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Amphibole Fibres in Chinese Chrysotile Asbestos

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Ten chrysotile bulk samples originating from six Chinese chrysotile mines were studied for amphibole fibres. Five of the mines operate on ultramafic rocks whereas one exploits a dolomite-hosted deposit. The asbestos fibre content in lung tissue was examined from seven deceased workers of the Shenyang asbestos plant using these raw materials. The bulk samples were pretreated with acid/alkali-digestion, and thereafter, scanning and transmission electron microscopy, X-ray microanalysis, selected area electron diffraction and X-ray powder diffraction were used to identify the minerals. Sample preparation of lung tissue involved drying and low-temperature ashing.

All of the bulk samples contained amphibole fibres as an impurity. The amphibole asbestos contents were between 0.001 and 0.310 w-%. Tremolite fibres were detected in every sample but anthophyllite fibres were present only in the sample originating from the dolomite-hosted deposit. In comparison, anthophyllite (71%), tremolite (9%) and chrysotile (10%) were the main fibre types in the lung tissue samples indicating faster pulmonary clearance of chrysotile fibres. The total levels ranged from 2.4 to 148.3 million fibres (over 1 µm in length) per gram of dry tissue, and they were consistent with heavy occupational exposure to asbestos. © 2001 British Occupational Hygiene Society. Published by Elsevier Science Ltd. All rights reserved.

Keywords: anthophyllite; tremolite; asbestos; lung tissue; China

INTRODUCTION

Tremolite fibres are common, natural impurities of chrysotile ore and various mineral products. Despite low concentrations, inhaled amphibole fibres tend to accumulate in the lung tissue and may contribute significantly to the mesothelioma risk of chrysotile miners and millers (Cass, 1991; McDonald *et al.*, 1997). This hypothesis may not extend to other health hazards of chrysotile exposure (Stayner *et al.*, 1996).

China is the world's third largest producer of asbestos following Russia and Canada (Kendall, 1998). China has recorded a total of 467 asbestos locations, the vast majority (95%) of which contain chrysotile asbestos (Lu, 1998). Eleven of the 14 major commercial deposits are hosted in serpentinised ultramafic rocks, while three of them are dolomite-hosted

and located in the north and northeast provinces of Liaoning and Hebei. The latter types occur as groups of tabular and lenticular ore bodies lying along the bedding plane of host rock and are far smaller in size than the ultramafic deposits. In eastern China the dolomite-hosted mines have been more accessible and thus exploited for thousands of years. The oldest artifact of asbestos cloth dates back to 1001-947 BC. The ultramafic chrysotile deposits are located in western China and they contribute 96% of the country's total resources and the bulk of the total production. In 1995 the Chinese production chrysotile from 140 quarries exceeded 440 000 tons per year, of which about 300 000-350 000 tons were consumed domestically (Li, 1986; Huang, 1986; Lu, 1998).

In many asbestos mines and manufacturing plants, air contamination exceeds the national standard of 2 mg/m³ in terms of total dust, with the worst levels in smaller enterprises (Cui, 1999). A national survey recorded 4289 cases of asbestosis in China during 1946-1986 (Anon, 1992). In 16 large asbestos plants, 10.6% of the workers had some form of asbestosis

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(Report on Compensating Asbestos Product Workers, China Nonmetallic Company, unpublished data, 1990). Several studies have reported dose-response relationships between asbestos exposure and asbestosis (Huang, 1993; Wang and Lu, 1985). Lung cancer and mesothelioma are known to occur excessively among Chinese chrysotile workers (Zhu and Wang, 1993; Zou *et al.*, 1990; Chen and Kong, 1992; Fang *et al.*, 1997).

