

# Lung-retained Fibre Content in Brazilian Chrysotile Workers

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**Introduction.** Beginning with observations in Québec in 1976, the study of lung-retained fibre has led to an increased understanding of exposure and disease in chrysotile mining areas. Until 2000 this was confined largely to Québec, but recent work has outlined exposure profiles for lung content in Russian, Chinese and South African chrysotile production and factory workers. We now report the first findings from Brazilian chrysotile production and asbestos cement workers with well characterized clinical, exposure and smoking histories.

**Methods.** Subjects working either in the chrysotile mining industry in Brazil ( $n = 6$ ) or in the asbestos cement industry ( $n = 4$ ) were admitted to hospital for investigation of pulmonary lesions. Six had benign infections and four lung cancer, while two also had pleural plaques (both miners) and one cement worker had asbestosis. Portions of formalin-fixed lung tissue obtained at surgery or autopsy were obtained for analysis. Asbestos bodies (AB) were quantified following bleach digestion using phase contrast optical microscopy at  $\times 312$ . Additional portions were examined using transmission electron microscopy at  $\times 14000$ . Seven hundred and four fibres (aspect ratio  $> 3:1$ ) were identified and quantified by X-ray energy dispersive spectrometry in three length classes,  $<5$ ,  $5$ – $20$  and  $>20$   $\mu\text{m}$ , each at a theoretical detection limit of 0.1 fibres/ $\mu\text{g}$  dry lung.

**Results.** AB counts confirmed occupational exposure as proportional to years of exposure ( $r = 0.72$ ,  $P < 0.05$ ), but were far lower than observed in workers elsewhere, possibly due in part to fibre type and to a mean duration of exposure of  $<10$  years. Conversely, non-asbestos 'fibres' were much more common than among chrysotile workers elsewhere, averaging 70% of all fibres counted. Chrysotile accounted for  $>90\%$  of asbestos fibres in all length classes (median 98% of all asbestos fibres; median 4.99 fibres/ $\mu\text{g}$  chrysotile versus 0.11 for amphiboles;  $P < 0.001$ ). As is generally the case,  $<5$   $\mu\text{m}$  fibres were most concentrated (86%). Tremolite was detected in the lung of two mining workers, but only  $>5$   $\mu\text{m}$  in one who worked in a now-abandoned mine known to contain that mineral.

*Keywords:* amphiboles; asbestos; Brazil; chrysotile; lung fibre analysis; tremolite

## INTRODUCTION AND BACKGROUND

Of chrysotile producing countries, Brazil is ranked fourth in the world with an estimated annual output of 170000 metric tons (Virta, 2001), half the production of Canada and  $\sim 25\%$  of Russian production. All has come from two mines, with 10157 persons employed for at least 1 month (Bagatin, 2000). The first, the São Felix mine near the remote location of Poçoões in the state of Bahia, opened in 1940 and closed in

1967. At this location there were never more than  $\sim 4000$  metric tons produced per year, but exposure levels were probably high as workers, often children, obtained the fibre by manual extraction. Overall, only 425 workers were employed at this location alone, with an additional 113 moving to the new location in or after 1967. Recent mineralogical characterization of this mine has demonstrated the presence of tremolite.

In 1967 the larger Cana Brava mine was discovered and developed at Minaçu in the province of Goiás. By 1990 production reached just under 250000 metric tons annually.

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About 90% of Brazilian production has been used in the asbestos 'fibre cement' industry, mostly to make roof tiles and water tanks. Imported fibre was also used in this industry, including crocidolite.

