HSCI 348
Industrial Hygiene Instrumentation Techniques
Laboratory No. 7

ASBESTOS ANALYSIS

INTRODUCTION

Asbestos sampling and analysis is an important topic for all industrial hygienists to understand. Airborne asbestos can be generated by any process that disturbs materials containing any of the various asbestiform minerals (primarily **chrysotil**e, **amosite** or **crocidolite**). The ability to determine the presence of asbestos and the airborne concentration is necessary to be able to estimate exposures and health risks as well as to comply with government regulations.

The identification of a bulk sample of a material (pipe insulation, ceiling tile, etc.) to determine if it contains asbestos, and if so, what type of asbestos, can be done with a **Polarized Light Microscope (PLM).** The identification process follows a flow chart which includes fiber morphology, refractive light index, isotropic or anisotropic, dispersion staining and sign of elongation. Although these characteristics will not be covered in this lab, bulk asbestos samples will be shown.

Airborne samples of asbestos fibers collected on filters (usually .45um or .8 um MCE) can be analyzed with a **Phase Contrast Microscope (PCM)**. After a section of the filter is "cleared" so that light can pass through it, areas or "fields" of the filter are examined optically to obtain a representative fiber count per unit area of filter surface. This count combined with the collected sample volume is then translated into fiber concentration. The rules of counting and the method are stipulated by the NIOSH 7400 Method or the similar OSHA Reference Method (ORM).

Airborne samples of asbestos fibers collected on filters (usually 0.4 um PC - polycarbonate) can be analyzed by an electron microscope, typically a **Transmission Electron Microscope (TEM)** type. After the filter sample is specially prepared for insertion into the vacuum chamber of the TEM, it is examined to obtain a representative count of "structures" per unit area of filter surface by counting a number of "grid" squares. The method is as indicated in NIOSH 7402. The individual fibers can also be analyzed for chemical content with an **energy dispersive x-ray analyzer (EDX),** which looks at the element-characteristic x-rays given off by the excitement of the sample fiber due to the energy of the focused electron beam. The identity of the fiber can be confirmed using the unique properties of its crystalline structure by focusing the electron beam in such a manner as to create a unique dot pattern known as **Selected Area Electron Diffraction (SAED).**

OBJECTIVES

At the completion of this laboratory, the student should:

1) Be familiar with the OSHA Reference Method for asbestos fiber counting using Phase Contrast Microscopy (PCM).

2) Be able to determine an airborne asbestos concentration from counting fibers on a filter sample.

3) Be familiar with advantages and basic operating principles of the TEM for counting and identifying asbestos fibers.

EQUIPMENT

Phase Contrast Microscope	Acetone Vapor Generator
Walton-Beckett graticule	Asbestos bulk samples
Test slide	Transmission Electron Microscope, with EDX

PROCEDURE

A. PHASE CONTRAST MICROSCOPY (PCM)

Part 1. Preparation of Filter for Analysis

1) Become familiar with filter preparation techniques by cutting a pie shaped wedge out of a filter cassette containing an MCE filter.
2) With tweezers, place the cut piece of filter on a microscope slide and place it in the acetone evaporator. With a syringe, inject a small amount of acetone into the evaporator to "clear" the filter.
3) Place a small amount of triacetin onto the cleared wedge to fix the filter.

4) Place a cover slip over the cleared filter section and glue it to the slide with nail polish. Mark the outline of the filter section with a marking pen on the under side of the glass slide to aid in microscopic analysis..

Part 2. Analysis of Sample Slide for Asbestos Fibers

1) Become familiar with the PCM and the eyepiece. Calibrate the Walton-Beckett graticule with the stage micrometer.

2) Use the OSHA Reference Method to count fibers. Each person should count enough randomly selected fields and record them on the group lab data sheet so that there will be a total of 100 fibers of 100 fields, whichever comes first.

B. TRANSMISSION ELECTRON MICROSCOPY (TEM)

Part 3. Demonstration of TEM for Asbestos Fiber Counting

1) Observe the vacuum evaporator chamber which is used to prepare samples for the TEM with a fine coating of carbon or gold.

2) Observe the operation of the TEM and the increased magnification potential in order to "see" smaller fiber diameters and for determination of a count by grid squares.

3) Observe the operation of the TEM with regard to the use of Selected Area Electron Diffraction (SAED) to identify asbestos by crystallography.

Part 4. Demonstration of EDX for Asbestos Fiber Identification

Observe the EDX and its ability to identify different types of asbestos by chemical content.

