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CHRYSOTILE AND CROCIDOLITE ASBESTOS PULMONARY FIBRE CONCENTRATIONS AND DIMENSIONS AFTER INHALATION AND CLEARANCE IN FISCHER 344 RATS

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Abstract—Following inhalation exposure to aerosolised chrysotile (CH) or crocidolite (CR) asbestos, Fischer 344 rats were found to have similar lung fibre concentrations and size distributions. Average chamber concentrations were 10-15 mg/m^3. Exposures were nose only, 7 hrs/days, 5 days/week, 6 weeks for CH and whole chamber, 6 hrs/day, 5 days/week, 90 days for CR. To examine clearance from the lungs, animals were also sacrificed 90 days following end of exposure. Fibre number and dimensions in chamber aerosol and hypochlorite-digested lung tissues were measured on nuclepore filters using scanning electron microscopy. Both CH and CR aerosols showed similar size data. In the lungs of both CH and CR animals, we found longer and narrower fibres with greater aspect ratio than in the chamber aerosol. Ninety days after cessation of exposure, striking clearance of CH was seen (95%) but no measureable clearance of CR (by numbers of fibres). However, a mass loss was observed by X-ray diffraction and could be calculated from changes in fibre dimensions. The CH which was retained appears to be the longer, thinner fibres. This selective clearance is not seen with this particular CR exposure in the 90 days clearance time studied. Such differences between fibre types are likely to be important in interpreting observed epidemiological differences in response to different exposures. These studies reinforce the facts that nominal short fibre preparations may lead to retention of long fibres in the lung, even when the long fibres make up only a small fraction of the initial aerosol, and that electron microscopic counting and sizing of fibres in both aerosol and tissues are needed to optimally interpret fibre clearance data. The underlying mechanisms are incompletely understood. Further studies of comparative exposures using different exposures, varying times and different sized preparations of asbestos and other fibres will be informative.

INTRODUCTION

ASBESTOS-RELATED pulmonary fibrosis (asbestosis) is well recognised. Yet precise understanding of the relationships between fibre characteristics, tissue burden and inflammatory mechanisms remains elusive. This report presents comparative data on two types of asbestos fibre (chrysotile and crocidolite) clearance in the Fischer 344 rat.

Although the exposure conditions for chrysotile (nose only) and crocidolite (whole chamber) were different, the findings of closely matched lung fibre burden and dimensions at the end of exposure in the same species of rat suggested that comparison of fibre clearance would be both interesting and valid as an opportunity to observe clearance of different fibre types starting from a nearly common reference point. The clearance after 90 days of recovery was examined for this report.

Of course, the range of fibre lengths and diameter for chrysotile and crocidolite are not matched precisely. Ideally, monodisperse fibre preparations and/or aerosols would permit an exact match for everything but fibre chemistry. These results may have important implications regarding human disease. For example, if short asbestos fibres cause fibrosis but are nearly completely cleared, one could expect asbestosis without increased fibre burden. Conversely, if pulmonary retention of fibres is required for

development of asbestosis (which is implied by the definition of pneumoconiosis), the fibre burden will depend on relative clearance.

METHODS

Chrysotile (NIEHS, Jeffrey Mine) and crocidolite (NIEHS) asbestos were obtained from NIEHS (National Institute of Environmental Health Sciences, courtesy of Dr. A. Brody). Fischer 344 male rats were used for either exposure. Chrysotile exposures were performed in a nose-only chamber for 7 hrs/day, 5 days/wk for 6 weeks (210 hours total) (SMITH *et al.*, to be published). Crocidolite exposures were in chambers 6 hrs/day, 5 days/wk, 90 exposure days (540 hrs total) (HEMENWAY and MacASKILL, 1982). Both exposures were at nominal 10–15 mg/m^3.

