Erionite exposure in North Dakota and Turkish villages with mesothelioma

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Edited by David M. Jablons, Surgery; Cancer Center, San Francisco, CA, and accepted by the Editorial Board June 23, 2011 (received for review April 13, 2011)

Exposure to erionite, an asbestos-like mineral, causes unprecedented rates of malignant mesothelioma (MM) mortality in some Turkish villages. Erionite deposits are present in at least 12 US states. We investigated whether increased urban development has led to erionite exposure in the United States and after preliminary exploration, focused our studies on Dunn County, North Dakota (ND). In Dunn County, ND, we discovered that over the past three decades, more than 300 miles of roads were surfaced with erionite-containing gravel. To determine potential health implications, we compared erionite from the Turkish villages to that from ND. Our study evaluated airborne point exposure concentrations, examined the physical and chemical properties of erionite, and examined the hallmarks of mesothelial cell transformation in vitro and in vivo. Airborne erionite concentrations measured in ND along roadsides, indoors, and inside vehicles, including school buses, equaled or exceeded concentrations in Boyali, where 6.25% of all deaths are caused by MM. With the exception of outdoor samples along roadsides, ND concentrations were lower than those measured in Turkish villages with MM mortality ranging from 20 to 50%. The physical and chemical properties of erionite from Turkey and ND are very similar and they showed identical biological activities. Considering the known 30- to 60-y latency for MM development, there is reason for concern for increased risk in ND in the future. Our findings indicate that implementation of novel preventive and early detection programs in ND and other erionite-rich areas of the United States, similar to efforts currently being undertaken in Turkey, is warranted.

asbestosis | cancer | carcinogenesis | environmental carcinogenesis | mineral fiber carcinogenesis

The malignant mesothelioma (MM) epidemic in Cappadocia, a region of Central Anatolia in Turkey, was first described in the villages of Karain, Tuzkoy, and Sarihidir (Turkish MM villages) in 1978 (1, 2). Subsequently, through mineralogy and analysis of lung content, MM was shown to be associated with exposure to erionite, a zeolite mineral with some physical properties similar to asbestos (3, 4). The potency of erionite in causing MM is underscored by the observation of MM in almost 100% of erionite-exposed rats compared with incidences of 48 and 0% following injection of chrysotile asbestos or inhalation of crocidolite asbestos, respectively (5). Other studies in animals showed that erionite was 500–800 times more tumorigenic than chrysotile asbestos (6) and 200 times more tumorigenic than crocidolite asbestos (7). The International Agency for Research on Cancer classified erionite as a group 1 known human carcinogen and concluded that erionite is the cause of the MM epidemic in Cappadocia (8). Since then, research has shown that erionite exposures result in pleural and interstitial fibrotic changes, similar to those observed with asbestos exposures (9, 10). Moreover, in vitro studies demonstrated that erionite exposure, but not asbestos exposure, is sufficient to cause malignant transformation of human mesothelial cells in tissue culture (11). As rural areas are being developed, environmental and occupational exposure to erionite may occur and reports describing cases of erionite-associated disease in North America have already begun to emerge (12, 13). Thus, geologic formations containing erionite with a potential for environmental exposures must be promptly identified to prevent the risk of "disturbing" erionite fibers and exposing the population, as has occurred in Cappadocia (14) and, as described here, in the United States. To determine whether erionite exposure occurs at carcinogenic levels, we compared the concentration and composition of erionite from North Dakota and Turkey. Composition was determined by electron beam analysis, as shown in Fig. S1 *A* and *B*. We also compared the biological activity of the fibers from both regions.

Results

Erionite Exposure Occurs in the United States. To test the hypothesis that erionite exposure might occur in the United States due to increased urban development, we conducted pilot studies in different areas in the United States known to contain geological deposits of erionite (15). In 2006, we learned that erionite-containing geologic formations in the North Killdeer Mountains in Dunn County, ND were being used since the 1980s (16) to produce gravel. Thus, we focused our studies in Dunn County and our research team determined that over the past two to three decades, more than 300 miles of roads, including 32 miles of school bus routes, parking lots, playgrounds, and baseball fields were paved with erionite-containing gravel (Fig. 1). We tested whether the use of erionite on these roads caused human exposure to this potent carcinogen. Air sampling was performed during activities that disturb gravel such as driving, raking, and sweeping, with air monitoring done in the breathing zone of the individuals performing the activities. We found that when erionite-containing gravel is disturbed, erionite fibers become airborne and can enter the personal breathing zone (Table 1).

