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# Epidemiological Significance of Mineral Fiber Persistence in Human Lung Tissue

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For the experimentalist, mineral fiber persistence may provide clues to disease mechanisms, for the epidemiologist, to the measurement of exposure. Qualitatively, this can be valuable when unsuspected exposures have been demonstrated as, for example, MMMF workers exposed to amosite or chrysotile workers to tremolite. Quantitatively, the potential of lung burden analyses to assess lifetime mineral fiber exposure has yet to be achieved with confidence. The difficulties are 2-fold, the first related to sampling and the second to the dynamics of biopersistence. Until some noninvasive method is found to identify and quantify numerically inorganic fibers in human tissue during life, epidemiological studies must depend on lung samples obtained at autopsy or thoracic surgery. This source is inevitably subject to seriously large and indefinable bias of various kinds. Of equal importance is the present uncertain state of knowledge concerning factors that determine what is present in the lung at any time. These determinants clearly include the dimensional features of airborne environmental particulates and characteristics that affect their durability in tissue. — Environ Health Perspect 102(Suppl 5):221–224 (1994)

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## Introduction

The significance of mineral fibers in lung tissue for epidemiological research depends on whether they can provide a valid indicator, qualitatively and quantitatively, of past environmental exposure, taking into account the feasibility of making such measurements. Since current techniques require lung tissue samples, seldom available except at autopsy or from biopsy taken during major surgery, this raises a critical question. As neither procedure is likely to be based on representative subjects, very serious and indefinable types of selection bias are virtually unavoidable, even in carefully controlled studies. The problem is perhaps reduced by the fact that, in epidemiological research, tissue burden is used to assess exposure and not for pathological diagnosis. Nevertheless, there can be no assurance that sampling efficiency of the lung is unrelated to disease susceptibility, indeed that would be unlikely. The problem would still apply even if all mineral fibers were equally persistent, but this is far from the case; in fact, variation in durability between fiber types may provide the answer to questions of both cause and effect and of disease mechanism.

At first sight the measurement of lung burden provides the ideal biological model whereby exposure, which has inevitably varied in intensity over time, can be integrated with duration. For several reasons, however, this is unreasonably optimistic. While particles identified in the lung at autopsy or biopsy have undoubtedly resulted from past environmental exposure, they are most unlikely to represent a complete or representative sample of what has been inhaled. There is good evidence of the apparently more rapid clearance of one type of fiber (e.g., chrysotile) than another (e.g., tremolite) (1), and perhaps of a differential rate of penetration and retention. There is also the question of exposure duration. Altogether the interactions of penetration, retention, and clearance with various times are complex. A different aspect of the problem concerns the nature of the pathogenic mechanism for the diseases of interest, together with the biological activity of potentially toxic or carcinogenic inhaled mineral particles. If their continued presence in the respiratory tract—lung in particular—directly affects disease incidence or rate of progression, measurements of lung burden would be directly relevant. If, on the other hand, the inhaled particles only transport pathogenic agents, for example chemicals, or react with an agent already present, such as a latent virus or bacterium, retention may be of only minor importance. It is further complicated by possible variation in pathogenic activity of the mineral particles over time, at entry, or after retention.

## Lung as an Air Sampler

In some ways the human lung is an excellent air sampling device and in others, a very bad one. Its strength lies in the ability to accumulate persistent particles selectively over a lifetime, in particular those whose size and shape reach and are retained in the alveoli and thus can damage the lung. On the other hand it is a poor sampling device—a leaking, idiosyncratic sieve that holds only a small, unrepresentative sample of inhaled particles seldom available for analysis until long after inhalation. It is next to useless for particles that, for whatever reason, are poorly retained, and it is, moreover, extremely difficult to calibrate for time variables. Furthermore, only a small, highly selective proportion of lung samples ever become available for analysis, a process that itself is tedious, expensive, and difficult to standardize. Were it not for certain unique qualities, this instrument—the human lung—would clearly have no future; let us therefore consider its merits.

## Sensitivity

In some circumstances the lung can detect airborne pollutants with greater sensitivity, and indeed specificity, than any other sampling device. Although geologists have long known that the host rock for chrysotile deposits in Quebec and elsewhere often was associated with a variety of amphibole minerals (2), the first indication of the potential biological importance of the fibrous tremolite content was obtained by lung tissue analysis (3,4). Later studies showed over time the concentration of

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tremolite fibers in lung tissue of miners and millers exceeded that of chrysotile; the same was true for certain textile workers (1). Similarly, in studies of mesothelioma cases and controls (5), 19 mineral fibers other than asbestos were identified, measured, and counted by electron microscope and X-ray spectrometer. Although none appeared important etiologically for mesothelioma, this may not be true of other diseases or in other circumstances. Conversely, although little convincing evidence was obtained on the pathogenic effect of man-made mineral fibers (MMMf) from lung analyses of former production workers, the unsuspected presence of amosite was demonstrated in a plant where the lung cancer standardized mortality ratio (SMR) was 200 and a case of mesothelioma had occurred (6).