For this study chrysotile samples currently used at the Shenyang Asbestos Material Plant were examined in order to determine the types of minerals present in raw asbestos originating from six Chinese mines. Also lung tissue samples of several workers from this asbestos material plant were analyzed. The level and type of asbestos fibres were determined by electron microscopy. The mortality of the plant workers had been studied earlier. Significantly elevated risks were observed for lung cancer (SMR=2.13) and pneumoconiosis (SMR=16.9) among 528 male workers followed for a period of 25 yr. There was also a positive gradient in the mortality rate for lung cancer according to the cumulative levels of dust exposure (Takahashi *et al.*, 1997, 1998).

MATERIALS AND METHODS

Bulk samples

Ten asbestos samples from the Shenyang Asbestos Material Plant were available for electron microscopic and X-ray diffraction analyses. These raw materials originated from the following six mines: Shimian mine of Sichuan province (Samples 1-3), Xinkang mine of Sichuan province (Sample 4), Mongyi mine of Qinghai province (Samples 5 and 6), Qilian mine of Qinghai province (Samples 7 and 8), Tuoliu mine of Xinjiang province (Sample 9) and Jinzhou mine of Liaoning province (Sample 10). All of these mines are located in western China and they exploit ultramafic rocks except the last sampling site (10), which situates in north-eastern China and belongs to the small dolomite-hosted type of asbestos deposits.

The samples were prepared for scanning electron microscope (SEM) viewing by mixing about 2-3 g of the material with distilled water and filtering a few drops of the suspension through a Nuclepore polycarbonate membrane filter (diameter 37 mm, pore size 0.2 µm). Each piece of the filter was coated with gold in a Bal-Tec SCD 005 Sputter Coater for 100 s. Routine SEM viewing was carried out with a JEOL JSM 6400 electron microscope combined with an energy dispersive X-ray microanalyzer (Tracor Northern TN 5500). The particles and fibres were identified by their relative elemental peak intensities in comparison to the respective mineral standards. The collection time of one spectrum was 20 s and the identification was based on the silicon, magnesium, aluminum, iron and calcium peaks.

For further analyses, the samples were treated with an acid/alkali digestion method (Addison and Davies, 1990) in order to dissolve chrysotile. This was necessary for the detection of any amphibole asbestos in the products. The acid treatment dissolves magnesium from chrysotile and strong alkali was then used to remove the remaining silica. Amphiboles are known to be more resistant chemically. The samples were pre-heated (600°C) before the treatment with acid (2 N H₂SO₄) and alkali (4 N NaOH). The remaining insoluble material was then prepared for the SEM viewing as described above.

The quantity of amphibole asbestos fibres in the residual material was determined in the following way. Approximately 0.5 mg of the dissolution residual was weighed, mixed with distilled water and filtered on a polycarbonate membrane filter. The filters were coated with gold and viewed with the JEOL JSM 6400 at a magnification of 3000×. Each amphibole fibre was identified with the energy dispersive X-ray microanalyzer and fibre lengths and diameters were measured. Only perfect fibres with parallel sides that were at least 5 µm in length, less than 3 µm in diameter and with a length/diameter ratio more than 3:1 were counted. At least 200 fields or 100 fibres were counted, on first to come basis. The weight percentage of the fibres was calculated as follows:

$$\text{Weight\%} =$$

$$\frac{\text{Volume of fibres } (\mu\text{m}^3) \times \text{Density } (\text{g}/\text{cm}^3) \times \text{Area of filtration } (\text{mm}^2)}{\text{Number of fields} \times \text{Field area } (\mu\text{m}^2) \times \text{Amount of sample } (\text{mg}) \times 10}$$

The detection limit of the method is below 0.01 w% (Hartikainen and Tossavainen, 1997).

The minerals present in the remaining material (after acid/alkali-treatment) were also identified by X-ray powder diffractometry (Philips X'PERT System) equipped with a Cu X-ray tube. The powder samples were analyzed at a power of 50 kV and 20 mA with a scanning speed of 1°/min for the diffraction angle from 5 to 70° as well as in a step scanning mode for the more accurate detection of amphibole phases and other minerals (Kohyama *et al.*, 1996). To identify the minor mineral phases, a computer was employed for matching the X-ray diffraction (XRD) peaks with the mineral powder diffraction file (JCPDS, 1989). X-ray diffraction does not differentiate fibrous and non-fibrous forms.

