Review: The Fiber Length of Coalinga Chrysotile: Enhanced Clearance due to Its Short Nature in Aqueous Solution with a Brief Critique on ``Short Fiber Toxicity''

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The Fiber Length of Coalinga Chrysotile: Enhanced Clearance due to Its Short Nature in Aqueous Solution with a Brief Critique on ''Short Fiber Toxicity''

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Key Words

Short fiber · Coalinga chrysotile · Length · Toxicity · Clearance

Abstract

''Coalinga'' chrysotile is a short fiber chrysotile that historically has been noted to be very different from other forms of asbestos. Under the microscope the ''extreme fiber length'', typical of most forms of asbestos, was said to be neither ''visible'' nor ''even measurable''. This morphology has been recognized for decades. ''Short'' fiber also refers to the predominant length distribution of a fiber population in a given sample. The US Government used Coalinga chrysotile as its ''standard'' short fiber sample preparation for animal testing by ingestion and inhalation more than 30 years ago. The ''standard'' preparations were largely composed of fibers less than 5 μm long. This was only demonstrable in aqueous solution because Coalinga chrysotiles are composed of loosely bound short fibers and fibrils that fall apart into their constituent components in an aqueous milieu. The short length is thus not an artifact of sample preparation but a reflection of the nature of the starting materials. However, currently used regulatory counting methods do not discriminate between Coalinga type and typical long chrysotile fibers even though their biological potencies differ radically. Consequently, these methods grossly overestimate the attendant health risks. Biologically relevant fiber length discrimination is determined by macrophage mediated clearance and, more specifically, macrophage diameter. The diameter of a human macrophage approximates 10–15 μm providing a logical biological foundation for differentiating ''short'' vs. ''long'' fibers. The findings of this report support the importance of fiber length cut-offs at this level.

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Nomenclature

Asbestos types [1]

Coalinga	Chrysotile from the New Idria deposit, California. also known as "Calidria" chrysotile (trademark of the Union Carbide Corp., Inc). Other deposits of this form of chrysotile are known, for example, from Stragari, Yugoslavia.
UICC B	Standard sample of a mix of Canadian chrysotiles prepared for the "Union Internationale Contre le Cancer" for research purposes.
Jeffrey	Chrysotile from the mine Jeffrey, Québec

Asbestos grades

JM Plastibest	Chrysotile asbestos, grade Plastibest No. 20 (trademark of Canadian Johns-Manville Co., Ltd.)
COF25, SG130, RG144, RG244, HPO	[1]

Analytical techniques

SEM	Scanning emission microscopy
TEM	Transmission electron microscopy
PCM	Phase contrast microscopy (light microscopy)
ISO method 13794, ISO method 10312	International Organization for Standardization reference methods using TEM for determination of the concentration of asbestos structures in ambient atmospheres.

Regulatory authorities and commercial companies

ATSDR	Agency for Toxic Substance and Disease Registry
EPA	Environmental Protection Agency
NTP	National Toxicology Program
NIEHS	National Institute of Environmental Health Sciences
IITRI	Illinois Institute of Technology Research Institute
ASTECO	Asbestos Testing Company
KCAC Inc.	The King City Asbestos Corporation. Successor to UCC. The last corporation to mine the New Idria deposit.
UCC	Union Carbide Corporation

Introduction

Fiber length was shown to be an important determinant of the pathogenicity of asbestos fibers more than 60 years ago (reviewed by Ilgren and Chatfield [2]). More recent studies, particularly those since the 1960s, have suggested that only fibers longer than 5 μm were particularly pathogenic [3]. Langer et al. [4] have argued that 5 μm was only employed for utilitarian purposes and that a 5 μm cut-off was not biologically relevant.[2] However, the weight of evidence indicates that 5μm does reflect biological activity and particularly macrophage clearance.[3] Fibers longer than the diameter of a macrophage that penetrate deep into the lungs cannot readily be cleared from the body. In fact, more recent evidence strongly suggests biological activity actually correlates with fibers at least 10 μm and probably 20 μm long [5–7]; lengths that more closely approximate the true diameter of a human macrophage (10–15 μm) ([6]; also discussed by Bernstein and Hoskins [7]). This proposal is thoroughly in keeping with the widely recognized fact that macrophages mediate asbestos related disease [8].

Berman et al. [9] found fibers less than 5 μm long lacked biological potency.[4] This was based exclusively upon direct analyses of different fiber types with proven biological activity. A "negative" control lacking biological potency was not included.[5] However, the availability of different types of chrysotile of known biological potential and the performance of large fiber counts done following direct and indirect preparation has enabled Chatfield to determine the fiber size populations most predictive of disease. Chatfield's very detailed data set will be presented in a forthcoming publication (Chatfield, in preparation). The present report summarizes a few of Dr Chatfield's preliminary findings[6] and extensively reviews work previously done by others in this field. The overall findings of this review strongly support a critical biological length cut-off in excess of 15 μm in keeping with the notion that structures longer than 20 μm are the most potent [7,10,11].

Langer et al. [4,12,13] were the first to refer to a "Coalinga type" chrysotile. They did so since its behavior not only differed from other types of chrysotile but also from asbestos fibers in general. Thus: "Asbestos minerals occur naturally. They are usually fibrous; have extreme length: width ratios; are flexible, possess high tensile strength, and are easily separated into filamentous strands [14]. It is important to note that although most exploited asbestos deposits throughout the world possess these characteristics, there are exceptions. For example, the definition does not hold for some chrysotile deposits in

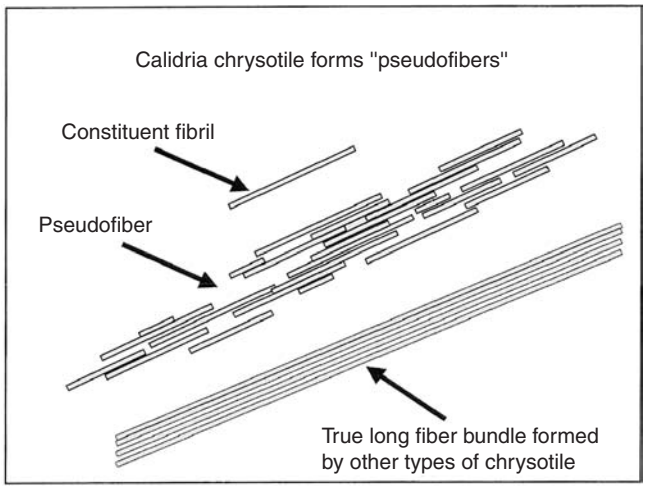

Fig. 1. Calidria chrysotile forms "pseudofibers" compared to true chrysotile fiber bundles. [17,31] Each fibril is a single crystal produced by unidirectional crystalline growth [15].

California that have been described as the 'Coalinga type'. In these, fibers occur as intergrown mats with few of the above characteristics visible or even measurable".

The present review presents information that confirms these claims. Thus, long Coalinga type fibers are actually "pseudofibers" consisting of assemblies of numerous overlapping, weakly bound short fibrils that each only extend over a small part of the length of the fiber. Each fibril is a single crystal produced by unidirectional crystalline growth [15]. By contrast, true long chrysotile fibers are composed of tightly bound fibrils oriented in parallel that run the entire length of the fiber[7] (Figure 1). This can be demonstrated by comparing the fiber size distributions of Coalinga type and non-Coalinga type chrysotiles after direct and indirect preparation. Thus, long Coalinga type pseudofibers readily disperse into their short (98% less than 5 μm) constituent fibrils in aqueous solution whereas true long chrysotile fibers fail to disperse.

Current regulatory methods for monitoring asbestos rely upon phase contrast microscopy (PCM). However, this method fails to discriminate between long Coalinga type pseudofibers and true chrysotile fibers. In consequence, the two are treated equally and so the risks attributed to Coalinga type chrysotiles have been greatly overestimated. Many types of short fiber chrysotile behave in a manner similar to Coalinga type chrysotiles. Thus, most exist in an aerosol as "long" clusters composed of weakly bound single short fibers and/or fibrils either bound to each other and/or to various types of nonfibrous materials. The risks attributed to products made from any of these weakly bound long pseudofibers, from whatever source, (e.g., those produced from friction materials [13]) are greatly overestimated by the use of PCM. Thus, the standard regulatory methods currently used to assess risk do not and cannot adequately represent the biological potency of many of the most commonly encountered types of chrysotile. This proposal has very broad implications for risk assessment since short fiber chrysotile is the most common form of asbestos frequently found in the air we breathe, the food we eat, and the water we drink. It is thus, not surprisingly, one of the common mineralogical constituents of the human lung. PCM counts of Coalinga type materials in air are highly misleading [16,17] since pseudofibers are counted as regulatory fibers irrespective of their origin or nature. The true biological potency of Coalinga type fibers is therefore only apparent on indirect examination. Future regulatory counting methods must use both direct and indirect analyses to assess accurately biological activity [18]. Such methods must require separate indirect analyses of the respirable and nonrespirable fractions found on direct examination.

Methods That Have Been Used For Fiber Length Measurements

Direct Measurements of the Length of Coalinga Pseudofibers and Non-Coalinga Fibers in an Aerosol

Pinkerton et al. [19] described the size characteristics of short Coalinga and long Canadian ("Jeffrey") chrysotiles in an aerosol prepared for experimental study by the National Institute of Environmental Health Sciences (NIEHS) [20] (Table 1). The criteria for "fiber–fiber cluster counting by scanning electron microscopy included fibers of all lengths and diameters so long as they possessed at least a 3:1 aspect ratio". The dust cloud was produced with a modified Timbrell generator [21] in a $5\,m^3$ stainless steel exposure chamber. Each asbestos preparation was hand compressed into a 3×9 cm cylinder to form a plug. This was mechanically advanced into the path of a blade rotating at 1500 rpms. The air flow was $200–400\,L \cdot min^{-1}$. "Fiber 'flocs' or clumps were occasionally present on filters and consisted of tangled fibers and fiber clusters. These were not analyzed because their complexity prevented accurate separation into individual fibers and fiber clusters". By light microscopy, the percentage of fibers present from 5 to 30 μm in length was said to be similar for all three aerosolized preparations. Using TEM, Pinkerton et al. [19] found "Combined fiber and fiber cluster length in Coalinga Mine chrysotile . . . showed a distribution with many fibers exceeding

Table 1. Fiber length distribution of coalinga chrysotile: aerosol

Study	Sample	Method	*n*	Percentage >5 μm	Percentage >10 μm	ISO (±)
Longo [23]	RG144	TEM	409[a,b]	56	32	(+)
Chatfield – Muhle (unpub.)	RG144	TEM	1083	>50[c]	30[c]	(+)
Pinkerton [19]	COF25	SEM	1,050[a]	48	34	(−)
Muhle [22]	RG144	SEM	200[a]	50[d]	10[d]	(−)
Bernstein [27]	RG144	TEM	600[a,e]	22	nd	(−)

n – number of fibers.
[a]Total number counted.
[b]Number greater than 0.5 μm long.
[c]Preliminary data, counts all greater than 0.5 m.
[d]There were no 5 and 10 μm cut-offs, only 6 and 14 μm. Therefore, the 10% figure is almost certainly an overestimation.
[e]The precise number counted was not actually stated: ''for fibers, the recording was stopped when 500 fibers with length greater than 5 μm, diameter less than 3 μm . . . were recorded . . . The evaluation of objects of length less than 5 μm was stopped when 100 objects were reached''.
nd – not given.

