new 0.45um 47mm PC filter and allowed to dry under a drying lamp for 20 to 30 minutes. This washing step was repeated two more times for the sample.

After drying, each of the final MCE filter/talc samples (heavy fraction or pellet) were provided to the PLM analyst. All the 47 mm MCE filter were weighed before HLS recovery process, then after the filtration and drying of the heavy fraction.

ISO 22262-1 PLM Analysis of the Samples Prepared by the CSMP & New York ELAP Method

Chrysotile Asbestos

Approximately 100 milligrams from each of the twelve muffled talcum powder samples (heavy fraction) were analyzed by the ISO 22262-1 PLM method. To determine the actual amount of talcum powder analyzed by this method, each sample was prepared as follows: two new glass slides that are used to analyze the talcum powder sample by PLM for this project were separately weighed and recorded (Sartorius Research Balance). Next, three talcum powder sample mounts were placed on the two glass slides (one talcum powder mount on one slide and two talcum powder mounts on the second slide). While each sample mount was transferred onto the glass slides, each of the glass slides were reweighed and recorded. Afterwards, a drop of either 1.550/1.560 (CSMP-chrysotile) and 1.605 (NYELAP-amphibole asbestos) refractive index liquid was placed on each sample mount and stirred with the point of a scalpel blade. The three sample mounts were then covered with an 18 x 18 mm glass cover slip.

Each sample was then examined under elongation PLM conditions, cross polars with the 530 nm analyzer plate inserted. 30 total fields per field of view (a single PLM field of view has an area of (0.785 mm^2) are examined (10 fields of view for each of the three mounts) for a total area examined of 23.55 mm^2.

Positive identification of chrysotile asbestos bundles was done by morphology, refractive indices, elongation, extinction angle, birefringence and pleochroism as described by the ISO 22262-1 PLM

method. The ISO PLM analysis protocol was used to show how the analysis is done. However, the range of acceptable RI's for the NIST 1866 chrysotile were not used. The reason for this will be discussed later in this report.

If chrysotile is present, the PLM analyst will count the number of positively identified chrysotile structures in each field of view based on the above criteria and record that number on the MAS PLM data sheet. In addition, up to three or four representative chrysotile bundles are photographed in both the parallel and perpendicular direction under dispersion staining, elongation, cross polars and with polarizers out. The detection limit for this method, as specified by the ISO 22262-1 method, is the finding of either 1 fiber or 1 bundle in the analysis.

Amphibole Asbestos

As described above, amphibole asbestos was also analyzed by the ISO 22262-1 PLM method. In addition to the determination of whether regulated amphibole asbestos structures are present in the sample, the sample was also examined for possible amphibole cleavage fragments in 1.605 RI liquid. The detection limit for this method, as specified by the ISO 22262-1 method, is the finding of either 1 fiber or 1 bundle in the analysis.

ATEM Sample Preparation: Amphibole Asbestos ISO 22262-2 (with HLS Sample Preparation)

The HLS sample preparation for the ATEM analysis was done by the ISO 22262-2 methodology. Approximately 25 to 30 milligrams (Sartorius Research Balance) from each muffled furnace talcum powder sample were placed into twelve separately labeled 15 ml centrifuge tubes (VWR 10026-076). Approximately 5 ml of heavy liquid (Lithium heteropolytungstates solution, GeoLiquids, Inc., Cat. No. LST010 (stated density 2.85 g/cc) was added into each of the twelve centrifuge tubes containing the talcum powder samples, that was then prepared and shaken vigorously by hand for 10 to 20 seconds. The twelve centrifuge tubes were placed in an Eppendorf micro-centrifuge (Model No. 2412D) set at 2000 RPM for 24 hours at room temperature. After removing the tubes from the centrifuge, the talc/heavy liquid (light fraction) was pipetted off the top of each centrifuge tube. Deionized water was added to each centrifuge tube to bring the volume to approximately 15 ml. The 15 ml centrifuge tubes were then capped and inverted by hand 2 times to distribute the collected material in the bottom of the tube tip. Next, the 15 ml mixture was immediately and continuously filtered through a separate 47 mm Polycarbonate filter (PC) with a 0.22µm pore size. After each mixture was filtered, the excess heavy liquid was washed through the filter with the addition of approximately 100 ml of deionized water. The prepared PC filter was placed in a new disposable plastic 47 mm petri dish and allowed to dry at ambient room temperature in a HEPA hood for a minimum of 2 hours. The processed PC filter sample was directly prepared onto 100 µm

