Assessment of Environmental Asbestos Exposure in Turkey by Bronchoalveolar Lavage

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Environmental or domestic exposure to asbestos fibers originating from local soil is responsible for a high incidence of diseases in large rural areas of Turkey. Bronchoalveolar lavage fluids (BALF) were obtained for 65 Turkish subjects originating from these areas and for 42 Turkish controls. Asbestos bodies (ABs) and uncovered fibers (UFs) were quantified by phase contrast light microscopy. Total fiber burden was determined by transmission electron microscopy. The main asbestos types disclosed were tremolite and to a lesser extent chrysotile. AB and fiber concentrations were higher in environmentally exposed subjects (geometric mean [geometric standard deviation]: 5.20 [6.22] AB/ml, 444 [11.6] tremolite fibers/ml) than in control subjects (0.22 [1.45] AB/ml, 12.0 [15.4] tremolite fibers/ml) ($p < 0.001$). In subjects environmentally exposed in Turkey, AB burdens on tremolite were in the same range as those on commercial amphiboles in subjects occupationally exposed in Belgium. In Turkish subjects, values above either 1 AB/ml, 3 uncovered fiber/ml in light microscopy, or 300 fibers/ml in electron microscopy indicated usually an abnormal alveolar retention reflecting a significant cumulative exposure from environmental or domestic origin. These observations are probably valid for other areas in the world where diseases associated with environmental exposure to soil-derived asbestos fibers occur and for immigrants originating from these areas. Dumortier P, Çoplü L, de Maertelaer V, Emri S, Baris I, De Vuyst P. Assessment of environmental asbestos exposure in Turkey by bronchoalveolar lavage. AM J RESPIR CRIT CARE MED 1998;158:1815–1824.

There are widespread natural asbestos deposits in rural parts of central and eastern Turkey. The main fiber type in these deposits is tremolite but the presence of chrysotile has also been reported (1). High incidences of both benign and malignant pleural and pulmonary diseases have been reported in these regions (1–4). Environmental tremolite–related diseases have also been reported from Greece, Cyprus, Corsica, Afghanistan, and New Caledonia (5–11). Erionite, a nonasbestos mineral fiber that belongs to the zeolite group which occurs in volcanic tuffs, has also been reported to cause high incidences of lung and pleural diseases in three villages of Cappadocia (12).

In Turkey, villagers use asbestos soil (white soil) as a whitewash or plastering material (white stucco), on roofs for insulation and prevention of water leakage and even as a substitute for baby powder (4). One of the most important sources of domestic exposure is dust originating from the walls of homes whitewashed with white stucco. Sweeping in such houses leads to elevated airborne fiber levels (11). The majority of the population are farmers, and farming activities in areas with asbestos-containing soils also generate airborne fibers. Exposure to amosite or crocidolite fibers has not been reported among Turkish villagers.

Environmental asbestos-related diseases are an important public health problem in Turkey (1–3), but there are relatively few data about the asbestos fiber burden in the lung or pleura from Turkish patients (13–17). This is mainly due to the low autopsy rate.

Bronchoalveolar lavage (BAL) is a well-established noninvasive method to study the alveolar retention of asbestos fibers (18–20). The aim of our study was to investigate the asbestos body (AB) and asbestos fiber levels, types, and dimensions in bronchoalveolar lavage fluid (BALF) from Turkish subjects with and without environmental exposure to asbestos, and to compare AB concentrations with those observed in Belgian subjects with and without occupational exposure to asbestos (Reference 18 and unpublished updated data).

METHODS

Patients

The study population included 107 patients who underwent diagnostic fibreoptic bronchoscopy at the Chest Department of Hacettepe University School of Medicine, between September 1991 and April 1996. Bronchoscopy was performed on one of the following clinical indications: a suspicion of an asbestos-related benign pleural or parenchymal disease, of malignant pleural mesothelioma (MPM), of lung cancer, or of other pulmonary or pleural diseases (sarcoidosis, tuberculosis, eosinophilic pneumonia, etc.). A written informed consent to perform BAL was obtained from all patients.

Data about BALF AB burdens from the Turkish subjects were compared with those of 600 Belgian subjects (230 with definite, 115 with suspected occupational exposure to asbestos, 126 unexposed blue-collar and 129 white-collars controls) (Reference 18 and unpublished updated data).

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Environmental Asbestos Exposure History

All Turkish patients filled in a questionnaire about their place of birth, type of house in which they had lived in childhood, use of whitewash or of possibly asbestos-contaminated material, and the localization of villages, towns, or cities where they lived in chronological order. This was followed by an interview carried out by a physician with experience in environmental asbestos–related diseases (L.Ç.). The subjects were categorized into two groups on the basis of probability of environmental asbestos exposure history. A group of 65 subjects who were born in areas where environmental asbestos diseases are endemic and/or had lived in a house built with asbestos-contaminated material and/or with walls covered by whitewash were considered to be exposed to environmental asbestos fibers. A group of 42 subjects without known environmental asbestos or erionite exposure was used as a control group. Figure 1 shows the area of origin of the subjects and controls.

Occupational History

On completion of the environmental history, a detailed chronological occupational history was obtained with a questionnaire. Among the subjects with environmental exposure, three subjects reported possible occupational exposure to asbestos (mixed exposure). According to the occupational history, environmentally exposed subjects and controls were categorized into four subgroups: farmers/villagers, housewives, blue-collar workers, and white-collar workers. Farmers/villagers subgroup comprises both men and women occupied in the fields or with known history of whitewashing activity.

BAL Sampling and Preparation

BAL was performed during fiberoptic bronchoscopy with successive 50-ml aliquots of saline. From 6 to 41 ml (mean $\pm$ SD: 23.9 $\pm$ 9.1) of the second and/or third fraction of recovered BALF were collected for mineralogical analysis. BALF was not filtered on gauze. Ten milliliters of formalin was added immediately to the BALF. The mixture was kept at room temperature and was shipped from Ankara to Brussels. All fluids used during sample preparation were filtered on 0.22-μm filters and all glassware was carefully cleaned in order to avoid contamination. The organic components were digested with commercial bleach and particles were collected on Millipore cellulose ester membrane filters (Millipore Corporation, Bedford, MA) (nominal porosity 0.45 μm, diameter 25 or 47 mm according to the amount of dust remaining after digestion) (18). The filters were clarified and fixed on glass slides with acetone vapors. As far as possible, only one filter is prepared for each sample in order to increase analytical sensitivity (the analytical sensitivity is the theoretical fiber concentration equivalent to counting one fiber in the analysis). The same filter is used for light microscopy (LM) and analytical transmission electron microscopy (TEM) examination.