30 μm in length. Less than 52% of the combined fibers and fiber clusters from the Coalinga chrysotile were less than 5 μm in length and only 66% of the combined fibers and fiber clusters in the Coalinga chrysotile aerosol were less than 10 μm. By contrast, for both the Jeffrey and the UICC B chrysotiles, approximately 75% of the combined fibers and fiber clusters were less than 5 μm in length while at least 92% of the combined fibers and fiber clusters in the Jeffrey and UICC B aerosols were less than 10 μm in length'' [19].

Muhle et al. [22] characterized the length of Coalinga pseudofibers in an aerosol using SEM. The RG144 Coalinga chrysotile sample they used was received directly from NIOSH. The material as received was treated with a knife mill for one minute before it was fed into the aerosol generator. The dust cloud was generated with a vibrating bed aerosol generator and static discharged with a ^{85}Kr source. The counting rules were not stated. Monthly samples were taken on polycarbonate filters. The detection limit was said to be 0.05 μm. Data were not given for the percentage of fibers longer than 5 μm and 10 μm: 90% of the fibers were longer than 2 μm; 50% longer than 6 μm; and 10% longer than 14 μm. Muhle et al. [22] wrote that the fiber dimensional analysis of airborne Coalinga chrysotile was ''not very reliable'' due to the preferential generation of thick bundles.

Longo [23,24][8] characterized the fiber lengths of Coalinga chrysotile fibers in an aerosol. The study was not intended to simulate any type of work activity [25]. The dust generation method initially involved placing 20 g of Calidria powder in a ''typical saltshaker''. Then, a small amount was shaken onto a 200 mesh sieve mounted on top of a 5 gallon bucket for ca. 25 s. The other edge of the sieve and the top of the bucket was sealed so that compressed air (90 PSI) could be blown in from the bottom of the bucket to cause some of the Calidria to become airborne in a $20 \times 15 \times 8$ ft chamber whose walls and ceiling were constructed of painted plastic laminate. To get an even dispersal, Longo blew the fiber sample with an air hose at 90 PSI using 2–3 s bursts through the bottom of a container during the 4 min test (see also Longo [23]). TEM analysis (of the direct filters) was performed using a modified Yamate[9] EPA Level II method [26]. Structures of any diameter longer than 0.5 μm were counted. Longo said a single fiber was a single fibril with at least a 5 : 1 aspect ratio that was greater than 0.5 μm long. A bundle was any structure with three parallel touching fibers. A cluster consisted of any three fibers that intersected at three points. A matrix was any asbestos structure associated with a nonasbestos particulate. The TEM data demonstrated that ca 56% of the fibrous asbestos structures measured in the four air samples were greater or equal than 5.0 μm in length and 32% were longer than 10 μm.[10]

Bernstein et al. [27] measured the length of Coalinga pseudofibers in an aerosol. Thus: ''The fiber was prepared for the exposures prior to the technical trials by grinding it in a Cylotec sample mill . . . by a high-speed action, rolling the sample against the inner circumference of a durable grinding surface and then passing it through a fine mesh screen''. The generation technique, described in detail by Bernstein et al. [28], was ''designed to maximize the number of long respirable fibers'' without ''breaking, grinding or contaminating'' them. Fibers were counted using TEM at a magnification of 10,000x ''with no lower or upper size limits imposed on either length or diameter.

Fibers were defined as any object that had an aspect ratio of at least 3:1''. Twenty two percent of the Coalinga fibers were longer than 5 μm; 0.4% were longer than 20 μm (range: 0.6–25 μm). The findings of Bernstein et al. [27] for Coalinga chrysotile were rather similar to those obtained by Bernstein et al. [29,30] using Canadian (13% greater than 5 μm; 1.4% greater than 20 μm: range: 0.5–110 μm) and Brazilian (23% greater than 5 μm; 22% greater than 20 μm: range 0.6–80 μm) samples, respectively; all assessed using identical analytical protocols.

Chatfield measured the length of airborne Coalinga chrysotile pseudofibers in collaboration with Professor Hartwig Muhle of the Frauenhofer Institute in an investigation that began in 1994 (Chatfield, in preparation). The filters originally used by Muhle et al. [22] for fiber counting by SEM were split in half. One half was retained by Muhle and his colleagues for re-analysis whilst the other half was sent to Dr Eric Chatfield for independent analysis. Chatfield and Muhle used identical TEM analytical techniques (ISO method 10312) (Table 1). Standard ISO counting rules were applied.[11] The details of the methodology are described by Chatfield (in preparation).[12] The dust cloud was generated as described by Muhle et al. [22] (see below). Chatfield and Muhle found the majority (greater than 50%) of Coalinga pseudofibers (Figure 2) (Table 1) and Canadian fibers (Figure 3) were longer than 5 μm. However, no Calidria fibrils were ever found to be longer than 30 μm in length whilst some Canadian chrysotile fibers were up to one inch long [31].

In summary, five groups have analyzed the length distribution of Coalinga pseudofibers in an aerosol. The results of four (Chatfield, Pinkerton, Muhle, and Longo) were similar and showed that the majority of Coalinga pseudofibers were longer than 5 μm (Table 1). The findings of Pinkerton et al. [19] were probably subject to a certain degree of sampling bias since the occasional fiber flocs or clumps present ''were not analyzed because their complexity prevented accurate separation into individual fibers and fiber clusters''. The findings of Bernstein et al. [27] were out of line with the others almost certainly because counting constraints were not imposed on the size of the structures counted and also possibly because of grinding that caused further distortion of the actual fiber size distribution. The inclusion of particles smaller than 0.5 μm may thus have seriously ''diluted'' the longer ones reducing the percentage of long fibers accordingly.

The length distributions of chrysotiles other than Coalinga have been measured by direct examination in an aerosol. The majority of these fibers were also longer than 5 μm [19,27,32,33] and their fiber length distributions

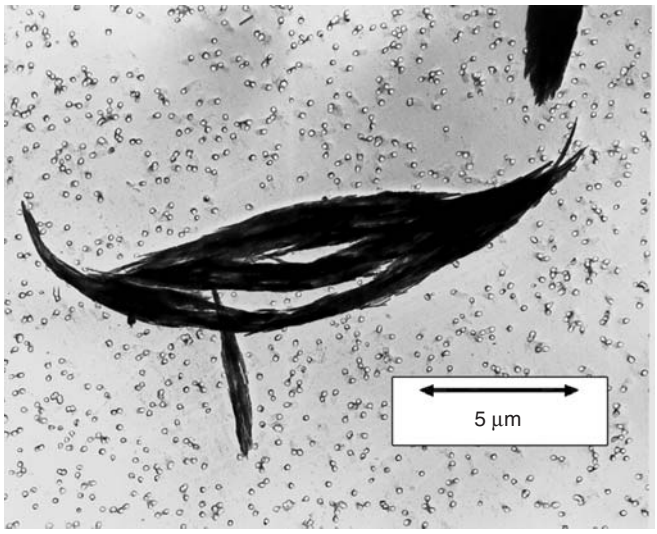

Fig. 2. Sample of Calidria chrysotile dispersed in air for animal experiments conducted by Dr H. Muhle.

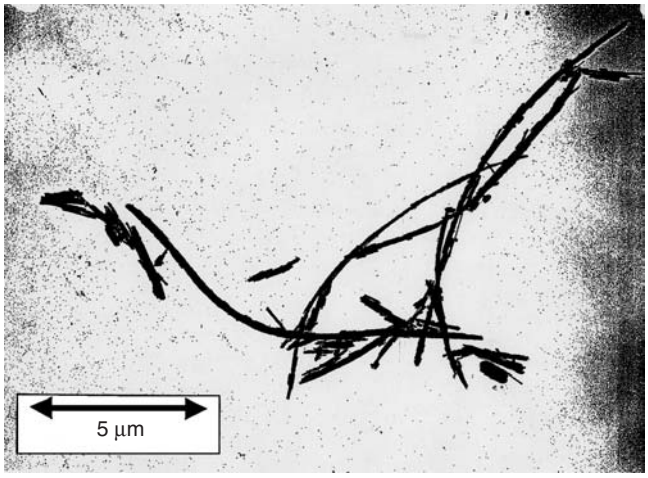

Fig. 3. TEM micrograph of airborne UICC-B (Canadian) chrysotile dispersed in air for animal experiments conducted by Dr J.M.G Davis.

did not differ significantly from those of Coalinga chrysotile. The detailed length distribution data of the different non-Coalinga chrysotiles will be presented by Chatfield (personal commun.) in a forthcoming publication (in preparation).

Measurements of the Length of Coalinga Chrysotile Fibers in Aqueous Solution

Langer et al. [4] analyzed the fiber length distribution of RG144 Coalinga chrysotile fibers (Tables 2 and 3) dispersed in nitrocellulose solution on precleaned glass slides by gentle stirring.[13] Sonication was not used. A second slide was placed over the first and the two slides were pulled apart producing a film on each. The film

Table 2. Fiber length distribution of Coalinga chrysotile: aqueous solution (ISO counts)[a]

Study	Sample	*n*1	*n*2	Percentage >5 μm	Percentage >10 μm
ASTECO [39][b]	SG130	453	269	0.4	0.4
ASTECO [39][b]	RG144	409	392	0.8	0
Chatfield[c] [unpub]	COF25	3489	3489	7.0	0.8
ASTECO [39][b]	RG244SS	420	406	10	2.0
Chatfield2[c] [unpub]	RG144	346	346	14	3.2
ASTECO [39][b]	RG244NS	417	352	18	5.0
ASTECO [39][b]	RG244SN	492	451	27	5.0
Longo[d] [23]	RG144	100	81	42	15

*n*1– all particles of any size.
*n*2 – only particles greater than 0.5 μm long.
[a]All samples are of bulk material except.
[b]The raw data used to re-calculate the percentage of fibers longer than 5 and 10 μm excluding particles less than 0.5 μm in length are not shown in Table 2 but are available upon request from the author.
[c]Preliminary unpublished data.
RG244 SS – This sample was silanized and ultrasonicated.
RG144NS – This sample was not silanized but was ultrasonicated.
RG244SN – This sample was silanized but not ultrasonicated.
[d]A total of 19 structures have been excluded from Longo's data for being less than 0.5 μm long.

