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Sent: Thursday, May 31, 2007 4:27 PM

To: NIOSH Docket Office (CDC)

Subject: Asbestos and Other Mineral Fibers: A Roadmap for Scientific Research, NIOSH Docket Number NIOSH-099

Attachments: NIOSH Comments Docket-099.pdf

Attached is information related to the asbestos content of a tremolite sample used in the Davis et al injection studies.

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RE: Comments on NIOSH Docket Number NIOSH-099
Composition of Ala di Stura Tremolite

NIOSH has requested comments regarding the draft document "Asbestos and Other Mineral Fibers: A Roadmap for Scientific Research"[1] which describes a suggested research program to improve the scientific understanding of asbestos minerals and the health effects of fibrous minerals. A public meeting was held on May 4, 2007 where comments were presented on this "roadmap"; a transcript of that meeting has been made available to the public.[2]

The "roadmap" is a broad overview of selected documents related to asbestos mineralogy, exposure assessment, and health effects. It is by no means inclusive of the vast amount of literature readily available on the topic. As part of the research program, a more thorough and unbiased review of the literature must be conducted.

One of the studies cited in the roadmap is that of Davis et al[3] study of six different samples of tremolite. Three of the samples (Jamestown, Swansea, and Korea) were identified as asbestiform minerals while the other three (Ala di Stura, Carr Brae, and Shinness) were identified as non-asbestos minerals. These tremolite samples were injected intraperitoneally into rats of the AF/Han strain. The asbestiform tremolite samples had significant mesothelioma mortality while the non-asbestos tremolite (with the exception of the Ala di Stura) had very low mortality. The Ala di Stura tremolite had a lower incidence of mesothelioma compared to the Jamestown tremolite (asbestos) with more than twice the mean survival time.

The mortality and survival times of the Ala di Stura tremolite are indicative of a low dose exposure to asbestiform tremolite. As noted by Davis et al, some very long, thick fibers were observed in the sample, but these were not represented in the fiber size statistics. A prior

[1] Accessed 5/31/07 at http://www.cdc.gov/niosh/review/public/099/pdfs/NIOSHAsbestosRoadmap.pdf.
[2] Accessed 5/31/07 at http://www.cdc.gov/niosh/docket/pdfs/NIOSH-099/0099-050407-transcript.pdf.
[3] Davis JM, Addison J, McIntosh C, Miller BG, and Niven K (1991). "Variations in the carcinogenicity of tremolite dust samples of differing morphology", *Ann NY Acad Sci*,, 643, p. 473-490.

analysis of this material has shown that less than 2% of the fibers 5 μm and longer are also thinner than 0.25 μm.[4] RJ Lee Group has begun a characterization of the Ala di Stura sample to document the mineralogy of the material. A sample of the Ala di Stura tremolite was obtained from one of the authors (Mr. John Addison) and was analyzed using transmission electron microscopy (TEM) and field emission scanning electron microscopy (FESEM) as follows:

A portion of the tremolite sample was placed in a beaker with a water/alcohol solution and was sonicated for 3 minutes. An aliquot of the suspension was removed and deposited onto a polycarbonate filter. The filter was carbon coated and then dissolved following the procedure of Burdette and Rood[5], leaving the particulate trapped in a carbon film supported by a 400 mesh locater TEM grid[6]. The samples were examined with JEOL JEM 1200EX and JEM 2000FX TEMs operating at a beam voltage of 120 kV. Particles having a 3:1 or higher aspect ratio or larger (the requirement that particles have parallel or substantially parallel sides was not enforced for the purpose of this analysis) were analyzed following an enhanced Yamate Level III protocol[7]. Upon completion of the TEM characterization, a sketch was made of the location of the particles on the TEM grid and the grid transferred to the FESEM for relocation and imaging of each particle analyzed by TEM.

Prior to FESEM analysis, the samples were platinum coated (to improve the imaging). The samples were mounted for FESEM examination in a dual grid holder (EM-Q2 Quick Change Retainer) designed for use with a JEOL TEM sample rod. Particles that were analyzed by TEM were relocated in the FESEM with the aid of TEM field images and TEM analyst sketches. Secondary electron images were collected using the "Through the Lens Detector" (TLD) on a FEI Sirion 400 FESEM operating at a beam voltage of 3 kV, a beam current of 200 pA and a 4.0 mm working distance. The FESEM protocol developed for this analysis required the collection of FESEM images of the full structure, structure ends, and structure surface. Stereo pair images were also formed to provide depth perception not obtained from a single secondary electron image.

Figures 1 and 2 show images of typical asbestiform and non-asbestos tremolite particles observed in the Ala di Stura tremolite sample. Figure 3 contains TEM images of four additional asbestiform fibers. Fewer than 10 percent of the fiber-like particles observed in this sample were asbestiform. Our analysis of this material is on-going.