TEM size grids (2 for analysis and 1 for archive) using the standard TEM filter preparation protocol for PC filters.[3, 4, 5]

ATEM Amphibole Asbestos Analysis: ISO 22262-1 & 2

For the ATEM analysis, 100 grid openings were analyzed between two grids (50 openings per grid). JEOL 1200EX ATEMs equipped with either a Noran or an Advanced Analysis Technologies (light element) energy dispersive x-ray analyzer (EDXA) were employed for this analysis. Each of the 12 samples were analyzed at a screen magnification of 20,000X. Verification of regulated amphibole asbestos structures is done in the ATEM by the following three steps:

Morphology (Step 1)

The determination of the fibrous morphology for any potential regulated amphibole asbestos structures in the TEM sample was done by the standard ATEM methodology.[3,] Morphology is identified when the fibers and bundles of potential asbestos structures have substantially parallel sides with an aspect ratio of 5:1 or greater, and at least 0.5 µm in length.

Regulated Amphibole Asbestos Verification (Steps 2 & 3)

Potential fibrous amphibole asbestos structures that fit the above morphology criteria are analyzed in the ATEM by EDXA for the fiber/bundle chemistry (Step 2) and selected area electron diffraction (SAED), for the appropriate crystalline lattice measurements for amphibole asbestos (Step 3) as described in the ISO 22262-1 & 2 methods. The detection limit for this method, as specified by the ISO 22262-1 method, is the finding of either 1 fiber or 1 bundle in the analysis.

Process Laboratory Blank

All 10 process laboratory blanks were run concurrently with each of the corresponding Ammens talcum powder sample preparations by the ATEM HLS method (amphibole asbestos). The process blank PC filter was prepared in the same exact manner as the ATEM talcum powder sample (with heavy liquid, filtration on PC filters, etc.) but without any talcum powder. For the ATEM analysis, 100 grid openings (two grids, 50 grid openings each) were analyzed for the process blank.

RESULTS

Ammens Powder Container Inspections

According to the chain-of-custody, eleven Ammens Powder samples were sent from the Lanier Law Firm, and one sample split was sent by Lee Poye. When inspected upon their received condition, all twelve powder samples were opened from the received original packaging and sampled. Images of

[3] D5755-09 "Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Loading.

[4] D5756-02 "Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust Loading by Transmission Electron Microscopy for Asbestos Mass Surface.

[5] U.S. Environmental Protection Agency (USEPA) 1987. Asbestos Hazard Emergency Response Act, 40 CFR Part 763, Appendix A to Subpart E, USEPA, Washington D.C.

each container can be found in section 16 of the notebook. There was no indication that any of the twelve Ammens containers were tampered with.

CSMP Sample Prep. (HLS)/ISO 22262-1 PLM Analysis Chrysotile Asbestos)

The amount of chrysotile found in the Ammens Powder samples had an average estimated volume weight concentration of 0.0003% to 0.004% (recovery weight corrected). The average amount of chrysotile bundles was 273,000 bundles per gram of talc (recovery weight corrected).

The average birefringence (BIR) of the chrysotile bundles was calculated from the refractive index measurements and found to have a BIR classification of **0.006** which is classified as a Low birefringence (<0.01). The CSMP/ISO-PLM data sheets can be found in Sections 3 through 14 of this report.

PLM – New York ELAP Method Sample Prep. (HLS)/ ISO-22262-1 PLM Analysis for Amphibole Asbestos

The analysis showed that one of the twelve Ammens powder samples (M71482-001) was positive for tremolite asbestos with a concentration range of 0.001 wt.%-0.002 wt.%. The average amount of tremolite bundles per gram was found to be 92,000. The ISONY-PLM data sheets can be found in Sections 3 through 14 of this report.