AB and Uncovered Fiber Counting (LM)

The filters were covered with 1.518 refractive index immersion oil and a cover glass. ABs were counted with phase contrast light microscopy at a magnification of $\times$250 (18). Only typical ferruginous bodies were considered as asbestos bodies. In previous studies, 95 to 98% of such ferruginous bodies were built on an asbestos fiber (19, 21). Because ABs represent only a fraction of the fibers detectable by LM, straight uncovered fibers (UFs) longer than 10 m were also recorded. Strongly birefringent fibers with high refractive index, which are mostly identified as titanium oxide (rutile) when analyzed by TEM, and curly fibers were not taken into account. Concentrations are expressed as number of AB and UF per ml of BALF fluid. The surface of the filter examined was calculated to reach an analytical sensitivity of approximately 0.1 AB/ml.

Fiber Counting and Analysis by Electron Microscopy

Asbestos and nonasbestos fibers concentrations were determined with analytical TEM. After ABs and UFs counting by LM, the slides were uncovered and the immersion oil rinsed with CCl_4. Pieces of filter were cut out, their particle-bearing surface was coated under vacuum with carbon, and they were placed on 300 mesh thin bar grids in a modified Jaffe washer. The filter material was dissolved with acetone. The grids were examined at a $\times$22,000 magnification in a PHILIPS EM 400T transmission electron microscope (Philips Analytical, Eindhoven, The Netherlands) operated at 80 kV. The microscope was fitted with a scanning attachment and an EDAX PV9900 (EDAX International, Mahwah, NJ) X-ray energy dispersive spectrometer (EDS). Each coated or uncoated fiber with a length $\geqslant$ 1 μm and an aspect ratio $\geqslant$ 3/1 was evaluated. The dimensions and the chemical composition of the fiber were determined. Identification of the fiber type was based on the combination of the data about their morphology, chemistry, and electron diffraction pattern. Chemical spectra were compared with reference spectra obtained from International Union Against Cancer (UICC) asbestos standards and tremolite reference samples. In order to keep the analysis time within reasonable limits, TEM fiber

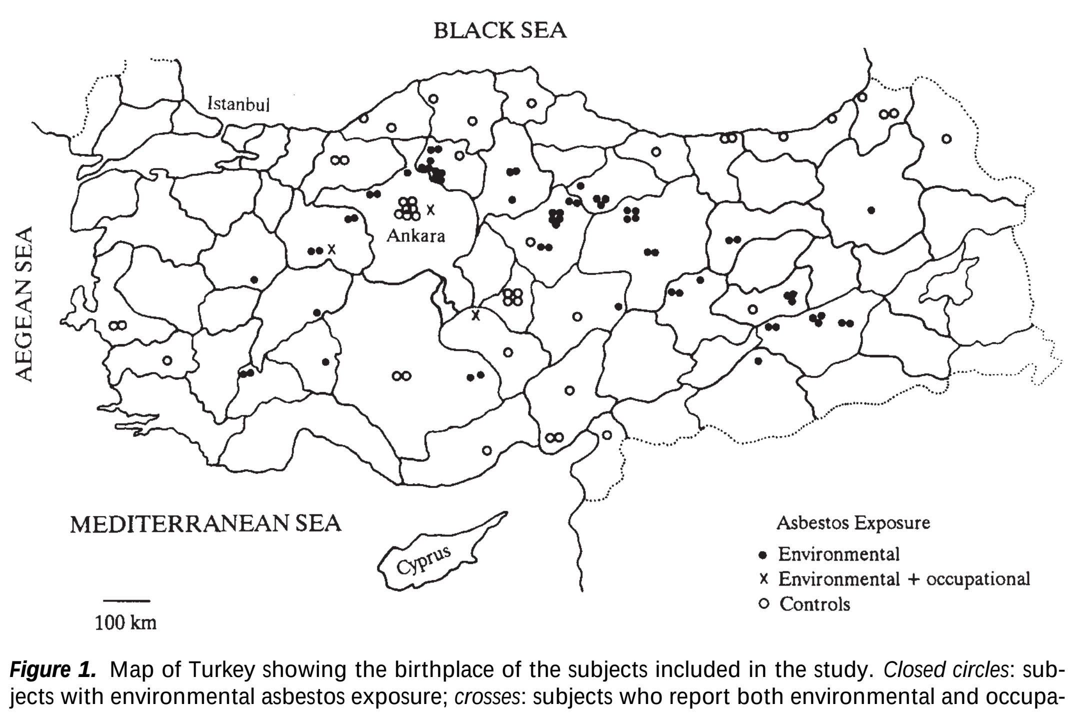

Figure 1. Map of Turkey showing the birthplace of the subjects included in the study. *Closed circles*: subjects with environmental asbestos exposure; *crosses*: subjects who report both environmental and occupational asbestos exposure; *open circles*: control subjects without environmental exposure.

counting was stopped after either of the following conditions was fulfilled: (*1*) the number of randomly selected grid openings examined corresponded to an analytical sensitivity just below 50 F/ml BALF, or (*2*) more than 50 consecutive asbestos fibers were analyzed and sized.

Quantitative Chemical Analysis of Fibers from BALF, Whitewash Raw Material, and Geological Samples

The quantitative chemical composition was determined by the k_{ASi} ratio method (22) for 61 tremolite fibers from 6 BALF and for 32 from whitewash raw material and soil samples from three villages (Kureysler, Yazir, and Capar Kayir) to determine if any major modification in the fiber chemistry resulting from contact with human body fluids or sample preparation media could be detected.

Statistical Analysis

The mean and the standard deviation (SD) are given for the time-related variables. Student's *t* test was used to compare age, duration of exposure, and delay since end of exposure between the different groups. As AB and fiber concentrations as well as size parameters (width, length, and aspect ratio) were approximately log normally distributed, geometric mean (GM) and geometric standard deviation (GSD) are presented (23). Tests were performed on log transformed concentration and size data. According to the number of groups to be compared, Mann-Whitney U test or Kruskal-Wallis test were used to compare concentrations and fiber sizes. For zero observations, values of half the analytical sensitivity of the method (0.05 AB or UF/ml BALF for LM, and 25 fiber/ml BALF for EM) were given in order to enable graphical presentation of concentrations on logarithmic scale. Spearman's correlation coefficients (r_s) were calculated to assess relationships between concentration variables. Data were analyzed with STATISTICA 5.0 statistical software package (StatSoft, Tulsa, OK) run on an IBM compatible personal computer.