Air Concentrations of Erionite in ND and Turkish MM Villages. Air concentrations of erionite associated with an increased risk of mesothelioma have not been established. Therefore, our team conducted an in-depth survey of the mineral fiber concentrations in the air of five villages in Cappadocia, Turkey, where some of us had previously observed an excess of MM caused by exposure to erionite (1–3, 14, 17, 18). These data allowed us to establish air concentrations of erionite that are associated with an increased risk of MM (Table 2). Next, the results of exposure-point

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Author contributions: M.C., G.G., H.I.P., H.Y., and A.M. designed research; M.C., Y.I.B., P.B., B.B., S.C., A.U.D., S.J., C.R.P., H.I.P., Z.S.R., I.S., M.T., S.W., H.Y., and A.M. performed research; M.C. and S.K. analyzed data; and M.C., S.K., and A.M. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. D.M.J. is a guest editor invited by the Editorial Board.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1105887108/-/DCSupplemental.

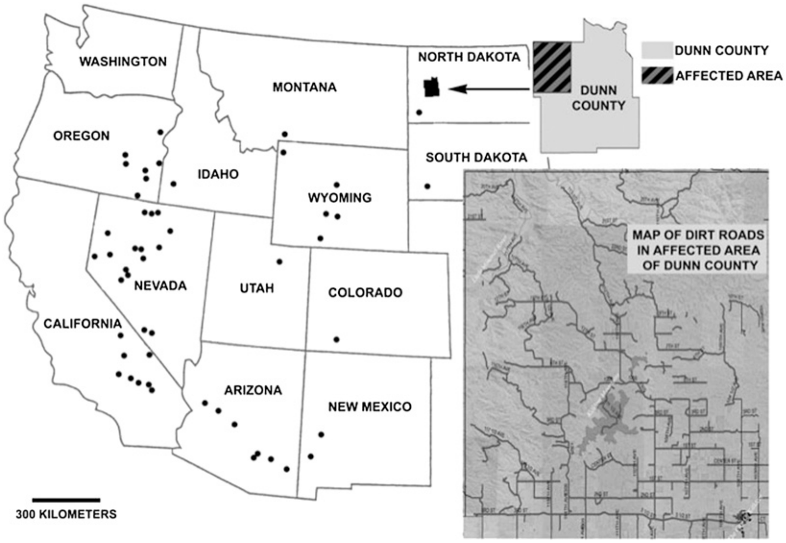

Fig. 1. Erionite deposits in the United States and roads with erionite-containing gravel in Dunn County, North Dakota.

measurements in ND, locations which are shown in Fig. S2 *A* and *B*, were compared with similar measurements performed in the Turkish MM villages. Results for erionite concentrations in personal breathing zones during outdoor activities involving raking of gravel in a community parking lot, ball field, and gravel pits in ND were lower than exposures measured during sweeping of roads in Turkish MM villages (Table 2). Erionite concentrations in stationary outdoor samples (defined in legends of Tables 1 and 2) taken along the road near a school bus stop in ND [0.11 structures/cubic centimeter (s/cc) total transmission electron microscopy (TEM), 0.01 s/cc phase contrast microscopy equivalent (PCME)] exceeded all stationary roadside samples collected along the main town roads in the Turkish MM villages (range: <0.00–0.01 s/cc total TEM, all <0.00 s/cc PCME) (Tables 1 and 2). As shown in Tables 1 and 2, results of indoor air samples in personal breathing zones collected in a road maintenance garage and a social services office in ND during sweeping and housekeeping activities (0.18 s/cc total TEM, 0.06 s/cc PCME) were comparable to those in Boyali and Karlik, villages that have MM mortality of 6.25 and 7.41%, respectively (17, 18). Elevated airborne erionite concentrations were also found within school buses (0.10 s/cc total TEM, 0.01 s/cc PCME), inside cars (0.27 s/cc total TEM, 0.02 s/cc PCME), and during bicycle riding (0.59 s/cc total TEM, 0.05 s/cc PCME) over erionite-containing gravel roads in ND. Peak concentrations of 2.74 s/cc total TEM (0.2 s/cc PCME) were measured inside cars and school buses during these driving scenarios (Table 1). Asbestos was also found in total TEM indoor samples in ND and in four of the Turkish villages (except for Tuzkoy, all indoor PCME samples were nondetectable; Table 2). Outdoor asbestos concentrations were observed most notably during activity-based air sampling in the Turkish villages and were not detected in ND (Table 2).

