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INTERPRETATION OF THE CARCINOGENICITY OF AMOSITE ASBESTOS AND FERROACTINOLITE ON THE BASIS OF RETAINED FIBER DOSE AND CHARACTERISTICS IN VIVO

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SUMMARY

Rats were exposed to amosite asbestos and ferroactinolite fibers by intrapleural inoculation and intratracheal instillation. The ferroactinolite sample was found to be more carcinogenic in both exposures than the amosite sample on the basis of total fiber dose or fiber dose expressed for any size category of hypothetical greatest carcinogenic potency. Quantitative transmission electron microscope analysis of low-temperature ashed whole lung samples collected at different times following intratracheal instillation of fibers demonstrated that concentrations and sizes of fibers retained in rat lungs were greatly influenced by the relative ability of each mineral to undergo longitudinal splitting as a consequence of dissolution in vivo. Ferroactinolite fibers rapidly split to produce many thin fibers so that the number of ferroactinolite fibers retained in the lung 2 years after intratracheal instillation was four times greater than the number of fibers originally instilled. The number of short, thin ferroactinolite fibers retained (10-fold more than amosite) after in vivo splitting best explains the greater lung carcinogenicity of ferroactinolite compared to amosite.

INTRODUCTION

A dose–response relationship for asbestos-induced malignant neoplasms in animals [1, 2] and man [3, 4] is generally recognized. Although animal exposures to different asbestos minerals have invariably been interpreted in terms of mass dose, currently popular theories for asbestos carcinogenicity are based on individual fiber characteristics such as size and shape. Pott [5] has suggested a hypothetical model for variable carcinogenic potency in relation to different fiber size categories and emphasized the importance of dose evaluation by measurement of fiber numbers in each size category. The term 'fiber' in this paper refers to mineral particles having

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parallel sides and a length to width (aspect) ratio ≥ 3. Transmission electron microscope analysis of exposure samples containing fibers, as received by the test animals, is required to obtain fiber dose information. Sample preparation procedures must insure that the fibers viewed on the electron microscope are representative of the original concentrations of fibers in each size category.

Evaluation of the carcinogenicity of fibers obtained from a sample of ferroactinolite, an amphibole mineral, relative to fibers of amosite, an amphibole asbestos with known human carcinogenicity, was accomplished by a detailed comparison of fiber doses used in rat intrapleural and intratracheal exposures [14]. The effective fiber dose which best explains differences in mineral fiber carcinogenic potencies may be quite different than the exposure dose. Retention of fibers in tissue over time is influenced by fiber deposition and clearance rates, movement of fibers in tissue, durability of the fibers in tissue, and changes in fiber morphology. Therefore, in this study the number and sizes of fibers retained in the lungs at different times following intratracheal instillation were determined for both ferroactinolite and amosite. We believe this represents the first successful attempt to link fiber carcinogenicity to quantitative measurements of fiber dose retained in the lungs during the lifetime of the test animals.

MATERIALS AND METHODS

The origin, mineral identity, and carcinogenicity of each mineral fiber test sample for intrapleural and intratracheal exposures to rats are described by Coffin et al. (submitted). Ferroactinolite and amosite fiber test samples were prepared for electron microscope analysis by suspending weighed amounts of the fine particles in filtered distilled water. Aliquots of these suspensions were filtered through 47 mm, 0.1 μm pore size Nuclepore* membrane filters. Electron microscope grids were prepared by direct transfer of carbon-coated filter pieces to Formvar-coated grids. The membrane filter and Formvar film were dissolved with chloroform by a wicking action leaving the fibers embedded in the thin carbon film suspended on the grid. This method is identical to the technique commonly used for analysis of particle concentrations in water samples [6].

Pairs of male Fischer-344 rats were killed at intervals of 1, 4, 12, and 24 months following 12 weekly intratracheal instillations of either 0.5 mg ferroactinolite or 0.25 mg amosite. Rats were also killed 1 day following single intratracheal instillations of either 0.5 mg ferroactinolite or 0.25 mg amosite and immediately following single intratracheal instillations of 5 mg of either test sample. The entire lower respiratory tract of each rat was removed and preserved in 10% buffered

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formalin. Electron microscope determination of the concentration and characteristics of fibers retained in the lung was accomplished by preparation of both lungs from each rat (trachea removed at the point where the bronchi enter each lung) as a single, homogenized sample. Each pair of lungs was freeze-dried and then low-temperature-ashed. The ash was suspended in filtered distilled water, aliquots were filtered through membrane filters, and electron microscope grids prepared with the technique previously described for the mineral fiber test samples.

Transmission electron microscopic (JEOL 100C) examination of randomly selected areas on several grids prepared for each sample involved the identification of mineral particles by morphology, selected area electron diffraction and energy dispersive X-ray spectroscopy. Particle size, shape, and mineral identification data were used for computer calculation of fiber size and shape distributions and concentrations. The incidence of large particles which are few in number but represent a large fraction of the sample mass was measured by examining larger areas of the grids for large particles only. The inclusion of this data created data sets which were based on observation of more than 1000 particles per sample. Five rat lung samples analyzed in duplicate produced an average coefficient of variation for fiber concentrations of 25%.

RESULTS

Preliminary electron microscope observation of ferroactinolite fibers obtained from rat lungs one year following intratracheal instillation indicated more fibers retained than expected from studies of the clearance of asbestos from lungs [7]. Many of the fibers present after one year appeared to be thinner than fibers in the exposure sample and chlorite plates common in the original exposure material were conspicuously absent. Larger ferroactinolite fibers frequently displayed numerous thin zones running parallel to the fiber long axis (Fig. 1). These zones are the apparent result of fiber dissolution in vivo. Occasional etch pits on fiber surfaces and leached zones perpendicular to the fiber's long axis were observed as described for acid leaching of mineral fibers [8]. Amosite fibers from lung tissue contained similar evidence of leaching but with fewer longitudinal thin zones.

Fiber concentrations (Table I) determined for lung samples from rats killed at different time intervals after intratracheal instillation of amosite and ferroactinolite confirmed the retention of large numbers of fibers throughout the lifetime of the animals. The four-fold increase in the number of ferroactinolite fibers 2 years after intratracheal instillation, $1.91 \cdot 10^9$ vs. $0.49 \cdot 10^9$ in the exposure, was unexpected. By contrast, the initial amosite dose was $1.71 \cdot 10^9$ fibers and the retained dose declined to $0.43 \cdot 10^9$ fibers 2 years later.

Mean fiber lengths, widths, and aspect ratios (Table I) describe changes in fiber morphology in vivo which parallel the changes in numbers of fibers retained in the lungs. Most striking is the dramatic 4-fold decrease in mean fiber width for

Fig. 1. Typical large fiber recovered from a rat lung 1 year after intratracheal instillation of ferroactinolite. Leaching in vivo produces longitudinal thin zones which act as foci for fiber splitting to produce very thin fibers also seen in this electron micrograph.

ferroactinolite fibers. This change cannot be explained by preferential lung clearance of thicker fibers since there is a 17-fold increase after 2 years in the numbers of ferroactinolite fibers with widths ≤ 0.1 μm. A doubling of the mean aspect ratio for amosite fibers after 2 years is a consequence of a slight decrease in fiber widths.

Both amosite and ferroactinolite fibers appears to undergo longitudinal splitting produced by fiber dissolution while residing in the lung. The ferroactinolite splitting reaction is more rapid and results in the formation of thinner and more numerous fibers than the amosite splitting reaction. The widths of the thin fibers produced correspond to the widths of longitudinal thin zones produced by fiber leaching on wider fibers (Fig. 1).

Fiber concentrations present at different intervals in the rat lungs following intratracheal instillation may be expressed in terms of different definitions for biologically active fibers. Evidence provided by Stanton [9] for greater carcinogenicity for fibers longer than 8 μm and thinner than 0.25 μm has received much attention [10–12]. Ferroactinolite and amosite concentrations for fibers of this

TABLE I

CHANGE IN MINERAL FIBER CONCENTRATIONS IN RAT LUNGS WITH TIME AFTER INTRATRACHEAL INSTILLATION

Time from end of intratracheal instillations	Fibers · 10^6/ mg of mineral instilled	Ferroactinolite			Fibers · 10^6/ mg of mineral instilled	Amosite		
		Mean length (μm)	Mean width (μm)	Mean aspect ratio		Mean length (μm)	Mean width (μm)	Mean aspect ratio
Exposure sample prior to instillation	81	3.18	0.41	9.0	570	3.44	0.29	11.8
0 days[a]	83	3.20	0.36	11.2	417	3.20	0.26	12.6
1 day[b]	107	2.86	0.37	10.8	585	2.60	0.24	10.9
1 month[c]	123	2.10	0.19	17.0	345	2.55	0.23	11.4
4 months[c]	262	2.00	0.17	22.3	393	3.02	0.22	15.7
12 months[c]	366	1.77	0.11	30.1	202	3.62	0.21	20.1
24 months[c]	313	1.64	0.10	25.4	141	3.61	0.20	18.4

[a] Single instillation (5 mg ferroactinolite and amosite).

[b] Single instillation (0.5 mg ferroactinolite, 0.25 mg amosite).

[c] 12 weekly instillations (total ferroactinolite = 6 mg, total amosite = 3 mg).

size category are plotted in Fig. 2. Lung concentrations of amosite fibers meeting Stanton's criterion for carcinogenic fibers decrease over the lifetime of the rats. The ferroactinolite sample has essentially no fibers meeting Stanton's criterion at the time of intratracheal instillation but fiber splitting produces $24.6 \cdot 10^6$ of such fibers in rat lungs 1 year after intratracheal instillation. The corresponding amosite dose at 1 year is $18.6 \cdot 10^6$ fibers. These numbers are calculated by multiplying the

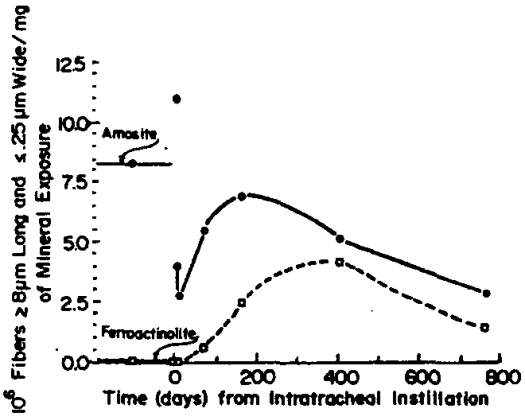

Fig. 2. Concentrations of long, thin fibers in rat lungs following intratracheal instillation. The number of fibers in the size range of length ≥ 8 μm and width ≤ 0.25 μm was determined by Stanton et al. [9] to best correlate with probability of pleural sarcoma following pleural implantation of fibers in rats.

intratracheal mass dose (6 mg for ferroactinolite and 3 mg for amosite) times the fiber concentrations reported in Fig. 2. At both 4 months and 2 years following the last intratracheal instillation of fibers the number of amosite fibers meeting Stanton's criterion slightly exceeds the corresponding number of ferroactinolite fibers.

DISCUSSION

The unexpected strong carcinogenicity of a ferroactinolite sample in comparison to amosite asbestos [14] cannot be explained on the basis of the fiber concentrations used in either the intrapleural or intratracheal exposures to rats. Expression of the fiber doses in terms of a currently popular theory which predicts greater carcinogenicity for long, thin fibers results in an even greater discrepancy between observed and predicted carcinogenicity. For example, if the dose is expressed as the number of fibers longer than 8 μm and thinner than 0.25 μm (Stanton's criterion [9]) and a linear dose response, no threshold relationship is assumed, the amosite sample would be predicted to induce 92 times more tumors in the intrapleural inoculation study and 46 times more tumors in the intratracheal instillation study.

Fiber concentrations retained in rat lungs (long after deposition and subsequent phagocytosis, muco-ciliary clearance, dissolution, splitting, lymphatic and hematogenous transport, and mechanical breakage) better explain the carcinogenic potency of the ferroactinolite. The actual time, during the sequence of events leading from exposure to observation of tumors, which provides the best point at which to measure the accumulated dose of fibers most indicative of a mineral's carcinogenicity is presently unknown. Since the time period from exposure to disease is much longer for man than for rodents, the kinetics of the various biological chemical and physical processes influencing fiber concentrations in vivo may dictate different relative carcinogenicities for mineral fibers in man.

The number of long, thin ferroactinolite fibers present in rat lungs 1 year after intratracheal instillations is approximately equal to the number of long, thin amosite fibers present after 1 year. Ferroactinolite, however, produced significantly more neoplastic lesions in the lung than amosite. In vivo fiber splitting of ferroactinolite produces many short, thin fibers. 1 year after intratracheal instillation there are 10 times more short, thin ferroactinolite fibers than short, thin amosite fibers residing in the lungs (Fig. 3). These data suggest that the number of very thin fibers of all lengths present in tissue is a better determinant of fiber carcinogenicity than the number of long fibers. This observation does not contradict the idea of increased carcinogenic potential of long, thin fibers, but supports and extends the concept of cumulative fiber carcinogenic potency proposed by Pott [5]. A recent statistical reanalysis of Stanton's data suggests that carcinogenicity may be a continuous function of aspect ratio and therefore short, thin fibers are carcinogenic [13].

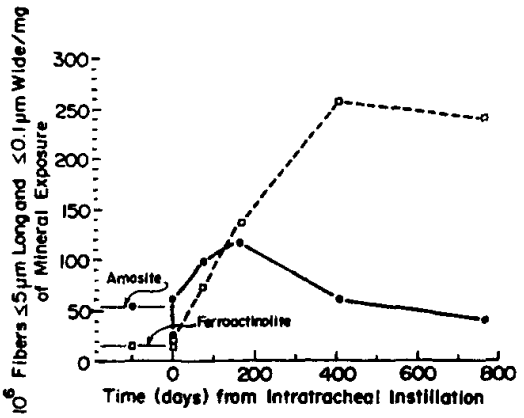

Fig. 3. Concentrations of short, thin fibers retained in rat lungs following intratracheal instillation. Fibers in this size range (length ≤ 5 μm and width ≤ 0.1 μm) are not observed by optical microscopic techniques used for occupational health monitoring of airborne asbestos.

Amosite produced 50% more pleural tumors than ferroactinolite following intrapleural inoculation [14], however, 600% more amosite fibers than ferroactinolite fibers were inoculated. Qualitative electron microscope examination of fibers removed from the pleural cavity months after inoculation indicates that fiber splitting occurs. The probable increase in the number of ferroactinolite fibers may be paralleled by an increase in the number of amosite fibers due to splitting. Very long amosite fibers, which tend to be much wider than shorter fibers, were placed in the pleural cavity and may have produced many very long, thin amosite fibers by splitting. The longest amosite fibers (100–600 μm) were not retained in rat lungs as a result of intratracheal instillation. Intrapleural inoculation studies could be more indicative of true carcinogenic potency if the size distributions of mineral fibers introduced are restricted to the size of fibers likely to be respireable and eventually transportable to the pleura.

We are examining chemical and structural determinants for in vivo fiber durability and dose alteration. We have observed the phenomenon of in vivo fiber splitting for other fibrous minerals such as crocidolite asbestos which produces fibers as thin as the ferroactinolite but at a slower rate. Chrysotile asbestos fiber bundles appear to rapidly dissociate to unit fibrils with diameters of approx. 300 Å and low durability in comparison to amphibole fibers. In addition to lack of durability, many mineral and synthetic fibers do not undergo splitting reactions and therefore are less likely to be human carcinogens when inhaled.

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REFERENCES

1 J.C. Wagner, G. Berry and V. Timbrell, Mesotheliomata in rats after inoculation with asbestos and other materials, Br. J. Cancer, 28 (1973) 173–185.
2 M.F. Stanton and C. Wrench, Mechanisms of mesothelioma induction with asbestos and fibrous glass, J. Natl. Cancer Inst., 48 (1972) 797–820.
3 V.L. Henderson and P.E. Enterline, Asbestos exposure: Factors associated with excess cancer and respiratory disease mortality, Ann. N.Y. Acad. Sci., 330 (1979) 117–120.
4 J. Peto, The hygiene standard for chrysotile asbestos, Lancet (1978) 484–488.
5 F. Pott, Some aspects on the dosimetry of the carcinogenic potency of asbestos and other fibrous dusts, Staub-Reinhalt. Luft, 38 (1978) 486–490.
6 P.M. Cook, I.B. Rubin, C.J. Maggiore and W.J. Nicholson, X-ray diffraction and electron beam analysis of asbestiform minerals in Lake Superior waters, The Institute of Electrical and Electronics Engineers, Inc., New York, NY, Annals No. 75CH1004-1, 34-1 (1976).
7 A. Morgan, R.J. Talbot and A. Holmes, Significance of fibre length in the clearance of asbestos fibres from the lung, Br. J. Ind. Med., 35 (1978) 146–153.
8 T. Zoltai, Amphibole asbestos, Chap. 7, Reviews in Mineralogy, Mineralogical Society of America Vol. 9, 1981.
9 M.F. Stanton, M. Lazard, A. Tegeris, E. Miller, M. May and E. Kent, Carcinogenicity of fibrous glass: Pleural response in the rat in relation to fiber dimension, J. Natl. Cancer Inst., 58 (1977) 587–603.
10 J.M.G. Davis, S.T. Beckett, R.E. Bolton, P. Collings and A.P. Middleton, Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats, Br. J. Cancer, 37 (1978) 673–688.
11 R.C. Brown, M. Chamberlain, R. Davies, J. Gaffen and J.W. Skidmore, In vitro biological effects of glass fibers, J. Environ. Pathol. Toxicol., 2 (1979) 1369–1383.
12 A. Morgan, Fiber dimensions: Their significance in the deposition and clearance of inhaled fibrous dusts, in R. Lemen and J.M. Dement (Eds.), Dusts and Disease, Pathotox, Park Loust South, IL, 1979, pp. 87–96.
13 R. Bertrand, and H. Pezerat, Fibrous glass: carcinogenicity and dimensional characteristics, in J.C. Wagner and W. Davis (Eds.), Biological Effects of Mineral Fibres, IARC Scientific Publications No. 30, Lyon, France, 1980, pp. 901–911.
14 D.L. Coffin, L.D. Palekar and P.M. Cook, Tumorigenesis by a ferroactinolite mineral, Toxicol. Lett., 18 (1982) 143–150.

risk of lung cancer. The experience of per-

TABLE 1

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MORTALITY OF RESERVE MINING COMPANY EMPLOYEES IN RELATION TO TACONITE DUST EXPOSURE[1]

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Analysis of mortality among men who were employed by Reserve Mining Company from 1952 to 1976 has been carried out. Follow-up was conducted with standard methods, including searches by the Social Security Administration. Occupational exposures to dust were based on personal samples taken over the past five years by the industrial hygiene department of the company. Smoking habits were obtained by mailed questionnaires or telephone interviews. A modified life table method was used to compare death rates of the employees with those expected for white males in the state of Minnesota. Comparisons were also made with US rates for white males. The results showed that the death rates for all causes were significantly below expectation. Deaths from malignant diseases were marginally below those expected for the state. Exposures to total dust, to silica dust, or to fiber were low. There was no relationship between mortality and estimated lifetime dust exposures, nor was there any suggestion that deaths from malignant neoplasms were increased after 15 to 20 years latency. In contrast, there was a strong relationship between smoking habits and mortality from all causes, from cardiovascular diseases, and from cancer. This study does not suggest any increase in cancer mortality from taconite exposure.

environmental exposure; mortality; occupational diseases; smoking

Considerable anxiety was created during the 1970s by suggestions that exposure to taconite dust might pose a risk to the health of the general public. This anxiety was focused mainly on the possible risk of taconite in drinking water; but there was also some concern that airborne dust, from industrial plant emissions and from the use of taconite tailings as a surface material for roads, might also be hazardous.

Taconite is a hard, dense rock, composed of silica, silicates, and iron. The iron is in the form of magnetite or hematite and may be either magnetic or nonmagnetic. Taconite from the eastern tip of the Mesabi Range contains amphibole in the cummingtonite-grunerite series, a mineral that is related to amosite asbestos (1). The fibers of taconite are short in length, the vast majority being less than 10 μm. A fiber is defined as any particle with a length-to-width (or aspect) ratio of 3 or greater. Current evidence suggests that short fibers do not cause fi-

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TABLE 2

Hiring periods and ages at first hire for 5751 men who had worked one year or longer for Reserve Mining Company (1952–1976)

Age (years) at hire	1952–1955	1956–1959	1960–1963	1964 or later	Total
<25	275	594	411	1399	2679
25–34	489	560	296	421	1766
35–44	267	287	208	139	901
45–54	58	66	89	128	341
55–64	13	13	7	31	64
65+	0	0	0	0	0
Total	1102	1520	1011	2118	5751
Mean age	31.0 ± 8.2	28.8 ± 8.5	29.6 ± 9.7	25.2 ± 9.3	28.0 ± 9.3
Age range (years)	18–64	17–63	18–57	18–62	17–64

whose vital status was unknown to the company. The vital status of 75 per cent of the former employees who had worked one to four years was established in this way.

In addition, information on vital status was obtained through the Social Security Administration. A list of Reserve Mining Company employees was submitted to that agency. Two separate searches were made during an interval of one year.

Persons not located and not identified as deceased either by the various methods described or through Social Security records were considered to be alive as of July 1, 1976. Of the 5751 men in the group, 59 (1.0 per cent) were not located but were presumed alive, 298 were dead, and thus 5453 were either known alive or presumed to be alive. Death certificates were obtained for all those who had died. Causes of death were categorized by qualified and highly experienced nosologists according to the Eighth Revision of the *International Classification of Diseases, Adapted* (Public Health Service, 1968).

Calculation of expected numbers of deaths

The expected numbers of deaths were estimated by a modified life table method. Person-years of observations were accumulated for five-year age groups over five-year time periods from the date of entry into the life table. Death rates were obtained by dividing the average numbers of deaths which had occurred in the three years around 1955, 1960, 1965, 1970, and 1975 among white males in the state of Minnesota by the census populations (or their linear interpolations for mid-decade years) and these rates were applied to the appropriate time periods. The number of deaths actually observed divided by the number of deaths expected for all causes and for specific causes is expressed as a percentage or standardized mortality ratio (SMR) by multiplying the quotient by 100; thus,

$$\text{SMR} = (\text{Observed deaths/expected deaths}) \times 100.$$

The significance of a difference from 100 was estimated from the confidence limits assuming a Poisson distribution (2). Selected causes of death used in this analysis are shown in table 3.

Assessment of dust exposures

Concentration of total respirable dust ranged from a low of about 0.02 mg/m^3 in a heavy-duty truck driver engaged in surface maintenance to 2.52 mg/m^3 in a balling line operator in the pelletizing plant and 2.75 mg/m^3 in a pan feed operator in the fine crusher. Values of 1–2 mg/m^3 were reported occasionally for men who worked in the powerhouse, on the

TABLE 3

Selected causes of mortality in the state of Minnesota with accompanying codes from the Eighth Revision of the International Classification of Diseases, Adapted *(ICDA)*

Description of cause	ICDA code	Comment
All causes	000–E999	
Cardiovascular	402, 404, 410–429	
All cancer	140–209	
Respiratory cancer	160–163	
Digestive cancer	150–159	
Urinary cancer	188, 189	
Genital cancer	180–187	
Selected respiratory diseases	470–474, 480–486, 490, 491, 493, 510–519	Influenza, pneumonia, bronchitis, emphysema, asthma, other respiratory
Most trauma	E800–E978	Excludes undetermined and war-related injury
Motor vehicle	E810–E823	

coal conveyors, and for conveyor men in the yards and docks. Values above 1 mg/m^3 were, however, infrequent, the modal range in most occupations being 0.2–0.6 mg/m^3.

Threshold limit values depend on both dust concentrations and silica content of the dust. These values were sometimes exceeded in samples from the crushing departments. The relatively few measurements of fiber which had been made indicated that fiber concentrations were usually low. Concentrations greater than 0.5 fibers per ml were occasionally observed, mainly in the crushing departments, but none approached the threshold limit value of two fibers per ml.

Estimates of dust exposure have been based on personal samples, which were collected between 1975 and 1978. This was considered to be a valid procedure since analysis of fixed-site dust samples over the whole period, from 1952 to 1976, did not indicate any marked trends in dustiness over the years. Furthermore, there were no major processing changes which might have led one to expect any marked changes. All samples were collected during four to eight hours of continuous air sampling, most of them being

TABLE 4

Mortality for selected causes for men who had worked one year or longer for Reserve Mining Company, compared with white males in Minnesota, 1952–1976

Selected causes of mortality	Deaths		Standardized mortality ratio	95% confidence limits
	Observed	Expected		
All causes	298	343.65	87*	77, 97
Cardiovascular	112	123.79	90	74, 109
All neoplasms	58	63.38	92	69, 118
Respiratory cancer	15	17.94	84	47, 138
Digestive cancer	20	17.57	114	70, 176
Urinary cancer	3	2.97	101	20, 295
Genital cancer	3	3.31	91	18, 265
Selected respiratory diseases	4	6.80	59	16, 151
Most trauma	76	72.76	104	82, 131
Motor vehicle	38	31.12	122	86, 168

* $p < 0.05$.

full eight-hour shift samples. For each sample, total respirable dust and silica dust were expressed in mg/m^3.

Personal samples had been taken for 38 per cent of all coded jobs. Jobs which had not been sampled (19 per cent) but which shared the same work code area as a sampled job, based on time and motion studies, were assigned the same dust concentration as the appropriate sampled job. Two procedures were used to estimate the concentrations of the remaining jobs. For those occupations for which the extent of involvement in production was uncertain (carpenters, painters, supervisors—which comprised 36 per cent of all jobs), the mean value of the department was assigned. For those jobs outside the high production area (clerical and office workers—which comprised 8 per cent of all jobs), the lowest value of the appropriate department was assigned.

Once an estimate of dustiness of each job had been made, dust exposure was calculated from the occupational histories of each employee by summing the years of exposure in each job over a working lifetime and dividing by the total number of years worked to obtain a time-weighted lifetime average dust exposure. For each employee, estimates were therefore available of total respirable dust exposure and of silica dust exposure.

Results

Observed and expected deaths and standardized mortality ratios (SMRs) for all men who had worked for one year or longer for Reserve Mining Company from 1952 to 1976 are shown in table 4. The standardized mortality ratio of 87 for all causes of death is significantly below that expected for the state of Minnesota ($p < 0.05$). This is most likely because of the "healthy worker" effect which occurs when essentially healthy working people are compared with all persons in the general population, which will include persons who are too ill or otherwise unable to work. Cardiovascular disease (SMR = 90) was the main cause of death contributing to the low overall standardized mortality ratio; however, there was also a deficiency of deaths due to selected respiratory diseases. Whereas seven deaths might have been expected from these causes, only four were observed. This suggests that respiratory disease is not a problem among employees of Reserve Mining Company. The standardized mortality ratio for all malignant neoplasms was low, 92 when compared with the state and 79 when compared with the United States. Ratios for cancers at particular sites were close to expectation, with respiratory cancer slightly below and digestive tract cancer slightly above expectation. These deviations from expectation were statistically insignificant.

Mortality in relationship to time since first exposure (latency)

Various periods since first hire have been examined to test the possibility that neoplasms may be increasing after a certain latency. Thus, standardized mortality ratios after 10, 15, and 20 years since first hire have been explored. Table 5 summarizes the findings assuming a latency of 15 years. There is no indication that cancer in general or cancer at any particular site is increasing after such an induction period. For all neoplasms, the standardized mortality ratio was lower in those surviving 15 years since first hire than in those surviving less than 15 years. Furthermore, the upper limit of the 95 per cent confidence band was 117, which is reasonably low. For specific sites, agreement with expectation is remarkably close. For those with a latency of 20 years and over (not shown), there were of course very few deaths, but again the numbers observed were close to expected. For all cancers, 5.97 deaths were expected, whereas five were recorded, giving a standardized mortality ratio of 84 with a 95 per cent confidence band of

TABLE 5

Mortality for selected causes according to years of survival since first hire for men who had worked for Reserve Mining Company one year or longer, compared with white males in Minnesota (1952–1976)

Selected causes of mortality	Survival since first hire							
	Less than 15 years (no. = 5751)				15 years or more (no. = 2893)			
	Deaths		Standardized mortality ratio	95% confidence limits	Deaths		Standardized mortality ratio	95% confidence limits
	Observed	Expected			Observed	Expected		
All causes	195	228.93	85*	74, 98	103	114.63	90	73, 109
Cardiovascular	66	76.37	86	67, 110	46	47.42	97	71, 129
All neoplasms	40	39.12	102	73, 139	18	24.26	74	44, 117
Respiratory cancer	7	10.07	70	28, 143	8	7.87	102	44, 200
Digestive cancer	12	10.79	111	57, 194	8	6.78	118	51, 233
Urinary cancer	2	1.71	117	13, 422	1	1.26	79	1, 442
Genital cancer	3	2.04	147	30, 430	0	1.27		0, 289
Selected respiratory diseases	2	4.54	44	5, 159	2	2.26	88	10, 320
Most trauma	64	59.48	108	83, 137	12	13.28	90	47, 158
Motor vehicle	33	26.77	123	85, 173	5	4.35	115	37, 268

*$p < 0.05$.

from 27 to 195. For respiratory cancer, two cases were expected and two cases were found, while for digestive cancer, 1.7 cases were expected and three were found. Such agreement with expectation makes any increase in cancer after a 20-year latency very unlikely.

Mortality was uninfluenced by age at first hire, time of first hire, and by the division of the company (Babbitt or Silver Bay) in which the man worked. (Additional tables to support these statements are available on request from Dr. Ian T. T. Higgins.)

No case of mesothelioma either of the pleura or of the peritoneum was recorded. All diagnoses that could conceivably have been confused with mesothelioma were carefully scrutinized. We do not believe that any such cases were missed. One man was reported as having died of a retroperitoneal tumor. However, in his case, a biopsy had confirmed this to be a leiomyosarcoma.

Mortality by cumulative lifetime dust exposure

Standardized mortality ratios for all causes and for several specific causes according to cumulative exposure to total dust and to silica dust are shown in tables 6 and 7, respectively. There are no striking trends. Deaths from all causes among men with a cumulative exposure of 1000–3000 total dust-years or of 500–1000 silica dust-years was significantly low ($p < 0.05$). This may be partly due to the concurrent measurement of exposure and mortality. Clearly, increasing exposure depends on survival. Those who die early do not have the same opportunity to accumulate dust as those who die later or who survive throughout the observation period. An inevitable bias will occur in which those who survive longest and experience the lowest mortality may acquire the greatest dust dosage. One solution to this problem is to separate the period when dosage is being assessed from the period when mortality is being measured. We have attempted to do this in the present analysis by categorizing dust exposures during the first year, the first two years, the first five years, and the first 10 years of employment with Reserve Mining Company. We then measured mortality during the remainder of the follow-up period. For those with 10

TABLE 6

Mortality for selected causes according to cumulative total dust exposure (mg/m³ × years) for men who had worked for Reserve Mining Company one year or longer, compared with white males in Minnesota (1952–1976)

Cause of death	Not exposed		<100		100–		1000–		3000+		Total	
	Deaths	SMR†	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR
All causes	34	90	10	90	103	101	87	77*	64	80	298	87*
All neoplasms	5	70	1	53	19	109	21	102	12	74	58	92
Respiratory cancer	0		1	219	5	110	4	69	5	98	15	84
Gastro-intestinal cancer	0		0		6	128	9	157	5	110	20	114
Cardio-vascular	15	101	3	80	35	107	29	72	30	94	112	90
Person-years	6284		2798		29199		25895		13224		77400	

* $p < 0.05$.
† SMR, standardized mortality ratio.

years of dust exposure, a maximum of about 15 years of follow-up was possible. We did not consider that a shorter follow-up period than this would be worthwhile and have therefore restricted the time for dust accumulation to a maximum of 10 years.

Observed deaths and standardized mortality ratios by categories of dust exposure and duration of survival since first hire based on total respiratory dust exposure in the first year of employment and to silica dust exposure in the first year are shown in tables 8 and 9, respectively. Neither these tables, nor the additional ones for the longer exposure periods (not shown), suggest that there was any association between mortality and exposure to dust.

Smoking and mortality

Using mailed questionnaires, we tried to obtain information on smoking habits from all persons who were included in the

TABLE 7

Mortality for selected causes according to cumulative silica dust exposure (mg/m³ × years) for men who had worked for Reserve Mining Company one year or longer, compared with white males in Minnesota (1952–1976)

Cause of death	Not exposed		<100		100–		500–		1000+		Total	
	Deaths	SMR†	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR
All causes	34	90	91	90	115	86	43	74*	15	104	298	87*
All neoplasms	5	70	17	98	22	91	11	94	3	97	58	92
Respiratory cancer	0		4	88	3	44	6	167	2	193	15	84
Gastro-intestinal cancer	0		9	192	6	89	4	124	1	117	20	114
Cardio-vascular	15	101	31	94	40	84	18	79	8	136	112	90
Person-years	6284		27789		31427		9889		2015		77400	

* $p < 0.05$.
† SMR, standardized mortality ratio.

TABLE 8

Mortality for selected causes according to cumulative total dust exposure and duration of survival since first hire for men who had worked for Reserve Mining Company one year or longer, compared with white males in Minnesota (1952–1976)

Selected causes of mortality	Survival since first hire											
	Less than 10 years						10 years or more					
	1-year average dust exposure						1-year average dust exposure					
	Low or none (no. = 2235)		Medium (no. = 2372)		High (no. = 1094)		Low or none (no. = 1693)		Medium (no. = 1869)		High (no. = 770)	
	Deaths	SMR†	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR
All causes	52	84	52	84	15	87	87	94	77	86	15	69
Cardiovascular	17	86	18	93	3	90	35	93	34	94	5	64
All neoplasms	11	109	9	90	1	45	18	96	16	87	3	72
Respiratory cancer	1	42	0		1	271	5	87	7	122	1	80
Digestive cancer	5	175	3	109	0		5	94	5	98	2	183
Urinary cancer	1	224	1	244	0		1	105	0		0	
Genital cancer	2	347	0		0		1	97	0		0	
Selected respiratory diseases	0		0		0		0		3	172	1	272
Most trauma	17	100	24	132	10	123	15	123	7	55	3	68
Motor vehicle	8	102	13	155	6	143	8	160	0	65	1	58

† SMR, standardized mortality ratio.

TABLE 9

Mortality for selected causes according to cumulative silica dust exposure and duration of survival since first hire for men who had worked for Reserve Mining Company one year or longer, compared with white males in Minnesota (1952–1976)

Selected causes of mortality	Survival since first hire											
	Less than 10 years						10 years or more					
	1-year average silica exposure						1-year average silica exposure					
	Low or none (no. = 1043)		Medium (no. = 3736)		High (no. = 972)		Low or none (no. = 850)		Medium (no. = 2706)		High (no. = 776)	
	Deaths	SMR†	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR
All causes	27	85	72	82	20	94	46	83	111	94	22	75
Cardiovascular	13	119	22	86	3	51	20	86	47	100	7	63
All neoplasms	6	112	11	80	4	122	9	79	25	104	3	52
Respiratory cancer	0		1	32	1	136	1	23	11	148	1	55
Digestive cancer	3	192	3	81	2	240	4	122	6	90	2	128
Urinary cancer	0		2	359	0		0		1	84	0	
Genital cancer	2	688	0		0		0		1	84	0	
Selected respiratory diseases	0		0		0		0		2	86	2	392
Most trauma	6	76	34	121	11	150	8	119	13	74	4	78
Motor vehicle	3	87	19	141	5	143	3	128	5	78	13	157

† SMR, standardized mortality ratio.

TABLE 10

Mortality for selected causes according to reported smoking habits for 3439 men who had worked for Reserve Mining Company five years or longer, compared with white males in Minnesota (1952–1976)

Selected causes of mortality	Nonsmokers (no. = 498)		Smokers						Ex-smokers (no. = 1125)		Not known (no. = 552)	
			Cigarette smokers (no. = 1056)		Other smokers (no. = 208)		Total (no. = 1264)					
	Deaths	SMR†	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR	Deaths	SMR
All causes	11	39**	80	149**	12	71	92	130*	32	37**	44	131
Cardiovascular	4	37*	38	190**	1	14*	39	145*	17	49**	17	131
All neoplasms	1	18	14	137	8	236*	22	162*	7	40**	11	169
Respiratory cancer	0		5	167	2	193	7	174	1	19	2	105
Digestive cancer	0		3	106	4	409*	7	184	5	101	4	217
Urinary cancer	0		0		1	591	1	154	0		2	635
Genital cancer	1	345	1	206	0		1	154	0		0	
Selected respiratory diseases	0		1	96	1	280	2	142	1	56	1	143
Most trauma	3	60	9	88	2	82	11	87	3	24**	11	196
Motor vehicle	2	100	5	124	0		5	101	1	21	2	93

* $p < 0.05$.
** $p < 0.01$.
† SMR, standardized mortality ratio.

personal follow-up study. Those men who were alive completed the questionnaire themselves; for those who had died, a proxy, usually the next of kin or a close relative, completed the questionnaire. In an earlier paper on another occupational cohort, it was shown that information taken from a proxy about a man's smoking habits agreed fairly well with information taken one year previously from the man himself (3). Similar conclusions about the validity of proxy smoking habits have been reached by others (4–6). Observed deaths and standardized mor-

TABLE 11

Comparative digestive system cancer rates for white persons, 1950–1969, in the state of Minnesota, St. Louis County and Lake County (7)

Cause	Rate/100,000					
	Minnesota		St. Louis County		Lake County	
	Male	Female	Male	Female	Male	Female
Esophageal	3.5	0.9	5.4	1.3	5.7	1.0
Gastric	18.4	9.2	25.8	11.6	30.9	14.0
Colonic	15.3	15.0	15.2	13.7	13.2	19.7
Rectal	7.6	4.1	9.2	5.0	6.5	3.0
Biliary	4.2	5.8	4.4	6.5	4.4	6.1
Pancreatic	9.5	6.1	10.9	7.4	7.8	7.0
Total	58.6	41.0	70.9	45.5	68.5	50.8
Standardized mortality ratio	100	100	121	111	117	124

tality ratios according to smoking categories are shown in table 10. The high ratios for all smokers are evident. The ratios for respiratory cancer are high for those reported as smoking tobacco other than cigarettes. This rather unusual finding may be due to the use of a proxy to obtain smoking habits of those who had died. Such a person may not have a sufficiently detailed knowledge of the subject's past smoking habits, with the result that cigarette smoking earlier in life may be underestimated. This table, which shows a strong effect of smoking on mortality in this group of employees, contrasts strikingly with those relating occupational exposure to mortality.

Discussion

Follow-up of men employed by the Reserve Mining Company from January 1, 1952, to July 1, 1976, does not suggest that working for the company posed any increase in risk of mortality. Deaths from all causes during this period were significantly fewer than would have been expected for white males in the state of Minnesota. Most of this effect was accounted for by a deficiency of deaths attributed to cardiovascular diseases. This is probably an example of the so-called "healthy worker" effect, which reflects the fact that persons who are able to work are generally fitter than those who are not. Deaths from all malignant diseases were marginally fewer than expected with deaths from cancer of the respiratory system minimally below and deaths from cancer of the digestive system minimally above expected. An increase in the latter category is to be expected, since the counties in which the Reserve Mining Company facilities are situated have higher than average digestive cancer rates and this excess applies to women as well as to men (table 11). There was no suggestion that after a latency of 15 years or more cancer was beginning to increase in these employees.

The apparent absence of any increased risk of cancer among employees of a taconite industry is not surprising. Although estimates of dust concentrations are less comprehensive and complete than we should like, the dust measurements available indicated that exposures to total dust, silica dust, and fibers have been low. Moreover, review of the occupational records indicated that men tended to spend relatively short periods in the dustier jobs, moving to less dusty jobs as they became promoted. Furthermore, the average latency of the whole cohort was 14.7 years with a maximum of 24.6 years. Conclusions should, therefore, be tempered with caution, in that the duration since first exposure, and hence the latency period for the development of cancer, is still relatively short. It does not seem likely, however, that any exposures to taconite which may have occurred in the general community will result in an increased risk of lung cancer.

References

1. Gunderson JN, Schwartz GM. The geology of the metamorphosed Biwabik iron-formation, eastern Mesabi district, Minnesota. Minneapolis: The University of Minnesota Press. Minnesota Geological Survey Bulletin No. 43, 1962.
2. Bailar JC, Ederer F. Significance factors for the ratio of a Poisson variable to its expectation. Biometrics 1964;20:639-43.
3. Higgins I, Welch K, Oh M, et al. Influence of arsenic exposure and smoking on lung cancer among smelter workers: a pilot study. Am J Industr Med 1981;2:33-41.
4. Haenszel W, Loveland DB, Sirken MG. Lung cancer mortality as related to residence and smoking histories: white males. JNCI 1962; 28:947-1001.
5. Haenszel W, Taeuber KE. Lung cancer as related to residence and smoking histories: white females. JNCI 1964;2:803-38.
6. Rogot E, Reid DD. The validity of data from next-of-kin in studies of mortality among migrants. Int J Epidemiol 1975;4(1):51-4.
7. Mason, TJ, McKay FW, Miller RW. Asbestos-like fibers in the Duluth water supply: relation to mortality. JAMA 1974;228(8):1019-20.

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INVESTIGATING POSSIBLE EFFECTS OF ASBESTOS IN CITY WATER: SURVEILLANCE OF GASTROINTESTINAL CANCER INCIDENCE IN DULUTH, MINNESOTA

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Levy, B. S. (Minnesota Dept. of Health, Minneapolis, MN 55440), **E. Sigurdson, J. Mandel, E. Laudon and J. Pearson.** Investigating possible effects of asbestos in city water: Surveillance of gastrointestinal cancer incidence in Duluth, Minnesota. *Am J Epidemiol* 103:352–368, 1976.

The recent discovery of over one million asbestos-like fibers per liter of Duluth tap water and the suggestive evidence of a link between certain gastrointestinal (GI) cancers and work exposure to asbestos fibers in the air prompted this study. GI cancer incidence data were gathered for Duluth in the same manner as data previously gathered for comparison cities, Minneapolis and St. Paul. Although some differences in GI cancer incidence occurred among the three cities in 1969–1971, there was no consistent pattern of statistically significant differences observed. The number of GI cancers diagnosed in Duluth residents in 1972 was similar to that in each of the previous three years. This study represents the start of ongoing cancer surveillance in Duluth.

asbestos; cancer; gastrointestinal neoplasms; water pollution

Human exposure to asbestos has increased greatly in recent years, yet the hazards of this exposure to human health are not understood completely. Occupational exposure to certain concentrations of asbestos fibers in the air has been linked with pulmonary asbestosis, lung cancer, and pleural and peritoneal mesotheliomas (1–6). There are also data suggestive of a link between such exposure and development of cancers of the stomach, large intestine, and rectum many years after initial exposure (1, 6–9). This possible link, presumably the result of swallowing initially inhaled fibers, raises the possibility that drinking water with high concentrations of asbestos or asbestos-like fibers for sufficiently long periods may lead to an increased incidence of gastrointestinal (GI) cancers among those who drink it.

In June 1973, the US Environmental Protection Agency (EPA) reported the recent discovery of large amounts of asbestos in Lake Superior, the source of municipal water for Duluth, Minnesota (1970 population: 100,578), and five smaller communities on the lake shore. Subsequent electron

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Abbreviation: GI, gastrointestinal.

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Mortality of Workers in Two Minnesota Taconite Mining and Milling Operations

W. Clark Cooper, MD; Otto Wong, ScD; and Robert Graebner, MS

Mortality during the years 1947 to 1983 was studied in 3,444 men employed for at least 3 months in Minnesota taconite mining operations during the years 1947 to 1958. During 86,307 person-years of observation, there were 801 deaths for a standardized mortality ratio (SMR) of 88 (US white male rates) or 98 (Minnesota rates). The 41 deaths from respiratory cancer were fewer than expected, the SMR being 61 ($P \leq .01$) (US rates) and 85 (Minnesota rates). There were 25 respiratory cancers 20 or more years after first taconite employment, for an SMR of 57 ($P \leq .01$) (US rates). SMRs for colon cancer, kidney cancer, and lymphopoietic cancer were elevated, but below the level of statistical significance. There was one death from pleural mesothelioma, 11 years after first taconite employment, in a man with long prior employment as a locomotive operator. The pattern of deaths did not suggest asbestos-related disease in taconite miners and millers.

Taconite is a hard, fine-grained, iron-bearing rock which after World War II became the principal form of iron ore mined in the United States, as natural hematite reserves became depleted. By 1978 it provided nearly 90% of the iron ore used in US iron and steel industries. Of this, more than 60% came from the Mesabi Range in Minnesota, where a deposit about 110 miles long and 1 to 3 miles wide extends roughly east to west from Babbitt to Grand Rapids.

The taconite ore body in the eastern end of the Range contains amphibole minerals, ie, actinolite and cummingtonite-grunerite. In 1973 fibrous grunerite particles, reported as similar to amosite, were found in the water supply of Duluth, 50 miles southwest from the point where mine tailings from one of the taconite processing plants were being deposited. There was justifiable concern as to possible effects on health. Concurrent with drastic measures to prevent effluents from entering Lake Superior, studies were begun to determine whether there was any evidence of effects on the health of Duluth residents, with concurrent studies of workers in the taconite mines and mills. So far such studies have been negative.[1-7]

Two of the investigations of taconite workers involved analyses of mortality. The first, by Higgins et al,[6] was based on 5,751 men employed by the Reserve Mining Company for 1 year or more in the period 1953 to 1976, and observed through July 1, 1976. There were 298 deaths during 77,400 person-years of observation, with no significant excess deaths from any cause. Respiratory tract cancers were slightly fewer than expected, and no mesotheliomas were observed. The second study, by Cooper,[7] involved 3,444 men who had worked in either the Erie or Minntac taconite operations for at least 3 months prior to Jan 1, 1959. They were observed through Dec 31, 1977, and there were 489 deaths during 69,306 person-years. There were no excess deaths from lung cancer or any other major cause. It was believed, however, that the results should be interpreted with caution, since only 8% of the person-years of observation were 20 or more years since first opportunities for exposure in the taconite industry, which did not begin to any major extent until 1947.

The present report is based on observation of the Erie-Minntac cohorts for an additional 6 years, through 1983, increasing the person-years of observation to 86,307 and the total number of deaths for analysis to 801.

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MORTALITY OF RESERVE MINING COMPANY EMPLOYEES IN RELATION TO TACONITE DUST EXPOSURE[1]

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Higgins, I. T. T. (Dept. of Epidemiology, The U. of Michigan, School of Public Health, Ann Arbor, MI 48109), J. H. Glassman, M. S. Oh and R. G. Cornell. Mortality of Reserve Mining Company employees in relation to taconite dust exposure. *Am J Epidemiol* 1983;118:710-19.

Analysis of mortality among men who were employed by Reserve Mining Company from 1952 to 1976 has been carried out. Follow-up was conducted with standard methods, including searches by the Social Security Administration. Occupational exposures to dust were based on personal samples taken over the past five years by the industrial hygiene department of the company. Smoking habits were obtained by mailed questionnaires or telephone interviews. A modified life table method was used to compare death rates of the employees with those expected for white males in the state of Minnesota. Comparisons were also made with US rates for white males. The results showed that the death rates for all causes were significantly below expectation. Deaths from malignant diseases were marginally below those expected for the state. Exposures to total dust, to silica dust, or to fiber were low. There was no relationship between mortality and estimated lifetime dust exposures, nor was there any suggestion that deaths from malignant neoplasms were increased after 15 to 20 years latency. In contrast, there was a strong relationship between smoking habits and mortality from all causes, from cardiovascular diseases, and from cancer. This study does not suggest any increase in cancer mortality from taconite exposure.

environmental exposure; mortality; occupational diseases; smoking

Considerable anxiety was created during the 1970s by suggestions that exposure to taconite dust might pose a risk to the health of the general public. This anxiety was focused mainly on the possible risk of taconite in drinking water; but there was also some concern that airborne dust, from industrial plant emissions and from the use of taconite tailings as a surface material for roads, might also be hazardous.

Taconite is a hard, dense rock, composed of silica, silicates, and iron. The iron is in the form of magnetite or hematite and may be either magnetic or nonmagnetic. Taconite from the eastern tip of the Mesabi Range contains amphibole in the cummingtonite-grunerite series, a mineral that is related to amosite asbestos (1). The fibers of taconite are short in length, the vast majority being less than 10 μm. A fiber is defined as any particle with a length-to-width (or aspect) ratio of 3 or greater. Current evidence suggests that short fibers do not cause fibrosis and are unlikely to increase the

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TABLE 3

Selected causes of mortality in the state of Minnesota with accompanying codes from the Eighth Revision of the International Classification of Diseases, Adapted *(ICDA)*

Description of cause	ICDA code	Comment
All causes	000–E999	
Cardiovascular	402, 404, 410–429	
All cancer	140–209	
Respiratory cancer	160–163	
Digestive cancer	150–159	
Urinary cancer	188, 189	
Genital cancer	180–187	
Selected respiratory diseases	470–474, 480–486, 490, 491, 493, 510–519	Influenza, pneumonia, bronchitis, emphysema, asthma, other respiratory
Most trauma	E800–E978	Excludes undetermined and war-related injury
Motor vehicle	E810–E823	

coal conveyors, and for conveyor men in the yards and docks. Values above 1 mg/m^3 were, however, infrequent, the modal range in most occupations being 0.2–0.6 mg/m^3.

Threshold limit values depend on both dust concentrations and silica content of the dust. These values were sometimes exceeded in samples from the crushing departments. The relatively few measurements of fiber which had been made indicated that fiber concentrations were usually low. Concentrations greater than 0.5 fibers per ml were occasionally observed, mainly in the crushing departments, but none approached the threshold limit value of two fibers per ml.

Estimates of dust exposure have been based on personal samples, which were collected between 1975 and 1978. This was considered to be a valid procedure since analysis of fixed-site dust samples over the whole period, from 1952 to 1976, did not indicate any marked trends in dustiness over the years. Furthermore, there were no major processing changes which might have led one to expect any marked changes. All samples were collected during four to eight hours of continuous air sampling, most of them being

TABLE 4

Mortality for selected causes for men who had worked one year or longer for Reserve Mining Company, compared with white males in Minnesota, 1952–1976

Selected causes of mortality	Deaths		Standardized mortality ratio	95% confidence limits
	Observed	Expected		
All causes	298	343.65	87*	77, 97
Cardiovascular	112	123.79	90	74, 109
All neoplasms	58	63.38	92	69, 118
Respiratory cancer	15	17.94	84	47, 138
Digestive cancer	20	17.57	114	70, 176
Urinary cancer	3	2.97	101	20, 295
Genital cancer	3	3.31	91	18, 265
Selected respiratory diseases	4	6.80	59	16, 151
Most trauma	76	72.76	104	82, 131
Motor vehicle	38	31.12	122	86, 168

* $p < 0.05$.

to sell its electricity to the U.S. at a lower cost than the U.S. could produce it.

Roberts Wants 'Environmentally Acceptable' Exports

In Washington, DC, May 5, Roberts disputed that point of view. He said he is concerned that any exports be done in an "environmentally acceptable" manner. If that can be done, however, he said he is in favor of sales of surplus Canadian power to the U.S. if the power is needed.

Nearly half of Canada's members of Parliament signed a petition protesting the proposed sales early this year. Roberts was willing to concede the NEB's argument that the proposed sales will not increase acid rain emissions after the first few months of the deal. That argument may be valid, he indicated, because new coal plants will not have to be built in the U.S. to supply the export electricity.

However, Environment Canada's point is that controls on new power plants in the U.S. are much stricter than those in Canada.

The department said Canadian plants producing export electricity should be at least as clean as new U.S. generators, which must control 80 percent to 90 percent of sulfur dioxide emissions.

Ontario Hydro officials said they were "pleased" by the energy board's ruling. Hydro stands to make about $1 billion on the export contract. However, environmental groups were up in arms about the board's move. Rick Pratt of the Canadian Nature Federation said he had trouble following the board's "twisted logic." Pratt said the move will blacken Canada's image in the U.S.

Energy Probe, which said the export plan will result in the death of 60 Canadian lakes a year, plans to pressure the federal Cabinet to block the sale. Greenpeace indicated its displeasure with Ontario Hydro even before the NEB decision was announced.

Two protestors spent several days atop a 650-foot Ontario Hydro smokestack to protest Ontario Hydro's policy.

United States

ADDITIONAL PROVISIONS TO COMBAT ACID RAIN URGED BY NEW ENGLAND CAUCUS

HARTFORD, Conn — (By an IER Staff Correspondent) — Members of the New England Congressional Caucus will try to add provisions dealing with acid rain to legislation reauthorizing the *Clean Air Act*, according to Representative Barbara Kennelly (D-Connecticut).

Caucus members held six hearings throughout the region April 16 to 26 to gather data from state government and industry officials on acid rain. At the April 19 hearing in Hartford, Kennelly said the caucus will try to develop a united regional position before the reauthorization bill comes to the House floor.

Connecticut's Environmental Protection Commissioner Stanley J. Pac testified that the Air Act "is ill equipped to deal with the transport of pollutants from one state to another." He said the two sections of the Act that deal with interstate pollution problems, Sections 110 and 126, "place an impossible burden on a receptor state to prove that a stationary source in another state is emitting air pollutants in amounts that prevent us from attaining or maintaining national primary or secondary standards."

Pac supported a uniform "emissions cap" of 1.2 pounds of sulfur dioxide per million British thermal units for utilities, so that there would be "an equality of sacrifice between all the states within a regional air shed." He asserted that "the states that are the worst polluters are also the ones that are trying to delay this roll back."

Support for including additional interstate pollution transport protection in the Air Act also came from John Rathgeber, vice president of the Connecticut Business and Industry Association. Rathgeber testified that the association would support legislation to require Midwestern states to move to sulfur dioxide emission levels comparable to those reflected in some New England standards.

He said the industry group is opposed to S 1706, which, he said, "would penalize the region for taking early steps to responsibly limit fossil fuel burning emissions" and other measures that "would unreasonably restrict New England's fuel choices."

Connecticut Air Quality Director Leonard Bruckman testified that only federal legislation and Environmental Protection Agency enforcement actions can stop out-of-state sources from causing air pollution problems like acid rain.

Current bills to amend the *Clean Air Act* — HR 5252, S 2266, and S 2307 — "would result in a significant weakening of our nation's clean air efforts," Bruckman said. "But as bad as these bills are for the provisions they contain, the areas that aren't addressed, such as toxic air pollutants, interstate transport, and acid rain, are potentially more damaging to Connecticut's air quality," he testified.

United States

FIRM TO PAY $1.84 MILLION TO FILTER ASBESTOS TAILINGS FROM LAKE SUPERIOR

A U.S. federal judge April 23 dismissed a case against the Reserve Mining Company and its parent companies for dumping asbestos fibers into Lake Superior from 1955 to 1980 in violation of the *Federal Water Pollution Control Act*, since all 23 parties have agreed to a "fair settlement," according to a Justice Department attorney (*U.S. v. Reserve Mining Co.*, DC Minn, No. 5-72-Civ-19).

The defendants will pay $1.1 million to Minnesota to reimburse it for grants to four North Shore communities. These communities built water filtration systems under a 1974 court order, which found that the asbestos fibers being emitted by Reserve Mining posed a potential health hazard. The communities subrogated their claims to the state, and it sought reimbursement from Reserve Mining.

Duluth will receive $740,000 from the defendants as reimbursement for building its filtration system under the same court order.

Justice Department lawyer Dean Dunsmore told IER April 27 that the Federal Government has not been participating in the litigation for the last three years and that Minnesota has been doing most of the arguing in the last several years.

All parties have signed the stipulation to the settlement figures discussed above, but not all have signed the stipulation to end the litigation and the Minnesota district court's continuing jurisdiction. None of the attorneys contacted felt there would be any last minute problems with the latter stipulation and dismissal.

Firm In Compliance

Reserve Mining is currently operating its taconite operations under state permits and appears to be in complete compliance with the terms of those permits, according to Jim Schoessler, Minnesota special assistant attorney general. The taconite ore is processed into pellets, which eventual-

0149-8738/82/$00.50

MORTALITY OF WORKERS IN TWO MINNESOTA TACONITE MINING AND MILLING OPERATIONS

W. Clark Cooper, MD, Otto Wong, ScD, and Robert Graebner, MPH

SYNOPSIS

Mortality during the years 1947-1983 was studied in 3,444 men employed for at least 3 months in Minnesota taconite mining operations during the years 1947-1958. During 86,307 person-years of observation there were 801 deaths for a standardized mortality ratio (SMR) of 88 (U.S. white male rates) or 98 (Minnesota rates). The 41 deaths from respiratory cancer were fewer than expected, the SMR being 61 (p 0.01) (U.S. rates) and 85 (Minnesota rates). There were 25 respiratory cancers 20 or more years after first taconite employment, for an SMR of 57 (p 0.01) (U.S. rates). SMR's for colon cancer, kidney cancer and lymphopoietic cancer were elevated, but below the level of statistical significance. There was one death from pleural mesothelioma, 11 years after first taconite employment, in a man with long prior employment as a locomotive operator. The pattern of deaths did not suggest asbestos-related disease in taconite miners and millers.

Footnote for page 1:

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INTRODUCTION

Taconite is a hard, fine-grained, iron-bearing rock which after World War II became the principal form of iron ore mined in the United States, as natural hematite reserves became depleted. By 1978 it provided nearly 90% of the iron ore used in U.S. iron and steel industries. Of this, over 60% came from the Mesabi Range in Minnesota, where a deposit about 110 miles long and 1 to 3 miles wide extends roughly east to west from Babbitt to Grand Rapids.

The eastern end of the taconite ore body has been found to contain amphibole minerals in the cummingtonite-grunerite series, some of which are fibrous and similar to amosite, a type of commercial asbestos. When such fibers were found in the water supply of Duluth, 50 miles southwest from the point where mine tailings from one of the taconite processing plants were being deposited, there was concern as to possible effects on health. Concurrent with drastic measures to prevent

effluents from entering Lake Superior, studies were begun to determine if there was any evidence of effects on the health of Duluth residents, with concurrent studies of workers in the taconite mines and mills. So far such studies have been negative (1,2,4,5,6,7).

Two of the investigations of taconite workers involved analyses of mortality. The first, by Higgins (6), was based on 5,751 men employed by the Reserve Mining Company for one year or more in the period 1952 to 1976, and observed through 7/1/76. There were 298 deaths during 77,400 person-years of observation, with no significant excess deaths from any cause. Respiratory tract cancers were slightly fewer than expected, and no mesotheliomas were observed. The second study, by Cooper (7) involved 3,444 men who had worked in either the Erie or Minntac taconite operations for at least 3 months prior to January 1, 1959. They were observed through December 31, 1977, and there were 489 deaths during 69,306 person-years. There were no excess deaths from lung cancer or any other major cause. It was felt, however, that the results should be interpreted with caution, since only 8% of the person-years of observation were 20 or more years since first opportunities for exposure in the taconite industry, which did not begin to any major extent until 1947.

The present report is based on observation of the Erie-Minntac cohorts for an additional 6 years, through 1983, increasing the person-years of observation to 86,307 and the total number of deaths for analysis to 801.

MATERIALS AND METHODS

Choice of study population. When the study was being planned in 1978, descriptive data from all operating taconite mines in the U.S. was reviewed. There were only five which had begun operations before 1958. One of them, Reserve Mining Company, was already being studied independently. Two were relatively small and were located in Michigan. It was decided to limit the study to the Erie Mining Company (Erie) and U.S. Steel Corporation (Minntac), which would provide populations based in Minnesota, and which, when added to the Reserve mining population, would encompass a large proportion of those engaged in early taconite operations.

Data collection. Demographic and work history information from Erie was obtained from individual forms supplied by the company, which were based on Social Security Administration (SSA) and plant records. Four thousand forms were received giving name, social security numbers, hire dates, termination dates and successive job assignments for all former and current

employees (as of December 31, 1977) who had worked prior to January 1, 1959. After exclusion of female employees, those with missing birth or hire dates, and those having less than 90 days employment in a job with potential taconite exposure before 1/1/59, a population of 2,764 men qualified for the study. Four of these had also worked at Minntac.

Data from the Minntac mine and mill were collected in a more conventional way, being based on summary cards on file in the plant for all individuals employed since operations began. An experienced research analyst from EHA microfilmed 800 such cards for all individuals who appeared to meet the study criteria. From these, 680 ultimately qualified, including the four who had also worked for Erie.

Classification of jobs and exposures. The occupational exposures which were of most interest were those to particulates which might be classified as asbestiform, i.e. with 3 to 1 aspect ratios. However, there were no industrial hygiene data upon which to make direct estimates of past exposures to such particulates. There was quantitative information, however, on non-fibrous airborne dust which had been collected to evaluate quartz exposures. These provided a foundation for ranking the relative dustiness of work areas and jobs, when supplemented by subjective appraisals from plant personnel and industrial hygienists.

The work areas or departments in which employees worked were coded as follows: Mining - 01; coarse grinding - 02; fine grinding - 03; concentrating - 04; pelletizing - 05; general plant - 06; pellet handling - 07; maintenance - 08; service - 09; non-taconite operation - 10; non-study plants - 11; power plant - 12; preliminary taconite plant - 51; Erie's Hibbing laboratory - 52; taconite contracting company or TCC - 53.

Each job title within a work area was also assigned a two digit code. Each work area job title combination was characterized in terms of relative dustiness, 00 = non dusty; 01 = least, 02 = intermediate and 03 = highest. For analysis, it was therefore possible to describe each individual's periods of exposure both in terms of relative dust exposures and in terms of the stage in mine operations.

Followup. Initial followup through 1977 was based on determining cohort members known by the plants to be alive because they were still employed, receiving pensions, or living in the local communities. Rosters of those whose status was unknown to the plants were sent to the Social Security Administration (SSA) for followup through 1977 and again through 1983. SSA provided lists of those presumed (1) to be alive because of continued contributions or payment of benefits; (2) to be dead because of notification of death

having been received. There remained a residual group whose vital status was unknown to SSA or to plant personnel.

Determining and coding causes of death. For all who were reported dead, requests for death certificates were sent to the offices of vital statistics in the states of residence or death. The certificates which were obtained were filed and utilized with careful consideration of restrictions imposed by many of the states.

Causes of death were coded by a certified nosologist according to the 7th or 1955 Revision of the International Classification of Diseases. This involved translation, when necessary, of causes coded by the 8th (1965) and the 9th (1975) revisions to correspond to the rubrics in the earlier classification which had been the basis for the initial study of the cohort.

The numbers of dead workers for whom death certificates were not obtained is shown in individual tables. Based on the assumption that causes of deaths in these individuals had the same distribution as those that were certified, the cause-specific mortality ratios should be increased proportionately.

Analysis of data. Cohort members were considered as under observation from three months after their first exposure in a taconite operation until December 31, 1983, or the date of

death, whichever occurred first. Those whose vital status was unknown as of the study cut-off date of December 31, 1983 were considered under observation through the last date they were known to be alive, which could be the date of termination or, in those alive at the conclusion of the previous study, December 31, 1977. This is a compromise between dropping those of unknown status from the study, which would underestimate person-years of risk, or treating them as alive until 12/31/83, which would overestimate person-years.

The numbers of deaths observed were compared with the numbers expected, and expressed as standardized mortality ratios (SMR's) by use of the Occupational Cohort Mortality Analysis Program or OCMAP (8). The U.S. white male population was the basis for comparison, since it was known that there were very few non-whites in the study population. Limited comparisons were also made with the numbers of expected deaths based on Minnesota death rates.

Tests for statistical significance. The statistical significance of deviations of SMR's from 100 were tested by a method assuming a Poisson distribution (9,10,11); 95% (p 0.05) and 99% (p 0.01) confidence limits were calculated. Significantly high or low SMR's will be indicated in text and tables, by an asterisk (p 0.05) or double asterisk (p 0.01).

A two-tailed test of significance was used, as there was no a priori justification for assuming that the effect of working in a particular occupation could only have the effect of increasing an SMR.

RESULTS

Description of the study population. There were 3,444 men who met the study requirement of having been employed for 3 months or more in taconite operations at some time prior to January 1, 1959. Of these, 2,764 were Erie employees, 676 Minntac employees and 4 had worked in both plants. More than half were born before 1925, with the mean birth year being 1922. The average age at the beginning of followup was 33.2 years.

Table 1 summarizes the distribution by the year of first occupational exposure to taconite, from 1947 through 1958. By December 31, 1983, 25 years had elapsed for the 1958 hires and 36 years for the 1947 hires. For 19.8% of the population, those hired in 1953 or earlier, 30 years had elapsed. Thus there were ample opportunities for diseases with long latency to become manifest.

