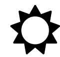
POLICIES & PROCEDURES



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INTRODUCTION

The first part of this manual is expressly written for compliance with the guidelines for medical laboratories as described by the Department of Health & Human Services/Health Care Financing Administration (HHS). Meixa Tech fulfilled all the requirements for certification (CLIA # 05D0565549) except one: HHS required that the laboratory manager have a MD or Ph.D. Clearly an impossible requirement at this time – certification has not been pursued further. Until this final requirement is met, certification of Meixa Tech will require application resubmission to the HHS.

The first part of this manual also addresses the handling of evidence in criminal and other civil cases at Meixa Tech.

The second part of this manual is a description of the techniques used to determine the inorganic and organic particle burden of tissue. The equipment necessary for the data acquisition is an scanning electron microscope (SEM) equipped with secondary electron imaging (SEI), backscatter electron imaging (BSEI) and X-ray analyzer (energy dispersive X-ray spectroscopy, EDS) for elemental analysis. A computer file record of the number of counts/second for each element is necessary.

The second part of this manual also describes special techniques for examining and processing evidence in criminal cases when required.

The third part of this manual contains the description of the database organization and the computer programs used for the analysis. These programs are in three parts: data management (dBASE and BASIC language), analysis programs (BASIC language) and summary programs (BASIC language). The intent of these programs is to provide estimates of particle burden from quantitatively derived data and to provide listings of individual particle types.

SECTION I - POLICIES

POLICIES & PROCEDURES REGARDING HUMAN TISSUE TESTING & EVIDENCE. The HHS has mandated that procedures where human tissues are analyzed in a diagnostic setting be described in a procedures manual. The procedures for handling case material by Meixa Tech comply with these guidelines. The number in brackets following the section titles are the HHS guideline provision numbers.

ASSURANCE OF POSITIVE ID OF PATIENT SAMPLES. [D3001]

The forms of human tissue received directly from a pathologist are: a) a tissue section mounted on a 1/2 inch graphite disk for direct viewing in the scanning electron microscope, b) tissue section ribbon for mounting on a graphite disk, c) paraffin embedded tissue, or d) wet tissue fixed in formaldehyde.

Tissue is received directly from the pathologist usually by Federal Express. The tissue always has associated a number that was applied by the pathologist when he or she received the case. On a document accompanying the material usually is the patient's name and the phone number/address of the party responsible for the charge for the analysis.

Upon receipt of the tissue:

1) An entry is place in the daily log book with the pathologist's acquisition number. If the tissue is from Dr. Jerrold Abraham, a "JA" number, which was assigned by him is applied to the case. If the material is from another pathologist or hospital, a Meixa Tech ("MT") number is recorded along with the pathologist's acquisition number. The full patient's name is recorded. The Federal Express (or other express company) tracking number is recorded. Additional information is recorded when necessary.

2) A file is opened with the acquisition number ("JA" or "MT") as well as the patient's name on the tab.

3) A 1" x 3" card is posted on the "CASE STATUS" bulletin board in the category of "New Cases." with the acquisition number (JA or MT number) as well as the patient's name and date the case is received.

4) A contract is faxed to the party responsible for paying for the analysis. A small tag is applied to the 1" x 3" card with the date the contract was sent.

5) The case is inputted in a computer file as a job number within the data file maintained by Intuit's Quick Books by both acquisition number and name. This is done at the time of receipt of the signed contract to perform an analysis.

6) When the signed contract is received, the 1" x 3" card is moved to the "Contract Received" column on the bulletin board.

7) Material is then analyzed on a first come first served basis unless a rush status is selected. When material is being analyzed, the 1" x 3" card is posted in the "Cases Being Analyzed Today" column.

8) At the completion of the SEM analysis, the 1" x 3" card is posted in the "Cases Ready for Computer Input" column.

9) At the completion of data input on the case, the data are then analyzed by a series of computer programs (see below) which prints out the analysis of the data for the pathologist. The 1" x 3" card is then posted in the "Reports Ready to Send" column.

10) Reports that are signed by the laboratory director are sent to the case pathologist by Federal Express or US Mail. Occasionally the report is faxed to the pathologist.

Return of the analysis tissue to the pathologist occurs every eight months to two years depending on the amount of case material on hand at Meixa Tech. The case numbers and associated patient's names are recorded in the laboratory log prior to sending the material by Federal Express. The Federal Express number is also recorded.

IDENTITY OF THE PERSONNEL PERFORMING THE ANALYSIS [D3042]

The identity of the person performing the analysis is (and always has been) required input for the computer-generated report. The full name of the person is at the head of the report document. Please see the example report (Appendix C) accompanying this protocol document.

SIGNATURE AND DATE OF THE SIGNING OF THIS LABORATORY MANUAL BY THE DIRECTOR [D4065]

The end of this protocol document shows the signature and the date of signing by the laboratory director.

LABORATORY DIRECTOR/ PERSONNEL RESPONSIBILITIES [D6107]

Director. All duties pertaining to the recording, storing, preparation, analysis, reports, computer operation, patient and /or client contact are performed by Bryan Burnett, Director of Meixa Tech.

Clinical Consultant. The clinical consultant, Dr. Jerrold Abraham (Department of Pathology, Health Science Center, Syracuse, NY), reviews most of the reports generated by Meixa Tech in both clinical and legal cases. In cases that Dr. Abraham does not review he usually does not see the report. If an error is noted (e.g. misspelling of patient's name, computer input error, etc.), he or the transmitting pathologist, reports the error to the director and appropriate changes are made. A updated (corrected) report is then issued. The old report is voided and retained in the patient's file. In addition, Dr. Abraham continuously monitors the status of the field of pneumoconiosis and makes suggestions for procedural changes. Most changes suggested by Dr. Abraham are implemented.

QUALITY ASSURANCE [D7001]

a) Patient Test Management. Dr. Jerrold Abraham continuously evaluates the patient test management. There are ongoing discussions in which various aspects of the test procedure. Changes in the test procedure are implemented when it is agreed that there is a need.

b) Monitoring and Evaluating Corrective & Remedial Action. The clinical consultant to Meixa Tech, Dr. Jerrold Abraham, re-evaluates any corrected reports or tests requested by him or in rare circumstances, by the client. All instances of corrective action have satisfied the clinical consultant.

c) Evaluation of Proficiency Testing Corrective Action. The clinical consultant to Meixa Tech, Dr. Jerrold Abraham, has evaluated the proficiency of Meixa Tech on several occasions. Meixa Tech has taken corrective action when necessary to the satisfaction of the clinical consultant.

d) Assuring Employee Competence. The clinical consultant, Dr. Jerrold Abraham, continuously monitors test results for reliability. This evaluation is based on 1) expectation (when possible) of particle burden based on the pathological features of the tissue and 2) case comparisons with another laboratory. In asbestos burden evaluation of tissue, Meixa Tech has in the past been part of an international study comparing laboratory results. In silica and silicates burden evaluation, Meixa Tech was part of a study with another laboratory (noncommercial). Results have been published in a reviewed journal.

e) Evaluation & Assurance for Corrective Action for Complaints. In the years (since 1982) that Meixa Tech has been in business, no complaints against the laboratory have been received.

For any complaints received by Meixa Tech:
1) Evaluate the nature of the complaint.
2) Take corrective action.
3) Be sure that the client is satisfied with the action.

Meixa Tech has always made every effort to assure that the client/patient is satisfied that they are receiving the highest quality performance possible. This guarantee is expressed in the contract that must be signed before work starts on a case.

f) Documentation of Quality Assurance Activities Reviewed with Laboratory Personnel. Quality assurance issues have always been discussed between the Director (the only employee of Meixa Tech) and it clinical consultant, Dr. Jerrold Abraham. These ongoing discussions are usually summarized in the daily log book or email printouts maintained by the Director.

CRIMINAL CASES

a) New Cases. Upon agreement to take a case, either a retainer agreement will be signed by the client or a letter or a court order will be sent from the client to Meixa Tech outlining the details of the retention of Meixa Tech. Meixa Tech will open a file on the case and assign a case number upon the submission of the retainer and the signed agreement or court order.

b) Evidence. All portable evidence, including firearms, will be examined at Meixa Tech's laboratory. Evidence located in San Diego County can be picked up by Meixa Tech. Evidence located outside of San Diego county will be sent to Meixa Tech, Cardiff, CA 92007 (complete physical address upon request) by FedEx or other premium delivery service. A court order directing Bryan Burnett to examine evidence elsewhere will be considered a breech of contract with the client and any agreement between Meixa Tech and the client will be void. In some circumstances, this position can be negotiated. At least two weeks are required for the examination of evidence at Meixa Tech and two additional weeks for a written report, if requested.

c) Evidence handling. Although this manual is mostly geared toward the handling of human tissues and its analysis in a diagnostic milieu, evidence in a criminal or civil case is handled in a manner in concordance with the spirit of this manual.

d) Photographic Evidence. Rarely does the defense in a criminal case receive all the image evidence from the prosecution or crime laboratory. Whole blocks of images are often missing. Meixa Tech requires all images in a case at the original digital resolution, preferably on DVD. All submitted CDs and DVDs must be closed. Photographic prints or 35mm slides must be digitized at high resolution (minimum 1500x1500 pixels) and provided on a DVD.

e) Outsourcing. For automated gunshot residue assessment of hand samples in criminal cases and perhaps other evidence, samples might be sent to another laboratory that is known by Meixa Tech to do reliable work. The client will be involved with any decision to outsource. Outsourcing may extend the report timeframe.

f) Report. As noted in b), the report for the work in a criminal cases requires two weeks from the return of the evidence, although this time frame might require lengthening depending on the complexity of the case. Reports are submitted in pdf (Acrobat) format by attachment to an email to the client. The client can then provide that document to other parties, at his/her discretion.

g) Quality Assurance. See the Quality Assurance section on this page.

SECTION II - PREPARATION AND ANALYSIS BY SEM/EDS

PREPARATION OF PATHOLOGY MATERIAL

There are two techniques for preparation of the tissue for analysis of particles. The selection of the proper technique depends upon the type of information about the patient's exposure that is desired. The tissue-section analysis is usually performed when the suspected exposure is metals, silica or silicates. The digestion technique for fibers is used to ascertain the tissue burden of asbestos and other fibers. The digestion technique for all particles is another method to estimate tissue burden of silica and silicates which might provide more reliable estimates of these particles than the tissue-section technique. The all particle digestion technique is not as reliable as the tissue section technique for the estimation of metal burden.

Previously, we have had mercury contamination, which could have originated from the tissue fixation with Zenker's fixative or contaminated containers. Metal contaminations often make a particle analysis impossible. Silver (used as a pre-stain or mordant) also can present a problem.

The graphite disks that are used to affix the paraffin sections must be as particle free as possible. Often these disks have embedded silicon particles, which comes from the manufacturing of these disks. Specially manufactured contaminate-free graphic disk are obtainable through various vendors, but even these may be contaminated and they are expensive. New batches of disks always need to be checked before use. Reasonably-priced disks are obtainable from Ladd industries where they are polished by diamond rather than silicon or aluminum dioxide sanding material. These Ladd graphite disks need to be treated for a few hours with 10% sulfuric acid to remove iron-rich particles.

1) **TISSUE SECTION**:

STANDARD PARAFFIN SECTION. As described in Abraham and Burnett (1983 & 1989), a 5 μm thick section is taken from paraffin embedded tissue and is floated in a warm-water bath the same as standard histological procedure. Only, instead of using a albumin-coated glass slide, the section is placed on an albumin-coated graphite disk and dried. Upon drying, the paraffin is extracted from the section with immersion of the disk in toluene. Care must be taken not to contaminate the sections on the graphite disk or the tissue.

TRANSFER SECTION. (performed at Dr. Abraham's laboratory). The transfer method consisted of removing the cover slip (by immersing the slide in xylene or toluene) and placing a few drops of Permount (a standard mounting medium) on the section. The permount is allowed to harden for at least 2 days. The hardened plastic containing the section is peeled from the slide after chilling the opposite side of the slide with ice. The removed plastic is placed onto the carbon disc (make sure that the same side of the plastic peeled off the glass slide is placed in contact with the graphite disc). This is allowed to dry and heated in a standard slide warmer or oven for several hours. The plastic is then removed from the graphite disc with toluene and the adhering tissue section adhered is then dried. No metal or carbon coating is necessary for analysis in the SEM.

Refer to Abraham and Burnett (1983) for more details on the procedure.

2) **TISSUE DIGESTION FOR FIBERS** This procedure was developed over the past twenty-five years. The technique was partly published in Scanning Microscopy, 5(1):95-108. A more detailed description is given here.

a) **Summary**. The reason for digestion is to be able to detect the concentration of fibers below that capable of being observed by the tissue section technique. In order to do this, a tissue block of 15 to 100 mg wet weight is place in *sodium hypochlorite* and digested for a minimum of 2 days. Thirty percent *hydrogen peroxide* is then added drop-wise (until bubbling stops = 1 ml of solution) to further digest the tissue. A minimum of two days for the hydrogen peroxide step is allowed before filtration. The tissue digestate is filtered through a 0.4 μm pore NUCLEPORE filter (as of ~ Jan 2007, the 0.2 μm pore filters are no longer used). The filter is dried and mounted on a graphite disk with carbon paint. After August, 2004: the filter is carbon coated. The search is conducted by SEM on an filter surface.

Note: Some laboratories use low temperature ashing or some other tissue dissolution technique followed by ultrasonication. Ultrasonication causes breaking and bundle disassociation of the asbestos (both chrysotile & amphiboles) and results in overestimates of tissue fiber burden (see **Appendix A-1).** For chrysotile asbestos ultrasonication can also result in underestimating burden due to fiber destruction (see **Appendix A-2**).

b) **The Digestion Solutions**. Reagent grade *sodium hypochlorite* (Baker Analyzed - **not commercial bleach!** New batches of NaOCl are checked for contamination before use.) is the primary digestion solution. The *sodium hypochlorite* is stored refrigerated.

H*ydrogen peroxide* (J.T.Baker, 2190-01, Hydrogen Peroxide, 30%) is reagent grade.

O*xalic acid* (J.T.Baker, 0230-01, Oxalic Acid, Dihydrate, Crystal) reagent grade. An 8% solution is used.

S*ulfuric acid,* reagent grade. A 10% solution is used.

All solutions are filtered through 0.1 or 0.2 μm pore polycarbonate filters (0.1 μm pore for the all particle digestion analysis - see below) before introduction into the reaction container.

c) **Digestion container.** The digestion containers are new glass borosilicate glass centrifuge tubes. Each tube is rinsed in 0.1 or 0.2 μm pore filtered distilled water. Prior to February 5, 1999,

polystyrene 15 ml centrifuge tubes were used. For the polystyrene digestion tubes, each was soaked for one day in 10% H_2SO_4 followed by one day in commercial *sodium hypochlorite* (bleach). The polystyrene digestion tubes were then soaked for one day distilled water. Both the polystyrene and glass tubes are used only once and then discarded. The polystyrene tubes were abandoned due to the propensity for scratching and liberating plastic debris into the digestate by the sharp edges of the Pasteur pipettes.

d) **Controls**. A control for each digestion series is often made by adding the same filtered solutions to the container and filtering in the same apparatus as is used for the digested tissues. The filters created with the control solutions and containers should not be stored with the digestate filters (to avoid cross contamination). After the quantitative run with the digestate filter, the control filter may be examined by an equal number of fields as that of the digestate filter.

e) **Digestion and Sample Preparation.**

1A. *Predigestion Tissue Preparation* - *Wet Tissue*. The tissue is first imaged with a digital camera then imaged again after the removal of tissue. No more than half of the tissue is use for the fiber analysis unless otherwise instructed by the client or pathologist. Prior to digestion, the tissue is dehydrated in ethanol, cleared in toluene and rehydrated, in an ethanol series of solutions.

1B. *Predigestion Tissue Preparation* - *Paraffin Embedded* Tissue. In most cases, the only available tissue for digestion is paraffin embedded tissue. The paraffin must be removed from the tissue before attempting digestion of the tissue. The paraffin block is first imaged with a digital camera, both sides of the block then imaged again after the removal of tissue. No more than half of the tissue in the paraffin block is removed unless otherwise instructed by the client or pathologist. The tissue is trimmed of paraffin. The trimmed tissue block is then immersed in toluene (about 20X the tissue-paraffin volume) and the toluene is changed every 2 or so hours for 3 changes. By warming the solution to approximately 45C, the paraffin removal can be quickened. The tissue is then hydrated to water through a graded ethanol series.

2. The tissue is cut into cubes, avoiding cancerous tissue if possible. Pleura is included with each tissue piece if possible. The three dimensions of each block is measured and the tissue pieces are weighed to the nearest 0.1 mg. Blocks are cut that weigh from 15 mg to 100 mg. In cases after December, 1998, for the LM mount, the filter of the 10,000X analysis is cut in half where the half is mounted for the SEM analysis and the other half for LM (see below). Up to 3 (usually 2) pieces of tissue (5 to 40 mg) are weighed, dried and weighed again. These are for estimates of particle burden per dry weight.

3. The filtration apparatus is ultrasonicated in fresh distilled water at least four times prior to use and is then thoroughly rinsed the with 0.1 or 0.2 µm filtered distilled water.

4. Hypochlorite digestion. Each tissue block is placed into a new borosilicate centrifuge tube and 10 ml of 6% *Sodium hypochlorite* added. (All solutions are filtered through 0.1 µm pore polycarbonate filters). The digestion of the tissue with sodium hypochlorite is a minimum of 48 hours, but more hours may be added to this step if it is difficult (i.e., slow dissolution of the tissue). The digestion solution is shaken one or two times during the digestion process in order breakup tissue debris that accumulates at the bottom of the container and to mix the hypochlorite.

CAUTION: the polycarbonate filters used for filtering the reagent solutions can be contaminated with silica and sub-micron diameter mineral fibers. Rinse the filter prior to use by injecting one syringe full of solution through that filter.