Table 3. Fiber length distribution of Coalinga chrysotile: aqueous solution (non-ISO counts)[a]

Study	Sample	*n*1	*n*2	Percentage >5 μm	Percentage >10 μm
Nolan [35]	RG144	2939	?	2.3	0.5
Langer [4]	RG144	1014	309	4.5	2.3
Campbell [20]	COF25	1080	?	7.9	2.1
Muhle [22]	ns	?	?	12	ns**

[a]All samples are of bulk material.
RG244 SS – This sample was silicon treated and ultrasonicated.
RG144NS – This sample was not silicon treated but was ultrasonicated.
RG244SN – This sample was silicon treated but not ultrasonicated.
*n*1 – all particles of any size.
*n*2 – only particles >0.5 μm long.
? – no information about the number of particles less than 0.5 μm long.
**90% greater than 0.4 μm; 50% greater than 1.2 μm; 10% greater than 5.9 μm.

was floated on water and carbon coated grids placed on the film. One thousand objects were counted for each preparation; a particle was composed of at least two fibrils. There were no size constraints on counting making the data difficult to compare with others. However, since particle numbers were given per length class, it was possible to calculate the number less than 0.5 μm long. Thus, discounting 43% of the fibers and 76% of the fibrils less than 0.5 μm long, 4.5% (14/309) were longer than 5 μm and 2.3% (7/309) were longer than 10 μm fiber diameter measurements were not recorded. Yeager et al. [34] re-presented the data of Langer et al. [4] where the two tables presented by Langer et al. [4] were combined and the length classes in each were simplified.[14] Nolan et al. [35] also presented Coalinga length data[15] but it was not clear if these were from Langer et al. [4] or if they were the results of a new analysis. Nolan et al. [35] did not specify the method used to measure the Coalinga sample. Thus, two methods were mentioned: the "wipe out" technique (that had been used by Langer et al. [4] and, ironically, seriously criticized by Langer [36] for "creating diameter artifacts so that fiber counts and width distribution measurements were not made")[16] and an "ultrasound water dispersion" method. The one used to examine Coalinga was not specified. No counting rules were given only: "The shortest chrysotile fibrils detected were ca. 0.1 μm long". Nolan et al. [35] said: Some 2.3% of the fibers were longer than 5 μm and 0.5% were longer than 10 μm. (Table 3)

Wylie [37] measured the size of COF 25 Coalinga chrysotile and JM Quebec Plastibest 20 fibers in aqueous solution using SEM. No counting rules were specified. The samples had been processed to remove impurities but were not milled. A few milligrams were agitated in distilled water with a small amount of detergent added to facilitate dispersion. The suspension was filtered onto a 0.1 μm polycarbonate filter and washed several times with distilled water to remove the soap. Segments of the filters were then mounted on aluminum specimen tabs, a drop of

a suspension that contained 1.1 μm latex spheres was added and allowed to dry. The tabs were then coated with either copper or carbon. Size measurements were made directly on the cathode ray tube by a ruler calibrated to the 1.1 μm latex spheres. The data for each mineral represented 1200–2000 individual particle measurements taken from 12 to 20 separate sample preparations. No width data were given. Length data for Coalinga and Quebec chrysotiles are given in Wylie [37] as aspect ratio frequency diagrams for all particle lengths. However, the number and percentage of fibers in each individual size class cannot be determined from these. If figures showing data from the two chrysotile types are superimposed, it is possible to virtually "exclude" particles greater than 0.5 μm long. Such a comparison initially suggests a very radical difference in the fiber length distributions of these two samples. This is particularly so for fibers greater than 20 μm long since these are virtually absent from the COF25 preparation but present in very large numbers in the JM Plastibest. Unfortunately, comparison of the log width distributions by SEM and TEM of the COF25 sample performed in a subseqent companion study Siegrist and Wylie [38] clearly demonstrated that SEM does not "see" most of the fibrils. Since the long Plastibest sample was not analyzed by TEM (Wylie, pers. commum., 2004), failure to see most of the fibrils probably made this sample appear much longer overall than it really was.

Campbell et al. [20] measured COF25 Coalinga chrysotile under an inter-agency agreement between the NIEHS and the Bureau of Mines with support through contracts with the University of Maryland [37]. The fine particle lab, IITRI, provided the NIEHS with size characterization data on Coalinga and "long fiber" chrysotile [20]. TEM was used to measure COF25. There were no length cut-offs (Wylie, pers. commun., 2004). Aspect ratios, even in the smallest size categories (1 : 1–2.9 : 1), were reported (Wylie, pers. commun., 2004). The only size limitations were on long particles since they did not fit on a TEM grid if they were longer than about 75 μm (Wylie, pers. commun., 2004). The TEM preparation used a 5% colloidal solution which was then added to distilled water that was stirred vigorously in a glass jar and ultrasonicated for 10 min. The COF 25 data involved area counts of 1080 particles. Campbell et al. [20] concluded that "COF 25 is comprised of very short fibers, with 7.9% longer than 5 μm and 2.1% longer than 10 μm". It was not possible to recalculate the data excluding particles less than 0.5 μm since the numbers of particles of this length were not given (Table 3).

Muhle et al. [22] measured the size of Coalinga chrysotile fibers in aqueous solution. The specification of the sample used was not stated only that it "was normally used for paper production"...(and) "pre-treated by the producer for manufacturing asbestos paper"[17]. Fibers were sonicated for about 1 min. The counting rules were not stated, only that the limit of detection for SEM was ~0.05 μm. Since the original particle counts were not given, the percentage of fibers longer than 5 μm with a length cut-off of 0.5 μm could not be calculated. Twelve (12%) of the fibers were longer than 5 μm (Table 3).

ASTECO [39] measured the length and width distributions of three different commercial samples of Coalinga chrysotile for KCAC Inc: SG130, RG144, and RG244 (Table 2). The samples were prepared "by water filtration while trying to disturb them as little as possible and thus attempting to keep any change in the fiber size of the sample to a minimum". The samples were mildly shaken by hand, put through serial dilutions and then filtered through 0.1 μm pore size Mixed Cellulose Ester (MCE) filters. Only one type of Coalinga chrysotile (one of the three samples of RG 244 on test) was subjected to ultrasonication (see legend to Table 2). "Analysis was continued until 400 fibrils were counted. The lengths and widths of the fibrils counted were measured at 20,000 and 60,000 magnification, respectively". The size constraints imposed by these counting methods were not stated. There was significant variation in the fiber length distributions depending on the type of sample. Thus, the percentage of fibers longer than 5 μm ranged from 0.4 to 27% whilst those greater than 10 μm ranged from 0.4 to 5.0%. Non-Coalinga chrysotile samples were not analyzed.

Longo [23] measured the size of RG144 Coalinga chrysotile fibers in aqueous solution. "A 1–3 mg sample of RG144 was suspended in 200 ml of distilled water. The pH was adjusted to 4.0 with dilute acetic acid. The mixture was vigorously hand shaken for 1–2 min. The mixture was then placed in a table top sonicator for 15 min. A 2.0 ml aliquot (sample) was filtered through a 0.20 μm polycarbonate filter and dried in a table top dessicator. A section of this filter was coated with Au/Pd and mounted on a SEM stub with carbon paint."..."For TEM analysis, another section of the polycarbonate filter was prepared for analysis by the Jaffee Wick Washer method. For the TEM analysis, 100 Calidria structures were precisely sized by length and width at a magnification of 25 KX to 150 KX and recorded". The counts were said to have been done using the Yamate method [26] with a length cut-off of 0.5 μm. However, if this was so, it is

difficult to understand why 19 structures less than 0.5μm were included in the counts. "At 150 KX, the width of the fibers was measured. If more than one fiber was in a structure, then each fibril was sized. Typical fibers and structures greater and less than 5 μm were recorded". TEM examination demonstrated "35% of Calidria fibers were longer than 5.0 μm in the 100 fiber count"[18] (max. 22 μm; average 4.1 μm). Recalculating the data without these short (less than 0.5 μm) particles, demonstrates that 42% were greater than 5 μm in length and 14.8% longer than 10 μm (Table 2). SEM analysis was said to demonstrate that Calidria "contained a significant amount of single chrysotile fibers longer than 5 μm" which was estimated to be ca 5–10% of all structures observed.[19] Longo did not analyze non-Coalinga chrysotile.

Chatfield [17,31] measured the fiber length distribution of a processed ore sample of COF25 Coalinga chrysotile in aqueous solution[20] using ISO method 13794. The bulk COF25 Coalinga chrysotile was available from the Chatfield lab stocks both as dried material and in aqueous suspension within sealed ampoules at known concentrations. Chatfield used these samples for more than 25 years to calibrate TEM-based measurements conducted in his laboratory. RG144 bulk material was obtained from KCAC in its commercially processed form.[21] The COF25 length measurements conducted at three different times (1980, 1994, 2000) were nearly identical and demonstrate that more than 90% of the fibrils were shorter than 5 μm. Only 0.8% were longer than 10 μm (Chatfield pers. commun., paper in preparation) (Table 2).

Chatfield also determined the fiber length distributions of RG144 Coalinga chrysotile that had initially been aerosolized by Muhle et al. [22] in their collaborative study that began in 1994 (see above). The fibers were scraped off the filters from Muhle, dispersed in water, and analyzed using ISO method 13794. Chatfield [17] said the "very big clusters taken off the Muhle filters (Figure 2) and placed into water dispersed completely into very, very short fibers"[22] (Figure 4) Fourteen percent of these were longer than 5 μm whilst 3.2% were greater than 10 μm in length (Table 2).

Summary of Indirect Analyses

Fiber length distributions of Coalinga chrysotile in aqueous solution have been determined by seven different research groups. Six groups studied bulk ore samples. The seventh examined an aerosolized preparation. All were done by TEM. Data from these studies have been summarized in Tables 2 and 3. The findings are presented in order of increasing percentage of fibers greater than 5 μm in length and displayed according to those that used ISO criteria and those that did not.