In addition amphibole asbestos fibres were identified by transmission electron microscopy (TEM) and selected area electron diffraction (SAED) (JEOL JEM 1220). Also the differentiation between antigorite and chrysotile was confirmed with TEM observation and SAED patterns (Middleton, 1982).

The presence of amphibole fibres was also verified with a dispersion staining method which is based on the differences between the refractive indices of a particle and the immersion liquid (Anon, 1989). A

polarization microscope (Leitz Laborlux 12 POL S) equipped with a phase contrast system was used for this purpose.

Lung tissue samples

From seven deceased workers of the Shenyang Asbestos Material Plant, several grams of formalin-fixed lung tissue was available for electron microscopic analysis and mineral fibre counting. One worker of the seven was female. The workers were aged from 51 to 72 yr (mean 60 yr) of age at the time of autopsy. The workers had been employed at the plant within the years 1950–1985 with a range of 6–31 working years (mean 21 yr). Three were smokers. All of them were diagnosed with asbestosis (Chinese grade I). These workers worked only in Shenyang asbestos material plant, except for S66, who had also worked in another asbestos plant in the same city for 6 yr. There was no evidence that anyone had an exposure history of anthophyllite asbestos.

Interview data and company records were compiled to establish a lifetime job history for each subject. Environmental monitoring data were available for these workplaces in terms of gravimetric units. Cumulative exposure index (CEI) is defined as the product of exposure duration (yr), exposure level (mg/m^3), the proportion of working hours with asbestos exposure and asbestos percentage in the product, summed over each job (or job site) of the worker.

The preparation of lung tissue samples involved drying, low-temperature ashing, dispersion in 0.5 N hydrochloric acid and filtration on a Nuclepore polycarbonate filter with a pore size of 0.2 μm (diameter 25 mm). A sector of the sample was coated with gold in a sputtering device (Balzers Union SCD004). A blank was prepared and analyzed as a means of contamination control. In addition, formalin fixative was examined to exclude contamination (Tossavainen *et al.*, 2000).

Mineral fibres were counted on the screen with a JEOL 100 CX-ASID 4D electron microscope in the SEM mode at an acceleration voltage of 40 kV. All inorganic particles having a length to width ratio at least 3:1 and parallel sides were defined as perfect fibres and counted (Hartikainen and Tossavainen, 1997). Cleavage fragments were not included. At a magnification of 5000 $\times$, 13–25 fibres were counted or a minimum of 400 viewing fields were analyzed in order to reach an analytical sensitivity of 0.1 million fibres/g. All inorganic fibres longer than 1 μm were recorded. An energy dispersive X-ray microanalyzer (Noran Instruments 5502N) was used to determine the fibre type by comparing peak ratios (Mg, Si, Ca, Fe) to standard spectra. The presence of anthophyllite asbestos was confirmed with X-ray spectra and SAED pattern with analytical TEM. The spectrum of the anthophyllite fibres was similar to the UICC standard. These measures were necessary in order to distinguish fibrous talc from anthophyllite. The concen-

trations of anthophyllite, tremolite and chrysotile fibres per gram dry tissue are reported in the results. The category of chrysotile includes some fibres, which were too fine to be identified for certain. Some of them might be other inorganic fibres and so the subclass 'chrysotile fibres' should be interpreted as the maximum concentration of chrysotile in the samples. 'Total silicate fibres' sums up all the counted fibres (also including some AlSi fibres).