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[4] Bailey, K. F., Kelse, J., Wylie, A. G., and Lee, R. J. (2003). "The Asbestiform and Nonasbestiform Mineral Growth Habit and Their Relationship to Cancer Studies: A Pictorial Presentation", National Stone, Sand and Gravel Association, Arlington, VA.

[5] Burdett, G., and Rood, A. (1983). "Sample Preparation for Monitoring Asbestos in Air by TEM - Analytical Chemistry." *Anal. Chem.*, 55, p. 1642-1645.

[6] Burdett, G. J., and Rood, A. (1983). "Membrane-Filter, Direct-Transfer Technique for the Analysis of Asbestos Fibers or Other Inorganic Particles by Transmission Electron Microscopy." *Environ. Sci. Technol.*, 17,p. 643-648.

[7] Yamate, G., Agarwal, S. C., and Gibbons, R. D. (1984). "Methodology for the Measurement of Airborne Asbestos by Electron Microscopy." EPA Contract No. 68-02-3266, July 1984.

The techniques described in this letter and presented at the May 4, 2007 public meeting[8] can be used to accurately characterize the mineral fibers described in the "roadmap". With generally accepted analytical procedures, the TEM provides information on each particle to indicate its size, crystal structure (using selected area electron diffraction), and elemental composition (energy dispersive x-ray spectroscopy) while the FESEM provides information related to the morphology and orientation of the particle on the filter surface.

RJ Lee Group has a large database of images obtained in the manner described above from a variety of mineral standards. RJ Lee Group has previously offered this database to NIOSH for use in evaluating proposed asbestos characterization procedures.

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[8] Accessed 5/31/07. http://www.cdc.gov/niosh/docket/pdfs/NIOSH-099/0099-050407-leestrohmeier_presentation.pdf.

