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Relationship Between Lung Asbestos Fiber Type and Concentration and Relative Risk of Mesothelioma

A Case–Control Study

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Lung tissue from 221 definite and probable cases of malignant mesothelioma reported to the Australian Mesothelioma Surveillance Program from January 1980 through December 1985 and from an age–sex frequency matched control series of 359 postmortem cases were examined by light microscopic (LM) and analytical transmission electron microscopic (TEM) analysis and energy dispersive x-ray analysis (EDAX). Concentrations of total fibers (coated and uncoated) (LM), crocidolite, amosite, chrysotile, and unidentified amphibole (TEM) (fibers/g dry lung tissue) were measured. Fiber concentrations < 10 μm in length and ≥ 10 μm in length were separately quantified. By comparing cases (221) and controls (359 LM, 103 TEM), odds ratios for increasing fiber concentrations compared with < 15 000 fibers/g (LM) and < 200 000 fibers/g (TEM) (the respective detection limits) were calculated. Univariate analyses showed statistically significant dose–response relationships between odds ratio and fiber concentration for all fiber concentration measures. The relationship between log(odds ratio) and log(fiber concentration) was linear. Multiple logistic regression analysis showed that a model containing crocidolite ≥ 10 μm, amosite < 10 μm, and chrysotile < 10 μm as explanatory variables best described the data. The odds ratios for a ×10 increase in fiber concentration (fibers/μg) were as follows: crocidolite ≥ 10 μm, 29.4 (95% confidence interval [CI], 3.6 to 241); chrysotile < 10 μm, 15.7 (95% CI, 6.1 to 40); amosite < 10 μm, 2.3 (95% CI, 1.0 to 5.3). An additive risk model gave similar results. In a subgroup of cases and controls with only chrysotile in the lungs, a significant trend in odds ratio with increasing fiber content was found. *Cancer* 67:1912–1920, 1991.

AFTER THE ORIGINAL OBSERVATIONS of Wagner *et al.*,[1] subsequent research in animals and humans has now shown a clear causal link between exposure to certain types of asbestos fiber and malignant mesothelioma of the pleura and peritoneum. In reviewing the literature, Seaton[2] noticed an increasing gradient of potential to cause mesothelioma in humans from chrysotile (least) through amosite to crocidolite (greatest). Animal studies suggest that fiber geometry rather than chemistry is the significant factor in carcinogenic activity, with fibers greater than 8 μm in length and less than 0.25 μm in diameter being the most carcinogenic.[3,4]

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TABLE 2. Distribution of Fiber Concentration by Fiber Length: Light Microscopic Study, Uncoated Fibers

		<10 μm					≥10 μm				
		Mesothelioma cases		Controls		Odds ratio	Mesothelioma cases		Controls		Odds ratio
		No.	Percent	No.	Percent	95% Cornfield CI	No.	Percent	No.	Percent	95% Cornfield CI
f/g	0–<15,000	21	9.5	224	62.4		34	15.4	187	52.1	
$\log_{10}$ (f/g)	4.2–4.5	21	9.5	59	16.4	3.8 (1.9–7.8)	22	10.0	68	18.9	1.8 (0.9–3.4)
	4.5–5	53	24.0	63	17.5	9.0 (4.9–16.7)	68	30.8	78	21.7	4.8 (2.9–8.1)
	5–5.5	64	29.0	9	2.5	75.9 (31.1–191.2)	48	21.7	22	6.1	12.0 (6.2–23.6)
	5.5–6	30	13.6	1	0.3	165.3 (50.9–597)	26	11.8	1	0.3	67.4 (21.4–236)
	6–6.5	16	7.2	3	0.8		11	5.0	3	0.8	
	6.5–7	4	1.8	0			4	1.8			
	7–8	9	4.1	0			7	3.2			
	8–9	3	1.4	0			1	0.5			
		$X^2_1 = 244.9$ ($P < 0.0005$) (trend)					$X^2_1 = 148.3$ ($P < 0.0005$) (trend)				

CI: confidence interval; f/g: fibers per gram of dried lung tissue.

The current study further quantifies the dose–response relationship between risk of mesothelioma and asbestos exposure by comparing lung fiber content, by type, and length, in a large series of cases collected through a very complete national surveillance program,[20] with that in a control group of lung tissue samples collected over the same period as the cases. The method is similar to that used in previously reported studies but a much larger series of cases (221) is analyzed and only definite or probable mesothelioma cases are accepted.