From June 1997 to December 2000 a multi-disciplinary team based at the State University of Campinas (UNICAMP), University of São Paulo and the Federal University of São Paulo conducted a multi-faceted clinical and epidemiological study of the workforces of the two asbestos mining areas. Results of these studies will be published elsewhere. Follow-up for workers at the old mine (São Felix) was limited to 35% of those known to have been employed for at least 1 month. Of these, 28% (54 of 195) had asbestos-related pathology, including 16 cases of asbestosis, one of mesothelioma, one of lung cancer and 36 with pleural plaques. Follow-up has been more complete for those working only in the larger and more recently exploited Cana Brava mine, being 74–95% for workers with at least 5 yr exposure. To date, asbestos-related pathology has been less [1.4% of those followed, of which the majority (0.9%) had pleural plaques on X-ray alone]. Mineralogical analysis using multiple techniques at this mine site has to date not identified amphiboles of any type (M.Cruzen, Institute of Technological Research, São Paulo, personal communication).

Although chrysotile is by far the most used form of asbestos and although attention to differential effects of fibre types was recommended by the UICC in 1964 (UICC, 1965), until very recently most studies of exposure and disease were limited to Canada (see e.g. Gibbs and Lachance, 1972; McDonald *et al.*, 1980; Churg *et al.*, 1993; Dufresne *et al.*, 1996; Case *et al.*, 2000). More recently it has been established that among Canadian miners and millers of chrysotile mesothelioma risk is concentrated among those with co-exposure to tremolite fibre (McDonald *et al.*, 1997) and there has been interest in whether chrysotile mined in other countries showed the same pattern for non-commercial amphibole co-exposure. Studies of a group of chrysotile miners and plant workers in Russia (Tossavainen *et al.*, 2000) and of seven chrysotile plant workers in one location in China (Tossavainen *et al.*, 2001) have demonstrated low, although detectable, tremolite content in lung tissue from the former and very high tremolite and, especially, anthophyllite lung fibre content for the latter. A recent study of South African chrysotile workers reported tremolite levels as 'unremarkable', although in fact it was identified in eight of nine cases and at what we would consider substantial levels in two (Rees *et al.*, 2001).

The current study has as its principal aim a first characterization of the lung content of Brazilian chrysotile workers employed in both the mines and the asbestos cement industry.

## MATERIALS AND METHODS

### *Subjects*

Surgically resected lung tissue samples were evaluated at the University of São Paulo Hospital Pathology Department for 10 workers. These were referred to McGill University in consultation for mineralogical analysis. Six had been employed in the chrysotile mines and four were asbestos cement workers. Age, exposure histories, clinical presentation, pathological findings and smoking habits are outlined in Table 1.

### *Lung samples*

For eight of the cases, two formalin-fixed samples were available from different lobes of the same lung. For the other two a single sample was available. For each sample, lung tissue was dried and the wet:dry ratio obtained as a denominator. For subsequent analysis in the cases having two samples, each was processed separately but the arithmetic mean values for each subject were used.

### *Asbestos body determinations*

A portion of each lung sample was bleach digested as described previously (Case and Sébastien, 1987) and a count of asbestos bodies (AB) obtained from the digestate. AB concentrations are expressed as AB/g dry lung, counted by light microscopy at  $\times 312$  magnification. 'Background' values are unknown for Brazilian subjects: generally an upper limit of 500–1000 AB/g dry lung has been found in previously published work using our methods in North American populations.

### *Lung-retained fibre determination*

The methods have been described previously (Case and Sébastien, 1987; Case *et al.*, 2000), consisting of bleach digestion and low temperature ashing followed by the use of a carbon replica technique. Counts were performed at  $\times 14000$  on a JEOL 100CX transmission electron microscope (TEM) using X-ray energy dispersive spectrometry (EDS) and morphology to identify fibres. For each sample in the current study three separate counts were performed: one of all fibres (aspect ratio  $>3:1$ )  $<5 \mu\text{m}$  in length; a second for fibres between 5 and  $20 \mu\text{m}$  in length; a third for fibres  $>20 \mu\text{m}$ . A total concentration was obtained by summing the three counts and, where applicable, taking the average of the two resulting values for two lung samples per subject. For each of the three length categories in each sample the theoretical detection limit was 0.1 fibres/ $\mu\text{g}$  dry lung (100000 fibres/g).