### Accurate Measurement

Lung burden analysis (below) can be used epidemiologically, either to estimate past exposure in individual cases of disease and comparable controls in survey designs, or to assess the nature and intensity of environmental exposure in population studies. In either case the approach is akin to biological monitoring, more typically in the former than the latter where, in effect, human lungs are used as a rather unusual instrument for area sampling. The advantage of using human lung tissue rather than the normal personal or area sampler lies in the collection of what actually enters and is retained in the lung rather than what ought to do so. Subject therefore to the reservations that must be made because of the serious problems listed earlier, lung burden analysis can yield accurate measurements which reflect in some way, as yet incompletely understood, accumulated past exposure to mineral fibers and probably a variety of other mineral particles. This might appear as "damning with faint praise," were it not that most epidemiological research to date on dust diseases of slow progression or long latency has been forced to use estimates of past exposure that are crude and unreliable, both qualitatively and quantitatively.

The main practical difficulties to be overcome are associated with the representativeness of subjects examined, lung sampling methods; and the technical aspects of identification, counting and measurement of particles. These difficulties can be reduced by study design and protocol, but in practice, they largely remain. The declining frequency of autopsy examination and the highly biased nature of tho-

racic surgery widen the gap between what is needed and what is wanted. With care and some luck, serious bias may be avoided but usually at the price of loss in power and discrimination. Analytic methods can be standardized but the results from one laboratory are unlikely to be directly comparable with those from another, or even with those from the same laboratory at another time (7). While these points are not unique to tissue analysis, they are probably among the more difficult to overcome. They require rigorous application of "blind" testing and use of predetermined sequence schedules for the examination of samples.

### Research Experience

During the past decade our group has given considerable priority to lung burden studies in epidemiological research, mainly because of concern for objective and discriminatory measurements of past exposure. We have emphasized elsewhere the uncertainties of this approach and that the results do not necessarily outweigh other types of evidence (8). A brief review of the six surveys we have undertaken may be illustrative and provide some guidance.

### Prevalence Studies

The first prevalence study (4) examined more fully the report by Pooley (3) that the lungs from a small sample of Quebec chrysotile miners who had died or suffered from asbestosis contained almost as much fibrous tremolite as chrysotile. In our survey, lung tissue was selected from 803 autopsies made on 4547 persons who had died in a cohort of almost 12,000 Quebec chrysotile miners. Because we knew full work histories and dust exposures, we were able to select subjects with a wide range of intervals from last employment to death and to exclude those who had lived within 20 miles of the mining area after leaving employment. Lung samples were requested from 140 autopsies (none for deaths from asbestosis or mesothelioma) and 78 were obtained. Initially 47 and eventually 55 (4,9) samples were examined successfully by electron microscopy. Tremolite and chrysotile were found in approximately equal quantities with counts which correlated significantly with accumulated dust exposure: chrysotile, 0.40 ( $p = 0.005$ ); tremolite 0.49 ( $p = 0.0005$ ). Despite unusually favorable circumstances, the autopsies comprised only 18% of all deaths. Of the 140 selected, results eventually were obtained for only 55 (39%), a loss probably less susceptible to serious bias

but, even so, findings based on only 7% (39%  $\times$  18%) success in sampling could well be misleading.

Our second prevalence survey (10) was designed to estimate the lung burden of Canadian males in the general population, by age and level of urbanization, a somewhat ambitious objective. Lungs were collected in 1983 to 1984 from 100 cases in 25 forensic centers—86% from violent deaths and the rest from unexplained causes—and sampled in a standard manner from the upper lobe. The analysis showed higher counts for asbestos bodies and for amphibole but not chrysotile fibers in samples from the larger cities, and an increasing concentration with age. In persons under 19, chrysotile was common, but the level was not related to age. This study avoided the biases of the first survey although accident victims may not have been wholly representative of the general population. The accumulation of amphibole fibers, in contrast to chrysotile, further illustrates the limitations of lung tissue concentrations as an indicator of exposure level.

### Case-Control Studies

We have conducted two investigations of similar design on the mineral fiber content of lung from mesothelial tumor cases and from matched controls; the first was reported in 1982 (11) and the second in 1989 (5). In both studies, case ascertainment aimed at comprehensive national coverage over defined periods with diagnosis based on autopsy or biopsy. Only autopsies were selected for study—172 in the first survey and 93 in the second—with controls matched for age, sex, date of death, type of tissue, and pathology department. In the first survey, lung tissue was obtained and examined from 99 case-control pairs; in the remaining 73 (42%) no tissue was available from case or control or both. In the second survey, requests for tissue were made earlier, with success in 78 of the 83 pairs. Both surveys found much higher concentrations of amphibole fibers in cases than controls, but little difference for chrysotile. In this survey, fiber length was estimated as greater or less than 8  $\mu$ m.

Doubts have been expressed about the interpretation of the findings for chrysotile in these two surveys, since it has been argued that chrysotile has low persistence and the absence of any difference in concentration was to be expected. Against this argument are the findings from our first prevalence survey (4) which suggested that, despite the time lapse and removal

from exposure, tissue concentrations of chrysotile correlated with estimates of accumulated exposure almost as well as those of tremolite. This issue is of major epidemiological importance, particularly in studies of man-made mineral fibers of even lower persistence. Data are very scanty and much more research in this area is needed.