Table 1. Summary of erionite concentration in Dunn County air samples

Scenario	Event	*N*	% Detect	Mean	Range
Transportation ABS, inside cars and school buses	TEM (s/cc)	41	90.24	0.235	2.74
	PCME (s/cc)	41	63.41	0.022	0.20
Transportation stationary, adjacent to roadway	TEM (s/cc)	3	100.00	0.108	0.16
	PCME (s/cc)	3	100.00	0.012	0.02
Transportation stationary, away from roadway	TEM (s/cc)	19	21.05	0.001	0
	PCME (s/cc)	19	10.53	0	0
Outdoor ABS	TEM (s/cc)	21	28.57	0.031	0.59
	PCME (s/cc)	21	14.29	0.003	0.05
Outdoor stationary	TEM (s/cc)	29	6.90	0	0
	PCME (s/cc)	29	6.90	0	0
Indoor (office) ABS	TEM (s/cc)	1	100.00	0.018	0
	PCME (s/cc)	1	0.00	0	0
Indoor (office) stationary	TEM (s/cc)	2	50.00	0.002	0
	PCME (s/cc)	2	50.00	0.001	0
Indoor (garage) ABS	TEM (s/cc)	5	80.00	0.207	0.50
	PCME (s/cc)	5	40.00	0.061	0.17

Transmission electron microscopy (TEM), phase contrast microscopy equivalent (PCME), structures per cubic centimeter (s/cc), nondetect values included as zero. ND activity-based and stationary air sampling was performed at differing locations. Outdoor activity-based samples (ABS) include personal breathing zone air samples collected while performing raking at two different gravel pits, a ball-field, and a community parking lot, and biking through residential alleys. Indoor activity-based samples included were collected while performing housekeeping activities, vacuuming in the office, and sweeping in the garage.

Physical Characteristics and Chemical Composition of ND and Turkish Erionite. Length and diameter of mineral fibers have been linked to carcinogenesis and only fibers with a diameter of about 0.3 µm or less reach the pleura (19). Scanning electron microscopy (SEM) and TEM analyses revealed that the average length of ND and Turkish erionite fibers was 2.20 and 3.57 µm, respectively. The average width of fibers was 0.31 µm for both locations (Table S1). Compositions of erionite from Old Sarihidir in Cappadocia, Turkey and from ND were compared using an electron microprobe with procedures to minimize loss of cations during analysis. Repeated scans for Sr and Ba showed them to be below the detection limit of 200 ppmw for erionite from both locations. The analysis included K, Na, Ca, Mg, and Fe and framework Al and Si. Accuracy of analysis was confirmed by the "balance error" calculation in which the sum of the nonframework cation charge should equal the charge loss in the framework due to Al^{+3} substitution for Si^{+4}. As shown in Fig. 2*B*, resulting atomic Si/(Si+Al) lie between 0.78 and 0.80, at the high end of erionite published values (20), but with ND values slightly lower than the Turkish ones. Atomic $Na/(Na^{+}\ Ca)$ values discriminate between ND (<0.10) and Turkish erionite (~0.5). On the conventional K-Mg-(Ca+Na) ternary diagram there appears to be a small difference (Fig. 2*C*). In summary, our data show that the physical and chemical characteristics of Turkish and ND erionite are very similar.

Biological Activity of ND and Turkish Erionite. To determine whether the minor differences in the physical properties or chemical composition between erionite from ND and Cappadocia could alter its capacity to induce transformation of human mesothelial cells (HM), we compared the biological activity of erionite from both sources in vitro and in vivo. HM were exposed to erionite in the presence of macrophages in a coculture system that mimics the process of macrophage recruitment and activation to sites of fiber

Table 2. Erionite exposure in the Turkish villages of Karain, Sarihidir, Tuzkoy, Karlik, and Boyali and in Dunn County, North Dakota