For these studies, aerosol chamber samples were collected onto 0.2 μm nuclepore filters using calibrated flows of approximately 1 litre per filter for optimal density for subsequent scanning electron microscopy (SEM) analysis. Samples of formalin inflated lungs at sacrifice were coded, weighed, measured and digested in pre-filtered hypochlorite (laundry bleach) in a 15 ml test tube. After 30–60 minutes digestion was complete. The entire content of the tube was decanted, after gentle mixing without sonication, into a Nuclepore filter apparatus containing an 0.2 μm nuclepore filter. The tube and apparatus were rinsed with prefiltered water several times with all fluid being put through the filter. The filter was mounted with carbon suspension onto a carbon disc for SEM observation. SEM analysis of uncoated filters was done at 20 kV, 1 nanoamp specimen current by counting random fields at 10 000 × screen magnification, and measuring 100 fibres. Length and diameter were measured to the nearest 0.05 micrometre. Unit chrysotile fibres were resolved. Elemental analysis of a sub sample was performed using energy dispersive X-ray analysis. Blank filters prepared with everything except tissue, and control (non-exposed) lung tissues were similarly prepared. No fibres were detected in blanks or control lungs. No tremolite or other contaminant fibres have been detected among 3000 chrysotile fibres, and only a rare contaminant ($<1\%$) aluminium silicate fibre was noted in some of the crocidolite analyses. A microcomputer was used to calculate the number of fibres on the filter, the number per volume of aerosol or weight of tissue, and the fibre length and diameter distributions.

RESULTS

The numbers and dimensions of fibres in the lungs studied are shown in Table 1. In both aerosols, the similarity in length, diameter, aspect ratio and percent of fibres longer than 5 μm is evident. The chrysotile lung burden and dimensions at the end of exposure can be seen to be similar to that in the crocidolite exposed rats, with the exception being a larger fraction (45.7%) of fibres >5 μm length in crocidolite exposed rats than in chrysotile exposed (26.7%). After 90 days of clearance, the lung burden (in fibre number) in the chrysotile exposed rats has fallen to approximately 5% of the burden at the end of exposure, while in the crocidolite exposed rats, the concentration of fibres is apparently unchanged. However, when one calculates the mass of asbestos, using the dimensional data, the loss becomes evident. This means that fibres must be fragmenting longitudinally to give an increasing number while mass is being lost. If the

TABLE 1. CHRYSOTILE AND CROCIDOLITE ASBESTOS FIBRE EXPOSURE AND CLEARANCE IN FISCHER 344 RATS

	Fibre Concentration*	Length	Diameter	Aspect Ratio	% Fibres > 5 μm length
Chrysotile					
Aerosol	5.5×10^3(3)	3.7(0.6)	0.18(0.03)	23(8)	21.5(5.1)
Lung at End of Exposure (6 wks)	2.1×10^8(0.7)	5.3(1.0)	0.13(0.01)	52(6)	26.7(4.2)
Lung after 90 days Clearance	1.0×10^7(0.5)	13.1(0.8)	0.09(0.006)	183(13)	78.7(3.5)
Crocidolite					
Aerosol	26.6×10^3(14.0)	3.7(0.7)	0.19(0.03)	22.6(3.3)	21.4(7.4)
Lung at End of Exposure (90 days)	3.5×10^8(1.2)	6.2(0.3)	0.12(0.02)	52(6)	45.7(2.3)
Lung after 90 days Clearance	3.5×10^8(1.0)	5.7(0.5)	0.10(0.00)	58(2)	37.0(4.4)

* Values are arithmetic mean (s.d.); Aerosol Values are number/ml in the aerosol ($n = 4$). Tissue values are number/gram wet weight of lung ($n = 3$). Length and diameter measurements are expressed in micrometers.

mass loss were due to dissolution alone, the fibre concentration would also be decreasing with clearance. Table 2 shows the calculated mass of asbestos from the fibre concentration and dimensional data (mass per fibre was calculated assuming a cylindrical shape and a density of 2.5). Additional data on crocidolite mass in the right lower lobe were determined by X-ray diffraction (XRD) (courtesy of L. Shatos, U. of Vermont).

