

E. Ilgren^a
E. Chatfield^b

^a Brvn Mawr, Pa., USA.
^b Toronto, Canada

Coalinga Fibre – A Short, Amphibole-Free Chrysotile

Part 1: Evidence for a Lack of Fibrogenic Activity

PLAINTIFF'S EXHIBIT

SA-474

Key Words

Short fibre chrysotile
Coalinga
Fibrosis
F344 rats

Abstract

Controversy continues to surround the biological activity of short fibre chrysotile. This is largely due to a lack of studies in which there has been 'pure exposure' to this material. Most of the exposures studied, whether in humans or animals, have been confounded by the additional presence of long fibre chrysotile and/or amphibole. This report presents the morphological and morphometric findings of a lifetime inhalation study of F344 rats exposed to three types of chrysotile. The first, from Coalinga, Calif., is comprised of fibres that are almost all less than 5 µm in length and is not contaminated with amphibole. The other two, the Jeffrey fibre and the UICC/B standard, are both Canadian long fibre preparations with a minor degree of amphibole contamination. Exposed animals displayed no fibrosis following exposure to Coalinga chrysotile but showed fibrogenic responses with both Canadian fibres.

Introduction

Two major theories are often cited to explain asbestos-related disease. Of these, the 'Stanton' hypothesis [1, 2] proposes that short fibres are less harmful than long whilst the 'amphibole' hypothesis [3, 4] claims that relatively soluble fibres, like chrysotile, are less injurious than highly durable amphiboles. If correct, these theories, taken together, would suggest that the least harmful type of asbestos would be uniformly short, amphibole-free, chrysotile. Short fibre chrysotile is the most common form of asbestos in air including the atmosphere of buildings [5–8], water, soil, and lung [9–11]. In fact, short fibre chrysotile is so ubiquitous that it can confound even the most carefully done analyses [12, 13]. Its large atmospheric contribution comes from numerous, naturally occurring

outcroppings as well as diverse commercial products such as brake linings, tiles, and cement in which it has been widely used [14].

This study and its companion reports describe the unique analytical and biological properties of Coalinga fibre, a short, amphibole-free, chrysotile, and demonstrate that it is largely devoid of biological activity. The proposal that this form of asbestos should be regarded as a nuisance dust and 'maybe innocuous in lung tissue' [5] is therefore put forth. This is not only consistent with the findings described herein but is also in keeping with the much larger body of scientific thought. Historically, supportive conclusions reached almost 50 years ago by Vorwald et al. [15] are consistent with the later work of Davis et al. [16] and Berman et al. [17]. Studies by all three groups concluded that the truly pathogenic fibres are

KARGER

E-Mail: karger@karger.ch
Fax: +41 41 506 12 34
http://www.karger.ch

© 1997 S. Karger AG, Basel
1420-326X/97/0005-0264\$15.00/0

Dr. E. B. Ilgren, MD, DPhil
Consultant Physician, Suite No 503
850 Montgomery Avenue
Brvn Mawr, PA 17801
Tel: +610 525 5960, Fax: +610 526 1156

those greater than 20 μm in length. Recent panels [6, 7] have also endorsed the increased pathogenicity of long fibres (i.e. > 5–8 μm in length). Although Lippmann [18] claimed that the 'critical fibre parameters for asbestosis' were surface area and fibre lengths greater than 2 μm , this was based largely on Timbrell's [19–22] lung burden studies of relatively few amphibole workers.

The inability to produce 'pure' short fibre preparations in large quantities, devoid of long fibres, has been a chronic problem when comparing the biological potential of long versus short fibres. For example, Wagner et al. [23] assessed the biological activity of the shortest commercial sample, a grade 7 fibre from the Bells mine (Québec), in relation to two experimental Canadian chrysotile preparations. Unfortunately, the Bells grade 7 material contained more long (> 5 μm) fibres than the other two [see fig. 1 in 23], and the resulting pathogenic responses were attributed to the high numbers of long fibres in each. More recently, Davis and Jones [24] administered 'short' and 'long' chrysotile preparations to rats by inhalation and found that the 'short fibre' produced significant pathogenic effects. This was again attributed to sample impurity due to long fibre contamination.

Aside from the study by Davis and Jones [24], the most recent short fibre chrysotile inhalation investigation was conducted at NIOSH by Platek et al. [25]. These workers exposed rats and monkeys via inhalation to a highly purified short fibre chrysotile sample and found neither fibrosis nor tumours in either species.

Historically, the findings of inhalation studies aimed at assessing the role of fibre length have been supported and clarified by numerous instillation and injection experiments [see 5, 24 for excellent review and 26 for critical discussion of method]. Injection studies using short fibre chrysotile preparations derived from ball milling [for citations see 24] have been consistent with those obtained with water sedimentation methods [27–30] in showing a general lack of biological effects with short fibres.

The present report is based upon a lifetime study of F344 rats exposed by inhalation to either Coalinga, Jeffrey or UICC/B chrysotile fibres that was performed at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, N.C., USA and the National Toxicology Program (NTP) between 1978 and 1980. Whilst this report is particularly concerned with fibrosis, the others in this series (see above) describe the implications of these findings for human fibrogenesis; the historical evidence in support of the greatly reduced pathogenicity of short fibre chrysotile; and the analytical properties, tumourigenic effects, differential deposition,

distribution, and clearance of Coalinga chrysotile which distinguish it from 'long' Canadian fibre. Taken together, the findings described in all these reports support the contention that short-fibred chrysotile is innocuous and should be regarded and regulated as a nuisance dust.

Materials and Methods

Source of Materials

The present study originated as part of a large, joint investigation undertaken by the NIEHS in the United States and the Medical Research Council (MRC) Pneumoconiosis Unit in the United Kingdom to compare the results of similar inhalation studies carried out at two different locations under nearly identical conditions [31, 32]¹. The comparative MRC – NIEHS study, as originally conceived, contained only one chrysotile exposure group, namely UICC/B². Two additional groups, the 'long' Jeffrey fibre (Jeffrey Mine, Asbestos, Que.), and the 'short' Coalinga, were added to the NIEHS protocol by the NIEHS investigators³.

None of the fibrosis or tumour pathology data have ever been published for Coalinga or Jeffrey fibre. Fibrosis score data have only been published for the untreated and UICC/B treated, interim sacrifice animals [31, 32] but not for the groups on lifetime test. None of the Coalinga and UICC/B morphometry data have been published in scientific, peer-reviewed journals though some have appeared in a thesis published by Pinkerton [33] and in several small abstracts by Crapo et al. [34], Pinkerton et al. [35, 36] and O'Neil et al. [37]. However, most of the morphometric data for the untreated control and the Jeffrey-treated animals have already been described [38–41]. All of the morphometric studies were performed by Professor Kent Pinkerton.

The records, histology slides, paraffin blocks, and wet tissues of the animals on lifetime test were located in November 1995 at the NTP archives by Drs Sils, Hamlin and Bridges as inventoried documents. All of these were reviewed solely by the senior author aside from 45 cases that were studied in conjunction with Dr J.C. Wagner.

¹ The NIEHS investigation was conducted at the NIEHS and funded by the NIH as an intramural project (NIEHS contract No. NO1-4-21-64BCD and EPA Coop. Agreement No. CR807255). Dr. Jack Moore was the supervisor and Dr. Arnold Brody, the Project Officer. The NTP pathologists, Dr. Eugene McConnell and Dr. Gary Boorman, oversaw the pathological study of the animals. Much of the routine work, including monitoring of fibre levels and daily animal health, was initially performed under contract with Becton Dickinson (BD) (vs 'BCD'), by Dr. Finas Cavender and Dr. Connie Stone (cf. BD Quarterly Reports: Effects of chronic exposure to airborne environmental agents (1 April–July 1978; 1 Aug–31 Oct 1978; and 1 Nov 1978–31 Jan 1979)) and finalised by Northrup, Inc. by Dr. Bernard Atkins and Dr. Robert O'Connor.

² 'Union Internationale Contre le Cancer' funded the preparation of 'standard' asbestos samples for research. UICC/B chrysotile was a mixture obtained from eight different Canadian chrysotile mines blended in proportion to their output.

