

Carcinogenic Implications of the Lack of Tremolite in UICC Reference Chrysotile

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Using light and electron microscopy analysis, as well as electron diffraction, and energy-dispersive x-ray analysis, an aliquot of UICC chrysotile B was analyzed with special attention given to any tremolite contamination. Polarized light microscopy, with its limit of detection of approximately 1 μm when using dispersion staining, revealed chrysotile as the only fibrous asbestos component. Analytical electron microscopy at 333,000 $\times$ of more than 20,000 consecutive fibers showed only the tubular morphology characteristic of chrysotile. These findings highlight that when this sample was used for exposure disease induced in animal models correlates with chrysotile-induced pathology, and does not support an explanation based on the "amphibole hypothesis." Thus, chrysotile should be considered as having the biologic ability to produce cancers, including mesotheliomas, based on the extensive use of this material as a standard reference material. Am. J. Ind. Med. 34:314-317, 1998.

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INTRODUCTION

Few now 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].

Another area of controversy has been over 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 does not cause these diseases. Central to this belief has been what has been called the "amphibole hypothesis" [Wagner, 1986; McDonald et al., 1989]. Other proponents of the "amphibole hypothesis" include Case [1991], Dunnigan [1988], Mossman and Gee [1989], and Mossman et al. [1990]. Certain policy questions may be influenced by the claimed lack of chrysotile's ability to

cause disease. The fact that there is no practical method by which tremolite could be removed from commercial chrysotile should make this a nonissue, but perhaps litigation-related matters have kept this controversy alive. One should recall that some have advocated that only certain fiber types, such as tremolite in chrysotile, play the singular role, or disproportionate share, in producing disease, especially cancer [Churg et al., 1984; Wagner, 1986], but other data have shown this to be untenable [Bégin et al., 1992; Stayner et al., 1996]. Karjalainen et al. [1994] have provided evidence of the ability of anthophyllite to produce mesothelioma, also negating the view that limited the fiber types responsible to crocidolite and, perhaps, amosite. Few populations have ever been identified in which only chrysotile exposure had taken place, but Mancuso [1988] documented mesotheliomas among such workers in the United States.

One possible reason for the conclusion that chrysotile does not cause mesothelioma may be that tremolite, as a contaminant of some asbestos samples, is more readily retained 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*, the conclusion that the fibers remaining caused disease is fraught with difficulty.

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The study reported here was undertaken to evaluate specifically the fibrous materials in UICC chrysotile B and to determine whether a statement could be made regarding the ability of chrysotile to produce mesotheliomas. The work does not look at the issue of the 8% of nonfibrous materials reported by Kohyama et al. [1996]. While the scientific literature is replete with references to tremolite contamination of chrysotile, it is by inference rather than by direct measurement. There have been no reports of specific searches for tremolite in standard specimens prior to 1996 [Frank et al., 1996; Kohyama et al., 1996]; more recently, there has been a further report of the chemical composition of UICC specimens [Bowes and Farrow, 1997].

The most widely used standardized preparations of asbestos are those of the International Union Against Cancer, better known by their French language initials, UICC, from the Union International Contre le Cancer. Timbrell and Rendall [1971/1972] reviewed their preparation, and Timbrell [1970] published on its characterization, but did not use some of the techniques more recently available for detailed and specific fiber-type analysis. 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 central to this paper is an inhalation experiment conducted by Wagner et al. [1974] that produced lung cancers and mesotheliomas and one reporting mesotheliomas after intrapleural inoculation [Wagner et al., 1973]. Others have made use of these materials in an attempt to understand mechanistic aspects of asbestos-related disease [Frank, 1980; Wade et al., 1976, 1979].

It is this finding of mesothelioma following UICC chrysotile B inhalation and inoculation that is of special relevance to this paper. We have been unable to locate any specific report of tremolite contamination of this material, and we are not aware of a systematic search for tremolite, using both light and analytical electron microscopy. The present report documents the findings of such a detailed evaluation of this specific specimen and extends the work of Kohyama et al. [1996] and Bowes and Farrow [1997], as well as our earlier report [Frank et al., 1996].