RESULTS

Demographic and exposure data are summarized in Table 1. Mean age and age distribution are similar between those with environmental exposure and the control subjects. At the time of BALF, supposed environmental exposure had ceased for more than 1 yr for 35 subjects (53.8%) who had moved from their village of origin.

TABLE 1

DEMOGRAPHIC, EXPOSURE, AND CLINICAL DATA OF THE TURKISH SUBJECTS

	Subjects without Environmental Asbestos Exposure (*n* = 42)	Subjects with Environmental Asbestos Exposure (*n* = 65)
Age		
Mean ± SD	52.0 ± 12.8	52.2 ± 11.4
(Min–max)	(23–73)	(27–77)
20–35 yr	5	5
36–55 yr	19	33
56+ yr	18	27
Sex ratio, M/F	2.81 (31/11)	2.25 (45/20)
Duration of exposure, yr (mean ± SD)		36.1 ± 19.2
Delay since end of exposure, yr (mean ± SD)		16.1 ± 16.7
Number of subjects with lifelong exposure		30
Occupation, M/F		
Farmer/villager	3/0	17/11
Housewives	0/9	0/9
Blue-collar workers	11/0	13/0
White-collar workers	17/2	15/0
Disease, M/F		
Malignant pleural mesothelioma	0/0	18/10
Bilateral benign pleural disease	4/0	23/4
Lung fibrosis	0/0	1/1
Lung carcinoma	8/2	2/1
Pleural effusion	1/1	2/1
Other	18/8	9/5
Smoking habits, M/F		
Nonsmoker	6/9	15/19
Smoker	18/2	19/1
Ex-smoker	5/0	10/0
No data	2/0	1/0

Light Microscopy Counting

Table 2 gives an overview of descriptive statistics for AB and UF counts. These concentrations range from < 0.1 to 4.6 AB/ml BALF and < 0.1 to 9.8 UF/ml BALF for control subjects and from < 0.1 to 723 AB/ml BALF and < 0.1 to 4,296 UF/ml BALF for environmentally exposed subjects. Concentrations exceeding 1 AB/ml were found in 7.1% of control individuals and in 64.1% of subjects with environmental exposure. Concentrations above 1 UF/ml were observed in 21.4% of control subjects and in 76.6% of subjects with environmental exposure and above 3 UF/ml in 7.1% and 53.1%, respectively. There was a good correlation between AB and UF concentrations (Figure 2, $r_s = 0.85$, $p < 0.001$), but the ratio UF/AB ranged from 0 to 185 (median 1.45).

Geometric mean concentrations of ABs and UFs in BALF differed ($p < 0.001$) between subjects with environmental exposure and controls. Among the latter, there was no detectable difference between males and females. In those with environmental exposure, both AB and UF concentrations were higher among men than women ($p < 0.01$). The maximal concentrations were 723 AB/ml and 4,296 UF/ml among men, and 165 AB/ml and 118 UF/ml among women. Differences in AB and UF concentrations between exposed men and women cannot be ascribed to different yield in BAL fluid recovery or to a dilution effect due to lavage of different lung volumes because BAL was always performed in the right middle lobe or in the lingula and the mean recovery is similar in both groups (mean ± SD [range]: 27.4 ± 8.6 ml [9–41] versus 22.9 ± 9.4 ml [6–40]). There were no differences in AB and UF concentrations between male villagers/farmers, blue-collar and white-collar workers, and between female villagers/farmers and housewives. Maximum counts for white-collar workers were 230 AB/ml and 772 UF/ml.

Among environmentally exposed men, there were no differences in AB and UF counts between ever-smokers (GM [GSD]: 8.98 [7.18] AB/ml and 17.55 [10.18] UF/ml) and never-smokers (GM [GSD]: 7.97 [5.02] AB/ml and 4.75 [2.94] UF/ml). For subjects with environmental exposure there was no correlation between AB or UF concentration and age, duration of exposure, or delay since end of exposure.

Environmentally exposed subjects with mesothelioma and benign pleural disease (BPD) showed similar AB and UF concentrations. Those without an asbestos-related disease had lower geometric mean concentrations but the differences were not significant.

Comparison of AB Counts in BALF from Turkish Subjects With Those from Workers Occupationally Exposed in Belgium

A summary of the population data and results of AB counts in BALF of Belgian subjects with and without occupational exposure to asbestos is given in Table 3. The AB concentrations among subjects with environmental exposure in Turkey were in the same range as among Belgian subjects with occupational exposure, but the cumulated concentration curve is closer to that of subjects with probable or suspected occupational exposure (NS) than to that of primary asbestos workers

TABLE 2

CONCENTRATION OF AB AND UF IN BALF OF TURKISH INDIVIDUALS WITH AND WITHOUT ENVIRONMENTAL ASBESTOS EXPOSURE (LIGHT MICROSCOPY)

Subjects	AB/ml*	UF/ml*
Control group (n = 42)	0.22 (1.45, 0.09–0.37)	0.51 (1.68, 0.28–0.77)
Asbestos-exposed group (n = 64)†	5.2 (6.22, 2.9–8.8)	7.9 (7.13, 4.45–13.55)
Sex		
Male	7.8 (6.18, 4.0–14.2)	11.6 (7.55, 5.8–22.3)
Female	1.9 (5.14, 0.35–5.2)	3.2 (5.10, 0.94–7.9)
Occupation		
Villagers/farmers (males)	7.6 (7.43, 2.0–24.1)	18.7 (14.32, 3.8–80.5)
Blue-collar	7.3 (5.26, 2.1–21.7)	5.4 (3.21, 2.2–12.0)
White-collar	8.3 (6.52, 2.3–25.3)	12.9 (5.89, 4.2–36.2)
Villagers/farmers (females)	2.0 (6.32, 0.0–9.4)	2.3 (5.37, 0.06–9.2)
Housewifes	1.8 (4.27, 0.0–7.5)	4.6 (5.01, 0.6–18.2)
Disease		
Mesothelioma	5.8 (7.72, 2.0–14.2)	11.1 (9.21, 4.0–28.2)
Bilateral benign pleural disease	7.9 (5.57, 3.5–16.5)	11.0 (8.55, 4.1–27.1)
Without asbestos-related disease	2.2 (4.46, 0.24–7.3)	2.3 (2.45, 0.85–4.8)

* GM (GSD, 95% confidence interval).