³ On 4 Sept 79, a Request for Proposal (RFP NIEHS-79-13) was sent out for bid by Dr. Arnold Brody to have morphometry performed in addition to histopathology. The RFP was awarded to Duke University under subcontract to Dr. James Crapo (NIEHS No. NO1-ES-0-0004). Most of the actual morphometry was done by Prof. Kent Pinkerton as Dr. Crapo's PhD student.

Table 1. Experimental design - NTP inhalation bioassay (1978-1980). Number of animals at each time point

Treatment groups	Time, months ¹			
	0	3	12	24
Controls, untreated	45m, 45f 36m, 36f 32m, 32f 28m, 28f			
Coalinga				
UICC/B				
Jeffrey				

¹ At each time point, 4 animals were sacrificed for morphometrical analysis. At time zero, 5 animals were sacrificed for morphological analysis.

The latter included most of the pulmonary neoplasms plus a random selection of diverse, non-neoplastic lesions. Other records from the Becton Dickinson Research Facility from Dr Steven Bernadyne and the NIEHS from Dr Elizabeth Ford and Mr Don Goula were also reviewed.

Animals

Four hundred 5-week-old, specific pathogen-free (SPF) male and female Fischer 344 rats (weighing 125-150 g), originally obtained from a single source (Chas. River, Inc. Wilmington, Mass.), were shipped to the NIEHS (Research Triangle Park, N.C., USA). Three hundred and thirty of these form the basis of this study. Upon receipt at the NIEHS, 5 animals of each sex in each exposure group of the 360 rats were randomly selected and killed for morphological study. This included total body necropsies which found these animals to be free of infectious and non-infectious disease of all major organs. The rats, thus found to be suitable for the inhalation study, were released to the Becton Dickinson contractor at 6 weeks of age and 40 animals of each sex were randomly assigned to either a control group or one of three exposure groups, at 7-8 weeks of age. A total of 96 rats out of the 320 were used for morphometric study: 4 males and 4 females were randomly selected from each group of 40 male rats and 40 female rats following 3 and 12 months' exposure to either: (1) Coalinga Mine chrysotile; (2) UICC/B chrysotile, or (3) Jeffrey chrysotile. Four additional males and 4 females from each exposure group were randomly selected from the surviving animals 1 year following the end of 12 months' exposure to each type of chrysotile. The remaining 224 animals not used for morphometry were kept on lifetime test as shown in table 1.

Due to the paucity of normal life-span data for F344 rats, 1,200 5-week-old SPF male and female animals were set up as lifetime controls to help interpret the NIEHS NTP study described in this report (see 42 for results and also 43 analysis of a subset (3,548/4,640) of the same 'historical' control group plus 44 for additional, relevant, concurrent control data).

Chrysotile Preparations

Pinkerton et al. [45] have described the origin and physical characteristics of aerosols produced from the 3 chrysotile samples. Chatfield and Ilgen [in preparation] have also provided further details of the physical and chemical properties of the Coalinga fibre, referred to

as NIEHS short range chrysotile and identified as COF-25 as obtained from Union Carbide [46]. The Coalinga chrysotile was subjected to additional milling and separation but these processes did not destroy the crystalline structure of the fibres [46]. Jeffrey chrysotile (NIEHS intermediate range) was prepared by roller milling using standard industrial techniques, followed by three cycles of 'Hurricane' pulverisation to open further the fibre bundles. UICC/B Chrysotile is a well described reference sample [47].

Exposure Conditions

Pinkerton et al. [39] and McConnell et al. [31, 32] have described in detail the exposure conditions, including inhalation chambers and dust generation. Briefly, exposures were of the 'occupational' type, 7 h/day, 5 days/week for 12 months, the fibres being aerosolised by a Timbrell generator. Total chamber dust mass concentrations were monitored daily with adjustments as required to maintain a constant dust concentration throughout the exposure period. The mean values ($\text{mg}\cdot\text{m}^{-3}$) for each chrysotile preparation ($n = 240$) were: 11.36 $\pm$ 2.18 for Jeffrey, 10.99 $\pm$ 2.11 for UICC/B, and 7.78 $\pm$ 1.46 for Coalinga as described by Pinkerton et al. [45]. Methods used to determine the mean chamber respirable dust concentrations and the complete results of these determinations, including PCM- and SEM-derived fibre size determinations of all three fibre types, have also been described by Pinkerton et al. [39, 45]. Direct and indirect TEM analyses of Coalinga have been described by Chatfield and Ilgen [in preparation].

Animal Sacrifice, Tissue Distribution, and Sample Preparation

Pinkerton et al. [39] and McConnell et al. [31, 32] have described the method of animal sacrifice, tissue distribution, and sample preparation. All animals had a complete post-mortem examination. The lungs of animals on lifetime test for morphological study were fixed in 10% neutral buffered formal saline. Random sections of the left lung; a slice from the right diaphragmatic lobe; and random sections through the rest of the right lung (apart from the cardiac and middle lobes) were taken and stained for cells with haematoxylin and eosin (H&E), for connective tissue with Van Gieson's stain (HVG), and in some cases, for mucin with Alcian blue. Lungs of interim sacrifice animals to be studied for morphological evidence of fibrosis using either the Wagner scoring method or a 'severity/extent' scoring system (see below), were also fixed in formalin. Replicate sections of the left lung were taken from the same area (mid-dorsal to ventral slices through the hilar region) of each animal and again stained with H&E and HVG. The methods used to fix and prepare the lung tissues of interim sacrifice animals (four randomly selected sites from the ventral and dorsal portions of the cephalic and caudal regions of the left lung of each animal) for morphometric examination have been described by Pinkerton et al. [39].

Histopathological Analysis

Animals on lifetime test were analysed histopathologically as described by McConnell et al. ([31, 32] (table 2) for the presence and degree of reaction to the fibres, using grades 1-8 as described by Wagner et al. [48]. The grading severity is: 1 = normal; 2-3 = cellular change (no fibrosis); 4 = minimal fibrosis; 5 = mild fibrosis; 6 = moderate fibrosis; 7-8 = severe fibrosis.

The interim sacrifices were scored for severity and extent based upon the number of cells and amount of matrix present within the interstitium of each particular structure. Severity scores ranged from 0 to 3+, 0 signifying no change and 3+ signifying the greatest change.

Table 2. Parenchymal fibrosis scores of rats on lifetime test treated with Coalinga, UICC/B, and Jeffrey chrysotile graded with the Wagner (severity) system

	Sex	n1 ¹	n2 ²	Av. fibrosis score
Control/NIEHS, 1996 ³	m	28	22	1.4
	f	26	21	1.6
Control/MRC, 1984 ⁴	m, f	34	ns	1.0
Coalinga/NIEHS, 1996	m	27	21	1.9
	f	24	17	2.0
UICC/NIEHS, 1996	m	30	25	5.4
	f	26	22	5.3
UICC/MRC, 1984	m, f	68	ns	4.3 ⁵ -4.7 ⁶
Jeffrey/NIEHS, 1996	m	26	25	5.6
	f	26	24	5.9

¹ n1 = Number of animals at risk at the start of the study.

² n2 = Number of animals that were/could be used for fibrosis scoring.

³ NIEHS, 1996 = present investigation.

⁴ MRC, 1984 = Wagner et al. [44].

^{5,6} UICC/B data for 500-749 days⁵ and 750+ days⁶ given but not stratified for sex.

Extent scores were based on involvement of less than 1/3 of the lung section, 1/3 to 2/3 of the lung section and greater than 2/3 of the lung section for scores of 1, 2 and 3 respectively. The results were expressed as the product of the scores for severity and extent [33].

Finally, although the difference in the percentage of Coalinga- and Canadian-treated animals (22%) which could not be scored due to age-related lesions was probably treatment-related, there is no reason to believe that the fibrosis scores of the Coalinga-treated animals which could not be scored would be higher than those which could be graded. Thus, there is little reason to question their representativeness.

Morphometric Analysis

This was performed as described by Pinkerton et al. [39].

Statistical Analyses

Statistical analysis of survival was performed using one-tailed tests for variance. Statistical analysis of morphometric findings was conducted using a two-way ANOVA test as described by Pinkerton et al. [39].