RESULTS

1. Determine the actual Walton-Beckett field area based on the calibration of this particular PCM.

2. Prepare a simple table comparing the average fibers/field count of each person's individual count.

3. Calculate the asbestos concentration in fibers/cc from the count of fibers in the fields *you* personally counted **and also** calculate the concentration for the fibers in the fields counted by the entire lab section. Estimate the statistically highest value expected with 95% confidence (the Upper Confidence Level) **or** the statistically lowest value expected (the Lower Confidence Level) (*NOT* both) based on the following equations:

   a) *Original NIOSH 239 Method*:

   UCL: $X + (1.64)(\text{Coef. of Var.})(\text{Stnd, f/cc})$

   or

   LCL: $X - (1.64)(\text{Coef .of Var.})(\text{Stnd, f/cc})$

   where:

X	=	concentration of fibers in your sample (f/cc)
1.64	=	constant associated with 95% confidence (one-tailed test)
Coef. of Var.	=	the relative variation associated with fiber counting (0.115 for a fiber count of 100 fibers; see graph for < 100 fibers)
Stnd, f/cc	=	the concentration level to which you are comparing your sample [for example, if your sample is *below* a standard, use the *UCL* to see if your results could possibly (statistically speaking) be exceeding the standard based on the method's variability; if your sample is below but close to the PEL, use Stnd = 0.1 f/cc (the PEL); if your sample is below but close to the 0.01 f/cc use the clearance value 0.01 f/cc as the "standard" for comparison.] [if your sample is *above* a standard, use the *LCL* to see if your results could possibly be below that standard; use the same criteria for selecting the correct reference standard as in the previous example]

b) *Current NIOSH 7400 Method:*

UCL: X + (2.13)X
or
LCL: X - (0.49)X

4. List the types of fibers identified for the unknown samples by the TEM and their constituent elements as identified by the EDX.

DISCUSSION

1. Compare the results of your personal PCM concentration to the entire lab section's concentration and to that determined by the consultant's lab staff. How variable was each student's individual fiber count. With a 95% UCL, did your own or the lab's results indicate an overexposure to asbestos in comparison to the PEL, considering both the NIOSH 239 *and* the NIOSH 7400 methods.
2. Was the Walton-Beckett graticule calibrated to within the acceptable field area range as specified in the NIOSH 7400 Method?
3. What is the difference in magnification between the PCM and TEM methods?
4. Why does the TEM operate in a vacuum?
5. Why does an asbestos sample have to be coated with carbon prior to examination in the TEM?
6. What types of asbestos fibers were identified by the EDX?
7. Discuss the advantages and disadvantages of the PCM and TEM methods.

CONCLUSIONS

Write five (5) good conclusions based on your observations.

Introduction:

The purpose of this experiment is to become familiar with asbestos counting and identification through a phase contrast microscope. In the first part we learned how to prepare a microscope slide which involved cutting the filter and placing it on a slide, and then we vaporized acetone and put it on the slide to dissolve the filter paper. Then we added two drops of solution to the slide, placed a slide cover over it and used nail polish to seal it and make it archivable. In part two, we looked at a slide that was prepared for us and counted asbestos fibers and kept count of how many fibers we saw. In the last part we went into The Armstrong Engineering building and were shown how a transmission electron microscope (TEM) works.

Results:

1. $A=1/4\ \pi d^2$
   $d= 100\ \mu m$
   $1mm^2 = 1000000\mu m^2$

   $A=(1/4)\ \pi(100\ \mu m)^2$
   $A=(1/4)\ \pi(1000000\ \mu m^2)$
   $A=7853.98\ \mu m^2$

   $(1mm^2/1000000\mu m^2) \times 7853.98\ \mu m^2 = .00785\ mm^2$

2. Counts per field by person

Initial	Fibers Counted	Fibers/fields counted
ZP[7]	2.5	.3571
EG[8]	1	.125
MS[7]	2.5	.3571
AH[8]	1	.125
JO[7]	10.5	1.5
RL[8]	3.5	.4375
EK[7]	3.5	.7
JS[8]	8.5	1.0625
BD[7]	4	.5714
KS[7]	7	1
LB[8]	7	.875
JG[7]	3.5	.5
HB[8]	2.5	.3125
CT[7]	2.5	.3571
[7]= 7 fields counted	[8]= 8 fields counted	

Total fibers counted= 59.5

The average number of fibers counted was about 4.25 fibers counted per person. The standard deviation of 2.87 and a relative standard deviation of 67.5. the range of fibers counted was from 0 to 10.5.