RESULTS

Bulk samples

All of the chrysotile samples contained amphibole fibres as an impurity. Tremolite was identified from every deposit, but anthophyllite was found only in dolomite-hosted chrysotile (Sample 10, Jinzhou, Liaoning). In addition to chrysotile, the samples contained small amounts of other minerals. These minerals included talc, antigorite, quartz, albite and iron oxide (magnetite, haematite). Chrysotile, tremolite, iron oxide and talc were identified with the routine SEM viewing without chemical treatment of the sample. The qualitative results of the bulk samples are summarized in Table 1.

The tremolite contents of the samples were 0.002–0.310 w-% whereas the anthophyllite content (except Sample 10) was below 0.01 w-% (Table 2). On an average 84% of the bulk chrysotile samples (range 78–95%) was leached in the acid/alkali digestion (Fig. 1).

Anthophyllite was the predominant fibrous amphibole in Sample 10 (0.040 w-% anthophyllite, 0.006 w-% tremolite). Between the samples, there was over 100-fold difference in the amphibole content in weight and about 60-fold difference in fibre number. In total 400 tremolite fibres (over 5 μm in length) were counted and measured. The mean length was 8.8 μm and the mean diameter 0.76 μm . The number of counted and measured anthophyllite fibres (over 5 μm) was 38 and their mean length was 14.9 μm and diameter 0.98 μm (Fig. 2).

Lung tissue samples

Anthophyllite and tremolite fibres were the main fibre types in the lung tissue samples. Anthophyllite fibres were detected in every sample and the levels were from 0.2 to 105 million fibres per gram of dry tissue (Tables 3 and 4). The fibre concentrations (over 1 μm in length) were from 0.5 to 11.7 million f/g for tremolite and 1.6 to 15.6 million f/g for chrysotile. Although the fibre levels are very high, there was no obvious correlation between mineral fibre concentrations and exposure doses, age or smoking habits in this series of exposed workers. On average, 71% of all mineral fibres were anthophyllite, 9% were tremolite and 10% chrysotile. The most significant result of the lung tissue analyses was the presence of antho-

Table 1. Qualitative results from bulk samples

Sample no.	Mine (U/O)*, province (type of deposition)	Findings (and analyses)*
1.	Shimian (U), Sichuan (ultramafic rocks)	Chrysotile (1), tremolite(1), antigorite(2,3,4,5), talc(1,2), quartz(3), albite(3)
2.	Shimian (U), Sichuan (ultramafic rocks)	Chrysotile (1), tremolite (2), antigorite(2,3,4,5), iron oxide (2), quartz (3), talc (2)
3.	Shimian (U), Sichuan (ultramafic rocks)	Chrysotile (1), tremolite, antigorite (2,3,4,5), quartz (3)
4.	Xinkang (O), Sichuan (ultramafic rocks)	Chrysotile (1), tremolite (1,2)
5.	Mongyei (O), Qinghai (ultramafic rocks)	Chrysotile (1,3), antigorite (2,4,5), iron oxide (2), talc (2)
6.	Mongyei (O), Qinghai (ultramafic rocks)	Chrysotile (1), tremolite (1,2,3), antigorite (2,3,4,5), quartz (3)
7.	Qilian (O), Qinghai (ultramafic rocks)	Chrysotile (1), tremolite (2), iron oxide (1), antigorite (2,3,4,5), quartz (3), magnetite (3)
8.	Qilian (O), Qinghai (ultramafic rocks)	Chrysotile (1), tremolite (2), antigorite (2,3,4,5), iron oxide(2), haematite (3), quartz (3)
9.	Tuofu (U), Xinjiang (ultramafic rocks)	Chrysotile (1), antigorite (2,3,4,5), tremolite (2,3), iron oxide (2), talc (2), nickel (3), quartz (3)
10.	Jinzhou (U), Liaoning (dolomite-hosted)	Chrysotile (1), anthophyllite (2), tremolite(1,2), iron oxide (2), talc (2,3), dolomite (2), quartz (3), antigorite (2,3,4,5)

*U=Underground mine; O=open casting mine.