Results

Morphological observations, based on histopathological study of animals on lifetime test treated with the three types of chrysotile, demonstrate that Coalinga chrysotile

Table 3. Parenchymal fibrosis (matrix) and cellular changes in rats sacrificed at 3, 12 and 24 months treated with Coalinga, Jeffrey, and UICC B chrysotile graded semi-quantitatively with a Severity/Extent scoring system

	n	Cells and matrix within the peribronchial interstitium	Cells and matrix around alveolar ducts
Controls			
3 months	8	0.00 ± 0.00	0.00 ± 0.00
12 months	8	0.03 ± 0.03	0.00 ± 0.00
24 months	8	0.00 ± 0.00	0.03 ± 0.03
Coalinga			
3 months	8	0.34 ± 0.24	0.88 ± 0.27
12 months	8	0.16 ± 0.05	0.75 ± 0.22
24 months	8	0.28 ± 0.16	0.75 ± 0.35
UICC/B			
3 months	8	0.53 ± 0.23	2.13 ± 0.40 ^a
12 months	8	1.75 ± 0.37 ^a	2.25 ± 0.16 ^a
24 months	8	1.03 ± 0.25 ^a	2.88 ± 0.52 ^a
Jeffrey			
3 months	8	0.81 ± 0.23	2.63 ± 0.60 ^a
12 months	8	2.38 ± 0.60 ^a	5.25 ± 0.49 ^{a,b}
24 months	8	1.75 ± 0.16 ^a	3.73 ± 0.42 ^a

^a p < 0.05 for comparison of the asbestos-treated groups to the corresponding age-matched controls;

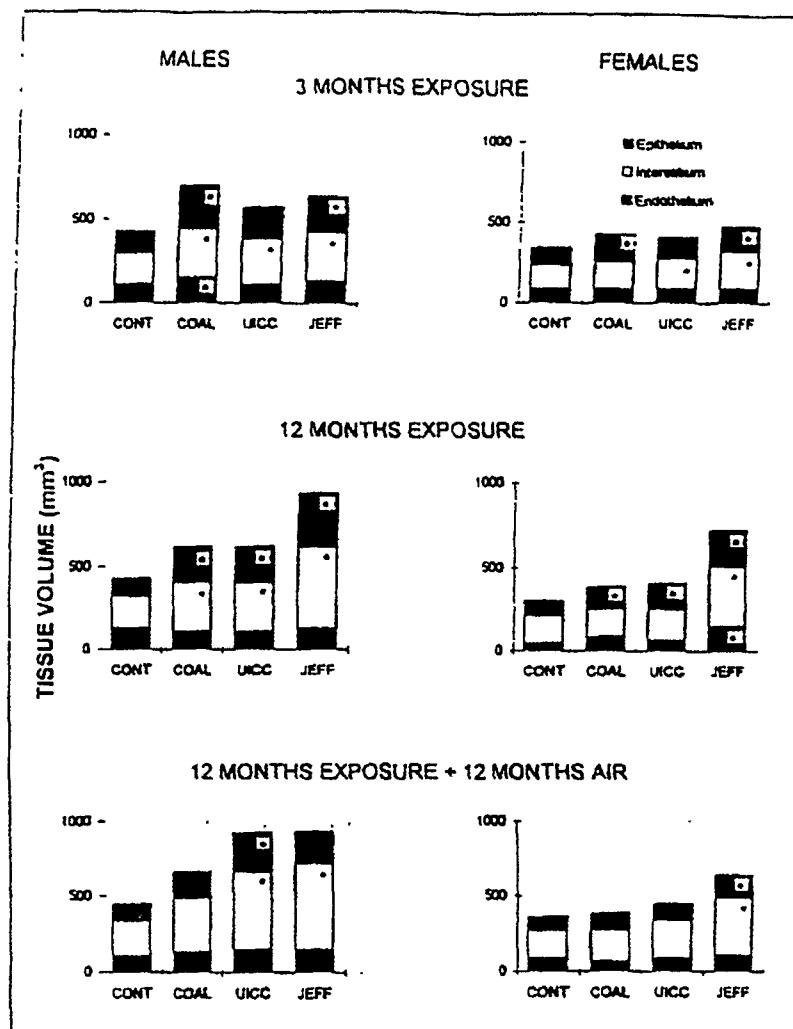
^b p < 0.05 for comparison of the group exposed to Jeffrey chrysotile to the corresponding age-matched groups exposed to UICC/B and Coalinga chrysotile; also see Pinkerton [33].

is not fibrogenic (table 2) in contrast to the Canadian fibres, both of which produced significant fibrosis.

Using a semi-quantitative, histological scoring system to assess the extent and severity of fibrosis found around bronchiolar walls and alveolar ducts, Coalinga chrysotile failed to produce fibrosis at any of the three sacrifice intervals, the measured parameters not being significantly greater than controls at any time point. In contrast, both Canadian fibres induced significant fibrosis (table 3) that was also associated with adenomatoid change or bronchiolar metaplasia [15] characterised by air spaces filled with a mucin like material [see also 33] and alveoli lined with a cuboidal to columnar epithelium. The interstitial matrix underlying these cells was thickened. These changes were not seen with Coalinga chrysotile [see also 33].

Electron microscopically, the lungs of Coalinga-treated rats sacrificed at 3-, 12-, and 24-month intervals did not display a thickened basement membrane or other atten-

Fig. 1. Changes in TITV in rats sacrificed at 3, 12, and 24 months treated with Coalinga, Jeffrey, and UICC/B chrysotile. CONT = controls; COAL = Coalinga; UICC = UICC/B; JEFF = Jeffrey. Each bar represents the mean of 4 animals and is subdivided into the epithelial, interstitial, and endothelial compartments; * $p < 0.05$ for comparison with age-matched controls: see also Pinkerton [33].



dant changes. By contrast, prolonged exposure to UICC/B and Jeffrey chrysotile clearly resulted in a generalised thickening of the basement membrane underlying much of the alveolar epithelium of the lung with notable membranous deposits of an amorphous, electron-dense material and concentrically layered, calcium-phosphorus bearing rings, along with marked collagenisation of the interstitial matrix [see also 33]. Such qualitative EM findings were morphometrically confirmed using data parameters related to the whole interstitium: the non-cellular and cellular components of the interstitium; and its individual cellular constituents. Changes in the thickness of the whole interstitial compartment are shown in figure 1.

These are referred to as alterations in 'total interstitial tissue volume' or TITV. The TITV has two major components: non-cellular (or 'interstitial (matrix) volume' - IMV) and cellular. Since the chief morphometric indicator of fibrosis is an increase in the non-cellular component, such IMV changes are of particular importance (table 4). Changes in the total volume of the cellular component of the interstitium are displayed in table 5 and are referred to here as alterations in 'cellular interstitial volume (CIV)'. Data are also presented for changes in the number and volume of the individual interstitial cells within the CIV. The former are presented in table 6 as interstitial cell number (ICN) while the latter (fig. 2) are

Table 4. Changes in noncellular IMV in rats sacrificed at 3, 12, and 24 months treated with Coalinga, Jeffrey, and UICC/B chrysotile

Time, months	Sex	Total volume, mm ³ /both lungs			
		Control	Coalinga	UICC/B	Jeffrey
3	m	126 ± 16	195 ± 22 ^a	177 ± 3 ^a	163 ± 13
	f	80 ± 10	82 ± 5	134 ± 11 ^a	98 ± 5
12	m	149 ± 13	239 ± 15 ^a	230 ± 25 ^a	362 ± 27 ^{a,c}
	f	109 ± 11	138 ± 9	146 ± 10	265 ± 21 ^{a,c}
12+	m	178 ± 20 ^d	287 ± 38	439 ± 5 ^{a,c}	422 ± 80 ^a
	f	153 ± 8 ^d	168 ± 15	211 ± 8 ^{a,c}	282 ± 22 ^{a,c}

All data mean ± SEM, n = 4 for each group and time period;
^a p < 0.05 when comparing the age-matched control values using Duncan's multiple comparison test after first testing for significance using a one-way ANOVA;
^b p < 0.05 when comparing groups exposed to Jeffrey chrysotile with all other asbestos-exposed groups of the same time period;
^c p < 0.05 when comparing one treatment group to its corresponding treatment group of the preceding time point;
^d p < 0.05 when comparing 24 month values to those at 3 months.