## RESULTS

Overall, subjects in the two industries did not differ in demographics or exposure history (Table 1). Results for each of the 10 cases are outlined in Table 2. Distribution of fibres by type and length category for all workers (geometric means) and for miners, millers and cement workers separately (arithmetic means and medians) are shown in Table 3. Although the four cement workers had overall arithmetic mean and median concentrations of chrysotile and total asbestos in their lungs exceeding those of miners and millers for every length category, none of the differences were significant. Overall, it can be seen that non-asbestos formed 70% of all fibres, but chrysotile was by far the most common asbestos fibre present in all but two cases. Tremolite was almost completely absent, as were all other non-commercial amphiboles, being identified as  $>5 \mu\text{m}$  in length only in the single case who had worked at the older São Felix mine, known mineralogically to contain tremolite.

Commercial amphibole fibres were present in relatively low concentration in the lungs of five of the 10 workers. These included three of the six miners and millers [two were known to have worked as mechanics at the mine and two had amosite asbestos lung contents in excess of 0.5 fibres/ $\mu\text{g}$  dry lung (500000 fibres/g)]. Two of the four asbestos cement workers had lung contents of crocidolite, a fibre reported by one of us (V.C.) to have been used in the asbestos cement industry in Brazil. In one of these (case 8, the only case with asbestosis) crocidolite was present at a concentration of 1.0 fibres/ $\mu\text{g}$  dry lung (1000000 fibres/g); in the other (case 9) the concentration was only 0.21 fibres/ $\mu\text{g}$ . However, all crocidolite fibres were  $>20 \mu\text{m}$  long. Of interest is the fact that the man with the shortest duration of exposure, only 22 days in the new mine 25 yr prior to surgery, nonetheless had the highest percentage (19%) of chrysotile fibres  $>20 \mu\text{m}$  long in the lung; a fact consistent with previous observations in

Quebec miners and millers (Finkelstein and Dufresne, 1999) and in textile workers exposed to a mixture of fibre types in Charleston, WV (Case *et al.*, 2000).

Fibre concentrations of chrysotile and non-asbestos were correlated (overall  $r$  for total concentrations 0.62,  $P = 0.05$ ), especially for fibres of both types in the 5–20  $\mu\text{m}$  length category ( $r = 0.99$ ,  $P < 0.0001$ ). Within fibre types all lengths of chrysotile were highly correlated ( $r$  all  $> 0.93$ ,  $P < 0.0001$ ), as were commercial amphiboles in the 5–20 and  $>20 \mu\text{m}$  length categories ( $r = 0.85$ ,  $P < 0.01$ ). No fibre type concentration was related to duration of exposure and although all chrysotile length categories were inversely correlated with years since last exposure, none of these correlations were statistically significant. Concentrations of short ( $<5 \mu\text{m}$ ) non-asbestos fibres were also inversely related to years since last exposure, suggesting a clearance effect ( $r = -0.63$ ,  $P = 0.05$ ); non-asbestos fibres in the 5–20  $\mu\text{m}$  range showed similar findings ( $r = -0.61$ ,  $P = 0.06$ ).

Asbestos body concentrations were significantly correlated with duration of exposure in years ( $r = 0.72$ ,  $P < 0.05$ ) and were negatively correlated with time since last exposure ( $r = -0.56$ ,  $P = 0.09$ ). This was true despite the relatively low level of AB concentrations (Table 3), perhaps consistent with the known poorer ability of chrysotile to form these structures.