*Analyses: 1. SEM=scanning electron microscope; 2. A/A=acid/alkali treatment; 3. XRD=X-ray diffraction of the residual after A/A treatment; 4. TEM=transmission electron microscope; 5. SAED=selected area electron diffraction.

Table 2. Tremolite and anthophyllite fibre (>5 µm) content of the bulk samples

Sample no.	Tremolite content w-%	Tremolite fibres/µg	Tremolite fibre length (µm)		Tremolite fibre diameter (µm)	
			Range	Mean	Range	Mean
1.	0.270	180	5-21	7.3	0.3-2	0.75
2.	0.110	28	5-18.5	9.8	0.3-2	0.85
3.	0.310	185	5-29	7.9	0.3-2	0.73
4.	0.060	38	5-41	9.4	0.5-1.5	0.73
5.	0.034	13	5-63	11.0	0.3-2.5	0.73
6.	0.220	39	5-49	9.9	0.5-2.5	0.90
7.	0.007	4	7-7	7.0	0.8-1.3	1.03
8.	0.002	3	5-6.5	5.8	0.5-0.8	0.60
9.	0.090	44	5-75	10.1	0.3-2	0.83
10.	0.006	5	5-22	9.4	0.3-1.5	0.67
10.	Anthophyllite content w-%	Anthophyllite fibres/µg	Anthophyllite fibre length (µm)		Anthophyllite fibre diameter (µm)	
	0.040	7	Range	Mean	Range	Mean
			5-70	14.9	0.3-2	0.98

phyllite and tremolite fibres, since the persons had been exposed only to Chinese chrysotile. No amosite or crocidolite fibres were detected. The mean values for fibre length/diameter were 9.97/0.14 µm ($N=6$) for chrysotile fibres, 9.01/0.67 µm ($N=121$) for anthophyllite and 8.13/0.44 µm ($N=17$) for tremolite.

DISCUSSION

Most of the fibres found in the lung tissue samples were amphibole asbestos fibres, while the bulk samples mainly consisted of chrysotile. In these Chinese chrysotile products the levels of amphibole impurities were substantial, although the differences between the mines were large. Tremolite was detected in every bulk sample, while anthophyllite asbestos

occurred only in one of the mines (Jinzhou, Liaoning). The geological setting of this mine differs from the others and evidently the dolomite-hosted mines in north and northeast of China are more likely to contain anthophyllite asbestos than the ultramafic chrysotile deposits. Jinzhou mine is located close to the material plant, and therefore its raw material has undoubtedly been extensively used in the past years. The proportions of the used asbestos from each mine are not known.

Few studies have examined impurities of Chinese chrysotile, except for qualitative analyses showing 'little amount' of amphibole in the chrysotile product of Qilian mine (Rong and Peng, 1997). A small amount of tremolite was detected in the chrysotile mine of Chaoyang mine, Liaoning province (Li,

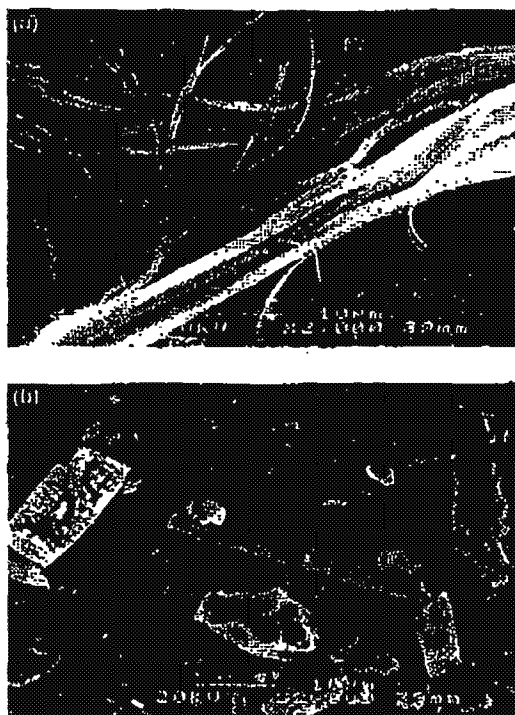


Fig. 1. Typical appearance of a bulk sample observed by SEM: (a) undigested, (b) acid/alkali-digested.