Prior occupational exposures. Only 1,968 cohort members had information on prior employment in their files. Of these,

1,223 had prior hematite mining experience. No separate analysis was made of this subcohort.

Followup and death certificate search. The vital status of 3,249 (95.8%) of the 3,444 cohort members was determined as of 12/31/83. As shown in table 3, 2,498 (72.5%) were known to be alive, 801 (23.3%) were dead, and the vital status of 145 (4.2%) was unknown. The latter fell into two categories: about 90 for whom neither SSA nor the plants could supply any information, and 55 in which there were discrepancies between names and social security numbers. Some of the latter might eventually be located.

The total number of person-years of observation was 86,307, of which 40,092 (46%) were after 1969 and 24,685 (29%) were after 1974. The 801 deaths represented 9.3 per 1000 person-years.

For the 801 deaths, certificates were obtained for 778, leaving 23 (2.9%) with unknown causes of death. This results in a slight understatement of cause specific mortality ratios. If one assumes that the unknown causes had the same distribution as those known, each cause specific SMR in the total cohort would be increased by 3.0% (23/778).

Standardized mortality for the total population. Table 3 summarizes deaths for major causes in all members of the cohort for the period 1959-1983, compared with deaths expected in U.S.

white males during the same years. The SMR for all causes was 88**, a highly significant difference from the U.S. average, but consistent with the healthy worker effect (i.e. those able to work usually have better than average health). There was a significant deficit in total deaths from malignancies (84*). Deaths from respiratory tract cancer (SMR = 61**), largely due to lung cancer (SMR = 59**), were strikingly low. There were elevations in SMR's for cancer of the large intestine (SMR = 128), kidney (SMR = 185) and the lymphopoietic system (SMR = 117), but none of these was statistically significant at the 95% level. Deaths from heart disease and other circulatory diseases were significantly fewer than expected (SMR = 89*). This largely represented a favorable pattern for arteriosclerotic heart disease (SMR = 85**), which is strongly influenced by the healthy worker effect. Deaths from non-malignant respiratory disease, which would include silicosis and asbestosis, were significantly below expected (SMR = 66*). As in the past, there was a non-significant excess of deaths from external causes, including accidents and suicide.

Deaths related to duration of employment and observation period. Table 4 summarizes the total deaths and SMR's based on the duration of employment in jobs with potential taconite exposures and periods of observation from the beginning of such

employment. Except for a slight upward trend in later observation periods, presumably attributable to the healthy worker effect, there was no striking pattern.

Table 5 similarly summarizes deaths from cancer of the respiratory tract, which showed no positive correlation with either length of exposure or time since exposure began. In the observation period 20 or more years since first exposure, the SMR was 57**, based on 25 deaths.

Deaths related to level and type of exposure. To determine if there was any suggestion of excess risk associated with level or type of exposure, the SMR's for a few causes of special interest were compared in several subcohorts. As can be seen in table 6, there was no evidence of any unusual pattern.

Occurrence of mesothelioma. There was one death attributed to pleural mesothelioma, in a 62-year-old white male. It had been classified as due to benign neoplasm, in conformity with the 1955 Revision of the ICD. Although the worker involved had been employed for 40 years, his exposures to taconite began only 11 years before his death. Prior employment had been principally as a locomotive fireman and engineer, in which there could have been exposures to asbestos from boiler insulation.

Standardized mortality ratios using Minnesota death rates. Minnesota death rates are lower than U.S. rates for a number of causes. When they were used for determining expected deaths (table 7), the SMR's were higher for total deaths (98), total neoplasms (95), gastrointestinal cancer (97), respiratory cancer (85), and trauma (123*). Only the last reached a level of statistical significance at the 95% level. The SMR for lymphopoietic cancer dropped to 108, as rates for this cause in Minnesota are higher than national rates. The available program did not separate kidney cancer from bladder cancer, but the two combined showed an SMR of 164, based on 15 cases; this was not a significant elevation. The corresponding combination in our study (not shown in tables using U.S. rates) had an SMR of 150.

When the analysis using Minnesota rates was limited to observation twenty years or more after first exposure, the findings were essentially the same, the SMR for all causes combined being 102, that for all neoplasms being 92, respiratory cancer 98 and lymphpoietic neoplasms 111.

DISCUSSION

The original study and the current extension were carried out because of the possibility that cummingtonite-grunerite, an

asbestiform mineral found in some Mesabi Range taconite deposits, might cause asbestos-related disease, such as lung cancer. There is no evidence of such an effect. Respiratory tract cancer deaths were 39% fewer than expected, based on comparison with U.S. white males, and 15% fewer than expected when compared with Minnesota white males. Even when analysis was limited to deaths 20 or more years after first exposure, which provided ample opportunity for the leading edge of any excess in latent tumors to appear, there was no excess. The number of expected cases of respiratory tract cancer was sufficiently high to give the study considerable power. One can say with 80% probability of being correct (alpha = 0.05 and beta = 0.2) that the SMR for this cause in the total cohort could have been no greater than 132 using U.S. death rates, and no more than 139 using Minnesota death rates. The corresponding SMR, using U.S. rates, for those with 20 or more years potential latency could have been no more than 141.

There were no deaths from asbestosis. The only death from mesothelioma was in a man whose exposure to taconite began only 11 years before his death; he had worked as a locomotive fireman and engineer for thirty years previously. Mesotheliomas rarely occur sooner than 20 years after exposure to asbestos and commonly occur after 30 or more years. It is therefore plausible to hypothesize that asbestos from

locomotive boiler insulation was responsible for this tumor. One can be fairly certain that it was not due to taconite.

The small excess of kidney cancer deaths (9 vs. 4.9 expected) is probably not related to occupational exposures. Because of the small numbers, there is at least a 1-in-20 likelihood that the excess occurred by chance. There was no apparent correlation with either type or duration of exposure. Higgins (6) reported 3 urinary tract cancers (bladder plus kidney) with 2.97 expected, for an SMR of 101, in men employed by Reserve Mining Company for at least one year. Although Lawler et al (7) found 15 kidney cancer deaths with 9.3 expected (SMR 161) in above-ground hematite miners in Minnesota, underground hematite miners had no excess (11 with 11.1 expected).

The pattern of mortality observed in these cohorts does not suggest that dust exposures associated with taconite mining and milling are having any adverse effects on health. The continued deficit in deaths from lung cancer is especially reassuring. If asbestiform minerals were initiating such tumors, one would expect some excess cases to have become apparent, since all members of the cohort had begun employment in the industry at least 25 years before the end of the observation period. Studies of workers exposed to commercial asbestos have shown that excess deaths from lung cancer usually

become apparent within 20 to 25 years after first exposure, although delayed effects may continue for many more years. If there were a positive association between taconite exposures and lung cancer, sufficient time had elapsed in the present study for it to have become apparent.

REFERENCES

1. Cook PM, Glass GE, Tucker JH. Asbestiform amphibole minerals: detection and measurement of high concentrations in municipal water supplies. Science 1974;185-853-5.

2. Masson TJ, McKay FW, Miller RW. Asbestos-like fibers in the Duluth water supply: relation to mortality. JAMA 1974;228:1019-20.

3. Levy RS, Sigurdson E, Mandel J, Laudan E, and Pearson J. Investigating possible effects of asbestos in city water: surveillance of gastrointestinal cancer incidence in Duluth, Minnesota. Am J Epidemiology 1976;362-368.

4. Sigurdson EE, Levy BS, Mandel J, McHigh R, Michienzi LJ, Jagger H and Pearson J. Cancer morbidity investigations: lessons from the Duluth Study of Possible effects of asbestos in drinking water. Env Res 1981; 25:50-61.

5. Clark TC, Harrington VA, Asta J, Morgan WKC and Sargent EN. Respiratory effects of exposure to dust in taconite mining and processing. Am Rev Resp Dis 1980;121:959-966

6. Higgins ITT, Glassman JH, Oh MS, and Cornell RG. Mortality of Reserve Mining Company employees in relation to taconite dust exposure. Am J Epidemiology 1983;118:710-719.

7. Cooper WC: An epidemiologic study of workers in the taconite mining and milling industry. Report prepared for American Iron Ore Association, March 26, 1984.

8. Marsh GM and Preininger M. OCMAP: a user-oriented mortality analysis program. Am Statistician 1980;34:245-246.

9. Mantel N and Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Nat Cancer Inst 1959; 32:719-748.

10. Mantel N. Appendix C in Haenszel W, Loveland DB and Sirken MG. Lung cancer mortality as related to residence and

smoking histories in white males. J Nat Cancer Inst 1962;23:947-997.

11. Bailer JC and Ederer F. Significant factors for the ratio of a Poisson varable to its expectation. Biometrics 1964;20:639-643.

12. Lawler, AB, Mandel JS, Schuman LM, and Lubin JH. A retrospective cohort mortality study of iron ore (hematite) miners in Minnesota. JOM 1985;27:507-517.

TABLE I. DISTRIBUTION OF STUDY POPULATION BY YEAR OF FIRST EXPOSURE TO TACONITE

Year	Number	Percent	Cumulative number	Cumulative percent
1947	2	0.1	2	0.1
1948	122	3.5	124	3.6
1949	41	1.2	165	4.8
1950	61	1.8	226	6.6
1951	57	1.7	283	8.2
1952	103	3.0	386	11.2
1953	295	8.6	681	19.8
1954	283	8.2	964	28.0
1955	425	12.3	1389	40.3
1956	308	8.9	1697	49.3
1957	1283	37.3	2980	86.5
1958	464	13.5	3444	100.0

TABLE 2. STATUS OF STUDY POPULATION AS OF DECEMBER 31, 1983

Total	3,444 (100%)
Alive	2,498 (72.5%)
Unknown	145 (4.2%)
Dead	801 (23.3%)
With certificate	778 (97.1%)
No certificate	23 (2.9%)

TABLE 3. OBSERVED AND EXPECTED DEATHS BY MAJOR CAUSES, 1948-1983 IN TACONITE MINERS AND MILL WORKERS EMPLOYED IN TACONITE-EXPOSED JOBS FOR 3 MONTHS OR MORE PRIOR TO JANUARY 1, 1959, COMPARED WITH U.S. WHITE MALES

	Deaths			Confidence limits	
CAUSE OF DEATH (ICD 7th Rev. 1955)	OBS	EXP	SMR	95%	99%
All causes (001-998)	801	914.0	88**	82-94	80-96
All malignant neoplasms (140-205)	158	188.2	84*	71-98	68-103
Digestive organs and peritoneum (150-159)	48	49.6	97	71-128	64-139
Stomach (151)	5	8.7	58	19-135	13-164
Large intestine (153)	21	16.4	128	79-196	68-220
Respiratory system (160-164)	41	67.7	61**	43-82	39- 89
Bronchus, trachea, lung (162-163)	38	64.3	59**	42-81	37- 89
Kidney (180)	9	4.9	185	85-351	64-411
Lymphopoietic system (200-205)	22	18.9	117	73-177	62-197
All diseases circulatory system (400-468)	360	406.4	89*	80-98	77-101
Arteriosclerotic heart disease (420)	289	339.6	85**	76-96	73- 99
Non-malignant respiratory disease (470-527)	34	51.7	66*	45-92	40-101
Cirrhosis of liver (581)	22	27.5	80	50-121	43-135
All external causes of death (800-998)	105	94.3	111	91-135	86-143
All accidents (800-962)	73	62.9	116	91-146	84-156
Motor vehicle accidents (810-835)	30	29.3	102	69-146	61-161
Suicides (963, 970-979)	29	22.6	128	86-184	75-203
Cause unknown	23	---	---	------	------
Number of workers			3,444		
Number of person-years			86,341		
Deaths per 1,000 person-years			9.3		
Adjustment of cause-specific SMR's for missing certificates			+3.0%		

* Significant at the 5% level, i.e. p =<0.05 ** Significant at the 1% level, i.e. p =<0.01

TABLE 4. DISTRIBUTION OF TOTAL DEATHS, WITH STANDARDIZED MORTALITY RATIOS (SMR's) BY DURATION OF EMPLOYMENT AND OBSERVATION PERIODS (COMPARISON WITH U.S. RATES)

Duration of Employment (Years)	Observation period (years from first taconite exposures)							
	10		10-19		20		Total	
	No.	SMR	No.	SMR	No.	SMR	No.	SMR
0.25 - 0.99	24	81	56	126	83	110	163	109
1 - 4.99	37	76	47	74*	132	85	216	81**
5 - 9.99	28	77	21	66	75	89	124	81*
10 or more	--	--	77	69**	221	95	298	87*
Total	89	77*	201	80**	511	93	801	88**

TABLE 5. DISTRIBUTION OF DEATHS FROM RESPIRATORY CANCER (ICD 160-65) STANDARDIZED MORTALITY RATIOS (SMR's) BY DURATION OF EMPLOYMENT AND OBSERVATION PERIODS (COMPARISON WITH U.S. RATES)

Duration of Employment (Years)	Observation periods (years from first taconite exposures) 10		10-19		20		Total	
	No.	SMR	No.	SMR	No.	SMR	No.	SMR
0.25 - 0.99	1	87	3	97	8	50	7	68
1 - 4.99	2	100	4	93	7	65	13	76
5 - 9.99	2	103	1	46	2	32	5	48
10 or more	--	---	3	34*	13	62	16	53**
Total	5	98	11	59	25	57**	41	61**

Table 6. OBSERVED AND EXPECTED DEATHS 1948-1983 FOR SELECTED CAUSES IN SUBCOHORTS OF TACONITE MINERS AND MILL WORKERS WHO EVER OR NEVER WORKED IN INDICATED AREAS (U.S. Rates)

Subcohort (work area code)		All deaths			Respiratory cancer			Kidney cancer		
		OBS	EXP	SMR	OBS	EXP	SMR	OBS	EXP	SMR
Mining (01)										
Ever	398	398	462	86**	24	34	70	5	2.4	205
Never	403	403	452	89**	17	33	51**	4	2.3	172
Grinding & Conc (02-04)										
Ever	243	243	297	82**	12	23	52*	4	1.7	241
Never	558	558	617	91*	29	45	65*	5	2.2	156
Pelletizing (05)										
Ever	245	245	281	87*	12	21	56*	3	1.5	198
Never	556	556	633	88**	29	46	62*	6	3.4	179
In any of above										
Ever	589	589	695	85**	30	52	58**	8	3.7	217
Never	212	212	219	97	11	16	69	1	1.2	84
In high or medium dust area										
Ever	539	539	571	94	27	43	63*	6	3.1	195
Never	262	262	343	77	14	25	56	3	1.8	170
In high dust area										
Ever	270	270	282	96	12	21	57*	0	1.5	---
Never	531	531	632	84	29	47	62*	9	3.4	265
Total population		801	914	88**	41	68	61**	9	4.9	185

* Significant at the 5% level, i.e. p = <0.05 ** Significant at the 1% level, i.e. p = <0.01

TABLE 7. OBSERVED AND EXPECTED DEATHS BY MAJOR CAUSES IN TACONITE MINERS AND MILL WORKERS WHO WORKED AT LEAST 3 MONTHS IN TACONITE-EXPOSED JOBS PRIOR TO JANUARY 1, 1959 COMPARED WITH MINNESOTA WHITE MALES

	Deaths			Confidence limits
CAUSE OF DEATH (ICD No., 7th Rev 1955)	Observed	Expected	SMR	95%
All causes (1-999)	801	820.5	98	91-105
All neoplasms (140-205)	158	166.0	95	81-111
All gastrointestinal (150-159)	48	49.5	97	71-129
Respiratory (160-165)	41	48.0	85	61-116
Lymphopoietic (200-205)	22	20.4	108	67-163
Kidney and bladder (180-181)	15	9.1	164	92-271
Cardiovascular (400-443)	342	345.0	99	89-110
Selected respiratory (241, 480-83, 490-493, 500-502)	20	21.4	94	57-145
Most trauma (800-965, 970-979, 980-985)	105	85.3	123*	101-149
Motor vehicle accidents (810-835)	30	29.9	101	68-144
Other causes	153	202.0	---	----
Cause unknown	23	---		----
Number of workers		3,444		
Number of person-years		86,342		
Adjustment of cause-specific SMR's for missing certificates		+3.0%		

* Significant at the 5% level, i.e. $p = <.05$ Significant at the 1% level, i.i. $p < 0.01$

MORTALITY PATTERNS AMONG HARD ROCK GOLD MINERS EXPOSED TO AN ASBESTIFORM MINERAL *

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Introduction

The first suggestion that asbestos could be a carcinogen was made by Lynch and Smith in 1935,[1] as they had noticed an association between asbestosis and lung cancer. Since then, all forms of *commercial* asbestos have repeatedly been shown to be carcinogenic in man, and data from animal studies have corroborated these findings. In 1960 Wagner *et al.*[2] reported that pleural and peritoneal mesotheliomas occurred among asbestos miners occupationally exposed to crocidolite in certain areas of South Africa. From 1960 to 1973, studies of anthophyllite miners in Finland by Kilviluoto,[3] Meurman,[4] and Meurman *et al.*[5] reported significantly increased proportional mortality from bronchogenic carcinoma. In 1972, Selikoff *et al.*[6] demonstrated a sevenfold excess of lung cancer among insulation workers with 20 or more years since their first exposure to amosite asbestos. In 1973, Wagoner *et al.*[7] reported results of a mortality study of asbestos workers occupationally exposed predominantly to chrysotile and demonstrated significant increases in lung cancer and asbestosis.

In 1972, the National Institute for Occupational Safety and Health (NIOSH) recommended an occupational standard for asbestos exposure of 2.0 asbestos fibers greater than 5 μm in length per cubic centimeter of air, as an 8-hr time-weighted average daily concentration.[8] This standard was recommended with the recognition that it would "prevent asbestosis and more adequately guard against asbestos-induced neoplasms."[8] In developing this standard, NIOSH recognized the need for additional research due to "the lack of epidemiological studies or clinical reports with supporting environmental data in the exposure range that must be considered" and "the lack of definite information on the biologic response of fibers of different size."[8]

Further need for research on possible adverse health effects of occupational and nonoccupational exposures to *noncommercial* asbestos fibers, and asbestos fibers *shorter than 5 μm*, was brought out at recent court hearings for a mining company in Minnesota and its disposal of taconite tailings.* Expert testimony

* A synopsis of this paper, along with comments and rebuttal, are available from the National Technical Information Service, Springfield, Va. 22151.

Results

As shown in TABLE 1, from April 1960 through December 31, 1973, a total of 71 deaths occurred among these gold miners, as contrasted with 52.9 expected deaths, an excess significant at $p < 0.05$. The distribution of these deaths was such that the only significant excess of mortality occurred in the respiratory system category. This excess was not specific to any response. Rather, it showed up both for respiratory malignancies (10 observed vs 2.7 expected, $p < 0.01$) and for respiratory nonmalignancies (8 observed vs 3.2 expected, $p < 0.05$). A significant excess of deaths also occurred in the category "other accidental deaths" ($p < 0.05$). Of the nine observed deaths in the "all other causes" category, 3 (33%) had mention of pneumoconiotic disease on the death certificate.

A more detailed analysis of respiratory disease mortality, both malignant and nonmalignant, by time interval since onset of underground employment at the mine, appears in TABLE 2. The mortality pattern in this Table shows an excess of nonmalignant respiratory disease in general (8 observed vs 2.56 expected, $p < 0.01$), and of pneumoconiotic disease specifically (5 observed vs 1.57 expected, $p < 0.05$), to have occurred after 20 years since onset of under-

TABLE 1

OBSERVED AND EXPECTED DEATHS ACCORDING TO CAUSE AMONG WHITE MALES WHO HAD 5 OR MORE YEARS OF UNDERGROUND MINING EXPERIENCE BY APRIL 1960, HARD ROCK GOLD MINE, SOUTH DAKOTA

Cause of Death	ICD	Total Obs.	Total Exp.
Malignant neoplasms	140–205	15	9.7
Respiratory system	160–164	10	2.7 *
Other	140–159, 165 170–205	5	7.0
Vascular lesions of central nervous system	330–334	6	3.2
Diseases of the heart	400–443	25	25.2
Nonmalignant respiratory diseases	470–493 500–527	8	3.2 †
Influenza and pneumonia	480–493	3	1.3
Other respiratory diseases	470–479 500–527	5	1.9
Accidental deaths	800–962	8	5.2
Motor vehicle	810–835	0	2.5
Other	800–809 840–962	8	2.7 †
All other causes		9	6.5
Total		71	52.9 †

* Significant at $p < 0.01$.
† Significant at $p < 0.05$.

TABLE 2

OBSERVED AND EXPECTED DEATHS DUE TO MALIGNANT AND NONMALIGNANT RESPIRATORY DISEASE AMONG WHITE MALES WHO ACHIEVED 5 OR MORE YEARS OF UNDERGROUND GOLD MINING EXPERIENCE BY APRIL 1960, HARD ROCK GOLD MINE, SOUTH DAKOTA

Cause of Death	ICD	Number of Years Since Onset of Underground Employment			
		5–19 Years		≥20 Years	
		Obs.	Exp.	Obs.	Exp.
Malignant neoplasms	140–205	4	2.14	11	7.59
Respiratory system	160–164	3	0.56 *	7	2.18 †
Other	140–159, 165 170–205	1	1.58	4	5.41
Nonmalignant respiratory diseases	470–493 500–527	0	0.59	8	2.56 †
Influenza and pneumonia	480–493	0	0.31	3	0.99
Other respiratory diseases	470–479 500–527	0	0.28	5	1.57 *

* Significant at $p < 0.05$.
† Significant at $p < 0.01$.

ground gold mining. No such excess of nonmalignant respiratory disease risk was noted during the first 19 years after first exposure. A significant excess of respiratory tract cancer was demonstrable at each time interval since onset of underground mining: 3 observed vs 0.56 expected ($p < 0.05$) at less than 20 years and 7 observed vs 2.18 expected ($p < 0.01$) at 20 or more years since onset of such employment.

Analysis of the site of the 10 respiratory cancer deaths revealed that eight were primary bronchogenic carcinomas, one was a carcinoma of the maxillary sinus, and one was a mediastinal carcinoma (otherwise unspecified).

INDUSTRIAL HYGIENE STUDY

Occupational Exposures

In assessing what possible etiologic agent(s) contributed to the observed excess of deaths due to malignant and nonmalignant respiratory disease in this population, the environmental contaminants to which these miners were exposed must be considered. The ore body of the mine under study lies within metamorphosed precambrian sedimentary formations, in which the major mineral components are quartz, cummingtonite-grunerite, and sulfides, including arsenopyrites.[4] Other minor constituents of the ore are siderite, biotite, ankerite, chlorite, and hornblends.

Environmental conditions at this mine have been the subject of several industrial hygiene investigations. In 1960, the Bureau of Mines, during its silicosis study, collected impinger samples to evaluate free silica exposures to

underground miners.[13] These samples showed an average airborne dust concentration of 1.7×10^6 particles per cubic foot in miners' breathing zones, whereas airborne and settled dust samples showed an average free silica content of 39%. Given a 39% free silica content, the Occupational Safety and Health Administration (OSHA) 8-hr time-weighted average standard for occupational exposure to free silica would be approximately 5×10^6 particles/ft³. Thus, free silica exposures were well below the present applicable OSHA standard. Semiquantitative x-ray spectographic analysis of an underground settled dust sample taken during this survey for arsenic, chromium, and nickel showed trace concentrations (<0.01%). Radon daughters were not detected as sampled with instruments that had a lower limit of detection of 0.01 working levels, a level within the range of general ambient indoor residential exposure.

In 1973, the Bureau of Mines,[13] under its Mine Enforcement and Safety Administration (MESA) program, conducted further environmental studies at this mine. With personal samplers, a total of seven working shifts were sampled for respirable and total dust concentrations. X-ray diffraction analysis of the respirable dust showed an average free silica content of 13.1%. All samples of mining personnel exposures showed average breathing zone dust concentrations

TABLE 3

RESULTS OF MICROSCOPIC IDENTIFICATION OF AIRBORNE FIBERS BY MEANS OF ELECTRON DIFFRACTION AND X-RAY SPECTROMETRY, HARD ROCK GOLD MINE, SOUTH DAKOTA

Amphibole by Electron Diffraction (%)	Fibrous Grunerite (Amosite) (%)	Fibrous Cummingtonite (%)	Fibrous Hornblend (%)	Unknown (%)
80–90	60–70	1–2	10–15	≈20

below the OSHA standard for free silica. In addition to air samples, material samples of the ore were analyzed for asbestos by scanning electron microscopy by means of energy-dispersive x-ray techniques. Numerous fibrous particles were observed. Their identification was not determined, however.

Concern for possible worker exposure to asbestiform minerals at this mine then spurred a 1974 investigation by MESA.[13] During that study, approximately 200 personal membrane filter samples were collected over full working shifts. Fiber concentrations were determined with the NIOSH phase-contrast optical microscope analytic method for asbestos fibers. The average fiber concentration was found to be 0.25 fibers greater than 5 μm in length/cm³; the highest single concentration found was 2.8 fibers/cm³.

In addition, the content of arsenic, chromium, and nickel compounds of the ore at this time was quantified by x-ray fluorescence analysis to be less than 0.015, 0.035, and 0.016%, respectively.

To perform further analysis of the fiber exposures at this mine, portions of all 200 air samples collected by MESA during this environmental survey were made available to NIOSH. Twenty-five of these 200 air samples with the highest fiber concentrations, as determined by optical microscopy, were selected

TABLE 4

AIRBORNE FIBER CONCENTRATIONS AS DETERMINED BY ELECTRON MICROSCOPE FIBER COUNTS AT 10,000 × MAGNIFICATION,* HARD ROCK GOLD MINE, SOUTH DAKOTA ($N=22$)

Exposure Measure	Fiber Concentrations (fibers/cm³)	
	Total Fibers	Fiber >5 μm
Mean ± SE	4.82±0.68	0.36±0.08
Range	0.66±11.79	0.07±1.29

by NIOSH and were analyzed by analytic transmission electron microscopy via selected-area electron diffraction and energy-dispersive x-ray analysis to determine fiber identification, concentration, and size distributions.

TABLE 3 shows the results of the NIOSH electron microscope identification of airborne fibers present in this mine. As can be seen, 80–90% of the airborne fibers were fibrous amphiboles, and 60–70% of the latter were fibrous grunerite (amosite). Fibrous cummingtonite comprised 1–2% of the amphibole fibers, and fibrous hornblends constituted an additional 10–15% of these fibers. Approximately 20% of the fibers were either too thin or too thick to give definitive amphibole diffraction patterns by direct observation on the electron microscope viewing screen. A large majority of these fibers, however, had x-ray spectra identical to those of amosite asbestos.

Average airborne fiber concentrations in this mine, as determined by electron microscopy, are shown in TABLE 4. With the transmission electron microscope, total fiber concentrations were found to average 4.82 fibers/cm³, whereas concentrations of fibers greater than 5 μm in length averaged 0.36 fibers/cm³. In addition, the median fiber diameter and length were found to be 0.13 and 1.1 μm, respectively; approximately 94% of the airborne fibers were less than 5 μm in length. Summary statistics for the fiber size analyses are shown in TABLE 5.

In addition to asbestos fiber analyses of these samples, energy dispersive x-ray analyses were performed on airborne particles to determine the form of arsenic present in the airborne dust. All particles so analyzed were found to be arsenopyrite (FeAsS) at extremely low concentrations.

TABLE 5

SUMMARY OF AIRBORNE FIBER SIZE DISTRIBUTIONS AS DETERMINED BY ELECTRON MICROSCOPY, HARD ROCK GOLD MINE, SOUTH DAKOTA

Summary Statistic	Diameter	Length
Count median size, $N=2111$ (μm)	0.13	1.10
Geometric Standard Deviation *	3.13	2.70
95% confidence interval for count median size (μm)	0.128–0.141	1.07–1.15

* Data fitted to log normal distributions by probit analysis and linear regression ($r>0.90$).

Discussion

Engineering controls in this hard rock gold mine have been quite good for many years. During its 1960 study,[13] the Bureau of Mines observed extensive use of mine ventilation, wet drilling, and wetting of the work site to control dust exposures. Ore pockets and transfer points were also equipped with water sprays. The effectiveness of these controls is apparent by observation of the low free silica exposures and radon daughter air levels measured during the 1960 survey [13] and the MESA 1973 study.[11]

These methods would have been equally effective in controlling exposures to all dusts, including asbestos fibers, compounds of arsenic, and trace metals. Based on observations of present and past use of engineering controls and their effectiveness, as demonstrated by the Bureau of Mines 1960 [13] and 1973 [11] dust surveys, it may be adjudged that past exposures to asbestos fibers were not significantly different from those found by the 1974 MESA survey.[15] Further evidence to support low present and past airborne exposures to arsenic and trace metals are the low arsenic and trace metal contamination levels found in mine ore and settled dust samples [13, 11] and the low respirable mass concentrations measured by the Bureau of Mines in 1973.[14]

The role of such extremely low levels of radon daughters in the etiology of respiratory tract cancer has been found to be noncontributory, as demonstrated previously among underground potash miners.[16]

The role of cigarette smoking also must be taken into account. Smoking histories obtained during the 1960 silicosis study of this hard rock gold mine indicated that these miners smoked far less than did underground uranium miners. Although a known factor in the causation of lung cancer, it has been estimated in studies of underground uranium miners that such smoking by itself would increase the expected lung cancer death risk by no more than 49%.[17] Cigarette smoking, therefore, could not account per se for the observed increased respiratory cancer risk among these gold miners. Consequently, exposure to fibrous grunerite (amosite) stands out as the prime etiologic agent for the increased risk of respiratory cancer among this study cohort.

In addition to the occupational health implications of the above findings, parallels may be drawn between these findings and potential health effects on general, nonoccupational populations as a result of exposure to similar industrial waste and out-plant process effluents. At hearings for a Minnesota mining company and its disposal of taconite tailings and process discharges, data were presented on airborne asbestos concentration measurements made in the vicinity but not in the property of that company. These samples had been collected by the U.S. Environmental Protection Agency and analyzed by the Mount Sinai School of Medicine, City University of New York by means of electron microscopy.[18] Table 6 presents a summary of these results and their comparison to the airborne concentrations found in the hard rock gold mine in South Dakota. The similarity both of the concentrations observed in these studies and of the relative prevalence of short fibers is striking: a mean concentration of 4.8 fibers/cm^3 was noted in the South Dakota mine and 4.7 fibers/cm^3 in the vicinity of the Minnesota company. Six percent of the fibers in the latter mine were longer than 5 μm in length, whereas 5–10% were longer than 5 μm in the former area. In addition, the fibers from both facilities have been shown to have identical compositions (fibrous grunerite).

Conclusions

A study among a group of miners exposed to amphibole fibers (amosite) in the cummingtonite-grunerite series, airborne concentrations less than 2.0 fibers/cm³ as determined by the NIOSH phase-contrast counting technique, and fibers shorter than 5 μm in length has demonstrated significant risks of mortality from both malignant and nonmalignant respiratory disease. Exposures to known carcinogens in the mine, other than asbestos, did not exceed normal ambient residential levels for radon daughters or were adjudged to be negligible for arsenic, chromium, and nickel. The observed excess of malignant respiratory disease can, therefore, be attributed to asbestos, singly or in combination with cigarette smoke, and that of nonmalignant respiratory disease can, therefore, be ascribed to asbestos, with a possible additive role from low exposures to free silica dust.

The findings of this study point to the need for reevaluation of the adequacy of the OSHA standard of two fibers longer than 5 μm/cm³, of the deleterious health effects of asbestos fibers shorter than 5 μm vis-à-vis any standard for occupational or environmental exposure to asbestos, of the adequacy of the present NIOSH asbestos sampling and analytic methods, and of the potential adverse health effects among general, nonoccupational populations exposed to industrial waste disposals and industrial process effluents that contain trace concentrations of short-fiber noncommercial asbestos minerals.

Table 6

Comparison of Airborne Asbestos Fiber Concentrations in the Vicinity of a Minnesota Mining Company with Those Observed in the Study Mine, South Dakota

Source	Total Fiber Concentration (fiber/cm³) Mean	Range	% >5 μm
Study mine ($N=22$)	4.8	0.7–11.8	6
Silver Bay ($N=6$)	4.7	0.5–11.0	5–10

References

1. Lynch, K. M. & W. A. Smith. 1935. Pulmonary asbestosis III—carcinoma of lung in asbestos-silicosis. Amer. J. Cancer 24: 56–64.
2. Wagner, J. C., C. A. Sleggs & P. Marchand. 1960. Diffuse pleural mesothelioma and asbestos exposure in the North-Western Cape Province. Brit. J. Ind. Med. 17: 260–271.
3. Kiviluoto, P. 1960. Pleural calcification as a roentgenologic sign of non-occupational endemic anthophyllite-asbestosis. Acta Radiol. Suppl. 194: 1–67.
4. Meurman, L. O. 1966. Asbestos bodies and pleural plaques in Finnish series of autopsy cases. Acta Pathol. Microbiol. Scand. Suppl. 181: 1–107.

5. MEURMAN, L. O., P. KILVILUOTO & M. HAKAMA. 1974. Mortality and morbidity of employees of anthophyllite asbestos mines in Finland. Brit. J. Ind. Med. 31: 105–112.
6. SELIKOFF, I. J., E. C. HAMMOND & H. SEIDMAN. 1972. Carcinogenicity of amosite asbestos. Arch. Environ. Health 25: 183.
7. WAGONER, J. K., W. JOHNSON & R. LEMEN. 1973. Malignant and non-malignant respiratory disease mortality patterns among asbestos production workers. Congressional Record-Senate, 14 March 1973, 54660–54662.
8. NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH. 1971. Occupational exposure to asbestos-criteria for a recommended standard. Publication HSM–1026. U.S. Government Printing Office. Washington, D.C.
9. U.S. DISTRICT COURT FOR MINNESOTA. 1973. United States versus Reserve Mining Comany. No. 5–72–Civil–19.
10. MASSON, T. L., F. W. MEKAY & R. W. MILLER. 1974. Asbestos-like fibers in Duluth water supply: relation to cancer mortality. J. Amer. Med. Ass. 228: 1019, 1020.
11. U.S. DISTRICT COURT FOR MINNESOTA. 1974. United States versus Reserve Mining Company. No. 5–72–Civil–19. Feb. 25, 1974. Reserve Exhibit 466.
12. U.S. DISTRICT COURT FOR MINNESOTA. 1974. United States versus Reserve Mining Company. No. 5–72–Civil–19, Feb. 25, 1974: 16909 Testimony by Raymond Erickson, Attorney for Reserve Mining Company.
13. U.S. DEPARTMENT OF THE INTERIOR, BUREAU OF MINES. 1960. Environmental study—Mine 30, Jan. 17–Feb. 6, 1960. Internal report.
14. U.S. DEPARTMENT OF THE INTERIOR, BUREAU OF MINES. 1973. Onsite dust assessment at the Homestake Gold Mine, Lead, South Dakota. Internal report.
15. U.S. DEPARTMENT OF THE INTERIOR, MINING ENFORCEMENT AND SAFETY ADMINISTRATION. 1974. Fiber survey, Homestake Gold Mine, Homestake Mining Company, Lead, Lawrence County, South Daokta, Sept. 23–Oct. 3, 1974. Internal report.
16. WAXWEILER, R. J., J. K. WAGONER & V. E. ARCHER. 1973. Mortality of potash workers. J. Occupat. Med. 15: 486–489.
17. LUNDIN, F. E., J. K. WAGONER & V. E. ARCHER. 1971. Radon daughter exposure and respiratory cancer—quantitative and temporal aspects. National Institute for Occupational Safety and Health-National Institute for Environmental Health Sciences Joint Monograph No. 1. U.S. Government Printing Office. Washington, D.C.
18. U.S. DISTRICT COURT FOR MINNESOTA. 1973. United States versus Reserve Company. No. 5–72–Civil–19, Sept. 6, 1973. Exhibit 62.

MORTALITY AFTER LONG EXPOSURE TO CUMMINGTONITE-GRUNERITE

by

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Mortality after Long Exposure to Cummingtonite-Grunerite[1-3]

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SUMMARY

Ore containing cummingtonite-grunerite has been mined to extract gold since 1876 in Lead, South Dakota. Each of the 1,321 men who were recorded as having worked 21 years or more with the Homestake Mine was allocated to one of 5 dust-exposure categories on the basis of work history and available information on environmental conditions. All except 9 men were traced to the end of 1973, when 652 were still living; the cause of death was ascertained for 657 of the 660 men who had died.

Deaths from cerebrovascular accidents and malignant disease were close to the numbers expected and from accidents and other causes were fewer than expected, but in each of the 3 diagnostic groups—pneumoconiosis (mainly silicosis), tuberculosis, and heart disease—there were more than 30 excess deaths. A clear dust-exposure relationship was found for pneumoconiosis and respiratory tuberculosis—with relative risks for the 2 groups with greatest exposure to dust as compared to the 2 with least exposure, of 19.9 and 16.0, respectively, but there was no convincing evidence of an increase in respiratory cancer.

Introduction

Cummingtonite-grunerite is the name given to a range of amphibole minerals. One of these is a naturally occurring, fibrous silicate mined at Penge, in the Transvaal, South Africa, and given the trade name "amosite" asbestos. Cummingtonite-grunerite also occurs in a crystalline form that when crushed divides by cleavage into fragments defined as fibers, because they are at least 3 times as long as they are broad. These resemble amosite fibers in appearance and chemical constitution, although lacking their strength and elasticity; they are mostly less than 5 μm in length.

Ore containing the crystalline form of cummingtonite-grunerite has been mined since 1876 at the Homestake Mine in Lead, South Dakota, for the extraction of gold and an apparently similar ore at Silver Bay, Minnesota, and elsewhere during the past 20 years for the extraction of iron. Although the host rock at Lead and Silver Bay is said to be similar, no comprehensive comparison of the mineralogies of airborne contaminants encountered in mining in the 2 areas has been reported. Pulmonary fibrosis and malignant disease of the lung or pleura can be caused by exposure to various mineral types of asbestos, including amosite (1). Because of its relationship to amosite, cummingtonite-grunerite may be similarly pathogenic. Disposal of tailings from the mines at Silver Bay into Lake Superior has been the subject of legal action on the grounds that a potential health hazard was being created and also on aesthetic grounds.

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[2] Presented at the American Thoracic Society Annual Meeting, San Francisco, May 1977.

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Clearly, therefore, it is important to determine whether long exposure to cummingtonite-grunerite carries a carcinogenic risk. Because the experience of the Homestake miners has been long enough to show whether such a risk exists, we embarked in October 1973 on a mortality study of the 1,358 persons who had worked 21 or more years with the mining company and virtually completed the follow-up in 1974. We intended that this should be followed by a larger-scale survey, but were unable to proceed because of lack of support. At about this time, in 1974, a study was initiated by Gillam and co-workers (2) of 440 men who, by 1960, had worked at least 5 years underground at the Homestake Mine. They reported 10 deaths from neoplasms of the respiratory system between 1960 and 1973, whereas 2.7 deaths would have been expected from rates in South Dakota. This indication of risk and the importance of the question prompted us to complete and analyze our initial survey with our own resources, without proceeding to the more extensive study originally planned.

Materials and Methods

Mining environment and concentrations of dust. The gold-bearing rock of Homestake was originally mined in open cuts, but underground methods were soon introduced. Pneumatic drilling was begun in 1900 and was performed dry until about 1920. Primary crushing of the ore was carried out underground until the mid-1930s, when the operation was transferred to the surface. Gold was at first extracted by amalgamation, later by a combination of amalgamation and cyanidation, and in recent years by cyanidation alone. It had long been known that exposure in the mine to silica dust involved a serious health hazard, but only recently was concern expressed with regard to effects attributable to cleavage fragments or fibers of cummingtonite-grunerite. These amphibole minerals, which are chemically and crystallographically similar to commercial amosite, have been shown by Gillam and co-workers (2) and Dement and associates (3) to be present in the mine. They reported that, by analytic transmission microscopy, 80 to 90 per cent of airborne fibers in the mine demonstrated an amphibole diffraction patern and that approximately 94 per cent of the airborne fibers were less than 5 μm in length.

Measurements of dust in samples collected mostly with midget impinger have been made frequently at various locations in the Homestake Mine since 1937. The median of readings in different places and on different occasions has varied considerably. In 1937, the median concentrations of dust ranged from 4.3 to 28.9 million particles per cubic foot (mppcf); in 1957, readings were approximately 1 or 2 mppcf in several areas, but the median reading in raises and drifts was 17.9 mppcf. In 1960, the average dust concentration in the working areas was reported by Gillam and co-workers (2) to be 1.7 mppcf, and the free silica content to be 39 per cent, less than the threshold limit value, which at this percentage of free silica, would be 6.1 mppcf. However, concentrations of free silica had been considerably greater than the threshold limit value in some workplaces as recently as 1957. In a recent paper by Swent and colleagues (4), approximate exposures to silica dust of employees working underground in the mine were presented for the years 1937 through 1974. Average counts (mppcf) ranged from 11.0 to 24.6 in the period before 1952, from 4.0 to 9.7 in the period 1952 through 1960, and from 2.0 to 5.0 thereafter.

Subjects and methods of study. The Homestake Veterans Association (HVA), of which all employees automatically become members after completion of 21 years service with the company, was founded in 1905. By the end of 1973, the HVA had registered 1,329 men and 29 women. The study was confined to 1,321 men, excluding 3 men with duplicated names, three with insufficient identifying information, and two whose work histories were unclear. Names, dates, of birth, and dates of first employ-

TABLE 1

AGE DISTRIBUTION OF MEN STUDIED AT DATE OF FIRST EMPLOYMENT

Age (years)	Before 1901		1901–1925		1926–1952		Total	
	(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.)	(%)
< 15	2	4.1	17	2.6	–	–	19	1.4
15–19	19	38.8	195	29.7	165	26.8	379	28.7
20–24	12	24.5	169	25.8	156	25.3	337	25.5
25–29	9	18.4	115	17.5	132	21.4	256	19.4
30–34	1	2.9	66	10.1	91	14.8	158	12.0
35–39	5	10.2	56	8.5	54	8.8	115	8.7
≥ 40	1	2.0	38	5.8	18	2.9	57	4.3
All ages	49	100	656	100	616	100	1,321	100

TABLE 2

MEN STUDIED ACCORDING TO DUST EXPOSURE CATEGORY AND RESULTS OF TRACING

Subjects	Dust-Exposure Category (*no.*)					Total Cohort	
	Very Low	Low	Moderate	High	Very High	(*no.*)	(%)
Total	159	459	178	274	251	1,321	(100.0)
Traced	159	457	178	272	246	1,312	(99.3)
Alive	86	226	91	183	66	652	(49.3)
Dead (total)	73	231	87	89	180	660	(50.1)
Cause of death ascertained	72	229	87	89	180	657	

ment were obtained from the HVA files, and occupational histories with starting date for each job were obtained from the mine personnel records. Eighty-seven per cent of the men were less than 35 years of age when they began work with the company (table 1); therefore, ample time remained even for diseases of long latent period to affect the pattern of mortality.

Each job was allocated a dust exposure category on the basis of the records of dust concentration, after consultation with the environmental engineer of the mine and a long-term retired employee who was familiar with past mining methods, locations, and conditions. Each man who had achieved 21 years of employment with the company, regardless of occupation, was then assigned to one of 5 dust exposure classes, taking into account how long he had spent in the various jobs. This procedure was carried out without knowledge of the results of the follow-up. As can be seen in table 2, 525 men (40 per cent) were assigned to the "high" and "very high" exposure groups. Almost all of these men had spent most of their working lives underground, as indeed had some in the groups with lower exposure. Although our estimates of the dust concentration to which the men were exposed are approximate, we believe that those men in the "high" exposure group experienced average concentrations in excess of 6 mppcf during many years and that those in the group with "very high" exposure experienced even greater concentrations. The "very low" and "low" exposure categories comprised men who were believed never to have worked in dust concentrations of more than 1 mppcf.

From the archives of the personnel department and of the HVA, the names of members known to be alive through December 1973 or who had died were ascertained. Most deaths of members were known because a death benefit was payable. Information concerning men with whom contact had been lost was sought from relatives, friends, and by correspondence. Of the 1,321 men listed, 652 were still alive in 1974, 660 had died, and 9 (0.7 per cent) remained untraced (table 2). Death certificates were sought for those who had died and were obtained for all except 6 men; for three of the latter, a reliable cause was obtained from other sources, leaving 3 deaths from unknown causes. Most deaths had taken place in South Dakota, and International Classification of Disease (ICD) codes for these were obtained from the State Department of Vital Statistics. Approximately one-half of the death certificates in other states also carried an ICD code, and this was accepted; the remaining certificates (codes illegible or absent) were coded for us by the South Dakota Department of Vital Statistics, except for 12 early deaths that were coded by the U.S. Department of Vital Statistics, all in accordance with the ICD Revision in effect at the time of death. Comparative composite cohort analysis (Case and Lea [5]) was used to make comparisons with external and internal reference populations (see Liddell and associates [6] for a discussion of the pros and cons of this approach). For the external comparison (table 3), observed deaths by cause in the cohort were compared with expected figures obtained by applying age/year cause-specific death rates for the reference population to the man-years accumulated by the study population from 21 years after start of first employment through 1973. The external reference population was that of South Dakota, 1937 to 1973, and man-years before 1937 were excluded. The internal reference was the total cohort, from the first death (1931) through 1973 (table 4). The analysis was based on the 7th ICD Revision (1955); codes for deaths and death rates on the basis of other revisions were allocated to the appropriate categories of the 7th Revision. The 7th Revision was selected because it and the closely similar 6th Revision were in effect for most of the deaths in the cohort; the 7th Revision was also used by Gillam and co-workers (2). Death rates for respiratory cancer were available for South Dakota only from 1942 and thereafter. Rates for the previous years were estimated from U.S. rates and from South Dakota/U.S. ratios for the years 1948 to 1950.

TABLE 3

NUMBER OF OBSERVED AND EXPECTED DEATHS BY CAUSE ACCORDING TO SOUTH DAKOTA RATES FOR VARIOUS PERIODS, 1937–1973

Cause of death	ICD	Period								
		1937–1973			1937–1955			1956–1973		
		O	E	O/E	O	E	O/E	O	E	O/E
Malignant neoplasms, total	140–205	93	90.5	1.03	41	30.2	1.36	52	60.3	0.86
Respiratory	160–164	17	16.5	1.03	6	3.4	1.76	11	13.1	0.84
Gastrointestinal	150–159	39	35.1	1.11	23	14.5	1.59	16	20.6	0.78
Other	140–149, 165–205	37	38.9	0.95	12	12.3	0.98	25	26.7	0.94
Vascular lesions of the CNS	330–334	64	63.0	1.02	24	22.6	1.06	40	40.3	0.99
Diseases of the heart	400–443	264	232.5	1.14	107	75.7	1.41	157	156.8	1.01
Pneumoconosis	523–524	37	–	–	17	–	–	20	–	–
Respiratory tuberculosis (including silico-tuberculosis)	001–008	39	3.6	10.83	31	2.8	11.07	8	0.8	10.00
Accidents	800–999	19	28.3	0.67	8	11.9	0.67	11	15.8	0.70
All other causes		115	131.8	0.87	42	50.9	0.83	73	81.6	0.89
Total		631	549.7	1.15	270	194.1	1.39	361	355.7	1.01

Definition of abbreviations: ICD = International Classification of Disease; O = observed; E = expected; CNS = central nervous system.

Results

As shown in table 3, the cohort experienced 81 excess deaths in the period 1937 to 1973, giving a standardized mortality ratio of 115. This excess was more than explained by excess deaths in 3 diagnostic categories: pneumoconiosis, respiratory tuberculosis (including silico-tuberculosis), and heart disease. The account was squared by deficiencies under accidents and other causes. Deaths from cerebrovascular disease and malignant neoplasms were very close to expectation; this remained true for the subcategories of respiratory, gastrointestinal, and other malignant disease.

Taken at face value, this pattern of mortality is characteristic of hard-rock mining with a severe silicotic risk. This is supported by the fact that silicosis was stated as the cause in 35

TABLE 4

NUMBER OF OBSERVED DEATHS BY CAUSE AND DUST EXPOSURE CATEGORY AND OF THOSE EXPECTED USING THE WHOLE COHORT AS REFERENCE POPULATION

Cause of Death	Dust-Exposure Category (*no.*)									
	Very Low		Low		Moderate		High		Very High	
	(O)	(E)	(O)	(E)	(O)	(E)	(O)	(E)	(O)	(E)
Malignant neoplasms, total	9	15.8	34	37.9	16	12.3	16	11.9	20	17.1
Respiratory	0	2.7	7	6.3	3	2.3	5	2.7	2	3.1
Gastro-intestinal	4	6.9	15	16.9	8	4.9	4	4.2	9	7.1
Other	5	6.2	12	14.7	5	5.1	7	5.1	9	7.0
Vascular lesions of the CNS	6	12.3	39	29.8	4	6.9	5	6.6	11	9.3
Diseases of the heart	39	47.0	108	112.9	34	33.0	34	34.2	56	43.9
Pneumoconiosis	1	6.4	2	15.6	2	5.1	9	5.0	26	7.9
Respiratory tuberculosis (including silico-tuberculosis)	1	7.6	3	18.2	3	6.4	11	6.1	31	10.8
Accidents	3	2.8	6	7.0	4	2.7	2	3.0	4	3.6
All other causes	13	20.7	37	49.0	24	14.4	12	13.5	32	20.6
Total*	72	112.5	229	270.3	89	80.8	89	80.2	180	113.2

Definition of abbreviations: O = observed; E = expected; CNS = central nervous system.

*The total number of deaths observed (667) exceeded the total shown in table 2 (631) because 26 deaths that occurred before 1937 are included.

of the 37 pneumoconiotic deaths in this period and was mentioned on the certificates in 28 of the 264 deaths coded to heart disease; in almost one-half of those coded to tuberculosis, silico-tuberculosis was specifically recorded. One of the 17 respiratory malignancies was certified as (*1*) bilateral pulmonary metastases, (*2*) mediastinal mesothelioma. This was in a man in the "low" dust exposure category who had worked all his life on the surface, mainly as a press operator and mechanic. He began work in 1917 and died in Homestake Hospital in 1968, at the age of 76 years; there was no autopsy, but a needle biopsy performed shortly before death was reported by the Mayo Clinic as "consistent with a mesothelioma."

Also given in table 3 are the results for the 2 periods: from 1937 to the end of 1955 (270 deaths), and from 1956 to 1973 (361 deaths). The standardized mortality ratio for all causes of death was much greater during the first period than the second, mainly because respiratory tuberculosis and most other causes of death, including cancer, were more common before 1956. There was no evidence in the more recent period of a decrease in the ratio of observed to expected deaths from pneumoconiosis or tuberculosis, although there was such evidence for respiratory and other cancers. In comparison to South Dakota, there was an excess of respiratory and gastrointestinal cancers only until 1955 and a deficiency thereafter; there was no excess of "other cancers" in either period.

Deaths by cause and dust group as compared to those expected on the basis of the whole cohort as a reference population are listed in table 4. The "very low" and "low" exposure groups were combined for use as a base for calculating relative risks in the categories with greater exposure (table 5). The relative risks for pneumoconiosis and respiratory tuberculosis were clearly and directly related to increasing dust exposure and, because there were 7 deaths attributed to these causes in the "low" and "very low" exposure groups, risks in the groups with greater exposure may have been underestimated. Taking the 2 categories of greatest dust exposure together, 7 cases of respiratory cancer, 13 cases of gastrointestinal cancer, and 14 cases of cancer of other sites were observed, compared with 5.8, 11.3, and 12.1 cases, respectively, expected on the basis of the whole cohort. A similar difference was present for heart disease, and there was a larger one for "all other causes."

Discussion

Past dust exposure in this mine produced substantial excess mortality, primarily from silicosis, tuberculosis, and silico-tuberculosis, but not any significant increase in malignant disease. There is nothing inherently improbable about this conclusion. More than 75 per cent of the 660 men who had died had started work before 1925 and had been exposed to free silica at concentrations sufficient to cause this rate of mortality. The observed mortality from respiratory, gastrointestinal, and other malignant dis-

TABLE 5

RELATIVE RISKS AND DUST EXPOSURE*

Cause of death	Very Low–Low	Moderate	High–Very High
Malignant neoplasms, total	1	1.6	1.6
Respiratory	1	1.7	1.6
Gastro-intestinal	1	2.0	1.4
Other	1	1.2	1.6
Vascular lesions of the CNS†	1	0.5	0.9
Diseases of the heart	1	1.1	1.3
Pneumoconiosis	1	2.9	19.9
Respiratory tuberculosis (including silico-tuberculosis)	1	3.0	16.0
Accidents	1	1.6	1.0
All other causes	1	2.3	1.8
All causes	1	1.4	1.7

* Relative risks are defined as ratios of the standardized mortality ratio (SMR) for the moderate and 2 greatest exposure groups to the SMR for the 2 groups with least exposure, obtained from table 4.

† Central nervous system.

eases was somewhat greater than expected in the groups with greater dust exposure taken together. This appeared to be part of a general trend affecting most causes of death, which was perhaps due to a background effect of silicosis. There was no evidence of a separate and specific increase in respiratory cancer, such as that reported by Gillam and co-workers (2).

There remains the question of diagnostic accuracy. It is conceivable, although surely improbable, that patients whose disease was diagnosed during the years by physicians, radiologists, and pathologists as silicosis or silico-tuberculosis were in fact suffering from asbestosis or lung cancer. Preconceptions can be misleading, but the 2 pneumoconioses, silicosis and asbestosis, differ clinically, radiologically, and in their relation to tuberculosis. In the earlier years, autopsy was uncommon (9 per cent of deaths in the period up to 1956). However, 21 per cent of deaths thereafter were autopsied, and of 28 deaths ascribed to pneumoconiosis or tuberculosis from 1956 to 1972, there were autopsies in 10 (36 per cent). We did not have the resources to check whether the autopsy findings were always taken into account in certifying the cause of death, but it seems probable to us that they usually were.

The death certified, without autopsy, to mediastinal mesothelioma must give pause for thought. One case in 631 deaths is certainly greater than one in 4,000, the estimated proportion of mesothelioma deaths in the United States in 1969 (7); nevertheless, this case is difficult to interpret for at least 3 reasons: (*1*) the pathologic report on the biopsy specimen left the diagnosis in doubt, (*2*) the man was employed at the surface throughout his working life and was subjected to comparatively little cummingtonite-grunerite dust, and (*3*) during the war years, 1942 to 46, when the mine was shut down, he was employed by the company on machine maintenance; thus, at a very relevant time (22 to 26 years before death) he was conceivably exposed to insulation materials.

It is in the nature of observational epidemiology—occupational cohorts included—that biases due to various kinds of selection are inevitable and that their effects are difficult to assess. Employed persons usually have a longer life expectancy than does the general population, which includes persons unfit for work (8). In jobs that are physically demanding, this advantage may persist and even grow with increasing length of employment, but will not affect all types of disease equally. Thus, men who survive 21 years in a mining company will not include those killed or disabled during the qualifying period or men who for physical or psychologic reasons found the work unattractive. On the other hand, occupational cancers, especially those associated with asbestos, seldom kill within 21 years of first exposure. Thus, a cohort such as the one we studied should be at least as likely to demonstrate excess mortality due to malignant disease as that of Gillam and associates (2), which included men with as little as 5 year's employment. Conclusions from the latter study are weakened by the authors' observation that the odds ratio for death from respiratory malignances was greater within 20 years of first employment (5.4) than after it (3.2).

We have considered whether the high mortality from silicosis and respiratory tuberculosis in our cohort (completely absent for men within 20 years of first employment in the study by Gillam and co-workers [2], could have obscured the picture for malignant disease. This problem of competing risks, reviewed recently by Enterline (9), occurs to the extent that occupationally induced respiratory disease prematurely terminated the lives of men who would otherwise have survived to die of cancer caused by the same or closely correlated environmental factors. This possibility seems remote for 2 reasons: (*1*) virtually every longitudinal study of asbestos workers has shown excess mortality from both pneumoconiosis and respiratory cancer, and (*2*) if silica rather than cummingtonite-grunerite were primarily responsible for the excess mortality, there remained an exposed population of substantial size to demonstrate any carcinogenic effect from the amphibole component. Moreover, the interval between date of first employment and death in the 76 fatalities from tuberculosis or pneumoconiosis ranged from 22 to 61 years (median, 35 years). It is difficult to believe that deaths with so wide a distribution could have systematically blocked the appearance of respiratory cancer.

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References

1. Selikoff, I. J., and Hammond, E. C.: Multiple risk factors in environmental cancer. Proceedings of Conference: Persons at High Risk of Cancer, December 10–12, 1974, Key Biscayne, Fla., pp. 467–483.
2. Gillam, J. D., Dement, J. M., Lemen, R. A., Wagoner, J. K., Archer, V. E., and Blejer, H. P.: Mortality patterns among hard rock gold miners exposed to an asbestiform mineral, NY Acad Sci, 1976, *271*, 336.
3. Dement, J. M., Zumwalde, R. D., and Wallingford, K. M.: Discussion paper: Asbestos fiber exposures in a hard rock gold mine, NY Acad Sci, 1976, *271*, 345.
4. Swent, L. W., Herrin, J. R., Waterland, J. K., and Bell, R. F.: Mortality patterns among hard rock gold miners exposed to an asbestiform mineral—a critique, American Industrial Hygiene Association Annual Conference, May 26, 1977, New Orleans, La.
5. Case, R. A. M., and Lea, A. J.: Mustard gas poisoning, chronic bronchitis, and lung cancer, 1955, *9*, 62.
6. Liddell, F. D. K., McDonald, J. C., and Thomas, D. C.: Methods of cohort analysis: Appraisal by application to asbestos mining, J R Stat Soc [A], 1977, *140*, part 4, 469.
7. McDonald, J. C., and McDonald, A. D.: Epidemiology of mesothelioma from estimated incidence, Prev Med, 1977, *6*, 426.
8. Fox, A. J., and Collier, P. F.: Low mortality rates in industrial cohort studies due to selection for work and survival in the industry, Br J Prev Soc Med, 1976, *30*, 225.
9. Enterline, P. E.: Pitfalls in epidemiological research, J Occup Med, 1976, *18*, 150.

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Mortality Study of Gold Miners Exposed to Silica and Nonasbestiform Amphibole Minerals: An Update With 14 More Years of Follow-Up

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We have updated a study of 3,328 gold miners who worked underground for at least 1 year between 1940–1965 in South Dakota, extending the follow-up from 1977 to 1990. The exposures of concern were silica and nonasbestiform amphibole minerals. The lung cancer standardized mortality ratio (SMR) was 1.13 (95% confidence interval [CI] 0.94–1.36, 115 observed) when the U.S. population was used as the referent group, increasing to 1.25 (95% CI 1.03–1.51) when the county was used as the referent, and to 1.27 (1.02–1.55) for person-time with more than 30 years potential latency. However, lung cancer mortality did not show a positive exposure–response trend with estimated cumulative dust exposure. Data on smoking habits suggested that the miners smoked slightly more than the U.S. population in a 1960 cross-sectional survey. In contrast to lung cancer, other diseases known to be associated with silica exposure (tuberculosis and silicosis) were significantly increased (SMR = 3.44 and 2.61) and exhibited clear exposure–response trends. Nonmalignant renal disease, also associated with silica exposure, was elevated for those hired in early years and showed a significant positive exposure–response trend. Multiple-cause analysis revealed significant excesses of arthritis, musculoskeletal diseases (including systemic lupus and sclerosis), and skin conditions (including scleroderma and lupus), diseases of autoimmune origin which have been associated with silica exposure in other studies. Multiple cause analysis also showed a significant excess of diseases of the blood and blood-forming organs.

Key words: silica, silicosis, lung cancer, asbestos, gold miners

INTRODUCTION

We have studied 3,328 gold miners who were exposed to several potential lung carcinogens. Of primary interest was exposure to silica and to nonasbestiform amphibole fibers (primarily cummingtonite-grunerite [CG] fibers). Silica has been shown to cause lymphomas after pleural injection and lung cancer after inhalation in rats [IARC, 1987]. Data from humans exposed to silica have been inconclusive for lung cancer, although cohorts of men with silicosis have shown consistent excesses of lung cancer [see reviews by Pairon et al., 1991; Goldsmith, 1994]. Nonasbestiform

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amphibole fibers have not been shown to cause lung cancer, but are suspect because of their similarity to asbestiform fibers.

Subcohorts of the cohort studied here have been studied previously. Gillam et al. [1976] studied 440 white males employed as of 1960 who had at least 5 years underground, while McDonald et al. [1978] studied 1,321 miners who were retirees in 1973, all with at least 21 years underground. Follow-up for these studies ended in 1973. Gillam et al. [1976] reported a lung cancer excess, while McDonald et al. [1978] found no excess and no indication of a positive trend with increasing exposure. The current cohort was identified and first studied by Brown et al. [1986], with follow-up through June 1977, partly to resolve this discrepancy. Brown et al. [1986] found a standardized mortality ratio (SMR) of 1.00 for lung cancer with no apparent trends with increased estimated cumulative exposure or latency. There were significant excesses of tuberculosis (largely silico-tuberculosis) and nonmalignant respiratory disease (largely silicosis) which exhibited a positive trend with increasing estimated cumulative dust exposure. Accidental deaths were significantly elevated, and there was an indication of an increasing trend in nonmalignant renal disease with increasing exposure.

The analysis by Brown et al. [1986] was based on follow-up through June 1977 with 861 total deaths observed (43 lung cancer deaths). We have extended follow-up of this cohort through 1990 and now have observed a total of 1,551 deaths (115 lung cancer deaths).

MATERIALS AND METHODS

Exposure Estimates

Most of the cohort (58%) was first employed before 1950. The silica content of respirable dust was estimated at 13% in a survey in the mid-1970s, while the silica content of settled dust was estimated to be 39% [Zumwalde et al., 1981]. The American Conference of Governmental Industrial Hygienists' [ACGIH, 1983] threshold limit value (TLV) in terms of millions of particles per cubic foot (mppcf) of dust (300/[%SiO_2 in settled dust + 10]) is 6.1 mppcf. Estimated dust levels were such that silica exposures exceeded this level prior to 1950 but were below this level after dust controls were implemented in 1950 [Zumwalde et al., 1981]. Based on average dust levels, prior to 1950 silica exposures would have ranged from approximately 10 to 30 mppcf. These are substantial levels (approximately one to three times current standards) although still somewhat less than other occupational cohorts of the time, such as granite cutters whose exposures prior to dust controls in the 1940s ranged from 20 to 50 mppcf [Steenland and Beaumont, 1986].

Asbestos refers to a variety of hydroxylated silicate minerals. These minerals are said to exist in an asbestiform or fibrous "habit" when the mineral has grown in one dimension to form long thin crystals. Nonasbestiform varieties of these minerals have crystallized in two or three dimensions and are generally composed of shorter fibers. When nonasbestiform minerals are broken up or crushed during mining operations, the fibers or cleavage fragments of nonasbestiform minerals are almost indistinguishable from asbestiform fibers under the light microscope. While the division between asbestiform and nonasbestiform minerals is not clear-cut, for the purpose of regulation the Occupational Safety and Health Administration (OSHA) has restricted its definition of asbestos to asbestiform fibers greater than 5 μm with aspect

(length to width) ratios of at least 3:1 [Code of Federal Regulations 29, 1990]. In general it is believed that the long thin fibers are more pathogenic [Stanton et al., 1981].

Asbestos minerals are also divided into two broad groups, serpentine and amphibole. The amphibole family includes CG (commercially called amosite), tremolite, and actinolite, all of which can exist in asbestiform or nonasbestiform varieties. The gold miners in this study were exposed to nonasbestiform CG (69% of all fibers) and tremolite–actinolite (15%), as well as other nonasbestiform varieties (16%) [Zumwalde et al., 1981]. The percentages of airborne fibers greater than 5 μm for CG and tremolite–actinolite fibers were 24% and 32%, respectively. The geometric mean of personal exposures to fibers greater than 5 μm in length in the mid-1970s was 0.44 fibers/cm^3, below the then-current OSHA standard (time-weighted average [TWA]) of two fibers greater than 5 μm in length/cm^3 [now reduced to 0.2 fibers, Code of Federal Regulations, 1990]. OSHA has recently exempted nonasbestiform amphiboles (tremolite, actinolite, and anthophyllite) from the asbestos standard [Code of Federal Regulations 30, 1992]. Levels of exposures to fibers in years prior to the 1970s were not measured, but presumably would have been somewhat higher, based on known higher levels of respirable dust.