Further insight into the dimensional changes taking place from aerosol to lungs at end of exposure and in lungs after 90 days clearance may result from inspecting data such as those in Table 3. A decrease in the fraction of shortest fibres (< 2 μm) is seen for both chrysotile and crocidolite. A trend toward retention of longest fibres (> 20 μm) is much more evident with chrysotile than with crocidolite. The fraction of chrysotile fibres in the 2–5 μm length category decreased during 90 days clearance, but no such change was noted in the crocidolite-exposed rats. In the 5–20 μm length group, the fraction increased markedly in chrysotile but not in crocidolite. The role of the diameter in the deposition and clearance appears to be major, with those fibres

TABLE 2. CALCULATED MASS FROM FIBRE NUMBER AND DIMENSIONS COMPARED TO XRD

	At End of Exposure	90 Days Clearance	Aerosol
Chrysotile			
SEM	37.6 μg/gm ww	2.6 μg/gm ww	1.4 mg/m³
Crocidolite			
SEM	113.5 μg/gm ww	73 μg/gm ww	6.5 mg/m³
XRD	115 μg/RLL	72 μg/RLL	N.A.

XRD: X-ray diffraction
RLL: Right lower lobe
Mass from SEM data calculated in Table 5.

TABLE 3. FIBRE DIMENSIONS (% OF TOTAL FIBRES)

		Chrysotile			Crocidolite		
		Diameter					
		<0.1	−0.2	>0.2	<0.1	−0.2	>0.2
	Length						
Aerosol							
	<2	14	15	6	11	18	3
	—5	12	17	15	6	28	13
	−10	5	5	7	2	9	5
	−20	1	1	3	1	3	1
	>20	0	1	0	0	0	1
At End of Exposure							
	<2	17	11	0	11	9	1
	—5	20	18	7	11	19	4
	−10	9	5	1	8	12	6
	−20	5	2	0	8	5	4
	>20	1	2	1	1	1	1
+ 90 Days							
	<2	2	2	0	11	7	0
	—5	14	3	1	19	21	4
	−10	19	1	0	11	9	1
	−20	36	4	0	7	4	2
	>20	10	6	2	1	2	0

Length and Diameter in micrometres
Percentages are the mean of 3 or 4 determinations

<0.1 μm showing the greatest fractional increase in both chrysotile and crocidolite animals.

Table 4 presents the calculated absolute pulmonary concentrations, and Table 5 the calculated aerosol and tissue mass of fibres in the same size ranges as in Table 3. The dramatic decrease in chrysotile fibres in all size ranges (95% overall decrease during 90 days clearance) can be contrasted with the shift in distribution of sizes seen with crocidolite. Increases in the numbers of fibres of 2–5 μm length and decrease in those 5–20 μm length are roughly equal. Also, the number of thin fibres <0.1 μm diameter increased while the number of thicker fibres (>0.2 μm diameter) decreased. This lends further support to a physical breakdown of crocidolite fibres into shorter and thinner fibres, plus clearance. Although the lung burdens of chrysotile and crocidolite were similar in fibre concentration at the end of exposure (2.1 vs 3.5 × 10^8 fibres/g wet lung), the distribution of mass by dimension shows that most of the difference was due to more crocidolite in the 5-20 μm length range and in the >0.2 μm diameter range.

DISCUSSION

There are relatively few comparative experimental studies of pulmonary clearance including fibre dimensions as well as concentration. Using exposure and analytic techniques nearly identical to ours, ROGGLI and BRODY (1984) have shown similar results for chrysotile clearance and dimensional changes after 1 hour exposures in CD-1 rats. No data on numbers of fibres in the chamber aerosol were given, but aerosolised

TABLE 4. MILLIONS OF FIBRES PER GRAM OF WET LUNG BY DIMENSIONS

	Chrysotile			Crocidolite		
	Diameter					
	<0.1	−0.2	>0.2	<0.1	−0.2	>0.2
Length						
At End of Exposure						
<2	35.7	23.1	0	38.5	31.5	3.5
−5	42.0	37.8	14.7	38.5	66.5	14.0
−10	18.9	10.5	2.1	28.0	42.0	21.0
−20	10.5	4.2	0	28.0	17.5	14.0
<20	2.1	4.2	2.1	3.5	3.5	3.5
+ 90 Days						
<2	0.2	0.2	0	38.5	24.5	0
−5	1.4	0.3	0.1	66.5	73.5	14.0
−10	1.9	0.1	0	38.5	31.5	3.5
−20	3.6	0.4	0	24.5	14.0	7.0
>20	1.0	0.6	0.2	3.5	7.0	0

Values calculated by multiplying tissue fibre concentrations in Table 1 by percentages in Table 3.