## DISCUSSION

In the last 2 yr the many studies of lung-retained fibre in Canadian chrysotile workers (including but not limited to Case and Sebastien, 1987; Churg *et al.*, 1993; Dufresne *et al.*, 1996; McDonald *et al.*, 1997; Finkelstein and Dufresne, 1999; Case *et al.*, 2000) have been joined by new studies in Russia (Tossavainen *et al.*, 2000), China (Tossavainen *et al.*, 2001), South Africa (Rees *et al.*, 2001) and now Brazil. It is difficult to compare results from different studies, not

Table 1. Demographics and disease status

	Miners/millers	Asbestos cement workers
<i>n</i>	6	4
Age (mean yr $\pm$ SD)	54.8 $\pm$ 6.7	60.5 $\pm$ 14
Years worked	8.8 (range 22 days–18.0 yr)	8.3 (range 3 months–11.9 yr)
Years since first exposure	29 $\pm$ 8.5 (range 17–39 yr)	24 $\pm$ 6.9 (range 14–30 yr)
Years since end of exposure	18 (range 9–31)	16 (range 14–18)
Smoking (pack-years)	40.5 $\pm$ 57	9.5 $\pm$ 15.7 (NS)
Mesothelioma		
Lung cancer	3	1
Asbestosis		1 (grade 2)
Pleural plaques	2	

Table 2. Data for 10 chrysotile production and cement workers<sup>a</sup>

ID	Job	Diagnosis and other lung lesions	Years worked (yr since first exposure)	Age (smoking) <sup>b</sup>	Chrysotile concentration <sup>c</sup>	Chrysotile in all asbestos fibres >20 µm	Tremolite concentration <sup>c</sup>	Total asbestos <sup>c,d</sup>	Total non-asbestos <sup>c</sup>	Asbestos in total (time since end of exposure)
1	Maintenance worker in both old mine and new mine	Carcinoma lung (small cell)	2.75 (39)	57 (54 py)	0.37	15.3	0	2.42 <sup>e</sup>	9.66	20 (31 yr)
2	Worked 22 days in new mine in 1975	Inflammatory pseudotumour; blastomycosis	0.06 (25)	46 (13 py)	1.11	100	19	1.11	43.06	2.5 (25 yr)
3	Worked in new mine only	Pulmonary nodule (adiaspiromycosis)	3.6 (17)	51 (21 py)	3.35	100	6	3.35	28.07	10.7 (13 yr)
4	Maintenance work only, in new mine only	Carcinoma lung (small cell); pleural plaques	11.9 (30)	64 (150 py)	4.58	98.7	14	4.99 <sup>e</sup>	7.48	40 (19 yr)
5	New mine only	Carcinoma lung (undifferentiated)	18 (38)	51 (5 py)	16.10	91	1	17.70 <sup>e</sup>	8.21	68.3 (12 yr)
6	New mine only	Subpleural nodule; blastomycosis	16.5 (25)	60 (non-smoker)	27.23	100	2	27.23	35.93	43.1 (9 yr)
7	Asbestos cement; also railroad	Adenocarcinoma lung	10 (16)	67 (2 py)	0			0.00	18.78	0 (26 yr)
8	Asbestos cement worker	TB subpleural nodule; Grade II asbestosis	11.9 (30)	69 (3 py)	5.39	84	3.9	6.39 <sup>e</sup>	13.05	32.9 (18 yr)
9	Asbestos cement worker	TB (subpleural nodule); 'lymphoid hyperplasia'	11 (15)	67 (non-smoker)	10.47	98	0	10.68 <sup>e</sup>	42.04	20.3 (15 yr)
10	Asbestos cement worker	Granulomatous lung disease NOS	0.25 (14)	39 (33 py)	91.74	100	4.0	91.74	55.19	62.4% (14 yr)

<sup>a</sup>All figures are in fibres/µg dry lung (million fibres/g) except as indicated.

<sup>b</sup>Smoking expressed in pack-years (py), self-reported to physician.

<sup>c</sup>Total of all three length categories (fibres < 5 µm plus fibres 5–20 µm plus fibres > 20 µm in three separate counts each at a detection limit of 0.1 fibres/µg).

<sup>d</sup>Sum of [chrysotile + tremolite + commercial amphibole (amosite/crocidolite)].