			Indoor activity-based air samples			Outdoor street-side stationary air samples			Outdoor activity-based air samples		
Mortality from MM	Fiber	Analysis method	*N*	% Detect	Mean	*N*	% Detect	Mean	*N*	% Detect	Mean
Karain											
51.5% (14)	Erionite	TEM (s/cc)	9	100	7.817	8	0	0.0000	6	66.6	0.0856
		PCME (s/cc)	9	77.7	1.737	8	0	0.0000	6	66.6	0.0106
	Total asbestos	TEM (s/cc)	9	55.5	0.167	8	0	0.0000	6	16.6	0.0024
		PCME (s/cc)	9	0	0.000	8	0	0.0000	6	16.6	0.0024
Sarihidir											
38.2% (14)	Erionite	TEM (s/cc)	10	100	3.589	7	57	0.0028	8	62.5	0.3739
		PCME (s/cc)	10	100	0.684	7	57	0.0017	8	50	0.1398
	Total asbestos	TEM (s/cc)	10	90	0.419	7	14	0.0004	8	37.5	0.2097
		PCME (s/cc)	10	0	0.000	7	0	0.0000	8	12.5	0.0262
Tuzkoy											
25.9% (17)	Erionite	TEM (s/cc)	5	100	7.324	4	25	0.0091	5	100	0.2854
		PCME (s/cc)	5	80	1.107	4	0	0.0000	5	60	0.0562
	Total asbestos	TEM (s/cc)	5	100	2.026	4	25	0.0167	5	80	3.6421
		PCME (s/cc)	5	20	0.053	4	25	0.0015	5	40	0.1556
Karlik											
7.41% (18)	Erionite	TEM (s/cc)	5	100	0.2221	4	50	0.0038	4	75	0.4464
		PCME (s/cc)	5	40	0.0157	4	0	0.0000	4	50	0.0552
	Total asbestos	TEM (s/cc)	5	80	0.1084	4	0	0.0000	4	75	0.0834
		PCME (s/cc)	5	0	0.0000	4	0	0.0000	4	0	0.0000
Boyali											
6.25% (17)	Erionite	TEM (s/cc)	3	100	0.0431	5	0	0.0000	4	75	0.0411
		PCME (s/cc)	3	100	0.0000	5	0	0.0000	4	0	0.0000
	Total asbestos	TEM (s/cc)	3	0	0.0000	5	20	0.0001	4	75	0.0515
		PCME (s/cc)	3	0	0.0000	5	0	0.0000	4	0	0.0000
Dunn County, North Dakota*											
Unknown	Erionite	TEM (s/cc)	6	83	0.1750	3	100	0.1082	20	25	0.0031
		PCME (s/cc)	6	33	0.0575	3	100	0.0122	20	10	0.0004
	Total asbestos	TEM (s/cc)	6	50	0.3054	3	0	0.0000	20	0	0.0000
		PCME (s/cc)	6	0	0.0000	3	0	0.0000	20	0	0.0000

Transmission electron microscopy (TEM), phase contrast microscopy equivalent (PCME), structures per cubic centimeter (s/cc), nondetect values included as zero.

Indoor activity-based air samples: These are air samples taken in personal breathing zones during sweeping, vacuuming, or routine activities inside of mosques, schools, homes, and public buildings in Turkish villages and in two locations in ND, a county garage shop and a social services building.

Outdoor street-side stationary air samples: These are stationary air samples collected alongside of the main streets in Turkish villages and alongside of a gravel road in ND by a school bus stop.

Outdoor activity-based air samples: These are air samples taken in personal breathing zones during sweeping of streets, public areas, and walkways in Turkish villages and raking of a community parking lot, ball field, and gravel piles in ND.

*Daily meteorological data during a large proportion of the outdoor ND activity-based sampling indicated winds between 10 and 20 mph. Although weather conditions appeared to be conducive to the release of particulate matter, including erionite from the soil and gravel surfaces, dispersion and transport of material before reaching the personal breathing zone were visually observed, potentially resulting in lower exposure than during quiescent periods such as those observed in Turkey.

deposition (21). After 8 wk in culture, high numbers of tridimensional foci developed in HM treated with erionite from ND (78.67 ± 3.480), Oregon (52.00 ± 5.686), and Turkey (24.33 ± 3.480) (Fig. 3 *A* and *B*). No foci developed in HM cultures with or without macrophages, unexposed or treated with glass beads as controls.

High mobility group box-1 (HMGB1) is localized in the nucleus of most cell types and it is released in the cytoplasm and the extracellular space during programmed cell necrosis, and it is actively secreted in the extracellular space by activated macrophages and by HM exposed to asbestos (21). Release of HMGB1 induces tumor necrosis factor-α (TNF-α) production and secretion by HM and macrophages (22), a process that has been linked to mineral fiber carcinogenesis (21). We tested whether, similar to asbestos, exposure to erionite could lead to the release of HMGB1 (21) and secretion of TNF-α (22). As shown in Fig. 3*C*, HMGB1 and TNF-α were specifically detected in the conditioned medium of HM and macrophages exposed to either US or Turkish erionite. To compare the hallmarks of MM pathogenesis in vivo (i.e., chronic inflammation in response to mineral fiber deposition that, over time, leads to MM development) (21) groups of three BALB/c mice were injected with 1 mg of ND or Turkish erionite and the organs were analyzed for signs of inflammation after 2 wk. In separate groups of three BALB/c mice, injection of 1 mg of asbestos was used as a positive control and vehicle injection was used as negative control. Inflammatory cell infiltration and mesothelial hyperplasia were detected in the peritoneum of mice injected with any of the mineral fibers (Fig. 3*D*). Immunohistochemistry showed that most cells surrounding tissue-embedded fibers were positive for the macrophage marker, F4/80. Some macrophages contained fibers or fiber fragments in their cytoplasm. Mesothelial cells were localized in the same areas, as verified by immunostaining for cytokeratin. HMGB1 staining was localized exclusively in both the nuclei and cytoplasm of reactive macrophages surrounding the fibers and in the nearby extracellular space, consistent with HMGB1 release. In vehicle-injected mice, neither chronic inflammation nor cytoplasmic or extracellular staining of HMGB1 was detected. These