Table 5. Changes in CIV in rats sacrificed at 3, 12, and 24 months treated with Coalinga, Jeffrey, and UICC/B chrysotile

Time, months	Sex	Total volume, mm ³ /both lungs			
		Control	Coalinga	UICC/B	Jeffrey
3	m	67 ± 5	124 ± 2 ^a	104 ± 5 ^a	131 ± 15 ^a
	f	59 ± 7	97 ± 9	72 ± 10	140 ± 24 ^a
12	m	57 ± 8	77 ± 3	80 ± 16	145 ± 1 ^a
	f	49 ± 4	47 ± 6 ^c	62 ± 10	138 ± 11 ^a
12+	m	57 ± 7	81 ± 21	127 ± 2 ^a	168 ± 54 ^a
	f	56 ± 6	53 ± 9	46 ± 4	107 ± 16 ^a

See text for explanation. All data ± SEM, n = 4 for each group and time period.
^a p < 0.05 when comparing the age-matched control values using Duncan's multiple comparison test after first testing for significance using a one-way ANOVA;
^b p < 0.05 when comparing groups exposed to Jeffrey chrysotile with all other asbestos-exposed groups of the same time period;
^c p < 0.05 when comparing one treatment group to its corresponding treatment group of the preceding time point.

Table 6. Changes in the ICN (× 10⁶) in rats sacrificed at 3, 12, and 24 months treated with Coalinga, Jeffrey, and UICC/B chrysotile

Time, months	Sex	Control	Ref. ¹	Coalinga	UICC/B	Jeffrey	Ref. ¹
3	m	161 ± 9	38, 39	251 ± 12 ^a	197 ± 9	274 ± 39 ^a	39
	f	131 ± 16	38, 39	170 ± 4 ^a	172 ± 4 ^a	187 ± 10 ^a	39
12	m	131 ± 9	38, 39	160 ± 12	165 ± 125 ^c	342 ± 14 ^{a,b}	39
	f	118 ± 4	38, 39	131 ± 65 ^c	118 ± 6	233 ± 22 ^{a,c}	39
24	m	130 ± 15	38, 39	140 ± 9	200 ± 13	317 ± 61 ^{a,b}	39
	f	136 ± 3	38, 39	132 ± 13	199 ± 13 ^a	191 ± 4 ^{a,c}	39

See text for explanation. All data mean ± SEM; n = 4 for each group and time period;
^a p < 0.05 when comparing to the age-matched control values using Duncan's multiple comparison test after first testing for significance using a one-way ANOVA;
^b p < 0.05 when comparing the group exposed to Jeffrey chrysotile with all other treatment groups;
^c p < 0.05 when comparing one treatment group to its corresponding treatment group of the preceding time point;
¹ References in which these data have previously been presented in part or whole replicated here for the sake of comparison: Pinkerton et al. [38, table 7.]; Pinkerton et al. [39, table 3].

referred to as interstitial cell volume (ICV). Finally, numerical changes in the seven interstitial cell types namely fibroblasts, monocytes-macrophages, lymphocytes, neutrophils, mast cells, plasma cells, and pericytes are displayed in table 7. The time course of events within the

interstitium and its compartments during and following exposure to each of the three chrysotiles is classified here as acute, subchronic, and chronic for alterations taking place after 3, 12, and 24 months' exposure, respectively [see also 33].

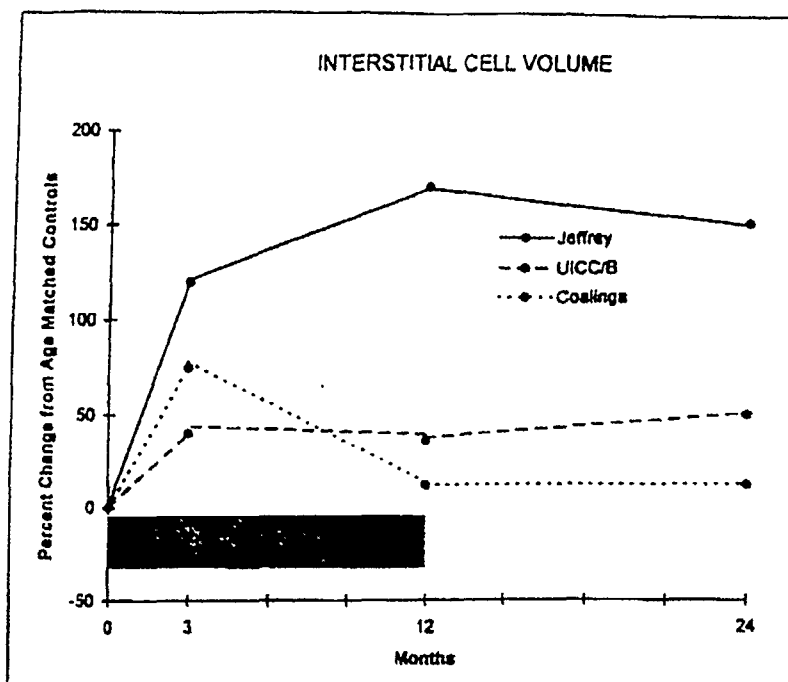


Fig. 2. Changes in ICV in rats sacrificed at 3, 12, and 24 months treated with Coalinga, Jeffrey, and UICC/B chrysotile. Each point represents the pooled mean of 4 males and 4 females; see also Pinkerton [33].

Over the first 3 months of exposure, acute changes were generally noted within the non-cellular and cellular interstitium in response to all three types of chrysotile. After 12 months' exposure, Coalinga failed to significantly increase the interstitial matrix volume over the IMV values noted after 3 months' exposure (table 4) even though these values were nonetheless significantly increased above controls for males, but not for females (table 4). By contrast, the IMV values noted in all Jeffrey-treated animals were significantly and dramatically increased above those seen at 3 months, over age-matched controls, and above the other two treatment groups as well. Subacute IMV changes in UICC/B-treated animals resembled those seen with Coalinga. The attendant cellular responses (see above) for the 3 fibre types at 12 months appeared to be less dramatic than those found in the non-cellular components. Still, the cellular changes (e.g. CIV and ICN) in the Coalinga-treated females, despite continuous exposure from 3 to 12 months, nonetheless fell very significantly during this time period. In contrast, highly significant and dramatic subacute cellular increases were noted following exposure to Jeffrey fibre though again most of the non-cellular and cellular interstitial responses elicited by UICC/B did not differ significantly from those produced by Coalinga. By 24 months, Coalinga was the

only fibre that failed to produce a persistent, significant increase in IMV over age-matched untreated controls. Similarly, the Coalinga associated cellular responses (see above) were also not significantly different from controls. In marked contrast to Coalinga, both Canadian fibre-treated groups displayed very marked, non-cellular and cellular interstitial changes by 24 months. Moreover, a unique change was observed in the IMV of the UICC/B-treated males during the post-exposure period with values 4–5 times greater than those seen in either untreated controls or in animals exposed to Coalinga or Jeffrey chrysotile. In a manner similar to IMV, the cellular responses (e.g. CIV, CIN and ICV) and the TITV remained significantly above control values in UICC/B- and Jeffrey-treated groups in almost every case. Finally, the quantitative changes in the individual, cellular components of the interstitium in rats sacrificed at 3-, 12-, and 24-month intervals assessed morphometrically (UICC/B data not available), generally paralleled those seen in the tissue components just described. Coalinga again failed to produce a significant increase in the number of any interstitial cell type by the end of the study whilst Jeffrey did so dramatically in virtually every case being significant for fibroblasts, monocytes-macrophages, lymphocytes, and mast cells but not for neutrophils and pericytes.