5. After at least for 48 hrs in the *sodium hypochlorite*, 30% *hydrogen peroxide* (reagent grade, 0.1 µm pore filtered) is added drop-wise. Initially, with each drop of *hydrogen peroxide*, the digestion solution actively bubbles (fume hood or good ventilation is absolutely necessary). The solution is carefully mixed with a pipette after each drop. When the bubbling ceases another drop is added. When the bubbling reaction no longer occurs four additional drops of hydrogen peroxide are added. Approximately one ml (~22 drops) of hydrogen peroxide is added to the digestion solution for a total solution volume of 11 ml.

6. Filter Prep for SEM Evaluation. The filtration with 13 mm polycarbonate filters of the digestate occurs from one to five days after the addition of the 30% *hydrogen peroxide*. The final fluid level of the solution is marked with a felt pen on the outside of the container. (The volume put through is needed to estimate the tissue volume and weight if not all the liquid in the reaction vessel is put through the filter.)

Set up the vacuum filtration unit, with a 13 mm D, 0.1, 0.2, or 0.4 µm pore polycarbonate filter. Cut a paper filter disc (Whatman Qualitative 4, Cat.No. 1004 090) smaller than the polycarbonate filter and place on top of the polycarbonate filter. Snap the filtration cassette together being careful not to dislodge the paper cut-out filter. (TEM? - see 8B below.) Add the digestion solution with a micropipette (about 2 droppers full). Be sure there are no air bubbles over the polycarbonate filter and start the vacuum pump at a low vacuum. You must gauge the amount that you are filtering. (If the filter plugs rapidly to the point of cessation of filtration, discard that preparation and start over with a lesser amount of digestate.) Increase the vacuum to maintain the flow, if necessary. Mark the final volume of the digestion container. Rinse the micropipette and the reaction if all the contents put through the filtration apparatus. Rinse the filter preparation with filtered distilled water with about the same volume of the material digested.

Pore size of the NUCLEPORE filters:

0.1 µm - used for "all" particles

0.2 µm - used for 5,000 X fiber analysis, prior to 2004 - [10,000 X on ETEC screen]. Currently rarely used.

0.4 µm - used for 2,000 X fiber analysis -[4,000 X on ETEC screen] or 4,000 X [8,000 X on ETEC screen].

7A. High Magnification Sample/ H_2SO_4 Treated Sample. The digestate is filter through a 0.4 µm pore polycarbonate filter. The digestate filter is then rinsed 2X with 0.1 µm pore filtered distilled

water, and 0.1 μm pore filtered 10% H_2SO_4 is added. The sample is allowed to be in contact with the 10% H_2SO_4 for approximately 3 minutes (although this can be longer by several minutes if the filtration is slow) during its vacuum filtration. Two final rinses with 0.1 μm pore filtered distilled water is followed by drying of the filter.

The reason for the short 10% H_2SO_4 exposure is to remove flocculent hemosiderin which may be present in these samples and whose presence interferes with the detection of small-diameter fibers. The short-duration H_2SO_4 exposure has very little or no effect on the Mg concentration of chrysotile fibers (tested at Meixa Tech).

7B. Low Magnification /Oxalic Acid Treated Sample. The identification of amphibole asbestos relies heavily on Fe CPS. Frequently, especially with amosite and crocidolite, there is a biological coating of Fe-rich material on the fiber. The recognition and noting of this coating in the data records provides interesting research information. However, the benefit of complete removal of the Fe outweighs this information in that the coating will often obscure fiber identities. Prior to April, 1990, partial removal of the coating was accomplished by a short exposure (3 to 5 min) of 10% *sulfuric acid* (see above). A more reliable technique for the biological Fe removal is reported by Dodson, Williams & Hurst (1983 - *Journal of Toxicology and Environmental Health*, **11**:959-966). They suggest an 8% *oxalic acid* at 75 C for four hours to completely remove the ferruginous coatings. Application of a modification of this procedure (8% *oxalic acid* @ 55 C for 4 hrs) accomplishes the Dodson et al. results.

The Dodson technique did not adequately test the effect of the 8% *oxalic acid* treatment on asbestos. Standard samples used for the FIBERID program (see below) were tested to see how they are affected by the oxalic acid treatment. The mineral samples tested: amosite, crocidolite, chrysotile, anthophyllite, talc (with & without Fe) and tremolite. The alteration of the elemental composition for the amphiboles was not detected (with the possible exception that crocidolite may lose a small amount of Mg). However, the chrysotile fibers remaining are quite distinctive after this treatment where they usually appear with a small amount of Mg or none at all. However, care must be made to confirm that fine contaminate mineral fibers are not mistaken for chrysotile. The estimate of chrysotile burden made using the oxalic-treated samples is likely below that of the sulfuric-acid treated samples. Chrysotile fibers are destroyed by the oxalic acid treatment.

Rinse the filter two times with 0.1 μm pore filtered distilled water. Allow some distilled water to remain over the filter in the cassette, and add 0.1 μm filtered 8% *oxalic acid*. Vacuum filter to be sure the *oxalic acid* is in contact with the filter. Keep the oxalic acid in the funnel part of the filtration apparatus. Float the entire filtration apparatus in the 55 C water bath. Replenish the oxalic acid in the funnel of the filtration apparatus if needed. Every hour or so vacuum filter a small amount (approx. 1 ml) of the oxalic acid and then replace the apparatus in the hot-water bath.

After four hours rinse twice with filtered distilled water and then vacuum filter to dryness. Remove the polycarbonate filter from the holder and dry on a Whatman paper filter in a Petri dish. If not all the material in the reaction vessel was used, calculate the tissue volume and wet weight that that represents.

8. Sample mounting for SEM. On the carbon disc, spread a smooth coat of carbon paint. Take the dried polycarbonate filter, and while the carbon paint is still evenly wet all over the disc, place the polycarbonate filter on top of the paint. Good contact of the filter with the paint should be over the entire extent of the carbon disc surface. To be sure of a good contact between the carbon disc and the stub, dab some carbon paint in this area. Prior to August 2004: dry for ~ 1 hr before placing in SEM. After August, 2004 coat the samples with carbon in a vacuum evaporator.

9. Make a control filter (all of the above except without tissue) prior to the filtration of the sample filters. A single control filter is usually made for several series of analyses.

PARTICLE ANALYSIS BY SEM/EDS

1) EQUIPMENT

SEM:
1980 to present: ETEC Autoscan scanning electron microscope.

EDS:
1980 to January 1990: Kevex Model 5500
January 1990 to December 31, 2001: Kevex Delta II
January 2002 to January 2004: IXRF Systems EDS2000 (includes imaging).
January 2004 to present: IXRF Systems EDS2004 (includes imaging)

2) TISSUE SECTION

The SEM examination was done basically as described in the 1983 report by Abraham and Burnett. Over twenty years' experience with this method of analysis of particles in tissues was reported in an updated description of the database containing results of similar analyses [Abraham, Burnett and Hunt, 1991].

The method is basically a morphometric point-counting method to determine the number concentration of inorganic particles in a tissue section. Using a standard magnification of approximately 3000X at an acceleration voltage of 20kV, sequential random fields of view of the tissue section are visualized using both secondary electron imaging (SE) and backscattered electron (BSE) imaging. The SE image shows the surface topography of the tissue and the carbon substrate. The BSE image uses atomic number contrast to reveal particles within the tissue. As the average atomic number of the tissue and the carbon support disc are low, inorganic particles which are retained in the tissue are revealed by their different brightness in the BSE image. Owing to penetration of the electrons into the tissue section (which, after paraffin removal, is mostly void volume) some particles can be detected through the entire thickness of the section. Particles as small as 0.15 micrometer are detectable, and some as small as 0.10 micrometer are detected. By searching a standard field size, the area sampled and the thickness of the section permits calculation of the volume of tissue searched for each field. Thus, the numbers of particles detected in a given number of fields represents the number concentration of particles per volume of tissue. [The number per cubic centimeter of tissue is roughly equivalent to the number per

gram of wet tissue.] In the majority of tissue section analyses, 100 to 200 fields of view are searched, with a resultant detection limit of between 1 million and 500,000 particles per cm3 tissue. This method is NOT used for fiber analyses (which need greater sensitivity (i.e., lower detection limits – see below) and cannot detect very light elements such as Beryllium, nor can it analyze organic, carbonaceous particles. It has been observed that this method's detection limit is adequate to find a particulate burden in most lungs with no exposures other than background [usually less than 20 x 10^6 particles/cm3]. In addition to the counting and measurement of the diameter of each particle, an energy dispersive x-ray spectrum of each individual particle detected is collected. [This is often referred to as Individual Particle Analysis (IPA).] The standard conditions utilize a 20 keV electron beam and approximately 1 nanoampere of absorbed beam current, with a 5 second analysis of each particle.

The analyses reported in this study were done using an ETEC Autoscan SEM and an IXRF energy dispersive x-ray analysis system. Each particle is classified as exogenous (foreign insoluble particulate material) or endogenous (formed in the body – a classification which includes calcium phosphates, calcium oxalates or carbonates (showing only Ca in the spectrum) or hemosiderin (with Fe associated with Ca or P). The endogenous particles are not tabulated as part of the exogenous inorganic particle burden. For each tissue section analysis, the results are presented as TOTAL exogenous particles, and the particles classified into one of a few major types: Silica (containing only Si in the EDS spectrum), Aluminum Silicates (containing Si, Al and often other elements, such as K, Ca, Fe, Mg), Talc (containing Si and Mg in characteristic ratio), Miscellaneous Silicates (containing Si with other elements but not matching aluminum silicates or talc), and Metals (containing one or more metallic elements). Only Silica, Aluminum Silicates and Metals are commonly found in the background population with this method, and the only metallic elements commonly found in the background population are Fe and Ti. It is important when analyzing the resultant data to look not only at the types of particles found, but at their concentrations. Also, among the metals, there are often associations of metallic elements in single particles – this may give a clue or 'fingerprint' of source materials, such as Fe with Cr or Fe with Cr and Ni, indicative of stainless steel; or Ba with S, documenting Barium Sulfate. It is also important to note that Aluminum may occur in Aluminum Silicates or as metallic Aluminum; these are separately tabulated in this method of analysis. Also, the element Silicon is carefully examined, and Silica particles are not misinterpreted as Silicates. Other methods, which do not quantify the particle composition or analyze individual particles, do not result in comparable data. If a number of particles are simultaneously analyzed by a method analyzing an area of tissue or a group of particles, the particle classification may be erroneous and individual particle types may be misclassified as a combined material [for example, in an analysis of more than one particle, the element silicon may be detected with other elements, such as Al, but there may or may not be a mixture of particles of crystalline Silica and metallic or oxide of aluminum, etc.] Similarly, for example, if the concentration is not measured, there may be no way to differentiate between a markedly elevated concentration of silica as seen with occupational exposure or even silicosis, and just the observation that some silica particles are present.

References

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Abraham JL, Burnett BR, and Hunt A. Quantification of non-fibrous and fibrous particulates in human lungs: Twenty year update on pneumoconiosis database. Ann Occup Hyg . 46 (Suppl.1):397-401, 2002.

Note. For the IXRF system, a text file (ASCII) created in Microsoft WordPad is where the element integrations are directly transferred to our standard ASCII data files used for analysis by the analysis programs (see page 19).

3) TISSUE DIGESTION FOR FIBERS.

To July, 2004: digestion analyses were performed at 30KV with the Be-window detector on the ETEC.

After July, 2004: analyses are performed on the carbon-coated samplers at 20KV with the thin-window detector. The asbestos standard values (see Appendix D) were adjusted to this new parameter.

a) Random Search. In order to see the very fine particles, the gain on the secondary image is turned high. This has the effect of intensifying the charging so that very fine particles can be observed (0.05 to 0.06 µm is possible). For the digestion samples, 10,000X (viewing magnification - images are 5000X) magnification to see the very fine fibers of chrysotile asbestos; 8000X (viewing magnification - images are 4000X) is used to detect amphibole asbestos & chrysotile; 4000X (viewing magnification - images are 2000X) is used for amphiboles. The 10,000X viewing magnification is currently rarely used. The particulars of the case determines which analysis magnification to use. The horizontal knob is rotated between fields without regard to features on the filter and the number of fields searched counted. When more than 30% of the screen becomes "burned out" as a result of a large particle, then the knob is rotated again without changing the field number on the counter.

b) Fiber Center Must Be in Field. When a fiber is found in a field, in order for it to be counted, its center point must be within the field for the quantitative analysis. Because the filter surface is at a 45 degree angle, the angle that the fiber is at is recorded (0,15,30,45,60,75 or 90 degrees). These data are later used to calculate the "true" lengths of the fibers (a computer operation).

4) TISSUE DIGESTION FOR ALL PARTICLES.

All particles are counted either in the entire field at 5000X or the reduced field at 5000X. For fields where there are more fifteen particles, five particles nearest the center point of the screen are X-ray analyzed by the technique described below. The total number in the field as well as the number analyzed are inputted into the database. The control preparation is analyzed by counting the particles in the same number of fields. The control sample is prepared by using the same volume of sample as the analyte sample. For all particle analyses on 0.1 µm pore filters, a special control sample is necessary from which a equal volume of sample

is filtered.

5) ACQUIRING TECHNIQUE AND DATA HANDLING.

The spectrum should be taken on the particle at the most distance spot from other particles or endogenous coatings as possible. Analyses after Dec. 2001: most fibers/particle analyses a model background is used to subtract from the spectrum. Previous to 2002 and some particle analyses in early 2002: A background spectrum taken close to a clear area on the filter and near the fiber. This is the standard background from which the particle spectrum is subtracted. As many as five spectra may be taken in order to obtain an adequate spectrum for thin fibers and as many as six spectra are sometimes performed for fibers < 0.15 µm in width due to the beam "slipping" off the fiber. If part of the spectrum is obtained off a fiber, then rather "odd" variations of the elemental composition may be observed.

Nov 2002 to date.. An image of the fiber(s) or particle(s) is taken at the analysis magnification via the IXRF system. The IXRF system has beam control where all that needs to be done is to position the cross-hair icon with the mouse on the particle in the image and click. Each spectrum is normally 2 to 5 sec. Instead of integrating each spectrum at that time, the IXRF system allows a batch store the image with all its spectra for later evaluation.

After the SEM analysis session, the batch stored images and spectra are recalled. The particles are measured and the spectra integrated for the significant elements. The IXRF system has a reliable automatic background subtraction routine which is now used. This system was extensively tested prior to use. The integrations of the spectra are transferred to an ASCII format (via the clipboard to WordPad). Particle number, size shape, and angle information is also added to the WORDPAD document. The ASCII WORDPAD document is converted to the analysis data format by the basic program IXRFSUM (see page 19).

ASBESTOS-BODY ANALYSIS BY LIGHT MICROSCOPY

During the processing of the tissue for asbestos fibers, a quantity of tissue usually is digested and filtered for a light microscopical search for asbestos bodies. The 0.2 µm pore 13 mm size polycarnonate filter is used for the filtration and mounting. The 0.2 µm pore prep is cut in half (see above).

Half of the filter is mounted for LM and the other half for possible SEM analysis. For the LM mounts, place a few drops of Permount on a clean glass slide or a Poretics Cyto-Clear Microscope Glass Slides (Catalog # 99030) and place the dry Nuclepore filter face up on the Permount. Put another few drops directly on the filter face, and hold the 22mm #1 cover glass with forceps and carefully lower it so that no bubbles form. Dry on a slide warmer at about 45 C for several days before counting.

All counts of asbestos bodies are made at a magnification of 400X on a Nikon compound microscope. A digital image of each asbestos body is captured with a digital camera unless there are a large number of asbestos bodies present. With a large number of asbestos bodies, representative bodies are imaged, usually not more than 15.

There are three counting techniques for the assessment of the asbestos body burden of the filter on the slide: 1) the entire filter can be counted or 2) half the filter counted or 3) if the burden is particularly heavy, asbestos bodies are counted by field for a total of 20 fields. A report of the estimated asbestos body burden is generated by a computer program (see below).

SECTION III - COMPUTER ANALYSIS

FILE NAME STRUCTURE

The data file names are either 7 or 8 characters:

1) 7 character data file: e.g.: 91226AT - the first two characters are the YR (91); characters 3,4 & 5 are the JA case number (226) character 6 (A) is the sample #; and the last character is the type analysis (see C, this page).

2) 8 character data file: 92135A2D - characters 1 through 6 are the same as above, character 7 is a special info character, usually the sample number) and character 8 is the type of analysis (see right column, this page).

Type Analysis. If the last character(s) is:

T - Tissue section analysis.
ZT - Tissue section analysis done at MT, (as of 2/91)
SZT - Tissue section analysis done at MT, silicates only.
MZT - Tissue section analysis done at MT, metals only.

Note: If there are additional analyses done from the same patient, then the last 3 letters will be TZT & NZT and so on.

D - Digestion, quantitative for fibers.
R - Nonquantitative (tissue or digestion).
A - Digestion, quantitative for all particles in field (5000X
B - Digestion, 5000X, reduced field, quantitative for all particles.
C - Control, for digestion/fibers: stand alone file. (For files A & B, the control records are appended at the end of these files.)
E - TEM Digestion, quantitative for fibers.
F - Tissue section analysis, fibers only.
G - Asbestos body analysis by LM only.
L - BAL (lav age) sample.
M - BAL, centrifuged, fixed, embedded & sectioned.
Q - Special circumstances data file:
- set up for a special presentation of existing data from regular data file(s)
- not to be used in the summary database

S - Air sample
X - Unused or obsolete file (not to be used in the summary database)

ENTERING THE DATA – FIRST RECORD

IXRF Systems: (as of Nov, 2002). For the both the digestion and tissue section data, the WordPad text data file created during the SEM analysis can be ported directly into a dBase –readable text file by **IXRFSUM.BB2**. See the program listing for details.

dBASE File initiation

To begin entering data:
1. Boot to DOS.
2. Go to dbase subdirectory
2. DBASE <enter>
3. When "." appears, enter "DO MENU"
4. A menu will appear:

0 - Exit to Dbase **
1 - Create new data file
2 - Add records to existing file
3 - Initialize data file for analysis by BASIC
4 - File check for editing

Item 0: Select this if you wish to exit data input. If you wish to exit dBASE then type "quit" <enter>. **NEVER, EVER exit by reset or turning off the computer!!** You will loose either part or the entire file that is open if you do.