Exposures also occurred to the potential lung carcinogens arsenic and radon. However, these exposures were below OSHA standards when measured in the mid-1970s (earlier measurements are not available). Radon daughters in the 1970s ranged from 0 to 0.17 working levels [Zumwalde et al., 1981; 0–2 working level months (WLM) per year]. The current Mine Safety and Health Administration (MSHA) standard is 4 WLM per year [Code of Federal Regulations 30, 1992]. With an average of 9 years exposure, these miners would have been exposed to average cumulative levels ranging from 0 to 18 WLMs, considerably below any levels associated with lung cancer in epidemiologic studies of uranium miners. Arsenic exposures in 1970s averaged under 5 μg/m^3 [Zumwalde et al., 1981]. The current OSHA standard is 10 μg/m^3. However, 16 of 51 samples exceeded the National Institute for Occupational Safety and Health (NIOSH) recommended 15-min standard of 2 μg/m^3.

Methods

A job–exposure matrix was created to estimate dust exposures for each job in the mine over time. Details are available in Brown et al. [1985, 1986] and Zumwalde et al. [1981]. Briefly, all full-time underground jobs were assembled into five major groups based on similarity in job function and dust exposures (laborers, miners, motormen, supervisors, and skip loaders). A sixth category grouped all jobs not considered full-time underground jobs (these jobs were considered nonexposed). Average dust exposures for the job categories were then calculated using existing measurements for each year from 1937 to 1975. The gold mine operated from the early 1900s, and prior to 1937 exposures had to be estimated. No job history data were collected after 1975. The mine continued to operate after that year, but with reduced numbers of miners and with low levels of exposure, so that there is little underestimation of cumulative exposure in our cohort by ignoring exposures after 1975 (only 15% of our cohort were still employed as of 1975).

The estimated daily dust exposures (constant over yearly intervals) for each of the five job categories were weighted (multiplied) by a factor estimating how much daily time was spent underground by miners in these jobs, with a factor of 1 assigned

to work done in the earliest years, decreasing in later years [Zumwalde et al., 1981]. For each miner, estimated daily dust levels were summed over time and this measure (dust-days, each dust-day is one day exposed to 1 mppcf of dust) was used as the estimate of cumulative exposure. Because this quantitative measure refers to total dust, it is not specific to either silica exposure or exposure to nonasbestiform minerals.

Mortality analyses were conducted using the Life Table Analysis System (LTAS) of NIOSH for [Steenland et al., 1990b]. Due to the cohort entry criteria, person-time for each miner did not begin to accumulate until he had spent 12 months in an underground job or in 1940, whichever came first. Person-time continued until date-last-observed or December 31, 1990, whichever was earlier. Follow-up for vital status was done via Social Security and the National Death Index (NDI), with the latter source searched through December 31, 1990. Men known to be alive after the beginning of NDI in 1979 were considered alive as of December 31, 1990 if not found via NDI.

Life-table analyses were conducted for 92 categories of death, using as the referent group either the U.S. population, the population of Lawrence County, South Dakota, where the mine was located, or the population of the entire state of South Dakota. Analyses using the Lawrence County or South Dakota referent population only consider person-time and deaths occurring after 1960, when county and state rates are first available (69% of the person-time and 82% of the deaths occurred after 1960). Analyses were also conducted by duration, time-of-hire, time-since-first-employment, and estimated cumulative exposure (dust-days). Categories of dust-days for cohort analysis were chosen a priori to conform to those used in the prior publication of these data.

Further analyses were conducted using only person-time after the end of employment ('inactive') person-years, which represented 74% of the person-time and 92% of the deaths). Such analyses may sometime reveal trends with cumulative exposure which are obscured when active and inactive person-years are combined [Steenland and Stayner, 1991]. Chi-square tests for trend in SMRs were done according to Breslow et al. [1983].

Analyses were also conducted using national rates for multiple causes of death, which include all causes of death listed on the death certificate and are particularly useful for diseases which are usually contributory rather than underlying causes. These analyses were restricted to post-1960 because U.S. referent rates are only available after 1960 [Steenland et al., 1992].

In addition, a nested case–control analysis of lung cancer, in which estimated cumulative exposure could be treated as a continuous variable, was also conducted. In this analysis, five controls were randomly selected from the risk set of all those who had achieved the age of the case at the time the case failed [incidence–density sampling, see Beaumont et al., 1989]. Work history and cumulative exposure were truncated for controls at the failure time of their index case. Analyses were conducted via conditional logistic regression using PHREG procedure of the Statistical Analysis System [SAS, 1991].

Smoking data for 602 men (volunteers) in the cohort were available from a 1960 Public Health Service silicosis survey [see Gillam et al., 1976], when these men were aged 35–64. Cigarette smoking categories included never/occasional (including smokers of only pipe or cigar), current, and former. Data were also available on

amount smoked for current cigarette smokers, never/occasional. Compatible age and race-specific data for U.S. white males were available from a 1955 survey of approximately 40,000 subjects, done as a supplement to the February 1955 Current Population Survey [Haenszel et al., 1956]. Age-adjusted smoking prevalences for the U.S. and the miners by smoking category were then compared, and the effect of smoking differences upon observed lung cancer rate ratios was estimated according to the technique of Axelson [Axelson and Steenland, 1988]. The assumed rate ratios for cigarette smokers vs. never smokers, used for this estimation, were 5 for former smokers and current smokers of less than 10 cigarettes, 10 for current smokers of 10–20 cigarettes, and 20 for current smokers of more than 20 cigarettes a day. These rate ratios were chosen to reflect average values from large prospective studies of smokers.

RESULTS

We observed 1,551 deaths among the 3,328 miners during 106,000 person-years of observation. Only 2% of the cohort were lost to follow-up. The average year of first exposure was 1945, the average length of follow-up was 37 years, and the average length of employment underground was 9 years. The median cumulative dust-days was 8,376, the mean was 23,569, and the range was 2,193–225,774. Seventy-five percent of the cohort had fewer than 26,823 dust-days. Based on data from Vermont where the silica content of respirable dust was similar, 10 mppcf of respirable dust would be approximately equivalent to 0.1 mg silica/m^3 of air. Using this conversion, the median average exposure level of these miners was 0.05 mg/m^3 (the current OSHA standard is 0.1 mg). However, this varied considerably by time period, with exposures being much higher in earlier years. The median average exposure for men hired prior to 1930, 1930–1950, and after 1950 was 0.15, 0.07, 0.02, respectively.

Table I presents SMRs for the entire cohort for a number of causes of a priori interest, as well as common causes and causes which showed marked elevations. Death from all causes was elevated 13%. Most of the excess overall death was accounted for by excess deaths from tuberculosis, nonmalignant respiratory disease, and accidents, causes which are associated with silica exposure and mining.

A review of death certificates showed that 21 of 39 tuberculosis deaths indicated silico-tuberculosis, while 40 of 92 deaths in the "other respiratory disease category" mentioned silicosis or unspecified pneumoconiosis (one death in this category was due to asbestosis). A review of all 1,551 death certificates for the entire cohort found that 140 men (9%) had some mention of silicosis on their death certificate, either as an underlying or contributing cause. The significant elevation for asthma may also have been the result of misdiagnosed silicosis, although silica also has pronounced effects on the immune system (see below).

Lung cancer was only marginally elevated for the cohort as a whole (SMR = 1.13). The SMR for person-time occurring more than 30 years since time of first exposure (first job underground) was 1.27 (95% CI 1.02–1.55, 90 observed), compared with an SMR of 0.82 (25 observed) for person-time with less than 30 years since first exposure. Trends with duration of exposure were inconsistent (SMRs of 1.02, 1.55, 1.01 for less than 10 years, 10–20 years, and 20+ years of exposure, based on 65, 35, and 15 observed deaths, respectively).

TABLE I. Overall Gold Miner Mortality Results (U.S. Referent Rates): Update of South Dakota Cohort to 1990

Cause (ICD9)[a]	Obs	SMR	(95%CI)
All deaths	1551	1.13	(1.07–1.19)
All cancers (140–208)	303	1.01	(0.90–1.13)
Ischemic heart disease (410–414)	431	0.94	(0.85–1.03)
Tuberculosis (010–012)	36	3.52	(2.47–4.87)
Cerebrovascular disease (430–438)	77	0.94	(0.75–1.18)
Cancers			
Digestive system (150–159)	69	0.85	(0.66–1.07)
Peritoneum, other (158–9)	4	2.81	(0.76–7.19)
Respiratory			
Larynx (161)	3	0.71	(0.15–2.07)
Lung (162)	115	1.13	(0.94–1.36)
Other respiratory (160, 3–5)	3	2.54	(0.52–7.43)
Urinary (188–189)	9	0.57	(0.26–1.08)
Hematopoietic (200–208)	35	1.29	(0.90–1.79)
Lymphosarcoma/reticulosarcoma (200)	8	1.72	(0.74–3.39)
Hodgkin's (201)	2	0.79	(0.09–2.85)
Leukemia/aleukemia (204–8)	14	1.24	(0.68–2.08)
Other (202–3)	11	1.26	(0.62–2.26)
Nonmalignant respiratory disease (460–519)	170	1.86	(1.58–2.16)
Emphysema (492)	23	1.39	(0.88–2.09)
Pneumonia (480–486)	40	1.27	(0.91–1.74)
Bronchitis (490–491)	6	1.66	(0.61–3.61)
Asthma (493)	7	2.61	(1.09–5.61)
Pneumoconioses and other respiratory (470–478, 494–519)[b]	92	2.61	(2.11–3.20)
Acute kidney disease (580–581, 584)	2	1.19	(0.14–4.29)
Chronic kidney disease (582–583, 585–587)	11	1.25	(0.62–2.23)
Accidents (E800–E949)	139	1.78	(1.49–2.09)
Falls (E880–888, E929.3)	15	1.55	(0.87–2.55)
Other accidents (E890–E928, E929.4–929.9)	67	2.98	(2.31–3.79)

[a]International Classification of Disease codes, 9th revision.
[b]includes chronic obstructive pulmonary disease and other disease besides pneumoconioses.

When local county rates were used as the referent rates, the SMR for lung cancer was 1.25 (1.03–1.51, 112 observed) and was 1.27 (1.02–1.57, 88 observed) for those with 30 or more years since first exposure. When South Dakota rates were used, the SMR for lung cancer was 1.59 (1.31–1.92), markedly increased from the SMRs using either U.S. or county rates. South Dakota has notably lower lung cancer rates than the rest of the United States, but is divided between the larger cities on the plains in the eastern portion and the mountainous western area where the gold mine is located. It may be that the county rates or the U.S. rates are preferable to the South Dakota rates for use as a referent population, although the county rates suffer from small numbers and instability. We have chosen to emphasize the U.S. rates throughout this discussion. Conclusions about the etiologic significance of findings are best based on biological plausibility and disease trends with calendar time or estimated exposure, rather than point estimates based on any set of referent rates.

Hematopoietic cancers were slightly elevated in the cohort as a whole, with the

TABLE II. Gold Miner SMRs for Selected Causes by Cumulative Dust Exposure: South Dakota Cohort Update to 1990

	Dust-days[a]				
Cause (ICD9)	<8,000 SMR(obs)	8,000–32,000 SMR(obs)	32,000–48,000 SMR(obs)	48,000+ SMR(obs)	Chi-square trend test
Respiratory tuberculosis	0.52 (1)	0.78 (2)	0.89 (1)	6.95[b] (32)	23.76[b]
Lung cancer	1.17 (44)	1.01 (35)	0.97 (8)	1.31 (28)	0.21
Pneumoconioses, other respiratory diseases	1.79[b] (19)	1.46 (18)	2.95[b] (10)	8.87[b] (45)	25.77[b]
Chronic renal disease	0.40 (1)	0.34 (1)	1.26 (1)	2.77[b] (8)	7.62[b]
Non-Hodgkin's lymphomas (ICD9 200, 202) (after 1960 only)	1.27 (4)	1.48 (4)	0.00 (0)	3.29[b] (5)	1.75

[a]One dust-day is one day with an exposure of 1 mppcf dust.
[b]Significant at the 0.05 level.

most pronounced excess (SMR 1.72, 0.74–3.39) occurring among the category lymphosarcoma/reticulosarcoma (ICD9 codes 200), which are non-Hodgkin's lymphomas. To explore this excess, we combined other non-Hodgkin's lymphomas (ICD9 code 202) from the category "other hematopoietic cancer" (composed of multiple myeloma, ICD9 203, and other lymphomas, ICD9 202) for the calendar time period after 1960 (the appropriate rates were available only after 1960). This combined category of non-Hodgkin's lymphomas had 13 observed deaths with an SMR of 1.63 (0.86–2.78).

Table I indicates nonsignificant excesses of cancers of the peritoneum (SMR 2.81, four observed) and of cancers included in the category "other respiratory cancers" (SMR 2.54, three observed), categories which might include mesothelioma. A review of these seven death certificates did not find any mention of mesothelioma. Furthermore, no mention of mesothelioma was found in a review of deaths from lung cancer or other nonspecified cancer, categories which at times include mesotheliomas [Lilienfeld and Gunderson, 1984].

Table II shows SMR analyses by cumulative exposure category for selected causes. Significant positive trends were observed for tuberculosis, pneumoconioses, and chronic renal disease, and a positive nonsignificant trend was observed for non-Hodgkin's lymphomas. All these causes were significantly elevated in the highest exposure category. Chronic renal disease has been associated with silica exposure in other epidemiologic studies [Steenland et al., 1990a] and some authors have suggested it may reflect an autoimmune process [Osorio et al., 1987]. There was little trend for lung cancer. Restriction of person-time to time after last employment ("inactive" person-time) did not alter the lack of a trend for lung cancer and cumulative dust exposure. Since dust exposure levels were estimated before 1937, we also conducted analyses restricted to the 76% of the cohort hired after that date, when actual dust levels were measured. These analyses also failed to exhibit any increased lung cancer with increased cumulative dust exposure.

A nested case–control analysis of the 115 lung cancers and a set of matched controls revealed a negative nonsignificant trend with either estimated cumulative dust exposure or the log of estimated dust exposure (the mean cumulative dust exposure for cases was 28,389 dust-days, while it was 31,060 dust-days for controls).

TABLE III. Gold Miner SMRs for Three Year-of-Hire Subcohorts: South Dakota Cohort

	Year of hire		
Cause (ICD9)	<1930 SMR(obs)	1930–1950 SMR(obs)	1951+ SMR(obs)
All cancers	1.11 (66)	0.95 (169)	1.12 (68)
Tuberculosis	7.72 (33)[a]	1.00 (6)	0.00 (0)
Cancer of peritoneum	6.09 (2)	1.20 (1)	3.92 (1)
Lung cancer	1.30 (21)	1.14 (71)	1.01 (23)
Pneumoconioses, other respiratory diseases	5.37 (36)[a]	2.12 (48)[a]	1.36 (8)
Chronic kidney disease	2.39 (7)[a]	0.80 (4)	0.00 (0)
Hematopoietic cancer	1.64 (8)	1.30 (21)	0.97 (6)
Lymphosarcoma and reticulosarcoma	3.24 (3)	1.45 (4)	1.04 (1)
Leukemia	1.72 (4)	1.36 (9)	0.43 (1)

[a]95% CI excludes 1.00.

Stratification by duration of exposure did not change these results. Duration of exposure itself was not a significant predictor of lung cancer. Neither year of first exposure nor year of birth differed between cases and controls, and these variables did not modify the relationship between estimated cumulative dust and lung cancer. Again, because dust levels had to be estimated prior to 1937, we also conducted the case–control analyses restricting cases and controls to those hired in 1937 or later (75 of the 115 cases were hired in 1937 or later). These analyses likewise failed to show any relationship between lung cancer and cumulative dust exposure.

Table III shows SMRs for selected causes after dividing the cohort by year of hire. Tuberculosis and chronic renal disease were significantly elevated only for those hired prior to 1930, and pneumoconiosis was significantly elevated only for men hired prior to 1930 and men hired from 1930 to 1950. These diseases are clearly related to cumulative exposure and exposures were known to be higher in earlier years. Lung cancer also shows the highest elevation in the earliest hire period, but is not significantly elevated.

Table IV shows SMRs by multiple cause mortality analysis, in which rates are calculated using any mention of a given disease on the death certificate. These analyses are particularly useful for diseases which may be prevalent at death but are not fatal. Categories which showed marked excesses here, but which were not revealed by underlying cause analysis, included arthritis, other musculoskeletal disease (including lupus and sclerosis), other diseases of the skin (including scleroderma and lupus), alcoholism, other diseases of the blood-forming organs, and other myocardial degeneration. Several of these disease (lupus, scleroderma, systemic sclerosis, rheumatoid arthritis) are autoimmune diseases, and are known to be associated with silica [Rustin et al., 1990; Haustein et al., 1990; Koskela et al., 1987; Sluis-Cremer et al., 1985; Klockars et al., 1987]. The cardiovascular disease elevations revealed by multiple cause analyses may have been due to the effects on the circulatory system of the pneumoconiotic lung disease which was so prevalent in this cohort.

A review of the multiple cause mortality data stratified by time-of-hire (<1930, 1930–1950, 1951+) showed that the excesses for chronic renal disease (10 observed, SMR 2.14, 1.05–4.04), myocardial degeneration (15 observed, SMR 9.20, 5.14–15.18), and other diseases of the blood (3 observed, SMR 4.71, 0.97–13.77) were

TABLE IV. Gold Miner SMRs for Multiple Cause Mortality (After 1960), Considering all Causes Listed on the Death Certificate: South Dakota Cohort

Cause (ICD9)[a]	Obs	SMR	(95%CI)
All causes listed	3038	1.05	(1.02–1.09)
All cancers (140–208)	465	1.08	(0.98–1.18)
Ischemic heart disease (410–414)	527	0.88	(0.80–0.95)
Tuberculosis (010–012)	27	4.72	(3.11–6.87)
Cerebrovascular disease (430–438)	128	0.95	(0.75–1.18)
Diseases of arteries, veins, circulation (415–7,440–59)	180	1.19	(1.02–1.38)
Other myocardial degeneration (429.0,429.1)	20	3.03	(1.85–4.68)
Cancers			
Lung (162)	121	1.16	(0.96–1.38)
Lymphosarcoma/reticulosarcoma (200)	6	1.37	(0.50–2.98)
Hodgkin's (201)	2	0.87	(0.10–3.14)
Leukemia/aleukemia (204–8)	10	0.82	(0.39–1.50)
Other (202–3)	15	1.43	(0.80–2.36)
Nonmalignant respiratory disease (460–519)	454	1.62	(1.47–1.78)
Emphysema (492)	61	1.42	(1.08–1.82)
Pneumonia (480–486)	119	1.20	(1.00–1.44)
Bronchitis (490–491)	11	1.41	(0.70–2.52)
Asthma (493)	8	1.70	(0.73–3.35)
Pneumoconioses and other respiratory (470–478, 494–519)	251	2.08	(1.82–2.35)
Acute kidney disease (580–581, 584)	8	1.03	(0.44–2.02)
Chronic kidney disease (582–583, 585–587)	34	1.27	(0.88–1.77)
Arthritis (711–6, 720–1)	17	2.19	(1.27–3.50)
Other musculoskeletal (710, 717–9, 722–9, 731–9)	10	2.14	(1.03–3.94)
Alcoholism (303)	22	1.79	(1.12–2.71)
Other disease of blood-forming organs (288–289)	9	2.31	(1.05–4.39)
Other diseases of skin (690–709)	10	2.45	(1.17–4.51)

[a]International Classification of Disease codes, 9th revision.

concentrated in those hired prior to 1930. The excesses for arthritis (12 observed, SMR 2.63, 1.36–4.59), other musculoskeletal disease (6 observed, SMR 2.14, 0.78–4.46), and other skin conditions (8 observed, SMR 3.28, 1.41–6.45) were concentrated in those hired between 1930 and 1950. The excess for alcoholism was concentrated in those hired after 1950 (11 observed, SMR 2.56, 1.28–4.58).

Among 602 miners participating in a 1960 U.S. Public Health Service survey, 23.4% had never smoked cigarettes or smoked only occasionally, 64.6% were current smokers, and 12.0% were former smokers. Among current smokers, 4.9% smoked less than 10 cigarettes a day, 76.6% smoked 10–20 per day, and 18.4% smoked 20 or more per day. Among white U.S. males surveyed in 1955, the age-adjusted prevalence of never/occasional cigarette smokers, current cigarette smokers, and former cigarette smokers was 32.8%, 56.6%, and 10.6%, respectively. Among U.S. current smokers, the percent smoking less than 10 cigarettes, 10–19 cigarettes, and 20+ cigarettes per day was 13.5%, 58.0%, and 28.5%, respectively. These data indicate that in 1960 more gold miners smoked cigarettes than U.S. white males of

TABLE V. Four Cohort Mortality Studies of Gold Miners*

Authors (year) (lung cancers)	Exposure–response for dust, comments	SMR or OR (95% CI)	Smoking control	Radon and arsenic
Hnizdo and Sluis-Cremer [1991; 77 lung cancers]	Positive and significant, good historical estimates, 30% respirable-free silica, silicosis generally not related to lung cancer	OR = 3.2(1.3–3.5) for highest exposed vs. lowest, nested case–control study	Yes, good data	Cumulative WLM average = 70, no data on arsenic
Kusiak et al. [1991; 378 lung cancers]	Positive and significant for duration, no detailed dose data, 6–12% respirable-free silica	SMR = 1.29 (1.2–1.5), risk only for mining <1946 (SMR = 1.40), Poisson regression used for exposure–response	Some, for a sample, not thought to explain excess	+ dose–response for both, not easy to separate effects from silica, average cumulative WLM ranges from 2 to 23 for 90% of cohort but some men had more than 50 WLM
Wyndham et al. [1986; 39 lung cancers]	Positive, p = .06, good historical estimates, exposure better predictor than duration	SMR = 1.61 (1.1–2.2), nested case–control used for exposure–response	Yes, good data	average WLMs (cumulative) = 36, no data on arsenic
Armstrong et al. [1979; 59 lung cancers]	No trend with years underground, no data on exposures, no risk for silicotics	SMR = 1.45, p<.01, internal comparison SMR = 1.40 (0.7–3.0)	Yes, good data	about 20 WLMs (cumulative average), arsenic levels about 50 ppm

*Excludes some autopsy case–control studies of gold miners, as well as some other publications referring to these same cohorts.

the same age, but that among smokers fewer were heavy smokers. Using these data and the technique described by Axelson and Steenland [1988], the estimated lung cancer rate ratio due to smoking alone, for the cohort vs. the U.S. population, would have been 1.07.

DISCUSSION

There have been four other cohorts of gold miners which have been studied (Table V). All these cohorts have been exposed to silica. In two [Wyndham et al., 1986; Hnizdo and Sluis-Cremer, 1991], positive trends between estimated cumulative dust and lung cancer were observed, another one [Armstrong et al., 1979] showed no such trend but had no data on exposure beyond duration of years underground, and the fourth [Kusiak et al., 1991] showed a positive trend but the effect was difficult to distinguish from the effects of radon and arsenic exposures.

The gold miners studied here exhibited excess mortality from silicosis and

silico-tuberculosis, which showed positive exposure–response trends. Chronic renal disease was nonsignificantly elevated, but also showed a significant positive exposure–response trend.

We found an excess of lung cancer in this cohort, the magnitude of which varied depending on which referent group was used. However, this excess was not related to estimated exposure to dust. The fact that silicosis and silico-tuberculosis showed clear positive exposure–response trends suggests that the estimated dust exposures used here were reasonably accurate. This would suggest that neither exposure to nonasbestiform amphiboles nor silica was likely to be responsible for the observed excess of lung cancer, at least not in a way related to quantitative exposure to dust.

There was only one death from asbestosis in this cohort (which could have been the result of asbestos exposure outside the mine). Assuming the overall lack of asbestosis is real, however, it would therefore appear that the nonasbestiform fibers in this mine did not cause any marked excess of either asbestosis or lung cancer.

There is strong evidence from numerous studies that silicotics suffer from high rates of lung cancer [see for example, Amandus et al., 1991]. Evidence that exposure to silica itself causes lung cancer has been less consistent to date, although the majority of the studies are positive, and two have shown a positive dose–response [Checkoway et al., 1993; Hnizdo and Sluis-Cremer, 1991]. In this cohort there was considerable mortality and morbidity from silicosis. Those who did have silicosis may have contributed to the observed moderate lung cancer excess. We did examine the presence or absence of a listing for silicosis (or silico-tuberculosis or pneumoconiosis) on the death certificate, although this may not be a very precise measure of which men had silicosis. There was no evidence that lung cancer deaths had more silicosis listed on their death certificates than other deaths. Only 3 of the 115 lung cancer deaths indicated silicosis (or silico-tuberculosis or pneumoconiosis) on their death certificates, while 140 of the 1,551 total death certificates indicated silicosis.

Levels of exposure to crystalline silica in our study were high, especially prior to the lowering of dust levels in the early 1950s, although they were not as high as some occupational cohorts, such as Vermont granite cutters. Vermont granite cutters were exposed to 20–50 mppcf of dust from granite which was composed of 10–30% silica prior to the installation of dust controls in the 1940s [Steenland and Beaumont, 1986]. The gold miners studied here were exposed to approximately 10–30 mppcf of dust prior to 1950, dust composed of approximately 13% free silica, and to under 10 mppcf thereafter. It is of interest to note that an analysis of lung cancer risk and the presence of silicosis on the death certificate was strongly positive in the granite cutters, while no such relationship was apparent in this study for gold miners.

The exposure to silica did lead to a variety of diseases besides the expected silico-tuberculosis and pneumoconiosis. Silica exposure has been associated with autoimmune disease, and the excesses found here for arthritis and musculoskeletal disease (the latter category included several deaths mentioning systemic sclerosis and systemic lupus) have been noted elsewhere in the literature for silica-exposed workers [Steenland et al., 1992; Koskela et al., 1987; Sluis-Cremer et al., 1985; Klockars et al., 1987]. The category "other skin disease," which was also significantly elevated, included several deaths with mention of lupus erythematosus and scleroderma on the death certificate. Renal disease also showed associations with high silica exposure, and this association may also reflect an autoimmune mechanism.

The elevation of "other diseases of the blood-forming organs" is interesting in light of the excess also observed for non-Hodgkin's lymphomas and the evidence from animal studies that injected silica can cause lymphoma [IARC, 1987]. Listings on the death certificates for this category included bone marrow depression, myelodysplasia, blood dyscrasia, and myeloid metaplasia.

In summary, the present cohort with substantial silica exposure exhibited large excess mortality from several causes of death known to be related to silica. Lung cancer, of interest because of a possible association with either silica, silicosis, or nonasbestiform amphibole minerals, showed some elevation but did not exhibit a positive exposure–response trend using estimated quantitative cumulative dust exposures. This cohort represents one of the principal cohorts in which the association between cumulative silica exposure and lung cancer was generally negative. The reasons for the discrepancy between our findings and the positive findings in other gold miner studies and in the silica literature in general remain unexplained. One possibility is that not all silica is alike, and different mineralogic varieties may have different effects. Another possibility is that the positive dose–responses for dust in other gold miner studies reflect to some degree uncontrolled confounding by radon or arsenic.

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REFERENCES

Amandus H, Shy C, Wing S, Blair A, Heineman E (1991): Silicosis and lung cancer in North Carolina dusty trade workers. Am J Ind Med 20:57–70.

ACGIH (1983): "Threshold Limit Values for Chemical Substances and Physical Agents in the Work Environment." Cincinnati, OH: ACGIH.

Armstrong B, McNulty J, Levitt L, Williams K, Hobbs M (1979): Mortality in gold and coal miners in Western Australia with special reference to lung cancer. Br J Ind Med 36:199–205.

Axelson O, Steenland K (1988): Indirect methods of assessing tobacco use in occupational studies. Am J Ind Med 13:105–118.

Beaumont J, Steenland K, Minton A, Meyer S (1989): A computer program for incidence density sampling of controls in case-control studies nested within occupational cohort studies. Am J Epidemiol 129:212–218.

Breslow N, Lubin J, Marek P, Langholz B (1983): Multiplicative models and cohort analyses. J Am Stat Ass 78:1–12.

Brown D, Kaplan K, Zumwalde R, Kaplowitz M, Archer V (1985): "Final Report: Retrospective Cohort Mortality Study of Underground Gold Mine Workers." Springfield, VA: IWSB/DSHEFS/NIOSH,NTIS.

Brown D, Kaplan S, Zumwalde R, Kaplowitz M, Archer V (1986): Retrospective cohort mortality study of underground gold mine workers. In Goldsmith D, Winn D, Shy C (eds): "Silica, Silicosis, and Lung Cancer." New York: Praeger, pp 311–336.

Checkoway H, Heyer N, Demers P, Breslow N (1993): Mortality among workers in the diatomaceous earth industry, Brit J Ind Med 50:586–597.

Code of Federal Regulations 29 (February 1990): "OSHA: Occupational Exposure to Asbestos, Proposed Rulemaking, Parts 1910 and 1926." Washington, DC: Government Printing Office.

Code of Federal Regulations 30 (July 1992): "MSHA: Safety and Health Standards for Underground Metal and Nonmetal Mines, Section 57.5038." Washington, DC: Government Printing Office.

Gillam J, Dement J, Lemen R, Wagoner J, Archer V, Blejer H (1976): Mortality patterns among hard rock gold miners exposed to an asbestiform mineral. Ann N Y Acad Sci 271:336–344.

Goldsmith D (1994): Silica exposure and pulmonary cancer. In Samet J (ed): "Epidemiology of Lung Cancer." New York: Marcel Dekker (in press).

Haenszel W, Shimkin M, Miller H (1956): Tobacco smoking patterns in the United States. Public Health Monogr 45:1–106.

Haustein U, Ziegler V, Herrman K, Mehlhorn J, Schmidt C (1990): Silica-induced scleroderma. Acad Dermatol 22:444–448.

Hnizdo E, Sluis-Cremer G (1991): Silica exposure, silicosis, and lung cancer: A mortality study of South African gold miners. Br J Ind Med 48:53–60.

IARC (1987): "The Evaluation of the Carcinogenic Risk of Chemicals to Humans, Monograph 42, Silica and Silicates." Lyon, France: IARC.

Klockars M, Koskela R, Jarvinen E, Kolari P, Rossi A (1987): Silica exposure and rheumatoid arthritis: A follow-up study of granite workers 1940–1981. Br Med J 294:997–1001.

Koskela R, Klockars M, Jarvinen E, Kolari P, Rossi A (1987): Mortality and disability among granite workers. Scand J Work Environ Health 13:18–25.

Kusiak R, Springer J, Ritchie A, Muller J (1991): Carcinoma of the lung in Ontario gold miners: Possible aetiological factors. Br J Ind Med 48:808–817.

Lilienfeld D, Gunderson P (1984): The 'missing' cases of pleural malignant mesothelioma in Minnesota, 1979–1981: Preliminary report. Public Health Rep 161:395–399.

McDonald JC, Gibbs G, Liddell W, McDonald A (1978): Mortality after long exposure to cummingtonite-grunerite. Am Rev Respir Dis 118:271–277.

Osorio A, Thun M, Novak R, Van Cura E, Avner E (1987): Silica and glomerulonephritis; case report and review of the literature. Am J Kidney Dis 11:224–230.

Pairon J, Brochard P, Jaurand M, Bignon J (1991): Silica and lung cancer: A controversial issue. Eur Respir J 4:730–744.

Rustin M, Bull H, Ziegler V, Mehlhorn J, Haustein U, Maddison P, Hames J, Dowd P (1990): Silica-associated systemic sclerosis is clinically, serologically, and immunologically indistinguishable from idiopathic systemic sclerosis. Br J Dermatol 123:725–734.

SAS (1991): "SAS User's Guide: Statistics (version 6.07)." Cary, NC: SAS Institute.

Sluis-Cremer GK, Hessel P, Hnizdo E, Churchill A, Zeiss E (1985): Silica, silicosis, and progressive systemic sclerosis. Br J Ind Med 42:838–843.

Stanton M, Layard M, Tegeris A (1981): Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. J Natl Cancer Inst 7:965–975.

Steenland K, Stayner L (1991): The role of employment status in occupational mortality studies. Epidemiology 2:418–423.

Steenland K, Thun M, Ferguson B, Port F (1990a): Occupational and other exposures associated with end-stage renal disease: A case-control study. Am J Public Health 80:153–157.

Steenland K, Beaumont J, Spaeth S, Brown D, Okun A, Jurcenko L, Ryan B, Phillips S, Roscoe R, Stayner L, Morris J (1990b): New developments in the Life Table Analysis System of the National Institute for Occupational Safety and Health. J Occup Med 32:1091–1098.

Steenland K, Beaumont J (1986): A proportionate mortality study of granite cutters. Am J Ind Med 9:189–201.

Steenland K, Nowlin S, Ryan B, Adams S (1992): Use of multiple-cause mortality data in epidemiologic analyses. Am J Epidemiol 136:855–862.

Wyndham C, Bezuidenhout B, Greenacre M, Sluis-Cremer GK (1986): Mortality of middle-aged white South African gold miners. Br J Ind Med 43:677–684.

Zumwalde R, Ludwig H, Dement J (1981): "Industrial Hygiene Report–Homestake Mining Company, Lead, South Dakota." Cincinnati, OH: IWSB, DHSEFS, NIOSH.

1981

Variations in the Carcinogenicity of Tremolite Dust Samples of Differing Morphology

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INTRODUCTION

Research on the harmful effects of mineral fibers has concentrated on the main commercially used varieties of asbestos—chrysotile from the serpentine group of minerals and crocidolite and amosite from the amphibole group. All of these have proved fibrogenic and carcinogenic in both humans and experimental animals.[1] However, the amphibole group of minerals is large and contains other examples that can exist as asbestos, including anthophyllite, actinolite, and tremolite.

Samples of tremolite have been examined in experimental studies using both inhalation and injection into the peritoneal and pleural cavities of rats and have been found to be extremely carcinogenic.[2-5] While the industrial usage of pure tremolite asbestos is very small (small deposits have been mined in Korea, India, and Pakistan), there is evidence that human disease may be associated with both environmental exposures to tremolite asbestos or exposures to other mineral forms contaminated with tremolite asbestos. Workers exploiting talc deposits contaminated with asbestiform tremolite fibers have been shown to have an excess of both pulmonary carcinomas and mesotheliomas,[6,7] and the use of lime containing tremolite asbestos for stuccoing houses in Greece and Turkey has also been associated with these tumor types.[8]

The importance that minor tremolite asbestos contamination of other commercially exploited minerals might have is demonstrated by findings from the Canadian chrysotile industry. While tremolite is a very minor contaminant of the chrysotile ore bodies, it has been found that tremolite fibers nevertheless make up the majority of the fiber burden in the lungs of workers examined at autopsy.[9,10] It is suggested that this tremolite asbestos may have been responsible for the very few cases of mesothelioma that have been associated with chrysotile mining. It is likely that the high tremolite content in the lungs of Canadian miners is due to differential retention, and a similar finding was reported from the Swedish Asbestos Cement Industry, where historically asbestos exposure was known to have been more than 90% chrysotile, with a small amount of amphibole asbestos, yet the lung fiber content at autopsy was more than 90% amphibole.[11] Experimental studies have also confirmed the rapid removal of chrysotile from lung tissues in comparison with the rate of removal of amphibole asbestos minerals.[12]

Since the amphibole minerals in general appear so durable in the lung, the contamination of any other product with an amphibole such as tremolite asbestos might prove hazardous. The problem of contaminated talc has been mentioned,

ıut other commercially used materials such as vermiculite and marble can also :ontain tremolite asbestos, and concern regarding the full significance of this :ontamination is increasing. Tremolite is a comparatively simple calcium magne-ium silicate with an ideal chemical formula of $Ca_2Mg_5Si_8O_{22}(OH)_2$. The Interna-ional Mineralogical Association guidelines on the nomenclature of amphiboles 'estricts the use of the term tremolite to compositions very close to the ideal 'ormula, although a certain amount of substitution of iron for magnesium, sodium 'or calcium, and aluminium for silica is permitted (similar minerals with progres-ively more iron in the formula are referred to as actinolite and ferro-actinolite). Tremolite is a common and widespread amphibole mineral simply because the ıssociation of the chemical components, calcium, magnesium and silica, is com-non within the metamorphic rocks of all of the major mountain belts of the world. For example, dolomite limestones ($CaMg(CO_3)_2$) in sedimentary rock sequences ›r the large serpentinite masses (high Mg content) of these regions were often :onverted to tremolite rocks by the great pressures and high temperatures of the nountain-building processes. The comparatively simple compositions and abun-lance of the parent materials lead to frequent occurrences of amphibole minerals very close indeed to the ideal tremolite composition.

While the different occurrences of tremolite may be remarkably consistent in :hemical terms, the mineral may show a very wide variation in morphological ıabit. Depending on the exact pressure, temperature and tectonic conditions ›revailing at the time of its formation, the tremolite may have crystallized as :oarse, prismatic, massive crystals or as fine asbestiform fibers. Dorling and Zussman[13] describe a very wide range of these different varieties, and, despite extremely careful evaluation using a variety of analytical techniques, including ıigh-resolution transmission electron microscopy and detailed chemical analysis, :hey were unable to provide an entirely satisfactory explanation of the different morphological varieties. More recent work[14] has suggested that asbestos could be differentiated on the basis of such features as the presence of polyfilamentous fiber bundles, the proportions of multiple crystal twins, and the frequency of :hain-width errors in the crystal structure.

Tremolite contamination of other materials can range from pure asbestiform ibers through irregular if elongated spicules to nonfibrous particles. While the ısbestiform fibers are known to be hazardous, the level of hazard associated with ›ther morphological habits is uncertain. In these circumstances concern has been expressed over the use of any product contaminated with any form of tremolite ınd a clear definition of the hazard to be expected from all tremolite types is greatly needed. Wagner *et al.*[3] commenced an examination of the relative carcino-genicity of asbestiform and nonasbestiform tremolite, but interpretation of the esults was made difficult by poor animal survival. The present study was planned :o examine this problem in more detail and the carcinogenicity of tremolite asbes-:os and nonasbestos varieties has been examined in rats using the technique of ntraperitoneal injection.

MATERIALS AND METHODS

The six tremolite minerals used in the present study are listed below.

(1) California tremolite from Jamestown
(2) Korean tremolite

(3) Tremolite from Swansea
(4) Italian tremolite (Ala di Stura)
(5) Tremolite from Carr Brae, Dornie, Scotland
(6) Tremolite from Shinness, Scotland

Specimens 1–3 were donated to the Institute of Occupational Medicine in Edinburgh as reference samples, and only with specimen 2 is their exact location of origin known. Sample 3 was donated by the British Coal Laboratory in Swansea and in this case even the country of origin is uncertain. All three specimens had a very distinctive asbestiform morphology, being flexible and strong and fairly elastic. The Californian tremolite was the finest and apparently the toughest, having fibers that were difficult to separate. The Korean tremolite differed in that it was already quite highly processed by milling when it was received and was also mildly contaminated by chrysotile.[4,5] The Swansea tremolite consisted of fine, easily separable fibers.

The tremolite from Ala di Stura (in a small valley in northwestern Italy) differed from the first three in very distinctive ways. It consisted of large bundles of very long (often >5 cm) needle-like fibers which were flexible and very elastic but quite brittle. It had a bright vitreous luster, unlike the more matt appearance of the first three tremolite samples. The tremolite from Dornie was similar to the Italian tremolite in its vitreous luster, but contrasted with it in being a fine powdery material. The original specimen was collected from a road cutting near Dornie in the Highland region of Scotland. The outcrop is one of a series where high-grade metamorphic marble, dolomite, and calcium silicates are found. The original material was extremely friable and the sample was soon reduced to a fine, soft powder in the course of handling when wet in a plastic bag.

The tremolite from Shinness was collected from a small, disused marble quarry on the shores of Loch Shin near Lairg in the Highland region of Scotland. It consisted of prismatic crystals up to approximately 10 mm × 1 mm × 0.5 mm in size in massive aggregates. The crystals were vitreous in appearance and very brittle.

For use in the injection studies, the three asbestiform tremolites (samples 1–3) were all prepared in exactly the same way. The materials were packed into the cylinders of Timbrell dust dispensers[15] and airborne dusts were generated. Respirable fractions of these dusts were collected using a vertical elutriator.[16] No further purification was required.

The Italian tremolite had to be broken by hand to separate the thicker, longer bundles before packing into the Timbrell dust dispenser, but the material was then treated in the same manner as the asbestiform varieties. Fortunately the large bundles of fiber were very easy to split and separate because of their brittle nature.

The Dornie tremolite differed again in the treatment required. The crumbled crystal powder was washed free of organic material (minor rootlets) by repeated sedimentation in distilled water. The tremolite was collected by decanting off the water and drying the powder at 110°C overnight. This loose material consisted mainly of small needle-like crystals which were used for dust preparation as above.

The prismatic crystals of the Shinness tremolite could not be used directly to generate airborne dust because of the high physical strength of the crystal mass. The individual crystals and clumps were hand-picked for purity from a roughly crushed preparation and these were then ground in distilled water for 5 minutes

using a corundum micronizing mill. The resulting powder was collected by filtration, dried at 110°C overnight, and then used for dust generation.

For the animal injection studies weighed amounts of the six tremolite dust samples were suspended in phosphate-buffered saline (PBS) so that the planned dose of 10 mg was contained in a saline volume of 2.0 ml. This dose was administered to groups of either 33 or 36 3-month-old rats of the AF/Han strain as a single intraperitoneal injection. For the injection, animals were anaesthetized with ether and after recovery were allowed to live out their full life span until they showed signs of debility or tumor formation. In most instances the diagnosis of mesothelioma was obvious at autopsy, but, in cases of doubt, appropriate tissue was taken for histological examination. The specimen was fixed in formal saline and embedded in paraffin wax. Sections were stained with hematoxylin and eosin for histological examination.

All of the minerals were analyzed qualitatively by X-ray diffraction using a Philips X-ray powder diffractometer, by infrared spectrophotometry using a Perkin Elmer spectrophotometer (PE 580), and by polarized light microscopy. Small samples of minerals were also evaluated by scanning electron microscopy (SEM) (Cambridge Instruments S250 Mark 2) and transmission electron microscopy (TEM) (Hitachi H7000), with individual particles analyzed semiquantitatively using energy-dispersive X-ray spectroscopy (Link Analytical 860.200,AN10).

For examination by scanning electron microscopy, small portions (0.4–0.8 mg) of the elutriated dusts were accurately weighed and made into suspensions with 100 ml of filtered distilled water (0.2 μm pore size filter). Aliquots of different volumes from 1–10 ml were taken from each suspension and filtered onto 0.2 μm-pore-size polycarbonate filters. For evaluation by scanning electron microscopy, segments were cut from each filter, mounted on a standard 13 mm sample stub, and coated with gold before evaluation.

Size distribution data for each of the tremolite samples were produced by counting and measuring fibers and particles directly on the screen at a magnification of × 10,500. All fibers and particles found in a standard search pattern were measured while recording the number of fields of view examined. Data were collected for fibers (aspect ratio >3 : 1) and particles (aspect ratio <3 : 1) of all sizes. Once sufficient data had been collected for description of the shorter fibers and the smaller particles, these were ignored so that the effort could be concentrated on counting and sizing of the larger material. Fifty particles of all sizes were counted and the number of fields recorded; thereafter a further 100 particles with their longer dimensions >0.3 μm were counted. Three hundred fibers of all sizes were counted, followed by a count of a further 100 fibers with lengths >5 μm. Data from these assessments were combined, weighted in proportion to the areas assessed to give estimates of fiber density in various size ranges. From the mass of dust known to have been deposited on the whole filter, these figures were then re-expressed in numbers of fibers or particles per unit mass.

Statistical analysis of the times at which the deaths associated with mesotheliomas were observed appealed to standard methods for analyzing survival data, treating deaths other than from mesothelioma as censoring events.[17] Survival curves were estimated by the Kaplan–Meier method, and analyses of the relationships between mesothelioma production and expressions of the doses received by each animal used Cox's proportional hazards regression model,[18] fitted by maximum likelihood. All computations used the package BMDP.[19]

RESULTS

Mineralogical Analysis

The X-ray diffraction patterns and the infrared absorption spectra of the si minerals were essentially identical, with only minor differences in some features

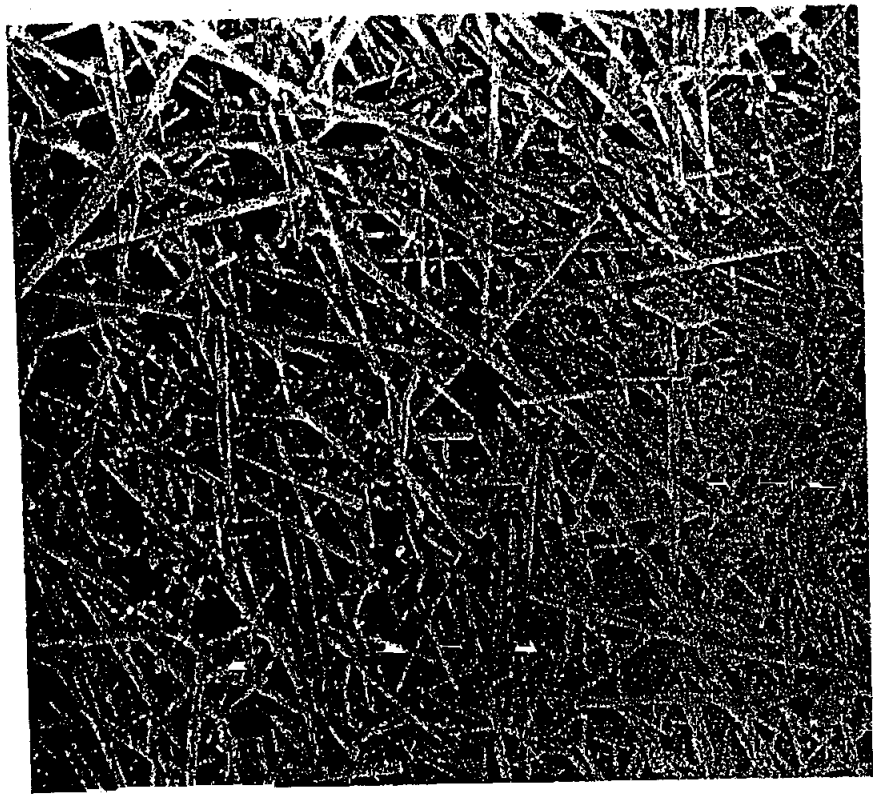

FIGURE 1. Tremolite sample No. 1: Californian tremolite (×10,500). FIGURES 1–6 a scanning electron microscope photographs of the tremolite dust samples used in the injection studies.

All of the XRD patterns were comparable with the ASTM powder diffracti pattern for tremolite from St. Gotthard.[20]

Although full quantitative chemical analyses were not carried out on the fiber the X-ray counts for the main elements were all very similar, as shown in TABL 1. These show higher iron and lower magnesium in the Swansea tremolite and, a lesser extent, in the Italian tremolite, but these differences are not considered be important.

ABLE 1. Proportions of Simple X-ray Counts for the Major Elements of the Six remolite Minerals

	Source of Tremolite Samples					
lement	California	Korea	Swansea	Ala di Stura	Dornie	Shinness
Si	65.8	65.9	66.3	66.3	65.3	65.6
Mg	11.3	12.0	8.5	11.6	11.8	11.8
Fe	2.0	0.9	4.0	2.3	1.5	0.6
Ca	20.8	21.1	19.9	20.5	21.0	21.4
Al	0.1	0.3	0.3	'0.3	0.5	0.7

The overall morphology of the tremolite samples as seen by SEM is illustrated ı Figures 1–6, all of which were produced at the same magnification. In the ptical microscopy and SEM examinations, the three asbestos tremolites (samles 1–3) were found to be typical of that form in displaying polyfilamentous fiber undles, curved fibers, fibers with splayed ends, and long, thin, parallel-sided bers. Most of the fibers showed straight extinction when observed with polarized

FIGURE 2. Tremolite sample No. 2: Korean tremolite (×10,500).

light under crossed polarizers, indicating the presence of multiple twinning of the crystals. All three asbestos samples did contain some elongated fragments of tremolite with oblique extinction, stepped ends, and nonparallel sides indicating that they were cleavage fragments. These were more abundant in the Korean and the Swansea minerals. The nonfibrous proportions of the dusts of these three minerals were very low. The tremolite from Italy contained mostly cleavage fragments, but some very long, thin fibers were observed. Similarly, the Dornie

FIGURE 3. Tremolite sample No. 3: tremolite supplied by the laboratory from Swanse (×10,500).

tremolite also contained a small proportion of long, thin asbestiform fibers, bu was predominantly made up of cleavage fragments. Both contained a high propor tion of nonfibrous tremolite particles. The Shinness tremolite dust was almo exclusively composed of cleavage fragments, only a small proportion of whic had an aspect ratio greater than 3 : 1.

The number of fibers >8 μm in length in the injected doses ranged betwee 13,000 × 10^6 for the California tremolite to 460 × 10^6 for the Shinness tremolit Complete fiber and particle sizing data are presented in TABLES 2 and 3.

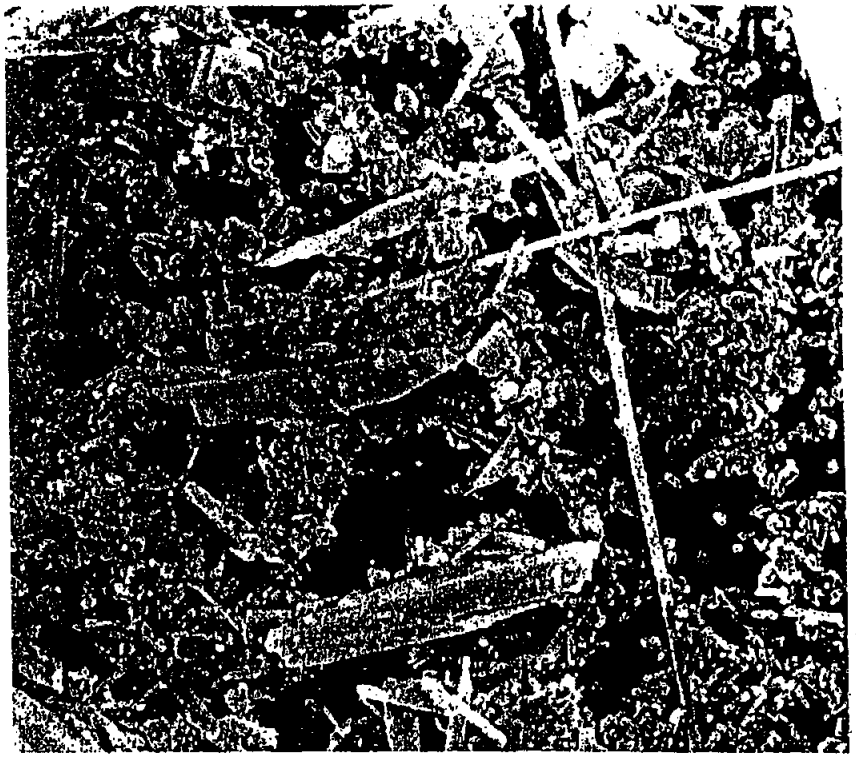

FIGURE 4. Tremolite sample No. 4: tremolite from Ala di Stura (Italy) (×10,500).

Injection Studies

The proportion of animals developing peritoneal mesotheliomas in each exper-ıntal group is illustrated in TABLE 4, together with the median survival time of ıor-bearing animals and condensed data relating to the numbers of fibers and ticles. Samples 1–3 produced tumors in almost all animals, although with ıificant differences in the distributions of the times at which deaths occurred. nple 4 produced tumors in 67% of animals, but with a longer median survival e than that of the previous groups, whereas samples 5 and 6 produced too few ıors for median survival times to be calculated.

The whole data set, with dose considered initially in terms of the mass of all ·rs, was analyzed by fitting Cox's proportional hazards model. Estimates of the ıtive hazards of the six dusts are illustrated in TABLE 4, with sample 3 chosen itrarily as the baseline. The estimated coefficients are on the log scale and .cate, for example, that the Californian tremolite (sample 1) is exp (0.8303) = times as hazardous as the Korean tremolite (sample 3).

For comparisons of the carcinogenicity of the six tremolite samples in relation he numbers of fibers of different dimensions, fibers were classified into ten ıps. Length groupings were 0–1 μm, 1–3 μm, 3–5 μm, 5–8 μm, and >8 μm.

Diameters were <0.25 μm and >0.25 μm. Fibers with dimensions exactly equal to the class boundary have been included with the larger fiber group. Logs of these numbers were used as explanatory variables in Cox's proportional hazards regression models. The model that fitted separate relative hazards yielded a log likelihood statistic for differences amongst the six types of 269.99 with five degrees of freedom. Fitting instead to a dose parameter will yield a smaller Chi-squared statistic, with a single degree of freedom, and the difference can be treated as a Chi-squared goodness-of-fit test, with four degrees of freedom, of the hypothesis that a single parameter regression is an adequate representation of differences between the types. Goodness-of-fit statistics have been calculated in this way for a variety of different fiber number parameters and these are summarized in TABLE 5. The smallest value, indicating the best fit, was achieved for the longest, thinnest fibers (length >8 μm, diameter <0.25 μm). However, with four degrees of freedom, a Chi-squared statistic of over 40 is very significant, indicating that even in this case the fit to a straight line is not good and that the carcinogenicity of the group of tremolite samples examined does not relate entirely to fibers of any one of the size ranges examined.

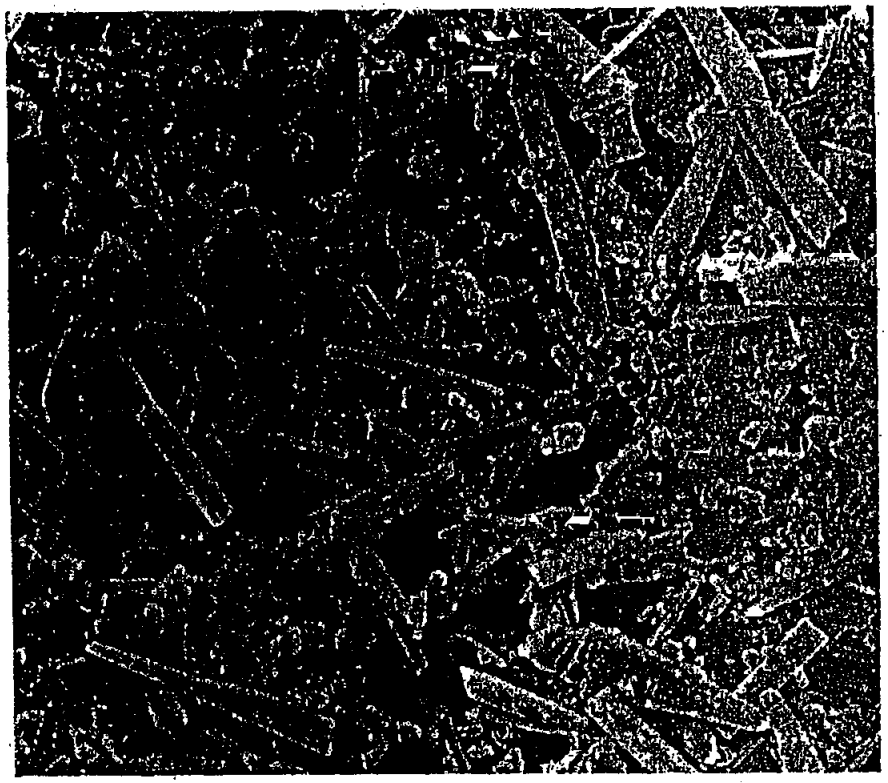

FIGURE 5. Tremolite sample No. 5: tremolite from Carr Brae, Dornie, Scotland (×10,500).

to Fiber Length and Diameter[a]

Diameter (μm)	Length (μm) 0.00–1.00	1.00–2.00	2.00–3.00	3.00–4.00	4.00–5.00	5.00–6.00	6.00–7.00	7.00–8.00	8.00–9.00	9.00–10.00	>10.00	Total
California												
0.000–0.125	56	226	85	0	0	0	0	0	0	0	0	367
0.125–0.250	1298	2794	847	452	282	179	108	27	54	9	27	6076
0.250–0.375	85	1073	1016	565	452	125	99	81	18	9	152	3673
0.375–0.500	0	123	226	198	85	108	45	36	54	45	36	1254
0.500–0.625	0	113	310	254	28	45	134	81	54	27	108	1154
0.625–0.750	0	0	85	56	0	9	9	0	0	0	90	249
0.750–0.875	0	0	56	113	0	0	0	9	0	0	27	205
0.875–1.000	0	0	0	0	0	0	0	18	0	0	0	18
>1.000	0	0	0	0	0	0	18	0	9	18	36	81
Total	1439	4629	2625	1637	847	466	412	251	188	108	475	13077
Korea												
0.000–0.125	420	404	65	32	32	13	0	0	0	0	7	973
0.125–0.250	549	1131	420	162	32	53	13	7	26	0	26	2420
0.250–0.375	0	1066	355	194	32	53	46	0	7	40	53	1846
0.375–0.500	0	339	258	210	32	53	20	20	0	7	92	1031
0.500–0.625	0	129	258	194	97	53	33	20	13	7	40	843
0.625–0.750	0	0	65	16	81	20	7	0	0	0	7	195
0.750–0.875	0	0	16	32	0	13	0	0	0	0	20	81
0.875–1.000	0	0	16	32	0	13	0	0	0	7	0	68
>1.000	0	0	0	0	32	26	0	0	20	7	53	138
Total	969	3070	1454	872	339	297	119	46	66	66	297	7595
Swansea												
0.000–0.125	26	0	0	0	0	0	0	0	0	0	0	26
0.125–0.250	31	62	15	15	0	12	0	0	0	6	3	144
0.250–0.375	10	144	88	15	36	0	6	3	3	0	12	317
0.375–0.500	0	57	98	10	51	26	6	9	15	0	12	283
0.500–0.625	0	57	165	72	134	38	35	20	20	9	23	573
0.625–0.750	0	0	41	36	26	12	15	6	0	0	23	158
0.750–0.875	0	0	21	26	15	15	18	0	3	12	23	132
0.875–1.000	0	0	10	21	5	15	0	9	9	6	26	100
>1.000	0	0	0	41	51	38	20	35	6	12	137	341
Total	67	319	438	237	319	155	99	82	55	44	260	2074

Ala di Stura (Italy)												
0.000–0.125	6	10	19	0	0	0	0	0	0	0	0	35
0.125–0.250	35	115	10	3	0	1	0	2	0	0	1	166
0.250–0.375	19	195	77	0	0	0	1	1	1	0	0	293
0.375–0.500	0	195	80	35	3	6	1	2	0	0	2	322
0.500–0.625	0	124	179	70	38	12	2	2	2	1	6	436
0.625–0.750	0	0	19	13	10	9	2	2	0	0	2	56
0.750–0.875	0	0	3	13	16	6	4	0	2	0	4	47
0.875–1.000	0	0	0	19	10	2	0	1	1	2	1	34
>1.000	0	0	0	16	26	10	13	8	6	2	26	107
Total	61	638	386	169	102	44	22	17	12	5	41	1496
Carr Brae, Dornie, Scotland												
0.000–0.125	24	24	10	0	0	0	0	0	0	0	0	58
0.125–0.250	14	51	10	5	2	0	0	1	0	0	0	83
0.250–0.375	5	89	14	10	0	7	2	0	0	0	2	129
0.375–0.500	0	53	53	27	7	1	4	0	6	1	1	153
0.500–0.625	0	68	101	36	17	9	2	6	0	0	5	244
0.625–0.750	0	0	41	5	17	3	2	3	6	3	4	84
0.750–0.875	0	0	14	14	2	1	3	0	2	0	5	42
0.875–1.000	0	0	7	0	12	4	4	1	2	3	5	38
>1.000	0	0	0	46	19	9	12	23	20	5	64	198
Total	43	285	251	142	77	34	29	34	36	12	86	1029
Shinness, Scotland												
0.000–0.125	4	0	0	0	0	0	0	0	0	0	0	4
0.125–0.250	14	41	6	0	0	0	0	0	0	0	0	60
0.250–0.375	15	60	10	1	3	2	0	0	0	0	0	91
0.375–0.500	0	60	8	4	2	0	0	0	0	0	0	73
0.500–0.625	0	37	56	9	7	0	0	0	0	0	1	109
0.625–0.750	0	0	14	7	3	0	0	0	0	0	0	24
0.750–0.875	0	0	7	9	4	3	1	0	0	0	1	24
0.875–1.000	0	0	0	10	2	1	1	0	0	0	1	14
>1.000	0	0	0	14	14	13	9	2	3	3	9	68
Total	33	197	100	52	34	19	11	2	3	3	11	467

to Fiber Length and Diameter[a]

Diameter (μm)	Length (μm) 0.00–0.30	0.30–0.60	0.60–0.90	0.90–1.20	1.20–1.50	1.50–1.80	1.80–2.10	2.10–2.40	2.40–2.70	2.70–3.00	>3.00	Total
California												
0.000–0.300	12750	4217	43	0	0	0	0	0	0	0	86	17096
0.300–0.600	0	3830	1850	86	258	0	86	0	86	0	0	6197
0.600–0.99	0	0	0	172	86	86	86	0	0	0	0	430
0.900–1.200	0	0	0	0	0	86	0	0	0	0	0	86
1.200–1.500	0	0	0	0	0	0	0	0	0	0	0	0
1.500–1.800	0	0	0	0	0	0	0	0	0	0	0	0
1.800–2.100	0	0	0	0	0	0	0	0	0	0	0	0
2.100–2.400	0	0	0	0	0	0	0	0	0	0	0	0
>2.400	0	0	0	0	0	0	0	0	0	0	0	0
Total	12750	8047	1893	258	344	172	172	0	86	0	86	23809
Korea												
0.000–0.300	7644	1421	0	0	0	0	0	0	0	0	0	9065
0.300–0.600	0	1184	908	395	118	0	0	0	0	0	0	2604
0.600–0.900	0	0	79	79	79	59	0	39	0	0	118	454
0.900–1.200	0	0	0	0	39	39	0	20	0	0	39	138
1.200–1.500	0	0	0	0	0	0	0	0	39	0	20	59
1.500–1.800	0	0	0	0	0	0	20	20	0	0	39	79
1.800–2.100	0	0	0	0	0	0	0	0	0	0	0	0
2.100–2.400	0	0	0	0	0	0	0	0	0	0	0	0
>2.400	0	0	0	0	0	0	0	0	0	0	0	0
Total	7644	2604	987	474	237	99	20	79	39	0	217	12399
Swansea												
0.000–0.300	3053	385	0	0	0	0	0	0	0	0	0	3438
0.300–0.600	0	602	735	325	0	0	0	0	0	12	0	1674
0.600–0.900	0	0	96	145	48	84	12	0	0	0	0	385
0.900–1.200	0	0	0	36	0	12	24	12	12	0	0	96
1.200–1.500	0	0	0	0	0	24	24	0	12	0	0	60
1.500–1.800	0	0	0	0	0	0	0	24	0	0	0	24
1.800–2.100	0	0	0	0	0	0	0	0	0	0	0	0
2.100–2.400	0	0	0	0	0	0	0	0	0	0	0	0
>2.400	0	0	0	0	0	0	0	0	0	0	24	24
Total	3053	988	831	506	48	120	60	36	24	12	24	5703
Ala di Stura (Italy)												
0.000–0.300	2564	641	0	0	0	0	0	0	0	0	0	3205
0.300–0.600	0	5609	5021	1442	267	0	0	0	0	0	0	12339
0.600–0.900	0	0	961	1496	481	53	107	0	0	0	0	3098
0.900–1.200	0	0	0	160	107	427	107	107	0	0	0	908
1.200–1.500	0	0	0	0	107	267	53	0	0	0	0	427
1.500–1.800	0	0	0	0	0	0	53	0	107	0	0	160
1.800–2.100	0	0	0	0	0	0	0	0	0	0	0	0
2.100–2.400	0	0	0	0	0	0	0	0	0	0	0	0
>2.400	0	0	0	0	0	0	0	0	0	0	0	0
Total	2564	6249	5982	3098	961	748	320	107	107	0	0	20137
Carr Brae, Dornie, Scotland												
0.000–0.300	3715	382	0	0	0	0	0	0	0	0	0	4097
0.300–0.600	0	1337	1359	510	191	0	0	0	0	0	0	3397
0.600–0.900	0	0	234	276	149	127	85	42	0	0	0	913
0.900–1.200	0	0	0	0	85	127	42	0	42	0	0	297
1.200–1.500	0	0	0	0	42	42	85	21	0	0	0	191
1.500–1.800	0	0	0	0	0	0	0	42	85	42	42	212
1.800–2.100	0	0	0	0	0	0	0	0	0	42	64	106
2.100–2.400	0	0	0	0	0	0	0	0	21	42	21	85
>2.400	0	0	0	0	0	0	0	0	0	0	191	191
Total	3715	1720	1592	785	467	297	212	106	149	127	318	9489
Shinness, Scotland												
0.000–0.300	4581	352	41	0	0	0	0	0	0	0	0	4975
0.300–0.600	0	1119	1099	415	124	0	0	0	0	0	0	2757
0.600–0.900	0	0	415	332	290	41	41	0	0	0	0	1?
0.900–1.200	0	0	0	83	83	83	83	0	21	0	0	?
1.200–1.500	0	0	0	0	21	104	62	0	0	0	41	228
1.500–1.800	0	0	0	0	0	41	0	41	21	41	83	228
1.800–2.100	0	0	0	0	0	0	0	21	0	0	104	124
2.100–2.400	0	0	0	0	0	0	0	0	0	0	41	41
>2.400	0	0	0	0	0	0	0	0	0	0	21	21
Total	4581	1472	1555	829	518	269	187	62	41	41	290	9845

[a] ... necessary at a magnification of ×10,500

TABLE 4. Summary of Survival Data and Comparison with Selected Re-expressions of 10-mg Dose as Numbers of Fibers and Particles

Sample No.	Type	No. of Animals	No. of Mesotheliomas	Median Survival Time (days)	Estimated Relative Hazard (log)	No. (*10^5) of Fibers in 1 mg of Injected Dust	No. (10^5) of Fibers of length ≥8 and diameter <0.25	No. (10^5) of Particles in 1 mg of Injected Dust
1	California	36	36	301	0.8308	13,430	121	18,375
2	Swansea	36	35	365	0.5593	2,104	8	4,292
3	Korea	33	32	428	0.0000	7,791	48	13,435
4	Italy	36	24	755	−1.6108	1,293	1	20,137
5	Carr Brae	33	4	*	−3.9043	899	0	9,490
6	Shinness	36	2	*	−4.7080	383	0	5,901

FIGURE 6. Tremolite sample No. 6: tremolite from Shinness, Scotland (×10,500).

