

Libby Superfund Site Operable Unit 3 Standard Operating Procedure

Date: November 20, 2007

SOP TREE-LIBBY-OU3 (Rev. 1)

Title: SAMPLING AND ANALYSIS OF TREE BARK FOR ASBESTOS

**APPROVALS:**

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Revision Number	Date	Reason for Revision
0	09/26/2007	--
1	11/20/2007	Modify procedure for sample preparation based on results of pilot-scale laboratory tests

## **1.0 PURPOSE**

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for collection and analysis of tree bark samples for asbestos. This procedure will be used by USEPA Region 8 for the Remedial Investigation work for Operable Unit 3 performed at the Libby Asbestos Superfund site.

## **2.0 RESPONSIBILITIES**

The Field Sampling Team Leader is responsible for ensuring that all bark samples are collected in accord with this SOP. The Laboratory Director is responsible for ensuring that bark samples provided to the laboratory for evaluation by this SOP are prepared and analyzed in accord with the requirements of this SOP. It is the responsibility of the Field Sampling Team Leader and the Laboratory Director to communicate the need for any deviations from the SOP with the appropriate USEPA Region 8 Remedial Project Manager or Regional Chemist.

## **3.0 EQUIPMENT**

### **3.1 Field Equipment**

- hole saw (2-inch diameter)
- battery-powered drill
- ½ inch chisel
- flathead screwdriver
- aerosol hair spray
- Ziploc plastic bags
- sample identification tags
- decontamination supplies
- trash bag
- GPS unit
- digital camera
- flagging tape or metal identification tag
- field log book
- field sample data sheet(s) for tree bark
- increment boring device (e.g., Hagloff)
- plastic sheath for age core
- ink pen
- clear packaging tape

### **3.2 Laboratory Equipment /Reagents**

- Disposable Filter funnels with Straight sides. VWR # 145-0020
- Culture Dishes. VWR # 25388-581 (case of 500)
- 47 mm 0.45 micron mixed cellulose ester (MCE) filters
- Glass Petri Dishes

- Glass microscope slides
- Low Temperature Plasma Asher
- Vacuum Evaporator (Carbon Coater)
- Graphite or Carbon rods
- HEPA Laminar Flow Hood
- Acetone Vapor Generator
- Grids
- Fine Forceps
- Grid Clips and Grid Storage Boxes
- Jaffe Wick or Sponge
- Kim wipes or alternative paper
- Transmission Electron Microscope with the following capabilities:
  - 100 Kev
  - fine probe size <250 nm
  - elemental Chemistry via X-Ray Detector
- Large ceramic crucibles (approx. 50 ml capacity or greater)
- Glass stirring rods
- Fumehood
- HEPA filtered Hood
- Ultrasonic Bath producing a rate of energy deposition in the range of 0.08-0.12 MW/m<sup>3</sup>
- Disposable plastic filter funnel apparatus
- Reagent Grade or better Acetone
- Reagent Grade or better HCl

#### 4.0 METHOD SUMMARY

One or more tree bark samples are obtained from selected trees by using a 2-inch hole saw to cut a circular ring in the bark, following by cutting/prying the circular piece of bark from the tree using a sharp chisel. The area to be sampled is sprayed with hair spray prior to sample collection in order to minimize the potential for loss of fibers from the tree bark. In some cases, a core may be obtained from the tree in order to allow verification of the age of the tree.

Tree bark samples are prepared for analysis by high temperature ashing to remove organic matter. The residue is then treated with HCl to dissolve any salts or carbonate component that may be present and applied to a filter which is examined for asbestos using transmission electron microscopy (TEM).

#### 5.0 SAMPLE COLLECTION

Bark samples should be collected from the sampling stations specified in the Sampling and Analysis Plan (SAP). At each specified sampling station, sample collection should be performed as follows:

## 5.1 Select Tree

The species and size of tree selected for sampling should be specified in the project-specific sampling and analysis plan. In the absence of specification, the tree selected for sampling should be a Douglas fir (*Pseudotsuga menziesii*) with a diameter (caliper) of about 8-10 inches. If there are multiple trees that meet these requirements in the vicinity of the sampling station, preference should be given to trees with rough bark, and trees that are in open areas.

## 5.2 Bark Collection

Collect the bark sample from the side of tree facing the mine and from a height of 4-5 feet above the ground.

Steps:

1. Spray the bark collection area with aerosol hairspray and allow to dry.
2. Use a 2-inch diameter hole saw and a battery-powered electric drill to cut a circle in the tree bark. Continue cutting until sawdust changes from red to cream, which indicates that the cambium has been reached (about ½ inch deep).
3. Using a sharp ½-inch metal chisel or flathead screwdriver, cut or pry the circular bark sample off the tree, attempting to maintain the sample in one piece.
4. Place the bark sample in a plastic Ziploc bag.
5. Label the bag with a unique sample identifier.
6. Place clear packaging tape over the sample identifier label.