Item 1: Create new data file. This will automatically set up the data file and display the entry form for the first record of the data file. See below for the information to input in this record. At the end of data entry, you will return to the main menu.

Item 2: Add records to existing file. The data can now be entered into the file by selection the proper file name (which was set up by #1). Files can be added to any dBASE III data file (of the MENU format) regardless of file size.

Item 3: Initialize data file for analysis by Better BASIC. The dBASE data files cannot be read by BASIC programs. Thus, the dBASE data files have to be translated to ASCII format. By selection of #3, the file name with the extension .TXT is created (dBASE data files have the extension .DBF) and the BASIC programs read the .TXT files.

Item 4: File Check for editing. Select this item when the basic program MASTER.BB2 informs you of an error. You can check for the error(s), and at the end of the data scrolling, by selecting "0" and typing in EDIT ## (or BROWSE), you can go to the offending item and quickly correct it. Move up or down records by pressing "PgUp" or PgDn". **DO NOT FORGET:** rerun item #3 to place the corrected dBASE data file into the ASCII data file!

** Upon pressing (1), enter the file name. The program with take you into the first record.

The First Record

The first record is reserved for data about the analysis (e.g # of fields searched...). The layout for these data:

FIELD 1: (e.g. 3A352) first field code (see below)

3A352 <== Magnification
3A35 <=== Analysis technique
3A3 <==== Minimum fiber length
3A <===== Filter size or amphibole only
3 <======= Instrument/operating KV

SAMPLE SIZE: e.g., 00030 (a sample size of 30 fields)
^---Operator code is first character

DGSTN SAMPLE #: - the designation of the digestion sample.

TISSUE BLOCK #: - tissue block # applied to the original tissue (usually paraffin).

INFO A: (e.g., 04.604.9) - first 4 character spaces is the volume of tissue; the second 4 is wet wt. Air Sample (S): amount of filtered air in cc, last character = exponent (e.g. 1.2000_6 = 1.2 x 10^6 cc). Tissue section data - leave blank.

INFO B: (e.g. 21.5:1.9) - the first 4 characters are the wet wt. of the first parallel piece; next is a colon separator, and the 3 follow characters are the dry wt of the same piece; the next 2 (spaces 10 & 11) are for % estimates of filter obscured when the debris covering the filter is heavy; the last character is for the pore size of the Nuclepore filter (1 = .1 um, 2 = .2 um, etc). For tissue section data - leave blank.

INFO C: (e.g. 22.5:1.7:15.8:1.2) The first 8 characters are for the second parallel piece (wet & dry wts) in the same format as INFO B; characters 10 through 17 are for the third parallel piece (wet & dry wts); and character spaces 19 through 23 are for special codes concerning the patient and how the tissue and sample was treated (see below). If Air Sample (S = final character on file name), the first ten characters are client's sample #. Tissue section data - only character spaces 19 to 23 are used as described for the digestion samples.

INFO D: (e.g. 292) for all analyses - Date (month , 1 character, & year, 2 characters) that the analysis was conducted. The first character is month (1: January, 2: February ... A: October, B: November, C: December). The next two characters are the year (e.g. 88:1988). So, May 2001 = 501 etc. **The analysis programs require a date here for the proper application of the field size to the analysis of the data. As of January 2001, all programs are Y2K compliant.**

Obviously, INFO A, B, and C are primarily for the digestion analyses. The tissue section analyses do not require input here. However, the last 4 spaces of Info C need to be filled in for both as well as the data in INFO D.

Details of the First Field of Record 1 (or 0)

1) the first character must start with a character that distinguishes the SEM instrument & KV:
* - MT-ETEC SEM, at 20KV.

! - MT-ETEC SEM, at 30KV.
2 - MT-ETEC/KEVEX DELTA SYS at 20KV (Be detector)*
3 - MT-ETEC/KEVEX DELTA SYS at 30KV (Be detector)*
A - MT-ETEC/KEVEX DELTA SYS at 20KV (Q detector)*
B - MT-ETEC/KEVEX DELTA SYS at 30KV (Q detector)*
T - Zeiss 10 TEM @ 80KV.
Q – MT-ETEC/IXRF System @ 20KV
R - " " " " " new detector window- (only one case)
S - " " etc: new detector window + 12um Mylar filter
V - " " etc: new detector window + 1.4 um Mylar filter (in current use)

* After January, 2002 "KEVEX DELTA SYS" replaced with the "IXRF EDS2000 SYS."

2) the next character usually is "0". However, in special cases, this character has the follow coding:

0 : 13 mm Nuclepore filter
L or **1**: 24 mm " "
M = L + C
A : Special amphibole analysis: Talc cases where only amphiboles are recorded, usually at 5000X.
B : Special amphibole analysis: Talc cases where only high aspect ratio fibers are recorded.
C : All fibers analyzed except chr, chd and probable chrysotile.
D: Ignores low aspect-ratio flat fibers.
E: Bubble formed over filter surface during filtration; particle pileup on filter edges.

3) The third character from the left is for digestion fiber analysis. The number here indicates the minimum length in microns of the fiber record in the data which follows..
3 - 3 um minimum fiber length

4) The fourth character indicates the type of analysis (see below).

5. Examples of this field of the first record (SEM machine reported magnification):
V0003: air sample on Nuclepore filter
V0013: non quantitative, tissue sect.
V0023: non quantitative., digestion at 3000X

V0033: quantitative tissue, 3000X
V0S33: Silicates only in this analysis - due to large number of silicates, metal particles are assessed in a separate file.
V0M33: Metals only (see above)
V0040 -> BAL sample (mag. not applicable)
V0352 : quantitative digestion fibers 2000X
V0355: quantitative digestion fibers 5000X
V0354: quantitative digestion fibers 4000X
V0352, **VA1554**: analysis selective for amphiboles (other fiber types are ignored).
V0064: quant. dig., reduced field, 4000X
V0074: quant. dig., all part., 4000X
V1074: same, 24 mm filter
V0090: material sample (nontissue, nonair)
V0052 Zeiss 10 TEM @ 25,000X.

The last character indicates the magnification, which must be 2 (for 2000X), 3 (for 3000X) or 5 (for 5000X) or 8 (for 8,000X [only Syracuse Hitachi SEM]). However, if the last character is 0, then the analysis was done at different magnifications (the 4th character in this case should be either 1 or 2, for non quantitative analysis). The underlined code examples above indicate standard analysis parameters for the MT-ETEC SEM.

5) The second field indicates both the sample size (number of fields scanned on the SEM) and the operator code.
The first character:
0 - no operator noted (some early cases)
1 - Jerrold Abraham
2 - Bryan Burnett
3 - Brian Powell
4 - Mike Poole
5 - Ellen Bradley
6 - Carey Merritt
7 - Andrew Hunt
The next four characters are reserved for the sample size (number of fields scanned).

INFO C

The last 7 characters of Info C are reserved for information about the patient and the treatment of the tissue.

1) CHARACTER 18:

N or ( ) [empty] - Membrane filter in the digestion analysis is NUCLEOPORE brand (if digestion analysis). After 4/2012 Whatman brand
P - Membrane filter in the digestion analysis is PORETICS brand (if digestion analysis). This brand is no longer used.
M - Tissue section analysis; - multiple sections analyzed. (Input the ID of the samples into INFO C - characters 1-15)
G - GE Poretics polycarbonate filter
S - SPI brand polucarbonite filter

2) CHARACTER 19:

A - tissue section mounted without the aid of albumin affixative
c or **C** - centrifugation at the completion of digestion
D - tissue rec'd dried - rehydrated for digestion
S - digestion sample, sonicated
T - tissue section sample, a transferred section
P - tissue extracted from paraffin block
p - tissue extracted from paraffin block in an outside laboratory & dried. The tissue was rehydrated at MT.
G - tissue associated with gun-shot wound
U - filter produced outside of MT or JA's lab
Z - Wet tissue - dehydrated, cleaned, rehydrated
2 - Two different stubs analyzed for one report (tissue section analysis).

3) CHARACTERS 20 AND 21:

L0 - lung, biopsy or autopsy (not known to analyst)
L1 - lung, standard biopsy
L2 - lung, autopsy
L3 - lung, transbronchial biopsy

ADDITIONAL OPTION: When two different stubs are analyzed ("2" at Character 19), place in the CPS field, starting with character 1, the ID of the two samples analyzed. The analysis should be about equally represented by the two samples.

L4 - lung tumor
L5 - liver
LA - cat lung
LC - control to digestion analysis
LD - dog lung
LN - lymph node
LR - lung, rat
LB - lung, baboon
LY - larynx, autopsy
MN - mineral sample
NB - non-biological
PT - Peritoneal tumor
PL - Pleura
BL - bronchial lavage
AP - appendix
OV - ovary
SK - skin
CO - Colon
AR - Air sample on Nuclepore filter

4) CHARACTER 22:

A - tissue section analysis, 5% acetic acid treated.
C - tissue block treated with 4% HCl for approx. 12 hr prior to digestion. **EXCEPTION**: In the filters mounted for LM/ asbestos body cnts, this indicates for some of the files that the filter was mounted on special slides made by Poretics (Cyto-Clear Preparations)
N - No special treatment (used up to 1988)
O - Sample on filter treated with 8% oxalic acid at 55 C for 5 hrs
o - Sample on filter treated with 8% oxalic acid at 55 C for 5 hrs
S - Sample sonicated => position 23:
2,3,4, or 5 = # min ultrasound @ 50 watts
a = 5 sec at 50 watts
b = 10 sec at 50 watts
c = 15 sec at 50 watts
d = 20 sec at 50 watts
U - Sample on filter treated with 10% H2SO4 for 3 min.
u - Sample on filter treated with 50% H2SO4 for 3 min
T - Tissue section analysis: graphite disk polished & checked for particle contamination prior to the mounting of the tissue section.
W - tissue section analysis - washed 1 hr with distilled water.
X - Sample centrifuged, washed with 0.2 um filtered distilled water, centrifuged, treated with 10% sulfuric acid prior to filtration.
Y - filter acid treated with 4% HCl
Z - tissue section analyses: endogenous particles ignored (character used by C. Merritt).

4) CHARACTER 23:

A - tissue sample contaminated by endo.Ca
B - " " " by Br
C - " " " by Cr
D - " " with heavy burden of diatom frustules and frustule fragments
E - tissue sample contaminated by endogenous Fe
e - many particles are coated by endogenous Fe
F - tissue sample contaminated nondigestible carbon (coal dust?)
G - " " " by glass
g - " " " by gypsum fibers
H - " " " by Hg & Ca
J - tissue sample contaminated by AlCuCl an apparent mordant
M - " " " by Hg
m - tissue sample contaminated by an occasional Hg particle found
N - tissue sample contaminated by Na from Clorox
O - " " " by Co
P - " " " by NaP particles
Q - Digestion analysis - filter heavily loaded with inorganic debris (may miss fibers)
q - Digestion analysis - filter heavily loaded with organic debris (may miss fibers)
S - tissue sample contaminated by Ag
T - " " " by talc
Z - " " " by ZnP
z - tissue sample contaminated by Zn
2 - Sample represents 2 pieces *
3 - Sample represents 3 pieces *

* Occasionally, the paraffin block will have more than one tissue piece or the pathologist will request pieces of wet tissue in the same digestion solution. This represents the number of tissue pieces in the single digestion solution. Note: Do not use "S" in position 22 with this notation.

FIELD STRUCTURE FOR THE REST OF THE DATA FILE

AUTOMATIC INPUT WITH THE IXRF SYSTEM (as of Nov, 2002)

The analysis system has become much more automated with the addition of the IXRF system. But, still during an analysis a handwritten paper is maintained for notes and comments during the SEM analysis. These handwritten items are added into the final data file.

TISSUE SECTION

In a TS analysis, the cross-hairs are used in the IXRF captured image to target particles in the image after a particle count in the ETEC backscatter field has been made. The field size of the ETEC CRT is larger than that of the captured IXRF image. If the particle counts (on backscatter CRT) are greater than the analysis counts (recorded on IXRF image), the counts on the backscatter CRT are recorded by hand. The IXRF has beam control and the cross hair system of the IXRF allows for individual targeting for EDS in the IXRF system. Most spectra are 5 sec. Instead of analyzing each spectrum at that time, the IXRF system allows a batch store of the image with all the spectra taken with that image. Each batch file has the field number as its name. The batch files will be recalled at the end of the SEM session and data files constructed as described below.

After the transect across a TS is finished, the SEM is turned off and then the batch files are retrieved. Analyses are still at 3000X. In the inputting of the ASCII file, the field/particle number is added as a hyphened number (e.g., 12-2 – field 12, particle 2). The particle type (1 character) is added if it is other than irregular (2). The size, as a direct screen measurement in mm (IXRFSUM converts this to microns). Each spectrum is closely examined and an element added to the spectrum's element list if needed. An automatic background subtraction is performed followed by a peak a Gaussian integration of the usual set of elements (Na, Mg, Al, Si, P, S, Cl, K, Ca, Ti, Cr, Mn and Fe). An ASCII data file in WordPad is set up to run to run simultaneously with the IXRF software. In the IXRF software, a copy the integrations to the clipboard is made and then paste with the field-particle info & size into the ASCII document. The ASCII record is then edited by removing element-integration data that are not significant. All but the most significant element integrations are left in the ASCII file (to fit in our database). From one to six elements are allowed. The Basic program IXRFSUM takes this ASCII file and creates a second ASCII data file in our database form. Those six or less elements-integration records/particle in the first ASCII data file determine the selection of the mineral code for the second ASCII file. For instance, SiP S ClCa would be automatically be classified as "sen" or silica-endogenous (see below for the designations). The criteria for selection of the mineral designation follows specific rules which were easy to program into IXRFSUM. After import of the second ASCII data file into the dBase file, the dBase file will need some edits before the final printout. A big advantage of IXRFSUM is that there will be no more of the difficult-to-catch CPS input errors either in value or in order.

FIBER ANALYSIS

The digestion/fiber cases are treated differently. The field/ particle input is the same as the tissue section analysis, but with this file type, the two-character fiber description, three-character angle and eight-character size (as the actual micrometer measurement) is inputted in the WordPad ASCII file. FIBERID is used for the final mineral classification If the elemental compositions has combinations of Si, Mg, Na, Ca, K and Fe, the mineral designation is "asb." Other designations as described above are applied.

FINAL SUIRVEY AND DATABASE PREPARTION

The final step before analysis is the entire data file needs to be carefully examined and changes made where for one reason or other the assigned mineral designation is incorrect. The user makes the final decision as to the mineral assignment. In dBase, after appending the ASCII file, the number of particles counted and the number of particles analyzed needs to be added (see below). Other additions and corrections will be required.

STRUCTURE OF THE DATABASE

FIELD 1 - "CTNS" (COUNTS)

There are five spaces available in this field. The first 3 indicate the number of particles in the filed, and the last 2 characters indicate the number analyzed. If the number of particles analyzed = the number in the field, then these last two spaces are left with "00". If the number of particles analyzed is less than that counted, then the number analyzed is inputted here (e.g. 02008 = 20 particles counted and eight analyzed). This data field is left blank for the other records from the same SEM field.

FIELD 2 - "FLD:PARTLE"

There are five spaces available in this field. The first three indicate the field #, and the last 2 the particle number (e.g. 03403 = field 34, particle 3).

FIELD 3 - "LOC" (LOCATION)

TISSUE SECTION: There are three spaces available in this field. The purpose of this field is to designate the location of the particle in the tissue (all in lower case):

dig - digestion on filter (obsolete usage - there should be no files which have this)
air - particle is in a macrophage in airway
int - particle is located in airway wall
plu - particle is in pleura
spl - particle is in subpleura

DIGESTION: For the digestion samples the designation in this field is a description of the fiber type:

ab - asbestos body (obsolete designation)
bh - bundle of flexed fibers
bu - bundle of straight fibers
cl - clumped (not bushy)
db - doublet, smooth (two smooth fibers stuck together that are the same length)
di - diatom frustule
sb - fiber branches, usually chrysotile
sf - smooth flattened fiber
sg - smooth droplet on fiber end (man-made fiber)
sl - smooth pleated fiber
sb - smooth flexed & branched fiber
sm - smooth (= rounded) fiber
sp - smooth spindle shaped fiber
sr - smooth fiber with parallel assoc. fiber fragments
st - smooth tapered fiber
sx - smooth flexed fiber
tr - triplet, smooth (as in db, but 3 fibers)
di - diatom frustule

The last space of the LOCATION field is for the digestion fiber analysis is reserved for indication special conditions associated with the fiber.

- **c** - fiber is likely coated with Ca (not used after Dec, 2001)
- **f** - fiber is likely coated with organic Fe
- **F** - fiber is likely coated on basis of parallel analysis with oxalic acid treated sample.
- **b** - a single ferruginous body or more (after Dec, 2001) is on the fiber.
- **n** - sodium left out due inadequate field space
- **t** - two or more ferruginous bodies are on the fiber (not used after Dec, 2001)
- **o** - fiber is obscured by large debris so length report is not accurate
- **?** - uncertain ferruginous body
- ***** - fiber is covered by small particles making a clean spectrum impossible
- **w** - object is blocking X-rays to the detector making an accurate spectrum impossible
- **x** - fiber is partly obscured by debris and X- rays are also being blocked making an accurate spectrum impossible

ir with **t** - ferruginous body, but no naked fiber observable thus making the identity of the core fiber impossible

i - discrete fiber, but fiber diameter is so small that an accurate X-ray sample is impossible.