† Subject exclusion: For the subject with the highest AB count (1,325 AB/ml BALF and 1,574 UF/ml BALF by LM, and 21,574 fibers with L ⩾ 1 μm/ml BALF by TEM), crocidolite accounted for 95.8% of the AB cores and 97% of the total fibers. The remaining were undifferentiated crocidolite or amosite. This subject reported that he stayed in his village (Kokacimen village, Erzingan district) until 18 and then moved to Erzingan city where he became a butcher. He had mesothelioma. He did not report any known occupational exposure to asbestos. As these results might reflect an unreported or unknown occupational exposure, it was decided to exclude this subject from further analysis of results to avoid bias.

($p < 0.001$, Figure 3). The duration of exposure among environmentally exposed subjects is approximately twice longer than that of subjects with definite occupational exposure ($p < 0.001$, Tables 1 and 3). There was no difference for AB concentration between the Turkish control group and the Belgian blue- or white-collar control subjects of the same mean age.

There were no differences in the cumulative AB concentration curves for environmentally and occupationally exposed subjects with BPD or mesothelioma (Figure 4). All these curves are statistically different from that of the group of occupationally exposed subjects with asbestosis ($p < 0.0001$). Mean age is similar for both groups of subjects with BPD, but duration of exposure is longer for environmentally exposed subjects (43.0 ± 22.3 versus 17.4 ± 10.8 yr, $p < 0.001$). Environmentally exposed subjects with mesothelioma are younger (50.1 ± 12.3 versus 61.4 ± 10.7 yr, $p < 0.005$) but were exposed for a longer time (38.8 ± 18.7 versus 15.1 ± 12.1 yr, $p < 0.001$) than occupationally exposed subjects with mesothelioma.

AB Core Fibers Types

The central fiber of 360 morphologically typical ABs from 32 environmentally exposed subjects was analyzed by TEM. Tremolite accounted for 81.6% of all AB cores analyzed and for 90% or more of the AB cores in 84% of the subjects with environmental exposure. The percentage of the other fiber types were as follows: crocidolite 7.8% (7.0% from two cases), chrysotile 3.1% (2.0% from one case), amosite 3.6% (3.1% from one case), and anthophyllite 0.3%. The remaining 3.6% were ABs built on an unusual fiber. The mean chemical composition (weight percentages of oxides) of this fiber obtained by EDS analysis is as follows: 9.2 ± 1.3% Na_2O, 17.8 ± 1.7% MgO, 0.6 ± 0.2% Al_2O_3, 59.1 ± 1.3 SiO_2, 1.1 ± 0.8% K_2O, 1.0 ± 1.0% CaO, and 11.2 ± 1.9% FeO. Although it contained more magnesium and less iron, this composition is close to that of magnesium crocidolite from Robertstown, South Australia or from Cochabamba province, Bolivia (24). The fiber showed an electron diffraction pattern similar to that of am-

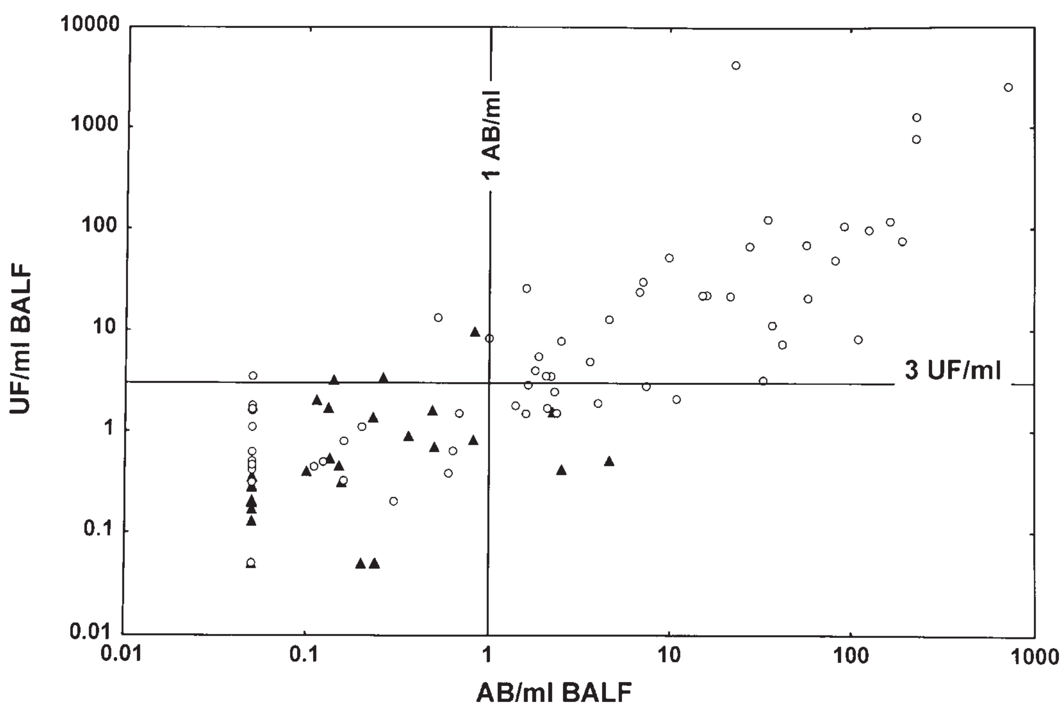

Figure 2. Scatterplot of asbestos bodies (AB) and uncovered fiber (UF) concentrations by light microscopy in the BALF of Turkish subjects with (*n* = 64, *circles*) and without (*n* = 42, *triangles*) environmental asbestos exposure.

TABLE 3

AGE, EXPOSURE DATA, ASBESTOS-RELATED DISEASES, AND CONCENTRATION OF AB IN BALF FROM SUBJECTS WITH AND WITHOUT OCCUPATIONAL EXPOSURE IN BELGIUM

	n	Age (*mean* ± *SD*)	Duration of Exposure* (*mean* ± *SD*)	AB/ml BALF *GM* (*GSD, 95% CI*)
Exposure				
Definite†	230	56.4 ± 11.3	17.4 ± 10.8	20.0 (9.84, 14.6–27.2)
Probable or suspected‡	115	55.3 ± 11.0		2.35 (3.25, 1.70–3.17)
Unexposed blue-collars	126	56.2 ± 9.9		0.37 (1.56, 0.27–0.48)
Unexposed white-collars	129	54.8 ± 14.8		0.13 (1.35, 0.08–0.19)
Disease, exposed subjects				
Asbestosis	61	58.1 ± 8.7	19.6 ± 9.4	123.5 (11.92, 65.0–233.9)
BPD	199	56.9 ± 10.8	17.8 ± 11.2	17.6 (10.09, 12.4–24.6)
Mesothelioma	38	61.4 ± 10.7	15.1 ± 12.1	12.1 (10.68, 5.0–27.6)
Healthy exposed	83	49.6 ± 10.9	15.2 ± 7.1	3.7 (4.22, 2.4–5.4)

* Only available for subjects with definite exposure.
† Primary asbestos workers.
‡ Welders, electricians, plumbers, construction workers, foundry workers, maintenance employees, garage mechanics, shipyard engineers.

phibole asbestos. The composition of this fiber was markedly different from that of current industrial grade crocidolite fibers. For the following discussion this fiber is called "crocidolite-like" fiber. These crocidolite-like fibers were all detected in the BALF of a 72-yr-old woman who always lived in Kirikol village (Erzingan province). She had 7.4 AB/ml BALF and presented with calcified pleural plaques.