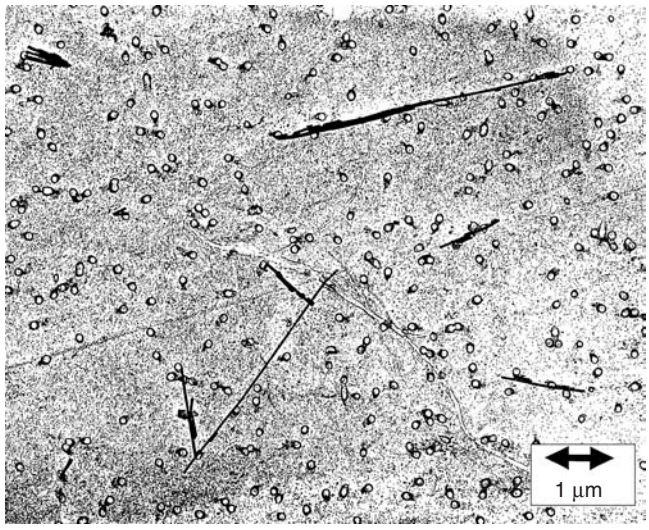

Fig. 4. Airborne Calidria chrysotile dispersed in water (Experimental Sample RG144). ("from Chatfield [17,31]")

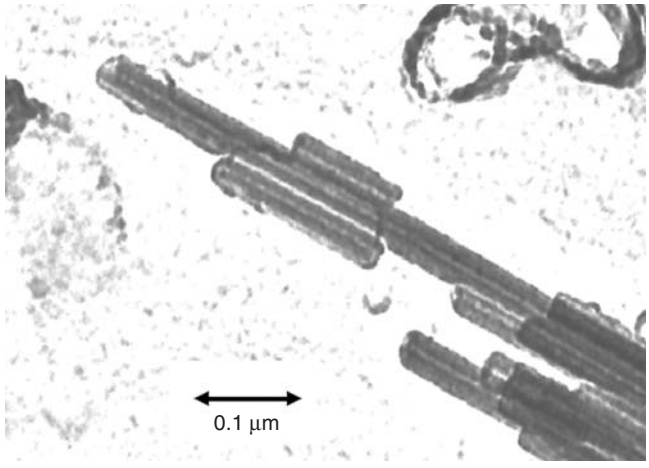

Fig. 5. Airborne Calidria chrysotile dispersed in water showing incomplete separation of fibrils. ("from Chatfield [17,31]")

Nine samples, including four different types of fiber preparation, were analyzed using ISO counting rules. Longo's [23] data: (42% greater than 5 μm) are those most out of line with the others. This is probably due to small sample size ($n = 81$) and poor dispersion. This is suggested by the photos appended to Longo's [23] report which show numerous overlapping short fibrils in many of the bundles (similar to some seen in Chatfield's studies as illustrated in Figure 5). These probably would have been released if a greater amount of force had been applied to disperse them.[23]

Two of the ASTECO [39] samples appeared to contain a particularly high percentage of fibers longer than 5 μm (18%, ultrasonicated, nonsilanized RG244; 28%, non-ultrasonicated, silanized RG244) probably due to limited dispersion. The data from Muhle et al. [22] (gave 12%), and Chatfield (paper in preparation) (see above and Table 2) probably also reflect a certain degree of incomplete dispersal (Figure 2). Their data, however, are not strictly comparable since their sample sources differed. Thus, the original Muhle et al. [22] sample was used for the production of paper. These came from an unknown source (see above). By contrast, the sample used by Chatfield (in preparation) was an RG144 preparation that had originally been experimentally aerosolized by Muhle et al. [22]. Chatfield's COF25 data set is almost 10 times larger than any other study ever performed and probably best approximates the actual fiber length distribution of well dispersed Coalinga chrysotile.

Chatfield [31] summarized important issues related to the fiber length distribution determinations of chrysotile in water including those of the so called "Coaling type". Thus, "stopping rules for TEM asbestos fiber counting methods generally specify termination of counting at 100 fibers (minimum length 0.5 μm); when only 100–200 fibers are counted all chrysotile length distributions look the same regardless of the origin of the chrysotile; even a count of 1000 fibers is often not sufficient to provide statistically valid information for the number of fibers longer than 10 μm; the differing capabilities of different instruments (optical microscopes, SEM, TEM) result in different size distributions; and the failure to establish a minimum length criterion for fiber counting makes much of the published size distribution data questionable".

Fiber Length Distributions of Coalinga and Non-Coalinga chrysotiles Prepared by Direct and Indirect Examination

Four studies: Longo [23]; Pinkerton et al. [19], Muhle et al. [22] and Chatfield (in preparation) present data that demonstrate that typical long, thick Coalinga chrysotile pseudo-fibers in an aerosol fall apart into their short, thin fibrillar constituents in aqueous solution (Table 4).

Chatfield has clearly demonstrated a marked reduction in the fiber length distribution of the Coalinga fibers but relatively little if any change in the majority of the non-Coalinga chrysotiles (Chatfield, in preparation).[24] Longo noted a relatively small reduction (14%) in the lengths of the Coalinga fibers measured in an aerosol versus aqueous solution[25,26] but this is clearly due to poor dispersion of the indirect sample.

Table 4. Fiber length distributions of Coalinga chrysotile: direct vs indirect analysis

Study	Sample	Percentage reduction (D-I)*	Percentage reduction (D-I)*
		>5 μm	>10 μm
Longo [23]	Bulk	14	17
Muhle [22]	Bulk	38	nd
Chatfield** (unpub.)	Aerosolized	41	11.8
Pinkerton [19]	Bulk	40	32

*The indirect figures are from Campbell et al. [20] as cited by Pinkerton et al. [19]. D – Direct; I – Indirect.

**Unpublished. Full data set to be published in a forthcoming report by Chatfield.

Table 5. Fiber length distribution: Coalinga vs non-Coalinga fibers[a]

	Coalinga[b]	Experimental long[c]	Commercial long[d]
% >11 μm	16	20	31
% >16 μm	2	9	14
% >23 μm	0	4	6

[a]Preliminary data: full data set to be published in a forthcoming report by Chatfield.

[b]Average RG144 and COF25; also note the findings of the Experimental "Short" preparation [11] were similar to those obtained with Coalinga (13, 3, and 1% ~ >1, 16, and 23 μm, respectively).

[c]Average UICC/A; UICC/B; and Long [11,95,96]. [1980]

[d]Average WDC and Factory preparations [97].

Comparison of the Length Distributions of Coalinga and non-Coalinga Chrysotiles – Demonstration of the Short Nature of Coalinga Chrysotile

Length discrimination between Coalinga and non-Coalinga chrysotile fibers can only be demonstrated in aqueous solution. Moreover, discrimination only becomes apparent at 10 μm or more in length becoming particularly clear by 15 μm[27] (Chatfield, in preparation) (Table 5).

Discussion

Airborne Coalinga chrysotile fibers are not true fibers but "pseudo-fibers" consisting of assemblies of numerous

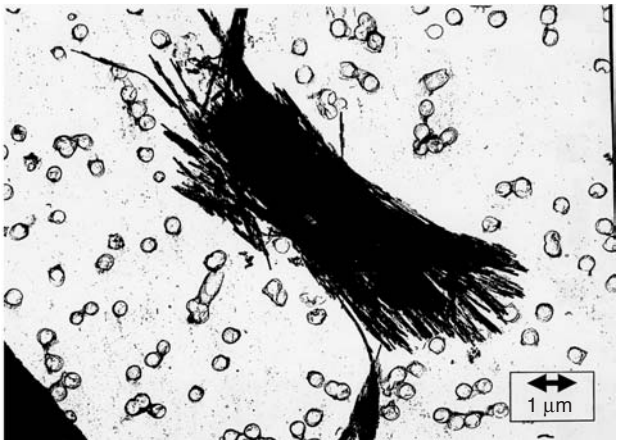

Fig. 6. Air sample collected from the King City mill. (''from Chatfield [17,31]'')

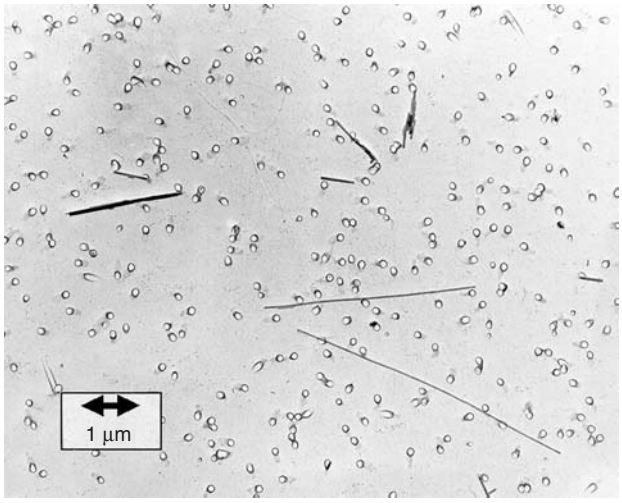

Fig. 7. King City mill air sample dispersed in water. (''from Chatfield [17,31]'')

overlapping, weakly bound short fibrils that only extend over a small part of the fiber's length (Figures 1 and 5). The ''short'' nature of Coalinga chrysotile only becomes apparent in aqueous solution whether the preparations were taken from experimental (Figure 2 compared to Figure 4) or human exposure (Figure 6 vs. Figure 7) settings. Thus, long Coalinga type pseudo-fibers readily disperse into their short (98% less than 5 µm) constituent fibrils in aqueous solution. By contrast, true long chrysotile fibers from non-Coalinga chrysotile samples fail to disperse extensively since they are composed of tightly bound fibrils that run, in parallel, the entire length of the fiber (also see Pinkerton et al. [19]).

Langer et al. [12] alluded to the difference between Coalinga and non-Coalinga type chrysotile saying: ''Although not included in this study, observations have been made that the Coalinga type chrysotiles tend to produce more fibril sized particles as compared with Canadian fibers, when reduced under identical conditions''. Pinkerton et al. [19] were the first to notice that the overall distributions of Coalinga chrysotile fibers in an aerosol and aqueous solution differed significantly. Thus: ''The results (of Campbell et al. [20]) demonstrated by both SEM and TEM more than 90–95% of the particles (in aqueous solution) were less than 10 µm in length... (whereas) ''In our study using aerosolized samples, 71.9% of the total number of particles were less than 10 µm in length''. Unfortunately, Pinkerton et al. [19] incorrectly attributed these observations to ultrasonication rather than clustering.

Claims that Coalinga''s Short Length is an ''Artifact'' of Sample Preparation

Egilman and Roberts [40] claimed the NTP – NIEHS inhalation studies described by Ilgren and Chatfield [2,41,42] were confounded by sample preparation. Thus:

''The asbestos types were prepared differently prior to testing. The Coalinga asbestos was water processed and ground three times while the Canadian fiber was passed through a hurricane pulverizer [43]. Unlike the Canadian fiber, which was a commercial sample, the Coalinga sample came from the cyclone overflow at the UCC mill [44]. The UICC-B was untreated [43]. Many studies have shown that grinding or manipulating the asbestos structure alters the toxicity and subsequent pathogenicity of asbestos [4,45]''.