TABLE 5. Chi-square (4) Statistics Comparing Goodness-of-Fit of Model Regressing Hazard on Dose Expressed as Logarithm of Numbers of Fibers in Different Size Ranges

Lengths (μm)	All	Diameters (μm)		
		<0.25	>0.25
ALL	72.63	76.10	74.32
0.0–1.0	67.55	74.31	63.78
1.0–3.0	86.51	80.72	92.34
3.0–5.0	73.90	109.31	72.83
5.0–8.0	68.97	71.62	72.97
Over 8.0	110.87	41.03	115.16
Over 5.0	89.22	67.96	94.11
Over 3.0	77.14	99.01	79.98

DISCUSSION

The results of the present study suggest that a wide-ranging group of tremolite samples all possessed some potential to produce mesotheliomas following injection into the rat peritoneal cavity. In general, carcinogenicity relates to the number of long, thin fibers rather than to any of the other dimensional characteristics of the dusts that were considered, but the relationship was by no means exact. Two of the dusts showed definite anomalies with this relationship. The tremolite from Swansea consisted of somewhat thicker fibers than those of the Korean variety, and therefore the number of long fibers in the injected dose was less. However, although both dusts produced a maximum response in tumor numbers, the Swansea specimen produced tumors with a significantly lower induction period. This resulted in a higher hazard factor's being calculated, indicating that the Swansea specimen was the more carcinogenic of the two dusts. These findings could suggest that some aspect other than the number of long fibers played a part in the carcinogenic effects of these dusts, but it is also possible that the relationship between long, thin fibers and mesothelioma production is blurred in the overdose situation that occurred with the asbestiform tremolite in the present study. The clarification of this problem would need a dose–response study to be undertaken with injected doses of dusts ranging from the 10 mg already used down to a level at which tumor production was relatively infrequent.

The other anomaly in the relationship of tremolite carcinogenicity to the number of long, thin fibers is potentially more important. The Italian tremolite sample produced tumors in nearly 70% of rats, although the dose injected contained only about one-third of the number of fibers >8 μm in length that were present in the specimen from Dornie, which produced only 12% of mesotheliomas. It is true that many of the long fibers in the Dornie specimen were >1 μm in diameter, but if only fibers >8 μm in length and <0.5 μm in diameter were considered, the two specimens have approximately equal numbers, which still does not conform to their very different carcinogenic potential. The problem of discriminating asbestiform fibers from elongated cleavage fragments has been discussed for some time. The overall impression gained from dense SEM preparations, as shown in this paper, is that the Italian tremolite specimen did contain a certain amount of what most observers would consider asbestiform fibers and that the Dornie specimen did not. This, however, was not demonstrated by fiber counts undertaken by routine procedures, possibly because the number of long "fibers" was only a small proportion of the total. In the present study, the observers sizing fibers by SEM were initially asked to differentiate between fibers with smooth parallel sides and those without. This proved impractical to do subjectively with any degree of reproducibility and had to be abandoned as a routine procedure. These two specimens, however, of the same mineralogical type with proven differences in carcinogenicity represent an opportunity to examine the importance of geometric differences in tumor production more critically. Perhaps the use of photographs would permit reproducible differentiation between asbestiform fibers and elongated cleavage fragments that would relate to carcinogenicity.

The part played by the nonfibrous particles of any basically fibrous material in the harmful effects of dust exposure is difficult to assess, but the results of the present study suggest that they are of little importance. Although quite high numbers of nonfibrous particles were present in the three asbestiform tremolite samples, they made up only an extremely small proportion of the total dust mass injected. With the other dusts the mass proportion was much higher. It could be postulated that since, after intraperitoneal injection, dusts osely aggregate into granulomas, a large amount of particulate material could mask the fibers fron the surrounding cells and thus reduce carcinogenicity. In the present study, how ever, the dust with the highest proportion of particles (by number) was the Italia tremolite, which had a higher carcinogenic potential than its overall fiber number would indicate.

Regardless of these difficulties the present study has demonstrated that a forms of the mineral tremolite have some carcinogenic potential. The intraperito neal injection test is, however, extremely sensitive, and it is usually considere that, with a 10-mg dose, any dust that produces tumors in fewer than 10% of th experimental group is unlikely to show evidence of carcinogenicity followin administration by the more natural route of inhalation. Human exposure to material such as that obtained from Shinness in Scotland, whether as a pur mineral dust or as a contaminant of other products, will almost certainly produc no hazard, and the material from Dornie is probably to be considered harmless t human beings as well. The problem lies in distinguishing these "harmless" cleav age fragments from more dangerous fibers, and routine fiber counting, using th current fiber definitions and counting rules with either optical or electronmicros copy, is probably inappropriate for this purpose.

SUMMARY

Six samples of tremolite of different morphological type were prepared a dusts of respirable size and used in intraperitoneal injection studies in rats. Thre "asbestiform" tremolites produced mesotheliomas in almost all animals, althoug with significantly different tumor-induction periods. A brittle type of fibrous trem olite which, when manipulated to prepare "respirable dust," produced a sampl with relatively few asbestiform fibers remaining nonetheless produced tumors i 70% of rats. Two samples of nonfibrous tremolite produced respirable dust sam ples containing numerous elongated fragments with aspect ratios greater tha 3 : 1, which therefore fitted the definition of respirable fibers. Both these sample produced relatively few tumors, although one had more long "fibers" than did th brittle tremolite that produced 70% of tumors. This study has therefore demor strated that different morphologic forms of tremolite produce dusts with ver different carcinogenic potential. Carcinogenicity does not depend simply on th number of elongated particles injected, and we need to develop methods of distir guishing carcinogenic tremolite fibers from relatively innocuous tremolite dust: with similar numbers of elongated particles of similar aspect ratios.

REFERENCES

1. SELIKOFF, I. J. & D. H. K. LEE. 1978. Asbestos and Disease. Academic Press. Ne York.
2. STANTON, M. F., M. LAYARD, A. TEGERIS, E. MILLER, M. MAY & E. MORGAN. 198 Relation of particle dimension to carcinogenicity in amphibole asbestos and oth fibrous minerals. J. Natl. Cancer Inst. 67: 965–975.
3. WAGNER, J. C., M. CHAMBERLAIN, R. C. BROWN, G. BERRY, F. D. POOLE R. DAVIES & D. M. GRIFFITHS. 1982. Biological effects of tremolite. Br. J. Canc 45: 352–360.

4. DAVIS, J. M. G., R. E. BOLTON, K. DONALDSON, A. D. JONES & B. MILLER. 1985. Inhalation studies on the effects of tremolite and brucite dust in rats. Carcinogenesis 6: 667–674.
5. DAVIS, J. M. G., R. E. BOLTON, H. COWIE, K. DONALDSON, I. P. GORMLEY, A. D. JONES & A. WRIGHT. 1985. Comparisons of the biological effects of mineral fibre samples using *in vitro* and *in vivo* assay systems. *In In vitro* Effects of Mineral Dusts. E. G. Beck & J. Bignon, Eds.: 405–411. Springer. Berlin.
6. KLEINFELD, M., J. MESSITE, O. KOOYMAN & M. H. ZAKI. 1967. Mortality among talc miners and millers in New York State. Arch. Environ. Health 14: 663–667.
7. KLEINFELD, M., J. MESSITE & M. H. ZAKI. 1974. Mortality experiences among talc workers. A follow-up study. J. Occup. Med. 16: 345–349.
8. YAZICIOGLU, S., R. IKAYTO, K. BALCI, B. S. SAYLI & B. YORULMAZ. 1980. Pleural calcification, pleural mesotheliomas and bronchial cancers caused by tremolite dust. Thorax 35: 564–569.
9. POOLEY, F. D. 1976. An examination of the fibrous mineral content of asbestos lung tissue from the Canadian chrysotile mining industry. Environ. Res. 12: 281–298.
10. CHURG, A., B. WIGGS, L. DE PAOLI, B. KAMPE & B. STEVENS. 1984. Lung asbestos content of chrysotile workers with mesothelioma. Am Rev. Resp. Dis. 130: 1042–1045.
11. GYLSETH, B., G. MOWE & A. WANNAG. 1983. Fibre type and concentration in the lungs of workers in an asbestos cement factory. Br. J. Ind. Med. 40: 375–379.
12. DAVIS, J. M. G. 1989. Mineral fiber carcinogenesis: Experimental data relating to the importance of fibre type, size, deposition, dissolution and migration. *In* Nonoccupational Exposure to Mineral Fibres. IARC Publication No. 90. 33–46. International Agency for Research on Cancer. Lyon, France.
13. DORLING, M. & J. ZUSSMAN. 1987. Characteristics of asbestiform and non-asbestiform amphiboles. Lithos 20: 469–489.
14. LANGER, A. M., R. P. NOLAN & J. ADDISON. 1991. Physico-chemical properties of asbestos as determinants of biological potential. *In* Mineral Fibers and Health. D. Liddell & K. Miller, Eds.: 211–229. CRC Press. Boston and London.
15. TIMBRELL, V., J. W. SKIDMORE, A. W. HYETT & J. C. WAGNER. 1970. Exposure chambers for inhalation experiments with standard reference samples of asbestos of the International Union against Cancer (UICC). J. Aerosol Sc. 1: 215–223.
16. BOLTON, R. E., J. M. G. DAVIS, K. DONALDSON & A. WRIGHT. 1982. Variations in the carcinogenicity of mineral fibres. Ann. Occup. Hyg. 26: 569–582.
17. GART, J. J., D. KREWSKI, P. N. LEE, R. E. TARONE & J. WAHRENDORF. 1986. Statistical Methods in Cancer Research. Vol. 3. The Design and Analysis of Long-term Animal Experiments. International Agency for Research on Cancer. Lyon.
18. COX, D. R. & D. OAKES. 1984. Analysis of Survival Data. Chapman & Hall. London.
19. DIXON, W. J., Ed. 1983. BMDP Statistical Software. University of California Press. Berkeley.
20. STEMPLE, I. S. & G. W. BRINDLEY. 1960. A structural study of talc and talc tremolite relations. J. Am. Ceramic Soc. 43: 34–42.

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Inhalation studies on the effects of tremolite and brucite dust in rats

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Samples of commercially used asbestos, especially chrysotile, are frequently contaminated by small amounts of other fibrous minerals. Among these are tremolite and brucite although pure tremolite is also produced commercially in relatively small quantities. In order to determine how harmful commercially exploited tremolite might be in comparison with other asbestos types and to explore the possibility that small amounts of tremolite and brucite as contaminants could significantly affect the pathogenicity of industrially used chrysotile, long-term animal inhalation and injection studies using rats were undertaken with what were considered to be mineralogically pure samples of these minerals. Rats treated with tremolite developed very high levels of pulmonary fibrosis as well as 16 carcinomas and two mesotheliomas in a group of 39 animals. Tremolite thus proved to be the most dangerous mineral that we have studied. Animals treated with 'brucite' developed moderate levels of pulmonary fibrosis and two carcinomas. Both tremolite and brucite produced mesotheliomas in >90% of animals following i.p. injection. However, it was found that the supposedly pure brucite in fact contained 10% chrysotile, a level of contamination that could well have been responsible for the pathological changes found in both inhalation and intraperitoneal injection studies. The greatest care should be exercised by industry in handling tremolite or materials contaminated with it.

Introduction

The fibrogenic and carcinogenic potential of the main commercially used varieties of asbestos have been well documented (1). Recently, however, attention has been drawn to the fact that asbestos ore bodies, particularly those of chrysotile, are not mineralogically pure. Mixed with the asbestos are other minerals, some of them fibrous, which remain as contaminants in the final asbestos products supplied to industry from the mines. Two such minerals are brucite and tremolite. Brucite, $Mg(OH)_2$, is quite distinct from all the asbestos varieties in that it does not contain silicon but it is commonly found in chrysotile and other serpentines since it often forms in similar geological conditions. It can occur in the form of solid fibres, as tubular crystals, or in large foliated masses. Tremolite, $Ca_2Mg_5Si_8O_{22}(OH)_2$, is a commonly occurring amphibole mineral which is only rarely found as a pure fibrous asbestiform variety in quantities large enough for commercial exploitation. It is found more frequently in both the asbestiform and non-fibrous states as a contaminant in other materials such as talc, chrysotile and vermiculite (2). The identification of any sample of tremolite as asbestiform depends among other things upon its length and diameter distributions. Campbell (3) and Campbell *et al.* (4) have shown that asbestiform varieties of minerals have significantly longer and finer size distributions than their non-asbestiform equivalents.

Environmental exposure to tremolite in Turkey has been implicated in the development of human bronchial carcinomas and mesotheliomas (5). It has also been reported that the lungs of chrysotile miners can contain as much tremolite at autopsy as chrysotile even though only a small percentage of tremolite was present in the original ore body (6,7). Mesotheliomas in workers exposed to chrysotile are extremely rare and it has been disputed whether or not chrysotile alone can produce these tumours in humans. A few well-documented cases of mesothelioma in chrysotile workers do exist, where exposure to crocidolite or amosite appears to have been excluded but now it would appear that tremolite rather than chrysotile itself could be the cause. Experimental *in vitro* and *in vivo* injection studies so far undertaken (8) confirm that tremolite fibres can be carcinogenic in experimental animals. So far there is no epidemiological evidence to suggest that brucite is implicated in human disease but animal injection studies by Pott *et al.* (9,10) show that brucite fibres can produce mesotheliomas if injected into experimental animals.

At the IOM in Edinburgh we have used both inhalation and injection studies to compare the biological effects of a number of samples of asbestos and other mineral fibres. The present studies were undertaken in order to compare the harmful potential of tremolite and brucite to that of these other fibrous materials and to obtain more information on the likely importance of their presence as contaminants in other products such as commercially used talc and chrysotile.

Materials and methods

Mineralogy

The sample of 'tremolite' used in this study was a commercial material from Korea. It contained ~95% by mass of pure fibrous tremolite which was chemically and structurally consistent with published data as confirmed by scanning electron microscopy (SEM)* and X-ray diffraction (XRD) analysis. However, a few XRD peaks were present which could not be accounted for by tremolite. Some of these impurities had a layer structure with a 7.2 A and 3.6 A spacing typical of minerals such as kaolinite, serpentine, or chrysotile. The presence of talc was also suspected. The tremolite itself was chemically pure, being composed almost entirely of calcium, magnesium and silicon, with minor amounts of iron, well within the limits of the compositional range permitted for the mineral.

The brucite used in this study was chemically and structurally consistent with published data. The specimen was 85–90% by mass of pure brucite as confirmed by SEM and XRD analysis. In addition to brucite, however, the bulk material contained Pyraurite ($Mg_3Fe_2CO_3(OH)_{10}4H_2O$). This appeared as mottled brown segments on a proportion of the brucite fibres under the optical microscope. The Pyraurite probably comprised 5–10% of the bulk material. Chrysotile was also present as confirmed by XRD. Since, however, this form of analysis gives poor quantitative results for chrysotile when small amounts are mixed with other minerals, the bulk sample was treated with glacial acetic acid to remove the brucite and the chrysotile content was estimated by i.r.

*Abbreviations: SEM, scanning electron microscopy; XRD, X-ray diffraction.

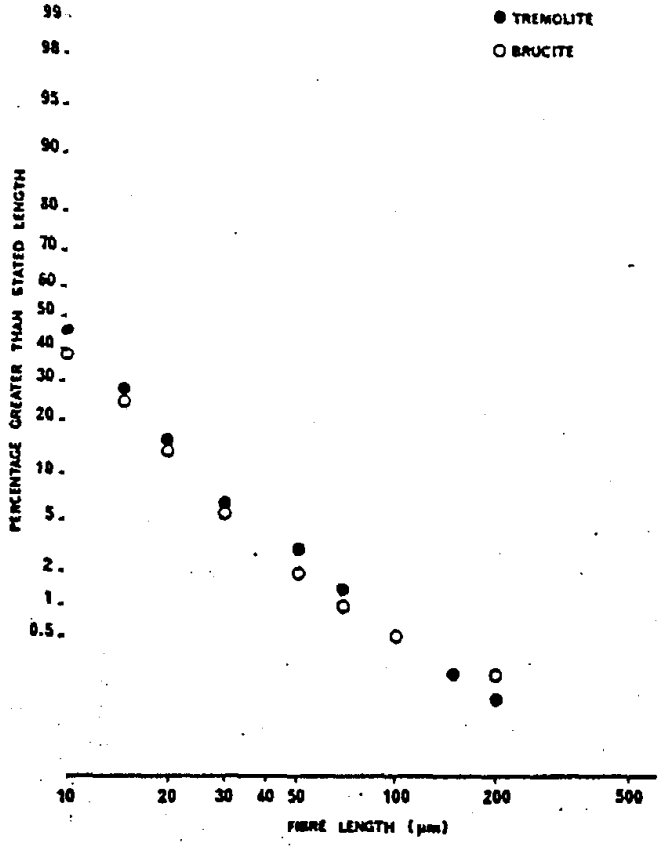

Fig. 1. Length distributions of fibres of tremolite and brucite >5 μm in length obtained by phase contrast light microscopy.

analysis. This technique showed that chrysotile comprised ~10% of the 'brucite' sample.

Dust generation and monitoring

The planned dust concentrations was 10 mg/m³ of respirable dust. The dust clouds were generated using a Timbrell dust generator and inhalation chambers as described by Timbrell *et al.* (11) but modified as described by Beckett (12). Selecting the airborne dust by a cyclone system prior to injecting it into the chamber airstream ensured a high proportion of respirable dust in the clouds. The dust generators were able to produce suitable clouds of tremolite dust from the bulk sample as it was received. Generation of a respirable dust cloud from the brucite sample required some preliminary breakage and dispersion by passing it through square-toothed intermeshed steel rollers prior to loading into the dust generator.

The mass concentration of dust in the chambers was measured daily by sampling throughout the 7 h of exposure using both an open filter holder facing vertically downwards and a Casella MRE113A dust sampling instrument. The former sampler monitored the total dust concentration and the later monitored the respirable dust mass concentration. The target mean respirable dust concentration of 10 mg/m³ was achieved by adjusting the equipment in response to each daily measurement.

The fibre number concentration was estimated from short period membrane filter samples taken on ~100 separate days. The number concentration for these samples was taken as being proportional to the mass concentration for that day, thus giving a mass/number ratio which was then averaged over 100 days. The membrane filter samples were examined by phase-contrast optical microscopy, following the normal practice for occupational hygiene measurements (13). This routine technique defines fibres to be included in the count by criteria of size; length >5 microns, diameter <3 microns, and aspect ratio >3:1.

The size distribution of fibres was examined by both phase-contrast optical microscopy and SEM. The optical studies were performed by a single observer, but all SEM studies were undertaken by two observers. Samples for electron microscopy were collected on Nucleopore filters. A portion of each filter was subsequently attached to an electron microscope stub with a conductive adhesive (carbon paste) and sputter coated with [illegible] gold. The samples were examined by SEM (Cambridge Instru[illegible]) 000x magnification and measurements taken on the length and diameter of those fibres with aspect ratio >3:1, length >0.4 microns and diameter >3 microns. The minimum length required arises from the combination of the aspect ratio definition and the resolution limit, which for the SEM corresponds to a fibre of ~0.1 micron diameter. Some fibres of <0.1 micron diameter were detected and reported and measured as closer to 0.05 microns but generally fibre diameters were measured to the nearest 0.1 micron.

Animal inhalation studies

For the inhalation studies groups of 48 SPF male Wistar rats of the AF/Han strain were exposed to either tremolite or brucite dust for 7 h each day, 5 days a week, for a total of 224 days, during a period of 12 months. The animals were 10 weeks old at the start of dusting. A batch of 36 undusted animals was maintained within the same unit as controls during the same overall time period.

Four animals from each group were killed at the end of the 12 months dusting period and four more were killed 6 months later. The remaining animals were left for their full life span except that the study was terminated when the number of survivors in one group dropped to six. Estimations of early pathological lesions were limited to the small groups of animals from the first two killing dates. However, for the more advanced lesions occurring in the oldest animals, it was decided to include all those dying within 2 months of the final killing date. In practice this produced groups of 12 animals for each dust treatment.

Tissue used for histological examination was fixed with 10% formol saline solution and embedded in paraffin wax. Lungs were fixed by inflation at a standard pressure of 30 cm of fixative. Subsequently the tracheas were ligated and the lungs excised and immersed in fixative. Sections were cut in the coronal plane at 1 mm intervals and were stained by either haematoxylin and eosin, Van Geison's method for collagen or Gordon and Sweet's stain for reticulin.

Measurement of pulmonary fibrosis was undertaken by similar methods to those previously published by Davis *et al* (14) except that an electronic image analyser (Graphic Information Systems Limited, GDS1) was available for use in conjunction with the light microscope. Single lung sections were examined with the section selected to contain the maximum area of lung parenchyma. As previously described, interstitial fibrosis was estimated using a 2x microscope objective lens and is expressed as a percentage of total lung-tissue area. Peribronchiolar lesions are more numerous and smaller and so the lung tissue was scanned with an eye-piece graticule covering an area of 2.9^2 mm and divided into 100 squares. A 4x objective lens was used. Peribronchiolar lesions were recorded as a percentage of squares containing lesions of this type. Small areas of irregular alveolar wall damage were measured by the same techniques. However, since these lesions were difficult in all cases to distinguish from fragments of peribronchiolar fibrosis included in the sections an overall measurement was undertaken initially to include both lesions, followed by a second one involving only definite peribronchiolar fibrosis. An estimate of lung tissue with irregular alveolar wall damage was obtained by subtraction.

Lung dust content

Lung dust estimations were performed on animals from the first two killing dates. Only the left lung was used so that the right lung was available for histological studies. Dust retained in the lungs was recovered by a low-temperature plasma ashing process using a Nanotech P100 apparatus. Studies in this laboratory have shown that the dust content ratio between left and right lungs following experimental inhalation of fibrous dusts such as asbestos in rats is 0.6:1 and this correction factor was therefore used to estimate the total pulmonary dust burden of each animal.

The tremolite residues were washed in 0.2 m HCl at room temperature before estimations of the amounts of retained fibre were made using the i.r. spectrophotometer techniques described by Middleton *et al.* (15). Since brucite is known to be highly soluble in acids, the brucite residues were washed only with distilled water initially. However, because the original dust cloud had contained a significant proportion of chrysotile asbestos, the analysis of these lung dust residues was repeated after washing with glacial acetic acid.

Animal injection studies

In addition to the inhalation studies, the ability of the tremolite and brucite to produce mesotheliomas was examined using the i.p. injection assay. A dose of 25 mg of dust suspended in 2 ml of Dulbecco's phosphate buffered saline was injected under ether anaesthetic into the peritoneal cavities of two groups of 32 rats of the AF/Han strain. The dust was collected from the animal inhalation chamber by an elutriation process and represented the respirable fraction of the dust cloud (16).

Results

Dust characterisation

The planned average mass dust concentration of both tremolite and brucite was achieved during the dusting period with >40% of the daily dust concentrations within 3 mg/m³ of the target concentration.

Fig. 3. SEM of the brucite dust used in the inhalation and injection studies. While the tremolite fibres are of uniform type the brucite is a mixture of relatively thick straight brucite fibres and thin curly ones which are chrysotile asbestos. (10 000x magnification)

phages and fibroblasts but a few foreign giant cells were also present. The deposits of granulation tissue tended to be both larger and more frequent in animals treated with tremolite than in those that had inhaled brucite. When the degree of involvement of the lung parenchyma was assessed (Table I), the difference between the two dust treatments at both 12 and 18 months after the start of dusting was statistically significant ($p<0.01$). During this period, the lesions did not increase in size and frequency and indeed in the tremolite-treated animals the 18-month group produced lower figures than those found at 12 months. With the small groups of animals examined, however, this reduction was not statistically significant. After 18 months from the start of dusting widespread interstitial fibrosis developed and this tended to obscure the earlier fibrotic deposits. For this reason, estimations of peribronchiolar fibrosis were limited to the first two killing dates. At 12 months after the start of dusting there was marked reticulin staining in the peribronchiolar deposits although relatively little collagen could be demonstrated by Van Geison's stain. As the animals aged, however, collagen staining became progressively more marked. Within the areas of peribronchiolar fibrosis, many tremolite fibres were clearly visible with the light microscope. Brucite fibres could not be seen, however, at any stage.

In addition to the peribronchiolar lesions, macrophages containing dust were visible in most alveoli at the end of the dusting period although they were more frequent close to the terminal bronchioles. In addition, however, there were small irregular patches of alveolar wall damage and thickening (Figure 6). It is possible that these small areas of damage represent precursors of the more widespread and distinct areas of interstitial fibrosis that developed in the older animals although this has not yet been proved. As shown in Table I, however, the extent of this type of pathological change in the individuals of any group of animals bears a close relationship to the amount of peribronchiolar fibrosis present. In the present study, animals treated with tremolite had significantly more of this alveolar damage than those treated with brucite ($p<0.01$).

From about 18 months onwards, areas of lung tissue in some animals showed a progressive thickening of alveolar septa. In its earliest form this thickening was caused almost entirely by hyperplasia of alveolar lining cells but later there was considerable deposition of reticulin and eventually collagen in the septal walls (Figure 7). Dust deposits were frequently visible among the fibrous tissues in the thickened septa in animals treated with tremolite dust. As shown in Table I, areas of interstitial fibrosis became more widespread in both treatment groups as the animals aged but far more was found in the tremolite-treated group than in the animals that had inhaled brucite ($p<0.01$). In some areas the interstitial fibrotic element of these lesions remained predominant throughout the study but in others the hyperplasia of alveolar epithelial cells became progressively more marked to produce a pattern of adenomatosis. Some

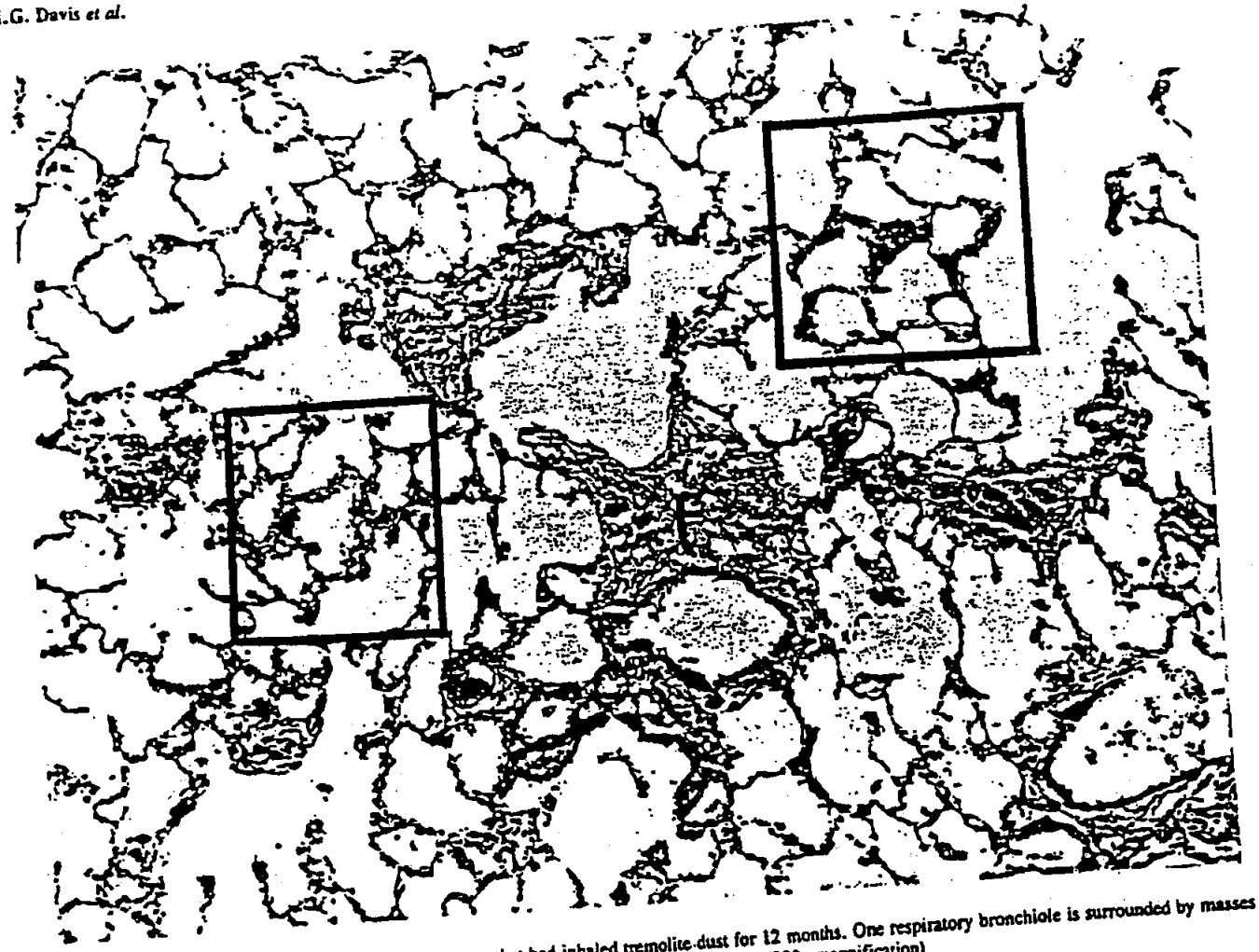

Fig. 6. A light microscope section of lung tissue from a rat that had inhaled tremolite dust for 12 months. One respiratory bronchiole is surrounded by masses of fibrous tissue (F). In addition, the alveoli in two areas (outlined) show irregular thickening. (200x magnification)

Table I. Levels of pulmonary fibrosis and irregular alveolar wall thickening produced by tremolite and brucite dusts

	Tremolite			Brucite		
Time after start of exposure (months)	12	18	27–29	12	18	27–29
Peribronchiolar fibrosis	23.0 (21.4–24.2)[b]	13.4 (9.7–18.9)	–	1.6 (0.5–2.7)	1.7 (1.0–2.9)	–
Irregular alveolar wall[a] thickening	35.2 (27.7–41.0)	27.7 (20.8–35.4)	14.5 (3.8–26.9)	5.8 (3.1–7.5)	7.6 (3.6–9.9)	2.9 (0.2–8.9)
Interstitial fibrosis	0	3.0 (0–5.6)		0.8 (0–3.3)	0	
Number of rats examined	3	4	12	4	4	12

[a]Estimates obtained by subtraction (see text).
[b]Figures in brackets are standard deviations.

mass of tremolite retained in the rat lungs was almost identical to that previously found with other amphibole dusts at both 12 and 18 months after the start of dusting so that the number of tremolite fibres present in the lung tissue would have been correspondingly high. Stanton's work was undertaken using the technique of intrapleural implantation and the injection studies reported in the present paper have confirmed that tremolite is very highly carcinogenic in this type of experiment. There is, however, no reason why the same criteria should not apply to the lung parenchyma and the carcinogenic potential of the tremolite dust used in our inhalation study is likely to have been due to its high content of long thin fibres. These studies would suggest that tremolite samples containing thin fibres should be treated with appropriate caution if used by industry. In addition, samples of chrysotile and talc contaminated with tremolite are more likely to be more harmful than uncontaminated material.

The results of the brucite studies indicate more complex patterns. The bulk brucite sample used for this work was considered to be as pure as can be obtained. However, both the

appropriate care by industry. In addition, the idea that the pathogenicity of the 'brucite' sample examined could be due to its chrysotile content highlights the problem of attributing observed pathological effects to individual components of a mixed dust sample.

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References

1. Selikoff,I.J. and Lee,D.H.K. (1978), *Asbestos and Disease*, Academic Press, NY.
2. Dement,J.H. (1977), *Asbestiform Minerals in Industrial Talcs: Commercial Definitions versus Industrial Hygiene Reality*, National Bureau of Standards, Special Publication 506, Proceedings of the Workshop on Asbestos: Definition and Measurement Methods held at NBS Gaithersberg MD, (Issue 1980), 313-323.
3. Campbell,W.J. (1977), *Identification of Selected Silicate Minerals and their Abestiform Varieties*, National Bureau of Standards, Special Publication 506. Proceedings of the Workshop on Asbestos: Definition and Measurement Methods held at NBS Gaithersberg MD, (Issue 1980), 201-221.
4. Campbell,W.J., Higgins,C.W. and Wylie,A.G. (1980), *Chemical and Physical Characterisation of Amosite, Chrysotile, Crocidolite and non-fibrous Tremolite for Oral Ingestion Studies by the National Institute of Environmental Health Sciences*, Bureau of Mines Report of Investigation RI.8452, United States Department of the Interior.
5. Yozicioglu,S., Ikayto,R., Bacli,K., Sayli,B.S. and Yorulmaz,B. (1980), Pleural calcification, pleural mesotheliomas and bronchial cancers caused by tremolite, *Thorax*, 35, 564.
6. Pooley,F.D. (1976), An examination of the fibrous mineral content of asbestos lung tissue from the Canadian Chrysotile Mining Industry. *Environ. Res.*, 12, 281.
7. Rowlands,N., Gibbs,G.B. and McDonald,A.D. (1982), Asbestos fibres in the lungs of chrysotile miners and millers – A preliminary report *Ann. Occup. Hyg.*, 26, Nos. 1-4, 411.
8. Wagner,J.C., Chamberlain,M., Brown,R.C., Berry,G., Pooley,F.D., Davies,R. and Griffiths,D.M. (1982), Biological effects of tremolite *Br. J. Cancer*, 45, 352-360.
9. Pott,F. and Friedrichs,K.H. (1972), Tumoren der Ratte nach i.p. Injektion faserformiger Staube. *Naturwissenschaften*, 59, 318.
10. Pott,F., Friedrichs,K.H. and Huth,F. (1976), Results of animal experiments concerning the carcinogenic effect of fibrous dusts and their interpretation with regard to the carcinogenesis in humans, *Zentralbl. Bakteriol. Microbiol. Hyg. Abt. I*, 162, 467-505.
11. Timbrell,V., Skidmore,J.W., Hyett,A.W. and Wagner, J.C. (1970), Exposure chambers for inhalation experiments with standard reference samples of asbestos of the International Union Against Cancer (UICC), *J. Aerosol Sci.*, 1, 215-223.
12. Beckett,S.T. (1975), The generation and evaluation of UICC asbestos clouds in animal exposure chambers. *Ann. Occup. Hyg.*, 18, 187-198.
13. Asbestosis Research Council (1971), *The Measurement of Airborne Asbestos Dust by the Membrane Filter Method*, Rochdale (Lancs) ARC Technical Note 1.
14. Davis,J.M.G., Beckett,S.T., Bolton,R.E., Collings,P. and Middleton, A.P. (1978), Mass and number of fibres in the pathogenesis of asbestos-related lung disease in rats, *Br. J. Cancer*, 37, 673-688.
15. Middleston,A.P., Beckett,S.T. and Davis,J.M.G. (1977), A study of the short-term retention and clearance of inhaled asbestos by rats using UICC standard reference samples, in Walton,W.H. (ed.), *Inhaled Particles IV*, Pergamon Press, 247-257.
16. Bolton,R.E., Davis,J.M.G., Donaldson,K. and Wright,A. (1982), Variations in the carcinogenicity of mineral fibres. In Walton,W.H. (ed.), *Inhaled Particles V*, Pergamon Press, 569-580.
17. Cherrie,J. (1983), An investigation of the reproducibility of counting and sizing of asbestos fibres by SEM. *Report of the Fourth International Colloquium on Dust Measuring Technique and Strategy, Edinburgh 1982*, Asbestos International Association, London, 369-378.
18. Stanton,M.F. and Wrench,C. (1972), Mechanisms of mesothelioma induction with asbestos and fibrous glass, *J. Natl. Cancer Inst.*, 48, 797-821.
19. Stanton,M.F., Layard,M., Tegeris,A., Miller,E., May,M. and Kent,E. (1977), Carcinogenicity of fibrous glass, *J. Natl. Cancer Inst.*, 58, 587-603.
20. Davis,J.M.G., Beckett,S.T., Bolton,R.E. and Donaldson,K. (1980), A comparison of the pathological effects in rats of the UICC reference samples of amosite and chrysotile with those of amosite and chrysotile collected from the factory environment. in Wagner,J.C. (ed.), *Biological Effects of Mineral Fibres*, International Agency for Research on Cancer, Lyon, I.A.R.C. Scientific Publication 30, pp. 285-292.
21. Bolton,R.E., Davis,J.M.G., Miller,B.G., Donaldson,K. and Wright,A. (1984), The effect of dose of asbestos on mesothelioma production in the laboratory rat, in *Report of the VIth International Pneumoconiosis Conference*, Bochum 1983. Published by the International Labour Organisation, pp. 1028-1035.

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ut other commercially used materials such as vermiculite and marble can also ontain tremolite asbestos, and concern regarding the full significance of this ontamination is increasing. Tremolite is a comparatively simple calcium magnesium silicate with an ideal chemical formula of $Ca_2Mg_5Si_8O_{22}(OH)_2$. The International Mineralogical Association guidelines on the nomenclature of amphiboles estricts the use of the term tremolite to compositions very close to the ideal ormula, although a certain amount of substitution of iron for magnesium, sodium or calcium, and aluminium for silica is permitted (similar minerals with progressively more iron in the formula are referred to as actinolite and ferro-actinolite). Tremolite is a common and widespread amphibole mineral simply because the ssociation of the chemical components, calcium, magnesium and silica, is common within the metamorphic rocks of all of the major mountain belts of the world. For example, dolomite limestones ($CaMg(CO_3)_2$) in sedimentary rock sequences or the large serpentinite masses (high Mg content) of these regions were often converted to tremolite rocks by the great pressures and high temperatures of the mountain-building processes. The comparatively simple compositions and abundance of the parent materials lead to frequent occurrences of amphibole minerals very close indeed to the ideal tremolite composition.

While the different occurrences of tremolite may be remarkably consistent in chemical terms, the mineral may show a very wide variation in morphological habit. Depending on the exact pressure, temperature and tectonic conditions prevailing at the time of its formation, the tremolite may have crystallized as coarse, prismatic, massive crystals or as fine asbestiform fibers. Dorling and Zussman[13] describe a very wide range of these different varieties, and, despite extremely careful evaluation using a variety of analytical techniques, including high-resolution transmission electron microscopy and detailed chemical analysis, they were unable to provide an entirely satisfactory explanation of the different morphological varieties. More recent work[14] has suggested that asbestos could be differentiated on the basis of such features as the presence of polyfilamentous fiber bundles, the proportions of multiple crystal twins, and the frequency of chain-width errors in the crystal structure.

Tremolite contamination of other materials can range from pure asbestiform fibers through irregular if elongated spicules to nonfibrous particles. While the asbestiform fibers are known to be hazardous, the level of hazard associated with other morphological habits is uncertain. In these circumstances concern has been expressed over the use of any product contaminated with any form of tremolite and a clear definition of the hazard to be expected from all tremolite types is greatly needed. Wagner *et al.*[3] commenced an examination of the relative carcinogenicity of asbestiform and nonasbestiform tremolite, but interpretation of the results was made difficult by poor animal survival. The present study was planned to examine this problem in more detail and the carcinogenicity of tremolite asbestos and nonasbestos varieties has been examined in rats using the technique of intraperitoneal injection.

MATERIALS AND METHODS

The six tremolite minerals used in the present study are listed below.

(1) California tremolite from Jamestown
(2) Korean tremolite

(3) Tremolite from Swansea
(4) Italian tremolite (Ala di Stura)
(5) Tremolite from Carr Brae, Dornie, Scotland
(6) Tremolite from Shinness, Scotland

Specimens 1–3 were donated to the Institute of Occupational Medicine in Edinburgh as reference samples, and only with specimen 2 is their exact location of origin known. Sample 3 was donated by the British Coal Laboratory in Swansea and in this case even the country of origin is uncertain. All three specimens had a very distinctive asbestiform morphology, being flexible and strong and fairly elastic. The Californian tremolite was the finest and apparently the toughest, having fibers that were difficult to separate. The Korean tremolite differed in that it was already quite highly processed by milling when it was received and was also mildly contaminated by chrysotile.[4,5] The Swansea tremolite consisted of fine, easily separable fibers.

The tremolite from Ala di Stura (in a small valley in northwestern Italy) differed from the first three in very distinctive ways. It consisted of large bundles of very long (often >5 cm) needle-like fibers which were flexible and very elastic but quite brittle. It had a bright vitreous luster, unlike the more matt appearance of the first three tremolite samples. The tremolite from Dornie was similar to the Italian tremolite in its vitreous luster, but contrasted with it in being a fine powdery material. The original specimen was collected from a road cutting near Dornie in the Highland region of Scotland. The outcrop is one of a series where high-grade metamorphic marble, dolomite, and calcium silicates are found. The original material was extremely friable and the sample was soon reduced to a fine, soft powder in the course of handling when wet in a plastic bag.

The tremolite from Shinness was collected from a small, disused marble quarry on the shores of Loch Shin near Lairg in the Highland region of Scotland. It consisted of prismatic crystals up to approximately 10 mm × 1 mm × 0.5 mm in size in massive aggregates. The crystals were vitreous in appearance and very brittle.

For use in the injection studies, the three asbestiform tremolites (samples 1–3) were all prepared in exactly the same way. The materials were packed into the cylinders of Timbrell dust dispensers[15] and airborne dusts were generated. Respirable fractions of these dusts were collected using a vertical elutriator.[16] No further purification was required.

The Italian tremolite had to be broken by hand to separate the thicker, longer bundles before packing into the Timbrell dust dispenser, but the material was then treated in the same manner as the asbestiform varieties. Fortunately the large bundles of fiber were very easy to split and separate because of their brittle nature.

The Dornie tremolite differed again in the treatment required. The crumbled crystal powder was washed free of organic material (minor rootlets) by repeated sedimentation in distilled water. The tremolite was collected by decanting off the water and drying the powder at 110°C overnight. This loose material consisted mainly of small needle-like crystals which were used for dust preparation as above.

The prismatic crystals of the Shinness tremolite could not be used directly to generate airborne dust because of the high physical strength of the crystal mass. The individual crystals and clumps were hand-picked for purity from a roughly crushed preparation and these were then ground in distilled water for 5 minutes

TABLE 1. Proportions of Simple X-ray Counts for the Major Elements of the Six Tremolite Minerals

	Source of Tremolite Samples					
Element	California	Korea	Swansea	Ala di Stura	Dornie	Shinness
Si	65.8	65.9	66.3	66.3	65.3	65.6
Mg	11.3	12.0	8.5	11.6	11.8	11.8
Fe	2.0	0.9	4.0	2.3	1.5	0.6
Ca	20.8	21.1	19.9	20.5	21.0	21.4
Al	0.1	0.3	0.3	0.3	0.5	0.7

The overall morphology of the tremolite samples as seen by SEM is illustrated in Figures 1–6, all of which were produced at the same magnification. In the optical microscopy and SEM examinations, the three asbestos tremolites (samples 1–3) were found to be typical of that form in displaying polyfilamentous fiber bundles, curved fibers, fibers with splayed ends, and long, thin, parallel-sided fibers. Most of the fibers showed straight extinction when observed with polarized

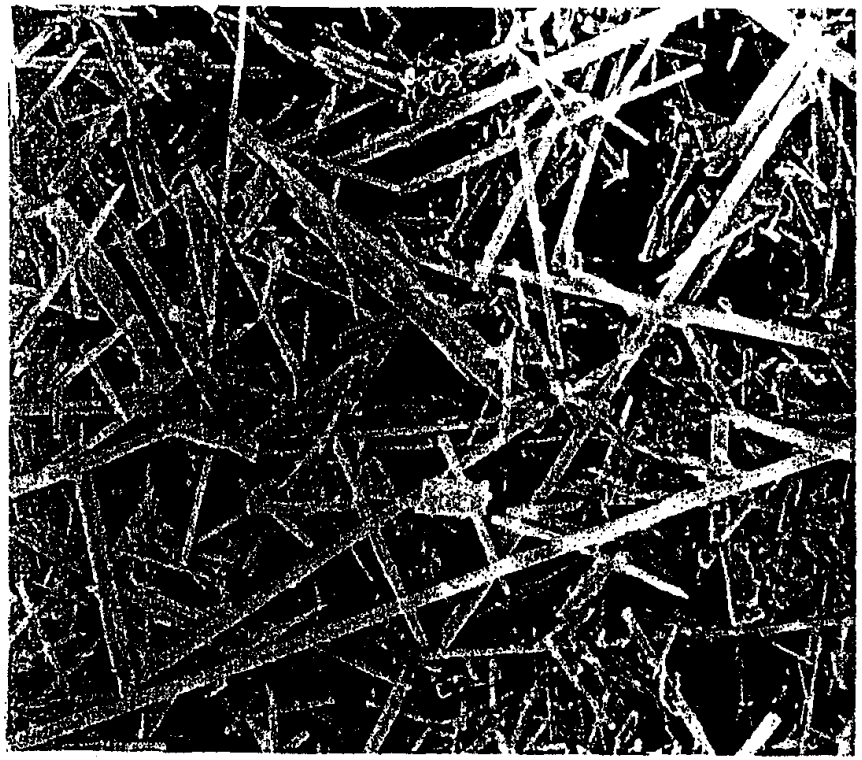

FIGURE 2. Tremolite sample No. 2: Korean tremolite (×10,500).

light under crossed polarizers, indicating the presence of multiple twinning of the crystals. All three asbestos samples did contain some elongated fragments of tremolite with oblique extinction, stepped ends, and nonparallel sides indicating that they were cleavage fragments. These were more abundant in the Korean and the Swansea minerals. The nonfibrous proportions of the dusts of these three minerals were very low. The tremolite from Italy contained mostly cleavage fragments, but some very long, thin fibers were observed. Similarly, the Dornie

FIGURE 3. Tremolite sample No. 3: tremolite supplied by the laboratory from Swanse (×10,500).

tremolite also contained a small proportion of long, thin asbestiform fibers, bu was predominantly made up of cleavage fragments. Both contained a high propor tion of nonfibrous tremolite particles. The Shinness tremolite dust was almo exclusively composed of cleavage fragments, only a small proportion of whic had an aspect ratio greater than 3 : 1.

The number of fibers >8 μm in length in the injected doses ranged betwee 13,000 × 10^6 for the California tremolite to 460 × 10^6 for the Shinness tremolit Complete fiber and particle sizing data are presented in TABLES 2 and 3.

TABLE 2: Number of Fibers/Milligram of Tremolite Dust Samples ($\times 10^3$) Used in Rat Injection Studies, Tabulated According to Fiber Length and Diameter[a]

	Length (μm)											
Diameter (μm)	0.00–1.00	1.00–2.00	2.00–3.00	3.00–4.00	4.00–5.00	5.00–6.00	6.00–7.00	7.00–8.00	8.00–9.00	9.00–10.00	>10.00	Total
California												
0.000–0.125	56	226	85	0	0	0	0	0	0	0	0	367
0.125–0.250	1298	2794	847	452	282	179	108	27	54	9	27	6076
0.250–0.375	85	1073	1016	565	452	125	99	81	18	9	152	3673
0.375–0.500	0	123	226	198	85	108	45	36	54	45	36	1254
0.500–0.625	0	113	310	254	28	45	134	81	54	27	108	1154
0.625–0.750	0	0	85	56	0	9	9	0	0	0	90	249
0.750–0.875	0	0	56	113	0	0	0	9	0	0	27	205
0.875–1.000	0	0	0	0	0	0	0	18	0	0	0	18
>1.000	0	0	0	0	0	0	18	0	9	18	36	81
Total	1439	4629	2625	1637	847	466	412	251	188	108	475	13077
Korea												
0.000–0.125	420	404	65	32	32	13	0	0	0	0	7	973
0.125–0.250	549	1131	420	162	32	53	13	7	26	0	26	2420
0.250–0.375	0	1066	355	194	32	53	46	0	7	40	53	1846
0.375–0.500	0	339	258	210	32	53	20	20	0	7	92	1031
0.500–0.625	0	129	258	194	97	53	33	20	13	7	40	843
0.625–0.750	0	0	65	16	81	20	7	0	0	0	7	195
0.750–0.875	0	0	16	32	0	13	0	0	0	0	20	81
0.875–1.000	0	0	16	32	0	13	0	0	0	7	0	68
>1.000	0	0	0	0	32	26	0	0	20	7	53	138
Total	969	3070	1454	872	339	297	119	46	66	66	297	7595
Swansea												
0.000–0.125	26	0	0	0	0	0	0	0	0	0	0	26
0.125–0.250	31	62	15	15	0	12	0	0	0	6	3	144
0.250–0.375	10	144	88	15	36	0	6	3	3	0	12	317
0.375–0.500	0	57	98	10	51	26	6	9	15	0	12	283
0.500–0.625	0	57	165	72	134	38	35	20	20	9	23	573
0.625–0.750	0	0	41	36	26	12	15	6	0	0	23	158
0.750–0.875	0	0	21	26	15	15	18	0	3	12	23	132
0.875–1.000	0	0	10	21	5	15	0	9	9	6	26	100
>1.000	0	0	0	41	51	38	20	35	6	12	137	341
Total	67	319	438	237	319	155	99	82	55	44	260	2074
Ala di Stura (Italy)												
0.000–0.125	6	10	19	0	0	0	0	0	0	0	0	35
0.125–0.250	35	115	10	3	0	1	0	2	0	0	1	166
0.250–0.375	19	195	77	0	0	0	1	1	1	0	0	293
0.375–0.500	0	195	80	35	3	6	1	2	0	0	2	322
0.500–0.625	0	124	179	70	38	12	2	2	2	1	6	436
0.625–0.750	0	0	19	13	10	9	2	2	0	0	2	56
0.750–0.875	0	0	3	13	16	6	4	0	2	0	4	47
0.875–1.000	0	0	0	19	10	2	0	1	1	2	1	34
>1.000	0	0	0	16	26	10	13	8	6	2	26	107
Total	61	638	386	169	102	44	22	17	12	5	41	1496
Carr Brae, Dornie, Scotland												
0.000–0.125	24	24	10	0	0	0	0	0	0	0	0	58
0.125–0.250	14	51	10	5	2	0	0	1	0	0	0	83
0.250–0.375	5	89	14	10	0	7	2	0	0	0	2	129
0.375–0.500	0	53	53	27	7	1	4	0	6	1	1	153
0.500–0.625	0	68	101	36	17	9	2	6	0	0	5	244
0.625–0.750	0	0	41	5	17	3	2	3	6	3	4	84
0.750–0.875	0	0	14	14	2	1	3	0	2	0	5	42
0.875–1.000	0	0	7	0	12	4	4	1	2	3	5	38
>1.000	0	0	0	46	19	9	12	23	20	5	64	198
Total	43	285	251	142	77	34	29	34	36	12	86	1029
Shinness, Scotland												
0.000–0.125	4	0	0	0	0	0	0	0	0	0	0	4
0.125–0.250	14	41	6	0	0	0	0	0	0	0	0	60
0.250–0.375	15	60	10	1	3	2	0	0	0	0	0	91
0.375–0.500	0	60	8	4	2	0	0	0	0	0	0	73
0.500–0.625	0	37	56	9	7	0	0	0	0	0	1	109
0.625–0.750	0	0	14	7	3	0	0	0	0	0	0	24
0.750–0.875	0	0	7	9	4	3	1	0	0	0	1	24
0.875–1.000	0	0	0	10	2	1	1	0	0	0	1	14
>1.000	0	0	0	14	14	13	9	2	3	3	9	68
Total	33	197	100	52	34	19	11	2	3	3	11	467

TABLE 4. Summary of Survival Data and Comparison with Selected Re-expressions of 10-mg Dose as Numbers of Fibers and Particles

Sample No.	Type	No. of Animals	No. of Mesotheliomas	Median Survival Time (days)	Estimated Relative Hazard (log)	No. (*10^5) of Fibers in 1 mg of Injected Dust	No. (10^5) of Fibers of length ≥8 and diameter <0.25	No. (10^5) of Particles in 1 mg of Injected Dust
1	California	36	36	301	0.8308	13,430	121	18,375
2	Swansea	36	35	365	0.5593	2,104	8	4,292
3	Korea	33	32	428	0.0000	7,791	48	13,435
4	Italy	36	24	755	−1.6108	1,293	1	20,137
5	Carr Brae	33	4	*	−3.9043	899	0	9,490
6	Shinness	36	2	*	−4.7080	383	0	5,901

FIGURE 6. Tremolite sample No. 6: tremolite from Shinness, Scotland (×10,500).

TABLE 5. Chi-square (4) Statistics Comparing Goodness-of-Fit of Model Regressing Hazard on Dose Expressed as Logarithm of Numbers of Fibers in Different Size Ranges

Lengths (μm)	All	Diameters (μm)	
		<0.25	>0.25
ALL	72.63	76.10	74.32
0.0–1.0	67.55	74.31	63.78
1.0–3.0	86.51	80.72	92.34
3.0–5.0	73.90	109.31	72.83
5.0–8.0	68.97	71.62	72.97
Over 8.0	110.87	41.03	115.16
Over 5.0	89.22	67.96	94.11
Over 3.0	77.14	99.01	79.98

4. DAVIS, J. M. G., R. E. BOLTON, K. DONALDSON, A. D. JONES & B. MILLER. 1985. Inhalation studies on the effects of tremolite and brucite dust in rats. Carcinogenesis 6: 667–674.
5. DAVIS, J. M. G., R. E. BOLTON, H. COWIE, K. DONALDSON, I. P. GORMLEY, A. D. JONES & A. WRIGHT. 1985. Comparisons of the biological effects of mineral fibre samples using *in vitro* and *in vivo* assay systems. *In* In vitro Effects of Mineral Dusts. E. G. Beck & J. Bignon, Eds.: 405–411. Springer. Berlin.
6. KLEINFELD, M., J. MESSITE, O. KOOYMAN & M. H. ZAKI. 1967. Mortality among talc miners and millers in New York State. Arch. Environ. Health 14: 663–667.
7. KLEINFELD, M., J. MESSITE & M. H. ZAKI. 1974. Mortality experiences among talc workers. A follow-up study. J. Occup. Med. 16: 345–349.
8. YAZICIOGLU, S., R. IKAYTO, K. BALCI, B. S. SAYLI & B. YORULMAZ. 1980. Pleural calcification, pleural mesotheliomas and bronchial cancers caused by tremolite dust. Thorax 35: 564–569.
9. POOLEY, F. D. 1976. An examination of the fibrous mineral content of asbestos lung tissue from the Canadian chrysotile mining industry. Environ. Res. 12: 281–298.
10. CHURG, A., B. WIGGS, L. DE PAOLI, B. KAMPE & B. STEVENS. 1984. Lung asbestos content of chrysotile workers with mesothelioma. Am Rev. Resp. Dis. 130: 1042–1045.
11. GYLSETH, B., G. MOWE & A. WANNAG. 1983. Fibre type and concentration in the lungs of workers in an asbestos cement factory. Br. J. Ind. Med. 40: 375–379.
12. DAVIS, J. M. G. 1989. Mineral fiber carcinogenesis: Experimental data relating to the importance of fibre type, size, deposition, dissolution and migration. *In* Nonoccupational Exposure to Mineral Fibres. IARC Publication No. 90. 33–46. International Agency for Research on Cancer. Lyon, France.
13. DORLING, M. & J. ZUSSMAN. 1987. Characteristics of asbestiform and non-asbestiform amphiboles. Lithos 20: 469–489.
14. LANGER, A. M., R. P. NOLAN & J. ADDISON. 1991. Physico-chemical properties of asbestos as determinants of biological potential. *In* Mineral Fibers and Health. D. Liddell & K. Miller, Eds.: 211–229. CRC Press. Boston and London.
15. TIMBRELL, V., J. W. SKIDMORE, A. W. HYETT & J. C. WAGNER. 1970. Exposure chambers for inhalation experiments with standard reference samples of asbestos of the International Union against Cancer (UICC). J. Aerosol Sc. 1: 215–223.
16. BOLTON, R. E., J. M. G. DAVIS, K. DONALDSON & A. WRIGHT. 1982. Variations in the carcinogenicity of mineral fibres. Ann. Occup. Hyg. 26: 569–582.
17. GART, J. J., D. KREWSKI, P. N. LEE, R. E. TARONE & J. WAHRENDORF. 1986. Statistical Methods in Cancer Research. Vol. 3. The Design and Analysis of Long-term Animal Experiments. International Agency for Research on Cancer. Lyon.
18. COX, D. R. & D. OAKES. 1984. Analysis of Survival Data. Chapman & Hall. London.
19. DIXON, W. J., Ed. 1983. BMDP Statistical Software. University of California Press. Berkeley.
20. STEMPLE, I. S. & G. W. BRINDLEY. 1960. A structural study of talc and talc tremolite relations. J. Am. Ceramic Soc. 43: 34–42.

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BIOLOGICAL EFFECTS OF TREMOLITE

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Summary.—Tremolite is an amphibole which has been implicated in a variety of disease patterns in different parts of the world. It occurs in a number of phases, which are chemically identical but have specific physical characteristics. In an attempt to clarify the epidemiological findings, tremolite fibres of 3 specific forms—A, B and C—were characterized and studied for biological activity by:

(i) *in vivo* intrapleural injection of rats (2 separate experiments—1 with poor survival).
(ii) *in vitro* enzyme release from mouse peritoneal macrophages
(iii) *in vitro* giant-cell formation in A549 cultures
(iv) *in vitro* cytotoxicity for V79-4 cells.

Sample C, which contained more long thin fibres than A and B, was alone in producing mesotheliomas. C, but not A or B, induced LDH and B-glucuronidase enzyme release, and induced giant cells. A was not cytotoxic, B moderately cytotoxic and C as highly cytotoxic as UICC crocidolite.

The *in vivo* studies were marred by being split between 2 experiments, of which the second had poor survival.

We are aware of the weakness of our *in vivo* data, but as Tremolite C was being considered for commercial use on the European market we felt it timely to submit our findings for publication.

TREMOLITE is an amphibole mineral (a chain silicate similar to asbestos) found in several countries. It has limited industrial value, but is used for stuccoing the exterior of buildings in the Middle East. It is frequently found as a contaminant of other minerals that are being exploited commercially.

Data on the health hazards of tremolite are currently being collected because it is an amphibole mineral and may be capable of causing diseases similar to those induced by amphibole asbestos (Wagner, 1980, 1982). A flake-like tremolite is found as a contaminant of talc in California but so far there is no evidence of disease which may be attributed to this tremolite. In the massive chrysotile ore bodies in Quebec Province in Canada, there are irregular deposits of a coarse-fibred tremolite. This material is found in the lungs of miners with pulmonary fibrosis and pleural plaques, but there is no correlation with mesotheliomas (Pooley, 1976). Further south in the northern part of New York State, a finer tremolite occurs as a contaminant of the talc deposits which are being exploited. From these mines there is evidence of pulmonary fibrosis, excess carcinoma of lung and pleura and a peritoneal mesothelioma (Kleinfeld *et al.*, 1967, 1974). Pleural plaques have occurred in the agricultural areas of Czechoslovakia and Yugoslavia and in the tobacco-growing regions of Bulgaria and Greece, all areas in which coarse tremolite fibres

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> 1 μm in diameter are found in the soil. In Cyprus, a few mesotheliomas have occurred among the people living in the vicinity of the asbestos mine. This mine has large chrysotile ore bodies associated with tremolite, some of which is fine-fibred. These people use the tremolite, which is separated from the chrysotile during the milling process, for stuccoing their houses. A similar situation exists in Turkey, where in some areas the tremolite used for stuccoing is associated with the incidence of pleural calcification, mesotheliomas and bronchial cancers (Yazicioglu *et al.*, 1980). A pure tremolite consisting of fine fibres is mined in South Korea.

The carcinogenic potential of mineral dusts can be assessed by depositing the dusts in the pleural cavities of rats, either by injection (Wagner & Berry, 1969) or implantation (Stanton & Wrench, 1972). Previous work has demonstrated that the ability of a dust to induce mesotheliomas is related to the content of fibres over ~8 μm long and thinner than ~1·5 μm (Stanton *et al.*, 1977). Mammalian cells in tissue culture have been shown to be sensitive to the toxic effects of fibres of similar dimensions to those which are carcinogenic *in vivo* (Brown *et al.*, 1978; Chamberlain *et al.*, 1979; Wade *et al.*, 1980). It was considered important to test 3 types of tremolite which would cover the spectrum of particle types found throughout the world, using both *in vivo* and *in vitro* tests as the first stage in assessing the potential health hazards.

MATERIALS AND METHODS

Dust samples

The three samples of tremolite available were the flake-like material from the Californian talc deposits, a medium-sized fibrous mineral from Greenland and the fine-fibred material from South Korea. The samples used in this investigation were selected because upon degradation they were found to form fibrous particles with very different size distributions both in length and diameter ranges. All samples were prepared by milling in a small agate mill and ultrasonic dispersion, large particles being removed by sedimentation in water.

Tremolite Sample A was prepared from a sample of Californian tremolitic talc which originally contained 62% talc and 38%

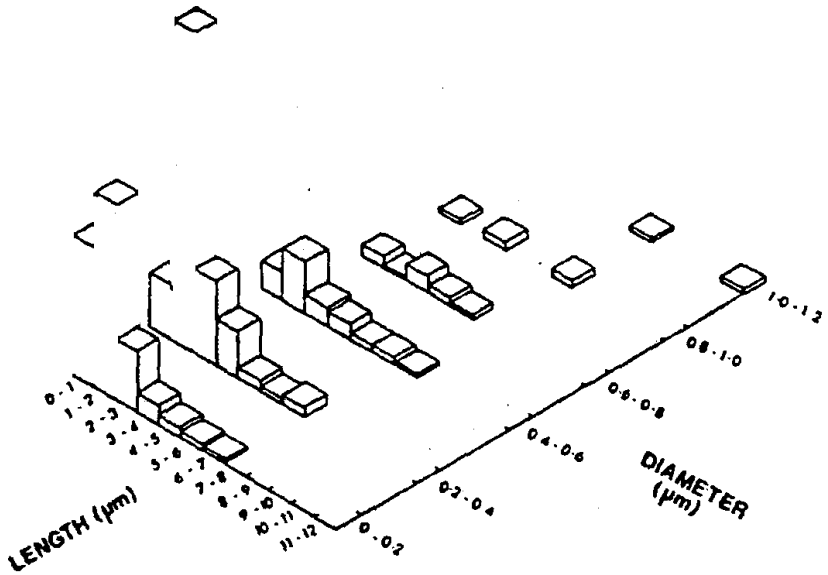

FIG. 1.—Length and diameter distribution of fibres in Tremolite Sample A.