TABLE 5. CALCULATED MASS OF FIBRES IN AEROSOLS AND TISSUES BY DIMENSION

	Chrysotile				Crocidolite			
	Diameter							
	<0.1	−0.2	>0.2	Totals	<0.1	−0.2	>0.2	Totals
Length								
Aerosol (mg/m³)								
<2	0.018	0.055	0.061	0.13	0.70	0.32	0.15	0.53
−5	0.026	0.10	0.25	0.38	0.063	0.82	1.06	1.95
−10	0.033	0.091	0.35	0.48	0.063	0.79	1.22	2.08
−20	0.013	0.036	0.30	0.35	0.063	0.53	0.49	1.08
>20	0	0.061	0	0.061	0	0	0.82	0.82
Totals	0.091	0.35	0.97	1.41	0.26	2.46	3.74	6.46
At End of Exposure (μg/g wet lung)								
<2	0.85	1.53	0	2.38	0.92	2.09	0.64	3.65
−5	1.67	4.17	4.51	10.35	1.53	7.34	4.30	13.17
−10	2.25	3.48	1.93	7.66	3.34	13.92	19.33	36.59
−20	2.50	2.78	0	5.28	6.68	11.60	25.77	44.05
>20	0.84	4.64	6.44	11.92	1.39	3.87	10.74	16.00
Totals	8.11	16.60	12.88	37.59	13.86	38.82	60.78	113.46
+ 90 Days (μg/g wet lung)								
<2	0.005	0.013	0	0.018	0.92	1.62	0	2.54
−5	0.056	0.033	0.031	0.12	2.64	8.12	4.30	15.06
−10	0.23	0.033	0	0.26	4.59	10.44	3.22	18.25
−20	0.86	0.27	0	1.13	5.84	9.28	12.89	28.01
>20	0.40	0.067	0.61	1.08	1.39	7.73	0	9.12
Totals	1.55	0.42	0.65	2.61	15.38	37.19	20.41	72.98

Values calculated from values in Tables 1, 3 and 4, assuming cylindrical fibres of density 2.5 g/cm³; and using values for diameters of 0.09, 0.15 and 0.25, and for lengths 1.5, 2.5, 7.5, 15 and 25 μm, respectively, for the cells in Tables 3 and 4.

fibre dimensions in their system were virtually identical to ours in the report by Pinkerton, *et al.* (1983).

More rapid clearance of chrysotile than crocidolite or amosite after similar inhalational exposure was reported by WAGNER and SKIDMORE (1968) but their work used a different method of quantitative analysis (silica content) and did not examine fibres by electron microscopy.

MORGAN, TALBOT and HOLMES (1978) used radioactive anthophyllite asbestos, selective bronchopulmonary lavage and light microscopy to study the relationship of fibre length to clearance in Albino rats. They observed a clearance half-time for all fibres of 76 days, whereas the half-time for asbestos in free macrophages (recovered by lavage) was 45 days. They also found that the percentage of long fibres remaining in the lung increased with time after exposure. This was noted in their size ranges for fibres greater than 10 μm length. Their absolute percentages cannot be directly compared to ours and those of ROGGLI and BRODY since MORGAN, *et al.* used anthophyllite and light microscopic analysis.

Our results showed that fractional retention of chrysotile fibres appears to be greatest in the 10–20 μm length category. At least two explanations for this are possible. The first is that clearance is minimal for the 10–20 μm range. The second is that the fibres 20 μm long are breaking down to shorter fibres during time. The available data do not discriminate between these possibilities. Studies with varying monodisperse fibre length aerosols will be needed.