ATEM ISO 22262-1 & 2 Amphibole Asbestos Method

Of the twelve Ammens powder samples, three (M71482-001, M71506-001 & M71507-001) reported positive for tremolite asbestos. All three samples had an average detection limit of approximately 48,000 tremolite fiber/bundles per gram, and had a range of tremolite asbestos from 36,600 to 56,100 fiber/bundles per gram. The ATEM data sheets can be found in Sections 3 through 14 of this report. The summary of the ATEM results are shown in Table 2.

ATEM Process Blanks

The analyzed ATEM process blank samples showed no asbestos structures, cleavage fragments or fibrous/platy talc detected. The ATEM data sheets can be found in Section 15 of this report.

Table 2
Overall Summary of the Ammens Powder Asbestos Sample Analysis Results

MAS Sample #	ATEM Amphibole Asbestos	ISO-NY PLM Wt. % Amphibole Asbestos	CSMP-PLM w/ HLS Chrys %	CSMP Weight Recovery Heavy Fraction	CSMP Chrys % Weight Corrected**
M71482-001	36,600	0.001 to 0.002	0.010-0.012	32.7%	0.003-0.004
M71483-001	<37,500	NSD*	0.007-0.010	28.2%	0.002-0.003
M71484-001	<161,000	NSD*	0.005-0.007	15.1%	0.0008-0.001
M71484-002	<152,000	NSD*	0.005-0.006	13.4%	0.0007-0.0008
M71485-001	<29,800	NSD*	0.010-0.011	30.0%	0.003-0.003

M71486-001	<52,000	NSD*	0.002-0.003	15.5%	0.0003-0.0005
M71487-001	<53,100	NSD*	0.005-0.007	38.7%	0.002-0.003
M71487-002	<48,000	NSD*	0.005-0.007	38.0%	0.002-0.003
M71501-001	<46,900	NSD*	0.005-0.006	49.7%	0.002-0.003
M71506-001	56,100	NSD*	0.004-0.006	52.3%	0.002-0.003
M71507-001	50,700	NSD*	0.005-0.007	55.8%	0.003-0.004
M71520-001	<54,200	NSD*	0.005-0.006	46.3%	0.002-0.003

***NSD: No Structure Detected **Weight Corrected**

The refractive index and calculated birefringence values are shown in Table 3.

Table 3
Overall Summary of the Calculated Chrysotile
BIR CSMP-PLM Data
(RI Fluid 1.550 & 1.605)

MAS Sample #	RI Fluid	Chrysotile RI Values CSMP-PLM	Birefringence Calculations
M71482-001	1.550	1.568-1.560 1.558-1.551	0.007-0.008 avg. = 0.008
M71483-001	1.550	1.568-1.563 1.566-1.552	0.005-0.014 avg. = 0.010
M71484-001	1.550	1.568-1.561 1.560-1.552	0.007-0.008 avg. = 0.008
M71484-002	1.550	1.568-1.565 1.560-1.552	0.003-0.008 avg. = 0.006
M71485-001	1.550	1.567-1.561 1.558-1.551	0.006-0.007 avg. = 0.007
M71486-001	1.560	1.569-1.564 1.563-1.558	0.005-0.005 0.005
M71487-001	1.560	1.568-1.564 1.562-1.558	0.004-0.004 0.004
M71487-002	1.560	1.568-1.565 1.566-1.558	0.003-0.008 avg. = 0.006
M71501-001	1.560	1.569-1.562 1.566-1.559	0.007-0.007 0.007
M71506-001	1.560	1.568-1.565 1.566-1.559	0.003-0.007 avg. = 0.005
M71507-001	1.560	1.570-1.566 1.562-1.557	0.004-0.005 avg. = 0.005
M71520-001	1.560	1.568-1.565 1.566-1.559	0.003-0.007 avg. = 0.005
		α range γ **1.566-1.551 1.570-1.558**	**1.550 avg: = 0.008** **1.560 avg. = 0.005**

Estimation of the Number of Chrysotile/Amphibole Bundles Detected for CSMP & ISO-NY PLM Methods

Using the number of chrysotile bundles counted during the PLM analysis, and the amount of talcum powder analyzed in a specified area on the cover slip mount per the two glass slides, the amount of chrysotile bundles per gram of talcum powder sample can be calculated. Total chrysotile bundles in the sample is calculated as shown in the following equation:

$$(A1 \div A2) \times (CB) \div W = TCB/W$$

Where:

A1: The total area (972 mm^2) that the talcum powder occupies on the two glass slides.