TABLE I.—*Particles/μg tremolite samples*

Sample	No. of non-fibrous particles ($\times 10^4$)	Total no. of fibres ($\times 10^4$)	No. of fibres > 8 μm long ($\times 10^2$) and < 1·5 μm diameter
A	6·9	5·1	1·7
B	20·7	4·8	0
C	3·3	15·5	56·1

tremolite. The talc in the sample was reduced by froth flotation to produce a tremolite sample >95% pure, the remaining material consisting mainly of talc together with minor magnesium and calcium carbonate material. The length and diameter distribution of the fibrous particles in this sample are illustrated by Fig. 1: most fibres were < 6 μm long and < 0·8 μm diameter. The number of particles/μg is shown in Table I. The chemical analysis is contained in Table II, from which it can be seen that the iron content of this tremolite is relatively low, but its distinguishing feature was that it contained significantly more potassium and sodium than the other samples.

Sample B was prepared from a tremolite rock specimen which originated from Greenland. On comminution the specimen was found to break down into fibrous particles, most of which were < 3 μm long and < 1·2 μm diameter (Fig. 2). The number of particles/μg is shown in Table I. In comparison with Tremolite A, this sample contained fibres which were on average both shorter and thicker. Sample B contained larger proportions of calcium and iron than Sample A and little potassium (Table II).

Sample C was prepared from a rock specimen which originated from South Korea. In appearance, the hand specimen contained no visual impurities, and on size reduction produced a dust which contained fibres up to 140 μm long, of which most were < 0·6 μm diameter (see Fig. 3). The fibres in sample C were very much longer and finer than those in samples A and B. The number of particles/μg is presented in Table I. The iron content of Sample C was lower than that of Sample B but higher than that of Sample A (Table II).

The chemical compositions of the 3 samples are very similar, the exceptions being the K and Na in Sample A, and the Fe in Sample B.

Analysis of fibre size distributions

The methods used for the preparation of mineral dust samples for viewing in the transmission electronmicroscope (TEM) have been described fully elsewhere (Brown *et al.*, 1978). Briefly, an appropriately diluted sus-

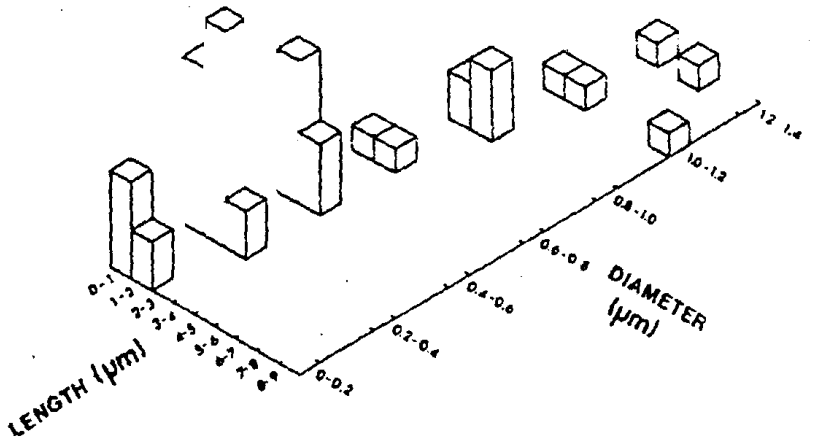

FIG. 2.—Length and diameter distribution of fibres in tremolite Sample B.

TABLE II.—*Oxide composition (g/100 g) of tremolite samples (water of crystallization not included)*

Sample	Na_2O	MgO	Al_2O_3	SiO_2	K_2O	CaO	FeO
A	3·0	24·5	1·2	59·8	1·1	9·6	0·3
B	0·5	23·9	0·3	59·3	0·1	13·6	2·0
C	0·6	24·9	0·4	58·8	0·2	13·9	0·9

pension of each dust was filtered on to a 0·1 μm-pore-size Millipore membrane filter. The filters were coated with carbon and placed over parallel-bar EM grids which were on an acetone-soaked sponge. As the membrane filters dissolved, the carbon coat, and the dust particles, were deposited on the grids which were then viewed by TEM. Overlapping fields were photographed and large mosaics constructed. The length and diameter of at least 300 fibres were measured where possible, and the numbers of fibres and non-fibrous particles determined. The rules advocated by Cooper *et al.* (1978) were used for counting the fibres on the photographs. Typical photographs of each dust are shown in Fig. 4.

In vivo *carcinogenicity*

The experimental animals used in this investigation were barrier-protected SPF rats of the Sprague-Dawley and Wistar strains. Each dust sample was prepared in physiological saline (0·9% w/v NaCl in distilled water) at 50 mg/ml and sterilized by autoclaving (15 lb/in² for 20 min). The dose of 20 mg of experimental material per rat was injected into the right pleural cavity (Wagner & Berry, 1969); animals receiving 0·4 ml saline served as controls. Equal numbers of males and females were used in each experimental group.

Sample A was injected into 32 Wistar rats 2 years before the rest of the investigation.

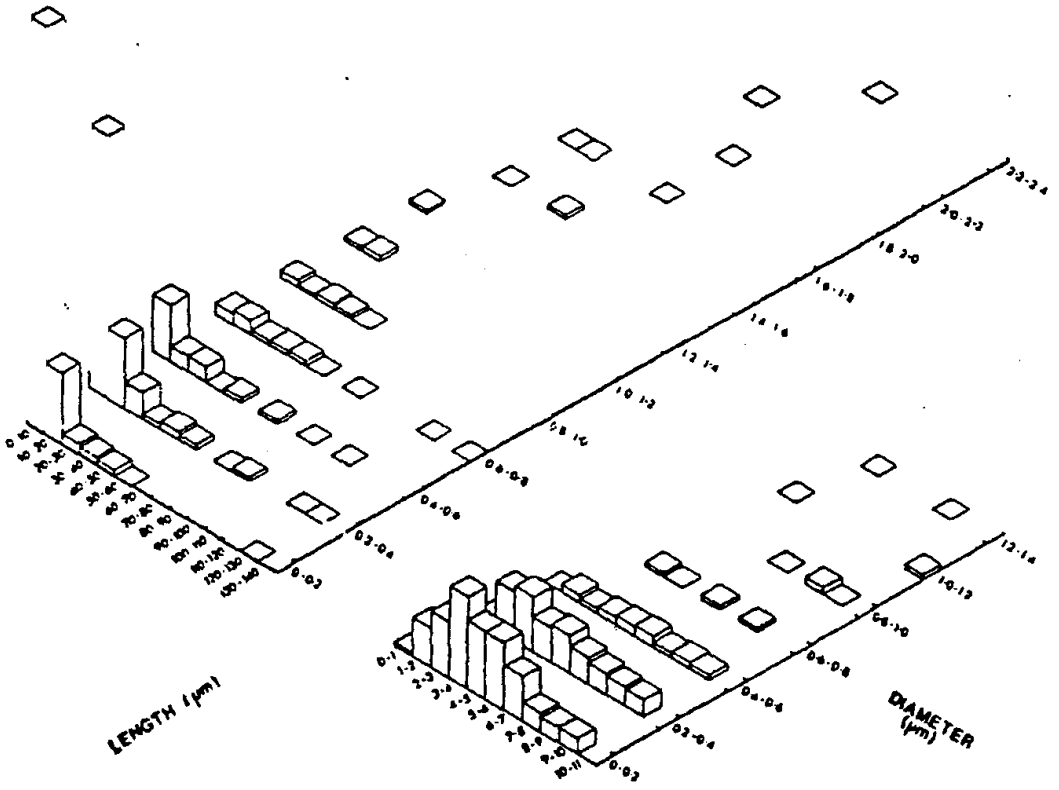

FIG. 3.—Length and diameter distribution of fibres in Tremolite Sample C.

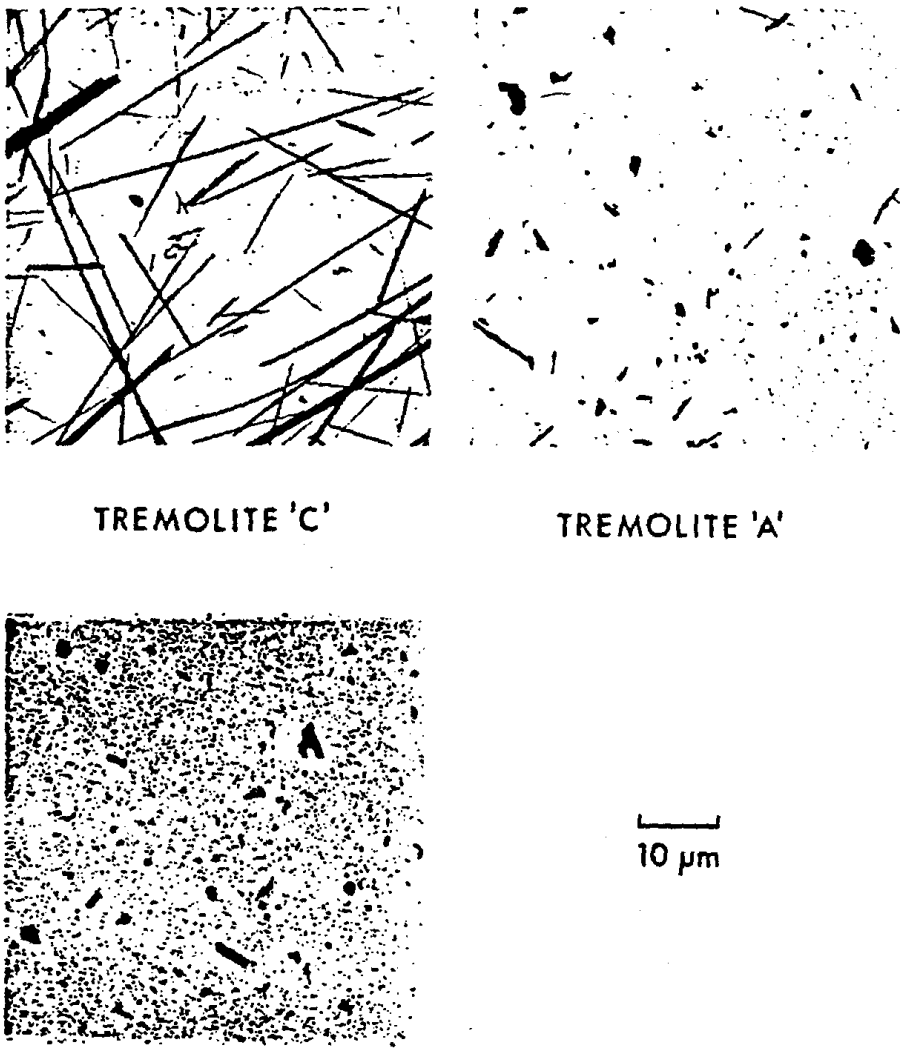

FIG. 4.—Electron micrographs of the 3 samples of tremolite.

Rats receiving SFA chrysotile served as positive controls in this experiment.

Samples B and C were injected into groups of 48 Sprague–Dawley rats; a group of 32 animals receiving UICC crocidolite served as positive controls.

Rats were 8–10 weeks old when injected and were allowed to live out their lives.

In vitro *toxicity*

Enzyme release from mouse peritoneal macrophages.—Mouse peritoneal macrophages were obtained from 22–27g Swiss TO mice (Tuck and Son Ltd, Battlebridge, Essex) by peritoneal lavage using 3·5 ml Medium 199 containing 5 i.u. heparin, 100 i.u. benzyl-

penicillin and 100 μg streptomycin per ml. About $1·5 \times 10^6$ cells in 2 ml of the above medium were placed in albumin-coated 35mm-diameter Petri dishes (Davies, 1980) and allowed to attach for 1 h at 37°C. The non-adherent cells were then removed by washing with phosphate-buffered saline. The remaining cells were cultured in 2 ml Medium 199 containing antibiotics and 10% heat-inactivated acid-treated foetal calf serum (Chamberlain *et al.*, 1979) in an atmosphere of 5% CO_2 in air at 37°C.

The medium of macrophage cultures prepared 24 h previously was replaced by 2 ml of medium containing the test dust at 50, 100 or 150 μg/ml; control cultures received medium without dust. After 18h incubation the medium was collected and the adherent cells disrupted by the addition of 2 ml saline containing 0·1% Triton X-100 and 0·1% bovine serum albumin and rubbing the Petri-dish surface with a silicone-rubber bung. Both the medium and the cell lysates were centrifuged at 800 *g* for 10 min and the supernatants assayed for lactic dehydrogenase (LDH) and β-glucuronidase (BGL) by the continuous-flow fluorimetric method of Morgan *et al.* (1978) using a Perkin Elmer Model 3000 fluorescence spectrophotometer.

Giant-cell formation in A549 cultures.—Type II alveolar cells (A549) derived from a human tumour (Leiber *et al.*, 1976) were obtained from Dr G. Todaro, NCI, Bethesda, Maryland, U.S.A. The cells were grown in Dulbecco's modification of Eagle's minimal essential medium supplemented with 10% heat-inactivated FCS and antibiotics in an atmosphere of 10% CO_2 in air at 37°C.

A standard inoculum of 10^5 cells was added to each of a series of 25cm² culture flasks along with an appropriate amount of dust suspension. Four flasks were used for each dust, 2 at a dust concentration of 100 μg/ml and 2 at 200 μg/ml. Flasks with no dust served as controls. All the cultures were incubated for 5 days, the cells detached using trypsin–EDTA and suspended in an appropriate volume of medium and photographed on a haemacytometer. The diameters of 200 cells from each treatment were measured as described in Chamberlain & Brown (1978).

Cytotoxicity to V79-4 cells.—Chinese hamster lung cells (V79-4) described by Chu & Malling (1968) were obtained from Dr C. F. Arlett, MRC Cell Mutation Unit, Brighton, and cultured in MEM supplemented with 15% FCS and antibiotics at 37°C in an atmosphere of 5% CO_2 in air.

This method has been reported in detail elsewhere (Chamberlain & Brown, 1978). Briefly, the survival of V79-4 cells in the absence or presence of a series of concentrations of each dust was determined by adding the appropriate amount of dust to a suspension of single cells. The cell/dust mixtures were then placed on 60mm-diameter Petri dishes (~200 cells/dish) and incubated for 6 days. After incubation the medium was removed, the cells fixed with 10% formal saline and stained with 1% methylene blue. The colonies on each dish were counted in an automatic colony counter (Micro Measurements Ltd, Cambridge).

RESULTS

Physical characteristics of the dusts

All of the dusts contained fibres; representative photographs are shown in Fig. 4. The size distributions of the fibres in each dust are shown in Figs 1–3 and the numbers of particles per μg are presented in Table I. Samples A and B contained relatively few fibres and Sample C contained many very long thin fibres.

Induction of mesotheliomas

The percentages of rats developing a mesothelioma following the various treatments are shown in Table III. The survival of the animals in Experiment II was poor because of infection, and is discussed later.

In vitro *toxicity*

Enzyme release from mouse peritoneal macrophages.—The release of both LDH and BGL from mouse peritoneal macrophages after 18h incubation with each of the dusts is shown in Table IV. Samples A and B had little effect on the cells, Sample C induced the release of 30% LDH and over 60% BGL.

Giant-cell formation in A549 cells.—The percentage of giant cells induced by each dust in cultures of A549 cells is shown in Table V. UICC crocidolite induced a significant percentage of giant

TABLE III.—*Carcinogenic activities of the dusts in experimental animals*

	No. of rats examined	Mean survival after injection (days)	mesotheliomas (%)
Exp I			
Saline control	32	717	0 (0)
Sample A	31	644	0 (0)
SFA chrysotile (+ve control)	32	612	20 (62)
Exp II			
Saline control	23	552	0 (0)
Sample B	48	549	0 (0)
Sample C	47	541	14 (30)
UICC crocidolite (+ve control)	31	557	2 (6)

TABLE IV.—*Activity of dusts against mouse peritoneal macrophages, measured by release of enzymes (mean of 4 cultures ± 95% confidence limits)*

Dust at 100 μg/ml	% LDH release	% BGL-release
Control	5·6 ± 0·4	3·6 ± 0·3
Tremolite A	10·4 ± 1·2	9·9 ± 0·8
Tremolite B	14·9 ± 0·6	14·9 ± 2·6
Tremolite C	28·5 ± 0·9	62·6 ± 1·0
UICC crocidolite	39·1 ± 1·7	48·5 ± 4·5

TABLE V.—*Activity of dusts against A549 cells (% of giant cells, with 95% confidence limits; giant cells defined as those > 25 μm diameter)*

Treatment			
	Control	1·47 (0·5–4·2)	
	Dose	100 μg/ml	200 μg/ml
Tremolite A		1·0 (0·3–3·6)	4·5 (2·4–8·3)
Tremolite B		5·3 (3·0–9·2)	3·0 (1·3–6·9)
Tremolite C		19·8 (14·9–25·8)	24·5 (19·1–30·9)
UICC crocidolite		14·4 (10·2–19·9)	26·3 (20·1–32·7)

TABLE VI.—*Cytotoxicity to V79-4 cells*

Dust	% survival at 50 μg/ml (± 95% confidence limits)
Tremolite A	101·0 ± 11·5
Tremolite B	36·7 ± 6·7
Tremolite C	3·5 ± 1·2
UICC Crocidolite	2·9 ± 1·2

cells, as reported by Chamberlain & Brown (1978). Of the test dusts, only Sample C induced giant cells, and it was as active as UICC crocidolite.

Cytotoxicity to V79-4 cells.—The cytotoxic potentials of the dusts towards V79-4 cells are shown in Table VI. Sample A was inert, B was moderately toxic, but C was as toxic as UICC crocidolite.

DISCUSSION

As indicated in the introduction, data on the human health hazards of tremolite are currently being collected. We report here experimental studies on both the carcinogenic effects *in vivo* and the cytotoxic effect *in vitro*, of 3 samples of tremolite.

Many inorganic dusts have been shown to be carcinogenic in experimental animals (for a review see IARC, 1977). Stanton *et al.* (1977) and Stanton & Layard (1978) demonstrated that the carcinogenic potential of a dust correlates with the number of fibres longer than ~8 μm and thinner than ~1·5 μm per unit mass. We have reported previously that fibres of very similar size are responsible for cytotoxic

effects in 3 types of mammalian cells (Brown *et al.*, 1978; Chamberlain *et al.*, 1979). Wade *et al.* (1980) have made similar observations. In view of the fact that fibres of similar size are both carcinogenic *in vivo* and cytotoxic *in vitro*, the use of certain mammalian cells for the detection of potentially pathogenic dusts has been proposed (Chamberlain *et al.*, 1979; Wade *et al.*, 1980; Brown *et al.*, 1980).

Only one of the tremolite samples, C, was carcinogenic. This sample was also consistently very active in the 3 *in vitro* systems. Sample C contained $5{\cdot}6 \times 10^4$ fibres >8 μm long and $<1{\cdot}5$ μm in diameter per μg. UICC crocidolite, used as a carcinogen-positive control dust, contained $6{\cdot}4 \times 10^4$ fibres of this size per μg. UICC crocidolite and Tremolite Sample C were found to be very similar in their activities in the *in vitro* systems (Tables IV, V & VI). However, Tremolite Sample C seemed to be more carcinogenic than UICC crocidolite (Table III).

It is a weakness that the animal data reported here though from 2 separate experiments, using 2 strains of rat, were impaired by the poor survival due to infection in the second experiment. In this experiment only 2 Sprague-Dawley rats (6%) injected with the UICC crocidolite positive control developed mesotheliomas. This is much lower than obtained previously with Wistar rats (Wagner *et al.*, 1973; Berry & Wagner, 1976; Wagner *et al.*, 1980*a*) which gave 46% mesotheliomas on average. The mean survival in these earlier experiments was over 4 months longer than in the experiment reported here, which partly explains the difference in mesothelioma rate. However, after allowing for survival, the mesothelioma rate in the second experiment reported here was only between 1/4 and 1/2 of the previous rates. The reason for this low rate is unknown, but an obvious possibility is that it is characteristic of the Sprague–Dawley strain that we used. However, in another experiment carried out during the same period, 6 Sprague-Dawley rats out of 48 developed mesotheliomas after injections with UICC African chrysotile (Wagner *et al.*, 1980*b*) with a mean survival only one month longer than with crocidolite in the experiment reported here. In Wistar rats UICC crocidolite produces more mesotheliomas than UICC chrysotile (Wagner *et al.*, 1973). Thus a low mesothelioma rate is not characteristic of the Sprague-Dawley strain that we used. A second possibility is that the low rate with crocidolite was a chance finding, and comparison of the present experiment with the earlier ones indicates that this possibility cannot be excluded ($P > 0{\cdot}1$). The present experiment is imprecise due to the poor survival: in terms of mesothelioma rate, there is an efficiency of only 40% of the earlier ones, *i.e.* the 31 rats with low survival are equivalent to only 12 rats with the longer survival previously obtained with Wistar rats.

Unsatisfactory though the experiment on carcinogenesis of Sample C may be, owing to the near failure of the positive controls, the fact remains that Sample C produced 14 mesotheliomas in 47 rats, whereas Samples A and B produced none.

In view of the foregoing remarks we think that it is wiser to interpret the data presented here in a qualitative rather than a quantitative manner. By analogy with other members of the amphibole asbestos minerals we suspect that Tremolite Sample C, originating from South Korea, would be a human health hazard if present in sufficient airborne concentration. An experiment exposing rats to airborne clouds of this tremolite by inhalation is now being planned as the next stage in assessing the potential health hazard.

REFERENCES

BERRY, G. & WAGNER, J. C. (1976) Effect of age at inoculation of asbestos on occurrence of mesotheliomas in rats. *Int. J. Cancer*, **17**, 477.

BROWN, R. C., CHAMBERLAIN, M., GRIFFITHS, M. & TIMBRELL, V. (1978) The effect of fibre size on the *in vitro* biological activity of three types of amphibole asbestos. *Int. J. Cancer*, **22**, 721.

BROWN, R. C., CHAMBERLAIN, M., DAVIES, R. & SUTTON, G. T. (1980) The *in vitro* activities of pathogenic mineral dusts. *Toxicology*, **17**, 143.

CHAMBERLAIN, M. & BROWN, R. C. (1978) The cytotoxic effects of asbestos and other mineral dust in tissue culture cell lines. *Br. J. Exp. Pathol.*, 59, 183.

CHAMBERLAIN, M., BROWN, R. C., DAVIES, R. & GRIFFITHS, D. M. (1979) *In vitro* prediction of the pathogenicity of mineral dusts. *Br. J. Exp. Pathol.*, 60, 320.

CHU, E. H. Y. & MALLING, H. V. (1968) Mammalian cell genetics: Chemical induction of specific locus mutations in Chinese Hamster cells *in vitro*. *Proc. Natl Acad. Sci.*, 61, 1306.

COOPER, D. W., FELDMAN, H. A. & CHASE, G. R. (1978) Fiber counting: A source of error corrected. *Am. Ind. Hyg. Assoc. J.*, 39, 362.

DAVIES, R. (1980) The effect of dusts on enzyme release from macrophages. In *The* In Vitro *Effects of Mineral Dusts*, (Eds Brown *et al.*) London: Academic Press. p. 67.

IARC (1977) The Evaluation of Carcinogenic Risk of Chemicals to Man. 14, Asbestos. Lyon: IARC.

KLEINFELD, M., MESSITE, J., KOOYMAN, O. & ZAKI, M. H. (1967) Mortality among talc miners and millers in New York State. *Arch. Environ. Hlth.*, 14, 663.

KLEINFELD, M., MESSITE, J. & ZAKI, M. H. (1974) Mortality experiences among talc workers: A follow-up study. *J. Occup. Med.*, 16, 345.

LEIBER, M., SMITH, S., SZAKAL, A., NELSON-REES, W. & TODARO, G. (1976) A continuous tumour-cell line from a human lung carcinoma with properties of Type II alveolar epithelial cells. *Int. J. Cancer*, 17, 62.

MORGAN, D. M. L., VINT, S. & RIDEOUT, J. M. (1978) Continuous flow fluorimetric assay of lysosomal enzymes. *Med. Lab. Sci.*, 35, 335.

POOLEY, F. D. (1976) An examination of the fibrous mineral content of asbestos lung tissue from the Canadian chrysotile mining industry. *Environ. Res.*, 12, 281.

STANTON, M. F. & WRENCH, C. (1972) Mechanisms of mesothelioma induction with asbestos and fibrous glass. *J. Natl Cancer Inst.*, 48, 797.

STANTON, M. F., LAYARD, M., TEGERIS, A., MILLER, E., MAY, M. & KENT, E. (1977) Carcinogenicity of fibrous glass: pleural response in the rat in relation to fiber dimensions. *J. Natl Cancer Inst.*, 58, 587.

STANTON, M. F. & LAYARD, M. (1978) The carcinogenicity of fibrous minerals. In *Proceedings of a Workshop on Asbestos: Definitions and Measurement Methods*. (Eds Gravatt *et al.*) National Bureau of Standards (U.S.A.) Special Publication. 506, 143.

WADE, M. J., LIPKIN, L. E., STANTON, M. F. & FRANK, A. L. (1980) P388D$_1$ *in vitro* cytotoxicity assay as applied to asbestos and other minerals: Its possible relevance to carcinogenicity. In *The* in Vitro *Effects of Mineral Dusts*. (Eds Brown *et al.*) London: Academic Press. p. 351.

WAGNER, J. C. (1980) Environmental and occupational exposure to natural mineral fibres. In *Biological Effects of Mineral Fibres*. (Ed. Wagner.) Lyon: IARC. p. 995.

WAGNER, J. C. (1982) *Mineral Fibre Carcinogenesis*. Am. Chem. Soc. Monogr. (in the press).

WAGNER, J. C. & BERRY, G. (1969) Mesotheliomas in rats following inoculation with asbestos. *Br. J. Cancer*, 23, 567.

WAGNER, J. C., BERRY, G. & TIMBRELL, V. (1973) Mesotheliomata in rats after inoculation with asbestos and other materials. *Br. J. Cancer*, 28, 173.

WAGNER, J. C., HILL, R. J., BERRY, G. & WAGNER, M. M. F. (1980*a*) Treatments affecting the rate of asbestos-induced mesotheliomas. *Br. J. Cancer*, 41, 918.

WAGNER, J. C., BERRY, G., HILL, R. J., MUNDAY, D. E. & SKIDMORE, J. W. (1980*b*) Animal experiments with man-made mineral fibres. In *The Biological Effects of Mineral Fibres*. (Ed. Wagner). Lyon: IARC. p. 361.

YAZICIOGLU, S., IKAYTO, R., BALCI, K., SAYLI, B. S. & YORTLMAZ, B. (1980) Pleural calcification, pleural mesotheliomas and bronchial cancers caused by tremolite dust. *Thorax*, 35, 564.

Mortality Among Talc Miners and Millers in New York State

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SINCE 1940, the Division of Industrial Hygiene has carried out a number of studies on talc miners and millers in the northern part of New York state. The prime purpose of these studies was to ascertain the health hazards associated with the exposure to dust in talc mining and milling. In 1940, a chest x-ray survey of 221 talc miners and millers employed in several plants in this region disclosed pulmonary fibrosis in 32 workers, 18 of whom had had no other occupational dust exposure. This survey clearly indicated that exposure in these talc mines and mills was capable of producing a fibrogenic type of pneumoconiosis.[1] In 1954, a follow-up study of the 32 workers with pulmonary fibrosis disclosed that the pneumoconiosis was progressive and disabling but that the progression was slow and the disability occurred primarily in the older age group.[2] A report on the clinical, roentgenographic, and pathological findings in six patients who had an average exposure to talc dust of 26 years with a range of 20 to 33 years was reported in 1963.[3] The major clinical features were chronic productive cough, dyspnea, diminished breath sounds, limited chest expansion, diffuse rales, and clubbing. The chest roentgenogram disclosed pulmonary infiltration both basally and in the midlung fields. In some instances obscuration of the cardiac borders or costophrenic sinuses, or both, were present. The most frequent pathological change was a diffuse fibrosis containing macrophages with absorbed dust particles. A pattern of endarteritis with intimal hyperplasia was frequently noted. Distension of bronchi and bronchioles with formation of cystic spaces was common. Rather characteristic was the presence of elongated, terminally clubbed bodies indistinguishable from asbestos bodies as seen in asbestosis. The pleura showed dense fibrotic thickening. Cor pulmonale was the major complication in four of the six cases and was the mechanism of death in three. A comparative clinical, roentgenographic, and physiologic study of workers exposed to fibrous and granular talc dust was made in 1964.[4] The two groups were similar with regard to age, sex, degree of exposure, and smoking habits. There were no significant differences in the clinical and roentgenographic findings between the two groups. However, the number of abnormal values for each parameter of pulmonary function measured (VC, VC_1, MBC, RV, TLC, RV/TLC and DL_{co}) was appreciably greater in those exposed to fibrous than to the granular variety of talc. In 1965, pulmonary function tests were performed on 43 workers engaged in the milling of the fibrous variety of talc.[5] The mean duration and weighted average of exposure was 19 years and 62.3 million particles per cubic foot, respectively. An appreciable number of the workers had abnormal pulmonary function measurements. This was particularly evident in the vital capacity, total lung capacity, and diffusing capacity. There were 16 who showed pulmonary infiltration on their chest roentgenograms as compared to 27 without such evidence. Those with positive radiologic findings had a proportionately greater number of respiratory symptoms and signs and abnormal pulmonary function values as compared to those with-

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out. In general, the correlation between the degree of pulmonary function impairment and the clinical and roentgenographic findings was poor.

The purpose of the present follow-up study was twofold: (1) to obtain an estimate of the incidence of disease among this select group of talc workers, and (2) to compare the observed mortality experience from specific diseases with the expected mortality among a similar basic population.

Materials and Methods

All talc miners and millers employed in 1940 who had 15 or more years of exposure to talc dust as well as those who achieved a minimum of 15 years of such exposure between 1940 and 1965 were included in this study. The group totalled 220 workers, and it is believed that the overall number of 220 constituted the total work force in the category under study.

Source of Data.—The data were obtained from a number of sources. Plant records were examined and the work history of each individual was detailed to include changes of job and specific years of employment. Death certificates were studied in all instances when available. The records of the physicians attending the workers privately were reviewed. Substantial medical information on the majority of the workers was available in the Division's files and was used as an additional source of data. Hospital records including autopsy reports and histological specimens were examined wherever available. Autopsy records were obtained in 35 of the 91 deaths.

Statistical Analysis.—The total number of deaths was distributed in the following categories: malignancies, cardiac, pneumoconiosis or complications or both, accidents or suicides, and all other causes. The proportions of mortality from cancer of the lung and pleura and of the gastrointestinal tract and peritoneum, out of the total mortality, were compared with the expected mortalities due to the above causes in the various age groups. The expected mortality represents the proportion of deaths among white men in the United States, due to the specific causes in the year 1957. This particular year was chosen as standard because it is the median year of death among the 91 deaths. Differences between observed and expected frequencies were tested for significance using the normal curve with large numbers and the Fisher's exact probability test for small frequencies.

Findings

Total Deaths.—There were 91 deaths in the group of 220 talc workers. The causes of death in the various categories are shown in Table 1. A breakdown of the deaths according to age group showed that two workers under 40 died, 38 workers who were between 40 and 59 years died, 47 between 60 and 79 years, and four died who were 80 years and over (Table 2). The average age at death of the 91 talc workers was 60.4 years with a range of 38 to 84 years.

Malignancies.—Of the 91 deaths, the observed and expected number of deaths due to cancers of the lung, pleura, gastrointestinal tract, and peritoneum are shown in Table 2. Of the ten malignancies of the lung and pleura, nine were carcinomas of the lung and one was a fibrosarcoma of the pleura. Of the seven malignancies of the gastrointestinal tract and peritoneum, two were cancers of the stomach, and one each of carcinoma of the colon, rectum, and pancreas. There was one primary hepatoma of the liver, and the remaining case was a peritoneal mesothelioma. The malignancies involving other sites included one case of retroperitoneal reticulum cell sarcoma and one of chronic lymphatic leukemia.

Cardiac Deaths.—Of the 25 cardiac deaths excluding cor pulmonale, 19 subjects had coronary artery disease, three had hypertensive heart disease, and three had rheumatic heart disease.

Pneumoconiosis or Complications or Both.—Of the 91 deaths, 28 were due to pneumoconiosis or complications or both. Nineteen of the 28 were due to cor pulmonale, five to advanced tuberculosis, and four

Table 1.—*Causes of Death in 91 Talc Workers During Period of 1940 to 1965*

Cause of Death	No.	%
Malignancies	19	20.9
Lung and pleura	10	11
Gastrointestinal and peritoneum	7	7.7
All other malignancies	2	2.2
Cardiac deaths (other than cor pulmonale)	25	27.5
Pneumoconiosis and complications	28	30.7
Accidents or suicides	8	8.8
All other causes	11	12.1
Total	91	100

Table 2.—*Deaths and Expected Mortality From Cancer of the Lung and Pleura and Gastrointestinal Tract and Peritoneum Related to Age in Talc Workers*

Age Group	Total Deaths	Deaths From Malignant Causes: Lung and Pleura	Deaths From Malignant Causes: G I* and Peritoneum	Proportional Mortality: Lung and Pleura: Observed	Proportional Mortality: Lung and Pleura: Theoretical	Proportional Mortality: G I and Peritoneum: Observed	Proportional Mortality: G I and Peritoneum: Theoretical
<40	2	0	0	0		0	
40-59	38	2	2	5.3	5.5†	5.3	5.5†
60-79	47	8	4	17	3.9‡	8.5	6.9†
80+	4	0	1	0		25	4.3
Total	91	10	7	11	3.2‡	7.7	5.3†

* Gastrointestinal tract.
† Difference between observed and theoretical values is not statistically significant.
‡ Difference between observed and theoretical values is statistically significant ($P = <0.01$).

to bronchopneumonia. The lapsed time from first talc exposure to death from pneumoconiosis or complications, or both, averaged 25.9 years, with a range from 15 to 39 years. In this group there were 11 talc millers, seven talc miners, and nine who had been both millers and miners. All 28 had their initial exposure before 1945 when more effective engineering controls, including wet drilling in the talc mines, were introduced.

All Other Causes.—Of the 11 deaths in this category, four were due to cerebrovascular accidents, two to lobar pneumonia, and the remaining five, to one of the following: bleeding duodenal ulcer, strangulated inguinal hernia, perforated diverticulum with peritonitis, acute glomerulonephritis, and mesenteric arterial occlusion.

Environmental Exposure.—Of the 91 death cases, data on environmental exposure were available in 80. The mean duration of exposure for this group was 24.7 years with a range of 15 to 47 years. The dust exposure consisted predominantly of talc admixed with other silicates such as serpentine and tremolite, carbonates, and a small amount of free silica. The comparative dust counts in the talc mines and mills prior to 1945 and between 1946 and 1965 are shown in Table 3.

Table 3.—*Comparative Dust Counts in Talc Mines and Mills, Northern New York**

Work Type	Before 1945: Low	Before 1945: Medium	Before 1945: High	Before 1945: Average	1946-1965: Low	1946-1965: Medium	1946-1965: High	1946-1965: Average
Mines								
Drilling	83	413	2800	818	0	3	10	5
Mucking	2	30	475	120	3	5	9	5
Scraping	No Dust Counts Made				5	8	13	9
Mills	(up to 1948)				(1949-1965)			
Crushing	22	69	690	180	3	13	360	42
Screening	43	61	136	69	8	37	68	37
Milling	32	75	271	92	5	20	70	25
Garners and separators	58	70	728	278	5	27	60	27
Pulverizers	No Dust Counts Made				25	28	31	28
Bagging	26	129	520	151	5	23	69	27
R R car and truck load	No Dust Counts Made				18	63	169	73
Blow Room	115	1196	2460	1227	Discontinued			
Open chutes	21	83	440	125	Discontinued			

* Concentration in millions of particles per cubic foot of air.

Comment

Malignancies.—The data on carcinoma of the lung and pleura shows an overall mortality from carcinoma of the lung and pleura to be approximately four times that expected. However, the significant increase appears to occur in the age group of 60 to 79 years rather than in the 40 to 59 year age group. This is at variance to what we have observed among workers exposed to asbestos dust where the observed values in the 40 to 59 as well as 60 to 79 year age groups were significantly different from the expected values (Table 4). The asbestos group consisted of 152 asbestos insulators who had 15 or more years of exposure in 1945 or achieved 15 years between the period of 1945 to 1965. The overall mortality was 46 or 30.3%. The reason for the earlier occurrence of an increased incidence of lung or pleural cancers in the asbestos workers com-

Table 4.—Deaths and Expected Mortality From Cancer of the Lung and Pleura and Gastrointestinal Tract and Peritoneum Related to Age in Asbestos Workers

Age Group	Total Deaths	Deaths From Malignant Causes: Lung and Pleura	Deaths From Malignant Causes: G I and Peritoneum	Proportional Mortality: Lung and Pleura: Observed	Proportional Mortality: Lung and Pleura: Theoretical	Proportional Mortality: G I and Peritoneum: Observed	Proportional Mortality: G I and Peritoneum: Theoretical
<40	1	1	0			0	
40-59	21	5	4	23.8	7.4*	19	5.6†
60-79	23	6	3	26.1	3.8*	13	5 †
80 +	1	0	0	0		0	
Total	45	12	7	26.1	3.1*	15.2	3.9*

* Difference between observed and theoretical values is statistically significant ($P = <0.01$).
† Statistical analysis not performed because of small number in group.

pared to the talc workers may be partly due to the greater carcinogenicity of asbestos dust or to an increased level of exposure to asbestos dust or both, as compared to commercial talc which is a mixture of silicates and carbonates with a small amount of free silica. In the absence of adequate smoking data one cannot assess the role played by smoking in the causation of the pulmonary carcinomas in both series. In three of the ten talc workers where a smoking history was obtainable, two smoked at least one pack daily for over 20 years and one smoked ten cigars daily for 55 years.

With regard to carcinoma of the gastrointestinal tract and peritoneum among the talc workers, we did not find any significant difference between the observed and expected values in the overall and specific age groups studied. However, in the asbestos group (Table 4) a significant difference between the observed and expected values was found in the overall categories. Our findings in the asbestos workers are similar to what has been reported by Selikoff, Churg, and Hammond.[6] If a clear-cut etiological relationship is subsequently established between exposure to asbestos and carcinoma of the gastrointestinal tract, then one may attribute the differences in incidence between the asbestos and talc groups to the reasons given for carcinoma of the lung and pleura.

Deaths Due to Pneumoconiosis or Complications or Both.—Previous studies have reported that cor pulmonale is a major complication of talc pneumoconiosis and is a major cause of death among talc workers.[3] The findings in this study are consistent with the previous reports.

Peptic Ulcer.—Among the death group of 91 cases, there were ten subjects who had duodenal ulcer, and in one of the ten the ulcer was the cause of death. In the 129 workers who are still alive, 19 had a diagnosis of duodenal ulcer. This is a total of 29, or 13.2%. According to the health statistics of the US Department of Health, Education and Welfare, the prevalence of peptic ulcer found between 1957 to 1959 for males of all ages is 21.4 per thousand population, or 2.1%.[7] Despite the fact that the figures obtained through the National Health Survey represent point prevalence in contrast to the period prevalence figures of our study, it appears that the incidence of peptic ulcer among talc workers is higher than among the general population. A significant increase in incidence of peptic ulcer varying between 20% to 25% has been found in patients with chronic obstructive pulmonary disease.[8] Since the talc workers have primarily a restrictive rather than an obstructive lung disorder, one may speculate that both types may be associated with an increased incidence of peptic ulcer.

Correlation of Carcinoma of Lung With Age, Duration of Exposure or Pneumoconiosis or Both.—Relating carcinoma of the lung with duration of exposure, it might be relevant that all the individuals with carcinoma of the lung or pleura had their initial exposure prior to the institution of wet drilling. The average duration of exposure prior to the introduction of wet drilling was 14.6 years. Although this was a period of heavy exposure (Table 3) there is no evidence to indicate that there was a direct relationship between the duration of exposure prior to the onset of wet drilling and the occurrence of pulmonary carcinoma. The mean age of

the group of ten workers who developed carcinoma of the lung or pleura was 63.6 years with a range of 45 to 75 years. This is slightly above the mean age of 60.4 years with a range of 38 to 84 years for the overall group of 91 death cases. Since the life expectancy of white men born in the United States in 1920 is given as 54.4 years and since these workers were born in 1920 or earlier, there does not seem to be a reduction in the life span from the exposure.[9] With regard to carcinoma of the lung and pneumoconiosis, our data indicated that eight of the ten persons with carcinoma of the lung had clearcut evidence of an associated pneumoconiosis. No confirmatory evidence was obtained in the remaining two, one of whom had a fibrosarcoma of the pleura and the other a bilateral massive pleural effusion.

Summary and Conclusions

Mortality data on 91 talc miners and millers in New York state who had 15 or more years of exposure to talc dust by 1940 or had achieved 15 years between 1940 and 1965 were reviewed. The average age at death was 60.4 years with a range of 38 to 84 years.

Proportional mortality from carcinoma of the lung and pleura among talc workers was four times that of the control population. However, the significant increase in incidence appeared in the 60 to 79 year age group rather than in the 40 to 59 year group. This was at variance with our findings in asbestos workers who had a similar duration of exposure covering a similar period where the increase in observed incidence was present in both age categories. The earlier occurrence in the asbestos workers was attributed to the greater carcinogenicity and possibly to exposure to asbestos dust of greater concentration as compared to commercial talc. Carcinoma of the gastrointestinal tract and peritoneum showed no significant difference in the talc and control populations. However, in the asbestos workers gastrointestinal and peritoneal malignancies were elevated significantly. Death due to pneumoconiosis or its complications, or both, was recorded in 28 individuals, 19 of whom died from cor pulmonale. The cause of death in the remaining nine was tuberculosis (5) or bronchopneumonia (4). The lapsed time from first talc exposure to death from pneumoconiosis or complications, or both, averaged 25.9 years, with a range from 15 to 39 years. Excluding cor pulmonale, there were 25 cardiac deaths, 19 of which were due to coronary artery disease, three to hypertensive heart disease, and three to rheumatic heart disease. Of the 91 death cases, data on environmental exposure were available in 80. The mean duration of exposure for this group was 24.7 years with a range of 15 to 47 years.

Previous studies have indicated that cor pulmonale was a major cause of death among talc miners and millers. This was also found in the present study. However, what has not been previously brought out is the increase in observed incidence of carcinoma of the lung among these workers as demonstrated in this study.

References

1. Siegal, W.; Smith, A.R.; and Greenburg, L.: The Dust Hazard in Tremolite Talc Mining, Including Roentgenological Findings in Talc Workers, *Amer J Roentgen* 49:11-29 (Jan) 1943.

2. Kleinfeld, M.; Messite, J.; and Tabershaw, I.R.: Talc Pneumoconiosis, *Arch Industr Health* 12:66-72 (July) 1955.

3. Kleinfeld, M., et al: Talc Pneumoconiosis: A Report of Six Patients With Postmortem Findings, *Arch Environ Health* 7:101-115 (July) 1963.

4. Kleinfeld, M., et al: Lung Function in Talc Workers: A Comparative Physiologic Study of Workers Exposed to Fibrous and Granular Talc Dusts, *Arch Environ Health* 9:559-566 (Nov) 1964.

5. Kleinfeld, M., et al: Effect of Talc Dust Inhalation on Lung Function, *Arch Environ Health* 10:431-437 (March) 1965.

6. Selikoff, I.J.; Churg, J.; and Hammond, E.C.: Asbestos Exposure and Neoplasia, *JAMA* 188:22-26 (April 6) 1964.

7. "Peptic Ulcers Reported in Interviews," in *US National Health Survey*, Washington, DC: US Department of Health, Education, and Welfare, Public Health Service, 1960.

8. "Chronic Obstructive Pulmonary Disease," in *Manual for Physicians*, National Tuberculosis Association 1966, p 71.

9. *Statistical Abstract of the United States*, ed 87, Washington, DC: US Bureau of the Census, 1966.

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Mechanisms of Induction of Ornithine Decarboxylase Activity in Tracheal Epithelial Cells by Asbestiform Minerals[1]

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ABSTRACT

Asbestos induces a constellation of biological responses in cells of the respiratory tract that are similar to those of classical tumor promoters. In this regard, induction of ornithine decarboxylase (ODC) activity and increased incorporation of [^{3}H]thymidine have been documented after addition of crocidolite and chrysotile asbestos to a hamster tracheal epithelial cell line (J. M. Landesman and B. T. Mossman, Cancer Res., *42:* 3669–3675, 1982). The objectives of studies here were to determine: (*a*) the importance of geometry, size, and/or chemical composition of asbestos fibers on induction of ODC activity; and (*b*) the possible involvement of calcium and/or protein kinase C in asbestos-induced ODC activity. After addition for 24 h to confluent hamster tracheal epithelial cells, fibers of crocidolite, chrysotile, and glass in medium containing fresh serum caused a significant increase in ODC activity. Stimulation of ODC was not observed when nonfibrous analogues (riebeckite, antigorite, and glass particles) were used. Sized preparations of long (>10-μm length) chrysotile fibers were more potent in enhancing ODC activity than shorter (≤2-μm length) fibers at similar concentrations. The mechanisms of ODC induction by asbestos were probed by adding the calcium channel blockers (verapamil and nifedipine) and inhibitors [10^{-5} to 10^{-7} M of 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine, of *N*-[2-(methylamino) ethyl]-5-isoquinolinesulfonamide, of TMB-8, and of palmitoyl carnitine] of protein kinase C simultaneously with chrysotile asbestos. These agents inhibited ODC activity by chrysotile in a dosage-dependent fashion. Results suggest that the fibrous geometry and length of asbestos fibers are critical in initiating ODC activity in airway epithelial cells. Moreover, they implicate the importance of calcium and protein kinase C in asbestos-induced mitogenic responses.

INTRODUCTION

Asbestos is a family of fibrous mineral silicates which possess great strength and flexibility. Although occupational exposure to asbestos is associated with an increased incidence of bronchogenic carcinoma, mesothelioma, and asbestosis (1, 2), the mechanism(s) responsible for the pathogenesis of asbestos-associated diseases is unclear. Various physicochemical properties of asbestos including its fibrous morphology (3, 4), surface properties (5–7), chemical composition (8, 9), and size (10) influence its cytotoxic or hemolytic activity *in vitro*. Moreover, longer, thinner fibers are more apt to cause sarcoma and mesothelioma when injected into the pleural cavities of rats (11–14).

Whether or not asbestos is a complete carcinogen in the development of bronchogenic carcinoma is unclear. Asbestos alone is neither mutagenic nor carcinogenic to a variety of cell types including hamster tracheal epithelial cells (15–18). However, many types of asbestos appear to augment the mutagenicity and carcinogenicity of polycyclic aromatic hydrocarbons (18–20).

The possibility that asbestos acts as a tumor promoter in respiratory epithelium was suggested by this laboratory after examination of the effects of these minerals on cell and organ cultures of tracheobronchial epithelium (reviewed in Ref. 18). When added to tracheal epithelium, asbestos causes a number of morphological and biochemical alterations similar to those seen with application of the tumor promoter TPA[3] to mouse skin. These include: stimulation of Na^+-K^+-ATPase in isolated plasma membranes (21); induction of cell division (22, 23); changes in normal cell differentiation (24); release of [^{3}H] arachidonic acid (25); production of active oxygen species (26); and increased activity of ODC (22), a highly regulated enzyme undergoing a rapid increase in activity in response to TPA and a number of growth-promoting stimuli (27–29).

Studies here represent a logical extension of these earlier findings in an attempt to determine the physical and/or chemical properties of asbestos that are responsible for induction of ODC. To this end, the activities of ODC in HTE cells exposed to nontoxic amounts of natural and man-made fibers and to their chemically identical, nonfibrous analogues were compared. In addition, the influence of fiber length on the activity of the enzyme was examined using sized preparations of long (>10 μm) and short (≤2 μm) chrysotile asbestos.

Recently, several investigators have demonstrated the involvement of both calcium and protein kinase C in TPA-induced ODC activity and tumor promotion in mouse skin and other cell types (30–33). To determine whether calcium and/or protein kinases are involved in induction of ODC by asbestos in tracheal epithelial cells, we used several inhibitors of protein kinases and calcium entry in an effort to ameliorate asbestos-induced ODC induction.

MATERIALS AND METHODS

Hamster Tracheal Epithelial Cells. Tracheal epithelial cells, obtained from neonatal Syrian golden hamsters, were isolated and cloned as described previously (34). Cultures of the clone B-cell line were maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY) and garamycin (100 μg/ml; M. A. Bioproducts, Walkersville, MD). For all assays, cells were grown to confluency before test materials were added for a 24-h period as described previously (22).

Preparation and Addition of Fibers and Analogues. Several types of fibers and their nonfibrous analogues were used to determine if geometry was important in ODC induction by asbestos. The appearance of minerals used in this study is shown in Fig. 1. The purity and size distributions of particulates have been reported previously (24). UICC reference samples of crocidolite ($Na_2O \cdot Fe_2O_3 \cdot 8SiO_2$) (Fig. 1*A*) and chrysotile ($3MgO \cdot SiO_2 \cdot 2H_2O$) (Fig. 1*C*) asbestos were used in comparative studies with riebeckite (Fig. 1*B*) and antigorite (Fig. 1*D*), their chemically identical, nonfibrous analogues (Wards Natural Science Est., Rochester, NY). Code 100 fiberglass (SiO_2), of comparable dimensions to asbestos, was obtained from Manville Corp., Denver, CO. Glass particles were produced by fusing Code 100 fibers at 750°C followed by grinding in a ball mill for 15 min (24). To determine if fiber length is important in induction of ODC, preparations of chrysotile asbestos containing long (>10 μm) (Fig. 1*G*) or short (≤2 μm) (Fig. 1*H*) fibers were made by a sedimentation-filtration procedure. Before introduction into cell cultures, suspensions of each material were dis-

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[3] The abbreviations used are: TPA, 12-*O*-tetradecanoylphorbol-13-acetate; ODC, ornithine decarboxylase; HBSS, Hanks' balanced salt solution with Ca^{2+} and MG^{2+}; HTE, hamster tracheal epithelial cells; PBS, phosphate-buffered saline with Ca^{2+} and Mg^{2+}, pH 7.4; UICC, International Union against Cancer; H-7, 1-(5-isoquinolinylsulfonyl-2-methylpiperazine) (10^{-5}–10^{-7} M); H-8, *N*-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide, (10^{-5}–10^{-7} M).

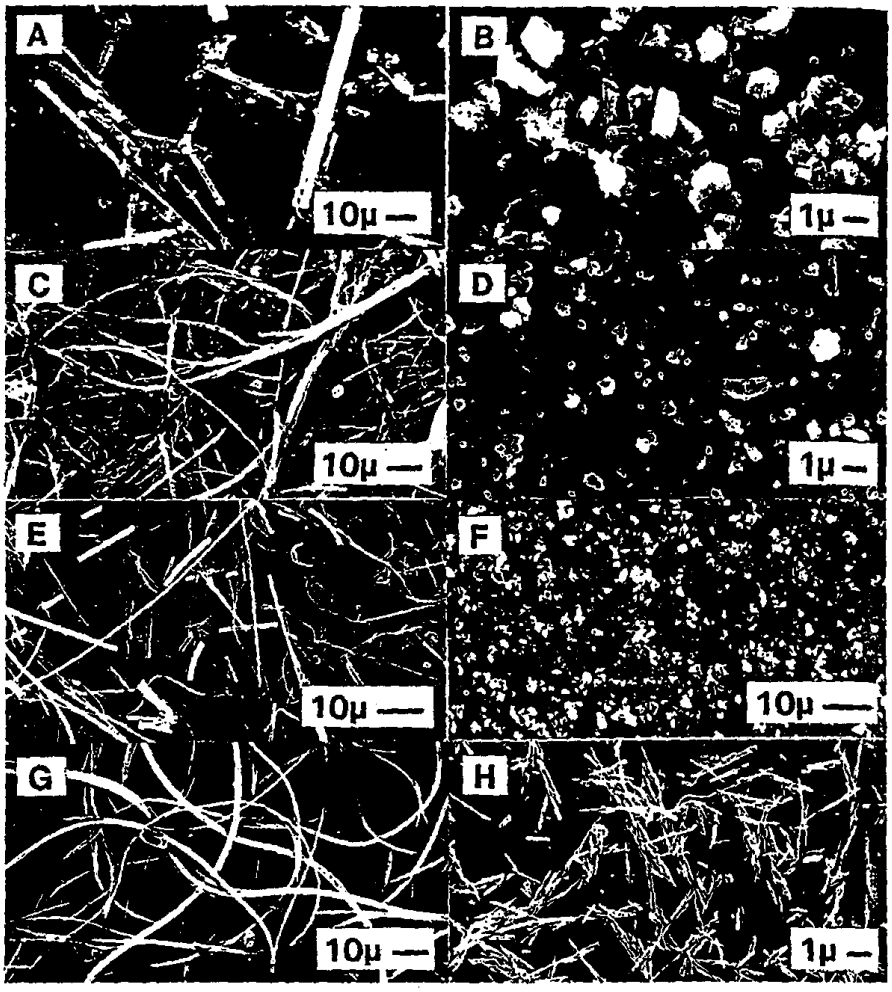

Fig. 1. Scanning electron micrographs showing appearance of fibers and their nonfibrous analogues. *A*, crocidolite asbestos consisting of straight, rod-like fibers; *B*, riebeckite, the nonfibrous mineral analogue of crocidolite; *C*, chrysotile asbestos fibers that are curly and pliable; *D*, antigorite, the amorphous analogue of chrysotile; *E*, Code 100 fiberglass consisting of finely extruded man-made fibers; *F*, Code 100 ball-milled to particles; *G*, Manville preparation of long chrysotile consisting of fibers ≥10 μm in length; *H*, Manville preparation of short chrysotile consisting of fibers ≤2 μm in length.

persed in HBSS. Riebeckite, antigorite, and glass particles were sonicated (Model B0220; Branson, Shelton, CT) for 5 min to ensure uniform distribution. Code 100 fibers, which are manufactured by extrusion, hence of infinite length, were broken up and homogenized using 10 passes with a Tenebrok glass homogenizer. Finally, all suspensions of minerals in medium were passed 5 times through a 22-gauge needle, in order to obtain a uniform suspension. The fibrous geometry of the various preparations was not altered by these manipulations (24).

Inhibitors of Calcium Entry and Protein Kinases. The calcium entry antagonists, verapamil and nifedipine, which act on voltage-dependent calcium channels (35), were obtained from Sigma Chemical Co., St. Louis, MO, as was palmitoyl carnitine, an inhibitor of protein kinase C. TMB-8, an antagonist of protein kinase C which also inhibits stimulus-induced release of calcium from the endoplastic reticulum (36), was provided by Dr. Kenneth B. Adler, Department of Pathology, University of Vermont College of Medicine. Additionally, two newly synthesized isoquinolinesulfonamide derivatives which bind directly, but with different affinities, to protein kinases (Seikagaku America, Inc., St. Petersburg, FL) were used. H-7 is a more potent and selective inhibitor of protein kinase C, whereas H-8 also inhibits cyclic nucleotide-dependent kinases in addition to protein kinase C (37). All agents at nontoxic concentrations were added to HTE cells with chrysotile asbestos (0.65 μg/cm² dish), and ODC activity was determined at 24 h thereafter. At the concentrations used here, inhibitors alone did not inhibit ODC induction in HTE cells.

^{75}Se Cytotoxicity Assay. A sensitive assay, based on uptake and incorporation of radiolabeled amino acid into cellular proteins and release into medium upon membrane damage, was used to quantitate mineral-induced cell injury and screen for nontoxic concentrations of inhibitors (26). HTE cells prelabeled for 16 h with 2.5 μCi/ml of [^{75}Se] selenomethionine (specific activity, 0.6 to 4 Ci/mmol; Amersham Corp., Arlington Heights, IL) were washed 3 times with PBS, scraped with a rubber policeman, and plated into 12-well dishes (10^5 cells/25-mm well). At confluency, fresh medium with or without various concentrations of fibers or particles or inhibitors was added. After 24 h, medium was removed, and aliquots of supernatant were counted in an Intertechnique gamma counter (Fairfield, NJ). The cells were washed with PBS and dissolved in 1.0 N NaOH, and aliquots were counted similarly. Results were expressed as a release index, calculated from the following equation.

$$\text{Release index} = \frac{\text{cpm medium}}{\text{total counts (cpm medium + cpm cells)}}$$

Assay for ODC. After incubation with nontoxic concentrations of test materials for 24 h, confluent cells were prepared and assayed as described previously (22). These prior studies had indicated maximal induction of ODC activity by asbestos at 24 h after addition of minerals (22). Activity of ODC was expressed as nmol CO_2/h/mg protein. Protein was determined by the method of Bradford (38).

Statistics. Data were analyzed using the Duncan test for multiple-range analysis or the *Z* test for variance of a ratio (39).

RESULTS

^{75}Se Cytotoxicity Assays. Before their introduction into HTE cultures, nontoxic concentrations of all fibers and analogues were determined using a ^{75}Se cytotoxicity assay (Table 1). Complete dose-response information on cytotoxicity of UICC crocidolite, UICC chrysotile, Code 100 glass fibers, and long

(≥10 μm) *versus* short (≤2 μm) Manville chrysotile in logarithmically growing HTE cells has been published previously (26).

Activity of ODC in HTE Cells after Exposure to Fibrous and Nonfibrous Minerals. The activity of ODC in quiescent HTE cells exposed for 24 h to nontoxic concentrations of various fibers and their nonfibrous analogues is presented in Table 2. Significant induction of ODC occurred after exposure of cells to all fibrous materials including both types of asbestos and Code 100 fiberglass, an amorphous fiber differing in chemical composition from asbestos. These enzyme effects were dose dependent. In contrast, addition of equal amounts of the nonfibrous, chemically identical analogues of fibers, *i.e.*, riebeckite, antigorite, and glass particles, did not increase ODC activity significantly.

Effect of Fiber Length on ODC Activity. To determine whether fiber length was important in the induction of ODC activity in tracheal epithelial cells, a range of concentrations of long (>10 μm) or short (≤2 μm) fibers of chrysotile was introduced into cultures for 24 h (Fig. 2). The nonfibrous analogue of chrysotile, antigorite, also was included in these experiments. Results indicated that the potency of ODC induction by chrysotile was related directly to fiber length. Maximal elevation of ODC activity was observed after exposure of cells to very low concentrations (0.72 μg/cm² dish) of long fibers, whereas a 4-fold higher concentration (2.9 μg/cm²) of short fibers was required to induce similar effects. ODC activity appeared to decline as the concentrations of fibers approached cytotoxic levels. No significant elevation of ODC activity resulted when antigorite was tested except at extremely high amounts (11.6 μg/cm²), a nontoxic concentration as determined by the ^{75}Se release assay.[4]

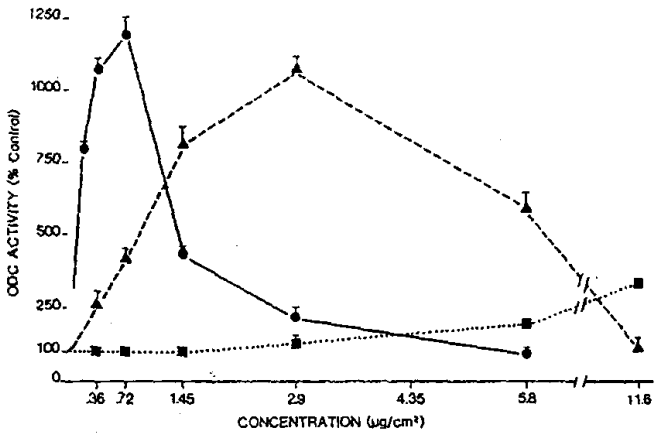

Fig. 2. Effect of fiber length on induction of ODC. Activity is expressed as a percentage of values observed in untreated, control cells. Various concentrations of long (●) and short (▲) fibers of chrysotile asbestos and the nonfibrous analogue of asbestos, antigorite (■), were assessed comparatively. One hundred ODC activity = 0.071 nmol CO_2/h/mg protein. Values (mean ± SE) are from a representative experiment (n = 6/group).

Table 1 *Effects of fibers and particles on release of ^{75}Se from confluent HTE cells after 24 h*

Group	Concentration (μg/cm²)	Release index[a]
Control	0	0.189 ± 0.007[b]
Crocidolite (UICC)	2.60	0.170 ± 0.010
Riebeckite	2.60	0.173 ± 0.014
Chrysotile (UICC)	0.65	0.172 ± 0.010
Antigorite	2.90	0.174 ± 0.009
Code 100 fibers	2.60	0.185 ± 0.010
Glass particles	2.60	0.175 ± 0.003
Manville chrysotile (>10 μm)	1.45	0.21 ± 0.010
	2.90	0.44 ± 0.017[c]
Manville chrysotile (≤2 μm)	5.80	0.180 ± 0.003

[a] Expressed as $\frac{\text{cpm medium}}{\text{total counts (cpm medium + cpm cells)}}$.

[b] Mean ± SE of triplicate determinations.

[c] $P < 0.05$ increased significantly in comparison to control untreated cells (Duncan multiple-range analysis).

Table 2 *ODC activity in HTE cells exposed for 24 h to nontoxic concentrations of fibers and respective nonfibrous analogues*

Group	Concentration (μg/cm²)	ODC activity (nmol CO_2/h/mg protein)	% of control activity
Control	0	0.041 ± 0.01[a]	
Crocidolite (UICC)	1.3	0.139 ± 0.02	339
	2.6	0.500 ± 0.09[b]	1220
Riebeckite	1.3	0.057 ± 0.008	139
	2.6	0.042 ± 0.005	102
Chrysotile (UICC)	0.325	0.793 ± 0.09[b]	1975
	0.65	1.007 ± 0.03[b]	2439
Antigorite	0.325	0.037 ± 0.004	90
	0.65	0.042 ± 0.004	102
Code 100 fibers	1.3	0.312 ± 0.04[b]	761
	2.6	0.810 ± 0.07[b]	2025
Glass particles	1.3	0.214 ± 0.02	522
	2.6	0.061 ± 0.004	149

[a] Mean ± SE of three experiments; n = 4/group.

[b] Increased significantly ($P < 0.05$) in comparison to control untreated cells (Duncan multiple-range analysis).

Studies Using Inhibitors of Calcium Entry and Protein Kinases. The mechanisms of asbestos-induced proliferation and induction of ODC in HTE cells are undefined. Since asbestos is a membrane-active agent (5), causing an influx of calcium into cells (40), we postulated the role of Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C) in asbestos-induced induction of ODC. To test this hypothesis, selective inhibitors were added to HTE cells simultaneously with chrysotile asbestos (0.65 μg/cm² dish, *i.e.*, a concentration causing maximal ODC induction). The highest concentration shown for each agent represents its maximal nontoxic concentration as assayed by the ^{75}Se method (data not shown). The calcium entry antagonists, verapamil and nifedipine, caused significant decreases in chrysotile-induced ODC activity at 24 h (Fig. 3). Whereas the inhibition by verapamil was dosage dependent, nifedipine caused a significant decrease of chrysotile-induced ODC activity at all concentrations tested (10^{-7} to 10^{-5} M). Several antagonists of protein kinase C including TMB-8, palmitoyl carnitine, H-7, and H-8 also were effective in inhibiting asbestos-associated increases in ODC activity (Fig. 4). For reasons that are unclear, H-8 at 10^{-7} M caused a consistent increase in asbestos-induced ODC activity.

DISCUSSION

ODC is a rate-limiting enzyme in the biosynthesis of polyamines, growth regulatory molecules necessary for the initiation of cell division (27). An increase in the rate of induction of ODC has been demonstrated consistently after application of a number of documented tumor promoters to mouse and human skin (28, 29). Thus, the regulation and induction of the enzyme are thought to be important in the progression of an initiated cell to a tumor cell. We documented previously (22) an en-

[4] Unpublished data.

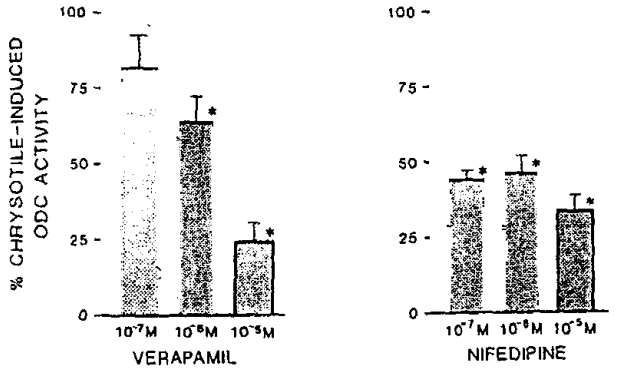

Fig. 3. Modulation of chrysotile asbestos-induced ODC activity by the calcium entry antagonists, verapamil and nifedipine. Values (mean ± SE) are expressed as a percentage of chrysotile-induced ODC activity (100% activity = 0.582 nmol CO_2/h/mg protein) and represent a typical experiment. Each experiment performed in duplicate or triplicate (n = 3–5/group/experiment). *, $P < 0.001$ in comparison to chrysotile-exposed group (Z-test for variance of a ratio).