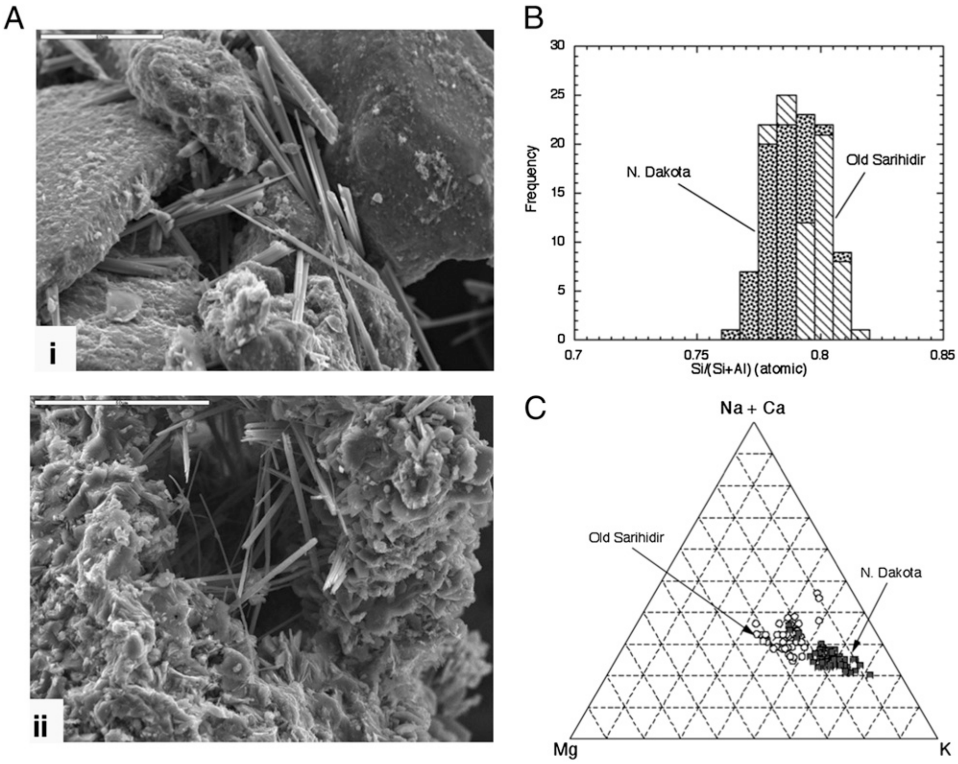

Fig. 2. Similar physical and chemical characteristics of erionite from North Dakota and Cappadocia, Turkey. (*A*) Scanning electron microscopy images with the 50-μm bar scale at *Upper Left* shows that fibers from (*i*) ND and (*ii*) Turkey are similar both in length and width. For both, maximum length is about 50 μm, whereas widths range well below 1 μm. Mechanical abrasion would be expected to reduce both dimensions. (*B*) Atomic Si/(Si+Al) vs. Frequency for ND and Turkey erionite. Turkey erionite appears to be slightly richer in Si but there is considerable overlap for the two locations. (*C*) A classic ternary diagram showing a compositional comparison of ND and Turkey erionite on the basis of atoms. These analyses have been adjusted for calculated Na and K loss and thus appear closer to the K vertex relative to no corrections.

data revealed no significant difference in biological activities of erionite from ND or Turkey.

Discussion

Asbestos and erionite fibers in rock, soil, or dust are not inherently hazardous to humans if left undisturbed. However, most soils and dusts are subject to disturbance by activities that are common for residents and workers. Fibers in surface soils, gravels, and indoor dusts contaminated with erionite become airborne when disturbed by foot traffic, automobile traffic, and a variety of other routine activities, as was clearly demonstrated by driving, raking, bicycle riding, and sweeping activities performed during this study. Exposures associated with roads surfaced with asbestos-containing gravel have caused a significant increase in the incidence of MM in New Caledonia (23). Erionite is a naturally occurring fibrous mineral hazard in the tristate area of North Dakota, South Dakota, and Montana (24). With expanded oil exploration in ND, use of erionite-containing gravel and traffic on the roads has increased significantly over the past few decades, leading to greater exposure. Although our studies were limited to Dunn County, ND, we are aware that similar erionite-contaminated gravel was and likely continues to be used in other places, for example in South Dakota.