In another portion of this study (SMITH *et al.*), the *in situ* chrysotile fibres were observed using SEM of 5 μm tissue sections. These observations also supported the differential clearance. At regions of alveolar duct and bronchiolar bifurcations, the interstitium could be seen to be full of fibres of lengths up to at least 15 μm. In contrast, the alveolar macrophages in the airway lumen were filled with tiny fibres, the longest of which was 3.5 μm.

The near absence of overlap of fibre lengths in such proximity reinforces the difference in handling of short and long fibres in the lung. Why longer fibres should preferentially penetrate into the interstitium at sites of deposition is not clear. One might hypothesise that shorter fibres would have an easier transepithelial passage. However, it seems likely from the work of BRODY *et al.* (1981) that the aerodynamic size is critical in determining the site of deposition, such that the smallest fibres might bypass the bifurcations and deposit on fluid lined zones where macrophages could be the first cells with which the fibres come in contact. Alternatively, one would have to develop what might seem to be a rather contrived explanation for mixed fibre length deposition, selective phagocytosis by fibre size and selective epithelial penetration and/or mixed epithelial penetration and selective clearance of short fibres with retention of longer fibres.

In summary, the chrysotile which was retained appears to be the longer, thinner fibres. This selective clearance is not seen with this particular crocidolite exposure in the 90 day clearance time studied. Such differences between fibre types are likely to be important in interpreting observed epidemiological differences in response to different exposures. These studies reinforce the facts that nominal short fibre preparations may lead to retention of long fibres in the lung, even when the long fibres make up only a small fraction of the initial aerosol, and that electron microscopic counting and sizing of fibres in both aerosol and tissues are needed to optimally interpret fibre clearance

data. The underlying mechanisms are incompletely understood. Further studies of comparative exposures using different exposures, varying clearance times and different sized preparations of asbestos and other fibres will be informative. Heppleston's observations (1969) that 'a prime requirement is quantitative studies on the deposition and retention of different forms and sizes of asbestos' still seems relevant in all possible experimental, epidemiological and clinical situations.

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REFERENCES

BRODY, A. R., HILL, L. H., ADKINS, B. Jr and O'CONNOR, R. W. (1981) Chrysotile asbestos inhalation in rats: deposition pattern and reaction of alveolar epithelium and pulmonary macrophages. *Am. Rev. Respir. Dis.* **123**, 670–679.

HEMENWAY, D. R. and MACASKILL, S. M. (1982) Design, development and test results of a horizontal flow inhalation toxicology facility. *Am. Ind. Hyg. Assoc. J.* **43**, 874–879.

HEPPLESTON, A. G. (1969) Pigmentation and disorders of the lung. In: Wolman, M. (Ed.) *Pigments in Pathology*, Academic Press, NY p. 33–75.

MORGAN, A., TALBOT, R. J. and HOLMES, A. (1978) Significance of fibre length in the clearance of asbestos from the lung. *Br. J. Ind. Med.* **35**, 146–153.

PINKERTON, K. E., BRODY, A. R., MCLAURIN, D. A., ADKINS, B., O'CONNOR, R. W., PRATT, P. C., CRAPO, J. D. (1983) Characterization of three types of chrysotile asbestos after aerosolization. *Env. Res.*, **31**, 32–53.

ROGGLI, V. L. and BRODY, A. R. (1984) Changes in numbers and dimensions of chrysotile asbestos fibers in lungs of rats following short-term exposure. *Exp. Lung Res.*, **7**, 133–147.

SMITH, C. M., BATCHER, S., CATANZARO, R., ABRAHAM, J. L. and PHALEN, R. (1987). Sequence of bronchoalveolar lavage and histopathological findings in rat lungs early in inhalation asbestos exposure. J. Toxicol. Environ. Health, **20**, 147–161

WAGNER, J. C. and SKIDMORE, J. W. (1968) Asbestos dust deposition and retention in rats. *Ann. N.Y. Acad. Sci.*, **132**, 77–86.