Table 7. Changes in number of the individual cellular components of the alveolar interstitium ($\times 10^4$) in rats sacrificed at 3, 12, and 24 months treated with Coalinga, Jeffrey, and UICC/B chrysotile

	Control ¹	Coalinga	Jeffrey
Fibroblasts			
3 months	94 $\pm$ 8	133 $\pm$ 7 ^a	108 $\pm$ 9
12 months	86 $\pm$ 9	93 $\pm$ 9	146 $\pm$ 16 ^{a,c}
24 months +	82 $\pm$ 9	86 $\pm$ 9	137 $\pm$ 19 ^{a,c}
Monocyte-macrophages			
3 months	21 $\pm$ 4	71 $\pm$ 4 ^{a,b}	106 $\pm$ 27 ^a
12 months	20 $\pm$ 2	39 $\pm$ 3 ^b	132 $\pm$ 12 ^{a,c}
24 months	19 $\pm$ 3	24 $\pm$ 2 ^b	89 $\pm$ 19 ^{a,c}
Lymphocytes			
3 months	8 $\pm$ 2	10 $\pm$ 2	5 $\pm$ 1
12 months	2 $\pm$ 1	5 $\pm$ 2	10 $\pm$ 4
24 months	9 $\pm$ 4	9 $\pm$ 3	61 $\pm$ 30 ^{a,c}
Neutrophils			
3 months	ND	5 $\pm$ 1 ^a	2 $\pm$ 1
12 months	ND	5 $\pm$ 2 ^a	8 $\pm$ 2 ^{a,b}
24 months	ND	2 $\pm$ 2	3 $\pm$ 1 ^b
Mast cells			
3 months	ND	ND	ND
12 months	ND	ND	10 $\pm$ 1 ^{a,c}
24 months	ND	1 $\pm$ 1	2 $\pm$ 1 ^{a,b}
Plasma cells			
3 months	1 $\pm$ 1	5 $\pm$ 2 ^a	8 $\pm$ 3 ^a
12 months	1 $\pm$ 1	1 $\pm$ 1	2 $\pm$ 1 ^b
24 months	1 $\pm$ 1	2 $\pm$ 1	4 $\pm$ 3
Pericytes			
3 months	20 $\pm$ 4	18 $\pm$ 5	12 $\pm$ 4
12 months	12 $\pm$ 3	7 $\pm$ 2	11 $\pm$ 1
24 months	13 $\pm$ 4	7 $\pm$ 2	10 $\pm$ 2

See text for explanation. All data are mean $\pm$ SEM; n = 4.

ND = not detected within the interstitium;

^a p < 0.05 for comparisons to the age-matched controls;

^b p < 0.05 for within group comparisons to the previous time point;

^c p < 0.05 for comparisons to animals exposed to Coalinga Mine chrysotile.

¹ Data previously presented in Pinkerton et al. [33, table 8] in part or whole.

Pleural Disease

Treatment-related pleural lesions, i.e. pleural plaques [6, 49], diffuse (visceral) fibrous thickening [6, 50], and benign pleural effusion / pleuritis [51] were not found in this study.

Survival Rates of Animals on Lifetime Test

Controls. Four (2 male and 2 female) out of 53 concurrent controls died spontaneously by 24 months on test, a mortality level consistent with that (10–15%) noted by Solleveld et al. [42]. The mean survival for untreated, concurrent controls dying after 24 months in this study was 809 days (604–967) for males (n = 28), 712 days (482–967) for females (n = 25), and 767 days for both sexes combined. The difference in length of survival between the concurrent males and females just failed to attain statistical significance (p = 0.07).

Treatment Groups. Eleven treated animals (Coalinga, 1 male and 2 females; UICC/B, 3 females; and Jeffrey, 2 males and 3 females) died spontaneously by 24 months for reasons unrelated to treatment. The difference in survival between males and females just failed to reach statistical significance (p = 0.06) for the Jeffrey-treated animals whereas no sex-related differences were noted in length of survival in the Coalinga- and the UICC/B-treated animals. Coalinga failed to reduce survival duration compared with controls whilst Jeffrey nearly did (p = 0.06).

Parenchymal Fibrosis and Survival

The survival times for control and Coalinga, male and female animals on lifetime test with combined scores of 1 and 2, were compared with UICC/B- and Jeffrey-treated rats with scores of 6 and 7. Cases with pulmonary tumours, severe leukaemic infiltration, or marked uraemic involvement of the lung were excluded from this analysis. The severe fibrosis due to the Canadian fibres thus significantly reduced survival (p = 0.04) whilst the minimal cellular changes associated with Coalinga exposure did not.

Age-Related Lesions

Uraemic pneumonitis and its attendant oedema, cellularity, and membrane formation were sufficiently severe in 1.8% (8/208) of cases so as to make reading of the slides impossible. Leukaemia, autolysis and congestion were also sufficiently severe enough to make scoring of the slides impossible in 6.0% (13/208), 2.0% (5/208), and 0.4% (1/208) of the animals respectively. The percentage of age-related lesions (leukaemia, uraemia, and congestion) severe enough to make reading of the slides impossible amongst the four groups was as follows: 22% (11/50) for Coalinga, 14% (7/53) for untreated controls, 8% (4/55) for UICC/B; and 0% for Jeffrey. There was no difference between the sexes. A small percentage of slides were missing 2.0% (5/208).

Table 8. Summary of selected inhalation bioassays using 'non-Coalinga' and Coalinga chrysotiles

Treatment	Average conc. ¹ mg·m ⁻³	Fibre, % > 10 µm	Fibre·ml ⁻¹ (>5 µm)	Fibre·ml ⁻¹ per average conc. ¹	Interstitial fibrosis (‘extent’ score) ²	Tumour response	
						%	n
Short ²	10	27	1,170	117	2.4 (0.3–6.7)	20	8/40
Long ²	10	35	5,510	551	12.6 (6.4–24)	53	22/40
Chry. yarn ³	4.3	40	428	100	8.8 (3.1–17)	40	16/40
WDC yarn ³	4.6	50	679	148	12.8 (2.2–41)	44	18/40
Fac WDC ³	4.8	35	468	98	12.1 (0.3–35)	51	21/40
Exp WDC ³	5.7	90	108	19	9.6 (0.5–22)	44	18/40
Exp WDC (lt. rev.) ³	5.6	90	111	20	10.8 (0.6–22)	44	18/40
Coalinga RG ⁴	5.9	(30) ⁵	131	22	0	0	0/50
Coalinga COF25 ⁶	3.3	(23) ⁵	100	30	0	1.8	2/90

¹ Average chamber dust mass concentration.² Davis and Jones [24].³ Davis et al. [52]; WDC is wet dispersed chrysotile: chrysotile treated with anionic surfactant to break down fibrils. Fac WDC was dust collected from the air in a WDC yarn factory; Exp WDC was an experimental sample not prepared for commercial use: (lt. rev.) in this experiment the animals were light reversed.⁴ Muhle et al. [51]; RG resin grade.⁵ The Coalinga samples contained long fibres artificially produced by a spinning process. These aggregates broke down when wetted to material containing 0% fibres >10 µm.⁶ The present study; COF25 cyclone overflow (25 mm final port): see Pinkerton et al. [45].

Note: RG and COF25 are dimensionally similar materials.

Discussion

In conclusion, our findings demonstrate that long-term, high-dose exposure to Coalinga fibre, a short, amphibole-free, chrysotile, does not induce parenchymal fibrosis in contrast to two long Canadian samples which are both clearly fibrogenic. These results were based upon our own analysis of animals on lifetime test plus careful review of interim sacrifice data provided by Dr. Kent Pinkerton. The latter had been analysed both morphologically using light microscopy and morphometrically with electron microscopic methods. The findings of previous studies, described below, support these conclusions.

Other Studies with Coalinga Chrysotile

The investigation by Muhle et al. [51] is the only other inhalation study of Coalinga chrysotile and it too failed to find fibrosis. In this investigation, fibrosis was assessed as 'septal thickening', the level (42%, 21/50) in the Coalinga-treated animals resembling that seen in the combined controls (36% i.e. 12% (6/50) for sham – and 24% (12/50) for untreated controls). The interpretation of these findings is made somewhat difficult by the fact that the authors failed to indicate whether cellularity or fibrosis

made up the greater part of the observed septal thickening. The starting materials used in this study (COF25 fibre, [45]) and those used by Muhle et al. [51] (RG144 preparation) were not identical. However, this is not confounding since extensive analyses of COF25 and RG144 have not demonstrated significant differences in their fibre size distributions. [Chatfield and Ilgren, in preparation]. The observed lack of fibrosis was not simply due to low [ca 131 fibres·ml⁻¹ >5 µm] concentrations of long fibre since very comparable fibre concentrations [111 fibres·ml⁻¹ >5 µm] of other chrysotile preparations [52], administered over identical exposure durations, have been very fibrogenic (table 8).

There are only two other investigations to assess specifically the fibrogenic potential of Coalinga chrysotile ([53, 54] for Union Carbide and [55] for Johns Manville). Both used injection methods and fibrosis was induced in each instance. However, the observed fibrotic lesions were clearly non-specific, being called 'artifactual (due largely to high doses and also) attributable to the technique' by Gross and de Treville [55]. Further confounding was created by inadequate controls; contaminated starting materials; pulmonary infection; a paucity of animals in each treatment group; the failure to study histologically all

but a few animals; and various non-specific histological reactions e.g. 'foreign body reactions' wrongly attributed to Coalinga fibre [53, 54].

