ERI ANALYTICAL

P. O. Box 2024, Tyler, Texas 75710
2026 Republic Drive, Suite A, Tyler, Texas 75701
Ph: (903) 534-5001 Fax: (903) 534-8701
www.ericonsulting.com

Date: March 15, 2024

File No: ERI AS # 240333 A

Case Name-Bryce Alan Zundel

Specimen: A package within which were twenty-two tubes containing pieces of tissue embedded in paraffin (C21-21748 A2 2 of 2; C21-21751 A2 2 of 2; S22-2438 A1 2 of 2; CLN21-3103 A1 2 of 3; CLN21-3295 A1 2 of 3; CLN21-3399 A1 2 of 3; SU14-5281 A1 2 of 3, A2 2 of 3, A3 2 of 3, A4 2 of 3; CLS21-64972 A1 2 of 3, A2 2 of 3, A3 2 of 3, B1 2 of 3, B2 2 of 3, B3 2 of 3, C1 2 of 3, D1 2 of 3, D2 2 of 3, D3 2 of 3, D4 2 of 3, D5 2 of 3) was received [redacted] Pictures showing the blocks and slides in the case were submitted [redacted] Since no slides were received in the case and the pieces of the tissue were already cut from the embedded blocks, it was not possible to evaluate the previous location of the samples in the undisturbed blocks nor could the quality of tissue in the cut samples be evaluated as to quality/morphology.

A communication [redacted]

What had been defined as approximately one third of the embedded tissue defined as upper left lobe/lung tissue from tubes SU14-5281 A1, A2, A3, A4 were used for digestion of lung tissue. This sample was designated as ERI AS #240333A and the digestate of the sample was represented by 0.4727 gm. tissue pool wet weight.

Tissue was sent by: See above.

Date received: March 5, 2024-twenty-two test tubes with pieces of embedded tissue

Date analyzed: March 11, 2024 –Sample 240333A d1 F4 -Light Microscopy for Ferruginous Bodies (RFD)
March 14, 2024-Sample 240333A d1 F1 (ATEM Eurofins/J3 Resources)

Morphology of Specimen: Pieces of embedded tissue defined as approximately one third of the original embedded materials from blocks of left lung tissue were assigned the designation as ERI A.S. # 240333A. As much paraffin as possible was cut from the edge adjacent to the tissue after which the respective sample of tissue underwent deparaffinization. The procedure for

deparaffinization was as follows: the tissue was put on a hot plate to melt some of the paraffin, after which the sample was put through 6 changes of xylenes and 6 changes of ethanol.

The deparaffinized samples from the left lung tissues were carried through the modified bleach digestion procedure and aliquots of the individual digestates were collected on either 0.22 μm pored mixed cellulose ester filters for analysis by light microscopy for the presence of ferruginous bodies and on 0.2 μm pored polycarbonate filters which was prepared for evaluation by ATEM for the presence of uncoated fibers/particulates and ferruginous bodies.

Light Microscopy: For this evaluation one fourth of the mixed cellulose ester filter from the representative sample of the left lung tissue was mounted on a glass slide, cleared (made transparent) using acetone vapor and then scanned by light microscopy at 200-400x in an AO light microscope.

Filter 240333A d1 F4 representing the digestate sample from the left lung tissue was scanned by light microscopy and represented by 0.18908 grams deparaffinized wet weight of tissue. [redacted]

ATEM Morphology: For electron microscopy analysis a strip was cut from the carbon coated polycarbonate filter representing the area sampled. These strips were mounted on 100 mesh copper grids and the filter matrix dissolved using chloroform vapor. This resulted in the production of a carbon extraction replica containing the entrapped fibers and other particulates. Scans were made at 15,000x with counts and analysis including all fibers greater than or equal to 0.5 μm in length and with an aspect ratio of greater than 5:1. A scan at lower magnification (2,000x) was made of additional grid squares with the emphasis to find ferruginous bodies. The cores of any ferruginous bodies found in the area scanned were analyzed as were any uncoated asbestos fibers (>3μm).

Filter 240333A d1 F1 represented a 0.07563 gm. deparaffinized wet weight aliquot of the digestate from the sample of left lung tissue. An area of the prepared grids from this sample consisted of 1.266 mm^2 was scanned at 15,000x. [redacted]

An additional scan at 2,000x was carried out on a total of twenty grid squares on three grids for the presence of ferruginous bodies and fibers (>3 μm). [redacted]

Background [redacted]

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Limit of detection: Limit of detection is defined as that concentration below which a single fiber or ferruginous body would not likely be detected.

Light microscopy analysis for the presence of ferruginous bodies performed by Ronald F. Dodson, Ph.D.

Analytical Transmission Electron Microscopy performed by Eurofins/J3 Resources

Final Report Approved:

_____ Date May 15, 2024

Ronald F. Dodson, Ph.D.
President: Dodson Environmental Consulting, LLC
Senior Consultant: ERI Consulting, Inc.