<sup>e</sup>Commercial amphibole (amosite/crocidolite) fibres were detected in lung samples from this worker (actinolite and anthophyllite were not detected).

Table 3. Arithmetic and geometric mean fibre concentrations in lungs of Brazilian chrysotile workers for three fibre length categories<sup>a</sup>

	Chrysotile miners and millers <sup>b</sup>	Asbestos cement workers <sup>b</sup>	All workers combined <sup>c</sup>
<i>n</i>	6	4	10
Asbestos bodies	2826 ± 3463 (median 963)	817 ± 603 (median 841)	916
Chrysotile <5 µm	7.0 ± 8.5 (3.2)	17.9 ± 26.1 (7.6)	11.36 (71%)
Chrysotile 5–20 µm	1.5 ± 2.2 (0.3)	8.0 ± 15.7 (0.2)	4.11 (25%)
Chrysotile >20 µm	0.3 ± 0.2 (0.2)	1.0 ± 1.8 (0.1)	0.57 (4%)
Amphiboles <5 µm	0.4 ± 0.6 (0.2)	0.2 ± 0.4 (0)	0.32 (57%)
Amphiboles 5–20 µm	0.2 ± 0.4 (0)	0.1 ± 0.3 (0)	0.15 (27%)
Amphiboles >20 µm	0.1 ± 0.3 (0)	0.1 ± 0.1 (0)	0.10 (16%)
Non-asbestos <5 µm	20.3 ± 14.6 (18.2)	25.3 ± 12.6 (25.5)	22.31 (85%)
Non-asbestos 5–20 µm	1.7 ± 2.8 (0.8)	7.0 ± 13.0 (0.6)	3.82 (15%)
Non-asbestos >20 µm	0 (0)	0.0 ± 0.1 (0)	0.02 (–)

None of the differences between groups of workers are statistically significant.

<sup>a</sup>Expressed as fibres/µg dry lung, aspect ratio >3:1, except for asbestos bodies expressed as asbestos bodies/g dry lung.

<sup>b</sup>Arithmetic mean ± SD; median concentration in parentheses.

<sup>c</sup>Geometric means; percentages in parentheses represent per cent of total fibres of that type in that size category.

only because of the differences in laboratory techniques and fibre length counting ranges, but also because of inherent differences between the populations under study. For example, this Brazilian group had working lives in these industries averaging <10 years, with only one man developing asbestosis, whereas the single study published from China was of seven long-term factory workers, all with asbestosis, and most of the Canadian studies are of long-term miners and millers. It is nonetheless of interest to compare the groups, at least in terms of the degree of lung non-commercial amphibole content. In that regard, the current series shows a low lung content of tremolite and absence of any other non-commercial amphibole fibre, when compared with any of the published data from other countries, especially Canada (Quebec) and China. Even for the Russian (Tossavainen *et al.*, 2000) and South African studies (Rees *et al.*, 2001), populations in which mesothelioma is reported not to occur to date, most reported lung analyses contain tremolite at levels higher than the detection limit in the Brazilian study. In all of these areas, however, scientific work is in its infancy. Further follow-up is needed both for disease (especially mesothelioma) and exposure assessment. Whether the apparent absence of non-commercial amphiboles remains the case after more prolonged exposures in what is a relatively recent Brazilian mine by international standards (the Cana Brava mine at Minaçu, opened in 1967) and whether the absence of mesothelioma there to date among the cohort of >10000 workers (most of whom are still living) continues will be of interest. The incidental finding not only in 'chrysotile' cement workers but even in miners and millers of some degree of commercial amphibole presence reconfirms the fact that in real work environments pure exposure to chry-

sotile is difficult to come by and further supports the continuing need for clinical follow-up. It is to be hoped that today's workers in Brazil are not subject to the same degree of mixed exposures, as it is well established that in addition to other factors there are important differences in cancer risk (especially for mesothelioma) as related to fibre type (Hodgson and Darnton, 2000).

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