This statement is replete with errors. In testimony Ilgren (author) never said UICC-B and COF25 were commercial samples. COF25 was developed by the Bureau of Mines and IITRI for experimental testing [19,20]. UICC-B is the prototype of an experimental preparation [1,46]. It has never been used commercially[28]. UICC-B was extensively milled and ground to separate the fiber bundles and shorten the material to standard lengths [46]. Coalinga samples, on the other hand, never underwent any form of commercial manipulation to shorten the fibers and alter its structure and thus bio-activity. Pinkerton [42] did not say: ''UICC-B was untreated''. Rather Pinkerton et al. [19] said: ''UICC-B chrysotile reference sample... No manipulation of these chrysotile preparations was done prior to aerosolizing the fibers''. Egilman and Roberts'' [40] statement was therefore totally out of context[29]: the sample was not treated after receipt at the NIEHS but severely manipulated before it arrived at that facility. Egilman and Roberts [40] also say: ''Many studies have shown that grinding or manipulating the asbestos structure alters the toxicity and subsequent pathogenicity of asbestos [4,45]''.[30] This incorrectly suggests any degree of grinding or manipulation

can alter a fiber's bio-activity. That is patently untrue. Langer et al. [4] never said any such thing and their data contradict that idea. Langer, in a later paper, [36] concurs. Unmilled RG144 was their baseline "control" sample. The unmilled RG144 was therefore used as such since it had not undergone any changes attributable to milling or grinding prior to being tested by Langer and his colleagues at Mt. Sinai. Langer et al. [4] presented no data to suggest the un-milled sample was damaged during processing. Indeed, Langer [47] said in testimony: "Coalinga has been naturally pulverized and crushed to such an extent that the fiber has been opened naturally" thus indicating little need to grind or mill Coalinga at all. Langer et al. [4] also indicated that in most cases brief milling for 60 sec of RG144 produced no changes compared to the un-milled samples. Indeed, in some cases, brief milling induced a "paradoxical" effect. i.e., "brief milling produced fibers with greater surface area, relatively unchanged in terms of chemistry. In this state, chemisorption is increased". Langer et al. [4] also found that when RG144 "was extensively ball-milled, the chrysotile patterns showed no appreciable line-broadening effects" suggestive of damage. Longo [25] also correctly points out that it is the degree of manipulation that is important and inferred that Calidria does not need much milling since the early work by Irving Selikoff showed it had already been "made" short "naturally". Similarly, Wylie [48] said: "The main dimensional characteristics of the material are retained unless the grinding is extremely severe".

Pinkerton et al. [19] said "grinding" was used to prepare COF25 but this is not correct. Coalinga chrysotile was never ground during processing (Myers, pers commun., 2004). It was only milled once with a hammer mill to crush waste rock early in the process of refining the fiber. There was no reason to grind or mill Calidria[31] since the fibers are already very small to begin with. Indeed, shortening was both unnecessary and undesirable (Chatfield, pers. commun., 2004). As a naturally defibrillated material, Coalinga chrysotile had already been "opened" by natural forces [49] so by the time it arrived at the mill there was no need to grind it further (Dresher, pers. commun., 2004). The main aim was to separate the fibrils that had already been opened naturally (Chatfield, Dresher, pers. commun., 2004) in order to get the waste rock out, not break up the fibers (Chatfield, Myers, Dresher, pers. commun., 2004). That is why a hydroclone (i.e., a cyclone in water) was used. "Very little energy was needed to separate the fibrils compared with any other form of chrysotile" since they are so weakly bound (Dresher, pers. commun., 2004). "The asbestos fibrils occur in the compacted form of flakes held together by relatively weak secondary forces. Therefore the energy we must expend in making colloidal material is small" [50].[32] "The separation process was a wet process and the fibrils were separated largely by micro-cavitation" (Dresher, pers. commun., 2004; Chwastiak, [50]). This did not entail grinding or milling. Thus, "cavitation was an effective way to separate the fibrils since it relied on hydraulic forces and if sufficient energy was put into the water, micro-bubbles formed. As these popped, "mini-dynamite" explosions took place near the clumped fibrils which served to separate them". Commercially, this was done with an extended Waring Blender type apparatus. "Separation was not brought about by the blades hitting the fibrils but by the heat energy introduced into the water". This caused micro-cavitation to occur. (Dresher, pers. commun., 2004). As discussed earlier in this report, effectively the only bonds holding the fibrils together were van der Waals forces. Martin and Phillips [51] said: "because the area of contact between adjacent fibrils is limited, the resultant binding forces would be fairly weak". Langer et al. [12] and Langer [47][33] noted the ease with which Coalinga fibrils could be "reduced" or separated due to the weakness of the bonds. In fact, the fibrils were so weakly bound they could be separated merely by adjusting the pH [50].

Pinkerton et al. [19] said: "The differences between COF25 and RG144 are a result of the differences in the processing of the raw material. COF25 has a finer particle size than RG144. This finer particle size is a result of differences in the pressure, vortex characteristics, and overflow port diameters used in the hydroclone. COF25 is not derived from RG144 by further grinding or pellet milling". There is no evidence to suggest COF25, as an "experimental" sample, is not representative in size and structure of the various standard commercial forms of Coalinga chrysotile sold by Union Carbide i.e., RG144, HPO, SG130, amongst others (Chatfield, pers. commun., 2004). Thus, there is nothing to suggest that the manner in which COF25 was produced created structural damage or caused its size distribution to differ significantly from commercial forms of Calidria. All of the various types of Calidria are derived from the same part of the ore body i.e., the Northern part originally mined by UCC, and this displays a high degree of homogeneity [49]. There appears to be very little chemical or structural variation in the source materials used to produce these different types of Calidria. The data presented in this report also enable one to assess potential size variation between COF25 and

other types of Calidria and this comparison demonstrates that there is no significant difference between them. Thus, one group, Pinkerton et al, [19], analyzed the size distribution of COF25 in an aerosol whilst four groups: Longo [23]; Muhle et al. [22]; Bernstein et al. [27] and in work by Chatfield (unpublished); assessed this for RG144 in air. On the basis of these studies, the length distributions of COF25 and RG144 pseudofibers appear to be comparable. The only outlier comes from Bernstein et al. [27] and this is due to the inclusion of all fiber sizes and possibly grinding effects. Three groups: Wylie [37]; Campbell et al. [20]; and unpublished work by Chatfield, measured the length distributions of COF25 in aqueous solution. The length distributions of various commercial types of Calidria were measured in aqueous solution by five groups: ASTECO [39]; Nolan et al. [35]; Langer et al. [4]; Longo [23]; Chatfield, unpublished; The COF25 data were comparable to the three types of Calidria chrysotile under study in the largest series of investigations. The least consistent comparisons arose with the samples that showed greater lack of dispersion i.e., those with 12–42% of the fibers greater than5 μm.

Claims that Short Fiber Chrysotile is Toxic on the Basis of Studies of Human Pleural Fiber Deposition. Pleural Burden Studies/"Translocation Investigations"

For more than 20 years, many people have claimed that the mere presence of short fiber chrysotile in human pleura and the peritoneum demonstrated short fiber chrysotile's ability to produce mesothelioma in those sites. This has served as one of the mainstays of Plaintiff's case for decades. Given the major financial impact these studies have had, particularly in the asbestos litigation, many "Plaintiff Experts" (e.g., Dodson [52–57]; Mancuso [58][34] and Lemon [59]) rely on them to support the notion that short fiber chrysotile is a substantial contributor to the production of mesothelioma. The following section briefly assesses the bases of such claims focusing chiefly on mesothelioma of the pleura.

The alleged predominance of short fiber chrysotile in the pleura was initially noted by French workers in the early 1970s. Their observations were extended by others in Japan and the United States particularly by Suzuki and his colleagues at Mt Sinai. Certain criticisms[35] apply to most if not all of these studies. Thus, none was adequately controlled.[36] Virtually none examined "normal" pleura.[37] Many of the structures counted were too small to be considered fibers under ISO counting rules. Contamination has probably accounted for many findings. "Contamination of specimens by fibers is almost impossible to avoid. It should be anticipated and corrected for. Autopsy and the storage of tissue present manifold opportunities for contamination and cross-contamination. Chemicals such as formalin contain fibers unless they are adequately filtered in a laminar flow clean room or similar facility. It is illustrative that a tendency has been shown for chrysotile concentrations to cluster around the same levels for certain hospitals. While premortem exposures cannot be ruled out, this finding is highly suggestive of a role for contamination of the specimens by autopsy processing or fluids.[38] In general, short chrysotile fibers have been found in a few pleural plaques and mesothelioma tissues [60]. The finding of short chrysotile fibers without other fiber types must always be regarded with suspicion, particularly when the study lacks controls and the finding is in what are in fact tissues of new growth such as plaques and tumor. In lung, tumor tissue is to be avoided for analysis for exactly this reason [61]." However, two other studies do show convincing evidence for short chrysotile fiber deposition in true parietal pleura [62,63]. In a heterogeneous group of subjects with varying disease and exposure, Sebastien et al. [62] found short chrysotile to be systematically present in the pleura over a wide range of concentrations-with no systematic relationship to lung content. Gibbs et al. [63] found that diffuse pleural fibrosis was associated with increased levels of amphibole fiber in lung, and with low levels of short chrysotile fibres in the pleura. In many studies sampling and selection bias have been enormous.[39] Methods used to count pleural fibers have generally been inadequate.[40] Long amphibole fibers were found in a few studies but these were largely ignored. Many cases especially the American ones were selected for litigation and tissues subjected to a nonstandard digestion analytical technique [64].[41]

Suzuki, a major proponent of the pleural translocation hypothesis, has even admitted [65] that the mere presence of short fiber chrysotile in "pleural tissue" did not prove causation. Various ASTDR-EPA Panelists agreed.[42] Suzuki has also admitted [65] that the short fibers could just have been in the fibrosis around a developing mesothelioma so the "mere finding (of short fiber chrysotile or) any other substance in the pleura did not prove it was capable of causing mesothelioma". In 2005, Suzuki's claims became seriously undermined when he admitted [65] that almost all of his work, including Suzuki and Yuen [66–68] had been done and paid for by Plaintiff. This testimony contradicted his earlier testimony and led to various admissions [65] including that a substantial part of his salary was also paid by Plaintiff.[43]; he never examined normal pleural tissue; he

had no controls; his sampling techniques were very limited; contamination could explain virtually all of his findings; amphibole could also be translocated to the pleura[44]; crocidolite was the most potent inducer of mesothelioma (followed by amosite and then chrysotile) and epidemiology was the 'gold standard'' for determining causation. He was unable to reconcile the views he held regarding chrysotile and mesothelioma based on his pleural burden studies with those he held regarding the potency of amphiboles based on the epidemiology. Despite all of this, Suzuki still believes short fiber chrysotile found in mesotheliomatous and plaque tissue serves as convincing evidence to show that short fiber chrysotile causes mesothelioma.