Probable asbestos – symbols at last CPS character (with MINERAL = "as?"):

- **A** – with ID= "as?" operator tentative assignment to amosite, but 1 or more elements out of range (see FIBERID).
- **C** – with ID= "as?" operator tentative assignment to crocidolite, but 1 or more elements out of range (see FIBERID).
- **c** – with ID= "as?" operator tentative assignment to chrysotile, Si only, but Mg not found, & fiber is distinctively chrysotile-like. (see FIBERID).
- t – with ID= "as?" operator tentative assignment to tremolite/actinolite, but 1 or more elements out of range (see FIBERID).
- a – with ID= "as?" operator tentative assignment to anthophyllite, but 1 or more elements out of range (see FIBERID).
- *No last character - with "as?" - the asbestos type is uncertain (rare).*

FIELD 4 - "TYPE"

There are three spaces available in this field. The first space is the type designation of the particle and the two remaining spaces are for the angle designation if the particle is a fiber. Leave last 2 spaces empty if analyte particle is not a fiber.

- **1** - particle is round
- **2** - particle is irregular in shape
- **3** - particle is a flake
- **4** - particle is a fiber. In the ETEC, since the sample is at 45°,
  - **400**- fiber at 0 degrees
  - **415**- fiber at 15 "
  - **430, 445, 460, 475, & 490**

FIELD 5 - "SIZE"

There are eight spaces in this field. The first 3 are for the smallest or only dimension of the particle and can be written 0.2 or .20. Space 4 is reserved for "x" and is used only if two dimensions are being used. The last 4 spaces are the for the largest dimension of the particle. For example: 0.2x01.2 or 0.4x00.6 or .15x12.8.

FIELD 6 - "ELEMENTS"

There are 12 spaces in this field. Each element occupies 2 spaces and is entered by its legal symbol (e.g. Na or Fe or Ti etc). If the element has only one character (e.g K or S or P), then the second space is left blank (e.g. SiAlK Fe). Six elements are permitted. If less than the 6 are entered leave the remaining spaces blank.

NOTE: If silicon (Si) is part of the composition of the particle and the mineral is a silicate, then the first two spaces in this field should be "Si". This is especially important for the FIBERID program.

FIELD 7 - "CPS" (COUNTS PER SECOND)

There are 23 spaces in this field. Enter the cps for each element entered in the element field. Separate the individual cps by ":". Leave the final entry without a ":". For example: SiAlK Fe has the entry 135:100:82:20. So, Si=135 CPS, Al=100 CPS, K=82 CPS, and Fe=20 CPS.

FIELD 8 - "ID" (MINERAL IDENTITY OF PARTICLE)

There are 3 spaces in this field. For each particle entered, you must make a decision as to what mineral it is and enter its abbreviation in this field.

- **sil** - silica : cps = 95% > other elements
- **als** - alumino-silicate: significant cps of Si & Al
- **mss** - misc silicate - not sil, but little or no Al
- **asb** - asbestos (unassigned - see below)
- **as?** - possible asbestos (odd composition or questionable fiber). See section 3 p. 15 for tentative fiber assignments.
- **tal** - talc (usually a Si:Mg of .25-.40)->Can ID by FIBERID.BB2
- **gyp** - gypsum (CaS)
- **met** - metal
- **enc** - endogenous, Ca rich (with very little or no Fe)
- **enh** - endogenous, Fe rich (with some or almost equivalent Ca counts)
- **org** - only small amounts of P or S or Cl or Mg or any combinations of these elements (Fe & Ca may be present, but at very low relative values). The X-ray count rate may also be very low considering the size of the analyte particle - placing it as mainly organic.
- **men** - metal associated with endogenous *
- **sen** - silica associated with endogenous *
- **aen** - aluminosilicate associated with endogenous *
- **msn** - misc. silicate associated with endogenous *

* - the association is so close that the spectrum cannot be separated.

DIGESTION/FIBER ANALYSIS

After the initial data entry with dBASE with all the fibers that are thought to be asbestos (mineral assignment: asb), the BASIC program "FIBERID.BB2" (in BASICD directory) is run. Each fiber is examined and probability values generated that each element is actually an asbestos component. This is made possible by "t" statistics and a number of asbestos and talc standards. After the program prints out all the possibilities for each fiber, the analyst goes through the print outs and assigns a mineral to each fiber. The mineral assignments are:

amo - Amosite
cro - crocidolite
acr - Amosite/crocidolite*
ant - Anthophyllite
chr - Chrysotile
chd - Chrysotile, Mg depleted
gla - Glaucophane
tre - Tremolite
act - Actinolite
as? - Uncertain affinity
mmf - man-made fiber (identified by the FIBERID program)**
mm? - man-made fiber (uncertain - i.e elemental composition does not quite match element relations of standards in the FIBERID program)**

* Amosite and crocidolite sometimes may have approximately the same proportions of Mg & Fe. Analysis conditions may be present where the distinguishing parameter(s) (usually the presence/ absence of Na) are obscured. Where there is this uncertainty the "acr" ID code is applied.

** The FIBERID program looks at all the "als" and "mss" mineral designations for a possible match to the elemental composition of man-made fibers. The "mmf" assignment is made based on a match and on fiber structure. If there is not quite an elemental match, then the "mm?" mineral designation is used.

After the fibers have been assigned (by dBASE edit function), the program is again run on these data and the fiber assignments are spelled out in the printout. This is the copy that the pathologist sees. The same elemental probabilities are given.

The following mineral designations are no longer used, but the analysis programs will accept (there should be no data files which have these left). These have been replaced by the coated fiber designations in the LOCATION field (space 3). See above.

abb - asbestos body
ab? - asbestos body (questionable)
abc - Ca coated asbestos fiber
abf - Fe coated asbestos fiber

DIGESTION/ALL PARTICLE ANALYSES

In these analyses, it is absolutely necessary to assess the control filter with a equal number of fields at the same magnification as the analyte filter. These records are then appended to the records of the analyte data file.

1) Enter the analyte data as described above (data file name ends in either "A" or "B").
   a) The 7-character file name should end in “D” when doing the file conversion. Then change to “A” or “B” for the data analysis.
   b) Add the control start record at eh end of the analyte data (see 2).
   c) Add the control records following the control start record.

2) The record after the last of the analyte should be:
   field 1 (COUNTS): CONTR
   fields 2 - 7 0's in all
   field 8 (the last one) - blank

3) Enter the control data as standard records. NOTE:
   A) All particles in a control field must be analyzed. (Particle cnts > analyzed not permitted.)

   B) The number of control fields searched must = analyte fields searched. If there is one control survey for two or more analyte surveys, then just apply control records where the number of fields equal.

ASBESTOS BODY ANALYSIS BY LIGHT MICROSCOPY

(For early cases, last record of 5000X survey)

The last record of the 5000X survey (up to approximately 1989 cases) has information regarding the analysis of the same case by LM for asbestos body estimates. After 1989, all those records with the last character "G" are asbestos bodies by digestion for LM. Information is applied to the various fields (second record in the "G" files):

FIRST RECORD - Set up as though a high-magnification fiber analysis.

SECOND RECORD - Set up as follows:

CNTS: AB___ -> The first field indicates that this record is an asbestos body analysis.

FLD:PRT: (5 spaces)
Space 1:

T - The total number asbestos bodies on filter counted.
H - Half of the filter counted: input the number counted, the program will calculate the actual number.
F - Counts by field (value in next four spaces must be the mean of 20 fields). Spaces 2-5: Actual number of asbestos bodies counted.

The first record, CNTS field, position 22 ("G" files only):
C - Fliter was mounted on Poretics Cyto-Clear slides.

LOCATION: (3 spaces) Give the sample #

SIZE: (8 spaces)
Spaces 1-4: estimated mm^3 of tissue
Spaces 5-8: mg of tissue in sample

ELEMENTS: Reserved for description of body types. Space 1:

A - Amorphous Fe coating on fibers - atypical of asbestos bodies.
B - Many single ferruginous bodies. Some or none may be organized onto fibers.
C - heavily encrusted bodies.
O or **N** - no fibrous bodies observed (space filler).
S - standard asbestos bodies (beaded).
W - Bodies are wheel-like.
F - Fibers are observed in sample, but none are coated.
G - Fibers observed in sample, but only a few are asbestos bodies.
P - Many ferruginous bodies organized into grape-bunch-like structures.

SPECIAL NOTE: From July, 1993 to present, the PORETICS CYTO-CLEAR slides have been used. These slides optically remove the pore diffraction seen in regular slides, thus allowing for more accurate body counts.

MISCELLANEOUS NOTES

ON ENTERING DATA (GENERAL). The menu program of dBASE is straight forward. Just follow the guidelines for data entry outlined above. If you realize that you made an entry error and have gone on to the next record, then as soon as you can leave the MENU program (by pressing "0" when you are given the opportunity). Enter EDIT ### where ### is the record #. Once in the edit mode of dBASE you can move around the data records quickly by pressing "PgUp" or "PgDn". When you are finished making the corrections press "Ctrl" and "W" at same time. Return to the MENU program and continue data entry.

The dBASE III data files cannot be read by BASIC I/O. DBASE, however, supplies a feature whereby the data files can be converted to ASCII format (makes each record 1 long string). On the data disk, each file is actually represented by 2 files; one with the extension "DBF" and the other "TXT". The "TXT" is the ASCII file. After entering all the data to a file by dBASE program "MENU", return to MENU and select item "3". This portion of the MENU program will copy to the data disk the data file in the compressed form.

ON ENTERING THE FIRST RECORD: Be sure the correct codes and spacing is present. If code is misaligned, the file cannot be read by the analysis program. FOR ALL THE DIGESTION FILES, ALL OF THE FIELDS MUST BE FILLED IN FOR THE ANALYSIS PROGRAMS TO WORK PROPERLY.

ON ENTERING THE REST OF THE RECORDS: Again, follow the guidelines. The first field of each record should only be filled in if it is the first record of a series of particle analyses from the same SEM field. In single particle/SEM field, the first data field should be "00100". In multiple particles/SEM field, e.g. "02005" which means that 20 particles were counted, and 5 analyzed in that particular SEM field. The remaining 4 records of that first field should be blank.

BASIC DATA CHECK

MASTER.BB2 (see below) also functions as a data checker. Although errors make for wasted paper, is easier to keep this program up to date than a separate data-checker program.

If errors are reported:
1. Note the record number of each error. Most errors reported by the *MASTER* program causes exit from the program.
2. Return to DOS by entering "BYE". Go to dBASE.
3. Input the case file at dot-prompt “USE ******)
4. type in “brow”. Edit the record that has the error.
5. After you are through editing, press "Ctrl W" and you will return to the dBASE "." mode. From there enter "COPY TO A:******* SDF", and then return to BASIC when finished.

BASIC ANALYSIS PROGRAMS

1. From Word Notepad (txt document) convert with BASIC program “IXRFSM” (DOS to BASICD ”begin”) to proper txt format.
2. Covert txt document to dBASE format
3. From dBASE go to DOS
4. From DOS - go to BASICD directory
5. BEGIN <enter>

The program "MENUP" will be automatically run. From here you can go to the other menus.

Analysis Programs: At the end of each of these programs, the menu will display. If during the running of the MASTER program, an error is reported, but the program is not exited, do not try to run the data set with the other programs. There are some errors which can really mess up these other programs. If errors are reported, you must return to dBASE and edit those records (see above).

BASIC PROGRAMS/DATA FILES - DESCRIPTION

MENUP To load the analysis programs. This automatically loads

after the Better Basic load.

DATAF.TMP All programs write the name of the selected data file to disk. Each of the analysis programs uses this file to automatically load the data file being analyzed. All programs allow the selection of a different file.

AREASUM.DAT is read by the analysis programs. This file is a historical record of field size data. This record will be used to apply the proper field size data to an old data file whose field size was different from the present field size. For the data files, the last field in record 1 must be filled in for the other analysis programs to correctly apply the proper SEM field size.

IXRFSUM.BB2 This program will take the text WordPad document that was created for the integrations of spectra from the EDS2000 and user inputted data (field-particle number, particle size, angle [if fiber] and fiber type [only if fiber] and reorganizes these text records to allow direct import into the dBase record structure used in these analyses. See page 19.

EXTENT.BB2 This program allows for the estimation of the number of fields to analyze at 2000X and 5000X in the digestion/ fiber analysis. For the 5000X analysis, the number of fields analyzed should be equal to the approximate minimal fiber burden estimate of 200,000 fibers/g dry wt. of tissue and for the 2000X . For *EXTENT.BB2* to work you need only the first record of a file. Fill it in as you normally would, but do not enter a sample size. Enter the dry/wet wt. data.

FIBERID.BB2 (SEE APPENDIX B, p. 43; fiber analysis asbestos and man-made fiber standard matching) This program is in two parts:

A) After the initial data entry with dBASE with all the fibers that are thought to be asbestos (mineral assignment: asb), this program is run on these data. The program looks at the elemental composition of the "asb" fibers ("mss" and "als" for man-made fibers) and then provides the minerals that have that elemental composition and for each elemental ratio to Si gives the probability that elemental is part of that asbestos mineral. The probability values are given in percent. This is made possible by "t" statistics and asbestos, talc, other minerals and made-made fiber standards. After the program prints out all the possibilities for each fiber, the analyst goes through the printouts and assigns a mineral to each fiber. See above for the correct mineral field assignments.

B) After the fibers have been assigned (by dBASE edit or BROWSE function), the program is again run on these data and the fiber assignments are spelled out in the printout. This is the copy that the pathologist sees. The same elemental probabilities are given.

MASTER.BB2 (SEE APPENDIX C, p 44) This is the general analysis program for all the particles entered in the file for the respective data type. It tabulates the IDs (field # 8), and provides statistics on exogenous particle distribution, estimates of endogenous particles, and summarizes the different mineral types. This program will not work with BAL samples and should always be run first. For the digestion/all particle analysis data, the control records at the end of the file are read by this program. For old data files, this program (and several others) looks at the date field at the end of the first record of a data file and applies the correct SEM field size to the data.

FIBQUANT.BB2 (SEE APPENDIX C, p. 48; supplementary program to MASTER.BB2 and accessed via MASTER.BB2.) This program conducts a quantitative analysis of fibers only (data file name must end in "D"). After running MASTER.BB2, this program allows you to select FIBQUANT for additional analysis (a summary of the individual fiber types).

METALS.BB2 This program gives a more detailed analysis of the types and distribution of the metal particles. This program will not work with BAL samples or fiber/digestion data.

MINERAL.BB2 This is a printout by mineral type. You can select individual mineral types or have the program automatically print out every record. Record #, field-particle #, CPS of first element, size (adjusted to stage tilt if fiber), elemental composition, and elemental ratios are displayed.

SIZE.BB2 ((SEE APPENDIX C, pp. 48; section & digestion analysis) This program prints out the size distribution of the various minerals either of regular particles or fibers. If regular particles, the program selects the first dimension of the SIZE field in a record, and if fiber analysis, the program selects the second dimension.

PRINTER.BB2 (VERBATIM OUTPUT - SEE APPENDIX C, p. 49) This prints out all the data files essentially unmodified.

ASBODY.BB2 This program analyzes asbestos body counts by LM only. ASBODY requires the first and second records to be filled in. See above for details.

SPECIAL PROGRAMS

SETAREA.BB2 This short program writes the current field size in um^2 to disk for the MT-ETEC (2000X,3000X & 5000X) and for the Syracuse Hitachi (2000X, 3000X, 5000X, and 8000X). The data file which has this file is *ARAESUM.DAT.*
analysis 10,000 fibers/g dry wt.

STRUCTURE OF THE ANALYSIS PROGRAMS

Generally, all the analysis programs have to line 5000 of each about the same structure. From lines 5000 to 10000 is where each program is unique. The follow is the general ASCII structuring.

```
INITIAL.BAS   |
DATALOAD.BAS |----> CORE1.BB2
```

PROGRAMS VIA MAIN (DEFAULT) MENU
- Press "2" to access
MENU2

```
MENU1.BAS        |

   INITIAL.BAS        |
   DATALOAD.BAS  |
   MENU1.BAS          |----> CORE2.BB2
   MAGCALC.BAS    |
   GENCALC.BAS    |

   INITIAL.BAS        |
   DATALOAD.BAS  |
   MENU1.BAS          |----> CORE3.BB2
   MAGCALC.BAS    |
   GENCALC.BAS    |
   PDISPLAY.BAS    |

   CORE3.BB2 + MASTER.BAS  = MASTER.BB2
   CORE2.BB2 + FIBQUANT.BAS = FIBQUANT.BB2
   CORE2.BB2 + METALS.BAS = METALS.BB2
   CORE1.BB2 + MINERAL.BAS = MINERAL.BB2
   CORE1.BB2 + SIZE.BAS = SIZE.BB2
   CORE1.BB2 + PRINTER.BAS = PRINTER.BB2
   CORE1.BB2 + FIBERID.BAS = FIBERID.BB2

   ASBODY.BAS + MENU1.BAS = ASBODY.BB2*
   EXTENT.BAS + MENU1.BAS = EXTENT.BB2*
   SETAREA.BAS + MENU1.BAS = SETAREA.BB2*
   PFONT.BAS + MENU1.BAS = PFONT.BB2*
```

- Compile first module and then merge MENU.BAS to it. Adjust the MENU lines if needed while in BETTER BASIC.

ANALYSIS WITH THE IXRF SYSTEM & THE PROGRAM *IXRFSUM.BB2*

The IXRF batch file system is used to create a series of analyses (spectra) for each SEM field. Each batch file contains one field of an analysis, either tissue section or digestion. A batch file name is the field number and it contains images and spectra. As previously described the program, IXRFSUM.BB2, was written to take the spectral integrations calculated by the IXRF software directly into a form to be read by the Meixa Tech analysis programs. The IXRFSUM program:

File Name structure to be read (input) by IXRFSUM:
  Tissue section: 6 characters: e.g., 99120A
  Digestion: 7 characters e.g., 02345A1

File name size is assessed by the program to select the proper data format. The IXRFSUM output file will be:
  Tissue section: 8 characters: e.g., 99120AZT
  Digestion: 8 characters e.g., 02345A1D

The WordPad text document is constructed for reading by IXRFSUM.BB2:

Header
File ID e.g., JA05-234 Smith 1-20-2005
CONV= 7.3435 (“**CONV=** ” MUST BE PRESENT) This number represents the mm to micron conversion value. For each particle, the mm value of that particle is posted as indicated in the following. IXRFSUM will convert to microns.