A2: The area (23.55 mm^2) in thirty fields of view that the talcum powder occupies on the two glass slides.

CB: Number of chrysotile bundles detected in a positive sample by PLM analysis.

W: Weight of total talcum powder placed on the two glass slides.

TCB/W: Total number of chrysotile bundles per weight (grams) of talcum powder.

The results of CSMP sample preparation analysis calculations are shown in Table 4. The calculations were weight corrected*.

Table 4
Summary of Estimated Chrysotile Bundles per gram Calculations For the CSMP PLM Results

MAS Sample #	wt. of sample grams	No. of Chrys Bundles counted	CSMP/ISO Chrysotile Bundles/g	CSMP/ISO* Chrysotile Bundles/g
M71482-001	0.0010	35	1,446,000	**473,000***
M71483-001	0.0010	24	991,000	**280,000***
M71484-001	0.0009	12	551,000	**83,000***
M71484-002	0.0008	11	568,000	**76,000***
M71485-001	0.0007	34	2,000,000	**600,000***
M71486-001	0.0004	4	413,000	**64,000***
M71487-001	0.0010	12	496,000	**192,000***
M71487-002	0.0008	14	723,000	**275,000***

M71501-001	0.0007	13	767,000	**381,000***
M71506-001	0.0008	9	465,000	**243,000***
M71507-001	0.0007	12	708,000	**395,000***
M71520-001	0.0007	11	767,000	**210,000***
			Avg. = 825,000	**Avg. = 273,000***

The average of the amount of chrysotile bundles for the CSMP sample preparation methods for the twelve Ammens powder samples was 273,00 bundles per gram of talc.

For the positive tremolite asbestos sample results by PLM, the amount of tremolite asbestos was calculated as described above and the results are shown in Table 5.

Table 5
Summary of Estimated Tremolite Bundles per gram Calculations
For the NYELAP PLM Results

MAS Sample #	Wt. of sample grams	No. of Tre/Act Bundles counted	NYELAP/ISO Tremolite Bundles/g*
M71482-001	0.0009	2	92,000

DISCUSSION/CONCLUSION

MAS' PLM analysis was able to both detect and determine the amount of chrysotile in the sample with HLS because MAS uses PLM microscopes that have higher resolution and analytical sensitivity capabilities than a standard PLM microscope (Olympus BH2), which is more suited for analyzing asbestos-added products (AAP), and for cosmetic talc samples.

AAP (chrysotile) samples, as compared to cosmetic talc samples, have a much higher population of very large size chrysotile bundles and orders of magnitude higher concentration levels of chrysotile in these types of products.

The PLM analysis of AAP samples does not challenge the resolution of the typical PLM microscope optics since most PLM labs are analyzing AAP samples that contain some very large bundles that are longer then the entire field of view of the PLM scope, or burden the microscopist with very long

sample analysis times. For example, in most PLM labs, including MAS's, the typical time required for an experienced PLM microscopist to analyze asbestos added products (AAP), where the majority of the AAP samples contain approximately 10 to 25 % asbestos, will only take about 15 and 20 minutes to complete the analysis.

With cosmetic talc samples on the other hand, a typical PLM analysis at MAS, for either chrysotile or amphiboles asbestos, would routinely take 2 to 4 hours for a positive sample and a minimum of 20 minutes to hour for a negative sample, if there are no pigments in the sample. If pigments are present in the sample, even a non-detect could take up to two hours. Additionally, during the analysis, the light intensity for our PLM microscopes are always kept at full brightness.

In order to both detect and analyze the small size of the chrysotile bundles (10 to 20 µm in length), that are typically found in cosmetic grade talcum powder, through the use of dispersion-staining, the PLM microscope must have "flat" objective lenses, and a HD video camera attached to the PLM microscope that is interfaced to a high definition monitor.