Preferential Pleural Fiber Deposition – Evidence Against the ''Suzuki'' Hypothesis

In 1996, Boutin et al. [69] in Marseille demonstrated preferential deposition of amphibole asbestos in human pleura. Since then evidence has continued to mount against claims that the mere finding of short fiber chrysotile in the pleura denotes its ability to produce mesothelioma. Indeed, in 2002, a series of panelists was assembled at the request of the EPA to assess potentially toxic short fiber effects [6]. They were highly critical of these ''pleural translocation'' (PTL) studies as were others.[45] A major premise underlying these studies is that fiber deposition in the pleura is random and thus non-preferential. However, there is now very strong evidence to suggest fibers are preferentially deposited in pleural ''hot spots'' [69]. Suzuki's failure to routinely find long thin amphibole fibers in the parietal pleura in cases of amphibole induced mesothelioma tissue was largely because he (and others that followed his sampling methods) never sampled such hot spots.[46] This was so even though the literature prior to and most particularly after 1996 suggested pleural fiber deposition in pleura was not random.

State of the Art Regarding Knowledge of Preferential Pleural Fiber Deposition

French and Belgian investigators convincingly identified long thin amphiboles in specific parts of the parietal pleura in asbestos exposed humans in 1996 [69][47] and in animal [70] tissues by 2002. However, the state of knowledge should have been sufficient to suggest preferential pleural fiber deposition long before that date. A brief historical account is therefore provided to support this idea. Some of the first studies to examine pleural fiber burden (done by the French and cited by Suzuki as ground breaking e.g., those by Le Bouffant et al. [71] and Sebastien et al. [62]) actually suspected the existence of pleural ''hot spots'' as ''stomata'' or sites of focal lymphatic pleural drainage. These had also been described by other workers decades before Boutin et al. [69] but were known by other names e.g., Kampmeirer foci, Milky spots [72]. Holt [73] (in discussing the translocation of inhaled dust to the pleura) said Beattie and Knox [74] and Hourihane [75] both remarked on the movement of dust and fiber through pleural or pulmonary lymphatics.[48] Hillerdal [76]; op. cit. Holt [77] believed the ''movement of dust in the lung toward the pleura had first been described by Arnold in 1885 and Von Recklinghausen in 1863 in workers who described the sites in the diaphragmatic mesothelium where dust particles entered the lymphatics''. Holt [73] said these ''loci probably represented the lacunae or stomata demonstrated electron microscopically by Wang [72] and Leak and Rahil [78]''. Taskinenen [79] said ''black linear streaks followed the course of the intercostal arteries in the parietal pleura'' indicating particulate transport from the lungs via lymphatics: ''This mechanism is supposed to be the same in the transport of small asbestos particles causing pleural plaques''.[49] Selikoff [80] reported pleural calcification in the anterior mediastinal pleura corresponding to the course of the lymphatic vessels... ''findings (that) support our hypothesis'' of pleural lymphatic transfer.[50] Herbert [81] also noted that lymphatic flow in the adult lung is directed by valves and that the distribution of the stomata is similar to the aggregates of macrophages and specialized mesothelial cells referred to as Kampmeier foci, structures not found in the visceral pleura.[51] Bignon [82] said the distribution of fibers in the parietal pleura was not uniform (citing Bignon et al. [83]) since some ''analyses revealed very high focal concentrations of chrysotile next to areas with no fibers. The role of these hot spots in the induction of mesothelial tumors is still unclear''. Sebastien et al. [84] said ''... the finding of many negative samples may be due to a heterogeneous topographic distribution of fibers in the pleural area. If fibers are concentrated in pouctal areas, they can be ignored by TEM which observes a very small sample size. This point is relevant to the possible short range inhomogeneity of concentration within the pleura which until now has not been investigated''. Sebastien et al. [62] repeated the same sentiments saying ''the finding of many negative samples may be due to a heterogeneous distribution of fibers in the pleural tissues''. Gibbs et al. [63] also found evidence of amphibole in pleural tissues and commented on the non-uniform distribution of pleural fibers. They said this was a ''recurring finding in studies of different asbestos related diseases'' citing Sebastien et al. [62], Churg [85,86], Morgan and Holmes [87], and Wright and Kuschner [88]. Dodson

et al. [53] was also cited by Suzuki [64] to support his views. Dodson et al. [53] said that amphibole fibers fitting the dimensions of the Stanton hypothesis do reach "the plaques". Dodson et al. [53] also suggested their processing methods might not have detected more of these fibers since "many of the uncoated fibers are such that they could pass through filters with larger pore sizes". Rey et al. [89] also said Sebastien et al. [62] had found fiber "hot spots" and noted that the anatomical structures represented by these did correspond to the stomata described by Wang [72], Kanasawa [90], Pereira [91], Takada [92], Moalli [93] and the others noted above. Boutin (pers. commun., 1996) said the "localization of (these) lesions generally correspond to the postero-lateral part of the vertebral gutter, precisely where the milky spots are most numerous".[52] The analyses of Mitchev et al. [94] of parietal pleural tissues from 150 consecutive autopsies from urban dwellers in Brussels revealed black spots in nearly all (93%) of the cases. These were mainly located in areas corresponding to the "anatomical distribution of structures of pleural cavity clearance". Mitchev et al. [94] and others such as Boutin et al. [69] used such structures as "sampling guides" to demonstrate the preferential accumulation of the longest amphibole fibers.[53] Where short fiber chrysotile fibers are found in black spots, Mitchev et al. [94] referred to them as reflections of a "clogged sewage system". Chrysotile has been found in black spots in workers with confirmed histories of occupational chrysotile exposure [69]. This could have been due to the existence of "large (60–70 μm in diameter) intensely active macrophage (giant cells)...(found)...in black spots (see Figure 4 in Boutin et al. [69])" that could most likely clear chrysotile fibers of almost any length.

The state of the art on this subject as summarized above makes it abundantly clear that Suzuki and his colleagues totally ignored the literature pointing to preferential pleural deposition when they published their own work between 1991 and 2005. Suzuki"s broad access to these writings from the time he arrived at Mt Sinai hospital in 1964 suggests he and his colleagues knew their studies were fatally flawed (particularly from 1996 onwards) but continued to conduct them irrespectively to suit their needs and those of others.[54]

Conclusions

Coalinga chrysotile is a form of short fiber chrysotile. It is prototypic of a variety of short fiber chrysotile based materials. Coalinga type chrysotiles have been recognized for decades as being fundamentally different from many "typical" forms of asbestos that display extreme length, flexibility, and marked tensile strength.

In an aerosol, Coalinga type chrysotiles form "long" aggregates known as "pseudofibers" but in an aqueous milieu these fall apart into their largely "short" constituent components. This forms an important basis for their lack of biological activity.

In order to assess the true biological activity of Coalinga type chrysotiles current regulatory methods must be modified. A combination of direct and indirect measurement methods will be required to update the regulations.

Claims that the short length of Coalinga chrysotile is an artifact of sample preparation are unfounded. Similarly, claims that the pleural translocation of short fiber chrysotile implicates this form of asbestos in the production of mesothelioma are seriously flawed.

Acknowledgments

I gratefully acknowledge the assistance provided by Dr Eric Chatfield over the last 13-years. During that time, Dr Chatfield and I have extensively reviewed and discussed his fiber size data for both Coalinga and non-Coalinga chrysotiles. Nonetheless, the conclusions reached in the present report are based on, amongst many other things, my own interpretation of the data Dr Chatfield has shared with me and our many detailed discussions. The views expressed in this report are not meant to represent those held by Dr Chatfield or anyone else cited herein. I am also very grateful to Dr Kent Pinkerton for sending me data in 1991 and to Drs Wylie, Myers, Dresher, Addison, Campbell, Camus, Elmes, Fripiat, Chwastiak, and Muhle for discussing various aspects of this paper with me.

Dedication

This report is dedicated to Dr Chris Wagner, Prof. Fred Mumpton, Dr Wayne Naumann, and Prof. Keiji Yada. Their pioneering investigations into many aspects of this work were fundamental contributions. I am very grateful to each of them for discussing this work with me and, in some cases, providing me with unpublished data.