## 5.3 Tree Age Core Collection

At locations where an age core is to be collected (as specified in the project-specific sampling and analysis plan), collect a core from the tree using a Hagloff manual increment borer or similar device. Place the core in a plastic straw. Crimp and tape the ends of the straw, and label the straw with the same sample identifier as the bark field sample. Place the straw into a Ziploc bag. Label the bag with the same sample identifier as the bark field sample, and place clear packaging tape over the sample identifier label.

## 5.4 Field Documentation

Complete the Tree Bark Field Sample Data Sheet (FSDS) form. Measure and record the diameter of the tree. Obtain and record the GPS coordinates of the tree on the Tree Bark FSDS. Mark the tree with flagging tape or a metal identification tag.

## 5.5 Equipment Decontamination

If dedicated sample equipment is not used, after each sample collection, manually remove any fibrous debris from the hole saw teeth. If resin or pitch is present, use WD40 to clear saw of any residue. Thoroughly clean all collection equipment with isopropyl alcohol wipes. Dry sampling equipment using paper towels. Any spent wipes, paper towels, or other decontamination waste materials must be disposed or stored properly as investigation-derived waste.

## 6.0 SAMPLE PREPARATION AND ANALYSIS

### 6.1 Tree Bark Preparation

#### *Drying and Ashing*

Measure and record the diameter and the thickness of the tree bark sample to an accuracy of  $\pm 2$  mm (about 1/16 of an inch).

Weigh and record the tare weight of a clean crucible. Add the entire tree bark core to the crucible. Place the crucible with bark sample in a drying oven. Heat to 80°C and hold at this temperature until weight stabilizes (at least 6 hours). Record the final weight and calculate the mass of the dried tree bark sample by difference.

Place a lid on the crucible and transfer to a muffle furnace. Ramp up the furnace from a cold start to 450°C. Hold at this temperature for 18 hours or until all organic matter is removed. Allow the crucible to cool. Remove crucible lid and weigh and record the mass of the crucible plus ashed residue. Calculate the mass of the ashed residue by difference.

#### *Acid Treatment*

To the ashed residue, add just enough filtered and deionized (FDI) water (approximately 1-2 mL) to cover the surface of the residue. Slowly add approximately 10-20 mL concentrated HCl to the wetted ash. Typically a visible effervescing is observed. Add the HCl slowly to keep this reaction controlled. A small glass stirring rod is useful at this point to gently stir the ash and expose all material to the acid.

If after 3-5 minutes there is no further visible reaction, proceed to the next step. If bubbling is still occurring, continue observation and gentle stirring for up to an additional 5 minutes.

Dilute the sample by adding FDI water directly to the crucible (approximately 20 mL) using a squirt bottle. Pour the sample into an unused disposable 100 mL specimen container with lid. Rinse out any remaining residue from the crucible into the specimen container. Do not exceed 100 mL total volume. Bring the total volume to 100 mL with FDI water.

Cap the specimen jar and agitate the sample by inversion 5 or 6 times. Loosen the cap slightly and sonicate for 2 minutes. After sonication, tighten the cap and then dry the exterior of the specimen container with kim wipe or equivalent.

#### *Filtration*

Agitate the sample by inversion 5 or 6 times. Withdraw an initial aliquot of 5 to 20 mL of sonicated sample. Transfer this aliquot into a new disposable specimen container with lid. Bring the volume up to approximately 100 mL with FDI water. Cap and agitate by inversion (5 or 6 times).

Filter this entire volume onto a 47 mm mixed cellulose ester (MCE) filter with 0.4 um pore size.

If the filter appears overloaded (overall particulate level > 20%), repeat the process above, selecting a smaller aliquot volume, as suggested by the degree of overloading. Likewise, if the filter looks too lightly loaded, remove and filter a larger aliquot.

Transfer the filter membranes to individual disposable labeled Petri dishes with lids. With the Petri dish covers ajar, dry the filters by air drying.

## 6.2 TEM Examination

Prepare 3 grids for TEM analysis as detailed in International Organization for Standardization (ISO) TEM method 10312, also known as ISO 10312:1995(E). Utilize 2 grids for analysis, and archive 1 grid.

### *Counting rules*

Examine the grids using TEM in accord with ISO 10312, with all relevant Libby site-specific modifications, including utilizing the most recent version of all relevant project specific modifications, including LB-000016, LB-000019, LB-000028, LB-000029, LB-000030, LB-000053, and LB-000066. All fibrous amphibole structures that have appropriate Selective Area Electron Diffraction (SAED) patterns and Energy Dispersive X-Ray Analysis (EDXA) spectra, and having length greater than or equal to 0.5 um and an aspect ratio (length:width)  $\geq 3:1$ , will be recorded on the Libby site-specific laboratory bench sheets. Data recording for chrysotile (if observed) is not required.