Materials and Methods

Cases

Cases were obtained from notifications to the Australian Mesothelioma Surveillance Program.[20] This program operated between January 1, 1980 and December 31, 1985. Formal voluntary notifications were sought from clinicians and pathologists throughout Australia. Cross-checks were made with registrations in statutory cancer registries in each state of Australia.

Diagnosis of mesothelioma was based on histologic grounds. A panel of five experienced pathologists appointed by the Royal College of Pathologists of Australasia made a histologic classification of definite, probable, possible, and not mesothelioma, scored as 1, 0.75, 0.50, and 0, respectively. The definitive diagnosis was regarded as the category with score nearest to the mean score. Only definite or probable mesotheliomas were included in the current study. The total number of definite or probable mesothelioma cases diagnosed in the period was 697. In 221 of these cases (209 definite, 12 probable) formalin preserved postmortem lung tissue material was available for fiber analysis. Postmortem material from five possible cases was also available but was excluded from the current study. Two hundred seven cases were pleural and 14 were

TABLE 3. Distribution of Fiber Concentration by Fiber Length: Light Microscopic Study, Coated Fibers

		<10 μm					≥10 μm				
		Mesothelioma cases		Controls		Odds ratio	Mesothelioma cases		Controls		Odds ratio
		No.	Percent	No.	Percent	95% Cornfield CI	No.	Percent	No.	Percent	95% Cornfield CI
f/g	0–<15,000	71	32.1	333	92.8		75	33.9	264	73.5	
$\log_{10}$ (f/g)	4.2–4.5	28	12.7	11	3.1	11.9 (5.4–26.9)	28	12.7	50	13.9	2.0 (1.1–3.5)
	4.5–5	47	21.3	11	3.1	20.0 (9.5–43.3)	50	22.6	30	8.4	5.9 (3.4–10.2)
	5–5.5	30	13.6	4	1.1	87.9 (29.7–293)	26	11.8	12	3.3	7.6 (3.5–16.9)
	5.5–6	26	11.8				25	11.3	2	0.6	44.0 (9.8–275)
	6–6.5	7	3.2				5	2.3	1	0.3	59.8 (8.2–1225)
	6.5–7	5	2.3				5	2.3			
	7–8	7	3.2				7	3.2			
		$X^2_1 = 231.0$ ($P < 0.0005$) (trend)					$X^2_1 = 126.8$ ($P < 0.0005$) (trend)				

CI: confidence interval; f/g: fibers per gram of dried lung tissue.

TABLE 6. Distribution of Fiber Concentration by Fiber Length: Transmission Electron Microscopic Analysis, Crocidolite

		<10 μm					≥10 μm				
		Mesothelioma cases		Controls		Odds ratio	Mesothelioma cases		Controls		Odds ratio
		No.	Percent	No.	Percent	95% Cornfield CI	No.	Percent	No.	Percent	95% Cornfield CI
f/g	0–<200,000	64	29.0	39	37.9		132	59.7	89	86.4	
$\log_{10}$ (f/g)	5.3–5.5	22	10.0	18	17.5	0.7 (0.3–1.7)	18	8.1	11	10.7	1.1 (0.5–2.6)
	5.5–6	54	24.4	28	27.2	1.2 (0.6–2.3)	31	14.0	2	1.9	10.5 (2.4–64.8)
	6–6.5	30	13.6	15	14.6	16.5 (4.6–70.1)	20	9.0	1	1.0	27.0 (3.9–537)
	6.5–7	21	9.5	3	2.9		9	4.1			
	7–8	17	7.7				7	3.2			
	8–9	10	4.5				4	1.8			
	9–10	3	1.4								
				$X^2_1 = 13.87$ ($P < 0.0005$) (trend)					$X^2_1 = 31.66$ ($P < 0.0005$) (trend)		

CI: confidence interval; f/g: fibers per gram of dried lung tissue.

Light microscopic fiber counts were made on all 221 cases and 359 controls. Electron microscopic fiber counts, EDAX, and fiber length measurements were made on all 221 cases and 103 male controls, drawn randomly from the total group of 276.

Results

Distributions of fiber content determined by light microscopic study and TEM (total and by fiber type and length) are shown for cases and controls in Tables 2 through 8.