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Notes

1. Dr. Ilgren has no affiliation with Bryn Mawr College.
2. Langer et al. [4] is occasionally contradicted by Langer [36].
3. Clearance may also be facilitated by nonpolar effects that keep fibrils separated *in vivo* [50]. Thus, dispersions in nonpolar liquids differ from those in water. ''non polar liquids'' may form protective layers ''around each fibril making it impossible for them to approach closely enough for these van der Waals forces to bind them . . . Individual fibrils in nonpolar fluids may therefore be easier to clear than in water'' [50].
4. Berman and Crump [10] said ''Short fibrous structures (less than 5 μm long) do not appear to contribute to risk'' whilst those ''longer than 20 μm are the most potent''. However, they appear to contradict themselves when they agree with the hypothesis of Pott et al. [98] which says ''relative potency is low for short fibers'' and Lippman's [99] proposal that the 5 μm cut-off is ''still sufficiently short to allow for the possibility that a slightly shorter set of structures contributes to the induction of mesothelioma.''
5. Berman et al. [9] concluded the ''best information regarding the effects of size and mineralogy on the relative potency of asbestos structures has come from animal studies'' (due to) ''limitations in the characterization of asbestos exposures in epidemiological studies''. The same investigators also stated that ''animal studies generally indicated that longer fibers tended to be more carcinogenic than shorter ones'' . . . (but) ''no measure of asbestos exposure that satisfactorily predicts tumor incidence was identified in those studies''. To identify such a ''predictive measure'', these workers statistically analyzed the combined data on tumor incidence found in rats from 13 inhalation experiments reported in a series of studies conducted by Davis et al. (see Berman et al. [9] for details) in relation to the fiber size distributions to which these animals were exposed. This was done to see ''if a measure of exposure could be identified that satisfactorily predicted tumor incidence''. Univariate analysis revealed that the concentration of structures greater or equal 20 μm was most highly correlated with tumor incidence. Multivariate analyses indicated that structures less than 5 μm long made no contribution to tumor risk whilst potency increased above this point with increasing length, structures longer than 40 μm being about 500 times more potent than those between 5 and 40 μm in length. The original Davis et al. studies used SEM and this precludes the observation of structures thinner than 0.2 μm that may be of biological importance. Berman et al. [9] therefore took archived samples of the original stock materials used by Davis et al. and regenerated asbestos dust clouds in the same institute (IOM, Edinburgh), with the same generators, and by the same scientific technical staff. Although the dust thus generated was then collected on filters and characterized by Chatfield and his staff in Toronto, the size characteristics of these samples was never presented. The characteristics of the seven samples that were regenerated with different forms of chrysotile, most of which are long and pathogenic, will be described by Chatfield (in prep.) for comparison with Coalinga.
6. These are largely from the data Chatfield [17,31] presented in testimony in Conwed v Union Carbide.
7. ''PCM doesn't tell the whole story'' [100]. A ''pseudofiber'' would be counted as an OSHA fiber''.
8. This was the first time Longo did an analysis where he was ''absolutely sure (the material being analyzed) was Calidria''. [24]. Longo completed the fiber size analysis in Sept., 2002. Longo testified in 2002 [24] that he had no opinions regarding the size distributions of UCC products and Calidria
9. The Yamate counting rules include all structures with an aspect ratio (AR) greater than3. Bundles are counted that meet the overall dimensional criteria generally counted as single entities and noted as bundles on the count sheet. Within a cluster, up to three individual fibers that meet the overall dimensional criteria are counted. Otherwise, clusters that contain more than three fibers that meet the overall dimensional criteria are counted as single clusters Longo [23] counted four structures in his direct examination that did not meet these counting criteria (Length × width = 5×2, 8×8, 22×8, 2×1). Longo [24] testified that the Yamate method [26] was never officially adopted by the EPA.
10. In deposition, Longo [25] was asked: ''Q. It is correct, is it not, that there were four different analyses of Calidria performed using TEM microscopy? A. That is correct. Q. And in three of those there were no fibers identified that were greater than 5 microns in length; is that correct? A. That is correct, no single fibers. Q. And in one of them, there was one fiber identified that was greater than 5 microns in length and that was a 6 micron fiber? A. Correct. Q. Do you know how many fibers were counted? A. I am going to estimate about 40''. . . . ''The TEM, you can go through the data. It is all bundles. There 50 percent of the bundles are greater than 5 microns''.
11. ISO counting rules indicate that all structures with L greater than 0.5 μm (or containing components with L greater than 0.5 μm that also exhibit an AR greater than 5) are counted. Separately identifiable components of parent structures that satisfy dimensional criteria are also separately counted. A similar count to that described above is also done for structures with L greater than 5 μm. Parent bundles with L greater than 0.5 μm containing at least one component fiber that exhibits an AR greater than 5 are also counted. Qualifying bundles that are components of other parent structures are also separately enumerated. For counts of structures with L greater than 5 μm, only bundles longer than 5 μm are included. ''Dispersed'' and ''Compact'' clusters are distinguished. Also, all clusters containing at least one component fiber or bundle satisfying appropriate dimensional criteria separately enumerate up to five component structures satisfying appropriate dimensional criteria.
12. Fibers, bundles, clusters, and matrices, as defined in the literature [101], were characterized separately along with measurements of the length and width of each such structure. Up to five fibers and bundles that were components of clusters or matrices were also characterized for those complex structures (i.e., clusters and matrices) where the individual fibers or bundles could be characterized [102]. Filters were obtained from Muhle since those originally analyzed by Pinkerton et al. [19] were not available. In the experimental protocol employed by Chatfield, TEM specimens were prepared from the polycarbonate filters by a direct-transfer method. A portion of the polycarbonate filter was then placed into filtered, distilled water, the pH of which was adjusted to between 3 and 4 by the addition of acetic acid. The suspension was treated in an ultrasonic bath to disperse the particulate material from the filter, and aliquots of the suspension were filtered using cleared, 0.1 μm pore size polycarbonate filters. TEM specimens were then prepared from these polycarbonate filters by a direct- transfer method. The fiber counting criteria used were those of ISO 10312. Direct-transfer TEM examination of airborne Coalinga chrysotile was performed jointly by Chatfield and Muhle. All indirect analyses and direct analysis of non-Coalinga chrysotile fibers were performed solely by Chatfield. The filters with chrysotiles other than Coalinga were largely from the ''regeneration'' studies of Berman et al. [9]. These were based on the earlier inhalation investigations of Davis et al. [95–97,103]; and Davis and Jones [11]. The details of the preparation of the different samples used in each study is described in these references. (also see Berman et al. [9] for additional methodological information).
13. Longo [100] has testified that the difference between the findings of Langer et al. [4] and his was due to differences in sample preparation methods i.e., ''dispersal in nitrocellulose may have only collected the smalls''. However, Addison (pers. commun., 2000) said that dispersion of bulk fiber in nitrocellulose should not introduce any size selection in the sample and all size ranges should be fully represented. Longo [100] also disagreed with the data of Langer et al. [4] since they were ''not consistent with some of the other data out there'' . . . ''others (like the

Union Carbide Company) do not find the majority of Calidria less than 5 μm in length."

14. Longo [25] testified "It is very hard for me to figure out where (Yeager et al. [34]) went wrong. If they were going to ignore the bundles and not try to look inside the bundles, then their data is actually higher than ours because out of some 400 odd structures, we only had 1 fiber greater than5 μ. If they are breaking down the bundles to see what is inside those fibers, then our data is not consistent. So, without knowing what he did, I can't tell you where he went wrong". Longo, however, appears to be confusing Yeager's indirect data with his own direct data.
15. Brownson [17] said one of the cases in Nolan et al. [35] was an analysis of Calidria in the lung tissue of a Calidria worker in which 1.8% of the 2900 fibers were less than 5 μm long. However, Nolan et al [35] never examined lung tissue from a Calidria worker, only Calidria fiber in aqueous solution [17].
16. The mineralogist Prof Teebor Zoltai (Univ. Minnesota) was asked to perform a series of site visits by the Minnesota Dept of Health further to the Taconite mining reserve matter. One such visit was to mount sinai school of medicine [104]. He reported: "At 9:30 a.m. on December 19, 1975, we met with the Mt. Sinai Group in Environmental Medicine. Present were Tibor Rosa, Ed Prvzina from the MPCA and Dave Gray and Kyle Bishop from the Dept. of Health. The Mt. Sinai group was represented by Bill Nicholson, Art Langer, Art Rohl and Steve Shirey. Tibor Kosa began with a summary of our objectives and a statement that in the absence of lawyers and judges, we hoped for a purely scientific discussion of important problems. Dr Nicholson began by summarizing their sample preparation procedures. He noted that serious fiber loss problems exist with all techniques presently used in labs across the country. His own previous analyses have had losses up to 90% due to re-suspension problems. His present "rub-out" technique loses 50% of total fibers during handling. He stated that many previous court samples have fiber concentrations so low due to analytical loss to be "essentially meaningless". Dr Nicholson further indicated that his current counting procedures are low by an additional factor of two because of loss due to re-suspension in the rub-out technique. He corrects for this in his final concentrations but does not correct for the exclusion of small fibers mentioned earlier. Nicholson also estimates that the loss of fibers by the acetone dissolution procedure used by NIOSH and IITRI could be as high as 75–90%. Colin Harwood at IITRI did say there was substantial loss of small fibers in his technique but would not estimate the percent until they had done further studies. Discussion then extended around the dependence of carcinogenic effects of amphibole fibers on dimensions of the fibers. Dr Langer alluded to one of is own studies in which it had been shown that amosite workers in the S. African mines exposed to smaller (less than 0.2 μm diameter) fiber dust have higher incidence of mesothelioma. Dr Langer drew a graph of the size distribution of fibers actually observed and counted in diseased lung tissue of Patterson, N.J. amosite workers. Dr Langer further stated that in all amosite factories in which lung tissue has been examined, 95% of the amphibole fibers are less than 5 μm in length, and therefore the present MESA Standard of so many fibers greater than 5 μm in length is rather ridiculous."
17. Efforts to obtain more information about the specifications of this sample were not successful.
18. Longo [100] also testified "Others found 30–50% greater than 5 μm" but he did not specify who these other workers were.
19. "There were also many bundles of chrysotile fibers greater than 5.0 (μm) in length. These bundles were made up of individual fibers that were both less than and greater than 5 μm. The fibers that made up these bundles had the appearance of being welded together (see photos in Section 5 of the MAS report)". However, the bundles do not appear to be reflected in Table 2 of the MAS [23] report since their widths suggest most are actually not bundles but individual fibrils. The text of the MAS [23] report on this point is confusing since it refers to the presence of numerous bundles and the photos appended to the report also show bundles with the multifibrillar arrangement typical of poorly dispersed Calidria in aqueous solution. The "welding" clearly reflects the overlapping of the short fibrils within the bundles.
20. Chatfield first studied the size characteristics of COF25 in 1975 to help standardize inter-laboratory analytical methods [17] when the EPA and the ASTM wished to develop techniques for measuring asbestos in water [31]. Calidria was chosen since "it had been shown that the majority of Calidria fibers were less than 5 μm which is why the NIEHS used it as their standard short fiber preparation" [17].
21. COF25 is not derived from RG144 by further grinding or pellet milling. It is an experimental preparation originally prepared for oral ingestion [105,106] and inhalation [19,107] in studies conducted by the NIEHS. Prior to use in these investigations, COF25 was extensively analyzed by TEM and XRD by IITRI [108] and the data from these analyses was summarized by the Bureau of Mines [20] and then further reassessed by different workers [19,37] who describe how COF25 was prepared for experimental research and note that the mode of preparation caused COF25 to have a finer particle size than RG144. This resulted largely because of differences in the "pressure, vortex characteristics, and overflow port diameters used in the hydroclone", the final hydroclone port external diameter for COF25 was 25 mm whilst that for industrial purposes was 152 mm (6 inches).
22. Chatfield's data clearly support the notion that the weakly bound fibrils within airborne Coalinga chrysotile pseudofibers rapidly fall apart in water. Chatfield [17] said that when you put Coalinga into water it "all comes apart" i.e., "the groups of fibrils that came together in the dry stage all separate when put in water again" . . . "When you put other types of chrysotile into water . . . the aggregates separate but the fiber bundles themselves will not separate but remain intact" . . . Chatfield [31] said he used Calidria since it "was known as a type of asbestos that disintegrated very rapidly into individual fundamental fibrils" and "no amount of additional treatment" could "reduce it beyond the individual fibrils" (though "some (bundles) do not break up; one can still see the odd pseudofiber"). By contrast, "aggregates of Canadian chrysotile fiber bundles . . . will not break down in water".
23. Longo put the preparation through sonication and then acidification so "you don't get continuing agglomeration of more fibers than what has happened . . . Typically, what happens is that when you put asbestos in water instead of breaking it apart, it starts sticking together. That is where you have to put surfactants and things to keep them separated or make the solution slightly acidic so you don't start growing structures" [24]. Chatfield (pers. commun.) found that bacteria in the water causes Calidria fibers to clump. Nonetheless, Longo [25] testified his dispersal method was more than adequate to separate the preparation to single fibrils. He even used the ASTM method for 15 min which he said was the "worst, most aggressive sample preparation method"; "normally (we) only sonicate for three minutes" [25].
24. The Chatfield – Muhle data demonstrate this change in fiber size distribution more rigorously than that produced by Longo [23] and Muhle et al. [22]. Thus, the Chatfield-Muhle work was done under controlled standardized conditions in the same laboratories using the same samples for the most part both in the direct and indirect phases of the investigation. By contrast, Muhle et al. [22] and Longo [23] studied two different samples at different times, one sample in air and the other in water, under conditions that were not always, if at all, standardized to ISO criteria. Muhle et al. [22] data are also not strictly comparable with those produced by Longo and Chatfield-Muhle for other reasons. Muhle et al. [22] used fiber length cut-offs that differed from those used by the others (i.e., of 6 and 14 μm, not 5 and 10 μm). Nonetheless, the data of Muhle et al. [22] should, if anything, underestimate the true difference in the size distribution of the air and water samples and thus still be very supportive of the notion that the behavior of the Coalinga and non-Coalinga preparations is very different following direct and indirect preparation. Chatfield [31] testified that there 'is only one type of chrysotile that is morphologically similar to that from Coalinga, namely Wet Dispersed chrysotile (WDC). This was produced by chemical dispersion of the ore into single fibrils using surfactant; WDC, like Coalinga chrysotile, disperses almost completely into single fibrils in water, but contains fibrils longer than the maximum found in Coalinga chrysotile".
25. Whilst Longo does not appear to have compared the two data sets, he still disagrees (apparently without foundation) with the idea that the data from the aerosol and the aqueous solution are different. Thus: "Q. And the size of the chrysotile fibers that you find in those two different methods are very different; is that correct? A. I don't know if they are very different. The size of the fibers are not different. We are seeing more fibers, if you want to look at it that way. What we did is the indirect preparation created larger fibers. So I would agree with that. But I am being facetious, it didn't create them, what it did, it opened the