MATERIALS AND METHODS

Aliquots of UICC chrysotile B obtained from the Pneumoconiosis Research Unit (PRU), Johannesburg, were used. A representative sample was obtained by combining 10 separate subsamples. The combined sample was reduced in size by chopping in filtered (0.1- μ m pore) deionized water. The homogeneous suspension was collected on a polycarbonate filter with 0.1- μ m pores. The filter was dried

and carbon coated, and extraction replicas were prepared on 200-mesh grids for electron microscopic examination. The replicas were examined at 330,000 $\times$ (33,000 $\times$ direct magnification $\times$ 10 $\times$ optical magnification) in a JEOL 100CX analytical electron microscope. The analysis consisted of three phases.

First, 500 randomly selected fibers were identified as chrysotile by morphology and electron diffraction (ED). X-ray energy-dispersive spectroscopy (XEDS) was used for additional confirmation. Next, 20,000 additional consecutive fibers were examined for morphology during linear scans across five 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 fibers were determined to be chrysotile. ED and XEDS were used to resolve any questionable morphology. The data were never consistent with those obtained from a NIST SRM 1867 tremolite asbestos standard.

During the third phase of electron microscopic analysis, 50 fibers, 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-W light source was used. Dispersion staining was performed at 100 $\times$ and 150 $\times$, while extinction angle, sign of elongation, and bright field microscopy were done at 100–400 $\times$.

Extinction angles were measured with a rotary stage scale and an ocular graticule calibrated with nylon (bright) and anthophyllite asbestos fibers. 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 $\pm$ 1°C.

Fibers that had a high possibility of being tremolite, on the basis of one or another of the battery of observations, were individually eliminated based on the complete array of tests run with polarizing light microscopy. The tests included refractive index, type of extinction (undulose), sign of elongation, morphology, and color.

RESULTS

No tremolite fibers were identified in the UICC chrysotile B standard among more than 20,000 fibers evaluated by transmission electron microscopy or in a 10-mg aliquot evaluated by polarized light microscopy techniques.

DISCUSSION

These results, using the same standard reference material reported to produce mesotheliomas, led to the conclu-

sion that chrysotile was uncontaminated by tremolite (at least below the limits discussed above) and that chrysotile therefore must have the capability of producing mesotheliomas. The linkage of animal data and the detailed analysis of the specific reference sample allows for this conclusion. This finding is in keeping with the weight of the evidence as reviewed by Stayner et al. [1996] and, to a lesser extent by Dement [1991].

As noted above, no systematic examination of possible tremolite content of chrysotile was found in the published literature. Wagner (1986) alluded to work that was 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, non-UICC, chrysotile laboratory test materials have also not been reported as containing tremolite. Specifically, tests of two National Institute of Environmental Health Sciences 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 and commented on. The confounding caused by such, now unsubstantiated, statements has been significant in terms of public health and public policy. The health of workers and those environmentally exposed has been potentially endangered by these views.

There are at least 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]. In Great Britain, the amphiboles and chrysotile have been regulated at different levels, while in the United States they are considered as one. The public's health will be best served by reduction of future exposure to the most stringent limits possible. Significantly, France has recently extended its 1977 limitations on the use of asbestos and its 1987 ban on its use for building interiors to a total ban on all uses of asbestos as of January 1, 1997. The British have considered adding their own ban in 1998.

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]. Although production and use move, knowledge about hazards and appropriate safeguards do not generally move in parallel, putting thousands of previously unexposed workers at risk. In addition, one should note the concomitant issue of the increased efforts to sell tobacco products in these same settings, as well as the health implications.

The lack of tremolite in one of the best characterized and most widely used chrysotile standards does not support the amphibole hypothesis. The hazards of chrysotile must be fully recognized, and we question considering its risk to health as "a material causing little disease" [Wagner, 1986].

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