Other Comparable UICC/B and Jeffrey Inhalation Studies

Although McConnell et al. [31, 32] examined animals from the same UICC/B-treated and untreated control groups as we did for this report, their findings for animals kept on lifetime test were never published, only those for the interim sacrifices. The 24-month fibrosis score (3.6) reported by McConnell et al. [31, 32] for UICC/B animals is thus not strictly comparable to the lifetime scores (5.3–5.4) we report here (see table 2). However, McConnell et al. [31, 32] did describe progression in their investigation (see below), thereby making it most likely that their 3.6 score would have increased further by the end of life. Although the extent to which this would occur is obviously uncertain, it could very well come to within one grade or less of that found by us in this report. This is suggested by the fact that the lifetime fibrosis score reported by Wagner et al. [44] for identically treated, UICC/B-exposed animals was 4.7, which is less than one grade lower than those noted by us. This level of variation is not uncommon when individual, age – matched cases, are compared [e.g. see 23, 31, 32, 44; also noted in MRC Expt. 121, 3 Oct. '80]. Observed differences in fibrosis score are probably not due to dose and fibre size but may be related to other factors such as interreader variation, the choice of fixative and/or the mode of fixation [31].

Overall Level of Parenchymal Fibrosis

In a manner similar to this study, a substantial percentage of the rats exposed to UICC/B chrysotile by McConnell et al. [31, 32] and Wagner et al. [44] also had grade 5 fibrosis scores (NIEHS-MRC raw data, not shown). Thus, 33% (5/15) of the UICC/B-treated males and 55% (5/9) of the UICC/B-treated females reported by McConnell et al. [31, 32] had grade 5 lesions (raw data, not shown). Similarly, 28% (7/24) of the UICC/B-treated males and 32% (8/24) of the UICC/B-treated females reported by Wagner et al. [44] had grade 5 scores, with one grade 6 lesion noted in each sex.

Wagner et al. [23, 48] are possibly the only other group to administer UICC/B chrysotile to rats via inhalation in approximately the same duration and intensity as was used in the present study. Wagner et al. [48] noted that both sacrificed and surviving UICC/B-treated rats ultimately developed fibrosis scores that averaged between grade 5.1 and 6.0 whilst Wagner et al. [23] found that

UICC/B-treated animals, 12 months after a 12-month exposure, had a mean fibrosis score of 5.0.

There appears to be only one other inhalation rat study of Jeffrey fibre [56]. In this study, McConnell et al. exposed F344 rats for the same duration and intensity as the present investigation. These workers stated that grade 4 lesions typified the fibrotic changes [see 56, pp. 329, 331, and 333 as well as fig. 5] thus produced. However, these were by no means characteristic of the changes noted in the Jeffrey-treated animals reviewed in the present study which were clearly grade 5 and grade 6 lesions. Although it is presently not possible to reconcile the observed differences, the high proportion of minimal fibrotic lesions is surprising given the rate of neoplasia (40%) reported by McConnell et al. [56] and the recognised association between the degree of asbestosis and extent of pulmonary neoplasia [57].

Whole body exposure was used in the experiments since the nose only technique was probably not in use at the time the study was being designed (ca 1976) and because the NIEHS study was to be modelled after the MRC unit which only used whole body exposures. Although whole body exposure has been criticized on several grounds including adventitious exposure, avoidance of exposure and ingestion of sample through grooming, the necessary comparative experiments have not yet been done to demonstrate whether this is actually the case. [Davis, personal commun.] Moreover, the daily restraint associated with nose only exposure may, in itself, significantly influence toxicity outcome [58].

Progression of Parenchymal Fibrosis

Comparison of our lifetime fibrosis scores for UICC/B treatment (table 2) with those noted at 12 and 24 months by McConnell et al. [31, 32] is consistent with minimal to moderate progression. This is in keeping with McConnell et al. [31, 32], who also observed a minimal amount of progression in the UICC/B-treated animals between 12 and 24 months. In contrast, progression was not reported during the post-exposure year in the UICC/B-treated animals studied at the MRC (grade 4.0 at 12 months; 4.1 at 24 months) by Wagner et al. [44]. Wagner et al. [23, 48] did note an approximate one grade increase in animals exposed for 12 months [0.8 increase i.e. 4.3 at 12 months and 5.1 in survivors, tables 5, 6 in 48]; [1.3 grade increase i.e. 3.7 at 12 months and 5.0 in the 24-month sacrifices, table 4 in 23]. The failure of Wagner et al. [44] to note progression might be related to other factors [e.g. interreader variation, McConnell et al. 31, 32], although the 2.5 grade increment found in those exposed only for

6 months (3.0 in the 6-month sacrifice and 5.5 in the those sacrificed 6 months after 18 months non-exposure [23]) tends to go against this idea.

Sample variation within the UICC/B stock might explain some of these inconsistencies. Thus, Elmes [58] noted that since the UICC/B sample 'was the most popular (research material, the) original stock ran out and was replaced by a new stock, which turned out to differ yet again from the original standards in the number and the distribution of fibres'. Others have found the UICC standard chrysotile samples to be less active than expected probably due to 'overmilling' making them 'too short to produce a maximal tissue response' [52]. Perhaps the more recent UICC/B samples were even more extensively milled than the earlier ones thus further reducing the number of long fibres and their attendant fibrogenic potency.

Pinkerton [33] proposed, on morphometrical grounds (see table 4), that progression only occurred in the UICC/B-treated animals possibly due to 'the presence of fewer long fibres in the lungs of animals exposed to UICC/B chrysotile (that) resulted in a slower rate of lung fibrosis which continued to progress during the post-exposure period'. This led Pinkerton to surmise that 'The greater exposure to very long fibres ($>50\text{ }\mu\text{m}$) in the animals exposed to Jeffrey mine chrysotile stimulated a much more intense initial inflammatory reaction which encased many of these fibres in layers of non-cellular, collagenous interstitial matrix thereby reducing their inflammation inducing potential. This would have allowed the inflammatory reaction in these animals to have decreased in intensity during the post-exposure year at an initially slower but steady pace.'

In contrast to the UICC/B-treated groups, Pinkerton [33] (see above) and Pinkerton et al. [39] failed to find progression with the Jeffrey fibre stating 'that the interstitial fibrotic reaction (with the Jeffrey fibre) did not significantly progress during the post exposure period'. We do not have the morphological data to confirm or refute this. However, it is consistent with the failure by McConnell et al. [56] to demonstrate progression with this fibre type at least for severity since they did not assess extent.

Morphological and Morphometrical Correlation of Parenchymal Fibrosis

This is the first standard, inhalation asbestos bioassay to be rigorously assessed both morphologically and morphometrically. The findings thus derived are, in turn, both complimentary and consistent, fully supporting the proposal that Coalinga chrysotile is not fibrogenic, in

clear contrast to the Canadian fibres. Crapo et al. [34] morphometrically analysed a subset of the same tissues we assessed in this study but concluded that Coalinga could be 'injurious', thus inferring that it was also fibrogenic. However, this claim was based solely on 12-month data neither stratified for non-cellular interstitial changes nor correlated with the histological absence of fibrosis.

By 24 months, the volume of non-cellular interstitial matrix, the chief morphometrical indicator of fibrosis, in the Coalinga-treated animals, fell to levels that no longer differed from those of the age-matched controls (table 6). The statistically significant, morphometrically detectable, increases in non-cellular IMV seen in the Coalinga treated males at 3 and 12 months were thus only transient in nature. These were therefore, most likely, non-specific, 'nuisance' dust type reactions [see 59].

The morphometric data almost certainly underestimate the observed differences between the Coalinga- and Canadian-treated tissues because of sampling bias due to exclusion of areas of bronchiolar metaplasia seen solely in the tissues treated with Canadian fibre. Selective sampling could therefore have seriously underestimated the actual amount of morphometrically detectable 'fibrosis' in the Canadian-treated groups since the excluded metaplastic regions 'were probably the areas of most intense injury' [39].

The observed differential response in the individual interstitial cell types was yet another important indicator of the difference in fibrogenic potential between the Canadian and the Coalinga fibres. The failure of Coalinga fibre to elicit a mast cell reaction is particularly relevant in this regard since it has been said to be of importance in the production of pulmonary fibrosis both in animals and humans [60].