The MAS PLM microscopes are state-of-the-art Leica DM2700P PLM microscopes, where all of the objective lens, including the 10X central stop dispersion lens are the flat type, also known as infinity lens, LED light source, and are coupled with a state-of-the-art HD digital camera and 37" HD monitor. To detect these size chrysotile bundles, it is highly recommended that this type of PLM microscope setup should be used for the PLM analysis of cosmetic talc samples.

It is also my opinion that the PLM analyst must first analyze prepared talcum powder standards, containing UCC SG-210 or RG-144 Calidria chrysotile, to become familiar with both the size of chrysotile structures found in cosmetic talc, as well as the difference in the refractive indices for the chrysotile as compared to chrysotile added products. However, our studies have shown, that the UCC SG-210 Calidria chrysotile standard structure size range is more in line with the size of the chrysotile detected in the cosmetic talc samples. If the PLM laboratory does not have excess to UCC's SG-210 or their RG-144 chrysotile, then the NIST 1866b standard can be used if it is first cryo-balled milled with liquid nitrogen then sieved to a minus 200 to plus 325 size range and analyze the milled chrysotile structures that are in the 5 to 20 µm length and 0.5 to 3 µm width. This will produce refractive induces in the 1.560 to 1.569 range with 1.550 RI fluid.

Both the RG-144 and RG-210 Calidria chrysotile and the chrysotile found in the talcum powder samples typically shows central stop dispersion colors (CSDS) from blues (α) to golden yellows (γ) in 1.550 liquid, and blue to a dark gold in 1.560 liquid. MAS has been reporting this range of CSDS colors for the chrysotile detected in the cosmetic talc samples for almost two years using 1.550 RI

liquid. During that time, defendant experts, retained by a number of cosmetic talc manufacturers, have repeatedly testified that MAS's CSDS findings are not appropriate for chrysotile, based on dispersion staining results (golden-yellow dispersion) for the parallel direction. Therefore, in their opinions, MAS was and has been misidentifying fibrous/platy talc edge or cellulose as chrysotile.

For the Ammens project, MAS used both 1.550 RI liquid and 1.560 RI liquid for analyzing this set of 12 samples. As discussed by Dr. Gunter, Alan Segrave (defense experts in the talcum powder litigation) in their expert reports, and Dr. Su's photo-shop expert report, where they stated that to verify MAS is identifying chrysotile, a higher RI liquid than 1.550 needs to be used. For this PLM analysis of the Ammens powder samples, 5 samples were analyzed with 1.550 while the other seven samples were analyzed with 1.560. Our results show that the primary difference between the two RI liquids is that the measured refractive indices for the 1.560 RI liquid was closer together for the alpha and gamma directions, which caused the BIR calculations to be more in the LOW range (1.550 RI 0.008 vs.1.560 RI: 0.005) with an overall total average of 0.006 (See Table 3).

Additionally, Dr. Gunter, while working as a defense expert for Gold Bond defense counsel, analyzed samples of RG-144 and SG-210 Calidria chrysotile that MAS provided to him, and confirmed in a recent deposition that "Calidria chrysotile can produce a range of CDSC colors from bluish to golden-yellow in 1.550 liquid, which would be higher RIs than found by the un-milled NIST 1866b chrysotile standard".[6] Dr. Gunter's Calidria chrysotile results are consistent with our laboratory's findings, which validates our PLM chrysotile findings in the cosmetic talc samples. Dr. Gunter's testimony about his Calidria CSDS results is in direct contradiction to his original criticism of the "yellow" dispersion color, as well as Dr. Sanchez's and Mr. Seagrave's past testimony on this issue.

It is now my opinion, that when these defense experts were testifying that our laboratory was misidentifying fibrous talc or talc plates on edge for chrysotile based on the CSDS "yellow to yellow-gold color", as it turns out, the opposite was true, they were the ones misidentifying chrysotile as fibrous talc or talc plates on edge. In fact, a recent article by Dr. Su, in The Microscopy Journal, states that "There are chrysotile minerals whose refractive indices are significantly higher than those of the NIST SRM 1866 chrysotile.[7]

[6] Deposition of Dr. Mickey Gunter, Woods, Jesse & Sarah vs. Kolmar Laboratories Inc. et al. Supreme Court in the State of New York, County of Monroe, #E202000384

[7] SHU-CHUN, SU PH.D. "The Dispersion Staining Technique and its Application to Measuring Refractive Indices of Non-opaque Materials, with Emphasis on Asbestos Analysis", The Microscope, Vol. 69, 2nd Quarter 2022

ISO-PLM Chrysotile Refractive Index Ranges

As shown in Table 3, the range of measured refractive indexes for the detected chrysotile bundles in the twelve Ammens powder samples was 1.558-1.570 (parallel) and 1.551 to 1.566 (perpendicular) for the average CSMP method.