1986). Wang *et al.* (1988) analyzed asbestos fibre types in the lung specimens of 15 Chinese asbestos workers exposed to chrysotile and reported 34 of 35 uncoated fibres to be of the amphibole type and one chrysotile fibre.

Chrysotile fibres tend to leach out from the lung tissue, while the amphibole asbestos fibres are more resistant and remain in the tissue (Finkelstein and Dufresne, 1999). Therefore the exposure to Chinese chrysotile might have resulted in the high level of amphibole fibres in the lung tissue. These amphibole concentrations may have accumulated over a long period of time through the primary exposure to chrysotile having a low percentage of amphibole asbestos as an impurity. Five of the seven samples exceeded the limit of 5 million amphibole fibres (>1 µm) per gram dry tissue which can be associated with a twofold risk of lung cancer (Consensus Report, 1997). In both bulk and lung tissue samples, anthophyllite fibres were somewhat longer and thicker than tremolite and clearly coarser than chrysotile fibres.

Earlier studies from Russia and Canada have suggested high pulmonary levels of chrysotile and tremolite fibres among chrysotile miners and millers. In the study of Asbest, Russia, both chrysotile and amphibole were detected in the lung tissue samples. In 24

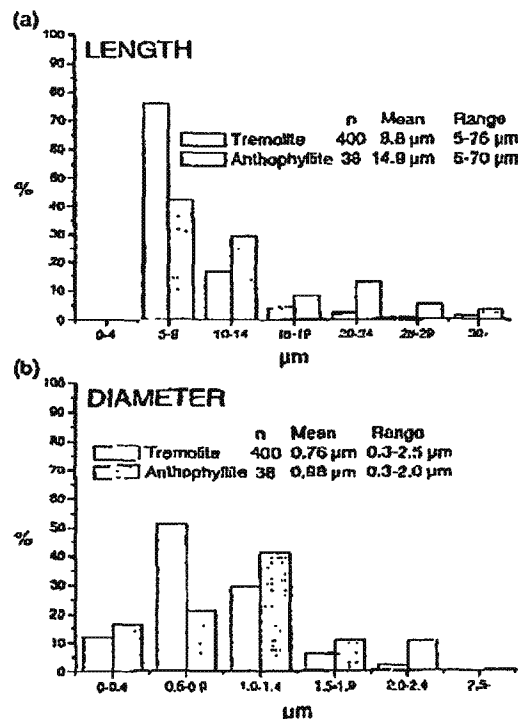


Fig. 2. Size distributions of tremolite and anthophyllite fibres in Chinese chrysotile samples.

Russian chrysotile miners, millers and product manufacturers, the pulmonary concentrations of retained fibres (over 1 µm in length) were 0.8–50.6 million f/g for chrysotile and <0.1–1.9 million f/g for amphiboles (tremolite and anthophyllite). The concentrations were lower in 23 persons without any known occupational contact with asbestos; 0.1–14.6 million f/g for chrysotile and <0.1–0.7 million f/g for amphiboles. On average, 90% of all inorganic fibres were chrysotile and 5% tremolite/anthophyllite (Tossavainen *et al.*, 2000).

In the Thetford area of Quebec, the mean concentration of tremolite in 22 male mesothelioma patients exceeded the chrysotile concentration (105 vs 12.9 million fibres of all lengths per gram dry tissue) whereas in Asbestos, Canada, the corresponding levels were almost equal (3.40 vs 3.54 million f/g) (Green *et al.*, 1997). The tremolite content of the Canadian chrysotile ore is less than 1%. It seems likely that even a small quantity of amphibole fibres in chrysotile asbestos ore constitutes an important source of exposure (Dufresne *et al.*, 1995, 1996; Case *et al.*, 1997).