EM Fiber Types and Concentrations

A total of 1,727 fibers were characterized by TEM in 16 controls and 59 environmentally exposed subjects. Fibers were counted for all subjects having asbestos-related disease and for randomly selected subjects without such disease. The maximal asbestos fiber concentrations were 1,627 fibers/ml BALF in control subjects and 111×10^3 fibers/ml BALF in environmentally exposed subjects. Asbestos fibers were not detected in the BALF of five (31.3%) controls and two (3.4%) exposed subjects. There were no fibers longer than 5 μm in the BALF fluid of 13 (81.3%) controls and 12 (20.3%) exposed subjects.

Tremolite and chrysotile were the most common types of asbestos identified. Tremolite was observed in 8 (50.0%) controls and 56 (94.4%) exposed subjects and chrysotile in 6 (37.5%) and 29 (49.2%), respectively. Tremolite was present in the BALF of all but one subjects with environmental exposure in whom asbestos fibers were detected. Chrysotile in concentration equaling the analytical sensitivity was the only type of fiber present in the remaining one. Anthophyllite was detected in four environmentally exposed subjects. Crocidolite was detected in four blue-collar workers and one white-collar worker. Amosite was associated with crocidolite in one blue-collar worker. Crocidolite or amosite were present in 2 of the 3 subjects who reported a possible occupational exposure in addition to their environmental exposure. The concentrations of crocidolite and amosite were close to the analytical sensitiv-

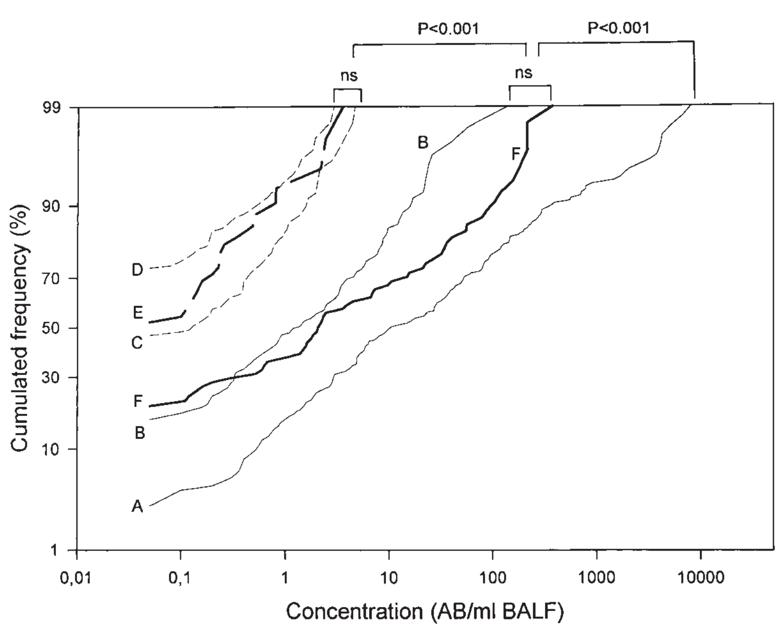

Figure 3. Cumulative frequency curves of AB concentrations in BALF of Turkish subjects environmentally exposed to asbestos (*F*), subjects with occupational exposure in Belgium (*A*: definite occupational exposure, *B*: suspected occupational exposure), and their respective control populations (*E*: Turkish controls, *C*: Belgian blue-collar controls, *D*: Belgian white-collar controls).

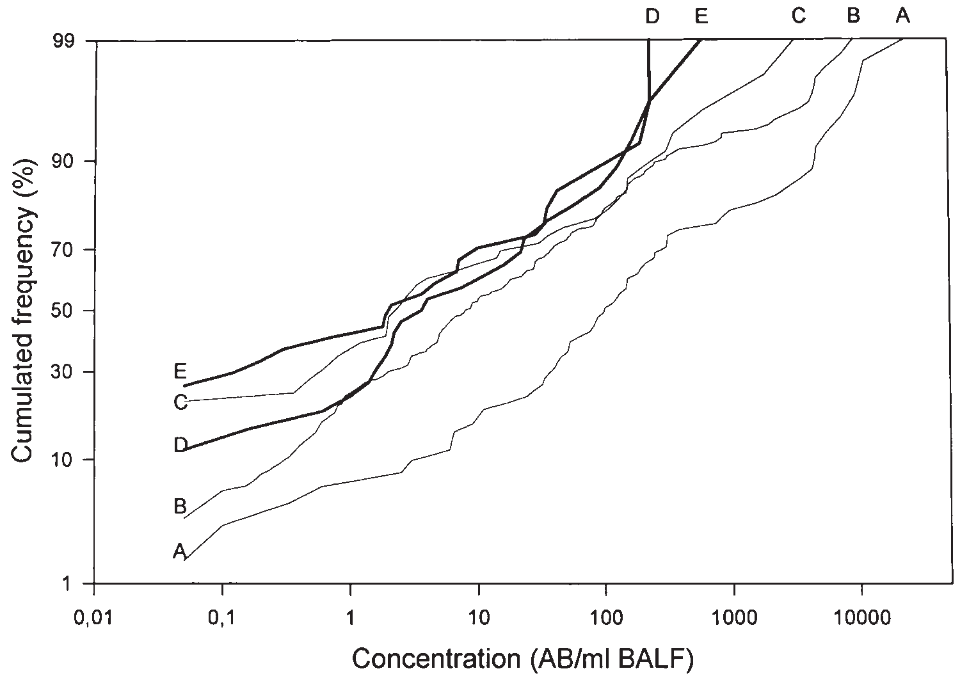

Figure 4. Cumulative frequency curves of AB concentrations in BALF of Turkish subjects with environmental asbestos exposure and mesothelioma (*E*) or benign pleural disease (*D*), compared with that of subjects with occupational exposure in Belgium and mesothelioma (*C*), benign pleural disease (*B*) or asbestosis (*A*).

ity, with a maximum of 270 fibers/ml BALF. GM concentrations of anthophyllite, amosite, and crocidolite were lower than 0.5 fiber/ml BALF both in controls and in environmentally exposed subjects, indicating a very low number of fibers detected when considering the whole groups.