In contrast to asbestos, there are no health benchmarks established in the United States to regulate erionite use and exposure. Until recently, erionite was erroneously believed to present a health risk only in Turkey. As erionite carcinogenicity is at least equal to that of asbestos, observed erionite exposures for individuals in ND such as school bus drivers and children, road workers, and others regularly using the roads, may exceed an estimated risk probability of one additional cancer per 10,000 people—a benchmark often used by regulatory agencies for risk management actions. It should be underscored here that the published literature indicates that erionite is much more potent than asbestos in causing MM (5–7); thus risk models developed for asbestos exposure, such as the US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) guidance, may underestimate the actual risks for erionite.

In addition to erionite, asbestos was observed during outdoor activity-based sampling in the Turkish MM villages, with the PCME asbestos findings most notable in Tuzkoy and Sarihidir (no PCME asbestos was found in ND, Karlik, or Boyali; Table 2). Previous studies have reported that tremolite and chrysotile asbestos are widespread in Cappadocian homes, including villages that do not experience a MM epidemic, mostly as contaminants in whitewash and stucco. There does not appear to be a correlation between asbestos use and the MM epidemic in specific Turkish villages, which is instead linked to the presence of erionite (1–3, 14, 25).

We found that the physical and chemical characteristics and the biological activity of erionite from ND and Cappadocia were very similar. The lengths and aspect ratios shown in Table S1 refer to erionite obtained from air samples, which necessarily requires mechanical removal from the rocks, dispersion into the air, and finally collection for measurement. The first two processes are not identical in the two locations, which may contribute to different size distributions. The width of the fibers is critical, as only fibers of about 0.3 μm or less are found in the pleura (19) and we observed that the width of the fibers from Cappadocia and ND was identical. The distribution of Si/(Si+Al) will be affected by the host rock composition and the physiochemical conditions leading to the growth of erionite. Although the measured Si/(Si+Al) ratio (Fig. 2*B*) is slightly different in Turkish and ND erionite, there is no a priori reason or experimental data to suggest that this small difference will affect the carcinogenicity of erionite. It can be expected, but has not been determined yet, that samples taken from many locations in either ND or Turkey will show a narrow range of values with varying amounts of overlap. Our data show that ND erionite was more potent than Cappadocian erionite in inducing foci formation, which is an early indicator of cell transformation. Focus formation is characterized by changes in cell morphology and loss of contact inhibition. This is one of the first steps in the process of carcinogenesis and cells derived from these foci have an extended life span in vitro. However, after a few passages in tissue culture the cells isolated from the foci entered into crisis and could not establish cell lines, supporting previous experiments showing that mineral fibers do not cause malignant transformation of mesothelial cells in culture in the absence of cofactors (26). It is only when macrophages, which are a source of HMGB1 and TNF-α are cocultured with mesothelial cells, or when these cytokines are added exogenously to the mesothelial