Survival, Parenchymal Fibrosis, and Competing Causes of Death

Fibrosis with the Canadian fibres significantly reduced survival but only in those animals with the highest fibrosis scores. The present report may actually be the first to demonstrate such a fibrosis-related reduction in survival. Thus, Wagner et al. [23, 44], McConnell et al. [31, 32], Davis and Jones [24], and Davis et al. [16, 52, 61, 62] do not discuss fibrosis and survival whilst Wagner et al. [48] state that 'the effect was very slight'. The natural incidence of pulmonary parenchymal fibrosis in untreated F344 rats is very low. No spontaneously occurring cases were noted in this study, in the investigations of Wagner et al. [44] and McConnell et al. [31, 32], or by others [42, 43, 63, 64]. Taken together, the above clearly suggests that

fibrosis, in itself, rarely, if ever, causes death in experimental animals exposed to asbestos. This is not too surprising, since it is not typically a cause of death in humans either, many of whom are also smokers. Progressive diffuse fibrosis, as produced by exceptionally high doses such as those used in the very early studies [65], could produce death directly by cor pulmonale. An indirect reduction in survival also might occur either by infection or an enhanced likelihood of tumour formation [57] though the lethality of such neoplasms has been questioned [31].

Acknowledgements

We are very grateful to Dr. Kevin Browne, MD; Professor John Craighead, MD; Dr. John Davis, DSc; Professor Edward Gaensler, MD; Professor Ulrich Gruber, MD; Dr. Arthur Morgan, DSc; Professor Brooke Mossman, PhD; and Dr. William Weiss, MD for kindly providing critical comments on this manuscript. Dr. Morgan also reviewed many of these data at an earlier time in great detail when he and the Senior author were kindly afforded the opportunity to discuss the morphometric findings with Professor Pinkerton in person at the University of California at Davis in June, 1995. We are also

grateful to Professor Kent Pinkerton and his staff for their hospitality during this visit. We would like to thank Dr. Gene McConnell for providing earlier versions of the pathology data and for discussing these with the Senior author. Dr. Gary Boorman, who with Dr. McConnell reviewed the earlier pathological analysis of many of these materials, also kindly discussed his earlier work with us. We would like to thank the NTP archive scientists including Dr. Melvin Hamlin, Mr. David Bridge, and Dr. Robert Sils and their staff who very graciously assisted in locating the lifetime test materials and allowed the Senior author to study these with their assistance, helpful comments and support. We would also like to thank Dr. Jerry Hardisty at the EPL laboratories, Dr. Bernie Atkins and Dr. Dan McLaurin of Becton Dickinson, Dr. Jack Moore of the Institute of Environmental Health, Mr. Dennis Munday of the MRC external unit, Dr. Finas Cavendar, and Dr. Elizabeth Ford and Mr. Don Goula of the NIEHS procurement division for their assistance in attempting to locate all of the original data underlying this study and its collaborative, comparative investigation. Finally, we would also like to thank Dr. J.C. Wagner for his very kind assistance in reviewing a number of the slides of the animals on lifetime test, for discussions about the study and its origins, and for insightful comments concerning the scientific bases of the observations made herein. Again, we are most grateful to all of those just mentioned for their help but it is we, not they, who will take responsibility for any errors or defects in the work. Similarly, acknowledgement of assistance does not necessarily imply agreement with the conclusions expressed.

References

- 1 Stanton M, Wrench F: Mechanisms of mesothelioma induction with asbestos and fibrous glass. *J Natl Cancer Inst* 1972;48:797-821.
- 2 Stanton M, Layard M, Tegeris A, Miller E, May M, Morgan E, Smith A: Relation of particle dimension to carcinogenicity in amphibole asbestos and other fibrous minerals. *J Natl Cancer Inst* 1981;67:965-975.
- 3 Port F: Asbestos use and carcinogenicity in Germany and a comparison with animal studies. *Ann Occup Hyg* 1994;38:589-600.
- 4 Mossman B, Bignon J, Corn M, Seaton A, Gee JBL: Asbestos: Scientific developments and implications for public policy. *Science* 1990;24:294-301.
- 5 Davis J: Mineral fibre carcinogenesis: Experimental data relating to the importance of fibre type, size, deposition, dissolution and migration; in Bignon J, Peto J, Saracci R (eds): *Non-Occupational Exposure to Mineral Fibres*. Lyon, Int. Agency for Research on Cancer, 1989, vol 90, pp 33-43.
- 6 HEI (Health Effects Institute - Asbestos Research): *Asbestos in Public and Commercial Buildings: A Literature Review and Synthesis of Current Knowledge*, 1991.
- 7 HEI (Health Effects Institute - Asbestos Research): *Asbestos in Public and Commercial Buildings: Supplementary Analyses of Selected Data Previously Considered by the Literature Review Panel*, 1992.
- 8 Corn M: Airborne concentrations of asbestos in non-occupational environments. *Ann Occup Hyg* 1994;4:495-502.
- 9 Schrier H: *Asbestos in the natural environment*; in: *Studies in Environmental Science*, No. 37. Amsterdam, Elsevier, 1989.
- 10 Langer AM, Wolff MS, Rohl A, Seikoff I: Variation of properties of chrysotile asbestos subjected to milling. *J Tox Environ Health* 1978;4:173-188.
- 11 Chung A: Asbestos lung burden and disease patterns in man; in Ribbe P (ed): *Health Effects of Mineral Dusts*. Reviews in Mineralogy, Washington, Mineralogical Society of America, Bookcrafters Inc., 1993, vol 28, chap 13, pp 410-431.
- 12 Case B: Biological indicators of chrysotile exposure. *Ann Occup Hyg* 1994;38:4503-518.
- 13 Lee R: Asbestos contamination in paraffin tissue blocks. *Arch Pathol Lab Med* 1995;10:528-532.
- 14 Pigg R: Uses of chrysotile asbestos. *Ann Occup Hyg* 1994;38:4453-458.
- 15 Vorwald AJ, Durkin TM, Pratt PC: Experimental studies of asbestosis. *AMA Arch Ind Hyg Occup Med* 1951;3:1-43.
- 16 Davis J, Beckett S, Bolton R, Collings P, Middleton A: Mass and number of fibres in the pathogenesis of asbestos related lung disease in rats. *Br J Cancer* 1978;37:673-688.
- 17 Berman W, Crump K, Chatfield E, Davis J, Jones A: The sizes, shapes and mineralogy of asbestos structures that induce lung tumours or mesotheliomas in AF/HAN rats following inhalation. *Risk Analysis* 1995;15:181-195.
- 18 Lippmann M: Asbestos exposure indices. Review. *Environ Res* 1988;46:86-106.
- 19 Timbrell V: Deposition and retention of asbestos fibres in the human lung. *Ann Occup Hyg* 1982;26:347-369.
- 20 Timbrell V: Fibres and carcinogenesis. *J Occup Health Soc Austral* 1983;3:3-21.
- 21 Timbrell V: Pulmonary deposition and retention of South African amphibole fibres: Identification of asbestosis-related measure of fibre concentration. *Proc 7th Int Pneumoconiosis Conf*, Bochum, 1983 vol 2, pp 998-1008.
- 22 Timbrell V, Ashcroft T, Goldstein B, Heyworth F, Meurman LO, Rendall REG, Reynolds JA, Shilkin KB, Whitaker D: Relationships between retained amphibole fibres and fibrosis in human lung specimens; in Walton W (ed): *Inhaled Particles VI*. Oxford, Pergamon, 1987.
- 23 Wagner JC, Berry G, Skidmore JW, Pooley FD: The comparative effects of three chrysotiles by injection and inhalation in rats: Biological Effects of Mineral Fibres. *LARC Sci Publ* 1980; 363-372.
- 24 Davis JM, Jones A: Comparisons of the pathogenicity of long and short fibres of chrysotile asbestos in rats. *Br J Exp Pathol* 1988;69:717-737.