Dose–response analysis of relative risk was carried out by grouping fiber content levels into strata and calculating the relative risk (estimated by odds ratio) for each stratum of exposure compared with zero exposure (defined as < 15 000 fibers/g by light microscopic study, or < 200,000 fibers/g by TEM). Where no controls were exposed, categories were pooled, as otherwise odds ratios are infinite. The Mantel chi-square test, with one degree of freedom for trend, was used to test the significance of linear trends of relative risk with dose. Tables 2 through 8 show univariate analyses of relative risk as a function of fiber content, type, and length.

A progressive increase in relative risk with increasing fiber content can be seen for all fiber content measures. Tests for trend are highly significant in all cases.

The increase in relative risk is apparent at the lowest dose levels but relative risk increased more rapidly for dosage greater than 3×10^5 ($10^{5.5}$) fibers/g (light microscopic study) and 10^6 to $10^{6.5}$ fibers/g (TEM). At the higher dose levels, there were often no exposed controls and many exposed cases.

The relative risks for ≥ 10 μm chrysotile, crocidolite, and total amphibole fibers are greater than the corresponding risks for fibers < 10 μm.

When cases and controls are compared in relation to exposure to single-fiber types only, the number of cases and controls for analysis is much lower. However, increased relative risks for lung fiber contents greater than 200,000 fibers/g (all lengths) are still seen for single-fiber type lung contents of chrysotile and crocidolite. Fifteen

TABLE 7. Distribution of Fiber Concentration by Fiber Length: Transmission Electron Microscopic Analysis, Unidentified Amphibole (<10 μm)

		Mesothelioma cases		Controls		Odds ratio
		No.	Percent	No.	Percent	95% Cornfield CI
f/g	0–<200,000	155	70.1	88	85.4	
$\log_{10}$ (f/g)	5.3–5.5	20	9.0	11	10.7	1.0 (0.5–2.4)
	5.5–6	24	10.9	3	2.9	4.5 (1.3–19.5)
	6–6.5	19	8.6	1	1.0	12.5 (1.7–253)
	6.5–7	2	0.9			
	7–8	1	0.5			
				$X^2_1 = 14.17$ ($P < 0.0005$) (trend)		

CI: confidence interval; f/g: fibers per gram of dried lung tissue.

TABLE 11. Multiple Logistic Regression Coefficients

Predictor variable (all fiber concentrations) $\log_{10}$ (f/μg)		Model 1			Model 2		
		b	SE	*P*	b	SE	*P*
Crocidolite	≥10 μm	3.25	1.19	0.006	3.38	1.07	0.002
	< 10 μm	−0.12	0.40	0.77			
Amosite	≥10 μm	0.45	0.90	0.62			
	<10 μm	0.57	0.61	0.35	0.84	0.42	0.05
Chrysotile	≥10 μm	6.40	5.16	0.22			
	<10 μm	2.70	0.54	0.00001	2.75	0.48	0.00001
Unidentified	≥10 μm	32.3	46.7	0.49			
Amphibole	<10 μm	1.29	1.11	0.25			
Age (yr)		0.003	0.02	0.81			
−2 log L			293.7			301.9	

SE: standard error; f/μg: fibers per microgram of dried lung tissue.

were not reported, nor were tests for trend carried out. Reanalysis of their data,[6] in fact, shows an odds ratio of 3.8 (95% CI 1.8 to 8.0) for > 10^6 fibers/g compared with ≤10^6 fibers/g amosite and crocidolite combined and an increasing gradient of odds ratio with fiber content for amosite, crocidolite, and anthophyllite in men and women. This gradient was apparent for chrysotile only in the high (> 10^6 fibers/g) amphibole subgroup of those exposed to both chrysotile and amphibole. The dose–response relationship for tremolite was U-shaped. Similarly, reanalysis[6] of the data of Wagner *et al.*[17] (a study of 86 mesothelioma cases and 56 age-matched controls) gave an odds ratio of 7.4 (95% CI 3.5 to 16) for > 10^6 *versus* 10^6 fibers/g combined amosite and crocidolite. A dose–response gradient was not reported or recalculated.

Mowé *et al.*,[8] in a study of 14 mesothelioma cases and 28 matched controls in Norway, showed an odds ratio of 8.5 (95% CI 2.3 to 31) for lung content > 10^6 fibers/g compared with ≤ 10^6 fibers/g.

Gaudichet *et al.*[18] compared fiber content in 20 mesothelioma cases to four different age–sex matched control sets (squamous cell carcinoma of lung, adenocarcinoma of lung, metastatic carcinoma in lung, cardiovascular disease). Increased amphibole content was found in cases compared with controls for each control comparison, particularly with crocidolite and amosite and especially crocidolite and amosite > 8 μm in length. No difference in chrysotile content between cases and any control series was found. There were increased numbers of coated fibers in cases compared with controls. Dose–response relationships were not reported.