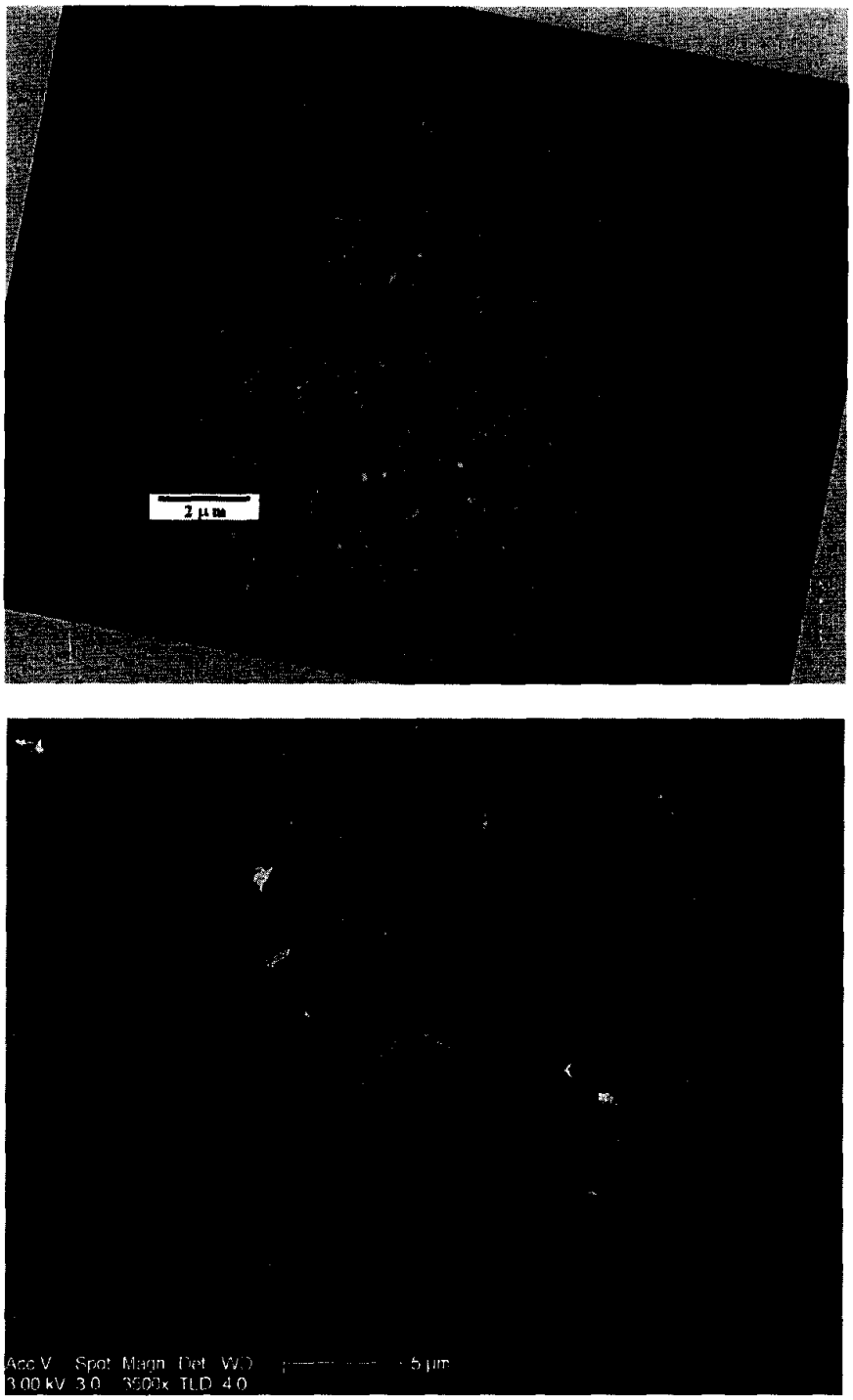


Figure 1. Photographs of an asbestiform tremolite fiber observed in the transmission electron microscope (top) and field emission scanning electron microscope (bottom). The fiber is 16.1 µm long and 0.15 µm wide.