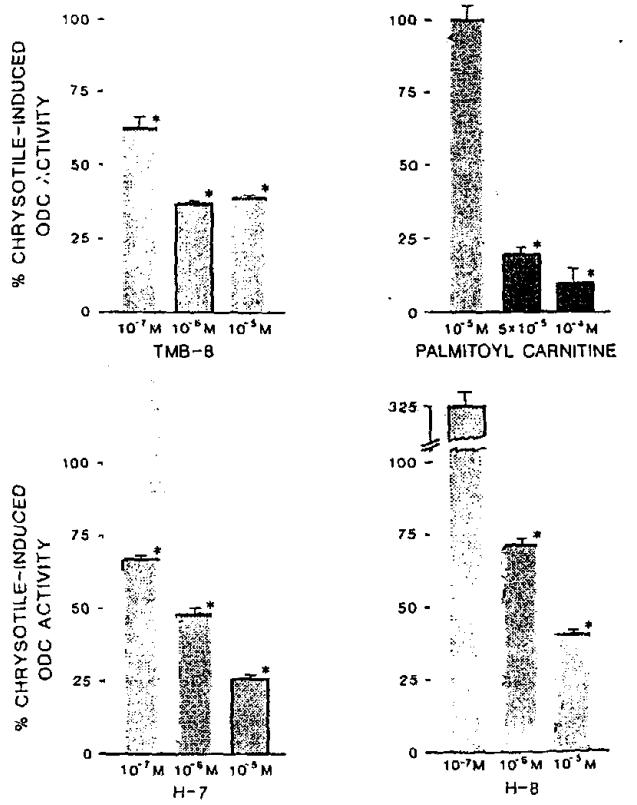

Fig. 4. Modulation of chrysotile asbestos-induced ODC activity by inhibitors of protein kinase C. Values (mean ± SE) are expressed as a percentage of chrysotile-induced ODC activity and represent a typical experiment. For TMB-8 and palmitoyl carnitine, 100% activity = 0.568 nmol CO_2/h/mg protein. For H-7 and H-8, 100% activity = 0.498 nmol CO_2/h/mg protein. Each experiment performed in duplicate or triplicate (n = 3–5/group/experiment). *, $P < 0.001$ in comparison to chrysotile-exposed group (Z-test for variance of a ratio).

hanced activity of ODC and increased uptake of [^{3}H]thymidine in hamster tracheal epithelial cells after exposure *in vitro* to crocidolite and chrysotile, chemically and physically distinct types of asbestos fibers associated with the development of bronchogenic carcinoma and mesothelioma in humans (1). In an attempt to determine those characteristics of asbestos responsible for the induction of ODC, other fibrous and nonfibrous particulates were examined here in comparative studies.

Results show that the fibrous morphology, rather than the chemical composition of asbestos, is integral to the stimulation of ODC in epithelial cells of the respiratory tract. The nonfibrous chemically identical analogues of crocidolite, chrysotile, and fiberglass (riebeckite, antigorite, and glass particles, respectively) were ineffective in increasing ODC activity, whereas fibers at comparable nontoxic concentrations induced the enzyme in a dosage-dependent fashion. Unlike asbestos, the crystalline fiber, Code 100 fiberglass, is an amorphous fibrous silicate; thus the nature of the structural matrix of the fiber does not appear critical to the induction of ODC.

Data here support published work by this laboratory demonstrating the importance of fibrous geometry and size in both the development of squamous metaplasia, a putative preneoplastic lesion, and increased uptake of [^{3}H]thymidine by tracheal explants (23, 24). In these studies, Code 100 fibrous glass, crocidolite, and long chrysotile fibers caused metaplastic and proliferative changes in the epithelium of tracheal explants, whereas growth alterations were not observed with corresponding nonfibrous analogue (glass, riebeckite, or antigorite). Ultrastructural studies indicated selective growth of dedifferentiated and keratinizing epithelial cells over fibers lodged on the epithelial surface, whereas particles were either cleared or taken up by epithelial cells. Thus, increases in ODC in HTE cells by asbestos can be linked to altered proliferation and differentiation phenomena important in tumor promotion in the respiratory tract (18, 23, 24).

Studies in this laboratory have focused on defining the mechanisms of action of asbestos in comparison to those caused by soluble phorbol compounds. Although asbestos-induced biological effects (*i.e.*, alterations in proliferation and differentiation, increased ODC activity, etc.) generally occur over a longer time course, they parallel many events observed in a variety of cell types after exposure to TPA (18). Accumulating evidence indicates that TPA-induced effects are mediated through its mimicry of diacylglycerol and consequent activation of protein kinase C (41, 42). Accordingly, several investigators have suggested the importance of calcium-activated protein kinase C in the induction of ODC activity associated with TPA (30–33). For example, exposure of lymphocytes to calcium ionophore in combination with TPA or diacylglycerol augments ODC activity above levels obtained with the use of ionophore alone (33). Recent work by Verma and coworkers shows that either diacylglycerol or phospholipase C, which enzymatically cleaves diacylglycerol from membrane phospholipids, induces ODC mRNA, an observation suggesting the importance of protein kinase C in gene transcription of ODC (30). Lastly, TPA-induced ODC activity and skin promotion can be blocked with the use of palmitoylcarnitine, an inhibitor of protein kinase C (30, 31).

To determine if calcium and protein kinase C are involved in asbestos-associated induction of ODC, we used calcium entry antagonists and a battery of inhibitors of protein kinase C to modulate typical chrysotile-associated increases in ODC activity. As can be seen in Figs. 3 and 4, test agents ameliorated chrysotile-induced changes in dosage-dependent fashion. Interestingly, the affinity of H-compounds for protein kinase C corresponded well with their ability to inhibit asbestos-associated ODC activity. H-7, the more potent and selective protein kinase C inhibitor (37), was effective at 10^{-7} M, whereas H-8 at 10^{-7} M not only failed to inhibit chrysotile-induced ODC, but actually augmented the stimulatory effect of the fiber. It is unclear at this time whether this stimulatory effect of H-8 is due to its modulation of protein kinase C or a cyclic AMP-dependent protein kinase (or both enzymes).

Although studies with antagonists provide only indirect evidence for the involvement of protein kinase C in asbestos-induced proliferative alterations, recent experiments show a

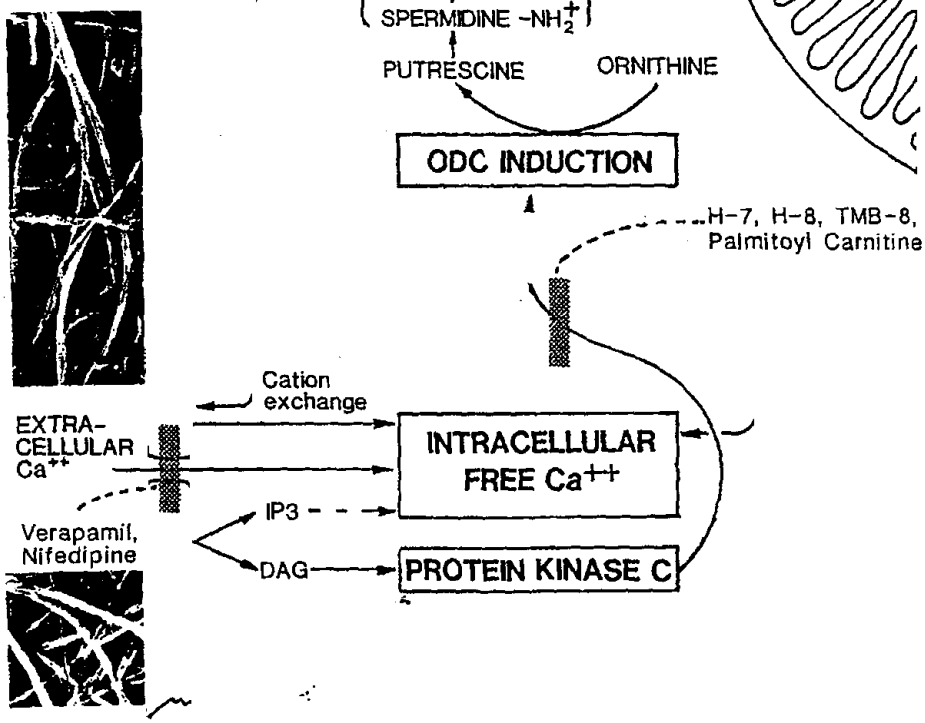

Fig. 5. Hypothetical schema of asbestos-stimulated signaling involved in induction of ODC activity.

rapid accumulation of diacylglycerol in HTE cells exposed to asbestos.[5] Clearly, since insoluble asbestos fibers are physically and chemically dissimilar to TPA, they cannot mimic diacylglycerol and activate protein kinase C directly. Therefore, other mechanisms, such as activation of membrane phospholipases, must be involved in cell signaling by asbestos. We are exploring these possibilities presently.

A hypothetical pathway of asbestos-induced signal transduction involved in stimulation of ODC activity is presented in Fig. 5. This schema is consistent with our observations to date using antagonists of calcium entry and protein kinase C. Asbestos fibers interact with the plasma membrane causing Ca^{2+} influx (40) and an increase in intracellular free Ca^{2+}. In addition, diacylglycerol accumulates, presumably due to activation of membrane phospholipases by asbestos. These events could stimulate protein kinase C and result in subsequent gene transcription of ODC (30). Since recent work by Hovis *et al.* (32) suggests ODC activity also can be regulated via mechanisms independent of protein kinase C, we are currently focusing on whether asbestos activates this enzyme directly.

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REFERENCES

1. Craighead, J. E., and Mossman, B. T. Pathogenesis of asbestos associated diseases. N. Engl. J. Med., *306:* 1446–1455, 1982.
2. Becklake, M. Asbestos-related diseases of the lung and other organs: their epidemiology and implications for clinical practice. Am. Rev. Respir. Dis., *114:* 187–277, 1976.
3. Pott, F. Animal experiments on biological effects of mineral fibers. *In:* J. C. Wagner (ed.), Biological Effects of Mineral Fibers, Vol. 1, pp. 261–269. Lyon: I.A.R.C., 1980.
4. Wright, G. W., and Kuschner, M. The influence of varying lengths of glass and asbestos fibers on tissue response in guinea pigs. *In:* W. H. Walton (ed.), Inhaled Particles, Vol. 4, pp. 455–474. New York: Pergamon Press, 1977.
5. Light, W. G., and Wei, E. T. Surface charge and hemolytic activity of asbestos. Environ. Res., *13:* 135-145, 1977.
6. Valentine, R., Change, M. J. W., Hart, R. W., Finch, G. L., and Fisher, G. L. Thermal modification of chrysotile asbestos. Evidence for decreased cytotoxicity. Environ. Health Perspect, *51:* 357–368, 1983.
7. Fisher, G. L., McNeill, K. L., Mossman, B. T., McFarland, A. R., and Hart, R. W. Investigations into the mechanisms of asbestos toxicity. *In:* E. G. Beck and J. Bignon (eds.), *In Vitro* Effects of Mineral Dusts, Vol. 3, pp. 31–38. Berlin: Springer-Verlag, 1985.
8. Monchaux, G., Bignon, J., Jaurand, M. C., Lafuma, J., Sabastian, P., Masses, R., Hirsch, A., and Goni, J. Mesotheliomas in rats following inoculation with acid-leached chrysotile asbestos and other mineral fibers. Carcinogenesis (Lond.), *2:* 229–236, 1981.
9. Morgan, A., Davies, P., Wagner, J. C., Berry, G., and Holmes, H. The biological effects of magnesium-leached chrysotile asbestos. Br. J. Exp. Pathol., *58:* 465–473, 1977.
10. Frank, A. L., Rohl, A. N., Wade, M. J., and Lipkin, L. E. Biological activity *in vitro* of chrysotile compared to its quarried parent rock (platy serpentine). J. Exp. Pathol. Toxicol., *2:* 1041–1046, 1979.
11. Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M., and Kent, E. Carcinogenicity of fibrous glass: pleural response in the rat relation to fiber dimension. J. Natl. Cancer Inst., *58:* 587–603, 1977.
12. Stanton, M. F., and Wrench, C. Mechanisms of mesothelioma induction. J. Natl. Cancer Inst., *48:* 797–821, 1972.
13. Wagner, J. C., Berry, G., and Timbrell, V. Mesothelioma in rats after inoculation with asbestos and other materials. Br. J. Cancer, *28:* 173–185, 1983.
14. Harington, J. S. Fiber carcinogenesis: epidemiologic observations and the Stanton hypothesis. J. Natl. Cancer Inst., *67:* 977–987, 1981.
15. Chamberlain, M., and Tarmy, E. M. Asbestos and glass fibers in bacterial mutation tests. Mutat. Res., *43:* 159–164, 1977.
16. Brown, R. C., Poole, A., and Fleming, G. T. A. The influence of asbestos dust on the oncogenic transformation of C3H10T½ cells. Cancer Lett., *18:* 221–227, 1983.
17. Reiss, B., Solomon, S., Tong, C., Levenstein, M., Rosenberg, S. H., and Williams, G. M. Absence of mutagenic activity of three forms of asbestos in liver epithelial cells. Environ. Res., *27:* 389–397, 1982.
18. Mossman, B. T., Cameron, G. S., and Yotti, L. P. Cocarcinogenic and tumor

[5] A. Sesko, M. Cabot, and B. T. Mossman. Accumulation of diacylglycerol precedes asbestos-induced proliferation in hamster tracheal epithelial cells, manuscript in preparation.

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Induction of Squamous Metaplasia in Organ Cultures of Hamster Trachea by Naturally Occurring and Synthetic Fibers[1]

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ABSTRACT

Asbestos exhibits many properties of classical tumor promoters. These characteristics include the ability to stimulate proliferation and inhibit normal differentiation of cells. In organ cultures of trachea, crocidolite and amosite asbestos stimulate squamous metaplasia, a pathological process in which a rapidly proliferating squamous epithelium replaces the normal epithelium. We hypothesized that the induction of metaplasia depends upon the fibrous nature of asbestos. Accordingly, several naturally occurring and synthetic fibrous materials and their nonfibrous analogues were assessed for their ability to induce metaplastic changes in tracheal mucosa of the Syrian hamster. Exposure to both crocidolite asbestos and fiberglass resulted in significant increases ($p < 0.05$) in squamous metaplasia over a range of dosages (1.0, 4.0, 16.0 mg/ml). Attapulgite (palygorskite) and both "long-" and "short-" fiber preparations of chrysotile asbestos had similar but less marked effects. Nonfibrous analogues of each material (riebeckite, antigorite, and glass particles) failed to produce metaplasia. Asbestos, and fibrous materials in general, appear to stimulate squamous metaplasia because of their fibrous geometry.

INTRODUCTION

Asbestos has enormous commercial importance (3, 21) because it is durable and resistant to both heat and fire. Asbestos acts as a cocarcinogen in the respiratory tract (6, 9, 24), and exhibits properties of classical tumor promotors (for review, see Refs 6, 17, and 28). These characteristics include the ability to stimulate proliferation of cells and alter their normal differentiation. For example, crocidolite and amosite asbestos induce squamous metaplasia in cultured tracheal epithelium (18). Although the chemical composition of asbestos minerals might influence pathogenicity (11), most experimental evidence indicates that physical parameters such as the length and diameter of fibers are important (10, 13, 26, 33).

Fiber geometry is critical in the experimental induction of mesotheliomas in rats (26). Intrapleural inoculation of materials comprised of fibers which are long and thin results in tumors, whereas short, thick fibers and particles are less tumorigenic. We hypothesized that fibrous morphology also might be important in the induction of proliferation and squamous metaplasia by asbestos. To test this, several different fibrous materials and their nonfibrous analogues were examined for their ability to induce these alterations in organ cultures of hamster trachea. We used chrysotile and crocidolite, the 2 types of asbestos of greatest commercial importance. Attapulgite, a naturally occurring fibrous clay mineral, and 2 forms of fiberglass also were examined.

MATERIALS AND METHODS

Preparation of Minerals. The sources of materials tested in these studies are listed in Table 1. Fibrous materials were prepared as follows. β-Fiberglass yarn was cut to varying lengths using a Sorvall TC-2 tissue sectioner. Code-100 fiberglass wool was ground gently in a tissue homogenizer since the random orientation of fibers made cutting ineffective. Attapulgite, crocidolite, and "long" and "short" chrysotile (Tables 2 and 3) were used without further modification. Samples of the minerals antigorite and riebeckite were prepared from rocks selected because of their mineralogical purity, and glass particles were produced by fusing code-100 fiberglass at 750°. These latter materials were ground in a ball mill for 15 min, yielding powders with heterogeneous size distributions. The large particles (>5 μm in diameter) then were separated by aqueous sedimentation (27). The suspensions of minerals were dried, sterilized (125° for 16 hr), and stored in powdered form.

Materials were dispersed in HBSS[3] (Grand Island Biological Co., Grand Island, N. Y.) by a 2-min bath sonication (Model B-22-4 Branson Ultrasonic Cleaner; Branson Cleaning Equipment Co., Shelton, Conn.) before addition to organ cultures. Chrysotile was not sonicated because this procedure alters the size of the fibers (25).

Characterization of Minerals. The mineralogical purity, surface charge, and size distributions of the particles were characterized. We used X-ray diffraction to assess mineralogical purity (Table 1). The electrophoretic mobility, an indication of net surface charge (22), was measured after suspending the particles in MEM (Grand Island Biological Co.) and the zeta potential (22) was assessed after dispersal of selected fibers in distilled H_2O (Table 1). Zeta potential and electrophoretic mobility are similar; however, the latter is applicable when particles are suspended in solutions containing organic compounds. When suspended in MEM, particles had no detectable net surface charge.

Size distributions were assessed using SEM (Tables 2 and 3). Suspensions of each material (10 μg/ml) were collected on Nucleopore filters (Nucleopore Filter, Pleasanton, Calif.) by pressure filtration. Regions from each filter, which appeared to represent the normal distribution of fibers, were photographed at magnifications ranging from ×100 to ×10,000. Photographs of adjacent regions were assembled as a montage to allow measurement of long fibers. A planimeter was used to calculate the length of long, curly fibers of chrysotile, and diameters of nonfibrous particles were determined by the technique of Cadle (5). Eight hundred fibers were counted, and particle size distributions were compiled after each experiment to standardize results.

Tracheal Organ Culture. The procedures used to prepare and maintain organ cultures of hamster trachea have been described previously (16). Briefly, random bred male Syrian hamsters between 6 and 10 weeks of age were sacrificed by an i.p. injection of 0.1 ml euthanasia solution (Taylor Pharmacal Co., Decatur, Ill.). Each trachea was isolated aseptically, and the adherent connective tissue was removed by dissection. The trachea was divided longitudinally at the cartilagenous discon-

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[3] The abbreviations used are: HBSS, Hanks' balanced salt solution; MEM, Eagle's minimal essential medium; SEM, scanning electron microscopy.

tinuity and then dissected into 14 to 16 explants measuring approximately 1.5 x 2.5 mm.

Experimental Design. The study was carried out as 14 separate experiments because of procedural considerations. In each, 2 materials and the appropriate controls were evaluated. Explants from 16 hamsters were divided randomly into groups and then transferred to 60- x 15-mm Petri dishes (approximately 36 to 40/dish). Suspensions of each material in HBSS (1.0, 4.0, and 16.0 mg/ml; 2 ml total volume) were added to the Petri dish and allowed to deposit onto the mucosal surface of organ cultures for 1 hr. The amounts of minerals used in our studies are substantially higher than those causing biological effects in monolayers of cells. However, the mucociliary clearance of tracheal organ cultures is very efficient, and only a small fraction of the original amount remains on the surfaces of explants after 1 hr. We chose the dosages above on the basis of previous experiments using crocidolite which indicated that a 4.0-mg/ml dose was most effective in inducing metaplasia (18). For each type of dust, 12 explants were assessed at each concentration. All experiments were repeated twice.

Table 1

Source and net surface charge of materials

Fibrous (A) and nonfibrous (B) analogues	Source	Net surface[a] charge
A. Crocidolite asbestos	Union Internationale Contre Cancer	0 (−42.0 ± 3.2)[b]
B. Riebeckite[c]	Wards Natural Science Est., Rochester, N. Y. (sample of fluor-riebeckite from El Paso county, Co.)	0
A. Chrysotile asbestos		
"Long" fibers "Short" fibers	Manville Corp., Denver, Co. (samples from the Jeffrey Mine in Quebec)	0 (+49.4 ± 2.1)
B. Antigorite[d]	Wards Scientific Est. (sample from Arizona)	0
A. *Fiberglass*		
β-fiber	Owens Corning Fiberglas, Toledo, Ohio	NA[e]
Code-100 fiber	Manville Corp.	0 (−48.6 ± 4.1)
B. Glass particles	Prepared from samples of code-100 fiberglass	0
A. Attapulgite	Clay Mineral Society, Columbia, Mo. (sample from Nevada)	0

[a] Electrophoretic mobility (22) of particles which were suspended in MEM.
[b] Numbers in parentheses, zeta potential (22) determinations of fibers in distilled H_2O ± S.E.
[c] Sample of riebeckite contained <1% fibers (aspect ratio >3).
[d] Antigorite contained <3% fibers and small amounts of the minerals picrolite and lizardite. No contamination was detected in samples of the other minerals.
[e] NA, not applicable. β Fiberglass rapidly settled from suspension due to the large diameter of fibers. This precluded accurate determinations of zeta potential.

After experimental treatments, 4 explants were transferred to 30- x 15-mm plastic Petri dishes (Costar, Cambridge, Mass.) the surface of which had been scored to facilitate attachment. We added 0.5 ml of MEM containing 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer (Sigma Chemical Co., St. Louis, Mo.), gentamicin (100 μg/ml), and nystatin (25 units/ml). This volume of medium was sufficient to wet but not submerge the mucosal surface of the explant. Cultures were maintained at 35–36° in an humidified atmosphere containing 5% CO_2, and the medium was changed every 3 days. Explants maintain normal mucociliary differentiation for at least 4 weeks under these conditions (16). Representative explants were examined at intervals of 2, 4, and 6 weeks after exposure to particles.

Assessment of Metaplasia. The extent of squamous metaplasia was evaluated by SEM. Explants were rinsed twice in HBSS to remove adherent mucin and were placed in modified Karnovsky's fixative for 12 hr. The specimens were dehydrated in ethanol, critical-point dried, sputter-coated with gold-palladium, and examined in a JEOL JSM 350 SEM.

The method used to grade the extent of the epithelium on the explants exhibiting squamous metaplasia has been described previously (32). An image of the entire mucosal surface was centered on the viewing screen of the SEM at a 0° tilt. A rectangular area (0.138sq mm, which comprised approximately 5% of the mucosal surface) in the center of each specimen then was defined after increasing the magnification 10-fold. The central region of the explant was analyzed since artifactual metaplastic changes usually develop at the cut margin (presumably due to traumatization of the tissue during preparation).

The percentage of mucosal surface within this area which showed either squamous differentiation or cytotoxic changes (cell necrosis and sloughing) was measured by placing a sheet of clear plastic over the viewing screen and outlining the distribution of lesions. The areas demarcated in this way were then converted to numerical values using a graphics tablet (Apple Computer, Inc., Cupertino, Calif.) and image analysis software (Optomax, Inc., Hollis, N. H.). Squamous cells were distinguished by their polygonal configuration and large diameter (>15

Table 2

Cumulative frequency distribution of fiber length

	% of particles at following fiber length[a]										
Test material	≤1 μm	≤5 μm	≤10 μm	≤20 μm	≤30 μm	≤40 μm	≤50 μm	≤100 μm	≤200 μm	≤300 μm	≤500 μm
Attapulgite	94	100									
"Short" chrysotile	59	100									
Crocidolite	21	69	87	95	98	99	100				
Code-100 fiberglass	2	31	54	78	87	92	94	100			
"Long" chrysotile	0	0	31	43	50	56	60	84	98	100	
β-Fiberglass	0	0	1	5	10	16	20	43	77	90	100

[a] Values indicate cumulative percentage of particles equal to or less than a given size.

Table 3

Cumulative frequency distribution of fiber/particle diameter

	% of particles at following fiber/particle diameter[a]								
Test material	≤0.2 μm	≤0.4 μm	≤0.6 μm	≤0.8 μm	≤1.0 μm	≤2.0 μm	≤3.0 μm	≤4.0 μm	≤5.0 μm
"Short" chrysotile	89	99	100						
Attapulgite	1	4	25	60	100				
"Long" chrysotile	65	82	90	93	96	98	99	100	
Antigorite	47	71	85	93	99	99	100		
Crocidolite	64	83	89		94	98	100		
Code-100	50	73	83	89	94	98	100		
Glass powder	7	20	33		51	91	98	99	100
Riebeckite	14	27		47	57	88	95	97	100
β-fiberglass	0	0	0	0	0	0	3	97	100

[a] Values indicate the cumulative percentage of particles equal to or less than a given size.

μm). Stratified squamous metaplasia was conspicuous because the superficial cells "heaped up" to form mounds. However, we did not attempt to distinguish simple squamous metaplasia from stratified squamous metaplasia by SEM.

Autoradiography. Explants were labeled for 5 hr with [^{3}H]thymidine (20 μCi/ml; specific activity, 49 Ci/mmol; Amersham-Searle Corp., Arlington Heights, Ill.) and then rinsed twice in HBSS at 37° before fixation. Using this protocol, approximately 0.5 to 2.0% of the tracheal epithelial cells are labeled in MEM after 2 weeks in culture (16). After examination by SEM, selected specimens were processed for light microscopy (2). Tissues were rehydrated and embedded in Paraplast (American Scientific Products, Bedford, Mass.), and sections of 5-μm thickness were cut. Sections mounted on glass slides were dipped in Kodak NTB immulsion (Eastman Kodak Co., Rochester, N. Y.) and then exposed at 4° for 1 week. Autoradiographs were developed in Kodak D-19 and stained with Harris hematoxylin.

One hundred epithelial cells (basal and suprabasal) on each side of the approximate center of the explant in 4 sections (a total of 800 epithelial cells per specimen) were counted to determine the labeling index (18).

RESULTS

Untreated explants exhibit normal mucociliary differentiation for 4 weeks *in vitro* although small foci of metaplasia occasionally develop over this period. In contrast, explants exposed to asbestos and other fibrous materials underwent both proliferative and metaplastic alterations. Analysis of variance and Fisher's least significant difference procedures were used to compare experimental groups (15). Crocidolite and fiberglass induced significant increases ($p < 0.05$) in the proportion of the mucosa involved by squamous metaplasia after both 2 and 4 weeks in culture (Chart 1). Metaplastic changes were most pronounced after 4 weeks whereas degeneration of the epithelium, characterized by cell sloughing and/or a spindly epithelium of one-cell thickness, often was evident after 6 weeks. Both the "long" and "short" fibers of chrysotile asbestos induced a significant increase in metaplasia at low dosages (1.0 and 4.0 mg/ml, respectively). Exposure to attapulgite resulted in similar effect although the increase was not statistically significant. High concentrations (16.0 mg/ml) of "long" chrysotile were markedly cytotoxic as determined by desquamation and necrotic alterations in epithelial cells.

The labeling index of epithelial cells was increased significantly in cultures exposed to both crocidolite asbestos and fiberglass for 2 weeks (Chart 2). Attapulgite and "long" chrysotile also caused increases in the labeling index, although the changes were not statistically significant. No change was observed after exposure for 2 weeks to "short" chrysotile. After 4 weeks in culture, labeling indices were low, and experimental groups did not differ from controls. The decrease in labeling of epithelial cells after extended periods *in vitro* has been observed previously in this system (16).

Nonfibrous analogues (riebeckite, antigorite, and glass particles) failed to induce significant increases in both the labeling index and extent of squamous metaplasia over a range of dosages and durations of exposure.

The pattern of deposition of materials on the mucosal surface was studied by SEM to determine the association of fibers with metaplastic lesions. Most fibers aggregated at the margins of the explant, although small numbers of individual fibers also were distributed randomly on the mucosal surface. These fibers either rested on nonciliated cells (Fig. 1) or protruded into the mucosal surface where they often were encompassed by accumulations of epithelial cells (Figs. 2 to 5). Metaplastic foci were usually

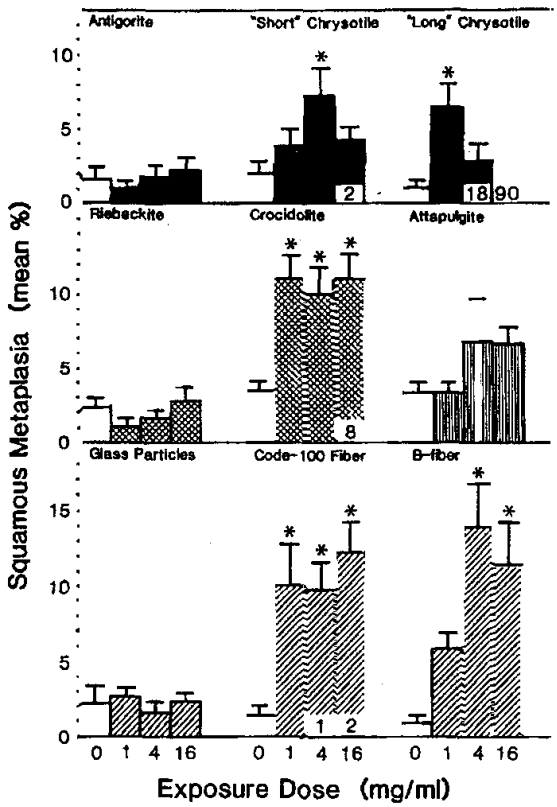

Chart 1. Development of squamous metaplasia 4 weeks after exposure of tracheal organ cultures to fibrous and nonfibrous materials. The percentage of squamous metaplasia represents the area of the mucosa showing squamous change divided by the total area examined. *, responses which are statistically different ($p < 0.05$) than the corresponding nonexposed controls (□); *bars*, S.E.; the number of observations varied between 19 and 26. *numbers* within *columns*, percentage of mucosal surface with cytotoxic alterations (*i.e.*, loss of epithelium).

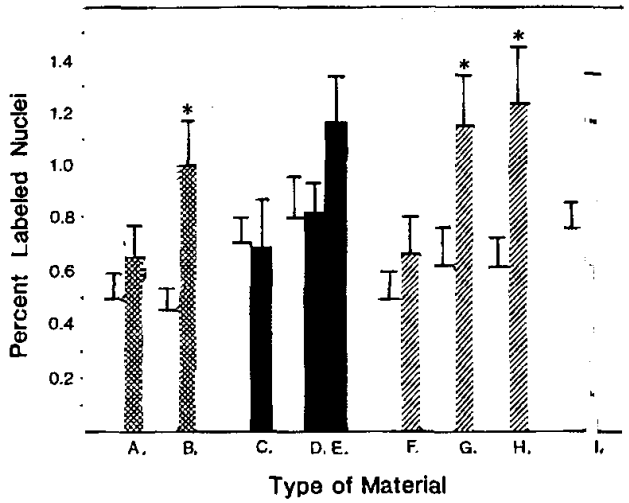

Chart 2. Percentage of epithelial cells with labeled nuclei on explants of hamster trachea 2 weeks after exposure to fibrous and nonfibrous materials. The explants were exposed to (*A*) riebeckite, (*B*) crocidolite, (*C*) antigorite, (*D*) "short" chrysotile, (*E*) "long" chrysotile, (*F*) glass particles, (*G*) β fiberglass, (*H*) code-100 fiberglass, and (*I*) attapulgite at 4.0 mg/ml medium. *, responses which were significantly different than the corresponding nonexposed controls (□); *bars*, S.E.; number of observations varied between 19 and 26.

small (<50 μm in diameter), but occasionally larger expanses of the mucosa were affected (approximate diameter > 300 μm; Fig. 5). Although many metaplastic lesions developed in association with fibers, foci also were located at sites where deposits of fibers could not be found.

As noted above, "long" fibers of chrysotile (in contrast to the other minerals) induced cell distortion and sloughing. These fibers often formed aggregates which were associated with clusters of necrotic cells (Fig. 6). Many fibers became coated with material having a smooth, bead-like appearance, suggesting deposition of mucin or proteinaceous material (Fig. 7). Cytotoxicity of naturally occurring asbestos has been described by others in a variety of cell types (11, 19).

The surface of explants treated with nonfibrous materials was relatively free of particles. When present, this material accumulated into aggregations which were scattered sparingly over the mucosal surface.

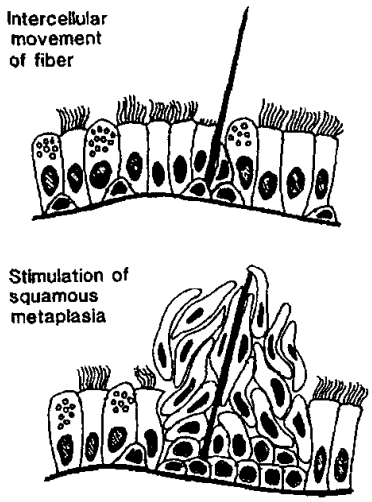

Chart 3. Hypothetical mechanism by which long fibers stimulate squamous metaplasia. Fibers occurred between cells of the mucociliary epithelium and appeared to interact with the underlying basal cells. This process causes injury and compensatory hyperplasia of adjacent basal cells (19). Alternatively, the long fibers which interact with the mucosal surface might serve as an artificial substrate which stimulates the outward migration of epithelial cells. Subsequently, these cells divide and differentiate into squamous cells.

DISCUSSION

Asbestos and cigarette smoke act synergistically in the development of bronchogenic carcinoma (9, 24). The stimulation of squamous metaplasia by asbestos might predispose epithelial cells to transformation by carcinogens in cigarette smoke. The studies recorded here were designed to investigate the role of particle geometry in the induction of squamous metaplasia by various materials. Fibers of differing physicochemical composition induced hyperplasia and metaplasia in cultured tracheal explants. Their nonfibrous analogues do not.

Hyperplastic and metaplastic lesions are found in the airways of rats after the inhalation of asbestos (4, 31). Therefore, our observations using cultured tissues are consistent with findings in animals.

The importance of fiber morphology in the induction of bronchogenic carcinoma is unclear, but fibrous shape is critical in the experimental production of both mesotheliomas (26) and pulmonary fibrosis (33). Intrapleural innoculation of materials comprised of long, thin fibers results in mesotheliomas, whereas short, blocky fibers are less tumorigenic (26). In the studies of Stanton *et al.* (26), maximum carcinogenic activity was observed with fibers <0.25 μm in diameter and >8 μm in length; however, we documented the metaplastic capability of β fiberglass, a preparation containing fibers >3 μm in diameter. Thus, fiber length in comparison to diameter seems more important in eliciting squamous metaplasia. Phagocytosis and subsequent elimination of short fibers might explain biological inactivity (13, 26, 33), but there has been no satisfactory explanation for the carcinogenicity of long fibers in the pleural and peritoneal cavity. Whether or not long fibers of types other than asbestos are carcinogenic in tracheobronchial epithelium is unproven in animals because of the difficulty in generating sized preparations of fibers for inhalation studies.

Our morphological observations suggest a hypothetical model by which long fibers stimulate squamous metaplasia (Chart 3). We have found that fibers occur between cells of the mucociliary epithelium where they appear to interact with the underlying basal cells (19, 32). This process is accompanied by necrosis of superficial epithelial cells (19). Although no definitive evidence exists to explain how fibers stimulate squamous metaplasia, the process probably involves more than compensatory changes resulting from cell death. For example, "long" chrysotile was the most cytotoxic material, but both crocidolite asbestos and fiberglass induced more extensive squamous metaplasia.

The nature of the substrate upon which cells rest influences their type of differentiation (8). Long fibers of glass provide a unique substrate for attachment of cultured fibroblasts and also act as a stimulus to promote cell division (13). Epithelial cells which interact with fibers might respond similarly. The failure of nonfibrous materials to stimulate metaplasia could reflect their rapid elimination from the mucosa by either phagocytosis or mucociliary clearance.

Squamous metaplasia in the human respiratory tract is topographically associated with squamous cell carcinoma (29). It might predispose the respiratory epithelial cells to malignant transformation by carcinogens in cigarette smoke. The rapid proliferation of cells which accompanies metaplasia (12) can enhance DNA damage by chemical carcinogens (14). Alternatively, chronic hyperplasia might promote neoplastic progression by previously initiated cells. Squamous differentiation also results in loss of protective mucociliary function, possibly leading to both the accumulation and prolonged interaction of carcinogens with epithelial cells (32). Cigarette smoking also causes squamous metaplasia (1) and modifies the deposition and clearance of inhaled particles (23). Hence, both substances could interact to increase retention of chemical carcinogens in the respiratory tract (6).

Because occupational exposure to asbestos is a recognized health hazard, a variety of nonasbestiform fibers recently have been promoted as substitutes (3, 21). These include both naturally occurring fibrous minerals (*i.e.*, attapulgite) and a number of man-made mineral fibers (*i.e.*, fiberglass). Although these materials are used widely, their biological effects are incompletely understood (7, 10, 20, 26, 30). Our results suggest that the ability of inorganic particulates to induce squamous metaplasia depends upon their fibrous geometry and not on any particular chemical or structural property unique to asbestos. Thus, other fibrous materials have the potential to induce hyperplasia and

metaplastic alterations in respiratory epithelium. Our results should be useful in predicting the potential of specific inorganic particulates to induce proliferative and metaplastic changes in epithelial cells.

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REFERENCES

1. Auerbach, O., Stout, A. P., Hammond, E. C., and Garfinkel, L. Changes in bronchial epithelium in relation to cigarette smoking and in relation to lung cancer. N. Engl. J. Med., *265:* 253–267, 1961.
2. Ayres, A., Allen, J. M., and Williams, A. E. A method of obtaining conventional histological sections from specimens after examination by scanning electron microscopy. J. Microsc. (Oxf.), *93:* 247–250, 1971.
3. Background information on substitutes for asbestos, Environmental Protection Agency, Office of Toxic Substances. Washington, D. C.: Government Printing Office, 1980.
4. Botham, S. K., and Holt, P. F. The effects of inhaled crocidolites from Transvaal and northwest cape mines on the lungs of rats and guinea pigs. Br. J. Exp. Pathol., *53:* 612–620, 1972.
5. Cadle, R. D. Particle Size: Theory and Industrial Applications, pp. 1–38. New York: Reinhold, 1965.
6. Craighead, J. E., and Mossman, B. T. The pathogenesis of asbestos-associated diseases. N. Engl. J. Med., *306:* 1446–1455, 1982.
7. Davis, J. M. G. The biological effects of mineral fibers. Ann. Occup. Hyg., *24:* 227–234, 1981.
8. Gospodarowicz, D. The extracellular matrix and the control of cell proliferation. *In:* J. B. Martin, S. Reichlin, and K. L. Bick (ed.), Neurosecretion and Brain Peptides, pp. 243–261. New York: Raven Press, 1981.
9. Hammond, E. C., Selikoff, I. J., and Seidman, H. Asbestos exposure, cigarette smoking, and death rates. Ann. N. Y. Acad. Sci., *330:* 473–490, 1979.
10. Harington, J. S. Fiber carcinogenesis: epidemiologic observations and the Stanton hypothesis. J. Natl. Cancer Inst., *67:* 977–988, 1981.
11. Harington, J. S., Allison, A. C., and Badami, B. V. Mineral fibers: chemical, physicochemical, and biological properties. Adv. Pharmacol. Chemother., *12:* 291–402, 1975.
12. Harris, C. C., Silverman, T., Smith, J. M., Jackson, F., and Boren, H. G. Proliferation of tracheal epithelial cells in normal and vitamin A deficient Syrian golden hamsters. J. Natl. Cancer Inst., *51:* 1059–1062, 1973.
13. Maroudas, N. G., O'Neill, C. H., and Stanton, M. F. Fibroblast anchorage in carcinogenesis by fibers. Lancet, *1:* 807–809, 1973.
14. Marquardt, H. Cell cycle dependence of chemically induced malignant transformation *in vitro*. Cancer Res., *34:* 1612–1615, 1974.
15. Montgomery, D. Design and Analysis of Experiments. New York: John Wiley & Sons, 1976.
16. Mossman, B. T., and Craighead, J. E. Long-term maintenance of differentiated respiratory epithelium in organ culture. I. Medium composition. Proc. Soc. Exp. Biol. Med., *149:* 227–233, 1975.
17. Mossman, B. T., and Craighead, J. E. Mechanisms of asbestos carcinogenesis. Environ. Res., *25:* 269–280, 1981.
18. Mossman, B. T., Craighead, J. E., and MacPherson, B. V. Asbestos-induced epithelial changes in organ cultures of hamster trachea: inhibition of retinyl methyl ether. Science (Wash. D. C.), *207:* 311–313, 1980.
19. Mossman, B. T., Kessler, J. B., Ley, B. W., and Craighead, J. E. Interaction of crocidolite asbestos with hamster respiratory mucosa in organ culture. Lab. Invest., *36:* 131–139, 1977.
20. Occupational exposure to fibrous glass-proceedings of a symposium. United States Department of Health, Education, and Welfare, Washington, D. C.: Government Printing Office, 1976.
21. Pye, A. M. Alternatives to asbestos in industrial applications. *In:* L. Michaels and S. S. Chissick (eds.), Asbestos: Properties, Applications, and Hazards, Vol. 1, pp. 339–373. New York: John Wiley & Sons, 1979.
22. Riddick, T. M. Zeta Meter Manual, pp. 43–73. New York: Zeta Meter, Inc., 1961.
23. Sanchis, J., Dolovich, M., Chalmers, R., and Newhouse, M. T. Regional deposition and lung clearance mechanisms in smokers and nonsmokers. *In:* W. H. Walton (ed.), Inhaled Particles III, Vol. 1, pp. 183–191. Surrey, England: Unwin Brothers Limited, 1971.
24. Saracci, R. Asbestos and lung cancer: an analysis of the epidemiological evidence on the asbestos-smoking interaction. Int. J. Cancer, *20:* 323–331, 1977.
25. Spurny, K. R., Opiela, H., and Weiss, G. On the milling and ultrasonic treatment of fibers for biological and analytical applications. *In:* J. C. Wagner (ed.), IARC Scientific Publication No. 30, Vol. 2, pp. 931–933. Lyon, France: International Agency for Research on Cancer, 1980.
26. Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M., Morgan, E., and Smith, A. Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. J. Natl. Cancer Inst., *67:* 965–975, 1981.
27. Tanner, C. B., and Jackson, M. L. Nomographs of sedimentation times for soil particles under gravity or centrifugal acceleration. Soil Sci. Soc. Am. Proc., *12:* 60–65, 1947.
28. Topping, D. C., and Nettesheim, P. Promotion-like enhancement of tracheal carcinogenesis in rats by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res., *40:* 4352–4355, 1980.
29. Valentine, E. H. Squamous metaplasia of the bronchus. A study of metaplastic changes occurring in the epithelium of the major bronchi in cancerous and noncancerous cases. Cancer (Phila.), *10:* 272–279, 1957.
30. Wagner, J. C., Berry, G., and Pooley, F. D. Carcinogenesis and mineral fibers. Br. Med. Bull., *36:* 53–56, 1980.
31. Wagner, J. C., Berry, G., Skidmore, J. W., and Timbrell, V. The effects of the inhalation of asbestos in rats. Br. J. Cancer, *29:* 252–269, 1974.
32. Woodworth, C. D., Mossman, B. T., and Craighead, J. E. Squamous metaplasia of the respiratory tract: possible pathogenic role in asbestos-associated bronchogenic carcinoma. Lab. Invest., *48:* 578–584, 1983.
33. Wright, G. W., and Kuschner, M. The influences of varying lengths of glass and asbestos fibers on tissue response in guinea pigs. *In:* W. H. Walton (ed.), Inhaled Particles IV, Vol. 2, pp. 455–474. New York: Pergamon Press, 1977.

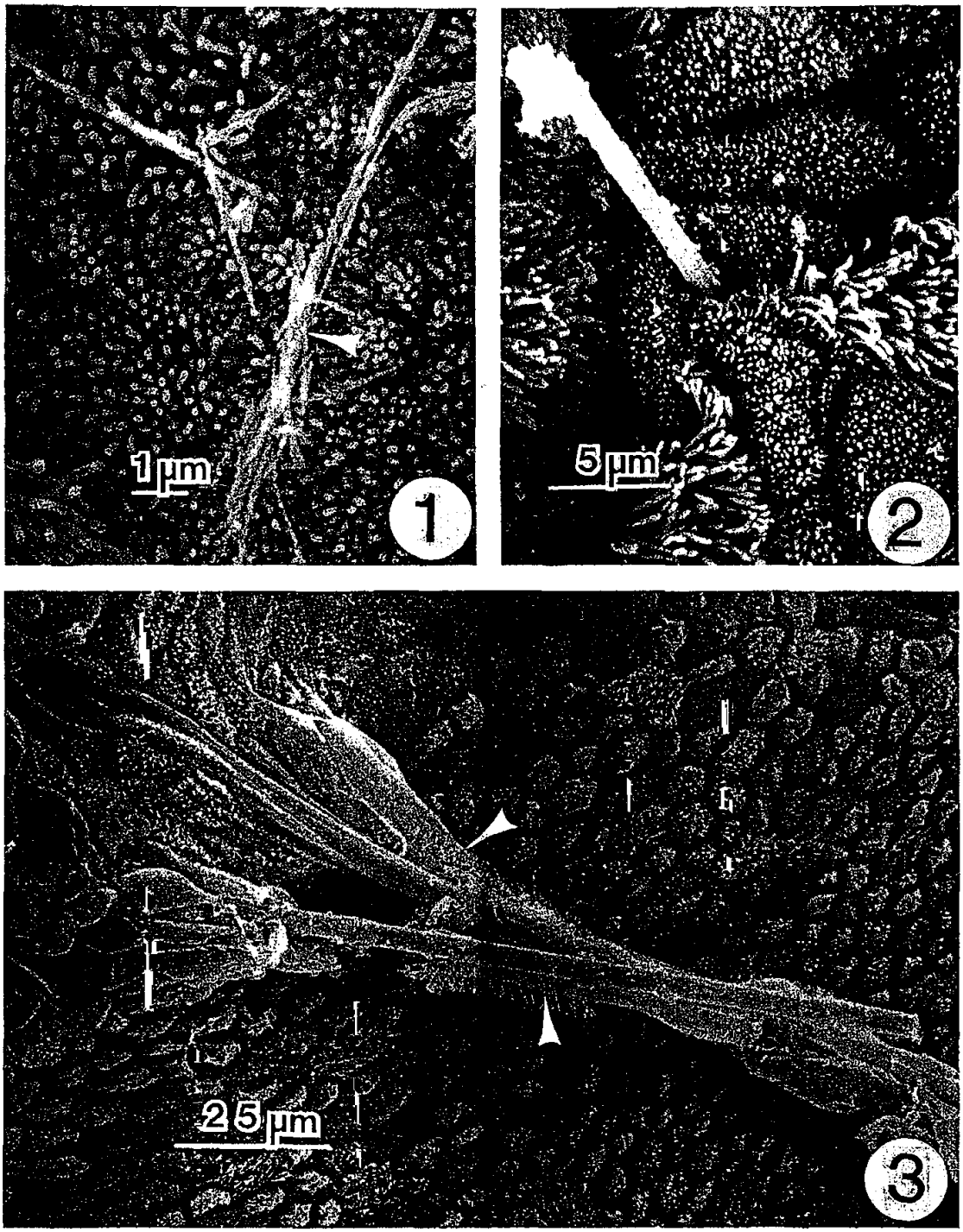
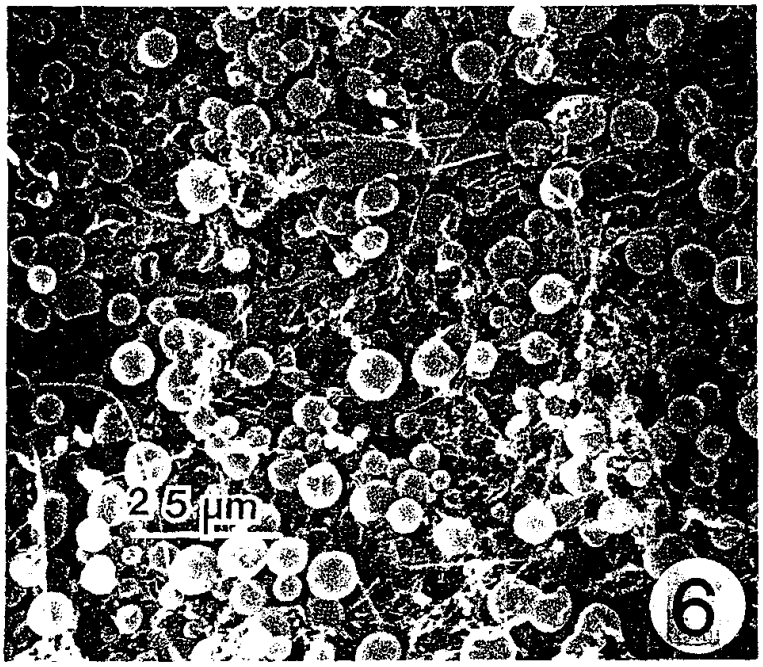


Fig. 1. Clusters of "short" fibers of chrysotile asbestos on the mucosal surface. After deposition, "short" fibers commonly form agglomerations which often resemble "long" fibers (*i.e.*, 10 µm long, *arrowhead*). Gold-palladium, × 8000.

Fig. 2. "Long" fiber of crocidolite asbestos protruding from the intercellular space between cells of the mucociliary epithelium. At 2 weeks after exposure, each type of fibrous material tested (asbestos, fiberglass, and attapulgite) often could be observed embedded similarly in the mucosa. Mechanisms involved in the uptake of such fibrous minerals have been considered previously by the authors (19, 32). Gold-palladium, × 4300.

Fig. 3. Large fibers of glass on the epithelial surface. Note how mucosal cells migrate to encompass these fibers. Cells appear to adhere to the fibers (*arrowheads*) and flatten out over their surfaces. The response of the tissue to crocidolite asbestos is similar. Gold-palladium, × 1000.



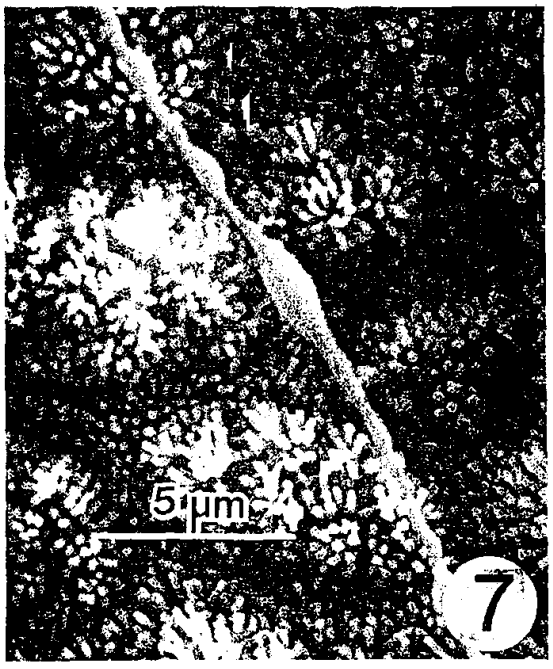


Fig. 4. An intercellular fiber of glass (*arrowhead*) which is associated with a small foci of metaplasia. This lesion is elevated above the surrounding epithelium and consists of cells which appear squamoid. Epithelial cells also phagocytize (*arrow*) short fibers of glass. Gold-palladium, × 1700.

Fig. 5. Large excrescence of epithelial cells on the mucosal surface of an explant exposed to code-100 fiberglass for 4 weeks. This lesion consists of numerous large, squamous-like cells. Many cells appear to encompass "long" fibers of glass (*arrowhead*). We believe these lesions may evolve by a process as described diagramatically in Chart 3. Fibers which interact with the mucosal surface in this manner might serve as an artificial substrate, stimulating the outward migration of epithelial cells. Subsequently, these cells divide and differentiate into squamous cells. Gold-palladium, × 450.

Fig. 6. Typical cluster of cells, apparently necrotic, associated with "long" fibers of chrysotile 2 weeks after deposition. These fibers are quite cytotoxic to the tracheal mucosa. Gold-palladium, × 850.

Fig. 7. Single "long" fiber of chrysotile, sitting atop the mucosal surface and coated with material demonstrating a "bead-like" appearance. This material might be mucus or protein deposits. Gold-palladium, × 6800.

INFLUENCE OF CRYSTALLIZATION HABIT OF MINERALS ON *IN VITRO* CYTOTOXICITY*

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The health hazards caused by exposure to commercial asbestos particles are well known. The question, however, still remains whether the nonasbestiform equivalents of these minerals to which people may be exposed in mining, quarrying, and other activities can also have adverse effects on human health. Identification of minerals in various crystallization habits and their interactions with biological systems is pertinent. Unfortunately, direct study of the physical, chemical, and biological properties of all minerals and their varieties would be an extremely expensive and time-consuming project. A more practical approach is to define the physical and chemical properties of minerals and their varieties in different categories and test them simultaneously in selected biological systems. It is conceivable that extrapolations can be made to relate specific mineralogical properties to specific biological activities.

Several attempts have been made by other investigators to relate physical and/or chemical properties of minerals with biological properties. While some studies revealed that hemolysis of mammalian erythrocyte is related to mineral surface area,[1,2] others have shown that it depends on surface charge,[3,4] and yet others have indicated a relationship to magnesium content.[1,5,6] In an earlier study, we demonstrated that still another mineral characteristic—namely, asbestiform crystallization habit—is responsible for sheep erythrocyte hemolysis and rabbit alveolar macrophage cytotoxicity.[7]

The study is designed in an attempt to define the roles of physical and chemical characteristics in the biological system. Four samples of cummingtonite-grunerite series in different crystallization habits are selected. In preparation for biological studies, these minerals are ground to obtain samples of various particle sizes. All samples are characterized for chemical composition, size distribution, and surface charge. Biological activity of the samples is assessed in terms of cell lysis of sheep erythrocytes and cytotoxicity to Chinese hamster ovary cells.

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Materials and Methods

Sample Selection

Asbestiform Grunerite

This is the classical South African asbestiform grunerite; the actual sample used is the UICC amosite standard.

Acicular Grunerite

This sample is obtained from Smallwood Mine (Wabush, Labrador, Canada) and is composed of parallel acicular crystals. The color of the semitranslucent crystals is yellowish green and their luster is subadamantine and silky.

Semiasbestiform Cummingtonite

This sample is obtained from Homestake Mine (South Dakota, U.S.A.). The crystals display a definitely fibrous appearance, but are less conspicuously asbestiform than amosite. The color of the semitranslucent crystals is greenish brown and their luster is adamantine and pearly, with some portions showing the silky luster of amosite. The length of the crystals ranges from a few millimeters to almost 10 cm; the width appears to be submicroscopic. The fibers can be separated by a needle with some difficulty (that is, not as easily as amosite fibers). The fibers are stiff in larger dimensions and become flexible as the size of the fibers is decreased. None of the fibers show the characteristic silky shine and high flexibility of amosite.

The term "semiasbestiform" is introduced to express this sample's intermediate asbestiform characteristics. Unfortunately, no quantitative expression is available at present to express the degree of asbestiform development of amphibole fibers. All that can be stated at this point is that the development of asbestos properties in this sample is somewhere between the lack of asbestos properties of the acicular variety and the highly developed asbestos properties of amosite.

Acicular Cummingtonite

This sample is obtained from Homestake Mine (South Dakota, U.S.A.) and is composed of radiating acicular crystals. The color of the opaque-to-semitranslucent crystals is similar to the semiasbestiform crystals but darker, and the luster is more dull than that of the semiasbestiform fibers. The length of the crystals ranges from 0.5 to 10 mm; the width is 0.1 mm or less. Most of the crystals are stiff and brittle; some, however, demonstrate a small degree of flexibility. These latter crystals are removed from the sample when noticed; however some are probably missed in this crude visual process. The sample is expected to have a small degree of asbestiform character.

Sample Analysis

Chemical Analysis

Electron probe microanalysis is performed with a MAC Model 5 probe. Approximately 10 mg of sample are mounted in the epoxy medium and the sample is viewed

optically in the instrument at a beam size of 5 μm in diameter. Silicate and oxide standards are used in calibration of the probe. The instrument is computerized and matrix corrections are made using the Bence-Albee recursive method. Approximately six analyses are made for several particles ranging from 10 to 20 μm in diameter. The average of these point analyses is generally within ± 5 percent, where there is no chemical zoning.

Measurement of Surface Area

Surface area is determined by the BET adsorption isotherm method using N_2 as the adsorbent. Determinations are made in an all-glass vacuum system operating at a pressure of less than 2×10^{-6} torr. An oxygen vapor thermometer is used to measure directly the partial pressure of nitrogen in the liquid nitrogen bath and hence that equilibrium value inside the sample bulb. This method gives a more direct, accurate value than would be obtained with a thermocouple.

Measurement of Zeta Potential

The surface charge of gross dispersion of the minerals in buffered solution is measured using a microelectrophoresis instrument. The instrument cell attached to the microscope is filled with 25 ml of freshly prepared suspension of particles. This, in turn, completes a circuit giving a potential difference of about 150 V across the cell. Measurements are by means of a null technique (stopping the "cloud" of particles which appear to move across the viewing field), rather than the commonly used timing of transit of an individual grain across a known distance.

Assessment of Hemolysis of Sheep Erythrocytes

The sheep blood is obtained in Elsevier's solution commercially. Before use, the erythrocytes are washed three times in veronal-buffered saline and a standard 2 percent suspension is prepared. As a standard reference, a lysate is prepared by adding 1 ml of 2 percent erythrocyte suspension to 3 ml of water. To standardize the Varian spectrophotometer, the lysate is read at 540 mM. Proper adjustments are made if the lysate absorbance is not between 0.700 and 0.750.

The test samples at a known concentration are added to a test tube containing 3 ml of Veronal-buffered saline and incubated at 37°C for 10 minutes and then 2 percent erythrocyte suspension is added. The tubes are then incubated at 37°C for 50 minutes. After this period, the tubes are centrifuged at 1500 rpm and the supernatant is read on the Varian spectrophotometer at 540 mM and percent hemolysis is calculated as follows:

$$\frac{\text{Optical Density of Sample}}{\text{Optical Density of Lysate}} \times 100 = \% \text{ Hemolysis}$$

Each sample is checked for surface adsorption of hemoglobin. Four ml of lysate are added to a known amount of test sample and incubated for 50 minutes at 37°C. The tubes are then centrifuged and the supernatant is read on the Varian spectrophotometer at 540 mM and percent adsorption is determined as follows:

$$\frac{\text{Optical Density of Lysate} - \text{Optical Density of Sample}}{\text{Optical Density of Lysate}} = \% \text{ Adsorption}$$

If a particulate sample is found to adsorb hemoglobin, the values of percent adsorption and hemoglobin are added to obtain actual hemolysis.

Assessment of Cytotoxicity to Chinese Hamster Ovary (CHO) Cells

The CHO cell line is obtained from the American Type Cell Collection. The cells are maintained in F-12 medium supplemented with 10 percent fetal calf serum, 100 units penicillin/ml and 100 μg/ml streptomycin. The cultures are incubated at 37°C and gassed with 5 percent CO_2 in air.

The cultures are seeded at 500 cells/25-mm² Corning flask in 4 ml of the nutrient medium and incubated at 37°C for 24 hours for attachment. After this period, a known amount of test sample is added to the cultures and incubated for 6 days; during this time, the cells divide and form separate colonies. The medium is then removed, and a mixture of 0.5 percent NaCl and 4 percent methanol in 10 percent formalin is added to fix the colonies. The colonies are stained with 0.04 percent crystal violet and counted (using a colony counter). The number of colonies is determined as a percent of control (cells without test particles).

Results

Hemolysis

Chemical analysis revealed that all minerals are iron-rich silicates (Table 1). The Fe/Mg ratios of 80/20, 90/10, 70/30, and 70/30 confirmed that the first two samples, UICC amosite and Labrador grunerite, are indeed grunerites and that the other two samples, semiasbestiform and acicular cummingtonite, are truly cummingtonites.

A comparison of hemolysis caused by these samples is indicated in Table 2. The degree of hemolysis caused by the asbestiform variety (UICC amosite) was much higher than that caused by other varieties of comparable surface area. Asbestiform particles with a surface area of 4.13 m²/g caused 53.3 percent hemolysis, whereas

Table 1

Electron Probe Microanalysis

Elements wt. % Oxide	Cummingtonite–Grunerite			
	Asbestiform Grunerite %	Acicular Grunerite %	Semiasbestiform Cummingtonite %	Acicular Cummingtonite %
SiO_2	43.13	48.34	52.36	52.77
FeO	29.95	43.93	33.76	34.02
MgO	3.50	3.04	8.10	8.16
Na_2O	0.21	0.03	—	0.40
K_2O	1.87	0.05	—	—
CaO	0.24	0.31	0.94	0.95
MnO	1.04	0.70	0.45	0.45
TiO_2	0.27	0.03	—	—
Al_2O_3	5.43	0.36	1.54	1.55
Fe/Mg	80/20	90/10	70/30	70/30

TABLE 2

HEMOLYSIS CAUSED BY CUMMINGTONITE-GRUNERITE MINERALS

Mineral	Hemolysis at 20 mg/ml %	Surface Area m^2/gm	Surface Charge mV
Asbestiform			
Grunerite	53.3 ± 4.5	4.13	−26.9 ± 3.1
(UICC Amosite)			
Semiasbestiform	32.48 ± 7.69	3.88	−22.5 ± 2.2
Cummingtonite	31.8 ± 7.19	1.61	
	32.3 ± 4.78	1.21	
Acicular	29.57 ± 7.43	3.76	
Cummingtonite	31.4 ± 4.2	2.45	−15.7 ± 2.4
	18.94 ± 2.52	.82	
Acicular	40.0 ± 7.1		
Grunerite	32.48 ± 4.6		
	15.83 ± 2.0		−26.2 ± 4.0
	10.0 ± 1.0		
	0	2.82	

acicular particles with a surface area of 2.82 m^2/g caused no hemolysis. The in-between samples, semiasbestiform and acicular cummingtonite, were found to be less hemolytic than the asbestiform grunerite but more hemolytic than the acicular grunerite. Semiasbestiform cummingtonite samples of surface area 3.88 m^2/g caused 32.48 percent hemolysis, whereas acicular cummingtonite samples of surface area 3.76 m^2/g caused 29.57 percent hemolysis. Although of different crystallization habit, these two samples did not exhibit any difference in hemolytic activity. The semiasbestiform samples of surface area 3.88 m^2/g and 1.61 m^2/g caused 32.48 percent and 31.8 percent hemolysis, respectively. The acicular samples of surface area 3.76 m^2/g and 2.45 m^2/g caused 29.57 percent and 31.4 percent hemolysis, respectively. At a lower surface area, however, the samples showed a significant difference in hemolysis. Semiasbestiform variety of surface area 1.21 m^2/g caused 32.2 percent hemolysis, whereas acicular samples of surface area 0.82 m^2/g caused only 18.94 percent hemolysis.

A relationship between the degree of hemolysis and particle size of the samples was demonstrated with acicular grunerite samples of a variety of size distribution. Although the sample of 2.82 m^2/g surface area caused no hemolysis, when the sample was ground further, 10 percent hemolysis resulted. Samples with increasingly finer particles caused 15.83 percent, 32.48 percent, and 40 percent hemolysis, respectively. Unfortunately, no surface area values were determined for this sample at this point.

In these data, no relationship between surface charge and degree of hemolysis is apparent. The surface charges of the asbestiform and acicular grunerite were found to be identical, yet their hemolytic activities were extremely different. On the other hand, the semiasbestiform and acicular cummingtonite had different surface charges but exhibited similar hemolytic activities.

