

Supporting Information

Carbone et al. 10.1073/pnas.1105887108

SI Methods

Fiber Counting and Identification. Transmission electron microscopy (TEM) was used to determine fiber concentration using a modified ISO 10312. Energy dispersive spectroscopy (EDS) data from verified erionite fibers were gathered and graphed and the appropriate chemical ratios determined to distinguish erionite from asbestos or other fibers by microscopy. Verified specimens of erionite were tested using X-ray diffraction (XRD) to determine spectral patterns. Methods 10312 and 13794 were modified to count all erionite fibers without regard to physical characteristics and to allow up to 25% loading of the filter with particulate. The analytical sensitivity limits specified was 0.003 structures per cubic centimeter (s/cc) of air. Overloaded filters (>25% loading) with extraneous particulate were analyzed by ISO method 13794 modified to identify erionite fibers. Phase contrast microscopy equivalent (PCME) and total TEM air concentrations were recorded in s/cc. PCME structures are defined as erionite or asbestos structures with the following dimensions: length greater than 5.0 μm , a width greater than or equal to 0.25 μm with an upper bound of 3 μm , and an aspect ratio (length/width) greater than 3–1. PCME results are a subset of the TEM results and generally include the longer fibers. Whereas erionite or asbestos fibers may have been present but not seen by the microscopist, using the detection limit may overestimate actual concentrations. Therefore, nondetects were assumed to be zero.

Analyses of Erionite Composition. The fibrous grains (up to 150 μm long and 7 μm in “diameter”) were dispersed on conducting tape and coated by evaporated carbon. Most grains lie flat on the tape and this mounting method is preferred over polishing. Compositions of Turkish and ND erionite were determined using an electron microprobe. Operating conditions were a 15 kV, 15 nA incident beam defocused to 3 μm that reduced, but did not eliminate, cation loss from the analysis volume. The defocused beam

allowed analysis of the largest erionite grains and the analysis area was confirmed by SEM imaging (Fig. S1A). There was a deficiency in nonframework charge attributed to loss of univalent cations. The univalent cations were confirmed to suffer large loss in a 15-s timescale (Fig. S1B). Values of Na and K (loss assumed equal) were analyzed first and adjusted to give charge balance.

Western Blotting. Briefly, HM or macrophages were cultured in T-75 flasks until 80% confluence in DMEM with 20% FBS. The medium was changed to DMEM with 2% FBS. One hour later, the cells were exposed to either crocidolite asbestos or erionite from North Dakota, Oregon, or Turkey (Karain) at a concentration of 5 $\mu\text{g}/\text{cm}^2$. At 48 h after exposure to the different fibers, the cell medium was collected and concentrated using an Amicon centrifugal filter (Millipore). The cells were also collected and protein was extracted from whole cell lysates. Protein concentrations were determined using the bicinchoninic acid (BCA) assay (Thermo Scientific). Twenty micrograms of protein from the whole-cell lysates and 25 μL from the media concentrates were used for Western blot analysis. Rabbit polyclonal antibodies against HMGB1 or TNF- α were purchased from Abcam and used at a dilution of 1:500 and 1:200, respectively. Antibodies against α -tubulin (Calbiochem) and GAPDH (Abcam) at a dilution of 1:1,000 and 1:5,000, respectively, were used to assess loading.

Immunohistochemistry. Briefly, immunohistochemistry was performed on tissues by rabbit polyclonal IgG purified anti-HMGB1 (Abcam), diluted 1:200. The inflammatory infiltrate around asbestos and erionite deposits was characterized by using antimacrophage markers F4/80 (MCA497; Serotec) and rat IgG2b (control, diluted 1:400). Rabbit polyclonal-wide spectrum cyokeratin antibodies (Invitrogen) were used to identify cells of mesothelial origin.

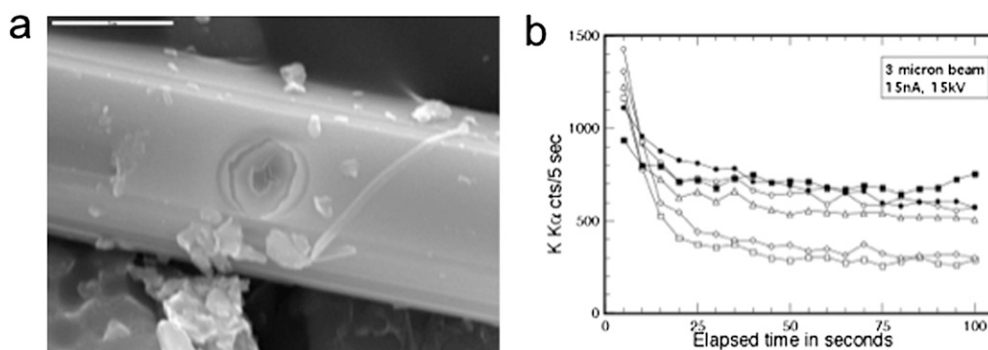


Fig. S1. Location of erionite composition analysis and loss of K during electron beam analysis. (A) North Dakota erionite fiber clearly showing the location of analysis with a ~ 3 - μm damaged area. Other small adhering grains (presumably clay material) are apparent but could not be avoided. Upper left corner shows 5- μm scale bar. (B) Within 15 s, the K K α intensity is reduced to an estimated 1/3 of the extrapolated intensity at “zero” time. A similar reduction would be expected for Na. This creates a major uncertainty for K and Na analysis in erionite. The above traces represent the observed decay in five different grains of erionite from North Dakota. The solid symbols show the decay in two analysis points in a single grain. Similar data for Na was not obtained due to its low concentration.

Table S1. Size distribution of erionite fibers

		North Dakota (μm)	Turkey (μm)
Length	Mean	2.20	3.57
	Median	1.61	2.24
	Minimum	0.56	0.56
	Maximum	16.80	38.08
Width	Mean	0.31	0.31
	Median	0.28	0.20
	Minimum	0.05	0.06
	Maximum	1.28	5.04
Aspect ratio	Mean	7.61	20.46
	Median	5.83	11.67
	Minimum	3.00	2.50
	Maximum	28.33	370.00

Only structures defined as fibers have been included. All fibers obtained from air samples. All sample scenarios and venues included.