For each particle, the following series of records are constructed:

Tissue section (only)
1-2 (the field number - particle number)
5.89 (the size in millimeters)

>> the IXRF integrations are transported to WordPad document via Clipboard from the EDS2000 application program; to include:
Elt. Line Intensity …. (list only 6 elements following—the 2-sig is not necessary)

Takeoff Angle 35.3 - Required: the final record for the particle. Absolutely needed to start record for a new particle.

Digestion (only)
1-2 (the field number - fiber number)
sf (fiber type)
445 (fiber angle)
5.8x12.4 (the size in millimeters, width is 3 characters, length 4 characters separated by “x”)

>> the IXRF integrations are transported to WordPad document via Clipboard from the EDS2000 application program; to include:
Elt. Line Intensity …. (list only 6 elements following this -the “2-sig” is not necessary)

Takeoff Angle 35.3 - Required: the final record for the particle. Absolutely needed to start record for a new particle.

When finished with the construction of the test document, run IXRFSUM (in the BetterBasic environment)

There are specific instructions with the program to follow. Additional features of IXRFSUM.BB2:

1. The program will printout the ASCII records which combines the text document records for each particle (from the WordPad text document) and if no errors (usually missing size data) are present in that document will then write the processed file to disk. This file that can be transported to dBase via the “APPEND FROM” + PROCESSED FILE + “ SDF”

2. The dBase file will need to have added for field 1 of each record the number of particles in the field (3 characters) and the number of particles analyzed if less than the number in the field (2 characters). All other fields in each dBase record should be in the correct position. This operation needs the dBase “BROWSE” feature.

3. IXRFSUM will apply a mineral type to the final ID field of each record. These are correct about 80% of the time—so these should be carefully checked and changed in the dBase environment. For asbestos files, the program usually applies “asb” to the ID field for the usual element combinations of Mg, Si and Fe. There may be some misapplied “mss” and “als,” so be certain to change these to “abs” prior to running FIBERID.

THE DIGESTION/FIBER SUMMARY PROGRAMS

INTRODUCTION

Virtually every aspect of the data in the digestion files are summarized by six programs described below.

The following series of programs have been extensively tested. I believe that there are few (if any) bugs remaining. If you think you have found a bug, please let me know.

Usually a program crash occurs when a data file has been incorrectly constructed or has been corrupted. In the testing of these programs, both problems were encountered. If you encounter a problem, check the offending file (the name will usually be displayed on the screen). The first record of a data file is often the cause of a crash (especially if you see error 5). Error 1001 is a file missing error message. Check to be sure that all the data files that a program needs are present.

ASCII DATA FILES --> dBASE

The ASCII data files created by the summary programs can be converted to dBASE. For example in dBASE "." mode:

```
.USE F:FIBSUM
.ZAP          (to remove old records)
.APPEND FROM E:FIBSUM.DAT SDF
```

All the summary data files on the Zip will have the up-to-date data files for both the .DAT and .DBF files.
NOTE: The file extensions indicate the origin of the file:
.DBF = a dBASE data file
.TXT = an ASCII data file created by dBASE
.DAT = an ASCII data file created by a BASIC program

MENU2 - All of the following programs can be easily accessed by this menu. RUN "MENU2" will display the following programs.

1- CREATE ASCII DATA-FILE NAME LISTING: **FILWRT.BB2**

DATA FILES:
DFILES.DAT - Digestion/fibers
TFILES.DAT - Tissue-section
GFILES.DAT - Asbestos body
AFILES.DAT - Digestion/all particles full field
BFILES.DAT - Digestion/all particles reduced field

This program is important for most of the programs that follow in that it creates an ASCII file of the file names resident in the CASES__ directories on the Zip disk. NOTE: The program requires the data files in the CASES__ directories be without the dBASE (.DBF) files.

Requires data file: Drive D:\CASES__\- Data files must be in the .TXT form only.

FILWRT.BB2 will write to the default directory the data file names in ASCII without the extension. This program must be run prior to use of most of the summary programs in that *FILES.DAT is relied upon by the other summary programs to accurately update the summary data files.

The analysis files must be on the Zip drive D: and must be organized by year in the directories named "CASES__", where the last two spaces are the year (e.g. "CASES89"). Further, the BetterTools keyword "DIRLOAD" is limited by 255 files. So, because some directories (e.g. CASES86) have more than 255 files of both *.DBF & *.TXT, the *.DBF files need to be removed. A Zip disk was set up with only *.TXT files in the CASES__ directories. None of these directories have more than 255 files.

Go through each directory/year on the Zip. The final file DFILES.DAT (for example) will be ASCII listing of the all the digestion/fiber files (for files that end with the letter "D". With a rerunning of FILWRT.BB2 other data file types can be listed.

RATIONAL OF FILWRT.BB2 - The reason for listing all of the data file names in this manner is due to the need to have such a file for the summary data programs. Doing this by computer, one avoids input errors that will occur by hand input.

NOTE: Upon the creation of a new directory (e.g. CASES91), line # 1720 in FILWRT.BB2 must have this datum inserted in the form of "91". In # 1700 update the last number to reflect the added number of DATA entries in lines # 1710 to 1730.

USE OF FILWRT.BB2 -
1) Place in Zip "JACASES BY .TXT FILES" in drive D:
2) BetterBasic: load as usual - access DIG/FIB SUMMARY MENU through the primary MENU or RUN "MENU2".
3) Run the program as instructed.

2- PRIMER PROGRAM: ***DSTART.BB2***

DATA FILE: **DATAF.TMP**

The purpose of this program is to "prime" MSTWRT.BB2, FIBANAL.BB2 & FIBANALA.BB2 so that these programs can read the *FILES.DAT files for an entire rewrite or append of their respective data files. See the following program descriptions for further information.

3- FIBER BURDEN DATA WRITE: **FIBANAL.BB2**

DATA FILE: **FIBSUM.DAT**

This program writes fiber burden estimates for all the fiber types to F:FIBSUM.DAT. It does this by accessing all the data files in F:CASES__. Total processing time is in excess of 1 hr for an entire rewrite of FIBSUM.DAT.

Requires data files:
1) Drive C: (default): DATAF.TMP
2) Drive C: (default): AREASUM.DAT
3) Drive C: (default): DFILES.DAT
(see Option 1 - FILWRT.BB2)
4) Drive D:\CASES__\- Data files must be in .TXT form only.

NOTE 1: Once the program is running, any interrupt will cause loss of data if you continue without returning to Option 2 (DSTART.BB2). In the append file name that you are requested in Option 2, supply the last file name to be printed.

NOTE 2: If after selecting Option 2 for append and imputing the file name, and upon running DFIBWRTF.BB2, the MENU quickly appears without printing or writing to disk. This means that the file name is probably incorrect.

NOTE 3: There are cases where there was not enough tissue to allow an estimate of the dry/wet ratio. For this reason all the fiber estimates are in # fibers/wet wt. Calculation of these value in dry wt can be accomplished by the "DRYPRCNT" Value (field 7). Please note that DRYPRCNT is (dry wt./wet wt)

DFIBWRTF.BB2 is constructed of modules derived from the standard data analysis programs (*.BAS = ASCII, *.BB2 =

CODE SHEET FOR FIBSUM.DAT:

	ASCII String Name	dBASE variable	# charac.	COMMENTS	
1	-	DUMMY	1	to take care of ASCII (")	
2	ANAFILED$	CASE	8	file name	
3	PORE$	PORESIZE	2	pore size of Nuclepore filter.	
4	AZ$	MAG	1	magnification code	
5	TISVOL$	TISVOL	4	tissue volume in mm^3	
6	TISWETWT$	TISWETWT	4	tissue wet wt in mg	
7	DRYRATIO$	DRYPRCNT	5	drywt/wetwt X 100	
8	SN$	NUMFIELDS	4	# of fields searched	
9	CASECODE$	ANALCODE1	1	anal code @ pos #19	
10	"	TISTYPE	2	tissue type @ 20 & 21	
11	"	ANALCODE2	1	analysis code @ pos #22	
12	"	ANALCODE3	1	analysis code @ pos #23	
13	MINEST$	MINESTIM	5	minimal estimate for 1 fiber *	
14	TOTPROB$	TOTPROBASB	5	total probable asbestos *	
15	POSSASB$	POSSASB	5	possible asbestos *	
16	AMOSITE$	AMOSITE	5	amosite *	
17	AMOSCRO$	AMOCRO	5	amosite/crocidolite(?) *	
18	ANTHO$	ANTHO	5	anthophyllite *	
19	CROCID$	CROCID	5	crocidolite *	
20	CHRY$	CHRY	5	chrysotile *	
21	CHRYMG$	CHRYMG	5	chrysotile -Mg *	
22	ACTIN$	ACTIN	5	actinolite *	
23	TREMO$	TREMO	5	tremolite *	
24	SILICA$	SILICA	5	silica *	
25	ALUMSIL$	ALUMSIL	5	aluminosilicate *	
26	MISCSIL$	MISCSIL	5	miscellaneous silicate *	
27	MANMADQ$	MANMADQ	5	man-made fiber ? *	
28	MANMADE$	MANMADE	5	man-made fiber *	
29	GLAUCO$	GLAUCO	5	glaucophane	
30	TALC$	TALC	5	talc*	
31	METAL$	METAL	5	metals	
32	CACOAT$	CACOAT	5	Ca coated fibers *	
33	FECOAT$	FECOAT	5	Fe coated fibers *	
34	COATANTH$	COATANTH	5	anthophyllite (coated)*	
35	COATCHR$	COATCHR	5	chrysotile (coated) *	
41	COATTAL$	COATTAL	5	talc (coated) *	
42	PROBASB$	PROBASB	5	probable asbestos *	(FIBANAL variable is “UNCERT *)
43	PROBAMO$	PROBAMO	5	probable amosite *	“
44	PROBANT$	PROBANT	5	probable anthophyllite *	“
45	PROBCHR$	PROBCHR	5	probable chrysotile *	“
46	PROBCRO$	PROBCRO	5	probable crocidolite *	“
47	PROBTRE$	PROBTRE	5	probable tremolite *	“
48	PROBACR$	PROBACR	5	probable amosite/crocidolite *	“
49	COATGLA$	COATGLA	5	glaucophane (coated) *	“
50	NUMFILE$	NUMFILE	5	JA file number	

* burden in #/g wet wt X 10^4

complied).

```
INITIALD.BAS    |
DATALD.BAS      |
MAGCALCD.BAS |    >  CORED.BB2
GENCALCD.BAS  |
MENU1.BAS       |
   +    DFIBWRTF.BAS = DFIBWRTF.BB2
```

4- FIBER BURDEN DATA WRITE: ***DFIBWRTA.BB2***

DATA FILE: **FIBSUMA.DAT**

This is the second half of DFIBWRTF.BB2 which because of space, could not be included in the first part. Essentially, the program is almost identical to DFIBWRTF.BB2, but shortened in places.

Requires data files:
1) Drive C: (default): DATAF.TMP
2) Drive C: (default): AREASUM.DAT
3) Drive C: (default): DFILES.DAT
(see Option 1 - FILWRT.BB2)
4) Drive D:\CASES__\- Data files must
be in the .TXT form only.

NOTE: See Option 3 for instructions/description

CODE SHEET FOR *FIBSUMA.DAT*:

	ASCII String Name	dBASE variable	# charac.	COMMENTS
1	-	DUMMY	1	to take care of ASCII (")
2	ANAFILED$	CASE	8	file name
3	TOTBURD$	TOTFIBER	5	total estimated fiber *
4	AH$	ANADATE	3	month & year of analysis **
5	NUMFILE$	NUMFILE	5	JA file number

* burden in #/g wet wt X 10^4
** with: A90=Oct 1990, B90=Nov 1990, C90=Dec 1990

DFIBWRTA.BB2 is constructed of modules derived from the standard data analysis programs (*.BAS = ASCII, *.BB2 = complied).

```
INITIALD.BAS    |
DATALD.BAS      |
MAGCALCD.BAS | > CORED.BB2
GENCALCD.BAS  |
MENU1.BAS       |
```

+ DFIBWRTA.BAS = DFIBWRTA.BB2

5- ASBESTOS BODY SUMMARY TO DISK:
SUMBODY.BB2

DATA FILE: **LMASBDY.DAT to 2000 cases**

LMASBDY2.DAT 2000 -> CASES

This program will access LMASBDY.DAT of the data files currently incorporated and then match the file names to the data file names in DFILES.DAT or GFILES.DAT. If a data file is found which has asbestos body data at the file end, and the file is not represented in LMASBDY.DAT then SUMBODY.BB2 will write that asbestos-body data to LMASBDY.DAT.

NOTE: <u>ASBODYS.DBF</u> was the original summary data file for asbestos bodies. There are records in this file which are from the Meixa Tech record books (They were never put on disk). LMASBDY.DAT was initially constructed from ASBODYS.DAT. Updating of this file is by direct reading of the original data files.

The two data file types that this program will search are those ending in "D" and "G". It is possible to have the same asbestos-body data in both file types. SUMBODY.BB2 will write to LMASBDY.DAT only one of these files.

Requires data files:

1) Drive D:\CASES__\- Data files must be in the .TXT form only.
2) Drive C: (default) GFILES1.DAT to 2000
   GFILES.DAT after 2000
   (see FILWRT.BB2!)
3) Drive D: (main directory)-
   LMASBDY.DAT

NOTE 1: Unlike the other summary programs under no circumstances is LMASBDY.DAT destroyed and reconstructed. One Record is added to LMASBDY.DAT at each cycling of SUMBODY.BB2 from the CASES__ directories in drive D:. Each time SUMBODY.BB2 adds to LMASBDY.DAT the <u>program must be rerun</u>. Thus, SUMBODY.BB2 is not very efficient at its task and will take about 2 seconds on a 95 MHz machine for each pass of the data.

NOTE 2: <u>Lines 517 & 557 change year value to last JA year where no new entries to the database occurred.</u>

NOTE 3: Rerun SUMBODY.BB2 until it returns to the MENU2. When it does so, all the data files have been checked and LMASBDY.DAT has been updated. Remember to use current GFILES.DAT in the updating process.

Once the LMASBDY.DAT is constructed:

1) dBASE "." mode
2) USE F:LMASBDY
3) ZAP
4) APPEND FROM F:LMASBDY.DAT SDF

STRUCTURE OF dBASE DATA FILE:

Field	Field Name	Type	Width	Comments
1	DUMMY	C	1	take care of ASCII (")
2	CASE	C	8	Data file name*
3	ASBODYCNTS	C	5	# ASBESTOS BODIES CNTED
4	CODE	C	5	T=tot,H=1/2,F=by field
5	MINWETWT	C	5	Min est bodies/g wet wt.
6	ASBWETWT	C	5	# bodies/g wet wt.
7	MINDRYWT	C	3	Min est bodies/g dry wt
8	ASBDRYWT	C	3	# bodies/g dry wt.
9	NUMFILE	C	8	JA Case #
			45	

*File type indicator ("D" or "G")(i.e., the last digit of a file name) is not present on file numbers 1 to 172.

6- MASTER WRITE: ***MSTWRTD.BB2*** ASCII: *MSTWRTD.BAS*

DATA FILES:
DMASTER.DAT - Digestion/fibers
AMASTER.DAT - Digestion/all particles full field
BMASTER.DAT - Digestion/all particles reduced field

MSTWRTD.BB2 writes all the records of the analysis files (except the first record of each file to a *MASTER file. The *MASTER files generated by this program replaces the DMASTER.DBF data file that was created by the dBASE program BUILD.

NOTE: The old DMASTER.DBF should not be used in future data analyses because most of it was constructed with archaic data files.

Requires data files:

1) Drive D:\CASES__\- Data files must be in the .TXT form only.
2) Drive C: (default) DATAF.TMP
3) Drive C: (default) *FILES.DAT (* = D, T, A, or B) (see FILWRT.BB2)

Writes data files: Drive D: (Main Directory):

DMASTER.DAT
AMASTER.DAT
BMASTER.DAT

MSTWRTd.BB2 either (1) appends the selected *MASTER.DAT or (2) erases the current selected *MASTER.DAT file and then reconstructs the file. If append is selected, then follow the procedure described above for appending the FIBSUM.DAT (see

MENU3 - All of the following programs can be easily accessed by this menu. RUN "MENU3" will display the following programs.

1- CREATE ASCII DATA-FILE NAME LISTING: ***FILWRT2.BB2***

DATA FILES:
DFILES.DAT - Digestion/fibers
TFILES.DAT - Tissue-section

DFIBWRTF.BB2). MSTWRT.BB2 writes to the main directory on the Bernoulli on drive D:. Be sure that enough space exists on the Bernoulli in drive D: for that file (especially TMASTER.DAT & DMASTER.DAT) to fit (together these files have an excess of 3 megs).

NOTE: MSTWRTD.BB2 adjusts fibers (type "4" particles) to compensate for stage tilt on the SEM.
Time: Approximately 70 minutes

The **MASTERD.DBF** found on the Bernoulli is a file that is constructed by combining all of the *MASTER.DAT types while in dBASE.

Once the *MASTER.DAT is constructed:

1) dBASE "." mode
2) USE D:MASTER (be sure you have a copy on the D: drive; ZAP it if you are reconstructing the file)
3) APPEND FROM D:*MASTERD.DAT SDF

NOTE: The first record is a DUMMY record and should be removed before adding to MASTER.DBF

For the ID field changes have been made in DMASTER: the probable designations: amosite: am?, crocidolite: cr?, anthophyllite: an?, chrysotile: ch?, tremolite: tr?