3. Fibers/CC= [(Fibers/Field) x 49]/(Q x t)

   Q= 2 lpm
   Time= 420 minutes
   Fiber count $_{group\ 2A}$ = 4.5
   Fields $_{group\ 2A}$ = 23
   Fibers/Field $_{group\ 2A}$= .196 fibers/ field
   Fiber Count$_{Class}$ = 59.5
   Fields $_{Class}$ = 100
   Fibers/Field $_{Class}$= .595 fibers/field
   Fiber Count $_{Lab}$ = 71
   Fields $_{Lab}$ = 100
   Fibers/Field$_{Lab}$=.71 fibers/field

   $X_{group\ 2A}$ =(Fibers/CC) $_{group\ 2A}$ = .0114
   X_{Class} =(Fibers/CC) $_{Class}$ = .0347
   X_{Lab} =(Fibers/CC) $_{Lab}$ = .0414

   a. Original NIOSH 239 Method Upper Confidence Limit
      UCL= X + (1.64)(Coef. Of Var.)(Std. f/cc)

      OSHA Std.= .10 f/cc
      Coef. Of Var. for Group 2A data = .27
      Coef Of Var. for Class data= .115

      UCL $_{group\ 2A}$=.05568 (f/cc)
      UCL $_{Class}$ = .05346 (f/cc)

   b. Current NIOSH 7400 Method
      UCL= X+ (2.13)X

      UCL $_{group\ 2A}$= .036 (f/cc)
      UCL $_{Class}$ = .109 (f/cc)

4. Chemical Components of Fiber types

Components	Fiber Type
Mg, Si	Chrysotile
Mg, Si, Fe	Amosite
Mg, Si, Fe, Na	Crocidolite
Mg, Si, Fe, Ca	Acenolite
Mg, Si, Fe	Anthrophylite
Mg, Si, Ca	Tremolite

Discussion

1. Our personal fibers/cc concentration was .0114 fibers/cc, this was lower than that of the class which was .0347 fibers/cc. in our group the amount of fibers seen in each field varied from .5 to 1.5. We believe that our concentration was so much lower because in 69% of our fields there were zero fields. In addition to this, 27% of our fields had .5 fibers, and 4% had 1.5 fibers. This accounts for all of the fibers we saw. The individual fibers counted by our group in all of the fields we saw range from1-2.5. in the entire class the counts range from 1-10.5, all of our counts were on the low end of this range. According to the NIOSH 239 method neither of the fiber counts (UCL2A=.05568 f/cc and UCL class= .05346 f/cc) were above the PEL of .1 f/cc. However, when it comes to the NIOSH 7400 method (UCL2A= .036 f/cc and UCL class= .109) the class data does have more than the accepted PEL of .1f/cc, but our group data is below the PEL.
2. Yes, the graticule was calibrated within an acceptable range. Using the equation for the area of a circle, A=1/4 π r^2, using the diameter of 100 micro meters (± 2 micrometers). After converting to millimeters and obtaining a value of .00785 mm^2, which is what the standard is.
3. The PCM magnifies about 450 times (about .25µm) while the TEM magnifies in a range from 1000 to 100,000 times (about .0025 µm) so it can identify thin fibers. The PCM does not identify the type of asbestos and only gives you the count. The TEM can tell you exactly what type of asbestos it is, by magnifying so much that you are able to decipher what atoms make up the fibers and then can figure out which type of fiber it is.
4. The TEM operates in a vacuum to increase the distance the electrons travel between impacts with other electrons and other things in its surroundings.
5. The asbestos sample has to be coated with carbon prior to the examination in the TEM because the surface of the asbestos must be electronically conductive so that it does not attract the electrons and they are able to pass through the screen.
6. The EDX identified chrysotile, and amosite based on their chemical compositions of Mg/Si, and Mg/Si/Fe respectively.
7. The advantages of the PCM are that it is cheap, quick, and readily available while some disadvantages are that the magnification is limited (450x), has some limits in wavelength, and it cannot identify asbestos fibers. Advantages of the TEM are that it has better resolving power (1000x -100,000x) and it can identify asbestos fibers. Some disadvantages are that it is a lot more expensive and takes longer to obtain the information you want.

Conclusion

1. We conclude that there this method of counting is not very consistent and leave great variability as to how many fibers there actually are.
2. We conclude that when using the NIOSH 239 method, the UCL of the fiber/cc count of both our group (2A) and the Class data are below the PEL of .1f/cc.
3. We conclude that when using the NIOSH 7400 method, the UCL of the fiber/cc count of our group data was below the PEL while the class data was at the PEL.

4. We conclude that the TEM is a better instrument if one wants to look at what the exact type of fiber being examined is. While the phase contrast can be used to simply see if fibers are present.
5. We conclude that the Walton-Beckett graticule was calibrated correctly; this is because the area of .00785 mm^2 matches the standard area of a graticule that we were given.