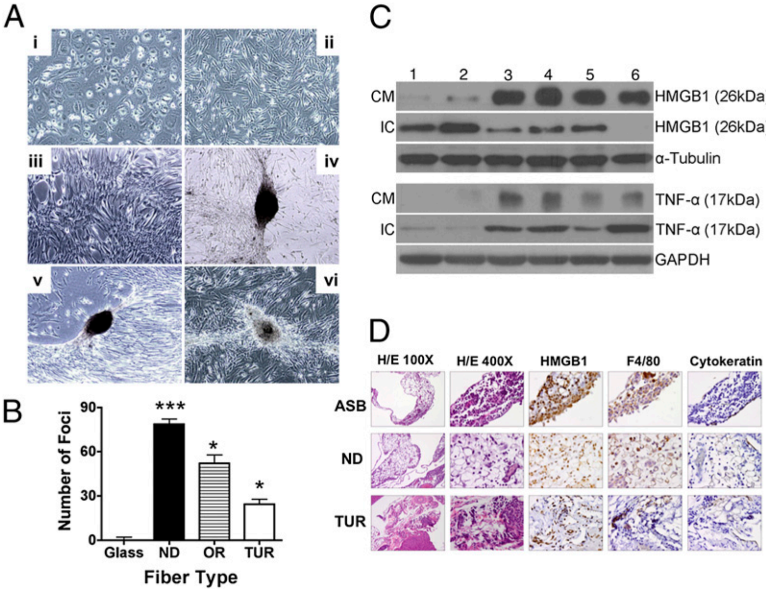

Fig. 3. Similar biological activity of erionite from North Dakota and Cappadocia, Turkey. (*A*) HM cells were cocultured with macrophages and exposed to either erionite or glass beads (control). After 6–8 wk of culture, foci were observed in erionite-exposed cells but not in glass beads-treated cells. (*i*) HM; (*ii*) HM cocultured with macrophages; (*iii–vi*) HM cocultured with macrophages and exposed to (*iii*) glass beads, (*iv*) US erionite from ND, (*v*) US erionite from Oregon, and (*vi*) Turkey erionite. (*B*) After 2 mo in culture, the numbers of tridimensional foci formed in HM cells under different treatment conditions were counted. Treatments were done in triplicates. OR, Oregon; TUR, Turkey. $^{*}P < 0.05$ and $^{***}P < 0.0001$ compared with glass beads using unpaired *t* test. (*C*) Western blot analyses show that exposure to erionite fibers induced the release of HMGB1 by HM and TNF-α by macrophages. In the untreated negative control (lane 1) or cells treated with glass beads (lane 2), HMGB1 is mostly retained intracellularly (IC). HMGB1 was released from the HM cells into the conditioned medium (CM) as the cells underwent programmed necrosis, which is induced by exposure to US erionite from ND (lane 3), US erionite from Oregon (lane 4), or Turkey erionite (lane 5). Crocidolite asbestos (lane 6) was used as a positive control. Macrophages produced and secreted TNF-α into the conditioned medium 48 h after being exposed to the erionite fibers. (*D*) Immunohistochemical analyses show HMGB1 staining around areas of inflammation caused by crocidolite and erionite deposits. At low magnification (100×) H&E staining shows areas of the greater omentum from the peritoneum of mice injected with ASB or ND erionite or TUR erionite. Analysis at higher magnification (400×) shows adipose tissues with asbestos and erionite fibers surrounded by inflammatory cells, macrophages, giant cells, and lymphocytes. On the same samples, HMGB1 is detected in both cytoplasm and extracellular space where F4/80 and wide spectrum cytokeratin antibodies identify macrophages and mesothelial cells, respectively.

cells in culture, that asbestos and erionite can cause focus formation (22). However, the same fibers can cause MM in animals. Thus, it is believed that the chronic inflammatory process and consequent release of HMGB1 and TNF-α over the course of years, and possibly the effect of other cofactors and cocarcinogens, are required to promote the accumulation of genetic damage that leads to the development of an immortal malignant clone. Therefore, the finding that ND erionite caused a larger number of foci to be formed in vitro than Turkish erionite should not be taken independently as evidence that ND erionite is more potent than Turkish erionite, because carcinogenesis is a much more complex phenomenon (27). However, focus formation is evidence that these two types of erionite have similar biological effects on human mesothelial cells.

Whereas a safe level of erionite exposure is not known, in general, increased intensity, frequency, and duration of exposure increase the likelihood for mineral fiber-related health problems (28). Erionite exposure in ND is of particular concern for children, where fibers lodged in the lungs may be able to exert their toxic effects for many more years compared with exposures during adulthood (29–31). Although our data do not allow for complete exposure comparisons, they show that exposure intensity in vehicles driving on erionite-containing roads in ND, including school buses, can equal or exceed measurements in Boyali and Karlik, villages with 6–7% MM mortality in which we have not identified genetically susceptible families (14, 17, 18). A recent investigation showing bilateral pleural plaques in 2 of the 15 individuals with high exposure to road gravel containing erionite and no history of asbestos exposure furthers our collective concerns for adverse health effects stemming from this exposure (32).

The annual incidence of MM in the US is $1–2/10^6$ in states with no exposure and $10–15/10^6$ in states with high asbestos exposure due to shipyards (33). In Cappadocia, the annual incidence of MM is about 1/100 in the villages of Karain, Sarihidir, and Tuzkoy, and about 1/1,000 in the villages of Boyali and Karlik. ND is not part of the Surveillance, Epidemiology, and End Results (SEER) database (33) and precise data about the incidence of MM during the past decades are not available. Current MM incidence in ND is estimated at about $12/10^6$, which is within the expected range of US states with higher exposure (32), despite, to the best of our knowledge, the lack of shipyards or asbestos-based trades in ND. The absence of a statistically observable MM epidemic in ND thus far is not surprising, given the lack of sensitivity of such mortality research and the relatively recent increase in erionite exposures. Prolonged exposure to erionite and asbestos are required to cause MM after latencies of 30–60 y (14). In the past, similar situations have often gone unrecognized for many decades in part due to long latency periods, small exposed populations, and undiscerning surveillance systems. For example, widespread asbestos contamination and associated disease in the community of Libby, Montana, went unnoticed by public health officials until 1999, despite warning signs decades earlier (34). Area residents in ND have expressed skepticism about the potential for health effects from erionite exposures (35). Similar skepticism prevailed in the first half of the past century about asbestos and it was only when the number of asbestos-related deaths increased to the magnitude of an epidemic that strict preventive measures were implemented (36). We hope that the lessons learned from such experiences will help to prevent a possible new wave of MM in the United States that could be caused by erionite.