In a more recent one–one matched case–control study on 78 cases identified by pathologists in Canada over the period 1979 through 1984, McDonald *et al.*[19] showed a clear dose–response relationship between mesothelioma risk and lung concentrations of long (≥ 8 μm) and short (< 8 μm) crocidolite, amosite, and tremolite. However, multiple binary regression analysis showed that the relative risk was related only to fibers ≥ 8 μm in length. From their Table 1, it can be computed that the relative risk (odds ratio) for total amphiboles in concentration ≥ 1 fiber/μg (10^6 fibers/g) compared with concentration less than 1 fiber/μg is 2.2 (95% CI 1.1 to 4.4) for fibers < 8 μm and 6.6 (3.0 to 14.6) for fibers ≥ 8 μm. Although a detailed matching procedure was used in this study, it is unfortunate that the criterion for inclusion of a case in the analysis on pathologic grounds was not more stringent. Cases classed as definite, probable, possible, doubtful, and unclassifiable were all included. Among these only 33 were definite and 25 probable. This wide inclusion criterion may have resulted in tumors which were not mesotheliomas, and with low lung fiber counts, being included, with a resulting bias in the results against any asbestos effects, thus underestimating relative risks.

In making comparisons with the slightly different analysis of McDonald *et al.*,[19] in which linear multivariate

TABLE 12. Coefficients in Model of Form: $R = 1 + \sum b_i x_i$

Predictor variable (all fiber concentrations in fiber/μg)		Model 3			Model 4		
		b	95% CI	*P*	b	95% CI	*P*
Crocidolite	≥10 μm	9.1	2.7–23.1	<0.0001	9.4	2.7–24.1	<0.0001
Amosite	≥10 μm	5.2	0.4–21.7	0.004			
	<10 μm				1.9	0.1–6.4	0.011
Chrysotile	<10 μm	7.0	3.5–12.3	<0.0001	7.6	3.9–13.3	<0.0001
−2 log L		305.3				307.1	

CI: confidence interval.

risk models for long (≥ 8 μm) and short (< 8 μm) fibers were separately fitted, it can be seen that we found lower coefficients for crocidolite and amosite and a higher coefficient for chrysotile. However, our coefficients are estimated with greatly shortened confidence intervals. We confirm that long amphibole fibers are more important than short, but differ somewhat in the relative effects of crocidolite, amosite and chrysotile.

There is a clear dose response relationship in the current study. Although the negative correlation of chrysotile < 10 μm with age is consistent with a clearance effect, there was a significant dose response effect for short chrysotile, independently of long amosite and long crocidolite. This is consistent with the analysis of the small group of cases and controls with only chrysotile found in the lungs which showed a statistically significant trend ($P < 0.005$) of relative risk of mesothelioma with lung fiber content (Table 9).

In the current study, we were also able to examine a much larger (light microscope) case–control analysis of coated fibers (asbestos bodies), and to examine long (≥ 10 μm) coated fibers separately. There was a very clear gradient of relative risk with coated fiber lung content, clearer still with fibers ≥ 10 μm (Table 3). This gradient was of the same order as for amosite and for crocidolite.

There are several possible selection biases operating in the current study. First, not all mesothelioma cases came to postmortem. The distribution by state of postmortem cases was closely comparable to the distribution by state of notification but there may have been other selection factors giving an increased chance of postmortem, *e.g.*, likelihood of compensation for a relative.

Secondly, the control group were patients coming to postmortem in one large referral hospital serving the whole of one state, New South Wales (NSW). New South Wales is the most populated state, with the largest number of mesothelioma notifications (426 in the study period). Whereas all controls would have been included as cases in the study, had they developed mesothelioma, their exposure background is more likely to have been that pertaining to NSW rather than to Australia as a whole. However, since most Australians live and work in cities with a similar mixture of industries and environments where asbestos exposure may occur, any bias due to this effect is likely to be small. Indeed, there is more opportunity for industrial and environmental exposure in NSW, so the restriction of controls to NSW would tend to underestimate relative risks.