Shown in Table 6 are the range of RI's for the 48 chrysotile bundles that were recorded as examples of the chrysotile detected in the twelve Ammens Powder samples that were prepared by the CSMP method (with HLS).

Table 6
Chrysotile
Range of Parallel and Perpendicular RI's

Chrysotile Bundle No.	RI Fluid	CSMP PLM (with HLS) Parallel RI	CSMP PLM (with HLS) Perpendicular RI
M71482-001	**1.550**		
1		Avg. 1.563	Avg. 1.552
2		1.565	1.560
3		Avg. 1.562	Avg. 1.557
4		Avg. 1.563	Avg. 1.559
		Avg. 1.563	**Avg.1.557**
M71483-001	**1.550**		
1		1.567	1.552
2		1.566	1.553
3		1.566	1.555
4		1.568	1.559
		Avg. 1.567	**Avg. 1.555**
M71484-001	**1.550**		
1		1.566	1.552
2		1.565	1.556
3		Avg. 1.564	Avg. 1.556
4		1.567	1.561
		Avg. 1.566	**Avg. 1.556**
M71484-002	**1.550**		
1		Avg. 1.563	Avg. 1.556
2		Avg. 1.566	Avg. 1.556
3		1.568	1.554
4		Avg. 1.566	Avg. 1.562
		Avg. 1.566	**Avg. 1.557**
M71485-001	**1.550**		
1		1.566	Avg. 1.554
2		1.566	Avg. 1.552
3		1.558	1.553
4		1.567	Avg. 1.554
5		1.566	Avg. 1.557
		Avg. 1.565	**Avg. 1.554**

M71486-001	**1.560**		
1		Avg. 1.565	Avg. 1.560
2		Avg. 1.567	Avg. 1.564
3		Avg. 1.568	Avg. 1.563
		Avg. 1.567	**Avg. 1.562**
M71487-001	**1.560**		
1		1.568	Avg. 1.563
2		Avg. 1.565	Avg. 1.562
3		1.567	1.560
4		1.568	Avg. 1.560
		Avg. 1.567	**Avg. 1.561**
M71487-002	**1.560**		
1		1.566	Avg. 1.560
2A		1.566	Avg. 1.563
2B		1.567	Avg. 1.560
3		1.568	1.558
4		Avg. 1.567	Avg. 1.560
		Avg. 1.567	**Avg. 1.560**
M71501-001	**1.560**		
1		Avg. 1.568	Avg. 1.561
2		1.566	Avg. 1.561
3		Avg. 1.567	Avg. 1.560
4		1.568	1.560
		Avg. 1.567	**Avg. 1.561**
M71506-001	**1.560**		
1		1.569	Avg. 1.560
2		Avg. 1.567	Avg. 1.560
3		1.568	1.560
4		1.566	Avg. 1.564
		Avg. 1.568	**Avg. 1.561**
M71507-001	**1.560**		
1		1.569	1.559
2		Avg. 1.568	Avg. 1.560
3		Avg. 1.564	Avg. 1.563
4		Avg. 1.566	Avg. 1.564
		Avg. 1.567	**Avg. 1.562**
M71520-001	**1.560**		
1		1.568	Avg. 1.560
2		Avg. 1.567	Avg. 1.561
3		1.567	Avg. 1.564
4		1.566	Avg. 1.561
		Avg. 1.567	**Avg. 1.562**

The individual chrysotile bundles that had a range of RI's for either the parallel or perpendicular direction were averaged.

Birefringence Measurements

The key optical property to differentiate fibrous talc from chrysotile asbestos, when using the PLM method, is determining the difference in the birefringence (BIR) value between these two elongated minerals. Most PLM analysts will just use the PLM cross-polar condition to visually estimate the magnitude of the BIR (Low, Moderate or High) by the amount of brightness and change in wavelength colors that are observed.