Cytotoxicity

Cytotoxicity to CHO was determined by counting the numbers of clones surviving after exposure to test substances as compared to the number of clones in the untreated

cultures. Asbestiform grunerite of surface area 4.13 m^2/g (FIGURE 1) was found to be the most toxic. At the dose of 0.05 mg/ml, less than 25 percent of the clones survived. At higher doses of 0.1 and 0.2 mg/ml, only 5 percent and 1 percent of the clones survived, respectively. Acicular grunerite of comparable surface area 2.82 m^2/g (Sample A, FIGURE 2) was nontoxic even at 0.50 mg/ml, a ten-times-higher dose. Acicular grunerite, when ground further (Sample B, FIGURE 3), was also nontoxic at the dose of 0.50 mg/ml. Samples with still finer particles (Sample C, FIGURE 4) were slightly toxic at 0.40 and 0.50 mg/ml, showing only 75 percent and 45 percent survival, respectively. In the samples with the most fine particles (Sample D, FIGURE 5), toxicity was observed at the 0.20 mg/ml dose. Seventy-five percent of the clones survived at 0.20 mg/ml, 55 percent at 0.30, 40 percent at 0.40, and 35 percent at 0.50 mg/ml. No significant toxicity was observed at lower concentrations of 0.05 and 0.10 mg/ml.

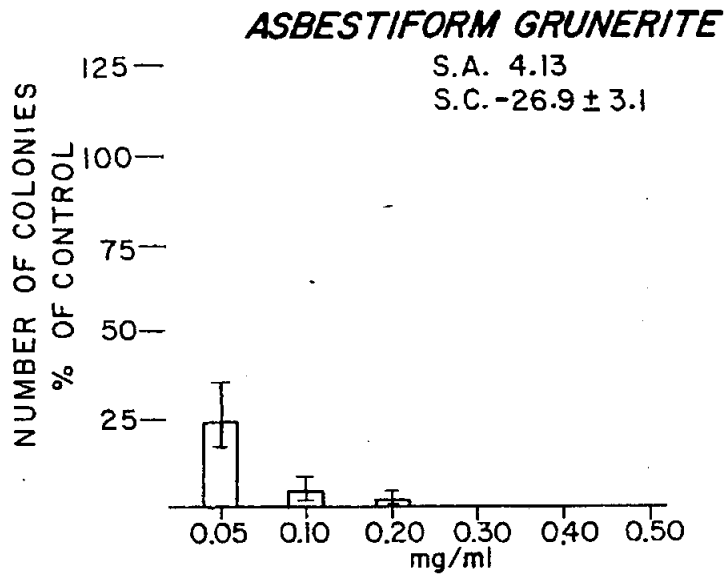

FIGURE 1. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of asbestiform grunerite, UICC amosite, (S.A. 4.13 m^2/g).

The levels of cytotoxicity caused by semiasbestiform and acicular varieties of cummingtonite were compared. Semiasbestiform of surface area 3.88 m^2/g was relatively more toxic than the acicular variety of comparable surface area 3.76 m^2/g (FIGURES 6 and 7). At the doses of 0.05 and 0.10 mg/ml, 85 percent and 70 percent of the clones survived, respectively, when exposed to the semiasbestiform variety. No toxicity was observed at these doses with exposure to the acicular variety. At higher concentrations, no significant difference in cytotoxicity was observed; however, in both instances the cytotoxicity levels were dose-dependent. Similar levels of cytotoxicity were caused by the semiasbestiform samples of surface areas 1.61 m^2/g and 3.88 m^2/g (FIGURES 8 and 6). The acicular variety of surface area 2.45 m^2/g (FIGURE 9) was relatively less toxic, however. No toxicity was obvious up to 0.30 mg/ml. At 0.40 and

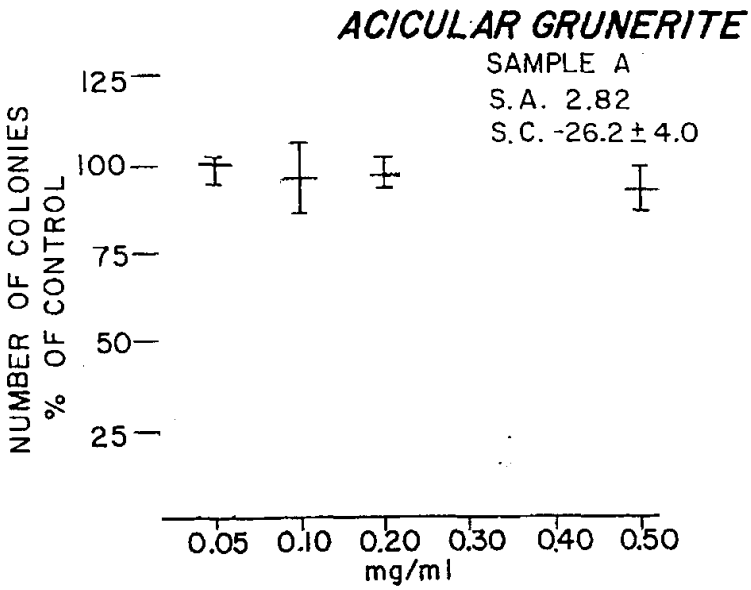

FIGURE 2. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of acicular grunerite, (S.A. 2.82 m^2/g, Sample A).

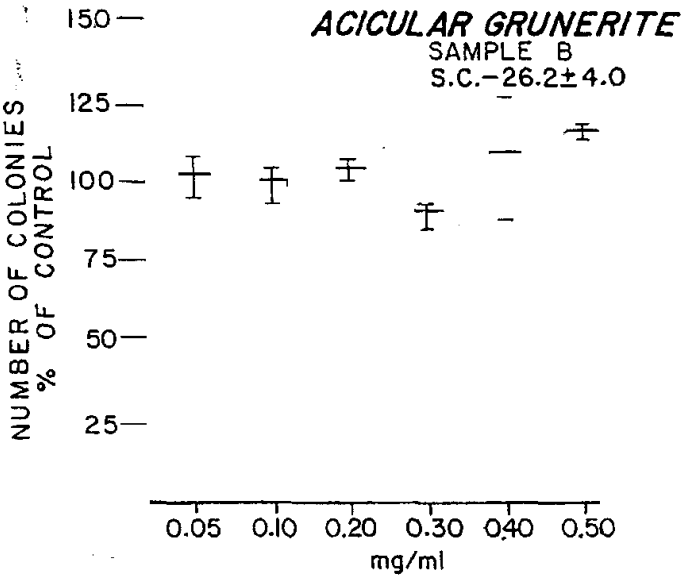

FIGURE 3. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of acicular grunerite (Sample B).

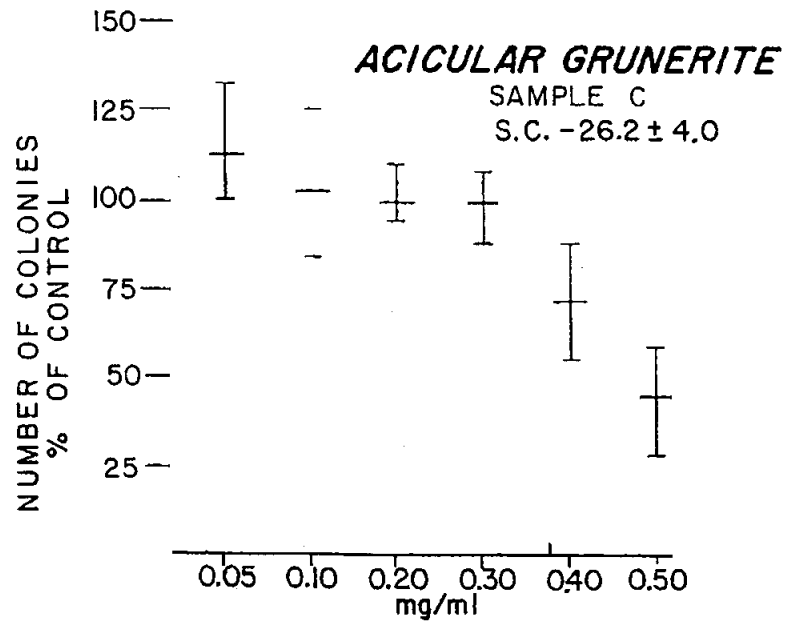

FIGURE 4. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of acicular grunerite (Sample C).

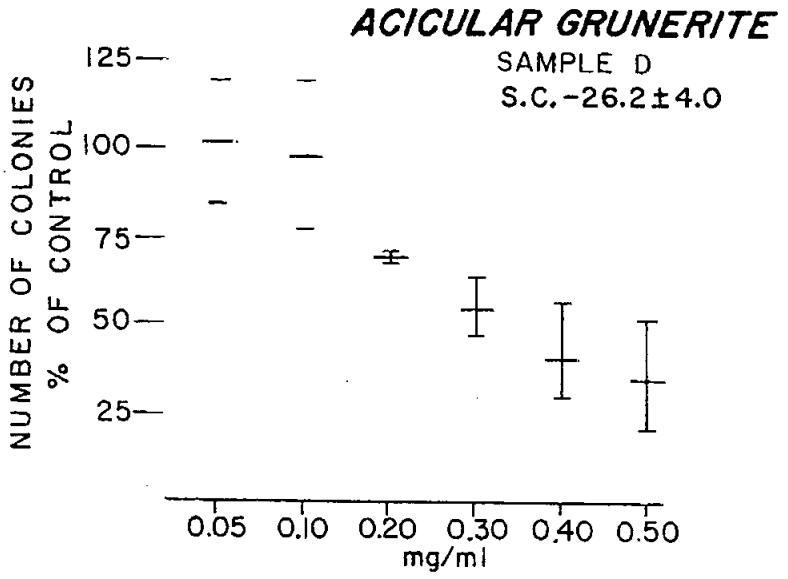

FIGURE 5. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of acicular grunerite (Sample D.)

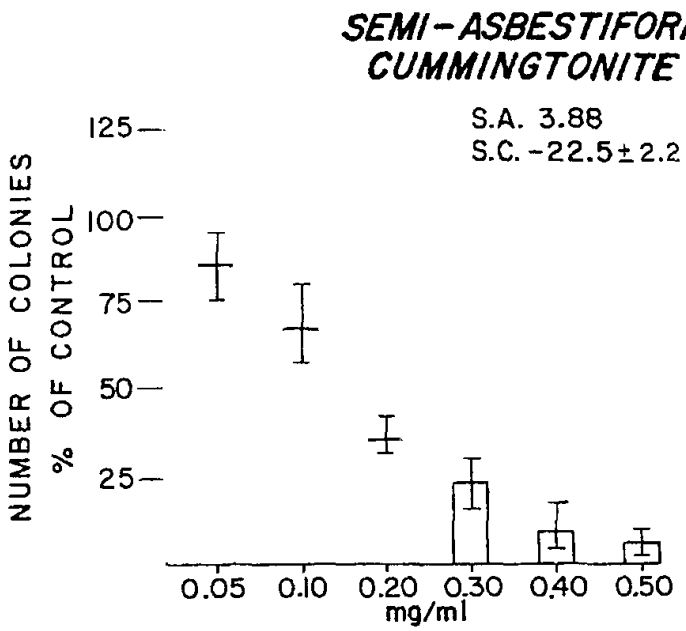

FIGURE 6. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of semiasbestiform cummingtonite, (S.A. 3.88 m^2/g).

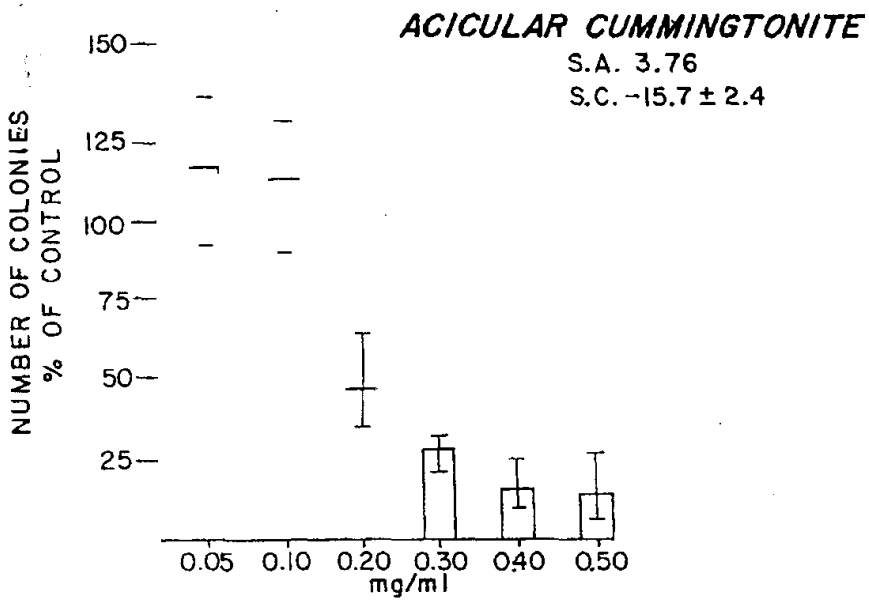

FIGURE 7. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of acicular cummingtonite (S.A. 3.76 m^2/g).

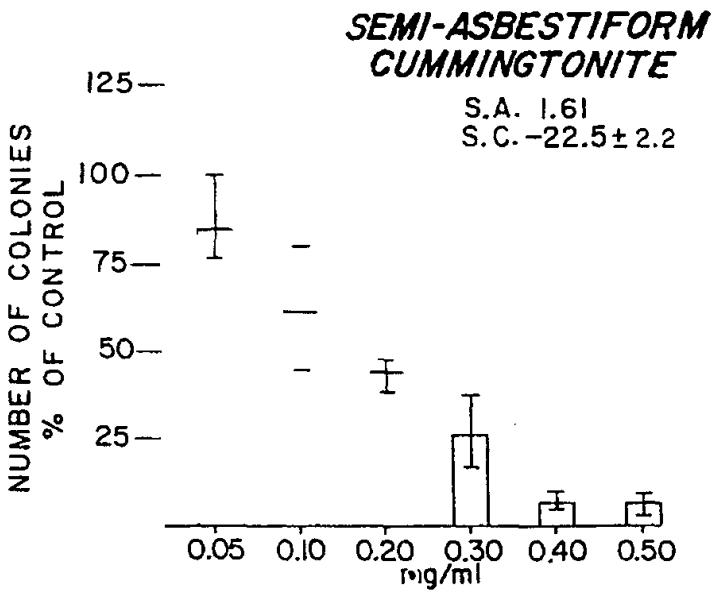

FIGURE 8. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of semiasbestiform cummingtonite (S.A. 1.61 m^2/g).

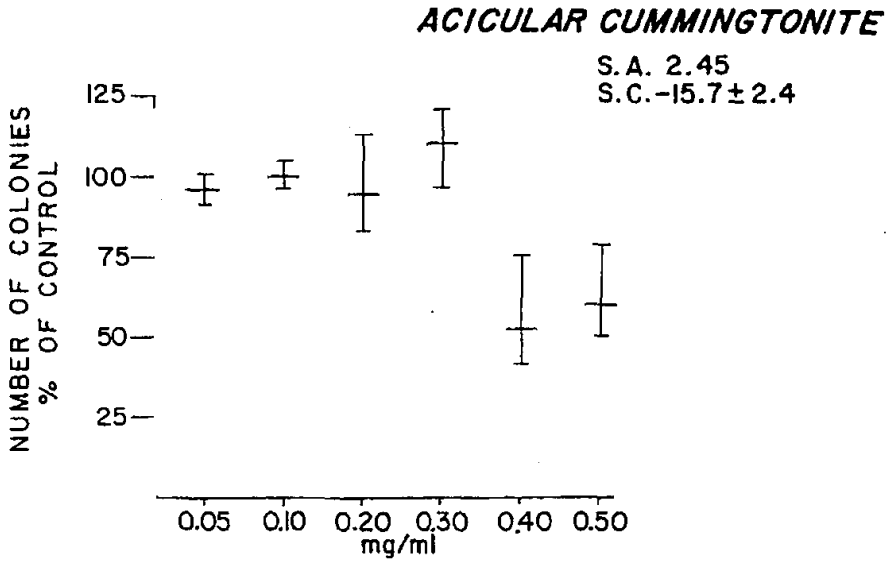

FIGURE 9. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of acicular cummingtonite (S.A. 2.45 m^2/g).

0.50 mg/ml, more than 50 percent of the clones survived. The semiasbestiform sample of surface area 1.21 (FIGURE 10) was slightly less toxic than the two other samples (surface areas 3.88 and 1.61 m^2/g). The acicular cummingtonite of surface area 0.82 m^2/g (FIGURE 11) was nontoxic even at a high dose of 0.5 mg/ml.

The relationship between surface area and toxicity was not quite obvious in the semiasbestiform samples. The cytotoxicity caused by acicular cummingtonite, however, was found to be related to surface area. Samples of higher surface area were more toxic than those of lower surface area.

In this study, there was no correlation between surface charge and cytotoxicity to CHO cells. Asbestiform grunerite and acicular grunerite with similar surface charges (−26.9 and −26.2 mV, respectively) caused cytotoxicity of extreme values. The

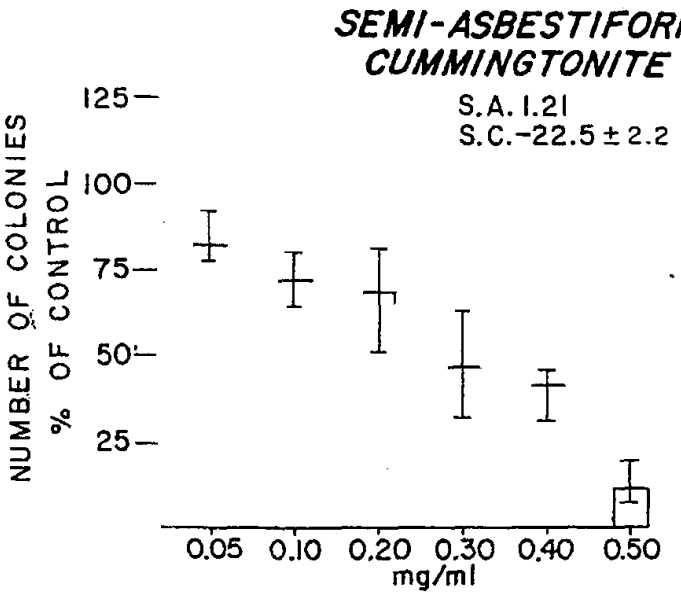

FIGURE 10. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of semiasbestiform cummingtonite (S.A. 1.21 m^2/g).

semiasbestiform and acicular cummingtonite were of different surface charge; the cytotoxicity caused by these samples was, however, found to be similar.

DISCUSSION

The asbestiform development of chain silicates is a continuous process depending on the earth's cooling history, temperature, pressure, presence of water, and other physical conditions. Since these conditions may be different in different geological regions, it is quite common to find silicates of similar chemical series in various developmental stages of crystallization habits. Some chain silicates may consist entirely of perfect long fibers with high tensile strength, some may consist of acicular,

noticeably weaker and brittle crystals, and yet others may consist of a mixture of high-tensile strength and acicular crystals with variable structural faults and surface defects.[8]

The chain silicate asbestos types exploited for commercial use are of the first category (perfect, long fibers of high-tensile strength). Epidemiological studies have clearly revealed that exposure to commercial asbestos is hazardous to human health. *In vitro* cytotoxicity studies and hemolysis studies have also shown that commercial asbestos is cytotoxic and hemolytic. In this study, we investigated the cytotoxicity and hemolytic activity of four samples of cummingtonite-grunerite series in four different crystallization habits.

The complexity of interaction of minerals with biological systems has been suggested by several investigators. The significance of surface area in hemolysis was

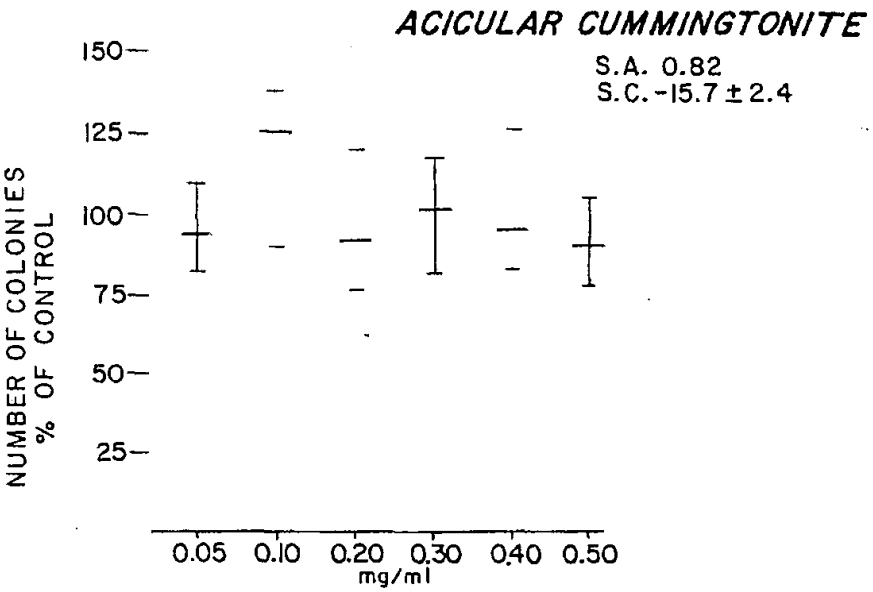

FIGURE 11. Surviving CHO clones (expressed as percent of untreated controls) following exposure to various doses of acicular cummingtonite (S.A. 0.82 m^2/g).

indicated by Harrington *et al.*,[1] who reported that the degree of hemolysis caused by several UICC asbestos samples was proportional to surface area. Light and Wei,[3,4] on the other hand, demonstrated that, at a dose compensated to provide an equal amount of surface area, the hemolysis caused by UICC asbestos samples was proportional to surface charge. The importance of Mg ions has also been investigated. When Mg was removed from UICC chrysotile by acid wash, it became inert,[1,5,6] pointing out the role of Mg in hemolysis; however, the investigators were unable to document this relationship with other UICC amphiboles. For these reasons, our samples were carefully characterized for surface area, surface charge, and chemical content. A careful evaluation was undertaken to determine whether there is indeed a relationship between the two biological systems studied and the crystallization habits.

The initial comparision between the four samples of different crystallization habits

was made at comparable surface areas of 4.13, 3.88, 3.76, and 2.82 m^2/g of UICC amosite, semiasbestiform cummingtonite, acicular cummingtonite, and acicular grunerite, respectively. The data indicate that hemolysis of sheep erythrocytes and cytotoxicity to CHO cells was inversely proportional to the degree of development of asbestos character in the minerals. Amosite from South Africa with high-tensile strength and practically no surface defects was most hemolytic and cytotoxic. The other three samples (semiasbestiform cummingtonite, acicular cummingtonite, and acicular grunerite) with limited or no asbestos character in increasing order were hemolytic and cytotoxic in decreasing order, the most nonasbestiform mineral being totally inert. The degree of hemolysis caused by UICC amosite is in agreement with the reports of other investigators.[1,2,5]

The relationship of hemolysis and cytotoxicity to particle size became clear when several samples of acicular grunerite were compared. Acicular grunerite of relatively low surface area (2.82 m^2/g) was totally inert; when the samples were then ground further, they were hemolytic and cytotoxic. This relationship was not quite apparent in the levels of hemolysis caused by semiasbestiform cummingtonite and acicular cummingtonite; it was, however, obvious in the cytotoxicity assay. Unfortunately, surface areas of the finer acicular grunerite samples were unavailable. Hence, conclusive correlation cannot be made between surface area and biological activities.

No relationship between surface charge and hemolysis (as well as cytotoxicity) was apparent. The complexicity of surface charge in biological systems has been indicated by Light and Wei.[3,4] Surface charge is known to vary according to pH, ionic strength, and the amounts of serum or surfactant present in the system. The zeta potentials for these studies were obtained in Veronal-buffered solution at pH 7.4, rather than in distilled water at pH 7.4. The surface charge value for UICC amosite in Veronal buffer was -26 mV as compared to -58.5 mV reported by other investigators.[3] However, since Veronal buffer was used for hemolysis assay, it seemed appropriate to use this system for determinations. Moreover, the hemolysis and cytotoxicity assays used in this investigation involved the presence of hemoglobin and serum, respectively. The fate of surface charge of the particles after being exposed to the sheep erythrocytes and CHO cells was not calculated. More work will be required to establish the role of surface charge in these biological systems.

Analysis of these data indicate that crystallization habit plays an important role in hemolysis and cytotoxicity. Although asbestiform minerals are known to be hazardous (and demand significant attention), other minerals of semiasbestiform and acicular variety should not be ignored. It seems possible that, at higher doses and surface areas, the nonasbestiform minerals could also be hazardous to human health. More epidemiological and experimental research will be required to fully understand minerals and their effects in biological systems.

Summary

Four samples of cummingtonite-grunerite series in various crystallization habits were tested *in vitro*. The cytotoxicity to Chinese hamster ovary cells and hemolysis to sheep erythrocytes was inversely proportional to the structural faults and surface defects of the minerals. At a comparable surface area, asbestiform grunerite (UICC amosite), semi-asbestiform cummingtonite, acicular cummingtonite, and acicular grunerite were found to be cytotoxic and hemolytic in a decreasing order. The influence of particle size on hemolysis and cytotoxicity was observed with acicular grunerite. Although samples of relatively large particle size were found to be inert, samples of smaller particle size were cytotoxic as well as hemolytic. No apparent

relationship between surface charge and hemolysis as well as cytotoxicity was observed.

ACKNOWLEDGMENTS

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REFERENCES

1. HARINGTON, J. S., K. MILLER & F. MACNAB. 1971. Hemolysis by asbestos. Env. Res. 4: 95–117.
2. SCHNITZER, R. J. & F. L. PUNDSACK. 1970. Asbestos hemolysis. Env. Res. 3: 1–13.
3. LIGHT, W. G. & E. T. WEI. 1977. Surface charge and asbestos toxicity. Nature 265:537–539.
4. LIGHT, W. G. & E. T. WEI. 1976. Surface charge and hemolytic activity of asbestos. Envtl. Res. 13:135–145.
5. DESAI, R., P. HEXT & R. RICHARDS. 1975. The prevention of asbestos-induced hemolysis. Life Sci. 16:1931–1938.
6. HARINGTON, J. S., A. C. ALLISON & D. V. BADAMI. 1975. Mineral fibers: chemical, physicochemical and biological properties. Advan. Pharmacol. Chemother 12:291–402.
7. COFFIN, D. L. & L. D. PALEKAR. 1977. EPA study of biological effects of asbestos-like mineral fibers. Natl. Bur. Std. Spec. Pub.
8. ZOLTAI, T. 1979. Asbestiform and acicular mineral fragments. Ann. N.Y. Acad. Sci. This volume.

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MORTALITY FROM LUNG CANCER AND RESPIRATORY DISEASE AMONG POTTERY WORKERS EXPOSED TO SILICA AND TALC[1]

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Thomas, T. L. (Environmental Epidemiology Branch, NCI, Bethesda, MD 20892) and P. A. Stewart. Mortality from lung cancer and respiratory disease among pottery workers exposed to silica and talc. *Am J Epidemiol* 1987;125:35–43.

A cohort mortality study of white men employed for at least one year between 1939 and 1966 at three plants of a single United States company was conducted to evaluate the risk of lung cancer and nonmalignant respiratory disease among workers exposed to silica dust and nonfibrous (nonasbestiform) talc in the manufacture of ceramic plumbing fixtures. Follow-up of 2,055 men through January 1, 1981, indicated a substantial excess of nonmalignant respiratory disease among those with high levels of exposure to silica dust (standardized mortality ratio = 2.26). The risk of nonmalignant respiratory disease rose with the number of years exposed, was not further enhanced by talc exposure, and appeared to be appreciably lower among those exposed in more recent time periods. For lung cancer, men exposed to high levels of silica dust with no talc exposure had a nonsignificant standardized mortality ratio of 1.37. However, those exposed to nonfibrous talc in addition to high levels of silica had a significant 2.5-fold excess risk of lung cancer. Among this group, the lung cancer standardized mortality ratio rose with increasing years of talc exposure to 3.64 among those exposed for 15 or more years. Although the role of silica as a cofactor cannot be ruled out, these data suggest that nonfibrous talc exposure is associated with excess lung cancer risk.

lung diseases; lung neoplasms; occupational diseases; silica; talc

Adverse health effects historically associated with employment in the pottery industry are toxicity from lead used in glazes (1) and silicosis and silicotuberculosis from silica found in clay (1–4). Recently, it has been suggested that silica dust might be a lung carcinogen, a cocarcinogen, or a promoter (5, 6). A proportionate mortality study of pottery workers in the United States (7) indicated an excess of deaths due to lung cancer, tuberculosis, and nonmalignant respiratory disease. While the excess of tuberculosis and nonmalignant respiratory disease occurred among workers in all subcategories of the industry, the lung cancer excess occurred exclusively among workers employed in the manufacture of ceramic plumbing fixtures. Silica is the major occupational exposure of these workers, but they may also be exposed to talc dust, pigments used in glazes, and other substances. A cohort mortality study was undertaken to further investigate the risk of nonmalignant respiratory disease, lung cancer, and tuberculosis among workers in the plumbing fixture industry.

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MATERIALS AND METHODS

Study population

Three plants of a single United States company producing ceramic plumbing fix-

tures were selected for study. The oldest of these plants, located in a small town in the midwestern United States, began production in about 1901. The second plant, located in an east coast urban area, was acquired by the company in 1929. The newest plant was built in 1957 in a rural area of the northeast.

Company personnel rosters and files were used to compile a list of all employees who had completed at least one year of employment with the company between January 1, 1939, and January 1, 1966. There is a slight overlap between this study group and the subjects in an earlier study by Thomas (7). The earlier study included all union members who died between 1955 and 1977 while they were actively employed or after they had retired, and therefore included some deceased employees of these three plants. Information abstracted from the service records of each employee included name, birth date, Social Security number, sex, race, dates employed by the company, and complete work history with the company. Work history information included the plant location, job titles, departments, and dates worked. Vital status of each study subject on January 1, 1981, was determined from company, Social Security Administration, credit bureau, and department of motor vehicles records. Death certificates for subjects known to be deceased were obtained from appropriate state vital records offices and were coded by a qualified nosologist according to the rules in effect at the time of death but using *International Classification of Diseases, Adapted,* Eighth Revision codes (8). Study subjects reported to have died after January 1, 1981, were counted as alive at the end of the study, and persons reported to be deceased for whom no death certificate was found were treated as vital status unknown. Nonwhite men ($n = 55$) and women ($n = 169$) were excluded from the statistical analyses due to their small numbers.

Industrial process and exposures

The industrial process in the manufacture of ceramic plumbing fixtures consists of mixing raw materials, casting liquid clay into molds, finishing, glazing, firing, inspecting, and packaging (table 1). Raw materials are mixed with water in the slip house to form a liquid slurry (slip). In the cast shop, casters dust plaster-of-paris molds with talc, pour slip into the molds, remove pieces from molds after they are set, and sponge and trim molded pieces. The pieces are held in a drying area for several hours prior to being sponged and sanded by finishers. The pieces are then sprayed with glaze before being loaded onto kiln cars and fired at high temperatures. The fired pieces are inspected, the hardware is affixed, and the finished pieces are packaged in cardboard boxes.

The predominant exposure of concern in this industry is quartz, a crystalline free silica. It is a major constituent of china clay, ball clay, feldspar, and flint used to make slip and is the primary exposure in numerous plant departments. In the production areas, silica becomes airborne when wet clay falls to the floor, dries, and forms a layer of dust, and when products are sanded and finished. Exposure to nonfibrous talc occurs almost exclusively in the cast shop. The Montana steatite talc, used to dust molds since about 1955, appears to contain no asbestiform fibers (9–11). Before 1955, flint and ground clay were used to dust molds. Glazes consist of raw materials similar to those in the slip, small amounts of talc, and various pigments. In the past, tremolitic (fibrous) talc was used in some glazes, but its use was discontinued in these plants in 1976.

No measurements of airborne silica or talc dust, current or historical, were available; however, the authors decided that a detailed knowledge of the industrial processes and job duties was sufficient for determining the exposure classifications used in this investigation. After conducting a walk-through survey of the plants, the industrial hygienist (P. A. S.) classified each job title-department combination listed in the work history of each of the study subjects according to its potential exposure to silica dust (none, low, or high). All jobs that

TABLE 1

Steps in the production of ceramic sanitary ware, 1940–1980

Process and description	Exposures
Slip making	High silica
Grind raw materials	
Mix with water	
Casting	High silica, nonfibrous talc (after 1955)
Dust molds	
Pour liquid clay into molds	
Release pieces from molds	
Sponge molded pieces and smooth rough areas	
Finishing	High silica
Dry pieces	
Sand and smooth dried pieces	
Spray glazing	High silica, fibrous talc (before 1976)
Spray dried pieces with glaze	
Firing	Low silica
Load glazed pieces onto kiln cars	
Send cars through kiln	
Inspecting	Low silica
Inspect fired pieces	
Packaging	Low silica
Attach hardware	
Package finished products	
Miscellaneous operations	
Glaze making	High silica, fibrous talc (before 1976)
Grind raw materials	
Mix with water	
Mold making	Plaster-of-paris, low silica
Make molds for large pieces	
Maintenance	High silica, fibrous talc (before 1976), nonfibrous talc (after 1955)
Maintain and repair equipment	
Clean production areas	
Clerical, administrative	No exposures
Office work	

involved exposure to talc had high silica exposure; thus, the jobs with high silica exposure were also classified according to whether there was exposure to no talc, nonfibrous talc, or fibrous talc. This was done without knowledge of the vital status of any study subject.

Statistical methods

Person-years at risk of fatal disease were accumulated for each study subject beginning with either January 1, 1940, or one year after the date of hire, whichever was more recent, and ending on the date he was last known to be alive. Observed numbers of deaths in the study group were totaled for specific causes. Corresponding expected numbers of deaths were calculated by multiplying cause-specific death rates for white men in the United States by person-years at risk in the study group with appropriate adjustments for age and calendar period (12). Standardized mortality ratios were calculated as the ratio of observed to expected deaths. Statistical significance of the standardized mortality ratios was determined at the 5 per cent level using a Mantel-Haenszel chi-square test with one degree of freedom (13). In the tables, standardized mortality ratios are shown in parentheses when both observed and expected deaths are less than five.

A hierarchy for exposure to silica and talc was created from the classifications assigned by the industrial hygienist. Jobs with no exposure to silica or talc were placed in the lowest category in the hierarchy, and jobs with low silica exposure

were placed second. The divisions of the high silica exposure category were rated in the hierarchy according to talc exposure, with no talc exposure as the lowest and fibrous talc exposure as the highest. Person-years at risk were accumulated in an exposure category until a study subject moved into a job in a higher category in the hierarchy or until he left the study. A subject did not contribute person-years to a classification lower in the hierarchy once he entered a higher exposure classification. Thus, the lowest category contained person-years during which a subject had never been exposed to silica or talc, while the highest category contained person-years that occurred only after a subject had ever been exposed to fibrous talc. In the analyses of lung cancer and nonmalignant respiratory disease by duration and latency of silica exposure, all subjects who had ever had a job with silica exposure were included regardless of their talc exposure.

Results

Vital status on January 1, 1981, was confirmed for 96 per cent of the cohort. Among the 2,055 white men in the study group, 1,394 (67.8 per cent) were known to be alive and 578 (28.1 per cent) were known to be deceased, while the remainder were unknown (2.3 per cent) or deceased without death certificate confirmation (1.7 per cent). Study subjects were followed for an average of 27.1 years, resulting in a total of 55,717 person-years at risk of fatal disease. More than 60 per cent were employed for 10 or more years.

The standardized mortality ratio for all causes of death was significantly less than 1.0 (table 2) because of significant deficits of infectious disease including tuberculosis, of digestive cancer, of other digestive disease, and of violent deaths. The number of deaths observed for all cancers was nearly the same as expected, but there was a significant excess of lung cancer deaths. The

TABLE 2

Mortality, 1940–1980, among white male pottery workers

Underlying cause of death (ICDA-8 codes)†	Observed deaths	Expected deaths‡	SMR§
All causes of death	578	645.1	0.90*
Infectious and parasitic disease (000–139)	5	12.9	0.39*
Tuberculosis (010–019)	3	7.9	0.38
Malignant neoplasms (140–209)	124	122.0	1.02
Digestive cancer (150–159)	19	36.5	0.52*
Lung cancer (162)	52	36.3	1.43*
Lymphatic and hematopoietic (200–209)	14	11.8	1.19
All other cancer	39	37.4	1.04
Ischemic heart disease (410–413)	231	222.1	1.04
Vascular lesions of the central nervous system (430–438)	34	45.7	0.74
Nonmaligant respiratory disease (460–519)	64	37.0	1.73*
Pneumonia (480–486)	16	14.3	1.12
Emphysema (492)	7	8.5	0.82
Other respiratory disease	41¶	14.1	2.90*
Digestive disease (520–577)	20	31.6	0.63*
Accidents, suicide, homicide (800–998)	33	64.2	0.51*
All other causes	67	109.6	0.61*

* Statistically significant at $\alpha = 0.05$.

† ICDA-8, *International Classification of Diseases, Adapted*, Eighth Revision.

‡ Expected deaths were calculated by multiplying cause-specific death rates for United States white men by person-years at risk in the study group with appropriate adjustments for age and calendar period.

§ SMR = standardized mortality ratio.

¶ Includes one case of influenza, one case of asthma, 23 cases of pneumoconiosis, eight cases of chronic interstitial pneumonia, and eight cases of "other respiratory disease."

magnitude of the standardized mortality ratio did not change significantly when lung cancer rates from the three counties in which the plants were located were used to calculate the expected number of deaths (expected = 38.0, standardized mortality ratio = 1.37). The standardized mortality ratio for nonmalignant respiratory disease was also significantly elevated. This finding can be attributed to a nearly threefold excess of nonmalignant respiratory disease deaths not classified as pneumonia or emphysema, which included 23 deaths nosologically classified as pneumoconiosis due to silica and silicates.

Table 3 shows mortality from lung cancer and nonmalignant respiratory disease by year of hire. Nonmalignant respiratory disease risk appeared to decrease over calendar time, while excess lung cancer mortality peaked among persons hired between 1940 and 1949. The latent period for persons hired in 1950 or later was probably not sufficient to observe whether a significant excess of lung cancer deaths occurred.

Excess risks of lung cancer and nonmalignant respiratory disease were observed exclusively among persons exposed to high levels of silica dust (table 4). Nonmalignant respiratory disease standardized mortality ratios were consistently elevated among workers with and without exposure to nonfibrous talc but not among those exposed to fibrous talc. Lung cancer mortality was slightly elevated among persons with exposure to fibrous talc and those with no talc exposure, but there was a significant 2.5-fold excess among workers with expo-

TABLE 3

Lung cancer and nonmalignant respiratory disease mortality, 1940–1980, among white male pottery workers by year of hire

Year of hire	Lung cancer			Nonmalignant respiratory disease		
	Observed deaths	Expected deaths†	SMR‡	Observed deaths	Expected deaths	SMR
<1940	23	19.7	1.17	55	24.5	2.24*
1940–1949	22	11.4	1.93*	7	9.0	0.78
1950–1965	7	5.3	1.33	2	3.4	(0.58)

* Statistically significant at $\alpha = 0.05$.

† Expected deaths were calculated by multiplying cause-specific death rates for white men in the United States by person-years at risk in the study group with appropriate adjustments for age and calendar period.

‡ SMR, standardized mortality ratio.

TABLE 4

Lung cancer and nonmalignant respiratory disease mortality, 1940–1980, among white male pottery workers by exposure category

Exposure category	Person-years†	Lung cancer			Nonmalignant respiratory disease		
		Observed deaths	Expected deaths‡	SMR§	Observed deaths	Expected deaths	SMR
No silica, no talc	2,452	1	1.6	(0.61)	1	1.8	(0.55)
Low silica, no talc	16,665	7	10.3	0.68	9	11.2	0.81
High silica	36,471	44	24.3	1.81*	54	23.9	2.26*
No talc	20,831	18	13.2	1.37	36	13.7	2.64*
Nonfibrous talc	12,369	21	8.3	2.54*	16	7.3	2.20*
Fibrous talc	3,271	5	2.9	1.74	2	3.0	(0.67)

* Statistically significant at $\alpha = 0.05$.

† Total excludes 129 person-years for jobs that could not be classified because job title or department were missing from the work record.

‡ Expected deaths were calculated by multiplying cause-specific death rates for white men in the United States by person-years at risk in the study group with appropriate adjustments for age and calendar period.

§ SMR, standardized mortality ratio.

sure to nonfibrous talc. All of the 21 lung cancer cases exposed to nonfibrous talc had worked as casters (expected = 7.7, standardized mortality ratio = 2.73, $p < 0.05$). Among casters who presumably had no exposure to talc, the number of observed lung cancer deaths was slightly greater than expected but not statistically significant (observed = 10, expected = 7.1, standardized mortality ratio = 1.46). One of the lung cancer deaths was attributed to the fibrous talc category because the study subject had worked as a maintenance man in the production area prior to 1976 for three months, but he was also exposed to nonfibrous talc for five years as a caster. None of the lung cancer deaths were attributed to malignant mesothelioma. In fact, only one death certificate listed malignant mesothelioma (without specification of site) as the underlying cause of death of the decedent. This subject had worked as a maintenance mechanic and had never had a production job while he was employed with the company.

Nonmalignant respiratory disease mortality increased by duration of exposure to silica and years since first silica exposure (table 5). The duration of exposure analyses were repeated by year of hire to determine whether the results seen in table 3 were confounded by the excess risk of nonmalignant respiratory disease among long-term employees. The magnitude of the standardized mortality ratio decreased over calendar time independent of the duration of exposure to silica. Lung cancer mortality did not increase by duration of exposure.

Mortality from lung cancer rose with increasing duration of exposure to nonfibrous talc (table 6). No lung cancer deaths occurred in the first five years after exposure, but mortality was significantly elevated after a latent period of five years. Mortality from nonmalignant respiratory disease was not affected by duration of exposure to talc.

Discussion

Silicosis was one of the earliest recognized occupational diseases and has been well-documented among miners, stonecutters, quarry workers, and other groups occupationally exposed to silica (14). Our cohort mortality study of workers in the production of ceramic plumbing fixtures indicated more than a twofold excess risk of nonmalignant respiratory disease among employees who had a job with high exposure to silica. The magnitude of the standardized mortality ratio increased both with duration of exposure to silica and with the length of time since first exposure. These findings are consistent with other studies of workers in the pottery industry and with studies linking nonmalignant respiratory disease risk with exposure to silica dust (1, 2, 4, 7, 14, 15). Risk of nonmalignant respiratory disease decreased over the calendar period independent of the duration of exposure, suggesting that efforts to control dust levels in these plants may have been successful in reducing the risk of nonmalignant respiratory disease.

Tuberculosis has often been diagnosed among workers exposed to silica dust as a complication of silicosis (15). It is thought that dust deposits in the lungs prevent defense mechanisms from stopping the growth of the tubercle bacteria, thereby making silicotics more susceptible than the population in general to the disease (15). Previous studies of workers in the pottery industry showed an elevated risk of tuberculosis (1, 3, 7, 16). In contrast, our study of workers in the ceramic plumbing fixtures industry showed a significant deficit of deaths from tuberculosis. Even among persons hired prior to 1940, there were fewer deaths from tuberculosis than were expected (standardized mortality ratio = 0.53).

The significant deficits of deaths due to infectious disease, digestive disease, violence, and "other causes" seen among pottery workers might be due at least in part to the "healthy worker effect," a phenomenon which causes the mortality experience of an employed population to appear more favorable than the population in general (17). General population mortality rates include deaths that occur among persons who

TABLE 5

Lung cancer and nonmalignant respiratory disease mortality, 1940–1980, among white male pottery workers ever exposed to silica by duration of silica exposure and years since first silica exposure

No. of years	Lung cancer						Nonmalignant respiratory disease					
	Duration of silica exposure			Years since first silica exposure			Duration of silica exposure			Years since first silica exposure		
	Observed deaths	Expected deaths†	SMR‡	Observed deaths	Expected deaths	SMR	Observed deaths	Expected deaths	SMR	Observed deaths	Expected deaths	SMR
<15	19	11.7	1.62	4	2.7	(1.46)	6	10.2	0.59	0	3.2	
15–29	19	11.3	1.68*	15	11.4	1.32	21	12.1	1.73*	13	9.9	1.31
30+	13	11.6	1.12	32	20.6	1.56*	36	12.8	2.81*	50	22.0	2.27*

* Statistically significant at $\alpha = 0.05$.

† Expected deaths were calculated by multiplying cause-specific death rates for white men in the United States by person-years at risk in the study group with appropriate adjustments for age and calendar period.

‡ SMR, standardized mortality ratio.

TABLE 6

Lung cancer and nonmalignant respiratory disease mortality, 1940–1980, among white male pottery workers by duration of nonfibrous talc exposure and years since first nonfibrous talc exposure

No. of years	Lung cancer						Nonmalignant respiratory disease					
	Duration of nonfibrous talc exposure			Years since first nonfibrous talc exposure			Duration of nonfibrous talc exposure			Years since first nonfibrous talc exposure		
	Observed deaths	Expected deaths†	SMR‡	Observed deaths	Expected deaths	SMR	Observed deaths	Expected deaths	SMR	Observed deaths	Expected deaths	SMR
<5	2	2.1	(0.95)	0	0.7		9	2.2	4.08*	0	0.7	
5–14	11	4.0	2.76*	8	2.9	2.81*	6	3.8	1.60	9	2.7	3.36*
15+	8	2.2	3.64*	13	4.7	2.75*	1	1.3	(0.75)	7	3.9	1.79

* Statistically significant at $\alpha = 0.05$.

† Expected deaths were calculated by multiplying cause-specific death rates for white men in the United States by person-years at risk in the study group with appropriate adjustments for age and calendar period.

‡ SMR, standardized mortality ratio.

never enter the work force or who leave early due to illness. This effect usually diminishes with age (17), and our data indicated that the deficits in total mortality occurred before the attained age of 60 years (observed deaths = 194, standardized mortality ratio = 0.72), while subjects who reached the age of 60 years had an overall mortality experience that was similar to that of the general population (observed deaths = 384, standardized mortality ratio = 1.04). There might also be other life-style differences between the study group and the general population that could affect mortality patterns (e.g., socioeconomic status, dietary habits, etc.).

Investigators have recently suggested that there is a relation between exposure to silica dust and excess lung cancer risk (5, 6). The excess mortality from lung cancer in our study occurred primarily among casters who had simultaneous occupational exposure to nonfibrous talc and silica; thus, the roles of the two agents cannot be entirely separated. If lung cancer and nonmalignant respiratory disease had a common causal factor, such as silica, both diseases might be expected to exhibit the same patterns; however, this did not occur. The risk of nonmalignant respiratory disease decreased over calendar time and may represent a reduction in exposure to silica, but a corresponding decrease in lung cancer was not seen. On the contrary, excess lung cancer risk occurred only among men hired after 1940. Nonmalignant respiratory disease was elevated among persons with exposure to high levels of silica dust regardless of nonfibrous talc exposure, while lung cancer was significantly elevated only among those who were simultaneously exposed to nonfibrous talc and silica. Risk of lung cancer increased with increasing duration of exposure to nonfibrous talc, but not with duration of silica exposure. The opposite effect was seen for nonmalignant respiratory disease risk. All of the workers exposed to nonfibrous talc who died from lung cancer had worked as casters and had had the greatest opportunity for exposure. Studies of steatite talc miners have also shown elevated risk of lung cancer (18–20), and the talc in two of the studies was known to contain no asbestos and very little quartz (19–21).

We had no information on smoking patterns in the cohort of pottery workers with which to examine possible confounding for lung cancer due to tobacco use; however, other smoking-related causes of death such as heart disease (observed deaths = 231, standardized mortality ratio = 1.04), cancers of the pancreas, kidney, and bladder (observed deaths = 13, standardized mortality ratio = 0.96), and emphysema (observed deaths = 7, standardized mortality ratio = 0.82) were not excessive. Furthermore, there were no emphysema deaths among casters who had exposure to nonfibrous talc; therefore, it is unlikely that our results were biased toward this particular occupational category due to smoking behavior.

In summary, our findings support the well-established association between silica dust exposure and nonmalignant respiratory disease risk but not tuberculosis risk. Findings suggest that there has been a decreasing risk of nonmalignant respiratory disease over calendar time in these plants, probably due to better dust control. The evidence suggests that exposure to nonfibrous talc is related to excess lung cancer risk; however, the role of silica as a cofactor or a promoting agent cannot be ruled out.

References

1. Flinn RH, Dreessen WC, Edwards TI, et al. Silicosis and lead poisoning among pottery workers. Public Health Bulletin no. 244, publication no. 1693. Washington, DC: US GPO, 1939.
2. Kolton R, Richter ED, Israeli R. Occupational exposure to dust containing free silica in a ceramics factory. Israel J Med Sci 1981;17:277–82.
3. Prowse K, Cavanagh P. Tuberculosis in the potteries 1971–74. Lancet 1976;2:357–9.
4. Office of Population Censuses and Surveys. Occupational mortality: the Registrar General's decennial supplement for England and Wales, 1970–72. Series DS no. 1. London: Her Majesty's Stationery Office, 1972.
5. Goldsmith DF, Guidotti TL, Johnston DR. Does occupational exposure to silica cause lung cancer?

Am J Ind Med 1982;3:423–40.
6. Goldsmith DF, Winn DM, Shy CM, eds. Silica, silicosis, and cancer: controversy in occupational medicine. Cancer Research Monographs, Vol 2. Philadelphia, PA: Praeger Press, 1986.
7. Thomas TL. A preliminary investigation of mortality among workers in the pottery industry. Int J Epidemiol 1982;11:175–80.
8. International Classification of Diseases, Eighth Revision. Adapted for use in the United States. US PHS publication no. 1693. Washington, DC: US GPO, 1967.
9. Minerals, pigments and metals division, Pfizer. Personal communication, 1985.
10. Gamble J, Greife A, Hancock J. An epidemiological-industrial hygiene study of talc workers. Ann Occup Hyg 1982;26:841–59.
11. Grexa, RW, Parmentier CJ. Cosmetic talc properties and specifications. Cosmetics Toiletries 1979;94:29–33.
12. Monson RR. Analysis of relative survival and proportional mortality. Comput Biomed Res 1974;7:325–32.
13. Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. JNCI 1959;22:719–48.
14. Jones RN. Silicosis. In: Rom WN, ed. Environmental and occupational medicine. Boston, MA: Little, Brown, and Company, 1983:197–205.
15. Worth G, Stahlmann W. Silicotuberculosis. In: Parmeggiani L, ed. Encyclopedia of occupational health and safety (third (revised) ed.) Geneva: International Labour Office, 1983:2041–3.
16. Guralnick L. Mortality by industry and cause of death among men 20 to 64 years of age: United States, 1950. (US DHEW Vital Statistics Special Reports, Vol 53, no. 4). Washington, DC: US GPO, 1963.
17. McMichael AJ. Standardized mortality ratios and the "healthy worker effect": scratching beneath the surface. J Occup Med 1976;18:165–8.
18. Kleinfeld M, Messite J, Zaki MH. Mortality experiences among talc workers: a follow-up study. J Occup Med 1974;16:345–9.
19. Selevan SG, Dement JM, Wagoner JK, et al. Mortality patterns among miners and millers of non-asbestiform talc. In: Lemen R, Dement JM, eds. Dust and disease. Park Forest South, IL: Pathotox Publishers Inc., 1979:379–88.
20. Katsnelson BA, Mokronosova KA. Nonfibrous mineral dusts and malignant tumors: an epidemiological study of mortality. J Occup Med 1979;21:15–20.
21. Boundy MG, Gold K, Martin KP, et al. Occupational exposures to non-asbestiform talc in Vermont. In: Lemen R, Dement JM, eds. Dust and disease. Park Forest South, IL: Pathotox Publishers, Inc., 1979:365–78.

A General Mortality Study of Production Workers in the Paint and Coatings Manufacturing Industry

A Preliminary Report

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Information has been obtained on a cohort of 16,243 men employed for at least one year in the manufacture of paint or varnish after January 1, 1946, and for seven subgroups on the basis of exposure. These workers experienced a level of mortality that compares favorably with that of the U.S. white male population. The workers' pattern of mortality differed somewhat from the U.S. pattern, with considerably reduced mortality from psychiatric, metabolic, respiratory, and violent causes. There was an increased mortality due to bowel and rectal cancer. While the numbers are smaller, there are also increased rates for liver and skin cancer. Lung cancer rates, while not in excess of the national average, did not match the low mortality from nonmalignant, noninfectious respiratory disease. The authors have concluded that work in this industry presents no major health hazard.

This study arose out of the concern of the National Paint and Coatings Association (NPCA) and its member companies for the health of workers employed in the manufacture of paint and coatings. At the time the study was initiated, little was known about the long-term health effects (if any) of this work. Also, unlike many industries where there are large numbers of workers in a single plant, the paint and coatings industry is characterized by a large number of small- to medium-size manufacturing plants, with the possibility that a real health hazard could exist and go undetected, because of small numbers of cases at any one site. Both considerations entered into the design and conduct of the study.

Given the nature of the industry, it was necessary to use a large number of plants for the study sample. In early 1976, SRI conducted a pilot study that involved mail inquiries to 18 of the largest manufacturing companies represented by NPCA, followed by two-day visits to six of the larger facilities. This pilot study showed that a full-scale cohort study was feasible.

The major objective of the cohort study was to be able to identify a doubling of the cause-specific mortality from diseases as rare as cancer of the pancreas among production workers in the paint and coatings industry exposed to substances and processes commonly used in that industry. Another objective of the study was to compare the numbers of observed and expected cause-specific deaths among various subgroups of the study population.

Materials and Methods

Since most occupational diseases affecting risk of death typically occur only after a long latent period, it was important to select a population in which there were persons whose exposure had begun long enough ago to permit assessment of risk. At the same time, it was important to select a population whose range of exposures was

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Table 1. — Subgroups Defined by Exposure.

Group	Description
1	Total cohort
2	At least one year of exposure to pigments
3	At least one year of exposure to solvents, excluding lacquer
4	At least one year of exposure to lacquer
5	At least one year in vehicle production
6	Groups 3, 4, and 5 together
7	At least one year in tub and tank cleaning
8	At least one year as a varnish cooker

reasonably representative of the entire industry. Because of the disruption of employment practices occurring during World War II, the start date for the study's cohorts was set at January 1, 1946. Also, since women represent such a small percentage of the work force in this industry, it was decided to limit the study to men.

The eligible population was defined as men who had (1) worked in the manufacture of paint or varnish for at least one year, (2) who had terminated their employment at some date after January 1, 1946, and (3) who had been employed in facilities that used processes typically found in paint and coatings manufacturing plants and that retained personnel records for terminated, retired, and deceased workers for at least 15 years. Such plants were identified from the questionnaires obtained during the pilot study. Forty-seven eligible plants were so identified, and for each of these plants, the estimated yield of eligible study population members was calculated.

To minimize the time and expense of traveling to plants, worker groups were entered into the study by plant, in order of diminishing size. Because larger plants were thereby overrepresented in the study population and, in addition, because the plants had been selected on the basis of age and record-retention policy, a random sample of 20 other general-product plants was selected from those NPCA plants not affiliated with the 20 to 30 largest companies in the industry. Walk-through surveys were carried out in each of these plants by industrial hygienists to verify that products and exposures found there were similar to those within the plants studied that had been selected previously for study. The industrial hygienists' judgment was that processes and exposures did not differ between the plants of different sizes.

The industrial hygienists then visited each plant used for data collection, obtained data on products and processes, and classified each job at the plants (present and past job titles) according to an exposure code developed by SRI in consultation with NPCA. Research assistants microfilmed the work records of present and past workers, together with whatever personnel documents were required to ascertain date of birth and social security number. Workers known to the plant to have died were identified and death certificates were obtained if they were available.

One of the study plants closed between the time that it was identified and the time a visit was planned. Records for that plant were retrieved and copied at the company headquarters, and the industrial hygiene information was

Table 2. — Vital Status and Follow-Up Rate by Plant.

Plant No.	Alive	Dead	Unknown	Total	% Followed
1	522	67	6	595	99.0
2	286	47	28	361	92.2
3	2,337	571	246	3,164	92.2
4	122	19	13	154	91.6
5	388	91	6	485	98.8
6	415	99	61	575	89.4
7	206	38	20	264	92.4
8	500	55	44	599	92.7
9	454	137	74	665	88.9
10	378	18	8	404	98.0
11	117	16	2	135	98.5
12	282	55	5	342	98.5
13	266	40	41	347	88.2
14	266	9	19	294	93.5
15	118	5	2	125	98.4
16	642	333	19	994	98.1
17	809	313	61	1,183	94.8
18	394	88	1	483	99.8
19	156	29	2	187	98.9
20	264	53	5	322	98.4
21	185	10	37	232	84.1
22	570	145	101	816	87.6
23	102	13	7	122	94.3
24	109	23	4	136	97.1
25	72	2	2	76	97.4
26	291	8	0	299	100.0
27	218	20	12	249	95.2
28	534	132	64	730	91.2
29	340	12	6	358	98.3
30	286	20	23	329	93.0
31	475	138	29	642	95.5
32	484	54	37	575	93.6
Total	12,588	2,670	985	16,243	93.9

Table 3. — Percent Distribution by Year of Hire for Those with Vital Status Known and Unknown.

Year of Hire	Vital Status Known	Unknown
Before 1945	26.4	12.2
1946-1954	26.3	33.1
1955-1964	19.1	24.3
After 1964	28.2	30.4
Total	100.0 (15,258*)	100.0 (985*)

*Number of men

reconstructed with the assistance of company personnel. After 32 plants had been visited, data had been collected on some 18,000 persons. Sampling of this group indicated that an adequate sample of persons who had incurred exposure in the course of employment had been attained, and data collection was therefore terminated.

Every job title that was found in the work histories of the study population was classified according to the product made and the basic function performed. There were four basic products: water-based paints, solvent-based paints, lacquer, and vehicle. Within each of the first three product categories, there were five functions: pre-batch assembler, mixer, tinter, filler, and tank and tub cleaner. However, the pre-batch assembly function has the same

Table 4. — Percent Distribution by Year of Employment for Those with Vital Status Known and Unknown.

Year of Employment	Vital Status Known	Unknown
< 5	31.8	70.4
5 - 9	18.2	17.8
10 - 19	17.3	8.1
20 - 29	16.3	2.3
30 +	16.4	1.4
Total	100.0 (15,258*)	100.0 (985*)

*Number of men

Table 5. — Number of Workers and Deceased Workers, by Exposure Group, Included in the Modified Life Table Analysis.

Group	Number	Deceased
1	15,951	2,633
2	3,612	651
3	4,731	744
4	2,932	485
5	1,725	302
6	8,200	1,307
7	617	159
8	238	64

Table 6. — Observed Deaths and SMRs — All Workers.

Cause	Deaths	SMR
All causes	2633	86
Malignant neoplasms	517	100
Malignant neoplasm of buccal cavity and pharynx	11	63
Malignant neoplasm of digestive organs and peritoneum	183	118
Malignant neoplasm of esophagus	13	106
Malignant neoplasm of stomach	33	101
Malignant neoplasm of large intestine, except rectum	65	138
Malignant neoplasm of rectum	26	139
Malignant neoplasm of biliary passages and liver	13	114
Malignant neoplasm of pancreas	30	104
Malignant neoplasm of respiratory system	160	98
Malignant neoplasm of larynx	8	99
Malignant neoplasm of bronchus, trachea, and lung	150	98
Malignant neoplasm of prostate	29	84
Malignant neoplasm of testis	3	79
Malignant neoplasm of kidney	5	39
Malignant neoplasm of bladder and other urinary organs	16	98
Malignant neoplasm of skin	12	126
Malignant neoplasm of brain and other parts of nervous system	10	165
Neoplasms of lymphatic and hematopoietic tissues	51	97
Lymphosarcoma and reticulosarcoma	15	125
Hodgkin's disease	7	92
Leukemia and aleukemia	20	92
Other lymphopoietic cancer	9	83
Infective and parasitic diseases	26	60
Allergic, endocrine system, metabolic, and nutritional diseases	35	68
Diseases of the blood and blood-forming organs	5	74
Mental, psychoneurotic, and personality disorders	5	37
Diseases of the nervous system and sense organs	206	90
Vascular lesions affecting central nervous system	189	92
Diseases of the circulatory system	1160	91
Diseases of the respiratory system	121	81
Diseases of the digestive system	121	88
Diseases of the genito-urinary system	25	57
Symptoms, senility, and ill-defined conditions	15	51
Accidents, poisoning, and violence	151	52
Person-years	274,881	
Unknown causes*	218	

*Distributed according to known causes (see text)

Table 7. — Observed Deaths and SMRs — Cohort 2 (Pigments).

Cause	Deaths	SMR
All causes	651	96
Malignant neoplasms	129	108
Malignant neoplasm of buccal cavity and pharynx	3	50
Malignant neoplasm of digestive organs and peritoneum	46	132
Malignant neoplasm of esophagus	4	139
Malignant neoplasm of stomach	9	125
Malignant neoplasm of large intestine, except rectum	11	104
Malignant neoplasm of rectum	6	144
Malignant neoplasm of billary passages and liver	7	273
Malignant neoplasm of pancreas	9	135
Malignant neoplasm of respiratory system	45	115
Malignant neoplasm of bronchus, trachea, and lung	43	117
Malignant neoplasm of prostate	5	70
Malignant neoplasm of skin	3	140
Neoplasms of lymphatic and hematopoietic tissues	10	84
Infective and parasitic diseases	3	31
Allergic, endocrine system, metabolic, and nutritional diseases	7	61
Diseases of the nervous system and sense organs	50	105
Vascular lesions affecting central nervous system	47	111
Diseases of the circulatory system	298	105
Chronic rheumatic heart disease	8	112
Arteriosclerotic heart disease, including coronary disease	242	106
Diseases of the respiratory system	20	60
Diseases of the digestive system	37	117
Gastric and duodenal ulcer	5	99
Cirrhosis of liver	19	117
Diseases of the genito-urinary system	5	55
Symptoms, senility, and ill-defined conditions	3	45
Accidents, poisoning, and violence	39	64
Person-years	58,135	
Unknown causes*	53	

*Distributed according to known causes (see text)

type of exposure in all processes. In vehicle production, there were four functions: reactor operator, varnish cooker, filter press operator, and filler.

Several complications arose in this categorization process. The first was that most plants made several products, either concurrently or sequentially, so that a given job title did not reflect a unique product exposure. A second complication was that in many plants more than one basic function was combined in a single job title. Finally, in a few plants, workers in paint production sometimes spent time in other production activities carried out at the same site. Additional categories were established to accommodate the combinations of basic categories and of paint and non-paint exposures at some plants. The fact that most jobs involved multiple functions and multiple products meant that it would be impossible to define a subgroup of any size with pure exposure to a single function or a single product.

For each plant every job in the job history of every study group member was coded with the month and year the job began and ended, and an exposure code number for each job title was assigned by the industrial hygienist who had visited the plant in question.* For persons whose job exposure during their tenure as salaried employees was unknown, the exposure was classified as unknown. In most plants job titles were found in the work histories which were not included in the lists of job titles from the plants. In the event of such questions (as well as problems), plant personnel were contacted for further information concerning the nature of exposure for a particular job.

The closing date of the study was fixed at December 31, 1976, the end of the year before data collection began. Although the starting date for cohort eligibility was January, 1946, in the sense that no one who left employment before that date was included in the study, for many plants the start date was actually later. If the period for which complete records of past employees was kept did not extend back beyond 1946, the "start" date for the plant was the earliest year for which complete records had been kept. If a plant began operations after the beginning of 1946, the earliest start date for any employee in that plant was the earliest year for which complete records were available. An individual from a given plant came under observation (that is, was counted as contributing person-years of risk) on the first anniversary of his hire or on the start date for the plant, whichever was more recent.

The cohort was divided into seven subgroups on the basis of exposure. Since most jobs involved multiple exposures, and since the workers generally moved among jobs with different kinds of exposures, these groups overlap, with consequent difficulties in interpreted findings. Table 1 identifies the groups analyzed.

In each of the subgroups, the individuals concerned came under observation not on the first anniversary of their hire dates, but on the date at which they had accumulated a year of exposure in one or more of the job categories listed. This could well have been several years after they had been hired, if they had moved in and out of

*A complete list of job codes will be provided upon request.

jobs in a given group. All the job histories of all the workers were therefore scanned by computer, the appropriate calculations were made in each case, and the resulting date used to determine when each person came under observation with respect to a particular exposure group.

The names and social security numbers of all those workers whose vital status was unknown were submitted to the Social Security Administration (SSA) for tracing. The names of those whom the SSA could not find were assembled into lists by plant, and returned to the plants with a request for whatever information was known to

Table 8. — Observed Deaths and SMRs — Cohort 3 (Solvents Excluding Lacquer).