In conclusion, this study has shown that, for Australia as a whole, the greatest risk of mesothelioma is associated with exposure to crocidolite ≥ 10 μm in length. It is difficult to assess the risks associated with chrysotile alone, due to almost universal mixed exposure to amphibole and chrysotile, and the possibility of differential clearance effects. In the current study there were two cases with only chrysotile found in the lungs and with an occupational history of exposure to chrysotile only. The results are generally consistent with the results of McDonald *et al.*,[19] except that a considerably greater relative effect of crocidolite ≥ 10 μm is apparent. This effect is very likely to be due to the wide distribution of Wittenoom crocidolite throughout Australia.

REFERENCES

1. Wagner JC, Sleggs CA, Marchand P. Diffuse pleural mesothelioma and asbestos exposure in the North Western Cape Province. *Br J Ind Med* 1960; 17:260–271.
2. Seaton A. Asbestos-related diseases. In: Morgan WKC. Seaton A, eds. Occupational Lung Diseases. Philadelphia: WB Saunders. 1984; 323–376.
3. Stanton MF, Layard M, Tegeris A *et al.* Relation of particle dimension to carcinogenicity in amphibole asbestoses and other fibrous minerals. *J Natl Cancer Inst* 1981; 67:965–975.
4. Davis JMG, Addison J, Bolton RE, Donaldson K, Jones AD, Smith T. The pathogenicity of long *versus* short fibre samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. *Br J Exp Pathol* 1986; 67:415–430.
5. Davis JMG, McDonald JC. Low level exposure to asbestos: Is there a cancer risk? *Br J Ind Med* 1988; 45:505–508.
6. Berry G, Rogers AJ, Pooley FD. Mesotheliomas: Asbestos exposure and lung burden. In: Bignon J, Peto J, Saracci R, eds. Non-Occupational Exposure to Mineral Fibres. IARC, Lyon, France. 1989; 90:486–496.
7. Rogers AJ. Determination of mineral fibre in human lung tissue by light microscopy and transmission electron microscopy. *Ann Occup Hyg* 1984; 28:1–12.
8. Mowé G, Gylseth B, Hartveit F, Skang V. Fiber concentration in lung tissue of patients with malignant mesothelioma. *Cancer* 1985; 56: 1089–1093.
9. Middleton AP, Beckett ST, Davis JMG. Further observations on the short-term retention and clearance of asbestos by rats, using UICC reference samples. *Ann Occup Hyg* 1979; 22:141–152.
10. Timbrell V. Deposition and retention of fibres in the human lung. *Ann Occup Hyg* 1982; 26:347–369.
11. Roggli VL, Brady AR. Changes in numbers and dimensions of chrysotile asbestos fibers in lungs of rats following short-term exposure. *Exp Lung Res* 1984; 7:133–147.
12. Roggli VL, George MH, Brody AR. Clearance and dimensional changes of crocidolite asbestos fiber isolated from lungs of rats following short term exposure. *Environ Res* 1987; 42:94–105.
13. Abraham JL, Smith CM, Mossman B. Chrysotile and crocidolite asbestos pulmonary fibre concentrations and dimensions after inhalation and clearance in Fischer 344 rats. *Ann Occup Hyg* 1988: (Suppl) 32: 203–211.
14. Wagner JC, Berry G, Skidmore JW, Timbrell V. The effects of the inhalation of asbestos in rats. *Br J Cancer* 1974; 29:252–269.
15. Middleton AP, Beckett ST, Davis JMG. A study of the short term retention and clearance of inhaled asbestos using UICC standard reference samples. In: Walton WH, ed. Inhaled Particles IV. Oxford: Pergamon Press, 1977; 247–257.
16. McDonald AD, McDonald JC, Pooley FD. Mineral fibre content of lungs in mesothelioma cases in North America. *Ann Occup Hyg* 1982: 26:417–422.
17. Wagner JC, Pooley FD, Berry G *et al.* A pathological and mineralogical study of asbestos-related deaths in the United Kingdom in 1977. *Ann Occup Hyg* 1982; 26:423–431.
18. Gaudichet A, Janson X, Monchaux G *et al.* Assessment by analytical microscopy of the total lung fibre burden in mesothelioma patients matched with four other pathological series. *Ann Occup Hyg* 1988; (Suppl) 32:213–223.
19. McDonald JC, Armstrong B, Case B *et al.* Mesothelioma and asbestos fiber type: Evidence from lung tissue analysis. *Cancer* 1989; 63: 1544–1547.
20. Ferguson DA, Berry G, Jelihovsky T *et al.* The Australian Mesothelioma Surveillance Program 1979–1985. *Med J Aust* 1987; 147: 166–172.