LACK OF TREMOLITE IN UICC REFERENCE CHRYSOTILE AND THE IMPLICATIONS FOR CARCINOGENICITY

A. L. Frank, R. F. Dodson and M. G. Williams
Department of Cell Biology and Environmental Sciences, University of Texas Health Center at Tyler, P.O. Box 2003, Tyler, TX 75710, U.S.A.

INTRODUCTION
In 1935, Lynch and Smith reported that in addition to causing asbestosis, asbestos might also cause lung cancer and soon after Heuper (1942) felt sufficient evidence existed to call asbestos a carcinogen. There is continuing controversy about aspects of asbestos-related disease. Few argue about the carcinogenicity of asbestos; disagreements exist regarding other issues, such as if asbestosis is needed before a lung cancer can be attributed to asbestos (Churg et al., 1984). Some of these controversial issues have been addressed elsewhere by Frank (1994).

One area of controversy has been the ability of chrysotile to produce lung cancer and mesothelioma. In spite of much evidence to the contrary, some still hold to the view that chrysotile, as chrysotile, does not cause these. Central to this belief has been what has been called the “amphibole hypothesis” (Wagner, 1986; McDonald et al., 1989). Certain policy questions may be influenced by the claimed lack of chrysotile’s ability to cause disease. One should recall that some have advocated that only certain fibre types, such as tremolite in chrysotile, produce disease, especially cancer (Churg et al., 1984; Wagner, 1986), but other data have shown this to be untenable (Begin et al., 1992; Stayner et al., 1996). There is now evidence by Karjalainen et al. (1994) of the ability even of anthophyllite to produce mesothelioma.

Few populations have ever been identified where only chrysotile exposure had taken place, but Mancuso (1988) documented mesotheliomas among such workers in the U.S.

Despite evidence to the contrary, some authors (Case, 1991; Dunnigan, 1988; Mossman and Gee, 1989; Mossman et al., 1990) have persisted in putting forth information that is not in keeping with most other scientific evidence.

One reason for the erroneous conclusion that chrysotile does not cause mesothelioma may be that tremolite is more readily found in lung tissue than chrysotile (Churg et al., 1984). Although tissue analysis has been useful for some issues, given the differences between amphibole and chrysotile persistence in vivo, concluding that fibres remaining caused disease is fraught with difficulty. A detailed analysis of the issue of the amphibole hypothesis has recently been published by Stayner et al. (1996), with the conclusion that dismissing chrysotile as a cause of mesothelioma and other asbestos-related diseases is inappropriate.
The study reported here was undertaken to address whether a statement could be made regarding the ability of chrysotile, considered solely as chrysotile, to produce mesotheliomas.

The most widely used standardized preparations of asbestos are those of the International Union Against Cancer. Timbrell and Rendall (1971/2) reviewed their preparation and Timbrell (1970) has published an assessment of their characterisation. There are five samples, one each of crocidolite, amosite and anthophyllite, and two of chrysotile, one from Zimbabwe (then Rhodesia) called chrysotile A and the other a mixture from eight Canadian mines, called chrysotile B.

These reference samples have long been used for in vivo and in vitro experiments. Among the relevant in vivo experiments that are central to this paper is an inhalation experiment of Wagner et al. (1974) which produced lung cancers and mesotheliomas and one reporting mesotheliomas following intrapleural inoculation (Wagner et al., 1973).

It is this finding of mesothelioma after UICC Chrysotile B inhalation or inoculation that is of relevance to this paper. We have not been able to locate any specific report of tremolite contamination of this material and we are not aware of a systematic search for such tremolite. The present report documents the findings of such a detailed evaluation of this specific specimen.

MATERIALS AND METHODS

Aliquots of UICC Chrysotile B obtained from the PRU Johannesburg were used. A representative sample was obtained by combining ten separate subsamples. The combined sample was reduced in size by chopping in filtered (0.1 µm pore) deionized water. The homogeneous suspension was collected on a polycarbonate filter with 0.1 µm pores. The filter was dried, carbon coated and extraction replicas were prepared on 200 mesh grids for electron microscopy.