The most frequent type of nonasbestos fibers was prismatic titanium oxide identified as rutile. Rutile is widespread as an accessory mineral in igneous, metamorphic, and sedimentary rocks and soils where it occurs often as microscopic crystals. The "other nonasbestos fibers" class includes mainly silica, feldspar, kaolin- and illite-like fibrous fragments, attapulgite, various silicates, and alumino-silicates, and a few nonsilicate fibers. Most of them are usual rock or soil components. Erionite fibers were not detected.

Total asbestos and tremolite concentrations are lower in controls than in exposed subjects ($p < 0.001$) (Table 4). Concentrations of chrysotile, titanium oxides, and other nonasbestos fibers were not significantly different in controls and exposed subjects.

For environmentally exposed subjects, there were no significant differences in the TEM fiber concentration related to sex, disease (mesothelioma, bilateral BPD, and no asbestos-related diseases), or smoking among males. There was no difference between male villagers and white collars, but both groups had higher GM fiber concentrations than blue collars ($p = 0.039$ and $p = 0.056$, respectively). GM (GSD) concentrations for chrysotile and tremolite fibers longer than 5 μm were, respectively, 0.7 (4.2) and 0.7 (4.3) fibers/ml BALF in control subjects and 1.8 (8.9) and 94 (21.1) fibers/ml BALF in environmentally exposed subjects.

No correlation could be found between the TEM fiber concentration and age, duration of exposure, or delay since end of exposure among environmentally exposed subjects. There were no differences in ratios of [tremolite longer than 5 μm]/[total tremolite] and [chrysotile]/[amphiboles] between environmentally exposed subjects with ongoing exposure and those whose exposure had ceased for more than 1 yr. However, out of the 29 exposed subjects in whom chrysotile was detected, 7 of the 8 with chrysotile concentration $\geqslant$ 500 fibers/ml BALF were still exposed. Among these, three had [chrysotile]/[tremolite] ratio higher than 1 (maximum 6.5). The maximal chrysotile concentration was 6,821 fibers/ml BALF. Twelve subjects

TABLE 4

EM FIBER CONCENTRATIONS* ACCORDING TO EXPOSURE AND GENDER IN TURKISH SUBJECTS

	Chrysotile	Tremolite	Ti oxide	Other Nonasbestos
Controls (n = 16)	4.4 (10.3; 0.6–17.8)	12.0 (15.4; 2.0–54.8)	13.9 (9.1; 3.6–47.5)	32.6 (9.0; 9.4–107)
Environmentally exposed (n = 59)	14.2 (19.6; 6.0–32.0)	444 (11.6; 234–842)	35.6 (14.5; 17.2–73)	52 (11.7; 27.0–100)
Males (n = 43)	12.2 (19.4; 4.3–31.8)	614 (10.1; 301–1,254)	66 (11.4; 30.6–140)	48 (14.4; 20.5–110)
Villagers (n = 16)	28.5 (26.6; 4.1–168)	1,005 (8.6; 318–3,169)	89 (11.8; 23.3–336)	68 (16.0; 14.9–303)
Blue-collars (n = 13)	13.6 (13.6; 2.0–70)	174 (10.5; 42–706)	24.9 (18.2; 3.5–149)	53 (13.0; 10.6–255)
White-collars (n = 14)	3.8 (16.4; 0–23)	1,124 (8.9; 317–3,981)	113 (5.6; 41.3–308)	28 (15.7; 5.0–144)
Females (n = 16)	21.2 (21.5; 3.3–113)	185 (14.4; 44–770)	6.3 (14.9; 0.7–29.6)	66 (6.3; 24–177)

* In fibers/ml BALF, GM (GSD; 95% confidence interval).

out of the 21 having less than 500 fibers/ml BALF had ceased environmental exposure.

Among environmentally exposed subjects, there was a good correlation between the concentration of tremolite fibers measured in TEM and the concentration of ABs and UFs measured in LM ($r_s = 0.68$ [$p = 0.001$] and $r_s = 0.82$ [$p < 0.001$], respectively). A total fiber concentration above 300 fibers/ml in TEM was found in 37 of 59 exposed subjects and 3 of 16 control subjects. Thirty-four of the 37 exposed subjects with such a concentration but none of the control individuals had also more than 1 AB/ml (Figure 5).

Fiber Dimensions

Table 5 reports the size characteristics for chrysotile, tremolite, titanium oxide, and other nonasbestos fibers. The low numbers of amosite, crocidolite, and anthophyllite fibers did not allow any statistical analyses.

Tremolite fibers were thicker ($p = 0.058$), longer ($p < 0.001$) and had higher aspect ratio ($p < 0.01$) in exposed subjects than in controls. Particularly, there was a higher proportion of tremolite fibers longer than 5 μm in exposed subjects than in controls (35% versus 7%: $p < 0.01$). The proportion of chrysotile fibers longer than 5 μm was similar for both groups (19% versus 25%: NS). There is no difference in the size of tremolite fibers and in the ratio [tremolite longer than 5 μm]/[total tremolite] when comparing subjects with BPD to those with mesothelioma. The percentages of long ($L \geqslant 8$ μm) and high aspect ratio ($\varnothing/L \geqslant 32$) fibers were similar in subjects with BPD and with mesothelioma (L: 12.6% versus 14.9%, NS; AR: 9.7% versus 11.9%, NS).

Fiber Composition

Quantitative chemical analysis showed that tremolite fiber composition is the same for fibers in BALF and in soil or whitewash raw materials. Mean composition (mean ± SD) was 21.9 ± 1.2% MgO, 59.9 ± 0.9% SiO_2, 13.9 ± 0.8% CaO, and 3.9 ± 1.3% FeO in BALF and 21.7 ± 1.5% MgO, 60.0 ± 1.2% SiO_2, 13.3 ± 1.4% CaO, and 4.8 ± 2.2% FeO in material samples. There is thus no detectable alteration of the tremolite fiber chemistry after their residence in the lungs or sample preparation.