This visual estimate of the amount of birefringence is typically done under cross-polar conditions and is a subjective interpretation by the PLM analyst, and therefore, can lead to errors. A more accurate determination of BIR is to calculate the numerical BIR value by simply subtracting the measured perpendicular RI from the measured parallel RI (n ‖ - n _|_).

The subtracted BIR results give the analyst a numerical birefringence (BIR) value that is either classified as **Low (<0.01)**, **Moderate (0.01 to 0.05)** or **High (>0.05)**.

Fibrous talc and/or talc plates on edge will have a calculated BIR value that is typically at the high end of Moderate (0.045) to greater than 0.05 which is in the High BIR range. Chrysotile on the other hand, will have BIR values that range from the upper end of the Low range to the lower end of the Moderate range. The overall average calculated range BIR's, for the detected chrysotile bundles from the twelve Ammens Powder samples for CSMP PLM method was **0.006**, which falls in the LOW end of BIR classifications. For just the samples that were analyzed with 1.550 RI liquid, the BIR calculation was 0.008 and for the 1.560- RI liquid, the BIR calculation was 0.004, both of the two BIR results are in the LOW category.

The BIR difference between fibrous talc and chrysotile, as demonstrated by MAS, is also verified by the EPA in their 600/R-93/116 PLM methodology document as shown in Table 2-2, page 21.

Table 2-2, "Optical Properties of Asbestos Fibers", provides four sets of refractive indexes measured from chrysotile bundles that have an overall average BIR of 0.011, and a published range of 0.004 to 0.017. This agrees with the overall **MAS BIR avg. of 0.006** for the chrysotile bundles detected in the twelve talcum powder samples for CSMP sample preparation methods.

Also, the range of BIR values calculated for the chrysotile refractive indexes shown in EPA's Table 2.2, supports MAS's PLM data that fibrous talc was not misidentified as chrysotile in the twelve Ammens Powder samples. The BIR calculations for the EPA's four sets of chrysotile RI measurements in their Table 2.2 are shown in MAS's Table 7.

Table 7
EPA-R93 Table 2-2 Chrysotile PLM RI Data
& Birefringence Calculations

Chrysotile RI's Direction Values	BIR Calculations for Chrysotile
1.517-1.493 1.557-1.546	0.024 – 0.011 **Avg. 0.018**
1.545-1.532 1.556-1.549	0.013-0.007 **Avg. 0.010**
1.537-1.529 1.567-1.559	0.008-0.008 **Avg. 0.008**
1.552-1.544 1.561-1.553	0.008-0.008 **Avg. 0.008**
Range 1.567 to 1.493	**Overall Avg. 0.011**

The EPA R93 protocol also provides RI and BIR data for both fibrous talc and flat cellulose ribbons that can be found in their Table 2.5. For the RIs of fibrous talc example, EPA reports refractive index 1.600-1.540 with a measured BIR of 0.06, and for cellulose ribbons, the reported EPA RI's are 1.580-1.530 with a measured BIR of 0.05 as shown in Table 8.

Table 8
EPA-R93: Optical Properties of Selected Fibers
Fibrous Talc & Cellulose Ribbons

Fiber Type	RI Parallel/Perpendicular	BIR Calculations
Fibrous Talc	1.600-1.540	0.06 "High"
Cellulose	1.580-1.530	0.05 high end of Moderate

In summary, this data demonstrates that the reported chrysotile bundles in the twelve Ammens Powder container samples analyzed by MAS have both the appropriate range of refractive indexes and BIR demonstrating that chrysotile asbestos was correctly identified in each container sample.

Potential Asbestos Exposure to Ammens Powders:

6.25 oz. Containers: M71484-001 &-002, M71486-001 and M71487-001 & -002 and M71520-001
The average chrysotile bundle results for PLM analysis for these six Ammens 6.25 oz. containers shows that one gram of Ammens body powder contained an average of 150,000 (weight corrected) chrysotile bundles per gram of talcum powder.

Six of the 12 Ammens containers contain 6.25 oz. (177.2 g) of talcum powder when full.