### *Stopping rules*

The target analytical sensitivity for sample analysis should be specified in the SAP. In the absence of such specification, the target sensitivity should be no higher than 100,000 cm<sup>-2</sup>. The analytical sensitivity is calculated using the following equation:

$$S = \frac{EFA}{GO \cdot Ago \cdot A \cdot F}$$

where:

S	=	Sensitivity (cm <sup>-2</sup> )
EFA	=	Effective filter area (mm <sup>2</sup> )
GO	=	Number of grid openings counted
Ago	=	Area of one grid opening (mm <sup>2</sup> )
A	=	Area of tree bark sample being analyzed (cm <sup>2</sup> )
F	=	Fraction of original sample deposited on the filter

Count the sample until one of the following occurs:

- The target sensitivity is achieved.
- A total of 50 or more LA structures are observed. In this case, counting may cease after completion of the grid opening that contains the 50<sup>th</sup> LA structure.
- A total of 100 grid openings are counted without reaching the target sensitivity or observing 50 LA structures. In this event, the laboratory should contact EPA asking for direction.

### **6.3 Electronic Data Deliverable**

All data on the number, type and size of LA fibers collected in the laboratory will be provided as an electronic data deliverable (EDD) using the most recent version of the spreadsheet developed for this purpose (“TEM Tree Bark.xls”).

### **6.4 Analysis of Core Sample**

The age of the tree will be determined from the core sample in accord with the method of Phipps (1985).

## **7.0 QUALITY ASSURANCE**

### **7.1 Field-Based Quality Assurance**

#### Field Duplicates

Field duplicate tree bark samples will be collected at a frequency specified in the SAP. Each field duplicate should be collected from the same tree at a location no further than 6 inches away from the original bark sample. In the absence of such specification, the rate should be no less than 5%. Field duplicate samples should be labeled with a unique identifier. Sample details should be recorded on the Tree Bark FSDS, including the unique identifier of the “parent” field sample.

#### Equipment Rinsates

If dedicated sampling equipment is not utilized, equipment rinsates should be collected after decontamination of field equipment as described above. The decontaminated equipment (hole saw, chisel) should be rinsed with about 25 mL filtered and deionized water into a glass container. The frequency of rinsate collection should be specified in the SAP. In the absence of such specification, one rinsate sample should be collected per sampling team per day. Equipment rinsate samples should be labeled with a unique identifier. Sample details should be recorded on the Surface Water FSDS.

### **7.2 Laboratory-Based Quality Assurance**

#### Laboratory Blanks

A laboratory blank is a filter that is prepared by processing a clean crucible in the same way that a bark sample is prepared. That is, a clean crucible is placed in the oven (with the sample set) at the

same time that tree-bark samples are undergoing ashing. After ashing, the blank crucible is treated by addition of water and HCl, as described above. The contents of the crucible are then rinsed out, diluted to 100 mL, and an aliquot at least as large as the highest volume aliquot for the sample set is removed and used to prepare a filter for TEM examination. This type of blank is intended to indicate if contamination is occurring at any stage of the sample preparation procedure.

Laboratory blanks should be prepared at a rate specified in the project-specific sampling and analysis plan. In the absence of a project-specific specification, laboratory blanks should be prepared at a rate of 3%.

#### Filtration Blanks

A filtration blank is a clean filter that is prepared by passing 100 mL of laboratory FDI water through it. The purpose of this type of blank is to ensure that the filters are not contaminated in the laboratory, and that fluids used for diluting and processing samples are fiber-free.

Filtration blanks should be prepared at a rate specified in the project-specific sampling and analysis plan. In the absence of a project-specific specification, filtration blanks should be prepared at a rate of 2%.

#### Laboratory Duplicates

Laboratory duplicates will be prepared by applying a second aliquot of ashed residue suspension to a new filter, which is then prepared and analyzed in the same fashion as the original filter. The frequency of laboratory duplicates should be specified in the SAP. In the absence of such specification, the rate should be no less than 5%. Laboratory duplicates should be recorded using the appropriate laboratory quality control field in the TEM EDD spreadsheet.

#### Recounts

The precision of TEM sample results should be evaluated by recounting selected grid openings in accord with the requirements specified in the most recent version of LB-000029.

## **8.0 REFERENCES**

International Organization for Standardization. 1995. Ambient Air – Determination of asbestos fibres – Direct-transfer transmission electron microscopy method. ISO 10312:1995(E).

Phipps, R.L. 1985. Collecting, Preparing, Cross-dating and Measuring Tree Increment Cores. U.S. Geological Survey Water Resources Investigations Report 85-4148

Ward TJ, T Spear, J Hart, C Noonan, A Holman, M Getman, and JS Webber. 2006. Trees as Reservoirs for Amphibole Fibers in Libby, Montana. Science of the Total Environment 367: 460-465.