STRUCTURE OF dBASE DATA FILE:

Field	Field Name	Type	Width	Comments
1	DUMMY	C	1	take care of ASCII (")
2	TISVOL	C	5	# particles .X 10^3/cm^3 tis.*
3	TISWET	C	5	# particles X 10^3/g wet wt
4	TISDRY	C	5	# particles X 10^5/g dry wt
5	COUNTS	C	5	
6	FLD_PRTLE	C	5	
7	LOCATION	C	3	
8	TYPE	C	3	
9	SIZE	C	8	
10	ELEMENTS	C	12	
11	CPS	C	23	
12	ID	C	3	
13	CASE	C	8	full file name
14	MAGCODE	C	1	magnification of analysis
15	ANALYST	C	1	analyst code **
16	SIZECODE	C	1	minimum fiber length
17	ANALCODE1	C	1	anal code @ position 19 **
18	TISTYPE	C	2	tissue type code **
19	ANALCODE2	C	1	analysis code @ position 22 **
20	ANALCODE3	C	1	analysis code @ position 23 **
21	KEYCODE	C	1	file designation code ***
22	YRANAL	C	2	year of analysis
23	NUMFILE	C	5	JA case number
		TOTAL	92	

** See pages 13 & 14 of the documentation for key
*** D=digestion/fiber, T= tissue section, etc.

Note 1: The tissue section MASTER, “TMASTER” has a different structure—see the Tissue Section summary files

THE TISSUE-SECTION SUMMARY PROGRAMS

GFILES.DAT - Asbestos body
AFILES.DAT - Digestion/all particles full field
BFILES.DAT - Digestion/all particles reduced field

This program is important for the programs that follow in that it creates an ASCII file of the file names resident in the CASES__ directories on the Bernoulli. NOTE: The program requires the data files in the CASES__ directories be without the dBASE (.DBF) files.

Requires data file: Drive D:\CASES__\- Data files must be in the .TXT form only.

FILWRT2.BB2 will write to the default directory the data file names in ASCII without the extension. This program must be run prior to use of most of the summary programs in that *FILES.DAT is relied upon by the other summary programs to accurately update the summary data files.

*FILWRT2.BB2 is the same as FILWRT.BB2 except for having MENU3 rather than MENU2 appended to it.

2- PRIMER PROGRAM: *DSTART2.BB2*

DATA FILE: **DATAF.TMP**

The purpose of this program is to "prime" MSTWRT.BB2, DFIBWRTF.BB2 & DFIBWRTA.BB2 so that these programs can read the *FILES.DAT files for an entire rewrite or append of their respective data files. See the following program description for further information.

*DSTART2.BB2 is the same as DSTART.BB2 except for having MENU3 rather than MENU2 appended to it.

3- PARTICLE BURDEN DATA WRITE: *TISECSUM.BB2*

DATA FILE: **TISSUM.DAT**

dBASE FILE: **TISSUM.DBF**

This program writes particle burden estimates for all the particle types to F:TISSUM.DAT. It does this by accessing all the data files in F:CASES__. Total processing time is approximately 100 minutes (May 1990) for an entire rewrite of TISSUM.DAT.

Requires data files:
1) Drive C: (default): DATAF.TMP
2) Drive C: (default): AREASUM.DAT
3) Drive C: (default): TFILES.DAT
(see Option 1 - FILWRT2.BB2)
4) Drive D:\CASES__\- Data files must be in the .TXT form only.

NOTE 1: Once the program is running, any interrupt will usually cause loss of data if you continue without returning to Option 2 (DSTART2.BB2). In the append file name that you are requested in Option 2, supply the last file name to be stored in TISSUM.DAT. You can determine this by listing the data in TISSUM.DAT by the DOS command "TYPE F:TISSUM.DAT" <ENTER>.

NOTE 2: If after selecting Option 2 for append and imputing the file name, and upon running TISECSUM.BB2, the MENU3 quickly appears without printing or writing to disk. This means that the file name is probably incorrect.

CODE SHEET FOR TISSUM.DAT:

ASCII String Name	dBASE variable	# charac.	COMMENTS
1 -	DUMMY	1	the ASCII (")
2 ANAFILED$	CASE	8	file name
3 AAA$	INSTCODE	1	Instrument code
4 OPCODE$	OPCODE	1	Operator (analyst) code
5 AZ$	MAG	1	magnification code
6 SN$	NUMFIELDS	4	# of fields
7 CASECODE#	ANALCODE1	1	analysis code @ pos #19
8 "	TISTYPE	2	tissue type @ 20 & 21
9 "	ANALCODE2	1	anal code @ pos #22
10 "	ANALCODE3	1	anal code @ pos #23
11 MINEST$	MINESTIM	5	minimum estimate for 1 particle *
12 TSLC$	TOTNUM	6	total number exogenous particles*
13 SILICA$	SILICA	6	silica *
14 ALUMSIL$	ALUMSIL	6	aluminosilicate *
15 MISCSIL$	MISCSIL	6	miscellaneous silicate *
16 TALC$	TALC	5	talc *
17 METAL$	METAL	6	metals *
18 AGM$	AG	5	Silver *
19 ALM$	AL	5	Aluminum *
20 ASM$	AS	5	Arsenic *
21 AUM$	AU	5	Gold *
22 BAM$	BA	5	Barium *
23 BIM$	BI	5	Bismuth *
24 BRM$	BR	5	Bromine *
25 CDM$	CD	5	Cadmium *
26 CEM$	CE	5	Cerium*
27 COBM$	CO	5	Cobalt *
28 CRM$	CR	5	Chromium *
29 CUM$	CU	5	Copper *
30 FEM$	FE	5	Iron *
31 HGM$	HG	5	Mercury *
32 IM$	I	5	Iodine *
33 LAM$	LA	5	Lanthanum *
34 MNM$	MN	5	Manganese *
35 MOM$	MO	5	Molybdenum *
36 NBM$	NB	5	Niobium *
37 NDM$	ND	5	Neodymium *
38 NIM$	NI	5	Nickel *
39 OSM$	OS	5	Osmium *
40 PBM$	PB	5	Lead *
41 RBM$	RB	5	Rubidium *
42 RUM$	RU	5	Ruthenium *
43 SBM$	SB	5	Antimony *
44 SEM$	SE	5	Selenium *
45 SNM$	SN	5	Tin *
46 TAM$	TA	5	Tantalum *
47 TIM$	TI	5	Titanium *
48 VM$	V	5	Vanadium *
49 WM$	W	5	Tungsten *
50 ZNM$	ZN	5	Zinc*
51 ZRM$	ZR	5	Zirconium*
52 ENC$	ENC	5	Endogenous, Ca rich*
53 ENH$	ENH	5	Endogenous, Fe rich*
54 ORG$	ORG	5	Organic (heavy BS)*
55 NUMFILE	NUMFILE	5	JA file number

* # particles X 10^6/cm^3 tissue

Once the TISSUM.DAT is constructed:

1) dBASE "." mode
2) USE F:TISSUM (be sure you have a copy on the F: drive;
3) ZAP it to remove old data
4) APPEND FROM F:TISSUM.DAT SDF

You now have the dBASE equivalent of the ASCII data file. All fields in dBASE are CHARACTER type.

TISECSUM.BB2 was constructed of modules derived from the standard data analysis programs (*.BAS = ASCII, *.BB2 = complied).

```
INITIALD.BAS     |
DATALD.BAS       |
MAGCALCD.BAS  | > TISECSUM.BAS
GENCALCD.BAS  |
```

However, because of differences in these modules from the digestion-summary programs, all of these modules were combined in TISECSUM.BAS. TISECSUM.BB2 is constructed from this TISECSUM.BAS file and MENU3.BAS

4-ENDOGENOUS CALCIUM PARTICLE BURDEN DATA WRITE:

TISCA.BB2

DATA FILE: **TISCA.DAT**

dBASE FILE: **TISCA.DBF**

This program writes particle burden estimates for all the elements associated with the endogenous Ca designation (enc) to F:TISCA.DAT. It does this by accessing all the data files in F:CASES__. Total processing time is approximately 100 minutes (May 1990) for an entire rewrite of TISCA.DAT.

Requires data files:
1) Drive C: (default): DATAF.TMP
2) Drive C: (default): AREASUM.DAT
3) Drive C: (default): TFILES.DAT
(see Option 1 - FILWRT2.BB2)
4) Drive D:\CASES__\- Data files must be in the .TXT form only.

NOTE 1: Once the program is running, any interrupt will usually cause loss of data if you continue without returning to Option 2 (DSTART2.BB2). In the append file name that you are requested in Option 2, supply the last file name to be stored in TISSUM.DAT. You can determine this by listing the data in TISSUM.DAT by the DOS command "TYPE F:TISCA.DAT" <ENTER>.

NOTE 2: If after selecting Option 2 for append and imputing the file name, and upon running TISCA.BB2, the MENU3 quickly appears without printing or writing to disk. This means that the file name is probably incorrect.

CODE SHEET FOR TISCA.DAT:

ASCII String Name	dBASE variable	# charac.	COMMENTS
1 -	DUMMY	1	the ASCII (")
2 ANAFILED$	CASE	8	file name
3 AAA$	INSTCODE	1	Instrument code
4 OPCODE$	OPCODE	1	Operator (analyst) code
5 AZ$	MAG	1	magnification code
6 SN$	NUMFIELDS	4	# of fields
7 CASECODE$	ANALCODE1	1	anal code @ pos #19
8 "	TISTYPE	2	tissue type @ 20 & 21
9 "	ANALCODE2	1	anal code @ pos #22
10 "	ANALCODE3	1	anal code @ pos #23
11 MINEST$	MINESTIM	5	minimum estimate for 1 particle *
12 CAM$	CALCIUM	5	calcium *
13 PM$	PHOSPHORUS	5	phosphorus *
14 SM$	SULFUR	5	sulfur *
15 MGM$	MAGNESIUM	5	magnesium *
16 KM$	POTASSIUM	5	potassium *
17 FEM$	IRON	5	iron *
18 NAM$	SODIUM	5	sodium *
19 ENC$	TOTENDOCA	5	total "endogenous" CA *
20 NUMFILE$	NUMFILE	5	JA file number

* # particles X 10^6/cm^3 tissue

Once the TISCA.DAT is constructed:

1) dBASE "." mode
2) USE F:TISCA (be sure you have a copy on the F: drive;
3) ZAP it to remove old data
4) APPEND FROM F:TISCA.DAT SDF

You now have the dBASE equivalent of the ASCII data file. All fields in dBASE are CHARACTER type.

TISECA.BB2 was constructed of modules derived from the standard data analysis programs (*.BAS = ASCII, *.BB2 = complied).

```
INITIALD.BAS     |
DATALD.BAS       |
MAGCALCD.BAS  | > TISECSUM.BAS
GENCALCD.BAS  |
```

However, because of differences in these modules from the digestion-summary programs, all of these modules were combined in TISCA.BAS. TISCA.BB2 is constructed from this TISCA.BAS file and MENU3.BAS

5-ENDO-CALCIUM PARTICLE BURDEN DATA WRITE:

TISCAFLT.BB2

DATA FILE: **TISCAFLT.DAT**
dBASE FILE: **TISCAFLT.DBF**

This program writes particle burden estimates for "pure" Ca and CaP particles. These particles are assigned as "enc" particles in the regular database. Data is written to the data file F:TISCAFLT.DAT. The program TISCAFLT.BB2 does this by accessing all the data files in F:CASES__. Total processing time is approximately 100 minutes (May 1991) for an entire rewrite of TISCAFLT.DAT.

Requires data files:
1) Drive C: (default): DATAF.TMP
2) Drive C: (default): AREASUM.DAT
3) Drive C: (default): TFILES.DAT
(see Option 1 - FILWRT2.BB2)
4) Drive D:\CASES__\- Data files must
be in the .TXT form only.

NOTE 1: Once the program is running, any interrupt will usually cause loss of data if you continue without returning to Option 2 (DSTART2.BB2). In the append file name that you are requested in Option 2, supply the last file name to be stored in TISCAFLT.DAT. You can determine this by listing the data in TISCAFLT.DAT by the DOS command "TYPE F:TISCAFLT.DAT" <ENTER>.

NOTE 2: If after selecting Option 2 for append and imputing the file name, and upon running TISCAFLT.BB2, the MENU3 quickly appears without printing or writing to disk. This means that the file name is probably incorrect.

CODE SHEET FOR TISCAFLT.DAT:

ASCII String Name	dBASE variable	# charac.	COMMENTS
1 -	DUMMY	1	the ASCII (")
2 ANAFILED$	CASE	8	file name
3 AAA$	INSTCODE	1	Instrument code
4 OPCODE$	OPCODE	1	Operator (analyst) code
5 AZ$	MAG	1	magnification code
6 SN$	NUMFIELDS	4	# of fields
7 CASECODE$	ANALCODE1	1	anal code @ pos #19
8 "	TISTYPE	2	tissue. type @ 20 & 21
9 "	ANALCODE2	1	anal code @ pos #22
10 "	ANALCODE3	1	anal code @ pos #23
11 MINEST$	MINESTIM	5	minimum estimate for 1 particle *
12 CAM$	CALCIUM	5	calcium *
13 CPM$	CaP	5	Calcium phosphorus *
19 ENC$	TOTENDOCA	5	total "endogenous" CA *
20 NUMFILE$	NUMFILE	5	JA file number

particles X 10^6/cm^3 tissue
Once the TISCAFLT.DAT is constructed:

1) dBASE "." mode
2) USE F:TISCAFLT (be sure you have a copy on the F: drive;
3) ZAP it to remove old data
4) APPEND FROM F:TISCAFLT.DAT SDF

You now have the dBASE equivalent of the ASCII data file. All fields in dBASE are CHARACTER type.

TISCAFLT.BB2 was constructed from TISCA.BB2 (see above).

6- MASTER WRITE: ***MSTWRT2.BB2*** ASCII: *MSTWRT2.BAS*

DATA FILES:
TMASTER.DAT - Tissue section

MSTWRT2.BB2 writes all the records of the analysis files (except the first record of each file to a TMASTER file. The TMASTER files generated by this program replaces the TMASTER.DBF data file that was created by the dBASE program BUILD. TMASTER.dbf has a different data structure than DMASTER.dbf.

Requires data files:
1) Drive D:\CASES__\- Data files must be in the .TXT form only.
2) Drive C: (default) DATAF.TMP
3) Drive C: (default) *FILES.DAT (* = T) (see FILWRT.BB2)

MSTWRT.BB2 writes to the main directory on the Bernoulli on drive D:. Be sure that enough space exists on the Bernoulli in drive D: for that file.

NOTE: MSTWRT2.BB2 adjusts fibers (type "4" particles) to compensate for stage tilt on the SEM.
Time: Approximately 70 minutes

Once the *MASTER.DAT is constructed:

1) dBASE "." mode
2) USE D:MASTER (be sure you have a copy on the D: drive; ZAP it if you are reconstructing the file)
3) APPEND FROM D:*MASTERD.DAT SDF

NOTE: The first record is a DUMMY record and should be removed before adding to MASTER.DBF

For the ID field changes have been made in DMASTER: the probable designations: amosite: am?, crocidolite: cr?, anthophyllite: an?, chrysotile: ch?, tremolite: tr?

STRUCTURE OF dBASE DATA FILE:

Field	Field Name	Type	Width	Comments
1	DUMMY	C	1	take care of ASCII (")
2	TISVOL	C	7	# particles .X 10^5/cm^3 tis.*
3	COUNTS	C	5	
4	FLD_PRTLE	C	5	
5	LOCATION	C	3	
6	TYPE	C	3	
7	SIZE	C	8	
8	ELEMENTS	C	12	
9	CPS	C	23	
10	ID	C	3	
11	CASE	C	8	full file name
12	MAGCODE	C	1	magnification of analysis
13	ANALYST	C	1	analyst code **
14	SIZECODE	C	1	minimum fiber length
15	ANALCODE1	C	1	anal code @ position 19 **
16	TISTYPE	C	2	tissue type code **
17	ANALCODE2	C	1	analysis code @ position 22 **
18	ANALCODE3	C	1	analysis code @ position 23 **
19	KEYCODE	C	1	file designation code ***
20	YRANAL	C	2	year of analysis
21	NUMFILE	C	5	JA case number
		TOTAL	84	

** See pages 13 & 14 of the documentation for key
*** D=digestion/fiber, T= tissue section, etc.

Note 1: The tissue section MASTER, “TMASTER” has a different structure—see the Tissue Section summary files

THE SECOND-ORDER SUMMARY PROGRAMS

6- MASTER WRITF: **MSTWRT2.BB2**

DATA FILES: **DMASTER.DAT** - Digestion/fibers
TMASTER.DAT - Tissue-section
AMASTER.DAT - Digestion/all particles full field
BMASTER.DAT - Digestion/all particles reduced field

MASTER.DBF = DMASTER.DAT + TMASTER.DAT + AMASTER.DAT +BMASTER.DAT

MSTWRT2.BB2 writes all the records of the analysis files (except the first record of each file to a *MASTER file. The *MASTER files generated by this program replaces the DMASTER.DBF data file that was created by the dBASE program BUILD. Total processing time is approx 60 minutes (04/30/91).

NOTE: The old DMASTER.DBF should not be used in future data analyses because most of it was constructed with archaic data files.

Requires data files: 1) Drive D:\CASES__\- Data files must be in the .TXT form only.
2) Drive C: (default) DATAF.TMP
3) Drive C: (default) *FILES.DAT
(* = D, T, A, or B)(see FILWRT.BB2)

Writes data files: Drive D: (Main Directory):

DMASTER.DAT
TMASTER.DAT
AMASTER.DAT
BMASTER.DAT

MSTWRT2.BB2 either (1) appends the selected *MASTER.DAT or (2) erases the current selected *MASTER.DAT file and then reconstructs the file. If append is selected, then follow the procedure described above for appending the FIBSUM.DAT (see DFIBWRTF.BB2). MSTWRT.BB2 writes to the main directory on the Bernoulli on drive D:. Be sure that enough space exists on the Bernoulli in drive D: for that file (especially TMASTER.DAT & DMASTER.DAT) to fit (together these files have an excess of 3 megs).