### Cohort Studies

There is probably no cohort study made so far with lung tissue analysis in the initial protocol; however, on two occasions we have made lung tissue analyses in an attempt to better understand the results from studies already completed. The first of these (12) aimed to explain the much greater risk of respiratory cancer observed in asbestos textile workers in Charleston, South Carolina, compared with Quebec miners and millers, both groups exposed to chrysotile from the same source. Lung tissue samples were sought from as many members as possible of both cohorts where an autopsy had been performed at death. Altogether 161 samples were obtained, 72 from textile workers and 89 from miners and millers. These represented only a limited proportion of men on whom an autopsy had been performed and a very small percentage of all deaths. In addition to the five main types of asbestos, many other mineral fibers were identified, counted, and measured. Despite their quality, these findings proved extremely difficult to analyze, mainly because the two case series differed so much in age at death, duration of exposure, and time from first and last employment to death. Efforts were made to deal with this problem by stratification and by selection of pairs matched for duration of and time since last employment. The latter method was perhaps the more convincing, but only 32 pairs met our matching criteria. An important by-product of this study was further evidence that, in both series, concentrations of both tremolite and chrysotile correlated with cumulative dust exposure.

Our most recent survey (6) aimed to assess the past exposure in the large cohort of nearly 17,000 American MMMF production workers (13). This was essentially a study of feasibility for, although MMMF had previously been identified at autopsy

(5,11,14), it seemed doubtful that they would be sufficiently persistent to allow any meaningful estimate of past exposure. In 652 (13%) of the 4840 deaths, an autopsy was recorded, but tissue was obtained from only 145 (28% of identified autopsies, 3% of all deaths). Tissue also was obtained from controls appropriately matched for 124 of the 145 cases. Aside from questions of selection bias, the results were informative in three respects: First, only 26% of workers' lungs contained any MMMF, almost all siliceous in nature and in low concentration. Second, MMMF were slightly more frequent, but not significantly so, in cases than controls. Third, amosite at  $>1.0$  fibers/ $\mu\text{g}$  was found in four of six workers, but in none of their matched controls, in a mineral wool plant, which had the highest lung cancer risk (SMR: 200) and a probable mesothelioma.

### The Tremolite Question

The etiology of mesothelioma in persons exposed to chrysotile is important. A recently completed follow-up of our cohort of nearly 11,000 Quebec miners and millers yielded 33 mesotheliomas in a total of over 7000 deaths, 20 at Thetford Mines, eight at the town of Asbestos, and five from a small factory in the same town (15). Preliminary analyses of incidence in relation to accumulated dust exposure suggest that the risk was higher in Thetford than in Asbestos and higher still in the factory workers. To test whether any difference in risk resulted from higher concentrations of fibrous tremolite in one mining area than in the other, researchers might consider lung tissue analyses of mesothelioma cases in the two areas. However, even if samples could be obtained, any difference in the relative quantities of chrysotile and tremolite in the two groups would be difficult to evaluate because of the clearance of chrysotile with time. A better strategy might therefore be to concentrate on analyses of lung tissue from deaths from other causes—preferably unrelated to asbestos exposure—selected in pairs from the two areas, matched for duration of exposure and time from first employment to death. Alternatively, some form of regression analysis using these same variables might prove more efficient and

avoid the wasteful and possibly biased procedure of matching. Regression analysis was used with apparent success to estimate the difference in tremolite content of the inhaled dust to which miners and millers and textile workers were exposed (12). The matching approach was therefore explored using data from our first prevalence study (4,9). In 13 adequately matched pairs, selected from the 55 lung analyses, the ratios of tremolite to chrysotile ranged from 0 to 8.3 at Asbestos (mean 1.5, median 0.7) and from 0 to 9.0 at Thetford (mean 4.9, median 1.7). The ratio of the means was thus 3.3 and of medians 2.4, suggesting that the level of tremolite exposure at Thetford Mines may have been two or three times higher than at Asbestos.

### Conclusion

Lung tissue analyses can provide information of great specificity on retained mineral fibers, natural or synthetic, in terms of concentration, mineral type and dimensions, which reflect in some almost indefinable way individual lifetime exposure. Such information is confounded by a very wide range in level of biopersistence and, less importantly, variation in penetration of and retention by the respiratory tract. A problem of similar magnitude stems from the selected and unrepresentative nature of lung tissue obtained at autopsy or thoracic surgery on which such studies must depend. Priority should be given to research designed to establish the extent to which mineral fiber concentrations in tissue correlate with lifetime or work-related exposure and in what circumstances. This is particularly important for fibers of low persistence, such as chrysotile and most varieties of MMMF. Without such evidence the use of tissue analyses for epidemiological research may be premature, but where such evidence exists, carefully matched case-control studies appear to be reasonably reliable for etiological investigation. In examining the relative contribution of specific types of fiber after mixed exposures, the use of lung tissue from deaths etiologically unrelated to the disease(s) of interest may be the best approach. Matching or regression analysis can then be used to allow for time-related variables.

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