Multiplying 150,000 chrysotile bundles by 177.2 grams would equal approximately 26,580,000 chrysotile bundles, on average, in the one (6.25 oz.) Ammens powder container.

5.5 oz. Container: M71482-001:
For the one 5.5 oz. (155.9g) container, the concentration of chrysotile bundles found in the analysis shows that one gram of this Ammens body powder sample contained 473,000 chrysotile bundles per gram of talcum powder.

In addition, sample M71482-001 had detectable amounts of tremolite asbestos at a concentration of 36,600 tremolite fibers/bundles per gram by TEM. Using the combined amount of chrysotile bundles and the addition of the tremolite asbestos would equal approximately 510,000 chrysotile/tremolite bundles and fibers per gram of talcum powder. Multiplying 510,000 chrysotile/tremolite asbestos by 155.9 grams would equal approximately 80,000,000 chrysotile/tremolite fibers/bundles, on average, in the one (5.5 oz.) Ammens body powder container.

11 oz. Container: M71483-001:
The chrysotile bundle results for PLM analysis shows that one gram of 11.0 oz. (311.9 g) Ammens body powder contained 280,000 chrysotile bundles per gram of talcum powder. Multiplying 280,000 chrysotile bundles by 311.9 grams would equal approximately 87,000,000 chrysotile fibers/bundles, on average, in the one (11.0 oz.) Ammens body powder container.

2.5 oz. Container: M71485-001:
The average chrysotile bundle results for PLM analysis shows that one gram of 2.5 oz. (70.9 g) Ammens body powder contained 600,000 chrysotile bundles per gram of talcum powder. Multiplying 600,000 chrysotile bundles by 70.9 grams would equal approximately 43,000,000 chrysotile fibers/bundles, on average, in the one (2.5 oz.) Ammens powder container.

4.5 oz. Container: M71501-001:
The chrysotile bundle results for PLM analysis shows that one gram of 4.5 oz. (127.6 g) Ammens powder contained 381,000 chrysotile bundles per gram of talcum powder. Multiplying 381,000 chrysotile bundles by 127.6 grams would equal approximately 49,000,000 chrysotile fibers/bundles, on average, in the one (4.5 oz.) Ammens powder container.

0.5 oz. Container: M71506-001:
The average chrysotile bundle results for PLM analysis shows that one gram of 0.5 oz. (14.2 g) Ammens body powder contained 299,000 chrysotile bundles per gram of talcum powder.

In addition, sample M71506-001 had detectable amounts of tremolite asbestos at 56,100 tremolite fibers/bundles per gram. Using the amount of chrysotile bundles and the addition of tremolite structures would equal approximately 355,000 chrysotile/tremolite bundles per gram of talcum powder. Multiplying 355,000 chrysotile/tremolite bundles by 14.2 grams would equal

approximately 5,000,000 chrysotile/tremolite fibers/bundles, on average, in the one (0.5 oz.) Ammens powder container.

2.5 oz. Container: M71507-001:
The average chrysotile bundle results for PLM analysis shows that one gram of 2.5 oz. (70.9 g) Ammens body powder contained 395,000 chrysotile bundles per gram of talcum powder.

In addition, sample M71507-001 had detectable amounts of tremolite asbestos at 50,700 tremolite fibers/bundles per gram. Using the amount chrysotile bundles detected and the addition of tremolite structures would equal approximately 446,000 chrysotile/tremolite bundles per gram of talcum powder. Multiplying 446,000 chrysotile/tremolite bundles by 70.9 grams would equal approximately 32,000,000 chrysotile/tremolite fibers/bundles, on average, in the one (2.5 oz.) Ammens powder container.

Based on these results, it is my opinion that the application of the talcum powder found in Ammens body powder containers will cause significant exposure, over background, to chrysotile/ tremolite asbestos to individuals, who used Ammens Powder brand talcum powder products for their intended purpose. Significantly over background is defined as 10 x 0.00005 f/cc or 0.0005 f/cc.

All of the opinions that I have stated in this report are held within a reasonable degree of scientific certainty and I reserve the right to supplement this report if any new information becomes available.

Sincerely,

William E. Longo, Ph.D.
CEO