* MSTWRT2.BB2 is the same as MSTWRT.BB2 except for having MENU3 rather than MENU2 appended to it.

NOTE: MSTWRT2.BB2 adjusts fibers (type "4" particles) to compensate for stage tilt on the SEM.

The **MASTER.DBF** found on the Bernoulli is a file that is constructed by combining all of the *MASTER.DAT types while in dBASE.

Once the *MASTER.DAT is constructed:

1) dBASE "." mode
2) USE F:MASTER (be sure you have a copy on the F: drive; ZAP it if you are reconstructing the file)
3) APPEND FROM F:*MASTER.DAT SDF

NOTE: The first record is a DUMMY record and should be removed before adding to MASTER.DBF

Field	Field Name	Type	Width	Comments
1	DUMMY	C	1	take care of ASCII (“)
2	TISVOL	C	5	# part X 10^5/cm^3 tis*
3	TISWET	C	5	$ (see below)
4	TISDRY	C	5	$ (see below)
5	COUNTS	C	5	
6	FLD_PRTLE	C	5	
7	LOCATION	C	3	
8	TYPE	C	3	
9	SIZE	C	8	
10	ELEMENTS	C	12	
11	CPS	C	23	
12	ID	C	3	
13	CASE	C	8	
14	MAGCODE	C	1	
15	ANALYST	C	1	
16	SIZECODE	C	1	
17	ANALCODE1	C	1	analysis code @ pos 19 **
18	TISTYPE	C	2	tissue type code **
19	ANALCODE2	C	1	analysis code @ pos 22 **
20	ANALCODE3	C	1	analysis code @ pos 23 **
21	KEYCODE	C	1	file designate code ***
22	YRANAL	C	2	year of analysis
23	NUMFILE	C	5	JA case number
		TOTAL	92	

* **NOTE!** This value is X 10^3/cm^3 tissue in the digestion analysis.
** See pages 13 & 14 of the documentation for key.
*** D=digestion/fiber, T= tissue section, etc.
$ There is no value for TISWET & TISDRY for tissue section analyses.

MENU4 - All of the following programs can be accessed by this menu. RUN "MENU4" will display the following programs.

IMPORTANT NOTE: Program #5 requires too much space to be accommodated by the regular computer memory. The virtual memory feature of Better Basic is needed. To implement:

a) Add the line to BEGIN.CNF: MODULES=VMM.BB
b) Add the line to BEGIN.CNF: MODULES=SYSCALL.BB

c) Change BEGIN.CNF: SHELL=&H800
d) For the program FIBRBODY.BB2, Line # 11005:

SHELL "SET EMM:0=G:\"

This means that the program is using a virtual disk with the designation G:. If your virtual disk has another name, then change it to that name (e.g. H:). Caution: the configuration of your computer in the allocation of drives and whether is has a virtual drive may impeded the operation of this program. Any computer crash will involve this aspect of the program.

1-OUTPUT TO THE PRINTER OF THE FIBER TYPES: **FIBTABL.BB2**

REQUIRES: **DMASTER.DAT**
OUTPUT: **Printer**

This program will calculate:

A) the total number of fibers in in the DMASTER.DAT database.
B) The number of fibers in the 2000x analyses as well as the types & percent composition.
C) The number of fibers in the 5000x analyses as well as the types & percent composition.

2- FIBER LENGTH SUMMARY PROGRAM: **FIBLNGTH.BB2**

REQUIRES: **DMASTER.DAT**
OUTPUT: To disk - file name structure depends upon the parameters selected in the program.

In this program the user can select the fiber type (based on the mineral field code, e.g. amo, als etc [lower case only]) and the magnification code (2 or 5 for 2000x or 5000x respectively). The program will then cycle through the DMASTER.DAT file and select the required mineral record. The program then puts it in a length category. After the final record is read and processed, the program then sequentially writes the categories to disk. These records can then be read by STATGRAPHICS and are of the structure:

```
"000512"
    +- -->the fiber length category (12 to 12.99 um)
 +---   -->the number of fibers in that category
```

The data file names are written to identify the contents of that file:

```
e.g.  AMO5LEN.DAT
        +--   --> Fiber length data
        +     --> Magnification code (2 or 5)
      +--     --> Mineral category (see p. 18)
```

3- FIBER ASPECT RATIONSUMMARY PROGRAM: **FIBASPCT.BB2**

REQUIRES: **DMASTER.DAT**
OUTPUT: To disk - file name structure depends upon the parameters selected in the program.

In this program the user can select the fiber type (based on the mineral field code, e.g. amo, als etc [lower case only]) and the magnification code (2 or 5 for 2000x or 5000x respectively). The program will then cycle through the DMASTER.DAT file and select the required mineral record. The program then puts it in an aspect-ratio category. After the final record is read and processed, the program then sequentially writes the categories to disk. These records can then be read by STATGRAPHICS and are of the structure:

```
"0005125"
    +-- -->the fiber aspect ratio category (120 to 130 [selects the centroid of the range])
 +---   -->the number of fibers in that category
```

The data file names are written to identify the contents of that file:

e.g. AMO5ASP.DAT
+-- --> Fiber aspect ratio data
+ --> Magnification code (2 or 5)
+-- --> Mineral category

4- FIBER NUMBER/PERCENTAGE PROGRAM: **FIBCOAT.BB2**

REQUIRES: **DMASTER.DAT**
OUTPUT: **Printer**

This program outputs to the printer the percentage of each fiber type that is endogenously coated with Fe or Ca. The program will skip records where the fiber was treated with oxalic acid.

5- FIBER BURDEN/ASBESTOS BODY SUMMARY PROGRAM: **FIBRBODY.BB2**

REQUIRES: **FIBSUM.DAT**
LMASBDY.DAT

OUTPUT: **FIBRBODY.DAT**
Printer (reflects the data being written to disk and the CASE/SAMPLE #)

This program takes the fiber burden estimates (# X 10^4/g wet wt) for each case (summarized in FIBSUM.DAT) and matches each file to the asbestos body data in LMASBDY.DAT. The wet wt values are converted to dry wt. FIBRBODY.BB2 ignores records of FIBSUM that are without dry wt estimates. The program then selects the fiber-burden estimates from matching files of the 2000X and 5000X analyses and adds the asbestos-body estimates. Usually the amphibole data is from the 2000X and the chrysotile from the 5000X analyses. If there is no 2000X analysis, then all the asbestos burdens written to disk are from the 5000X analysis.

FIBER BURDEN/ASBESTOS BODY CODE SHEET

	ASCII String Name	dBASE variable	# charac.	COMMENTS
1	-	DUMMY	1	
2	MINETS2$	MINEST1*	7	Minimum estimate for amphiboles*
3	AMOSITE$	AMOSITE*	7	
4	CROCID$	CROCID*	7	
5	AMOCRO$	AMOCRO*	7	Uncertain - either AMO or CRO
6	COMAMPH$	COMAMPH*	7	**
7	ANTHOPH$	ANTHO*	7	
8	TREMOL$	TREMO*	7	***
9	TALC$	TALC*	7	
10	MINEST1$	MINEST2*	7	Minimum estimate for chrysotile
11	CHRYSO$	CHRY *	7	****
12	ASBBODY$	ASBBODY	7	Asbestos body burden (#/g dry wt)
13	DCASE1$	NUMFILE	5	*****

* Number X 10^4/g dry wt tissue
** Commercial amphibole (amo+cro+acr)
*** Tremolite= tremolite + actinolite
**** Chrysotile= chrysotile+ chrysotile-Mg

PRIMER PROGRAM: **DSTART4.BB2**

DATA FILE: **DATAF.TMP**

The purpose of this program is to "prime" DATEFILE.BB2, so that this program can read the *FILES.DAT files for an entire rewrite or append of *DATEDAT.DAT. See the following program description for further information.

*DSTART4.BB2 is the same as DSTART.BB2 except for having MENU4 rather than MENU2 appended to it. This program does not have a .BAS form (modify by BASIC editor).

6- DATE OF ANALYSIS WRITE: **DATEFILE.BB2**

DATA FILE: ***DATEDAT.DAT**
dBASE FILE: **DATEDATA.DBF**

This program writes writes to a file in the B: drive the file name, month and year that that file was created using all the data files in F:CASES__.

Requires data files:
1) Drive C: (default): DATAF.TMP
2) Drive C: (default): *FILES.DAT
3) Drive D:\CASES__\- Data files must be in the .TXT form only.
4) Drive B: *DATEDAT.DAT (create or append)

Be sure that all the *FILES.DAT are current by going to MENU2 or MENU3 and running option 1 for all data file types.

NOTE 1: Once the program is running, any interrupt will usually cause loss of data if you continue without returning to Option 6 (DSTART4.BB2). In the append file name that you are requested in Option 6, supply the last file name to be stored in *DATEDAT.DAT. You can determine this by listing the data in *DATEDAT.DAT by the DOS command "TYPE F:*DATEDAT.DAT" <ENTER> (* = A, B, D, T, or G).

NOTE 2: If after selecting Option 6 for append and imputing the file name, and upon running DATEFILE.BB2, the MENU4

quickly appears without printing or writing to disk. This means that the file name is probably incorrect. A return to MENU4 means that that DATEFILE.BB2 is finished with the particular data type (see p. for the various data types).

NOTE 3: Be sure that you have enough room on the disk in drive B: for all the *DATEDAT.DAT files as well as the dBASE file DATEDATA.DBF

This information can be added to the dBASE file by:

1) ZAP the file FIBRBODY.DBF
2) MODIFY STRUCTURE: (NUMFILE to 6 characters)
3) APPEND from FIBRBODY.DAT SDF

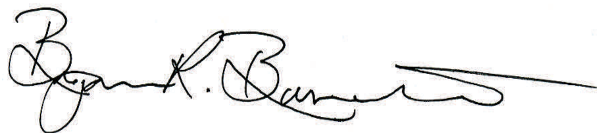
The printer output of these data is a direct representation of the data written to disk. If there is no 2000X analysis for any particular case, then MINEST1 = MINEST2 and all estimates of fiber burden are from the 5000X analysis.

CODE SHEET FOR *DATEDAT.DAT/DATEDATA.DBF

	ASCII String Name	dBASE variable*	# charac.	COMMENTS
1	-	DUMMY	1	to take care of ASCII (")
2	ANAFILED$	CASE	8	file name
3	MONTH$	MONTH	3	3 letter abbreviation of month
4	YRFILE$	YEAR	4	year of analysis

*** The dBASE file (DATEDATA.DBF) will be the combined files of the various data types.**

This manual written by:



Bryan R. Burnett
Director

AUGUST 17 2017

APPENDIX A-1

ANALYSIS OF THE EFFECT OF ULTRASONICATION ON THE ASSESSMENT OF FIBER BURDEN IN LUNG TISSUE. EXPERIMENTS ON ASBESTOS FROM RAT AND HUMAN LUNGS.

In 1985 I was a research participant in a series of experiments involving the exposure of adult rats to chrysotile asbestos. For estimating the effect of ultrasound on lung tissue preparations, some of this rat tissue (six months after a heavy chrysotile exposure) was selected. Human tissue was obtained from a case submitted to Meixa Tech. This tissue had a heavy burden of crocidolite asbestos.

Methods. Both the human and rat tissue were dehydrated in ethanol, cleared in toluene and rehydrated in an ethanol series. Lung tissue pieces that were approximately 20 mg wet weight were measured for volume and weighed to the nearest hundredth milligram. Three additional pieces were also weighed and dried for estimates of dry weight from each tissue source. The analyte tissue was dissolved in sodium hypochlorite for 24 hours followed by hydrogen peroxide treatment. The digestate fluid was divided in half where one of those halves was ultrasonicated. Each solution was then filtered through a 0.2 micrometer pore Nuclepore polycarbonate filter and the surface viewed in the electron microscope.

Results.

Sample	Lung ID	Amount Ultrasonicated *	Asbestos Type	#/g dry wt X 10^7
CS300B2b	rat	none	chrysotile	2.95
CS300B2a	"	20 sec	"	6.38
CS300C1a	rat	none	chrysotile	5.02
CS300C1b	"	5 min	"	14.07
JA88170/11a	human	none	crocidolite	1.29
JA88170/11b	"	4 min	"	5.28

* Branson Ultrasonicator, Model 1200 (rated at 50 watts)

From the first experiment (CS300B2), it is obvious even a small amount of ultrasound significantly increases the estimated asbestos burden. The other two experiments with longer ultrasound treatment also show increases in the estimated asbestos burden. The third experiment (JA88170/11) shows that amphiboles are also likely fragmented by ultrasound.

Ultrasound dissociates fiber bundles. For chrysotile asbestos, this effect appears to occur quickly after the start of the ultrasound treatment. Continued treatment does not seem to appreciably raise the estimated burden beyond that seen with a 20 sec exposure.

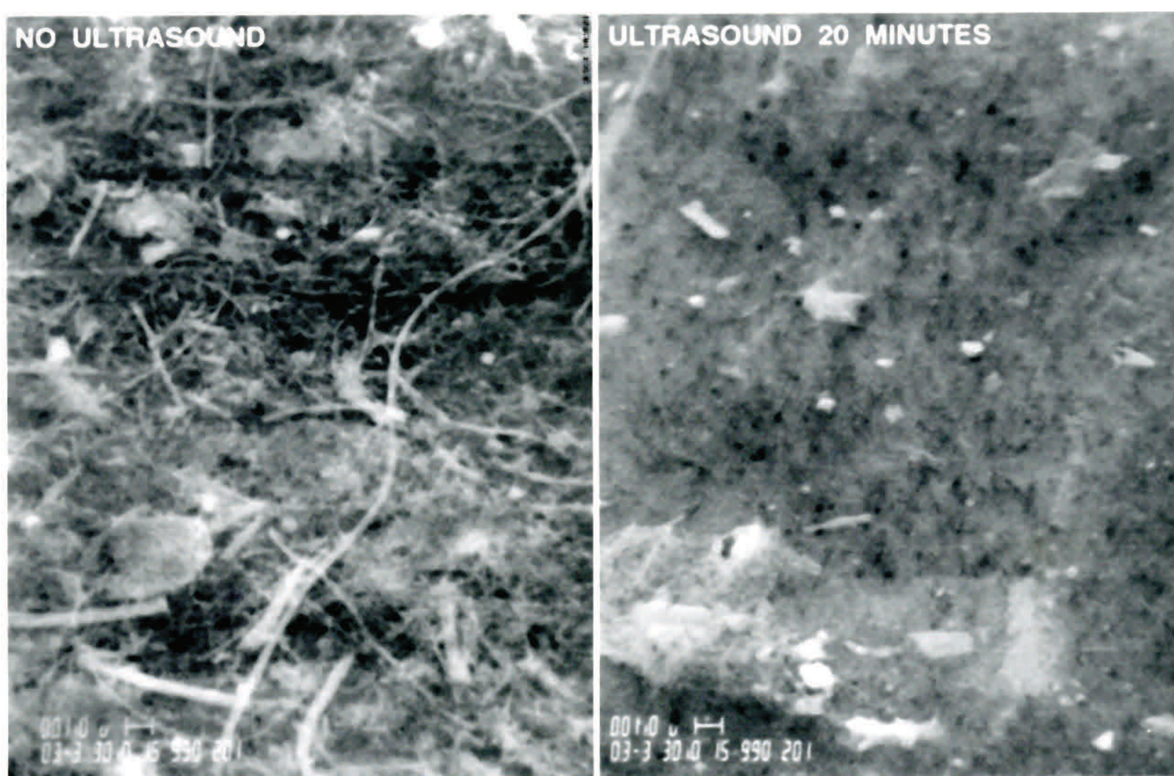
These experiments show that treatment of lung tissue samples with ultrasound prior to viewing in the electron microscope can increase the estimated asbestos burden of the tissue.

APPENDIX A-2

ANALYSIS OF THE EFFECT OF ULTRASONICATION ON THE ASSESSMENT OF FIBER BURDEN IN LUNG TISSUE. - MAGNESIUM LEACHING OF CHRYSOTILE ASBESTOS BY OXALIC ACID AND SUBSEQUENT DESTRUCTION BY ULTRSONICATION.

In the analysis of human lung tissue, chrysotile asbestos is frequently found. For those analyses on samples that have not been treated with oxalic acid, frequent apparent chrysotile fibers are found where magnesium is not detectable. These fibers are identified as questioned asbestos unless the distinctive morphology of chrysotile is present.

Treatment of UICC chrysotile by 8% oxalic acid @ 55° C for 11 hours renders the chrysotile without detectable magnesium by EDS. If the sample is subject to ultrasound (>10 minutes) by the same Branson Ultrasonicator as used in Appendix A-1, the chrysotile apparently is broken down into small pieces that pass through the 0.4 micron pores of the Nuclepore filter. The electron micrographs on page 41 illustrate this effect. Experiments are continuing in this area (dated October 6, 2000).



UICC CANADIAN CHRYSOTILE

SAMPLE SOAKED IN 8% OXALIC ACID 11 HRS @ 55 c.