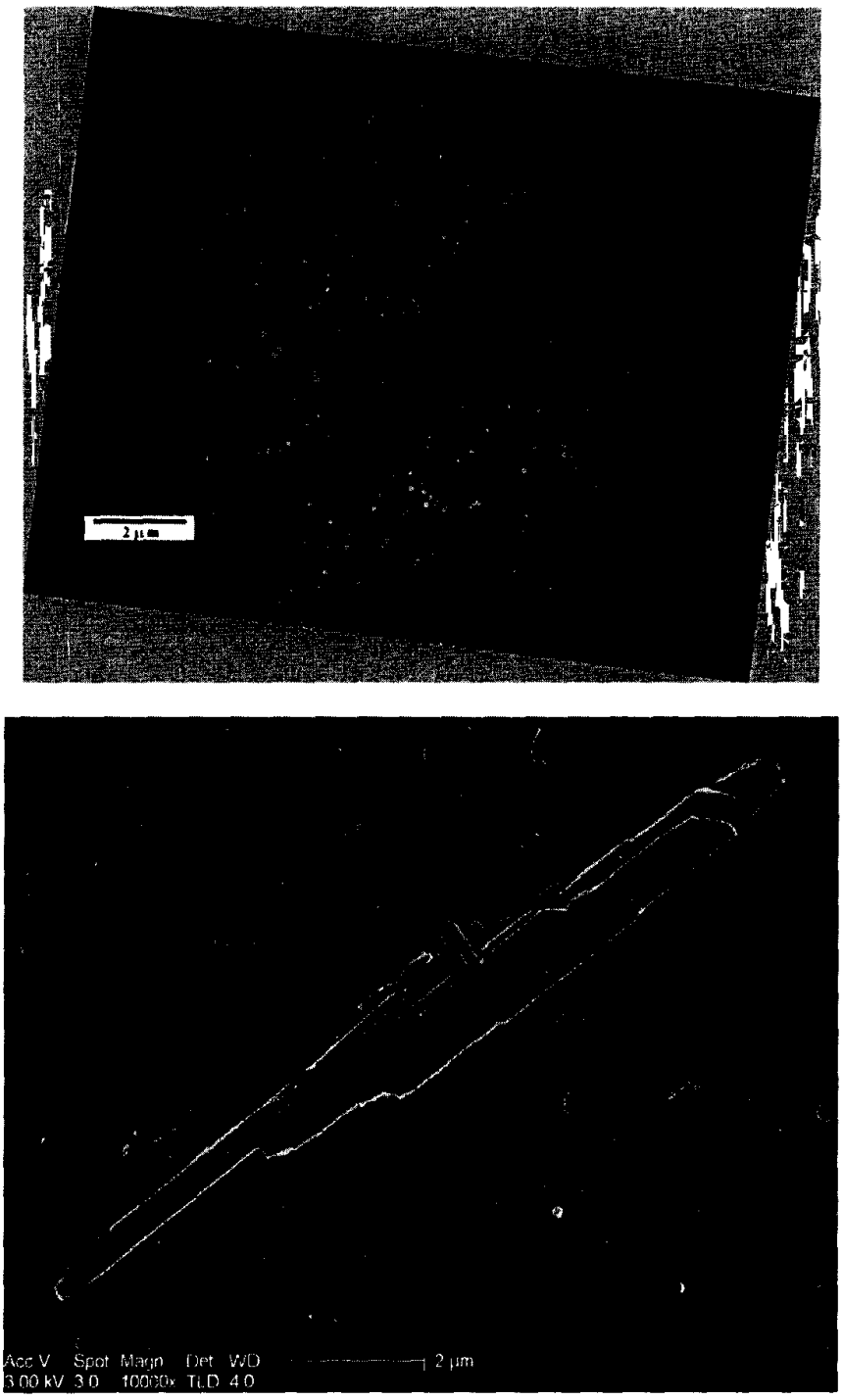
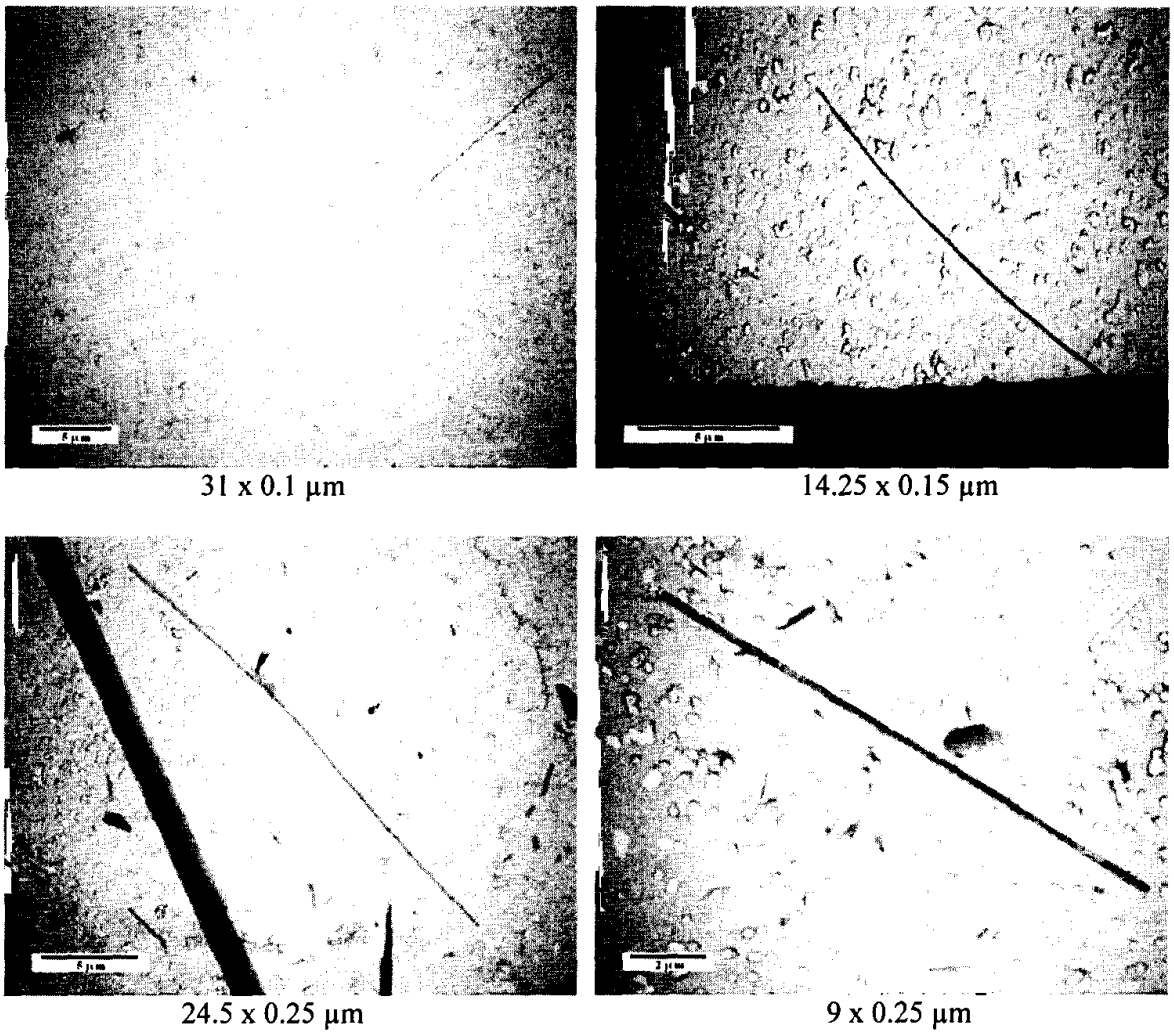


Figure 2. Photographs of a typical nonasbestos tremolite particle observed in the transmission electron microscope (top) and field emission scanning electron microscope (bottom). The fiber is 6.1 µm long and 0.25 µm wide.



31 x 0.1 μm

14.25 x 0.15 μm

24.5 x 0.25 μm

9 x 0.25 μm

Figure 3. Additional photographs of a asbestiform tremolite fibers observed in the transmission electron microscope.