DISCUSSION

This study is the first evaluation on a large scale of fiber retention in the lungs of subjects from areas of central and eastern Turkey with environmental exposure to asbestos. Fiber analyses in lung and BALF samples provides an individual estimate of past exposure to amphibole fibers. The value of this technique in assessing occupational exposures has been well established (20, 25). Elevated amounts of chrysotile are observed in cases with ongoing or recent exposures (19), but data accumulated over the past 25 yr demonstrate that it is difficult to relate chrysotile lung burden to estimated exposure owing to its faster clearance rate compared with amphiboles (26). Churg and Wright (26) estimated that the preferential clearance of chrysotile, leading to its lower biopersistence when compared with amphiboles, must occur and be completed within weeks to months of exposure.

Analysis of lung fibers has been published for only 36 subjects with environmental exposure to tremolite in various countries (5, 7, 10, 13–17, 27, 28). Lungs of local animals (sheep or goats) (7, 9, 12) have sometimes been used to evaluate the environmental fiber "contamination" in these areas, but the results are difficult to extrapolate to humans with regard to the effects of living activities and age. In humans, detailed and comparable data about fiber concentrations and fiber sizes are not available for all the cases. Concentrations up to 4,250 AB/ml BALF (10), 1.64 10^6 AB/g dry lung tissue (14), 100 10^3 F/ml BALF (17), and 221 10^6 F/g dry lung tissue (7) were reported. These data were obtained in very illustrative patients with obvious diseases. Our data indicate that these values are not representative of the mean but reflect the highest concentrations expected for populations environmentally exposed to tremolite. In the two lung tissue studies where fiber dimensions were reported as GMs, the mean lengths of tremolite fibers were, respectively, 3.7 and 4.3 μm, diameters 0.52 and 0.26 μm, and

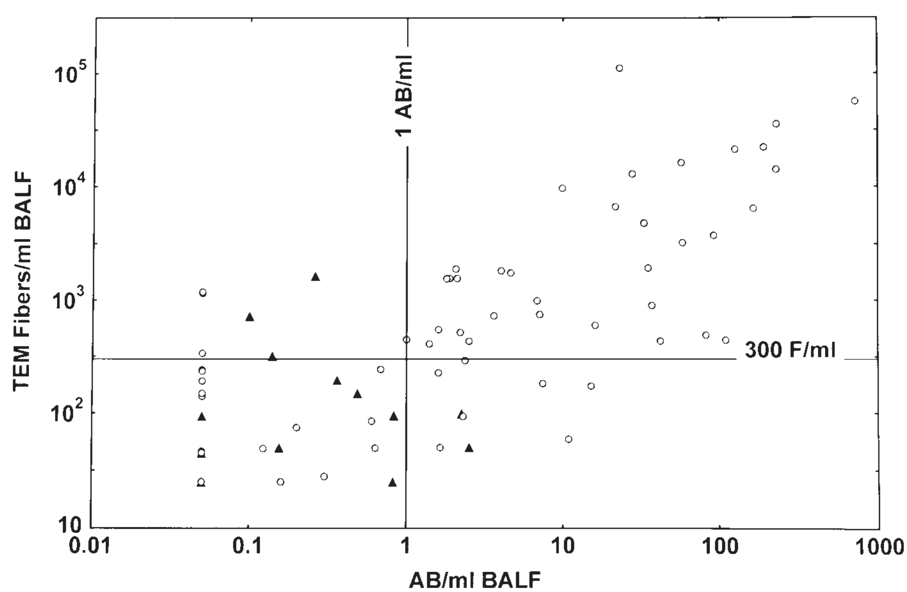

Figure 5. Scatterplot of asbestos body (AB) concentrations measured by light microscopy and total fiber concentrations measured by electron microscopy in the BALF of Turkish subjects with ($n = 59$, *circles*) and without ($n = 16$, *triangles*) environmental asbestos exposure.

TABLE 5

DIMENSIONS OF CHRYSOTILE, TREMOLITE, TITANIUM OXIDE, AND OTHER NONASBESTOS FIBERS IN TURKISH CONTROLS AND IN ENVIRONMENTALLY EXPOSED SUBJECTS*

	Chrysotile	Tremolite	Ti oxide	Other Nonasbestos
Controls				
Number of fibers examined	19	50	18	38
Diameter, μm	0.10 (3.7; 0.05–0.19)	0.22 (2.2; 0.18–0.28)	0.21 (1.8; 0.16–0.28)	0.24 (2.9; 0.17–0.34)
Length, μm	2.8 (1.8; 2.1–3.8)	2.3 (1.7; 2.0–2.7)	1.6 (1.6; 1.2–2.0)	2.1 (2.0; 1.6–2.6)
Aspect ratio, Ø/L	27.3 (2.7; 16.8–24.4)	10.6 (2.1; 8.6–13.0)	7.4 (1.7; 5.6–9.8)	9.3 (2.1; 7.3–11.8)
Environmentally exposed				
Number of fibers examined	177	1,034	156	214
Diameter μm	0.05 (2.2; 0.05–0.06)	0.28 (2.2; 0.26–0.29)	0.23 (2.0; 0.21–0.26)	0.17 (2.4; 0.15–0.19)
Length, μm	2.9 (2.2; 2.6–3.7)	3.8 (2.0; 3.7–4.0)	2.2 (1.7; 2.0–2.3)	2.0 (1.7; 1.8–2.1)
Aspect ratio, Ø/L	53.0 (2.8; 45.5–61.8)	13.8 (2.1; 13.2–14.4)	9.3 (1.7; 8.6–10.2)	12.2 (2.1; 11.0–13.5)

* Size data reported as GM (GSD; 95% confidence interval).

aspect ratios 7.0 and 17.0 (14, 27). Dimensions measured for tremolite fibers in BALF were in the same range.

The main type of asbestos fiber in BALF of Turkish subjects is tremolite. Together with tremolite, elevated concentrations of chrysotile were detected in a small number of Turkish subjects showing that for some cases environmental coexposure to chrysotile also occurs. Interestingly, the highest chrysotile counts were observed in subjects with current exposures. Nonetheless, because tremolite is predominant in most of the whitewash, soil, and air samples analyzed so far it is most likely that airborne chrysotile levels rarely reach those of tremolite and that only a minority of subjects experience significant retention of both fiber types.