LEFT IMAGE : 1/2 SAMPLE THROUGH 0.2 um PORE NUCLEPORE FILTER

RIGHT IMAGE: 1/2 SAMPLE ULTRASOUND 20 MIN & THROUGH NUCLEPORE FILTER

APPNEDIX B

97000A1D.TXT – FIBER IDENTITY BY RELATIVE ELEMENTAL COMPOSITION

10-15-1997
FIBER IDENTITIES
OPERATING VOLTAGE: 30KV

PLEASE NOTE:
1) Probability values for each standard given in %
2) Probability value of 0.00 means element is not present or element is not present in standard sample
3) Probability value of 5.00 means p <= 5%
4) Probability value of 95.00 means p => 95%
5) Single element fibers are not listed

#	Size*	Elements

00101 .10x 9.5 SiMgFe Identity: Amosite
Si CPS 79.0 Ratios: 1.0: 0.15 0.54
Smooth, round & straight fiber

AMOSITE

Standard ID	Mg	Fe
AMO1	5.00	8.88
AMO2	9.48	9.38
AMO3	5.00	8.53
AMO4	8.99	38.55
AMO5	35.90	29.81
AMO6	11.60	17.37

00102 .14x 5 SiMgFe Identity: Amosite
Si CPS 73.0 Ratios: 1.0: 0.13 0.61
Smooth, round & straight fiber

AMOSITE

Standard ID	Mg	Fe
AMO1	5.00	37.02
AMO2	35.48	37.83
AMO3	10.09	34.91
AMO4	31.19	80.74
AMO5	95.00	81.89
AMO6	37.44	54.59

00801 .28x 10 SiMg Identity: Fibrous talc
Si CPS 297.0 Ratios: 1.0: 0.43
Smooth, pleated & straight fiber

TALC

Standard ID	Mg	Mn	Fe
TAL1	44.41	0.00	0.00
TAL2	71.89	0.00	0.00
TAL3	15.23	0.00	0.00
TAL4	69.17	0.00	0.00
TAL5	47.28	0.00	0.00
TAL6	52.24	0.00	0.00
TAL7	37.35	0.00	0.00
TAL8	38.28	0.00	0.00
TAL9	50.06	0.00	0.00
TAL10	95.00	0.00	0.00
TAL11	76.83	0.00	0.00

01002 .40x 6.8 SiAlK Fe Identity: Alumino silicate
Si CPS 530.0 Ratios: 1.0: 0.48 0.06 0.04
Smooth, pleated & straight fiber

MAN-MADE FIBER

Standard ID	Mg	Na	Al	Ca

01801 .75x 7.7 SiAlNaK Identity: Alumino silicate
Si CPS 471.0 Ratios: 1.0: 0.26 0.07 0.08
Smooth, flattened & straight fiber

MAN-MADE FIBER

Standard ID	Mg	Na	Al	Ca

02801 .44x14.8 SiMgCa Identity: Tremolite
Si CPS 380.0 Ratios: 1.0: 0.36 0.22
Smooth, pleated & straight fiber

TREMOLITE

Standard ID	Mg	Ca
TRE1	32.25	9.37
TRE2	28.71	33.85
TRE3	71.65	25.79
TRE4	15.27	9.67
TRE5	5.00	6.54
TRE6	13.25	50.71
TRE7	62.48	86.74
TRE8	34.13	52.82
TRE9	5.00	56.60
TRE10	86.18	16.22

03201 .43x 9.1 SiMg Identity: Fibrous talc
Si CPS 1174.0 Ratios: 1.0: 0.39
Smooth, flattened & straight fiber

TALC

Standard ID	Mg	Mn	Fe
TAL1	95.00	0.00	0.00
TAL2	55.57	0.00	0.00
TAL3	59.38	0.00	0.00
TAL4	72.06	0.00	0.00
TAL5	86.66	0.00	0.00
TAL6	84.48	0.00	0.00
TAL7	89.82	0.00	0.00
TAL8	95.00	0.00	0.00
TAL9	16.89	0.00	0.00
TAL10	41.28	0.00	0.00
TAL11	65.53	0.00	0.00

05501 .31x 8.9 SiMgFe Identity: Amosite
Si CPS 179.0 Ratios: 1.0: 0.11 0.62
Smooth, pleated & straight fiber

AMOSITE

Standard ID	Mg	Fe
AMO1	40.69	39.44
AMO2	95.00	40.20
AMO3	51.09	37.26
AMO4	82.92	83.90
AMO5	36.38	86.11
AMO6	95.00	58.42

05601 .55x21.2 SiMg Identity: Fibrous talc
Si CPS 265.0 Ratios: 1.0: 0.48
Smooth, flattened & straight fiber

TALC

Standard ID	Mg	Mn	Fe
TAL1	8.95	0.00	0.00
TAL2	14.38	0.00	0.00
TAL3	5.00	0.00	0.00
TAL4	19.33	0.00	0.00
TAL5	9.59	0.00	0.00
TAL6	10.95	0.00	0.00
TAL7	9.58	0.00	0.00
TAL8	7.24	0.00	0.00
TAL9	88.02	0.00	0.00
TAL10	34.25	0.00	0.00
TAL11	25.17	0.00	0.00

07001 .12x12.5 SiMgFe Identity: Amosite
Si CPS 82.0 Ratios: 1.0: 0.14 0.55
Smooth, round & straight fiber

AMOSITE

Standard ID	Mg	Fe
AMO1	5.00	12.76
AMO2	24.65	13.56
AMO3	5.70	12.01
AMO4	21.36	42.71
AMO5	70.06	37.42
AMO6	29.16	23.39

07601 .54x 5.6 SiAlMg Identity: Alumino silicate
Si CPS 565.0 Ratios: 1.0: 0.22 0.04
Smooth, flattened & straight fiber

MAN-MADE FIBER

Standard ID	Mg	Na	Al	Ca

08601 .15x 8.2 SiMg Identity: Fibrous talc
Si CPS 32.0 Ratios: 1.0: 0.45
Smooth, flattened & straight fiber

TALC

Standard ID	Mg	Mn	Fe
TAL1	28.75	0.00	0.00
TAL2	39.76	0.00	0.00
TAL3	6.44	0.00	0.00
TAL4	42.67	0.00	0.00
TAL5	30.69	0.00	0.00
TAL6	32.58	0.00	0.00
TAL7	26.32	0.00	0.00
TAL8	24.21	0.00	0.00
TAL9	73.18	0.00	0.00
TAL10	69.71	0.00	0.00
TAL11	52.25	0.00	0.00

APPENDIX C

MT97-000/A1 * JOHN DOE
QUANTITATIVE ANALYSIS OF FIBERS IN DIGESTION MATERIAL

DATE: 10-15-1997 TIME:10:00:07
DATA DISK NAME: MT-5 FILE NAME: 97000A1D.TXT
Month & year of analysis: September, 1997
Name of Analyst: Bryan Burnett

Tissue from autopsy

Analysis conducted on the MT-ETEC @ 30KV & Kevex/Delta (Be-window detector)
Digestion sample # : C1
Minimum fiber length for analysis: 3 um

FILTER IS NULCEPORE BRAND
WET TISSUE ANALYSIS: DEHYDRATED-CLEARED-REHYDRATED PRIOR TO DIGESTION
SAMPLE ON FILTER TREATED WITH 8% OXALIC ACID (4 hrs @ 55 C)

Filter size is 13 mm. Filter surface area = 7.09 x 10^7 um^2.
Pore size of the polycarbonate filter = .4 um
Volume of tissue = 254. mm^3
Wet weight of tissue = 76.6 mg

ANALYSIS MAGNIFICATION = 2000X
Area of field at 2000X = 6045 um^2
Field size based on mag. calibration performed on 11/05/96
(Last mag. calibration performed on 11/05/96)

PARALLEL ESTIMATES OF TISSUE DRY WEIGHTS (in mg):
First parallel piece: wet wt = 18.8 dry wt = 3.3
Second parallel piece: wet wt = 28.8 dry wt = 5
*Mean ratio (dry wt/wet wt): 0.17

SAMPLE SIZE (# of fields searched) = 88

MEAN NUMBER EXOGENOUS FIBERS/FIELD= 0.17
Standard deviation = 0.530
Standard error = 0.057

NUMBER OF FIBERS ANALYZED WITH X-RAY = 15

TOTAL # EXOGENOUS FIBERS = 15

MASTER. BB2

Mineral type	Tot.#	%	#/field	Tissue Estimates #/cm^3	#/gWW	#/gDW
Total exo.particl	15	100.00	0.171	7,871	26,099	149,678
Silica	1	6.67	0.011	525	1,740	9,979
Alum-silicate	3	20.00	0.034	1,574	5,220	29,936
Tot.pos.asbestos	6	40.00	0.068	3,148	10,440	59,871
Prob. asbestos	6	40.00	0.068	3,148	10,440	59,871
Talc	4	26.67	0.046	2,099	6,960	39,914
Metals	1	6.67	0.011	525	1,740	9,979

MINIMUM ESTIMATES FOR 1 FIBER:

1 fiber =	133	fibers on filter
=	525	fibers/cm3 tissue
=	1,740	fibers/g wet weight
=	9,979	fibers/g dry weight

Mineral Category	# obs	Tissue Estimates #/cm3	#/g wet wt	#/g dry wt
ASBESTOS				
All poss. asbesto	6	3,148	10,440	59,871
Tot.prob.asbestos	6	3,148	10,440	59,871
Amosite	5	2,624	8,700	49,893
Tremolite	1	525	1,740	9,979
TOTAL SILICATE OTHER THAN ASBESTOS				
Total	8	4,198	13,920	79,828
Silica fibers	1	525	1,740	9,979
Alumino-silicat	3	1,574	5,220	29,936
Probable talc	4	2,099	6,960	39,914
METALS				
Total	1	525	1,740	9,979

NO COATED FIBERS OBSERVED

FIBQUANT.BB2

97000A1D.TXT

PARTICLE SIZE STATISTICS

FIBERS

All possible asbestos
Mean Adjusted Length = 15.62 um N= 6
Standard Deviation = 13.71 Standard Error = 5.598
Range: 5.02 to 42.77

Total probable asbestos
Mean Adjusted Length = 15.62 um N= 6
Standard Deviation = 13.71 Standard Error = 5.598
Range: 5.02 to 42.77
Number fibers above 5 um length = 6

Amosite
Mean Adjusted Length = 15.78 um N= 5
Standard Deviation = 15.32 Standard Error = 6.853
Range: 5.02 to 42.77
Number fibers above 5 um length = 5

Tremolite
Mean Adjusted Length = 14.84 um N= 1

SIZE.BB2

Silica fibers
Mean Adjusted Length = 4.06 um N= 1

Alumino-silicate fibers
Mean Adjusted Length = 6.73 um N= 3
Standard Deviation = 1.06 Standard Error = 0.612
Range: 5.60 to 7.70

Talc fibers
Mean Adjusted Length = 12.20 um N= 4
Standard Deviation = 14.08 Standard Error = 7.042
Range: 8.24 to 21.28

Metal fibers
Mean Adjusted Length = 4.34 um N= 1

SIZE.BB2 (CONT.)

VERBATIM OUTPUT - 97000A1D.TXT
CPS values were integrated from spectra taken at 30KV

#	quant	fld-prt	type	size	ID	elements	CPS
1	00200	00101	445	.10x07.8	amo	SiMgFe	79.3:12:42.5
2		00102	460	.14x03.8	amo	SiMgFe	73:9.8:44.5
3	00100	00801	430	.28x09.0	tal	SiMg	297:129
4	00200	01001	475	.24x03.1	met	Ti	418
5		01002	415	.40x06.5	als	SiAlK Fe	530:255:30:22
6	00100	01801	475	.75x05.5	als	SiAlNaK	471:121:31:36
7	00100	02801	415	.44x14.0	tre	SiMgCa	380:135:82
8	00100	02901	445	.23x03.3	sil	Si	485
9	00100	03201	430	.43x08.2	tal	SiMg	1174:460
10	00100	05501	460	.31x06.8	amo	SiMgFe	179:20:110
11	00100	05601	475	.55x15.2	tal	SiMg	265:128
12	00100	07001	430	.12x11.2	amo	SiMgFe	81.5:11.5:45.3
13	00100	07601	430	.54x05.0	als	SiAlMg	565:125:20
14	00100	08601	445	.15x06.7	tal	SiMg	31.5:14.5
15	00100	08801	460	.20x32.4	amo	SiMgFe	118:19:59

VERBATIM.BB2

APPROVED BY:

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APPENDIX D

ASBESTOS STANDARDS USED IN THE FIBERID PROGRAM

AMOSITE

AMO1: MT85-110: NIOSH 75-0043
AMO2: MT85-024: SDNHM 55OM55a
AMO3: MT85-022: SDNHM 926-75
AMO4: MT88-064: EPA 18-1024
AMO5: MT85-065: SDNHM 43M63 (Confirmed by Kellco)
AMO6: MT85-098: UICC (Obtained from McCrone)

CROCIDOLITE

CRO1: MT87-170: EPA 17-7016
CRO2: MT85-099: UICC (Obtained from McCrone)
CRO3 MT85-025: SDNHM 88-82
CRO4: MT85-112: NIOSH CR-37
CRO5: MT85-052: Obtained from Kellco
CRO6: MT89-053: Obtained from Kellco

CHRYSOTILE

CHR1: MT85-096: UICC-Rhod. (Obtained from McCrone)
CHR2: MT85-109: NIOSH CH-029
CHR3-D: MT85-079: SDNHM 122-86 (Mg-depleted, ID confirmed by TEM/SAED)
CHR4: MT85-095: UICC- Canada (Obtained from McCrone)
CHR5: MT87-062: Jeffery Mines, Asbestos, Canada (Obtained from Kenneth Cohen)
CHR6: MT85-043: SDNHM 172-81

ANTHOPHYLLITE

ANTH1: MT85-023: SDNHM 2OM63
ANTH2: MT85-014: SDNHM 2OM40
ANTH3: MT85-111: UICC (Obtained from McCrone)
ANTH4: MT87-065: Johns-Manville product (Obtained from Kenneth Cohen)
ANTH5: MT85-016: SDNHM 123OM50 (Confirmed by Kellco)
ANTH6: MT85-102: Obtained from Kenneth Cohen
ANTH7: MT85-033: SDNHM 18M64
ANTH8: MT85-097: UICC (Obtained from McCrone)
ANTH9: SDNHM 1231M50 (Confirmed by Kellco)
ANTH10: MT85-027; SDNHM 54OM51a (Confirmed by Kellco)

TREMOLITE

TRE1: MT85-049; SDNHM 4B-14-SY1/9
TRE2: MT85-057; SDNHM 786M51 (Confirmed by Kellco)
TRE3: MT85-019; SDNHM 93-79
TRE4: MT85-101: Baker Corp – commercial product (Obtained from Daniel Baxter, 1985)
TRE5: MT85-060; SDNHM 355M49
TRE6: MT85-054; SDNHM 4B-14-C7/6
TRE7: MT85-055: SDNHM 316-76
TRE8: MT85-058; SDNHM 1297M50
TRE9: MT85-041; SDNHM 24-85

TRE10: MT86-001; JA85-218, from Dannemova Mine, Uppsala Sweden.
TRE11: JA00-077; From patient's lung digestate – worked at Libby vermiculite expansion plant a few months, Office job only before death due to Mesothelioma (~50 yrs later).
ACT1: EPA 9209 17 2947; MT87-169 – Ferro-actinolite "...ferro-actinolite contains no magnesium" (ATSDR "Asbestos … Tremolite Asbestos and other related types of asbestos—http://www.atsdr.cdc.gov/asbestos/doc_tremolite.html) . Although a small amount of Mg detected by EDS in most (not all) of these fibers for this standard, the Mg concentration is quite small (average Mg/Si =0.05). Referenced in FIBERID (tremolite) only if iron is present in the analyte fiber.

TALC

TAL1: MT89-016; SDNHM 1229M5
TAL2: MT89-017; SDNHM 239M49
TAL3: MT89-018; 6M72 (Confirmed by Kellco)
TAL4: MT89-019; SDNHM 4B-14-SY2/36
TAL5: MT89-020; SDNHM 322M54
TAL6: MT89-21; SDNHM 375-50
TAL7: MT85-103; Johnson & Johnson Talc (Baby powder product)
TAL8: MT85-104; USP Talc
TAL9: MT85-105; Este Lauder (Cosmetic talc)
TAL10: MT85-107; Johnson & Johnson Talc (Baby powder product)
TAL11: MT85-108; Baby Magic Talc (Baby powder product)

ANTIGORITE

ANTI1: MT85-047; SDNHM 1128M50
ANTI2: MT85-044; SDNHM IOM49
ANTI3: MT85-045; SDNHM 4B-14-SYI1/16

LIZARDITE

LIZ1: MT85-064 SDNHM 242-76 (Confirmed by Alfred Petri of SDNHM in 1985) (Confirmed by Kellco)
LIZ2: MT87-064; "Hedmon Cationic Fiber 4-5-79" (Obtained from Kenneth Cohen)

GLAUCOPHANE

GLA1: MT85-056 SDNHM 21M55.
CLA2: MT89-014; SDNHM 273M50b

MAN-MADE FIBERS

Fiber Glass
GFB1: MT89-054; (Obtained from Kellco)
GFB2: MT90-004; (Obtained from McCrone/Atlanta)

Ceramic Fiber
CFB1: NT89-055; (obtained from Kellco)

Mineral Wool
MFB1: MT89-056; (Obtained from Kellco).
MFB2: MT89-057; Obtained from Kellco)

Glass Wool
GLW1: MT90-003; (Obtained from McCrone/Atlanta)

Slag Wool
SLW1: MT90-001; (Obtained from McCrone/Atlanta).

Rock Wool
ROW1: MT90-002 (Obtained from McCrone/Atlanta)

KEY

Daniel Baxter – Particle Diagnostics, San Diego, California
Kenneth Cohen – Consulting Health Services, El Cajon, California
EPA – Environmental Protection Agency (samples from certification tests)
JA – Jerrold Abraham, M.D., sample number
Kellco – Sample confirmed by PLM or obtained from Kellco Corp., 44814 Osgood Avenue, Fremont, California, 94539
McCrone – McCrone Accessories, Chicago, IL.
McCrone/Atlanta – Sample obtained from Thom Hopen, McCrone/Atlanta, 1412 Oakbrook Drive, STE 100, Norcross, GA 30093
MT – Meixa Tech sample number
NIOSH – National Institute of Occupational Safety & Health
SDNHM – San Diego Natural History Museum, Mineralogy Department, Balboa Park, San Diego, California
UICC – International Union Against Cancer