- 25 Platek S, Groth D, Ulrich C, Stettler L, Finnell M, Stoll M. Chronic inhalation of short asbestos fibres. *Fund Appl Toxicol* 1985;5:327-340.
- 26 Ilgen E, Wagner J. The value of experimental animals in analyzing the hazards of exposure to mineral fibres. II. Consideration of natural background incidence of mesotheliomas and non-physiological intraperitoneal technique. Submission to the German MAK Commission on Mineral Fibre Safety, 1989.
- 27 Lemaire I. Characterization of the bronchoalveolar cellular response in experimental asbestosis. *Am Rev Respir Dis* 1985;131:144-149.
- 28 Lemaire I. An assessment of the fibrogenic potential of very short 4-T-30 chrysotile by intratracheal instillation in rats. *Environ Res* 1985;36:134-136.
- 29 Lemaire I. Selective differences in macrophage populations and monokine production in resolving pulmonary granuloma and fibrosis. *Am J Pathol* 1991;138:487-495.
- 30 Lemaire I, Dionne P, Nadeau D, Dunnigan J. Rat lung reactivity to natural and man-made fibrous silicates following short-term exposure. *Environ Res* 1989;48:193-210.
- 31 McConnell EE, Wagner JC, Skidmore JW, Moore JA. A comparative study of the fibrogenic and carcinogenic effects of UICC Canadian chrysotile B asbestos and glass microfibre (JM 100). In: *Biological Effects of Man-Made Mineral Fibres* (Report on a WHO/IARC meeting). Copenhagen, 20-22 April 1982. (Annex 1984;45:234-242).
- 32 McConnell E, Wagner J, Skidmore J, Moore J. Two inhalation studies: A comparison. In: *Biological Effects of Man-Made Mineral Fibres* (Report on a WHO/IARC meeting). Copenhagen, 20-22 April 1982. (Annex 1984;38:118-120).
- 33 Pinkerton K. Lung reaction to chronic inhalation of three types of chrysotile asbestos during the lifespan of the Fischer 344 rat; PhD thesis Duke University, 1982.
- 34 Crapo J, Barry B, Brody A, O'Neil J. Morphological, morphometric and X-ray microanalytical studies on lung tissue of rats exposed to chrysotile asbestos in inhalation chambers. *IARC Sci Mono, Biol Effects Miner Fibres* 1980;1:273-283.
- 35 Pinkerton K, Pratt P, Brody A, Crapo J. Lung injury patterns as a result of exposure to different chrysotile asbestos fibres (abstract). *Am Rev Respir Dis* 1981;123:21.
- 36 Pinkerton K, Pratt P, Crapo J. Morphologic lung changes following exposure to chrysotile (abstract). *Am Rev Respir Dis* 1981;121:251.
- 37 O'Neil J, Pinkerton K, Crapo C. Lung volume changes in rats exposed to chrysotile asbestos (abstract). *Am Rev Respir Dis* 1981;123:146.
- 38 Pinkerton K, Barry B, O'Neil J, Raub J, Pratt P, Crapo J. Morphologic changes in the lung during the lifespan of Fischer 344 rats. *Am J Anat* 1982;164:155-174.
- 39 Pinkerton K, Pratt C, Brody AR, Crapo JD. Fiber localization and its relationship to lung reaction in rats after chronic inhalation of chrysotile asbestos. *Am J Pathol* 1984;117:484-498.
- 40 Pinkerton K, Plopper CG, Mercer RR, Roggli VL, Patra AL, Brody AR, Crapo JD. Airway branching patterns influence asbestos fiber location and the extent of tissue injury in the pulmonary parenchyma. *Lab Invest* 1986;55:688-695.
- 41 Pinkerton K, Young S, Fram E, Crapo J. Alveolar type II cell responses to chronic inhalation of chrysotile asbestos in rats. *Am J Respir Cell Mol Biol* 1990;3:543-552.
- 42 Solleveld H, Haseman J, McConnell E. Natural history of body weight gain, survival, and neoplasia in the F344 rat. *J Natl Cancer Inst* 1984;72:929-940.
- 43 Goodman DG, Ward JM, Squire R, Chu K, Linhart M. Neoplastic and non-neoplastic lesions in ageing F344 rats. *Toxicol Appl Pharmacol* 1979;48:237-248.
- 44 Wagner JC, Berry GB, Hill RJ, Munday DE, Skidmore JW. Animal experiments with VM(M)(V)F - effects of inhalation and intrapleural inoculation in rats; in: *Biological Effects of Man-Made Mineral Fibres* (Report on a WHO/IARC meeting). Copenhagen, 20-22 April 1982. (Annex 1984;42:210-235).
- 45 Pinkerton K, Brody A, McLaurin D, Adkins B, O'Connor R, Pratt P, Crapo J. Characterization of three types of chrysotile asbestos after aerosolization. *Environ Res* 1983;31:32-53.
- 46 Campbell W, Huggins C, Wylie A. Chemical and physical characterization of amosite, chrysotile and non-fibrous tremolite for oral ingestion studies by the National Institutes of Environmental Health Sciences. *US Bureau Mines Rep Invest No. 8452*, 1980.
- 47 Timbrell V. Characteristics of the International Union against Cancer standard reference samples of asbestos; in Shapero H (ed): *Pneumoconiosis Proc Int Conf*, Johannesburg, London, Oxford University Press, 1970, p 28.
- 48 Wagner JC. The effects of the inhalation of asbestos in rats. *Br J Cancer* 1974;29:252-269.
- 49 Gaensler EA. Asbestos related pleural plaques: Much ado about very little. *DRI Asbestos Medicine Seminar*, Scottsdale, 1991, section H.
- 50 Gibbs A, Stephens M, Griffiths D, Blight B, Pooley F. Fibre distribution in the lungs and pleura of subjects of asbestos related diffuse pleural fibrosis. *Br J Ind Med* 1991;48:762-770.
- 51 McLoud TC, Woods BO, Cammington CB, Epier GR, Gaensler EA. Diffuse pleural thickening in an asbestos-exposed population: Prevalence and etiologies. *Am J Roentgenol* 1985;144:9-18.
- 52 Muhle H, Poit F, Bellman B, Takenaka S, Ziem U. Inhalation and injection experiments in rats to test the carcinogenicity of VM(MF) Ann Occup Hyg 1987;131:755-764.
- 53 Davis J, Addison J, Bolton R, Donaldson, K, Jones, A. Inhalation and injection studies in rats using dust samples from chrysotile asbestos prepared by a wet dispersion process. *Br J Exp Pathol* 1986;67:113-129.
- 54 Mellon Institute: Report No. 29-55 produced for the Union Carbide company 1966 (accessed from Union Carbide on request).
- 55 Mellon Institute: Report No. 34-70 produced for the Union Carbide company 1971 (accessed from Union Carbide on request).
- 56 Gross P, de Treville L. The Pulmonary Response to Coal-tar Asbestos Dust: A Preliminary Investigation (Final Report). (Produced for the JM company) Ind Hyg Foundation of America, 7-1968.
- 57 McConnell E, Adkins B. Studies on the chronic toxicity (inhalation) of wollastonite in Fischer 344 rats. *Inhal Toxicol* 1991;3:323-337.
- 58 Davis J, Cowie B. The relationship between fibrosis and cancer in experimental animals exposed to asbestos and other fibres. *Environ Health Perspect* 1990;88:305-309.
- 59 Elmes P. Conflicts in evidence on the health effects of mineral fibres. Chapter 23; in Liddell D, Miller K (eds): *Mineral Fibres in Health and Disease*. Boca Raton, CRC Press, 1991, pp 321-337.
- 60 Oberdorster G. Evaluation and use of animal models to assess mechanisms of fibre carcinogenicity. *IARC Sci Publ* 1996;140:107-127.
- 61 Wagner M, Wagner J, Griffiths M. Mast cells and inhalation of asbestos in rats. *Thorax* 1984;39:539-544.
- 62 Davis JM. The effects of intermittent high asbestos exposure (peak dose levels) on the lungs of rats. *Br J Exp Pathol* 1980;61:272-279.
- 63 Davis JM. Experimental lesions in rats corresponding to advanced human asbestosis. *Exp Mol Pathol* 1986;4:207-221.
- 64 Sass B, Rabstein, Madison R, Nims R, Peters R, Kelloff G. Incidence of spontaneous neoplasms in F344 rats throughout the natural lifespan. *J Natl Cancer Inst* 1975;54:1449-1453.
- 65 Coleman G, Barthold S, Oshaldixon G, Foster S, Jones A. Pathological changes during ageing in Barrier-reared Fischer 344 male rats. *J Gerontol* 1977;32:258-278.
- 66 Wagner, J. Asbestosis in experimental animals. *Br J Ind Med* 1963;20:1-12.