The criterion for inclusion in the study was environmental asbestos exposure and not the type of disease. Nevertheless, the majority of the patients enrolled in the group with environmental exposure presented with asbestos-related diseases. Indeed, the recruitment of these patients depended on the population who underwent BAL for clinical purposes in Hacettepe University Hospital. The risk of a recruitment bias is however limited. First, within the environmental exposure group, there are no significant differences in the AB, UF, and TEM fiber counts between subjects with and without detectable asbestos-related diseases. Second, it may be expected from the exposure conditions that a large part of the population would anyway present with asbestos-related diseases. Yazicioglu and coworkers (2) reported that 290 of 1,243 (23.3%) individuals over 50 yr old from southeastern Turkey presented with pleural calcifications and 87 (7%) with fibrosis. Constantopoulos and coworkers (5) reported that 45.5% of the population exposed to tremolite in four villages in northwestern Greece had pleural calcifications. This percentage is raised up to 81% for the individuals over 70 yr old. It would thus have been difficult to find "healthy" volunteers from these areas. Furthermore, some control subjects also presented with pleural abnormalities compatible with asbestos-related lesions.

The absence of differences in the alveolar retention of nonasbestos fibers (rutile, silicates) between Turkish control subjects and environmentally exposed subjects is consistent with qualitative differences in exposure conditions to tremolite. The shorter length of tremolite fiber in control subjects could reflect an indirect environmental exposure to fibers originating from remote sources.

In Turkish people, AB or fiber burdens above 1 AB/ml BALF fluid, 3 UF/ml by LM, and 300 asbestos fibers/ml by TEM reflect environmental or domestic exposure with a high probability, even if the patient does not report or recall the exposure. Fiber chemical analysis is necessary to rule out or confirm additional occupational exposure, especially in blue-collar workers.

Electron microscopy analysis permitted detection of two unexpected exposures, one to crocidolite and another to a crocidolite-like fiber. There was no suspicion of occupational exposures and the subjects were natives of the same district (Erzingan); the composition of the crocidolite-like fiber differed from that of industrial crocidolite fibers. Small amounts of crocidolite have previously been reported in the analyses of sheep and human lungs (7, 12) and it seems possible that environmental exposure to crocidolite can occur in some places in Turkey, although an unreported or unknown occupational exposure cannot be ruled out for one of these subjects.

The AB burdens in BALF of Turkish control subjects without environmental or domestic exposure are in the same range as those observed in Belgian residents without occupational exposure (< 1 AB/ml BALF).

Results clearly demonstrate that environmental or domestic exposures should not be assimilated to low-dose exposures and that an elevated AB or fiber burden in BALF or lung tissue does not always results from an occupational exposure (14, 15). AB concentrations in BALF of subjects environmentally exposed in Turkey are close to those encountered in occupational settings, suggesting comparable cumulative exposures to biopersistent amphiboles. In occupational settings, exposures are limited to about 2,000 h a year, begin and end with employment in the exposing occupation whereas the theoretical exposure duration for environmentally exposed subjects is 8,736 h a year, beginning at birth and ceasing when leaving the village or at death. Whitewashing is thought to be the most important route of exposure to tremolite fibers. The raw material is quarried from the mountains by the male population (2). The application as whitewash is usually done by women who grind the material to a powder and suspend it in water. Whitewashing is repeated each year. Airborne fiber concentrations up to 200 fibers/ml air can be released (29). During sweeping in whitewashed houses, concentrations from 15 to 78 tremolite and up to 5.3 chrysotile fibers/ml air are reached (11). After housekeeping tremolite concentrations fall to 0.03 to 0.1 fibers/ml air. As whitewashing and housekeeping are usually performed by women, one can expect a higher fiber burden in women than in men; however, this is not the case. The contribution of circulation and farming of contaminated soils to the exposure may thus also be significant. The presence of fibers in the lungs of local animals supports this hypothesis as well as the absence of whitewashing or stuccoing practice in Corsica where environmental tremolite–related diseases are also endemic.

If all our study subjects had been exposed to similar fiber concentrations, one could have expected an increase of AB and fiber burdens in BALF with age or duration of exposure. The failure to demonstrate such an increase probably indicates differences in exposure between geographical areas and even between the inhabitants of the same village.

There is a good correlation between the concentrations of ABs in BALF fluid and in lung parenchyma (20, 30, 31). It is thus possible to evaluate the ABs concentrations that can be expected in the lung tissue of the Turkish subjects with environmental or domestic asbestos exposure. According to a pooling of the published correlations, about 70% of them have more than 1,000 AB/g dry lung tissue (LT), 44% more than 5,000, 34% more than 10,000 ABs/g dry LT and 12% more than 50,000 ABs/g dry LT.

All kinds of asbestos-related diseases have been reported after environmental exposure to tremolite. In some cases AB burdens may be very high, comparable to the mean of asbestotic patients (> 100 AB/ml BALF). However, intermediate AB burdens (1 to 10 ABs/ml BALF), in the range of those observed in patients with malignant mesothelioma or BPD without lung fibrosis, are mostly observed (18). The AB cumulative concentration curves in Turkish patients with environmental exposure are consistent with the epidemiological observations that benign and malignant pleural diseases are predominating so that only few cases of asbestosis are to be expected (1, 4).

The conditions in which environmental and/or domestic exposure occurs can be summarized as follows: the presence of tremolite- and chrysotile-bearing rocks (serpentines, ophiolites) and soils and/or the local use of "white soil" (6). The existence of dry or Mediterranean climate probably also plays a role. These conditions can be used as a preliminary basis for the identification of geographical areas at risk. In Turkey, they are probably around 1,000 villages and 5 to 10 million inhabitants potentially endangered by environmental or domestic exposure to asbestos fibers naturally occurring in the soil. In these areas, there is a need for preventive measures. These could include information disseminated to populations about the danger of the domestic use of soil-derived material for whitewash or stucco, avoidance of this practice, covering the walls of whitewashed houses with plastic paints, the identification of the contaminated fields to avoid farming or road building in the worst areas, their conversion into grazing or tree growing places, and the covering of the concerned roads with a safe material. Only such measures will lead to prevention of the numerous cases of environmental asbestos-related diseases in these areas.

In conclusion, our study confirms the geographical extent and the severity of the problem raised by environmental asbestos exposure in Turkey. The main types of asbestos fibers involved are tremolite and, to a lesser extent, chrysotile. Most of the observations on fiber burden in BALF are likely to be also valid for the other areas in the world where the development of asbestos-related diseases after environmental exposure to soil-derived fibers occurs or for migrants originating from these regions. Subjects with environmental or domestic exposure to soil-derived fibers are not exposed to low cumulated doses.

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