The long latency between exposure and disease provides a unique opportunity to implement preventive and early detection programs in the United States similar to those being implemented in Turkey. We have worked with the Turkish Ministry of Health to identify the villages at higher risk and relocate residents so as to reduce their exposure. For example, residents of Tuzkoy were moved to the new village of Tuzkoy that opened in 2009 and there is an ongoing project for relocation of residents of Karain (14). In addition, in 2009 the Turkish Ministry of Health opened MM centers nearby these villages to monitor the population for early signs of MM because early detection is associated with better survival (37). In 2011, we hope to start a prospective clinical trial at the MM centers in Turkey that is cosponsored by the Early Detection Research Network of the US National Cancer Institute and by the Turkish Ministry of Health to validate serological markers for early detection of MM. The results of these studies will be of direct relevance to the US population exposed to asbestos or erionite, such as in Dunn County, ND. Currently, studies of erionite-induced MM in the United States are limited by lack of resources. The availability of resources to study exposure, genetics, mechanisms of toxicity, and prevention strategies would be of immediate benefit to the population of ND and other erionite-rich areas of the United States to reduce the potential for disease and limit adverse effects among those already exposed.

Methods

Collection of Air Samples. Currently, there are no applicable or relevant and appropriate requirements or erionite-specific health benchmarks available to establish "safe" levels of human exposure to erionite fibers. Given the current lack of guidance regarding the nature and concentrations of erionite fiber exposure associated with adverse health effects in human populations and similarities with asbestos in physical characteristics, toxicology, and health effects, we used current approaches and benchmarks for asbestos exposures as a starting point for assessment of erionite-contaminated sites. Therefore, air samples were collected using a modified International Standards Organization (ISO) method 10312 "Ambient Air–Determination of Asbestos Fibers–Direct-Transfer Transmission Electron Microscopy Method" or 13794 (indirect method) at a nominal flow rate at or below 10 L per minute (l/min) using 0.8-μm pore size mixed cellulose ester (MCE) filters. Activity-based sampling (defined in the legend of Table 2) was performed to collect personal breathing zone air samples in a variety of settings both indoors and outdoors in Turkey and ND.

Fiber Counting, Identification, and Analysis of Erionite Composition. Fiber counting, identification, and analysis of erionite composition were performed according to standard procedures (*SI Methods*). Compositions of Turkish and ND erionite were determined using an electron microprobe according to standard procedures (*SI Methods*).

Coculture and Foci Formation. Primary HM were cultured and characterized as described in ref. 22 and used between passages 2 and 3. THP-1 human monocytes (American Type Culture Collection, ATCC) were differentiated into macrophages as described in ref. 38. Primary HM were seeded in 6-well plates until 80% confluence. Macrophages were cocultured in an insert chamber placed on top of the HM. The bottom of the insert chamber has 0.4-μm pores, allowing cytokines and growth factors produced by macrophages to reach the lower chamber where HM are cultured. Erionite (5 μg/cm^2) from North Dakota, Oregon, or Karain, Turkey were added into the culture and glass beads (3–10 μm; Polysciences) were used at the same concentration as control. Erionite fibers or glass beads were added once at the beginning of the experiment to both HM cells and macrophages. The cells were kept in culture up to 2 mo. The HM media, together with freshly differentiated macrophages were replaced two times a week. Data were compared between treatment groups by a two-tailed Student's t test.

Western Blotting. Western blotting was performed using standard procedures (21, 22) (*SI Methods*).

Animal Experiments. All procedures were performed in accordance with institutional guidelines and approved by the University of Hawaii Institutional Animal Care and Use Committee. Groups of three 21-d old BALB/c mice were injected intraperitoneally with 1-mg single injection of the following fibers: crocidolite asbestos (ASB), North Dakota erionite (ND), or Turkey erionite (TUR). Control group was injected with PBS. After 2 wk, animals were killed and the tissues and organs were evaluated histologically by hematoxylin-eosin (H&E) staining.

Immunohistochemistry. Immunohistochemistry was performed according to standard procedures (21, 22) (*SI Methods*).

Statistical Analysis. Data were analyzed using the two-sided Student's t test and considered statistically significant when the P value was <0.05. Bar graphs represent the mean, and error bars represent 95% confidence intervals. Statistical analyses were performed using GraphPad Prism version 4.0.

ACKNOWLEDGMENTS. This work was supported in part by the National Cancer Institute at the National Institutes of Health (P01 114047 to M.C.), the American Association for Cancer Research Landon Innovator Award, and a grant from the Butitta Mesothelioma Foundation (to M.C.). This work was also supported by grants from Mesothelioma Applied Research Foundation, Riviera Foundation, Aramec Foundation (to H.Y.), and the Hawaii Community Foundation (to H.Y. and G.G.). For B.B., C.R.P., S.W. (USEPA), and A.M. (NIEHS), this research does not represent policies or positions of the respective agencies.

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