Cause	Deaths	SMR
All causes	744	92
Malignant neoplasms	149	102
Malignant neoplasm of buccal cavity and pharynx	3	60
Malignant neoplasm of digestive organs and peritoneum	56	131
Malignant neoplasm of esophagus	5	143
Malignant neoplasm of stomach	9	101
Malignant neoplasm of large intestine, except rectum	16	124
Malignant neoplasm of rectum	10	196
Malignant neoplasm of biliary passages and liver	6	193
Malignant neoplasm of pancreas	10	123
Malignant neoplasm of respiratory system	53	112
Malignant neoplasm of bronchus, trachea, and lung	51	114
Malignant neoplasm of prostate	7	80
Malignant neoplasm of skin	4	148
Neoplasms of lymphatic and hematopoietic tissues	8	54
Infective and parasitic diseases	10	88
Allergic, endocrine system, metabolic, and nutritional diseases	8	57
Diseases of the nervous system and sense organs	72	122
Vascular lesions affecting central nervous system	66	126
Diseases of the circulatory system	333	96
Diseases of the respiratory system	25	62
Diseases of the digestive system	35	89
Diseases of the genito-urinary system	7	61
Symptoms, senility, and ill-defined conditions	6	71
Accidents, poisoning, and violence	43	51
Person-years	74,211	
Unknown causes*	44	

*Distributed according to known causes (see text)

Table 9. — Observed Deaths and SMRs — Cohort 4 (Lacquer).

Cause	Deaths	SMR
All causes	485	90
Malignant neoplasms	91	98
Malignant neoplasm of digestive organs and peritoneum	31	115
Malignant neoplasm of stomach	7	127
Malignant neoplasm of large intestine, except rectum	8	97
Malignant neoplasm of rectum	3	93
Malignant neoplasm of biliary passages and liver	5	255
Malignant neoplasm of pancreas	6	123
Malignant neoplasm of respiratory system	30	100
Malignant neoplasm of bronchus, trachea, and lung	28	99
Neoplasms of lymphatic and hematopoietic tissues	13	140
Lymphosarcoma and reticulosarcoma	3	140
Hodgkin's disease	1	78
Leukemia and aleukemia	8	212
Other lymphatic cancer	1	50
Infective and parasitic diseases	3	43
Allergic, endocrine system, metabolic, and nutritional diseases	8	89
Diseases of the blood and blood-forming organs	0	0
Diseases of the nervous system and sense organs	35	91
Diseases of the circulatory system	236	106
Chronic rheumatic heart disease	7	131
Arteriosclerotic heart disease, including coronary disease	187	104
Diseases of the respiratory system	15	57
Diseases of the digestive system	17	70
Symptoms, senility, and ill-defined conditions	4	75
Accidents, poisoning, and violence	23	47
Person-years	46,415	
Unknown causes*	42	

*Distributed according to known causes (see text)

plant personnel.

For those identified as deceased, death certificates were requested from the public health agencies of the states in which death was alleged to have occurred. When there turned out to be no record of death in the identified states, other states were queried (e.g., the state in which the plant was located, or the adjacent states(s) if the plant was near the state border). In all cases in which death certificates could not be found, at least two states were queried.

Cause of death was coded by a trained nosologist. Deaths occurring in 1967 or earlier were coded by the Seventh Revision of the International Classification of Diseases and Causes of Death. Those occurring after 1967 were coded according to the Eighth Revision. Observed deaths by cause were compared with the expected deaths that would have occurred in a similar population of U.S. white males, using a modified life table program adapted from the one described by Monson.[1] The computer program used is designed to handle entries from both Seventh and Eighth Revisions. Those men whose vital status was unknown were included as "alive" in the analysis and are mentioned later in this paper. In calculating the ratio of observed to expected deaths for specific causes, those deaths for which no certificates were found were assumed to have the same distribution by cause as those for which death certificates were available.

Results

The vital status and follow-up rate for each plant are shown in Table 2. Only one small plant (Plant 21) has less than 85% follow-up (84.1%). Four other plants have follow-up rates of under 90% (but all four are over 85.5%) while exactly half the plants have better than 95% follow-up. The total eligible cohort consisted of 16,243 men, of whom 2,670 were found to have died and 985 could not be traced. (The overall follow-up rate is therefore just under 94%.)

Table 3 shows the distribution by hire date for those of known vital status and those lost to follow-up. The men lost to follow-up seem to have been hired, on the average, slightly more recently than those who were successfully traced, the most striking find in the table being the differences between the percentages in the vital status categories of men who were hired before 1945 — i.e., the much higher percentage of those successfully traced.

Table 4 shows similar distributions by duration of employment. Not surprisingly, those lost to follow-up had a much shorter duration of employment, on the average, than those whose vital status was known.

There were 274,881 person-years of observation, about 14% of which was at ages 60 or older. Death certificates were obtained for all but 8.2% of the deaths. Race was known only for deceased individuals (from the death certificates). Of the deaths that occurred, 86.33% were whites, 8.80% were blacks, and 4.87% were other or unknown race. Thus, it was considered appropriate to use the white U.S. male mortality as the "expected" comparison group with the Monson Life Table Program. The person-years figure and the subsequent analysis do not include information on 292 individuals (1.8% of the entire cohort) who, because of missing information, could not be included in the modified life table analysis (Tables 5 to 13).

Table 5 shows the cohort size and number of deaths in each of the eight subgroups. Tables 6 through 13 summarize the mortality experience of various cohorts. The overall standardized mortality ratio (SMR) of 86 is about what might be expected as a result of the "healthy-worker effect," which results from the selective employment of men without disabling diseases or serious health problems.

One notes the excess mortality (Table 6) for cancer of the colon and rectum, with SMRs of 138 and 139 respectively, generated from 65 and 26 deaths. For these two conditions, there is an excess of nearly 20 deaths in the entire cohort over that expected which is statistically significant ($p < 0.05$). There is an increase of liver cancer,

Table 10. — Observed Deaths and SMRs — Cohort 5 (Vehicle Production).

Cause	Deaths	SMR
All causes	302	85
Malignant neoplasms	60	98
Malignant neoplasm of digestive organs and peritoneum	18	99
Malignant neoplasm of large intestine, except rectum	8	145
Malignant neoplasm of rectum	3	138
Malignant neoplasm of biliary passages and liver	2	151
Malignant neoplasm of pancreas	4	117
Malignant neoplasm of respiratory system	20	102
Malignant neoplasm of bronchus, trachea, and lung	20	109
Neoplasms of lymphatic and hematopoietic tissues	5	82
Infective and parasitic diseases	3	63
Malignant neoplasm of skin	2	181
Diseases of the nervous system and sense organs	32	121
Vascular lesions affecting central nervous system	31	131
Diseases of the circulatory system	131	88
Diseases of the respiratory system	17	97
Diseases of the digestive system	11	68
Accidents, poisoning, and violence	15	49
Person-years	29,010	
Unknown causes*	24	

*Distributed according to known causes (see text)

most markedly in cohorts 2 and 4 (SMRs of 273 and 255). Although numbers are small (13 in total work force), the increase in cohort 2 is statistically significant ($p < 0.05$). Cancer of the skin has an SMR of 140, based on 12 deaths for the total cohort, which is not a statistically significant increase ($p > 0.10$), but the total number of deaths is too small to permit a detailed analysis for specific cohorts. For the entire cohort, the SMR for leukemia was only 92 but in cohort 4 the SMR was 212 generated from eight observed deaths, although this was not statistically significant ($p > 0.05$). Although, for the entire cohort, vascular lesions of the nervous system generate an SMR of only 92,

Table 11. — Observed Deaths and SMRs — Cohort 6 (3, 4, or 5).

Cause	Deaths	SMR
All causes	1307	88
Malignant neoplasms	257	100
Malignant neoplasm of buccal cavity and pharynx	5	58
Malignant neoplasm of digestive organs and peritoneum	89	118
Malignant neoplasm of esophagus	8	131
Malignant neoplasm of stomach	13	83
Malignant neoplasm of large intestine, except rectum	28	122
Malignant neoplasm of rectum	13	144
Malignant neoplasm of biliary passages and liver	10	182
Malignant neoplasm of pancreas	17	119
Malignant neoplasm of respiratory system	86	104
Malignant neoplasm of bronchus, trachea, and lung	83	107
Malignant neoplasm of prostate	12	74
Malignant neoplasm of bladder and other urinary organs	6	77
Malignant neoplasm of skin	5	106
Malignant neoplasm of brain and other parts of nervous system	6	74
Neoplasms of lymphatic and hematopoietic tissues	24	93
Lymphosarcoma and reticulosarcoma	6	100
Hodgkin's disease	5	135
Leukemia and aleukemia	11	104
Other lymphopoietic cancer	2	37
Infective and parasitic diseases	14	69
Allergic, endocrine system, metabolic, and nutritional diseases	15	60
Diseases of the blood and blood-forming organs	1	20
Diseases of the nervous system and sense organs	116	108
Vascular lesions affecting central nervous system	108	102
Diseases of the circulatory system	580	94
Diseases of the respiratory system	55	76
Diseases of the digestive system	57	83
Diseases of the genito-urinary system	10	49
Symptoms, senility, and ill-defined conditions	8	54
Accidents, poisoning, and violence	72	51
Person-years	132,731	
Unknown causes*	102	

*Distributed according to known causes (see text)

Table 12. — Observed Deaths and SMRs — Cohort 7 (Tub & Tank Cleaning).

Cause	Deaths	SMR
All causes	159	87
Malignant neoplasms	30	98
Malignant neoplasm of digestive organs and peritoneum	15	155
Malignant neoplasm of stomach	3	142
Malignant neoplasm of large intestine, except rectum	4	136
Malignant neoplasm of rectum	2	169
Malignant neoplasm of biliary passages and liver	1	138
Malignant neoplasm of pancreas	4	229
Malignant neoplasm of respiratory system	5	53
Infective and parasitic diseases	3	128
Diseases of the nervous system and sense organs	17	112
Vascular lesions affecting central nervous system	17	121
Diseases of the circulatory system	71	90
Diseases of the respiratory system	4	42
Diseases of the digestive system	7	95
Symptoms, senility, and ill-defined conditions	3	186
Accidents, poisoning, and violence	4	34
Person-years	10,255	
Unknown causes*	15	

*Distributed according to known causes (see text)

Table 13. — Observed Deaths and SMRs — Cohort 8 (Varnish Cooking).

Cause	Deaths	SMR
All causes	64	97
Malignant neoplasms	10	95
Diseases of the nervous system and sense organs	5	100
Diseases of the circulatory system	29	108
Arteriosclerotic heart disease, including coronary disease	25	117
Diseases of the respiratory system	4	128
Accidents, poisoning, and violence	4	84
Person-years	4,589	
Unknown causes*	8	

*Distributed according to known causes (see text)

there are some differences by cohort. Cohorts 2, 3, 5, and 7 have SMRs of 111, 126, 131, and 121.

Tables 7 through 13 detail the experience of specific exposure cohorts. As mentioned earlier, most workers have multiple exposures so they appear in more than one cohort. Differences in cohort experience are small, but are examined further later in this paper.

Discussion

As already noted, those cohort members whose vital status was unknown were treated in the modified life table analysis as if they were alive, even though many epidemiologists suspect that persons lost to follow-up are, in effect, a different population than those who can be found and so have different mortality patterns. However, since the group was examined intensively to find any dead, the "unknowns" are likely to be alive. In any case, preliminary analyses performed after removal of unknowns gave results essentially identical to results reported here in which the unknowns have been assumed to be alive. Nevertheless, to the extent that deaths have been missed in those lost to follow-up, the SMRs are slightly underestimated.

At least one of the plants in the study had used each of the following toxic materials: asbestos (as a filler); chromium, lead, mercury and nickel compounds; silica; talc; benzene; and arsenicals. For this reason, particular attention was given to cancer of the respiratory, hematopoietic, and digestive systems; of the skin; diseases of the blood and blood-forming organs; and of the nervous, respiratory, and genito-urinary system. No deaths from mesothelioma were found.

Among cancers of the digestive system, the SMRs for colon and rectal cancers and for liver cancer, as discussed earlier, are noteworthy. There are over 90 observed deaths for colon and rectal cancer combined for an excess of nearly 20 deaths over those expected, which is statistically significant. In considering colon and rectal cancer, a certain amount of misdiagnosis or misclassification can blur the distinction between the two. However since both are increased, the pattern may not be simply one reflecting misclassification, although some of that error may occur coincidentally with an increase in one or both of the diseases.

There is an excess of cancer of the liver, particularly in cohorts 2 and 4, which for cohort 2 is statistically significant. However, the numbers are small, and liver cancer is notorious for problems of misdiagnosis. The pattern of deaths from lung cancer and deaths from other respiratory disease should also be noted. Since cigarette smoking is responsible for most deaths in both of these categories, the difference in SMRs of 98 for lung cancer and only 81 for respiratory disease is noteworthy, although there may be some worker selection. Within some cohorts (especially 2, 3, and 4) the difference in SMRs is even greater, leading one to suspect that the work force may smoke less than the national average, but have lung cancer rates as high. Without smoking data, however, only speculation can take place.

While the 12 deaths from skin cancer are too few to permit a detailed analysis for specific cohorts, the SMR of nearly 200 for cohort 5, while not statistically significant, is of interest. There are two further points that suggest that cancer of the skin merits further investigation. The first is that although there is a proportion of the total cohort who are black, rates for white males (as noted previously) were used to generate expected numbers of deaths in the cohort; hence, the expected deaths for skin cancer are somewhat overestimated, and consequently, the SMRs for skin cancer are underestimated. The second point is that of the 12 deaths from skin cancer, 10 are from malignant melanoma. While the life table program does not calculate expected numbers of deaths for malignant melanoma alone, the number of deaths from malignant melanoma in white males in the United States between 1950 and 1969 (23,417) was approximately the same as the deaths in white males in the United States during the same period from other skin cancer (21,722).[2] These two factors, in combination with the finding, are compatible with an increased risk of death from malignant melanoma in these workers. Given the notorious inaccuracy in recording of skin cancer, especially cancers other than melanoma, on death certificates, as well as the other uncertainties previously discussed, further investigation of the skin cancers would be required before any conclusions about their etiology could be drawn.

With the exception of cohort 4, no particular excess risk of cancer of the hematopoietic and lymphatic system is seen. In cohort 4, the SMR of 212 based on eight observed deaths in a cohort with past exposure to benzene raises serious concern and requires further investigation.

For diseases of the nervous system, vascular lesions have SMRs which, as noted earlier, are somewhat elevated, suggesting that there may be some effect of employment. An increased frequency of death from this cause might be due, for instance, to an environmental or

chemical exposure that raises blood pressure. The findings are inconsistent, however, and the diagnosis is subject to considerable physician error.

It is planned to validate — from autopsy, biopsy, or clinical data — all death certificates listing primary liver cancer and skin cancer as the cause of death. Detailed analyses of the existing data concerning bowel and rectal cancer and the discrepancy between lung cancer and respiratory disease — including a closer look at exposure categories, plant or geographic clustering, or both, and the relationship of SMRs for these conditions in other studies and situations — are also planned. Simultaneously, there will be an attempt to validate diagnoses of colon and rectal cancer to confirm that both conditions are in excess, rather than a case of only one affecting the other through misclassification. Finally, there will be a detailed investigation of leukemia in cohort 4 including a closer look at plant or geographic clusters and an investigation of exposure to known causes of leukemia such as exposure (both occupational and non-occupational) to ionizing radiation.

The authors conclude that there is no major mortality risk imposed by working in the production of paints and coatings, although certain diagnostic categories require further investigation.

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References

1. Monson RR: Analysis of relative survival and proportional mortality. *Comp Biol Res* 7:325-332, 1974.

2. Mason TJ and McKay FW: U.S. Cancer Mortality by County 1950-1969. Washington, D.C.: DHEW Publication No. (NIH) 76-615, U.S. Gov't Printing Office, 1974, pp 425, 445.

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Carcinogenicity studies on fibres, metal compounds, and some other dusts in rats

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Key words: carcinogenicity; metal compounds; dust; mineral fibres; vitreous fibres; plastic fibres; heavy metals; glass microfibres; asbestos; attapulgite; erionite; wollastonite; cadmium; nickel; ferric oxides; corundum; titanium dioxide; chrysotile; crocidolite; quartz; benzo(a)pyrene; polyvinylchloride; polyvinylpyridine-N-oxide; wood dust; inhalation experiments; intratracheal instillation; intraperitoneal injection

Summary

About 50 dusts were examined on their carcinogenicity in rats mainly after intraperitoneal injection and some after intratracheal instillation. In the i.p. test, very low doses between 0.05 and 0.5 mg asbestos led to tumour incidences of about 20 to 80%. Polyvinylpyridine-N-oxide prolonged the tumour latency after injection of actinolite. 60 mg attapulgite from three sources with short fibre lengths were not shown to be carcinogenic but an attapulgite sample with longer fibres had a moderate effect. Relatively thick rock and ceramic fibres (median $> 1\,\mu m$) induced tumours, but slag and wollastonite fibres did not, probably because of their better solubility. Intratracheal instillations of glass microfibres (20×0.5 mg) led to lung tumours in 5 of 34 rats (0 in control). The carcinogenic potency of an inorganic fibre depends on its size and persistency, and possibly also on other properties, especially on the surface.

Nickel powder, nickel oxide, nickel subsulfide and cadmium sulfide were all found to be carcinogenic in the two tests. Cadmium chloride and cadmium oxide could only be administered in very low doses because of their high acute toxicity. A high amount of magnetite (15×15 mg i.tr.) led to an unexpected lung tumour incidence of 69%.

The i.p. test in rats proved to be very sensitive for detecting the carcinogenic potency of non-acute toxic natural and man-made mineral dusts as well as metal compounds. This means that, if a high dose of one of these dusts does not induce tumours in this test, no suspicion of carcinogenic potency can be substantiated.

Introduction

When in 1972/73 the first experimental results on the tumour-inducing effect not only of asbestos but also of other fibrous dusts were published, the results strongly supported the old hypothesis that the elongated shape of asbestos particles may be the cause of their carcinogenicity (Nordmann 1938; Linzbach and Wedler 1941; Stanton and Wrench 1972; Pott and Friedrichs 1972; Wagner et al. 1973). Moreover the question was raised of a possible carcinogenic effect of non-asbestos fibres in humans, especially of man-made mineral fibres, because their production has risen steadily since the fifties. As a consequence, epidemiologic studies on the man-made mineral fibre industry were carried out over the past ten years. The results were reviewed recently by Saracci (1986) and Doll (1986). They support the hypothesis that man-made mineral fibres — as present in the workplace atmo-

Dedicated to Dr. A. Brockhaus on the occasion of his 60th birthday

sphere of early slag wool/rock wool production — may have played a role in the causation of lung cancer.

Up to now, in inhalation experiments with very fine man-made mineral fibres (glass microfibres 100 and 104), no statistically significant tumour rates have been detected (Le Bouffant 1986; Goldstein 1984; McConnell et al. 1984; Muhle et al. 1986; Smith et al. 1986; Wagner et al. 1984a). The strong carcinogen, crocidolite, has similarly produced only a low tumour rate in inhalation experiments. Therefore, this test model cannot be considered as sensitive for carcinogenic fibres. The positive results with chrysotile were observed after exposure to higher fibre concentrations (Davis et al. 1978; Davis et al. 1986; McConnell et al. 1984; Wagner et al. 1984a). Moreover it was demonstrated, that after a period of two years the number of chrysotile fibres deposited in the rat lung can increase more than tenfold by splitting (Bellmann et al. 1986). However, this multiplication cannot happen with crocidolite and vitreous fibres. Most carcinogenicity studies with mineral fibres in laboratory animals were carried out by means of intrapleural or intraperitoneal administration. By these routes of application, a high number of fibres can become active in the serosal tissue which is obviously very susceptible to fibre-related carcinogenic stimulation. This high sensitivity exists also in humans since the relation of lung carcinomas to mesotheliomas in asbestos workers amounts on average to about 3 to 1, although after inhalation contact of fibres with the thoracic or abdominal serosa should be much more difficult than with the bronchial epithelium.

The experimental results should lead to a generally valid definition of carcinogenic fibres. This aim is not yet reached. On the one hand there is a general consensus that length, diameter and bio-persistence represent the most important criteria for the carcinogenic potency of mineral fibres (Stanton et al. 1981; Wagner 1986; Davis 1986; Pott 1987). On the other hand, the relationship between the degree of these three characteristics and the expected tumour incidence is not sufficiently known. There is a continuous transition from the non-carcinogenicity of fibres which are too short, too thick, or too soluble to the maximum possible carcinogenic potency of fibres which have the "ideal" size and are persistent enough. However, it is an open question at which point the threshold is located between fibres which are too short, too thick or too short-lived in the tissue and fibres which are sufficiently long, thin and persistent regarding their carcinogenic potency for humans. Furthermore, for regulations, we need not only the distinction between carcinogenic and non-carcinogenic fibres but also criteria for a grading of carcinogenic potency.

For many years there has been the theory that the surface properties of fibres are the decisive agents for the tumour induction (Bignon and Jaurand 1983; Bonneau et al. 1986a, b; Cralley 1971; Cralley and Lainhart 1973; Dunnigan 1984; Fisher et al. 1985; Langer and Nolan 1985; Mossman 1983). This theory is mainly based on the results of *in vitro* testing where altered dust surfaces were seen to influence certain cytotoxic effects. But there are significant differences between a cytotoxic reaction *in vitro* and tumour induction *in vivo*. Often, good parallels were found between results from carcinogenicity tests and some *in vitro* tests (Davis et al. 1985). Nevertheless, up to now *in vitro* tests have not been able to provide reliable statements on fibre carcinogenicity (Wagner et al. 1985).

Recent results after combined injection of asbestos and the antisilicotic effective substance polyvinylpyridine-*N*-oxide indicate that the surface properties of asbestos fibres might not only be important for its cytotoxicity *in vitro* but also for its carcinogenicity (Pott et al. 1985).

Since the carcinogenic potency of fibres is composed of, at least, their length, diameter and durability, and as the spectrum of fibre dimensions in a single sample is wide ranging, it is very difficult to explain a particular tumour rate using only one specific parameter. With these difficulties always in mind, we are going to describe a number of results which may add some pieces to the puzzle. The experiments were carried out over the last 15 years and only some of them have already been published, although not with all the details now described.

A further question concerns the relation of the carcinogenic effect of fibres to chemically carcinogenic substances in the applied test systems. For comparison, a number of carcinogenic, non-carcinogenic and questionable carcinogenic substances were tested on their carcinogenicity after intraperitoneal injection and intratracheal instillation. In this context, additional carcinogenicity studies with some nickel and cadmium compounds are desirable because up to now there is no consensus on their carcinogenic potential. Of course, numerous reports on nickel carcinogenesis have been published in various reviews (IARC 1976; RAITHEL and SCHALLER 1981; SUNDERMAN 1981; SUNDERMAN 1984). Nickel powder and nickel oxide have been known to be chemical carcinogens for a long time. But recently, following a review of the epidemiologic and experimental results, it was proposed to classify these two substances as non-carcinogenic for humans and animals (LONGSTAFF et al. 1984). Cadmium is also suspected to be carcinogenic in the relevant compounds cadmium oxide and cadmium sulfide, since inhalation of relatively low concentrations of cadmium chloride induced lung tumours in rats (TAKENAKA et al. 1983).

To widen the basis of comparison, further dusts were included in the study e.g. corundum, titanium dioxide, ferric oxides, polyvinylchloride, and wood dust.

Materials and Methods

1. Laboratory animals and animal keeping

In most of the experiments female Wistar rats (Wistar-WU/Kißlegg) from the breeding farm S. Ivanovas (Kißlegg, Allgäu, F.R.G.) were used and only in experiments no. 10 and 12 Sprague-Dawley/SIV 50 rats from the same breeding farm. Before each experiment the animals were randomly allocated to plastic cages on wood granule bedding ("T-grob", Buntenbach, Solingen, F.R.G.), and numbered mostly 8—10 to a cage at the start. As male rats grow much bigger than female rats and need more space, in only one experiment (no. 7) male rats were also used.

The animals were maintained under conventional conditions and these could be improved in the late 70ies. A standard pelleted laboratory diet (RMH-TM, Fa. Hope-Farms Woerden, NL) and water were given *ad libitum*.

The weight of the animals was determined before the start of the experiment and the average age of the groups was calculated according to tables of the breeding farm. The average age of the animals is given for each experiment in the tables of results.

2. Substances used

Some of the dusts or chemicals administered were bought from manufacturers or their agencies and used in the condition delivered. Many materials, especially the man-made mineral wools, were delivered from manufacturers or agencies or other persons and prepared for the animal experiments by cutting and milling. Sometimes the milled dust was fractionated by sedimentation. This work and the measurement of the fibre sizes (mentioned only as median values in tables 1 and 3) was performed in several laboratories, especially by Dr. BELLMANN and Dr. MÜHLE (Fraunhofer Institut für Toxikologie und Aerosolforschung, Hannover, F.R.G.), Dr. FRIEDRICHS (Medizinisches Institut für Umwelthygiene an der Universität Düsseldorf, F.R.G.), Dr. RÖDELSPERGER (Institut für Arbeits- und Sozialmedizin der Universität Gießen, F.R.G.), and Dr. SPURNY (Fraunhofer Institut für Umweltchemie und Ökotoxikologie, Schmallenberg-Grafschaft, F.R.G.). The UICC samples were described by RENDALL (1970) and TIMBRELL (1970).

Actinolite, F.R.G. Origin: A diabas quarry near Schmallenberg, F.R.G. Preparation: Dr. SPURNY (method see SPURNY et al. 1979a). Measurement of fibre sizes: Dr. BELLMANN Dr. MÜHLE.

Actinolite, (granular). Origin unknown; a small rock was obtained from the Mineralienkontor Bonn, F.R.G. Preparation: Dr. FRIEDRICHS. The macroscopically fibrous structure was mainly destroyed by milling in an agate ball mill. A part of this sample had already been used in another experiment (POTT et al. 1976).

Anthophyllite, UICC. — Origin: Finland.

Attapulgite, Caceres. — Origin: Torrejon de Rubio Caceres, Spain. Preparation of a very fine fraction (reference No. 82 I) by H. PÉZERAT (Université P. et M. Curie, Paris, France). Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Attapulgite, Georgia. — Origin: Georgia, U.S.A. Delivered under the name "Pharmasorb colloidal" from Chemie-Mineralien KG, Bremen, F.R.G., and a product of Engelhard Minerals & Chemicals Corporation, Edison, N. J., U.S.A. Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Attapulgite, Lebrija. — Origin: Lebrija, Spain. Delivered with the label "Polvo fabrica" from Tolsa S.A., Madrid, Spain. According to the analysis of RÖDELSPERGER et al. (1987) the sample was identified as attapulgite with high probability. It was used in the condition obtained. Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Attapulgite, Mormoiron. — Origin: Mormoiron, France. The sample used was the drug "gastropulgite" which contains 83% attapulgite and is produced by Beaufour, Dreux, France. The fibre sizes measured came from a sample "Gastropulgite 50" which is produced under licence of Beaufour by Schwabe, Karlsruhe, F.R.G. Measurement of fibre sizes: RÖDELSPERGER et al. (1987).

Basalt wool. — Producer: Grünzweig – Hartmann und Glasfaser AG, Ludwigshafen, F.R.G. Preparation: Dr. SPURNY (method see SPURNY et al. 1979b). Measurement of fibre sizes: Dr. BELLMANN/Dr. MÜHLE.

Benzo(a)pyrene. — Delivered from Fluka AG, Neu-Ulm, F.R.G. Code-No. 12780. Purity 97%.

Brucite, $Mg(OH)_2$. — This mineral is the granular form of magnesium hydroxide (fibrous form: see nemalite). Preparation: Dr. FRIEDRICHS (FRIEDRICHS 1974).

Cadmium chloride ($CdCl_2 \times H_2O$). — Delivered from Merck, Darmstadt, F.R.G. Purity 99%.

Cadmium oxide. — Delivered from Merck, Darmstadt, F.R.G. Purity not indicated.

Cadmium sulfide. — Delivered from Aldrich-Chemie GmbH & Co. KG, Steinheim, F.R.G. Purity 99.999%.

Ceramic wool, Fiberfrax. — Producer: Carborundum, Düsseldorf, F.R.G. Delivered from Hecker Werke GmbH & Co. KG, Weil, F.R.G. Preparation: Dr. SPURNY (method see SPURNY et al. 1979b). Measurement of fibre sizes: Dr. BELLMANN/Dr. MÜHLE.

Ceramic wool, M.M.V. — Producer: Manville Corporation, Denver Co., U.S.A. Delivered from Gossler, Hamburg, F.R.G. Preparation: Dr. SPURNY. Measurement of fibre sizes: Dr. BELLMANN/Dr. MÜHLE.

Chrysotile, Calidria. — Origin: California, U.S.A. The material was prepared for the production of asbestos paper. The sample used was obtained from Dr. RONOCK, Asbest-Institut, Neuß, F.R.G. Measurement of fibre sizes: MÜHLE et al. 1986.

Chrysotile, UICC A. – Origin: Zimbabwe (formerly Rhodesia). Measurement of fibre sizes: FRIEDRICHS (1978).

Chrysotile, UICC A, HCl-treated. – The standard sample was boiled in 1 N HCl for 8 h. The magnesium content probably was leached almost completely. The loss of weight amounted to 46%. The fibres were shortened and partially split up.

Chrysotile, UICC A, milled. – The standard sample was dry milled in an agate ball mill for 4 h. Measurement of fibre sizes: FRIEDRICHS (1978). A part of this sample was used in larger doses in other experiments (PORT et al. 1972, 1976).

UICC B. – Origin: Canada. Measurement of particle sizes: TEMBERLL (1979). (It has to be underlined that the results of measurement of the twisted chrysotile fibres are rather doubtful. Extensive treatment with ultrasound homogenizes the suspension and changes the fibre number by splitting of the bundles.

Chrysotile, UICC B, milled. – The standard sample was dry milled in an agate ball mill for 3.5 h. Measurement of fibre sizes: Dr. BELLMANN/Dr. MÜHLE.

Corundum. — The aluminium oxide dust was delivered from Maschinen- und Schleifmittelwerke, Offenbach, F.R.G.

Crocidolite, South Africa. — Origin: South Africa (like UICC crocidolite but fibre lengths greater). Preparation: Dr. RENDALL, Johannesburg, South Africa. Measurement of fibre sizes: MUHLE et al. 1986.

Erionite, Oregon. — Origin: Oregon, U.S.A. The dust sample was supplied by Dr. WAGNER, Medical Research Council Pneumoconiosis Unit, Penarth, Wales, U. K., as representative of the material used by him in his experiments (WAGNER et al. 1985). Measurement of fibre sizes: Dr. RÖDELSPERGER.

Erionite, Turkey. — Origin: Karain, Turkey. A small rock of the fibrous material was sent from Dr. BARIS, Ankara to Dr. SPURNY and prepared by Dr. SPURNY. Measurement of fibre sizes: Dr. BELLMANN/Dr. MUHLE.

α-Ferric oxide hydrate. — The extremely short fibrous material was obtained from a producer of tapes. Estimation of the fibre sizes according to a microphotograph with a magnification of 1:50.000.

γ-Ferric oxide hydrate (1). — The fibrous material was obtained from a producer of tapes. Measurement of fibre sizes: Dr. MUHLE.

γ-Ferric oxide hydrate (2). — The fibrous material was obtained from a producer of tapes. Estimation of the fibre sizes according to a microphotograph with a magnification of 1:50.000.

Glass fibres, 100 Pen. — Producer: Manville Corporation, Denver, Co., U.S.A. The dust sample was supplied by Mr. SKIDMORE, Medical Research Council Pneumoconiosis Unit, Penarth, Wales, U. K., as representative of the material used in his animal experiments (WAGNER et al. 1984a). Measurement of fibre sizes: Dr. SPURNY.

Glass fibres, 100 L & V. — Producer: Manville Corporation. Delivered from Lehmann & Voss, Hamburg, F.R.G. After cutting with scissors, the fibres were ground in a knife mill for 20 min. It is not known whether this sample has the same chemical composition as 100 Pen mentioned above. Measurement of fibre sizes: Dr. SPURNY.

Glass fibres, 104/1974. — Producer: Manville Corporation. Delivered from Lehmann & Voss in 1974 (no further data). The chemical composition was analyzed with proton induced X-ray emission by E. BOMBELKA und Dr. F.-W. RICHTER, University of Marburg (published in BELLMANN et al. 1986); this analysis yielded about the same composition as reported by Manville Corporation for the glass fibre Tempstran E Glass Electrical Alkalifree. After cutting with scissors the wool was ground in distilled water in an agate mill for 1 h (charge 1) or 2 h (charge 2) and then dried at 80°C. Measurement of fibre sizes: Dr. SPURNY. These samples were also used in other experiments (PORT et al. 1980, 1984a, 1984b).

Glass fibres, 104/1974, HCl- or NaOH-treated. — 500 mg of the fibres (see above) were incubated in 20 ml 1.4 N HCl or 1.4 N NaOH for 2 or 24 h in a magnetic stirrer at room temperature. After 10 min centrifuging at 1.200 × g they were filtered through Nucleopore filters (pore diameter 0.1 μm). The sediment was washed twice with 20 ml distilled water in each case, the residue filtered and the fibres dried at 80°C for 16 h. The centrifuged particles and the particles on the filter were then weighed. The loss in weight 2 and 24 h after treatment with HCl amounted to 25 and 33%, respectively, after treatment with NaOH to 1.7 and 6.8%, and after treatment with distilled water to 1.7% (PORT et al. 1984a). Measurement of fibre sizes: Dr. SPURNY.

Glass fibres, 104 475. — Producer: Manville Corporation, Type: Tempstran 475. Delivered from Lehmann & Voss. After cutting with scissors, the wool was ground in a knife mill for 30 min. Measurement of fibre sizes: MUHLE et al. 1986.

Glass fibres, 104 475, HCl-treated. — The fibres mentioned before were treated for 24 h with 1.4 N HCl as described for glass fibres 104 1974. The loss in weight amounted to 0.5%. (The resistance of Tempstran 475 is much larger than that of the glass fibres 104 1974 mentioned above).

Glass fibres, 106. — Producer: Manville Corporation. Delivered from Schleicher und Schüll, Dassel, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS. The material was also used for other studies (POTT and FRIEDRICHS 1972; POTT et al. 1976).

Glass filaments, ES 3. — The (endless) textile fibre was delivered from Gewetex, Düsseldorf, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS. The variation in fibre diameters was in a limited range: $10\%_{o} < 3.3\,\mu m$, $90\%_{o} < 4.2\,\mu m$. Length: $10\%_{o} < 6\,\mu m$, $90\%_{o} < 50\,\mu m$ (FRIEDRICHS 1978).

Glass filaments, ES 5 and 7. — These (endless) textile fibres were obtained from Klöckner & Schott, Dortmund, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS. In these samples the diameter variations were in a limited range: ES 5: $10\%_{o} < 4.8\,\mu m$, $90\%_{o} < 6.3\,\mu m$; Es 7: $10\%_{o} < 6.8\,\mu m$, $90\%_{o} < 8.1\,\mu m$. Length Es 5: $10\%_{o} < 24\,\mu m$, $90\%_{o} < 80\,\mu m$; Es 7: $10\%_{o} < 23\,\mu m$, $90\%_{o} < 102\,\mu m$ (FRIEDRICHS 1978).

Glass (granular). — Pieces of glass milled to a fine dust. Preparation: Dr. FRIEDRICHS.

Kevlar (Trademark for aramide fibres). — Producer: E. I. du Pont de Nemours and Company, Newark, Delaware, U.S.A. It was not possible to make a suspension with separated fibres from the flaky material. The suspension of sample (1) for experiment 13 was prepared by ultrasonic treatment only. For sample (2) used in experiment 15 an attempt was made to get finer fibres and better suspension by drying, milling and ultrasonic treatment. This difficult preparation was carried out by Dr. SEIFERT. However, the suspension injected was not homogeneous like that of mineral fibres.

Magnetite. — Delivered under the name "Ferroso Ferric Oxide" from Research Organic Inorganic Chemical Corporation, Bellville, N.J., U.S.A. and obtained from Dr. OBERDÖRSTER, University of Rochester, N. Y. The particles are very small and cannot be measured by light microscopy.

Nemalite, $MgOH_2$. — This mineral is the fibrous form of magnesium hydroxide. It was obtained from the Mineralienkontor, Bonn, F.R.G. (Impurity with chrysotile possible.) Preparation and measurement of fibre sizes: Dr. FRIEDRICHS (FRIEDRICHS 1978).

Nickel oxide (NiO). — Delivered from Aldrich-Chemie GmbH & Co. KG, Steinheim, F.R.G. Purity 99.99%.

Nickel powder. — Delivered from Inco Metals Company, Missisauga, Ontario, Canada to Dr. MUHLE. Purity not indicated.

Nickel subsulfide (Ni_3S_2). — Delivered from Inco Metals Company, Missisauga, Ontario, Canada. Purity not indicated.

Polypropylene. — The fibrous dust was obtained ready milled by Rhodia AG, Freiburg im Breisgau, F.R.G. Measurement of fibre sizes: Dr. BELLMANN Dr. MUHLE.

Polyvinylchloride. — Produced by Chemische Werke Hüls, Marl, F.R.G. Particle sizes $90\%_{o} < 2.5\,\mu m$. Measurement: Dr. BELLMANN-Dr. MUHLE.

2-Polyvinylpyridine-N-oxide (PVNO). — Produced by Bayer AG, Wuppertal, F.R.G. as a 2%-solution probably in distilled water (Charge V 3504) for silicosis research in the sixties.

Quartz DQ12. — Origin: Dörentrup, F.R.G. The "Ground Product No. 12" (in short: DQ12) with a size distribution of $< 60\,\mu m$ was delivered from Dörentrup Sand- und Tonwerke GmbH of Dörentrup. A $< 5\,\mu m$ size fraction was prepared from this material by centrifugal separation in air (RONOCK 1973). The fine dust was obtained from Steinkohlenbergbauverein, Essen, F.R.G. This quartz specimen has often been used by German institutes involved in silicosis research.

Rock wool, Sweden. — Produced in Sweden. Prepared for animal inhalation studies at the Medical Research Council Pneumoconiosis Unit, Penarth, Wales, U.K. (WAGNER et al. 1984). A sample was obtained from Mr. SKIDMORE. One part was used in its delivered form, another was fractionated by Dr. SEIFERT to obtain a sample with finer fibres (method SEIFERT et al. 1979b). Measurement of fibre sizes: Dr. SEIFERT.

Slag wool, RH. — Produced by Rheinstahl, Gelsenkirchen, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS (FRIEDRICHS 1978).

Slag wool, ZL. — Produced by Zimmermann, Sprockhövel 2, F.R.G. Preparation and measurement of fibre sizes: Dr. FRIEDRICHS (FRIEDRICHS 1978).

Titanium dioxide. — Delivered from Degussa, Frankfurt, F.R.G. signed P25. The crystal form is anatas.

Volcanic ash, St. Helen's. — Obtained from Dr. RAABE, University of California, Davis, California, U.S.A.

Wollastonite. — Origin: India. A dust sample with relatively large particles signed D-1 was obtained from Osthoff-Petrasch KG, Hamburg, F.R.G. Preparation of a fine fraction by Dr. SPURNY. Measurement of fibre sizes: Dr. BELLMANN/Dr. MUHLE.

Wood dust, beech. — Signed "Buchenmehl Größe 00". Delivered from "brüder schulte", Olsberg, F.R.G. Commercially used for bread baking. Particle sizes about 10—100 μm.

3. Method of administration

The dusts were dispersed in 0.9% NaCl solution with ultrasound, most of them for about 1—3 min. The intraperitoneal injections were given without anaesthesia, the intratracheal instillations under CO_2 anaesthesia. The animals received 0.3 ml per i.tr. instillation and mostly 2 ml per i.p. injection. Only 0.8 or 1 ml was injected intraperitoneally in the very young rats in experiments 7, 11, 13, and in those groups of experiment 15 which received only a very small amount of asbestos. If more than one i.p. injection was given, it was done weekly. The 10 to 20 i.tr. instillations were also applied weekly. The large amounts of relatively thick glass filaments in experiments 4 and 5 were inoculated in 4 ml saline by laparotomia in nembutal anaesthesia.

Polyvinylpyridine-N-oxide (PVNO) was applied in experiments 13 (table 1) and 15 (table 3). Three groups received actinolite i.p. suspended in 1 ml PVNO-solution (2% or 0.4%). Two of these groups were not further treated: one group was additionally i.p. injected four times with 1 ml 2% PVNO-solution 4, 8, 12, and 16 months later. The other groups in experiment 13, labelled "PVNO separately", received 1 ml PVNO solution intraperitoneally five times: one day before the i.p. injection of asbestos and after 4, 8, 12, and 16 months.

4. Method of examination

The animals died spontaneously or were killed when in a bad health condition. In some experiments, the surviving animals were sacrificed about 2.5 years after treatment. Post mortem examination was made of the abdominal cavity of rats injected intraperitoneally. Parts of tumours or organs with suspected tumour tissue were fixed in formalin (6%) for histological examination. In the intratracheal experiments the lungs and tracheas were wholly fixed in formalin for histological tumour evaluation. The number of rats examined (listed in tables 1—4) includes all autopsied rats and does not include those animals lost through cannibalism or during anaesthesia. The percentage of dead rats does not comprise rats lost during anaesthesia but it does include those lost through cannibalism.

Results

Table 1 lists in chronological order the results of 13 experiments using the intraperitoneal test. All rats with sarcoma, mesothelioma or carcinoma of the abdominal cavity were counted as tumour-bearing rats. The most frequent diagnoses were sarcomas as in previous experiments (PORT et al. 1976; SCHEUER et al. 1973); only a few carcinomas were found. These three tumour-types could not always be differentiated with certainty histologically; sometimes two tumour-types occurred together. Since 5—10% of the control group showed malignant tumours of the uterus, part of them with metastases, rats with malignant uterine tumours were not counted as tumour-bearing rats. Thus it is possible that a tumour was classified as a spontaneous uterine tumour even though it was induced by the substance administered and occurred accidentally alongside with a malignant neoplasm of the uterus.

Table 1. Results after intraperitoneal injection of various fibrous and granular dusts

Dust	Fibre dimens. [μm]		Dose i.p. [mg]	Number of rats examined	Rats with tum. [%]	Life-span [weeks] after first treatment of					
	length 50%-	diameter 50%-				all rats				rats with tum.	
						20%-	50%-	80%-	100%-	first	average
Experiment 1 (Wistar, 12 weeks)											
Chrysotile, UICC A	9	0.15	6	31	77.1	58	71	79	101	49	71
Chrysotile, UICC A	9	0.15	25	31	80.6	19	58	73	100	39	60
Chrysotile, HCl-treated			6	38	0.0	15	78	133	133		
Chrysotile, HCl-trated			25	40	-	0.4	0.6	0.7	1		
Saline				70	0.0	78	87	87	124	-	-
						(life-span reduced by inf. in month 11)					
Experiment 2 (Wistar, 12 weeks)											
Glass filaments, ES 5	39	5.5	10	50	4.0	81	111	129	165	92	106
Glass filaments, ES 5	39	5.5	10 (2 - 20)	46	10.9	90	107	127	165	96	119
Glass filaments, ES 7	46	7.4	10 (2 - 20)	47	2.1	102	121	134	156	126	126
Glass	(granular)		10 (2 - 20)	45	4.4	102	119	136	165	121	129
Quartz, DQ 12	(granular)		10 (2 - 20)	41	22.0	88	101	115	133	15	91
Experiment 3 (Wistar, 15 weeks)											
Slag wool, RH	26	2.6	10 (2 - 20)	99	6.1	94	111	127	158	88	114
Slag wool, ZI	14	1.5	10 (2 - 20)	96	2.1	91	107	126	155	67	77
Nemalite, $Mg(OH)_2$	1.3	0.06	10 (2 - 20)	18	89.6	32	39	47	65	25	41
Ilmenite, $MgOH)_2$	(mainly gran.)		10 (2 - 20)	19	0.0	86	99	126	155		
Actinolite	(mainly gran.)		10 (2 - 20)	48	4.2	80	96	121	138	116	123
Saline			2 - 2 ml	48	0.0	85	101	129	150		
Experiment 4 (Wistar, 12 weeks)											
Glass filaments, ES 5	39	5.5	250 (lap.)	28	7.1	91	109	125	141	76	77
Experiment 5 (Wistar, 15 weeks)											
Glass filaments, ES 3	16.5	3.7	50 (lap.)	16	6.3	68	91	115	135	71	90
Glass filaments, ES 3	16.5	3.7	250 (lap.)	16	8.7	67	91	115	139	87	116
Glass	(granular)		50 (lap.)	18	8.3	70	88	115	139	62	91
Glass	(granular)		250 (lap.)	18	8.3	75	99	115	130	91	101
Saline			1 ml (lap.)	15	1.1	68	87	110	139	95	98
						(life-span reduced by inf. in month 15)					
Experiment 6 (Wistar, 12 weeks)											
Anthophyllite, UICC	2.6	0.61	2	37	10.8	71	84	105	139	102	112
Anthophyllite, UICC	2.6	0.61	10	39	15.6	71	84	105	133	67	91
Chrysotile UICC A milled	0.2	0.02	10	39	2.6	70	71	98	121	98	98

Experiment 6 (Wistar, 12 weeks)											
Anthophyllite, UICC	2,6	0,61	2	37	10,8	71	84	105	139	102	112
Anthophyllite, UICC	2,6	0,61	10	39	43,6	71	84	105	133	67	94
Chrysotile, UICC A milled	0,2	0,02	10	39	2,6	70	71	98	124	98	98
Glass fibres, 106	2,2	0,17	10	39	5,4	72	101	119	137	86	98
Nemalite	1,3	0,06	2	37	75,7	60	69	87	120	51	71
Nemalite	1,3	0,06	10	40	80,0	42	54	61	79	31	53
Quartz, DQ 12	(granular)		10	34	5,9	71	73	102	139	102	107
Corundum	(granular)		10	35	8,6	72	80	115	121	103	111
						(life-span reduced by inf. in month 16)					
Experiment 7 (Wistar, female (f) and male (m), 3 weeks)											
Glass fibres, 104 1974, Ch. 2	3,5	0,3	10	26 f	50,0	6	37	54	112	17	54
Glass fibres, 104 1974, Ch. 2	3,5	0,3	10	33 m	54,6	11	15	54	67	18	11
Experiment 8 (Wistar, 12 weeks)											
Chrysotile, UICC B milled	0,56	0,06	50	41	2,4	48	62	78	119	99	99
Actinolite, F.R.G.	1,9	0,17	2,5	45	66,7	40	56	62	71	38	53
						(life-span reduced by inf. in month 10 (14)					
Experiment 9 (Wistar, 9 weeks)											
Attapulgite, Mormoiron	0,7	0,07	60 (5 inj.)	114	3,5	92	116	138	164	47	92
Attapulgite, Lebrija	0,5	0,07	60 (5 inj.)	115	3,5	95	116	134	164	98	111
Attapulgite, Georgia	0,8	0,04	60 (5 inj.)	112	3,6	89	108	129	163	75	100
β-ferric oxide hydrate (1)	0,5	0,07	135 (5 inj.)	111	18,9	96	121	138	160	62	111
Corundum	(granular)		90 (5 inj.)	115	3,5	93	112	135	164	95	119
Titanium dioxide	(granular)		90 (5 inj.)	113	5,3	96	120	137	164	92	119
Experiment 10 (Sprague-Dawley, 8 weeks)											
Glass fibres, 104 1974, Ch. 1	4,8	0,29	5	54	81,5	52	64	79	108	40	67
Glass fibres, HCl-treated 2 h			5	54	59,3	75	88	105	133	68	93
Glass fibres, HCl-treated 24 h	5,3	0,5	5	54	7,4	78	93	108	142	102	111
Glass fibres, NaOH-treated 2 h			5	54	77,8	54	71	83	115	35	69
Glass fibres, NaOH-treated 24 h	5,4	0,5	5	53	86,8	61	72	84	106	42	72
Erionite, Turkey	2,9	0,38	1,25	53	71,7	64	83	97	130	41	82
Erionite, Turkey	2,9	0,38	5	53	81,1	45	52	68	115	32	54
Erionite, Turkey	2,9	0,38	20	53	69,8	35	41	51	72	30	41
Titanium dioxide	(granular)		5	52	5,8	77	99	109	142	86	97
Experiment 11 (Wistar, 1 weeks)											
Glass fibres, 104 1974, Ch. 1	4,8	0,29	5	45	44,4	7	34	53	65	31	19
Glass fibres, HCl-treated 24 h	5,3	0,5	5	45	4,4	104	113	131	146	112	126
Glass fibres, NaOH-treated 24 h	5,4	0,5	5	46	58,7	23	58	71	103	29	64
Erionite, Turkey	2,9	0,38	5	48	70,8	50	61	76	145	10	66
Actinolite, F.R.G.	1,9	0,17	0,5	59	91,5	57	66	80	122	45	69
Titanium dioxide	(granular)		5	47	0,0	84	102	120	145	-	- -

Table 1 (continued)

Dust	Fibre dimens. [µm]		Dose i.p. [mg]	Number of rats examined	Rats with tum. [%]*	Life-span [weeks] after first treatment of all rats				Rats with tum.	
	length 50% ≥	diameter 50% ≥				20% ≥	50% ≥	80% ≥	100% ≥	first	average
Experiment 12 (Sprague-Dawley, 8 weeks)											
Glass fibres, 100 Pen	2.4	0.33	2	54	38.9	79	98	112	134	53	99
Glass fibres, 100 Pen	2.4	0.33	10	53	45.3	68	79	91	126	53	83
Glass fibres, 100 E, A V	4.4	0.32	2	54	48.1	72	93	106	122	52	92
Rock wool, Sweden	23.0	1.9	75 (3 · 25)	63	71.4	61	77	96	134	38	79
Rock wool, Sweden, fine	4.1	0.64	10	45	13.3	88	97	115	134	88	109
Volcanic ash, St. Helen's	(granular)		10 (2 × 20)	54	5.6	74	93	116	134	79	87
NaCl-sol.	—	—	2 · 2 ml	54	5.6	79	94	109	134	91	100
Experiment 13 (Wistar, 5 weeks)											
Attapulgite, Caesey	1.3	0.07	10 (2 + 4 + 4)	30	40.0	94	109	132	142	71	116
Erionite, Oregon	1.8	0.21	0.5	31	48.4	81	108	120	142	63	98
Erionite, Oregon	1.8	0.21	2.0	31	90.3	60	79	93	118	51	80
Actinolite, E.R.G.	1.9	0.17	0.3	39	79.3	58	75	92	141	45	75
Actinolite, PVNO separately	1.9	0.17	0.3	32	65.6	61	80	106	142	39	77
Actinolite, in 1 ml 2% PVNO	1.9	0.17	0.3	29	48.3	87	117	135	142	95	121
PVNO separately											
Chrysotile, UICC B	0.9	0.11	1.0	32	84.4	41	51	68	134	30	56
Chrysotile, PVNO separately	0.9	0.11	1.0	30	80.0	46	66	90	136	38	66
Chrysotile, Calidria	1.2	0.03	0.5	32	6.3	93	116	135	142	81	106
Crocidolite, South Africa	2.1	0.20	0.5	32	56.3	84	103	132	141	70	110
Crocidolite, South Africa	2.4	0.20	2.0	32	87.5	58	71	81	103	32	71
Glass fibres, 104 475	3.2	0.18	0.5	30	16.7	95	116	138	142	86	113
Glass fibres, 104 475	3.2	0.18	2.0	31	25.8	81	110	127	142	84	107
Glass fibres, 104-treated 24 h			2.0	32	50.0	93	107	129	141	56	105
Kevlar fibres (1)			10 (2 + 4 + 4)	31	12.9	109	124	139	142	126	133
Titanium dioxide	(granular)		10 (2 + 4 + 4)	32	0.0	103	130	142	142	—	—
Saline	—	—	1 · 1 ml	32	0.3	97	120	142	142	113	117

* Animals with sarcoma, mesothelioma or carcinoma in the abdominal cavity excluding tumours of the uterus; percentage of rats examined

nickel compounds to Fischer rats. Cadmium sulfide also induced local sarcomas in rats after i.m. and s.c. injection (KAZANTZIS and HANBURY 1966).

All three nickel-containing dusts caused lung tumours after intratracheal instillation (table 4). In relation to the dose, nickel subsulfide had the strongest effect, nickel powder a slightly smaller one while nickel oxide was clearly the least effective.

Since cadmium sulfide has a considerably lower acute toxicity than cadmium chloride and cadmium oxide it could be dosed much higher and, depending on the dose level, led to lung tumours (table 4). The highest dose of 10 mg cadmium in cadmium sulfide caused early death of some animals. The tumour rate therefore appears to be too low. Also, after inhalation, cadmium sulfide seems to induce lung tumours in rats (OLDIGES and GLASER 1986) but not in hamsters and mice (HEINRICH et al. 1986c).

Most surprising was the high lung tumour rate of 69% after 15 intratracheal instillations of 15 mg each of very fine granular magnetite. This raises the question of whether an unspecific reaction of the rat lung to the large surface of the particles led to the tumours. This possibility is discussed for the inhalation of high concentrations of TiO_2 (LEE et al. 1985). But in contrast to other ferric oxides, magnetite could also have a chemically carcinogenic effect.

6. Other granular dusts

As non-carcinogenic control dusts we took corundum (experiments 6, 9), titanium dioxide (experiments 9, 10, 11, 13, 15), glass powder (experiments 2, 5) and volcanic ash (experiment 12). The tumour rates were between 0 and about 10%. Besides the above-mentioned metal compounds quartz was the only non-fibrous mineral dust which had a low tumour inducing effect after i.p. injection of a higher dose (40 mg, experiment 2). HOLLAND et al. (1986) found lung tumours in rats after inhalation of quartz dust. GROTH et al. (1986) after intratracheal instillation, and WAGNER and BERRY (1969) after intrapleural administration.

Polyvinylchloride (PVC) was injected in a very high dose (5 × 100 mg), but the preliminary result (experiment 15) shows no clear carcinogenic effect. Since the density of PVC is low, the animals received a high dust volume. In addition to a chemical carcinogenic effect, foreign body-induced tumours could be expected from the high mass which was deposited in clumps (OPPENHEIMER et al. 1948; BRAND 1986). The low tumour rate found after i.p. injection of high masses of granular dusts indicates that the carcinogenicity of fibres cannot be explained by a simple foreign body effect.

Beech and oak dusts are known to induce carcinomas in the nose in humans. In experiment 15 we tried to assess whether the i.p. test is sensitive for the unknown carcinogenic agent. The preliminary results (table 3) do not reveal an existing sensitivity.

Conclusions

The length and durability of fibres are of great significance for their carcinogenic potency. It should be re-examined how far this also applies to the diameter because the effect of relatively thick rock and ceramic fibres was unexpectedly strong. Further possible explanations could be surface properties or an especially strong carcinogenicity of very long fibres (>20 µm), which have been quite numerous in the samples used. A second measurement of several of the used fibre samples is necessary for a better evaluation of the relation between fibre dimensions and carcinogenic effect. The available data of the fibre size distributions are not comparable in all cases because measurements were carried out at different times using different methods and by different working groups.

The intraperitoneal test in rats is easy feasible and is proved to be very sensitive for detecting the carcinogenic effect of durable natural and man-made mineral fibres as well as metal compounds with low acute toxicity. This means that, if a high dose of one of these dusts does not induce tumours in this test, no suspicion of carcinogenic potency worth mentioning can be substantiated. Maybe the present examples are too few to be certain

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Tumorigenic Effect of Fibrous Dusts in Experimental Animals

by F. Pott,* F. Huth,* and K. H. Friedrichs*

Fibrous dusts (chrysotile, glass fibers, nemalite, palygorscite, and gypsum) and granular dusts (actinolite, biotite, hematite, pectolite, sanidine, and talcum) were injected intraperitoneally into rats. The fibrous dusts (other than gypsum) resulted in a high incidence of mesothelioma (30 – 67%). Gypsum produced only 5% and granular dusts none at all. It is suggested that the fibrous shape leads to a high multiplication rate of cells and predisposes to tumor formation. Fibrosis, in the other hand, does not so predispose. Milled chrysotile with 99.5% fibers than 5 μm in length are carcinogenic in our experience. The carcinogenicity of glass fibers in our experiments may have significance for occupational situations.

The starting point of our investigations was the question whether the tumorigenic effect of asbestos fibers depends on physicochemical properties of the fiber or the shape of the fibers which are characteristic for all kinds of asbestos. For this purpose chemically different asbestos forms were compared to chemically similar dusts having different forms.

Dusts Tested

Tables 1 and 2 list the dusts tested in the animal experiments with respect to their chemical composition, particle shape, fiber length, and particle size. The fiber length and particle size were estimated by evaluation of electron micrographs.

Experimental Methods

The dusts were injected intraperitoneally in Wistar rats. We could not see any difference between the reaction of peritoneum and pleura. In addition, the injection did not essentially disturb the general status of the animals.

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Table 1. Estimation of fiber length of fibrous dust by Electron microscopy.

Dust	Chemical composition	Fiber length	
		<2μm,%	<5μm,%
Chrysotile A	Mg silicate	78.7	93.9
Chrysotile (milled)	Mg silicate	97.4	99.8
Glass	Na, Ca borosilicates	49.9	72.6
Gypsum	Ca sulfate	65.0	95.0
Nemalite	Mg hydroxide	91.5	96.4
Palygorscite	Mg, Al silicates	37.5	70.0

Table 2. Estimation of particle size of grain dusts, by electron microscopy.

Dust	Chemical composition	Particle size	
		<2 μm, %	<5 μm, %
Actinolite	Ca, Mg, Fe silicates	a	a
Biotite	K, Fe, Mg, Al silicates	86.5	96.3
Hematite	Fe oxide (precipitated)	a	a
Hematite	Fe oxide (mineral)	a	a
Pectolite	Ca, Na silicates	86.2	98.4
Sanidine	K, Al silicates	85.6	97.2
Talc	Mg silicate	a	a

[a] No particle size analysis possible.

The best experimental method would be the inhalation of the dusts, but this method leads to

enormous technical difficulties. The time required to produce tumours following injection of dusts is already long. This time would be prolonged tremendously by an inhalation method, since it takes months to get an effective dose at the vulnerable cell structures. The necessary concentration time of the dusts and the time to produce the tumors may even exceed normal life span.

The test dusts were suspended in saline solution at concentrations up to 25 mg/2 ml. For higher dosages, the injections were repeated once a week. The different test groups consisted 40 rats. Pure saline was injected in 80 control animals. The rats were observed until spontaneous death or sacrifice. All tumors were studied histologically.

Results

The UICC standard asbestos as well as palygorscite, nemalite, and glass fibers induced tumors in the abdominal cavity of 30–67% of the rats. Several dusts chemically closely related to chrysotile (like actinolite, biotite, pectolite, talcum), but of granular or platy shape with only a few fibers did not lead to the development of tumors except for a few cases. Calcium sulfate (gypsum) did show a fibrous shape but dissolved in the animal tissue and induced tumors only in 5% of the animals. Table 3 shows the tumor rate within the different experimental groups. Histologically nearly all the tumors were sarcomatous mesotheliomata.

In current experiments the lowest effective dosage of glass fibers was 2 mg/rat. The glass fibers were uncoated. The average diameter of the glass fibers was about 0.5 μm. With this dosage the first mesotheliomata occurred at 17 months. It seems of importance that 2 mg of glass fibers induced only slight adhesions around the liver lobes; 10 mg led to slight or medium adhesions and fibrous alterations, especially around the liver and the stomach; 50 mg of fiber dust induced tremendous adhesions on all abdominal organs with strong fibrosis. The fibrotic alterations generally could be differentiated from the numerous tumors. With 100 mg glass fiber only 6.5 months were necessary for tumor induction in the first rat.

Activity of Fibers within the Tissues

We are confronted with the question: Why do special shapes of particles lead to formation of

Table 3. Tumor rate after intraperitoneal injection of fibrous and granular dusts.

Dust	Doses (i.p.), mg	Time required to produce first tumor in animals of group, days	Tumor rate, %
Chrysotile A	6	343	67.5
Chrysotile A	25	276	65
Chrysotile A	4 × 25	270	37.5
Chrysotile A (milled)	4 × 25	400	30
Glass fibers	4 × 25	197	57.5
Nemalite Mg_3O_4Fe	4 × 25	249	62.5
Palygorscite	4 × 25	257	65
Gypsum	4 × 25	546	5
Pectolite	4 × 25	569	2.5
Sanidine	4 × 25	743	2.5
Talcum	4 × 25	587	2.5
Actinolite	4 × 25	—	—
Biotite	4 × 25	—	—
Hematite (precipitated)	4 × 25	—	—
Hematite (mineral)	4 × 25	—	—
NaCl (control)	4 × 2 ml	—	—

tumors? We don't have a complete answer, but, we now make the following speculation. Animal experiments and the histological investigation showed formation of tumors in the mesothelium in an overwhelming degree. Some authors have reported lung tumors following asbestos application. In our experiments subcutaneous injection of chrysotile A in one case only led to tumors in the subcutaneous connective tissue space. In addition, the storage of fibers by the lymph nodes never induced tumors of the lymphatic system. These observations suggest that tissues have different vulnerabilities to fibrous dusts. Epithelial and epithelium-like cells obviously conflict with fibrous dusts in an intensive degree; this intensive contact can be well observed in cell cultures. Beck and Bruch *(1)* demonstrated by electron microscopy a partial invagination of long asbestos and glass fibers by fibroblasts (L-cells) of mice. Although these cells can not phagocytose the fibers completely they remain dense around the foreign bodies. Such a relation between fiber and cells could support a chronically enhanced reproduction of cells within quickly regenerating epithelial and mesothelial cells. For decades it has been known that in epithelial organs like the liver and the respiratory tract chronically enhanced regeneration can change to abnormal

[illegible] and finally the tumorous cell [illegible].

[illegible] for discussion the question of [illegible] the reported reaction of mesothelial [illegible] to fibrous particles could support the old [illegible] theory in carcinogenesis.

Finally, let us emphasize that the fibrosis induced by the fibrous dusts cannot be regarded as starting point of the malignant degeneration. Even around chrysotile A induced tumors in most of the rats: those animals of this group that died without any tumor showed only slight degrees of adhesion.

The Problems of Dose–Response Correlation

The carcinogenesis depends on the shape factor of the dusts. It would be ideal for animal experiments if the fibers were of identical diameter and of identical length within one specimen. Then the dosage could be determined by the amount of fibers instead of weight. This has so far been impossible to achieve, however. The question of minimal and maximal length and diameter of the fibers necessary for a carcinogenic effect can still not be answered completely. In contrast to Wagner, Berry, and Timbrell [2] we are inclined to believe that even short fibers less than 10 μm in length, can induce tumors. The milled chrysotile A contained only few fibers exceeding 10 μm in length, and most of the fibers were shorter than 5 μm. We also do not believe that the cancerogenic effect can be limited to fibers with a diameter less than 0.5 μm. Since 2 mg of fibers with a diameter of 0.5 μm and a length of 20 μm induced tumors in rats, 200 mg of fibers with a diameter of 5 μm would have to be injected to reach the same amount of fibers. We have not yet determined whether such a dosage can be survived by the animals long enough. We suppose that the maximum diameter of an effective fiber is limited by the length of the fiber that still can induce damage to the membrane of a cell. This size may range between 1 and 3 μm.

We believe that glass fibers are a better test dust for further investigations than asbestos, as glass fiber specimens with nearly identical fiber diameters can be better obtained.

To reach a better relation of the dosage and the amount of fibers we have developed a nomogram. Following measurement of sufficient fibers diameters and lengths, the number can be estimated by the nomogram.

Correlation of Experimental Results with Human Morbidity

Since glass fibers can induce tumors like those caused by asbestos fibers we would recommend precaution for all industrial plants in which there are high concentrations of fibers measuring less than 3 μm in diameter. To date, no tumors have been found in workers of the glass fiber industry, but, according to reports of the industry, thinner glass fibers (less than 5 μm in diameter) have only been in production for the last 10 years in Germany. Since the time of tumor manifestations is 20–40 years in asbestos workers, there is no epidemiological proof possible before 1985 that these experimental results apply to human morbidity.

REFERENCES

1. Beck, E. G., and Bruch, J. Effet des poussières fibreuses sur les macrophases alvéolaires et sur d'autres cellules cultivées in vitro. Rev. Franc. Mal. Resp. 2: 72 (1974).
2. Wagner, J. C., Berry, G., and Timbrell, V. Mesotheliomata in rats after inoculation with asbestos and other materials. Brit. J. Cancer 28: 173 (1973).