The use of commercial amphiboles (amosite, crocidolite) has also been related to tissue levels in Canada (Takahashi *et al.*, 1994). No amosite or cro-

Table 3. Background information for the studied asbestos workers in Shenyang asbestos material plant, China

Sample no.	Sex	Age (yr)	Smoking Y/N	Diagnosis of asbestosis (yr)	Cause of death* (ICD-9)	Years of employment	Occupation/work site (yr)	Concentration of airborne dust (mg/m ³)	Cumulative exposure index (CEI) (yr·mg/m ³)
S21	M	64	Y	1978	162	12	Weaving asbestos belt (1954-56)	8.6	25
S22	F	51	N	1978	162	19	Mixing asbestos (1957-66)	20.5	204
S26	M	52	N	1979	162	6	Weaving asbestos belt (1956-61)	15.6	139
S54	M	56	Y	1985	410	29	Splining asbestos thread (1966-75)	24.5	559
S66	M	72	N	1986	162	24	Hot shaping of brake (1959-60)	64.0	203
S68	M	68	Y	1987	434	29	Loading and unloading (1961-65)	20.0	729
S73	M	54	N	1978	491	31	Wind selection of asbestos (1956-63)	46.5	262
							Weaving asbestos cloth (1964-75)	40.0	
							Weaving asbestos belt (1975-85)	2.0	
							Mixing asbestos powder (1950-56)	124.0	
							Making asbestos rubber plates (1957-58)	13.0	
							Repairing machine (1959-74)	2.0	
							Grinding asbestos brake (1950-79)	54.3	
							Hot shaping of brake (1955-57)	64.0	
							Weaving asbestos rope (1956-77)	22.4	
							Storehouse worker (1976-86)	2.0	

*Cause of death: lung cancer (162); cardiovascular diseases (434); ischemic heart diseases (410); chronic obstructive lung disease (491).

Table 4. Mineral fibre concentrations in the lung tissue of asbestos workers in Shenyang asbestos material plant, China

Sample no.	Concentration, million fibres (over 1 µm in length)/g dry tissue			
	Anthophyllite	Tremolite	Chrysotile	Total silicate
S21	22.1	5.9	7.4	35.9
S22	100.4	5.6	5.6	122.8
S26	103.4	11.7	15.6	148.3
S54	7.4	2.0	<0.5	12.2
S66	1.3	2.8	1.6	7.3
S68	5.1	0.5	3.7	11.2
S73	0.2	1.5	<0.2	2.4

cidolite fibres were detected in any of the samples neither from the Russian chrysotile mining area (Tossavainen *et al.*, 2000) nor in this study from China. This could be expected since the geological processes involved in the serpentinization are such that they are unlikely to coexist geologically with chrysotile (Addison, 1999).

In 81 chrysotile samples from various sources, the mean tremolite content was 0.09% with a range from 0.1 to 0.6% in 28 samples where tremolite was detected (Addison and Davies, 1990). Anthophyllite fibres have been detected by XRD and TEM also in Zimbabwean chrysotile (Kohyama *et al.*, 1996).

The EU Regulations will prohibit the importation, use and sale of new and second-hand products containing chrysotile asbestos by the year 2005 (Commission Directive 1999/77/EC). There has been a concern about the health risks associated with amphibole contamination in chrysotile products and materials (Howie, 1999; Addison, 1999). Considering the proposed limit of 0.05% tremolite asbestos in chrysotile (Howie, 1999), the data obtained here (up to 0.31%) suggest that amphibole fibres are significant impurities in some grades of Chinese chrysotile, a fact that has been poorly documented until now.

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