The replicas were examined at 330 000 x (33 000 x direct magnification x 10 x optical magnification) in a JOEL 100CX analytical electron microscope. The analysis consisted of three phases.

First, 500 randomly selected fibres were identified as chrysotile by morphology and electron diffraction (ED). X-ray energy dispersive spectroscopy (XEDS) was used for additional confirmation. Next, 10 072 additional consecutive fibres were examined for morphology during a linear scan across 81 total grid squares on three grids. The electron beam intensity and diameter were limited to permit observation of the typical tubular morphology and of any subsequent characteristic beam sensitivity. All fibres were determined to be chrysotile. ED and XEDS were used to resolve any questionable morphology.

During the third phase of electron microscopic analysis 50 fibres, purposefully selected at lower magnification because of their "amphibole-like" shape, were sought out; all gave chrysotile electron diffraction patterns.

Polarized light microscopy techniques were used to examine an additional 10 mg aliquot. A Leitz Laborator Lux 12 POL microscope with a 100 watt light source was used. Dispersion staining was performed at 100 and 150 x, while extinction angle, sign of elongation and bright field microscopy were done at 100-400 x.

Extinction angles were measured with a rotary stage scale and an ocular graticule.
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Fig. 1. Transmission electron photomicrographs of (A) UICC Chrysotile B and (B) tremolite asbestos (McCrone). (Marker equals 2 μm; instrument magnification 5000×).

calibrated with nylon (bright) and anthophyllite asbestos fibres. The stated refractive indices of the oils (1.550 and 1.605, Cargile) were confirmed with a Fisher refractometer. Room temperature was maintained at 25°C ± 1°C.
NIST SRM 1867 Tremolite was used as a standard material. Fibres that had a high possibility of being tremolite based upon one or another of the battery of observations were individually eliminated based upon the complete array of tests run with polarizing light microscopy. The tests included refractive index, type of extinction (undulose), sign of elongation, morphology and colour.

Because of the known presence of trace amounts (0.01–0.1%) of calcium (Timbrell, 1970) further testing was done. A solution of 10% hydrochloric acid was placed on an aliquot of the standard and off-gassing was noted. This would lead to the conclusion that calcium carbonate might be present.

RESULTS

No tremolite fibres were identified in the UICC Chrysotile B standard among more than 10 000 fibres evaluated by transmission electron microscopy or in a 10 mg aliquot evaluated by polarizing light microscopy techniques.

DISCUSSION

These results, using the same material that has produced mesotheliomas, lead to the conclusion that chrysotile was uncontaminated by tremolite and chrysotile is capable of producing mesotheliomas.

As noted above, no systematic examination of this material related to possible tremolite content was found in the published literature. Wagner (1986) alluded to work to be undertaken by Pooley in a paragraph that began with the words “Our view that chrysotile, if uncontaminated, is probably a material causing little disease, . . .” has to our knowledge never been reported in print.

Other chrysotile laboratory test materials have not been reported as containing tremolite. Specifically, tests of two NIEHS chrysotile materials from California and Quebec did not report the presence of tremolite (Campbell et al., 1980).

With the use made of the “amphibole hypothesis” to minimize the health implications of exposure to chrysotile, it is curious that this finding, to our knowledge, has not been previously reported. The confounding caused by such, now unsubstantiated, statements have been significant in terms of public health and public policy. The health of workers and those environmentally exposed has been potentially diminished by these views.

There are two policy implications regarding this finding. The first is public policy regarding asbestos in public buildings. Chrysotile has been the major form of asbestos used in buildings and should be considered as a potentially significant public health hazard in that setting (Nicholson, 1991).

Similarly, the second issue also depends on an understanding of the hazards of chrysotile. There has been an effort to move asbestos use from the developed world to developing countries (Frank, 1993). In addition, one should note the concomitant issue of the increased efforts to sell tobacco products in these same settings and its health implications.

The lack of tremolite in one of the best characterised and most widely used chrysotile standards does not support the amphibole hypothesis. The hazards of chrysotile must be fully recognised, and it should not be considered as “a material
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causing little disease" (Wagner, 1986). Until other data to the contrary are offered, this work might even be thought by some to have relevance in the continuing medical and legal matters related to asbestos.

REFERENCES