bundles up so we could see what the longer fibers were in those bundles'' [25]. However, Longo is not being totally facetious since he does suggest that putting some forms of asbestos in water cause structures to ''grow''. Thus: ''Typically, what happens is that when you put asbestos in water instead of breaking it apart, it starts sticking together. That is where you have to put surfactants and things to keep them separated or make the solution slightly acidic so you don't start growing structures'' [24].

26. Longo has frequently stated that he is opposed to the notion that sample preparation can influence fiber size distribution. He has, for example, said that he does not agree with the statement of Professor Pooley that ''some samples of chrysotile can be reduced to their individual fibril state simply by immersion into water containing a surfactant'' [25]. He also said ''in the early years of developing the dust method'' (he tried) ''solutions laced with surfactant'' but never ''found a single instance where every sample we saw was single fibers''. He also stated: ''I don't think chrysotile structures can easily come apart like Pooley and others'' suggest and he ''never sees changes in the size distributions with different preparation methods'' [25]. Nonetheless, Longo admitted sonication could alter fiber size distribution.
27. Longo [100] has testified that the size distribution of Calidria was very close to that of the long chrysotile fibers from Vermont or Canada but admitted he had no data either for Vermont or Canadian chrysotiles to prove it.
28. Pinkerton et al. [19] also concur: ''UICC B chrysotile reference sample: This preparation is a grade 4 chrysotile [109]... approval of this preparation was made in 1966 by the Union Internationale Contre le Cancer (UICC) to standardize asbestos samples used in experimental research.''
29. Egilman said that Ilgren (author) ''admitted'' in deposition testimony [44] that he had no data to support his conclusion that almost all Coalinga fibers are less than 5 μm long. No such admission was ever made in that deposition. The relevant portions of the testimony read: Pg 716 - Q. ''Where in this paper is there any data that shows that the Coalinga fibre is indicated short? A. I don't believe there are any in this paper. A. I believe there is some reference to a future paper, which – again I have to go through this –(to see) if that makes reference to our forthcoming paper that would let the reader know that there is data to that effect. Q. And that's the part 4 that we spoke about a moment ago? A. Yes, sir. Q. Is Dr. Chatfield's electron microscope analysis now complete so that you have the data indicating that the Coalinga fibre is short? A. Yes, sir. Q. What does that data show in terms of the size of the Coalinga fibre? A. It demonstrates that (in) an aqueous solution Coalinga fibre is 96% or more less than 5 μm in length, and as I recall less than 1 percent shorter than 10 μ long''. Therefore, I do indicate that data exist to support my opinion and I even describe some of these supporting data. These data are part of Dr Chatfield's analysis as cited by Ilgren and Chatfield [42].
30. This is similar to Egilman's (26 Mar 04) [110] testimony that the milling or grinding COF25 underwent prior to its use in the NTP- NIEHS studies made it nontoxic.
31. It is not clear what effect grinding may have had in the study done by Bernstein et al. [27].
32. There may have been some chemical bonding in the early stages of processing ''since the ground water in the region where Coalinga asbestos is mined contains large quantities of sulfate salts, the processed asbestos has significant amounts of adsorbed sulfate anions and dispersions made for unwashed asbestos tend not to be stable over a long period of time''. It was necessary to carefully wash out the sulfate since ''divalent sulfate ions at $<10^{-5}$ molar) were enough to irreversibly flocculate asbestos suspensions'' [50].
33. Donnet and Cosme [111] op cit Hodgson, [16] also say the nature of the interfibrillar cement governs the ease with which fibers may be opened by grinding.
34. Mancuso, [58] said ''A series of experiments have consistently demonstrated that chrysotile, more than any other form of asbestos, gets to the pleura and is retained in the pleura as the basis of subsequent disease. Le Bouffant [112], in a study of human tissues, noted a preferential migration of chrysotile fibers to the pleura, with a significant increase and accumulation in the pleura, in comparison with the lung parenchyma. The median percentage of chrysotile fibers was 3% in the lung and 33% in the pleura.'' In one series of cases, the median concentration in the pleura was 3000 fibers (chrysotile) per gram of dry tissue. Sebastien et al. [62,113], in similar studies on human tissues, also demonstrated that the retention of asbestos fibers in the parietal pleura was type and size related, that inside the parietal pleura most of the fibers were short chrysotile fibers, and that lung retention was not a good indicator of pleural retention. Viallat et al. [114], with intratracheal injection of small amounts of UICC chrysotile, demonstrated that the ''shortest fibrils reach the pleura very rapidly and can be retrieved from the pleural fluid of rats within one month''.
35. An extensive critique of Suzuki and Yuen [66] regarding alleged short fiber chrysotile toxicity $\sim$ lung burden and the pleura can be found in FRAPR [115] (pp. 3–15 and 3–16).
36. See Suzuki dep [65]10 May 05, pp. 76–80; 102–122.
37. ''These studies are of questionable quality because they lacked matched controls or sampled tissue (such as tumors) other than the pleura''. [64]. The multitude of variables involved in the incorporation of fibers within these tissues can never be accounted for so adequate controls could never be selected. No one would ever compare fibers found within a lung cancer to those seen in nontumorous parenchyma. Fibers could be floating free within loose necrotic debris, tumorous secretions, or blood or trapped and sequestered within fibrotic/sarcomatous tumor or within fibrous tissue secondarily produced by the tumor itself. Lockey's [116], claim that Le Bouffant et al. [71] and Sebastien et al. [113] analyzed pleural tissue is therefore misleading; also see Suzuki dep 10 May 05 pp. 133 [65] re his opinion that the best place to sample is from the fibrous tissue near the mesothelioma. Churg [86] has suggested short chrysotile fibers most likely are ''buried in dense connective tissue'' via ''sequestration''.
38. In a study of the lung fibre content of American children [117], very low levels of fibres were found (32% asbestos; mostly chrysotile and tremolite cleavage fragments; geometric mean 0.10 f/mg/dry lung; fibres longer than 0.2 μm, aspect ratio greater than3 : 1). Asbestos bodies were absent in 57 of 60 cases (detection limit 25 AB per gram dry lung) and while those with levels of chrysotile, amphibole or talc above 0.1 f/mg/dry lung were on average older, the age-related excess was not statistically significant.
39. See Suzuki dep testimony pp. 138–143. [65].
40. Morinaga et al. [118] found an excess of mesothelioma cases over controls where chrysotile was identified. Twelve of 23 cases and one of 17 controls had unspecified levels of chrysotile; 6 of the cases had only chrysotile and no amphiboles. Both digestion and counting methods were unusual, not quantitative and highly insensitive for controls, and control selection was not followed by analysis. Only 17 of 46 ''matched hospital'' controls selected for 23 cases were actually analyzed; and only five of these 17 controls showed any asbestos fibers at all (this alone explains the apparent excess). Further, some of the six ''chrysotile-associated'' cases had no history of asbestos exposure, and are quite possibly background cases. Doll [119] as part of the summing up session of the 1989 IARC meeting, said that he thought Morinaga's findings ''provide perhaps the strongest evidence that mesotheliomas may be produced by pure chrysotile''. ''The dimensions of chrysotile fibre as observed in lung tissue are greatly affected by the methods used, particularly in fibre ''counting''. Indeed, the selection of what range of fibres to count is one of the first decisions made by the analyst, and it affects not only fibre dimension results but also exposure and disease inferences. One example is offered by contrasting findings from two groups (Churg et al. [120,121]; Sebastien et al. [122]; Case and Sebastien [61,123]; Case [124]) assessing the same autopsy population of chrysotile miners and millers in and near Thetford Mines and Black Lake, Quebec. Like many groups, that headed by Churg in this set of studies looks at fibers of all lengths down to that which is practically resolvable, the McGill group uses (in these studies) a method which counts only fibers longer than 5 μm. Both use the same aspect ratio cut-off (greater than 3 : 1), but there are naturally differences of about an order of magnitude in total tissue chrysotile fiber concentrations reported and geometric mean fiber lengths are by definition more than 5 μm for one group of investigators and much lower in the other. In general, however, both groups come to the same conclusions regarding disease etiology and systematic tendencies for fiber length to increase and fiber diameter to decrease with increasing exposure'' [125].
41. ''Examples of this are provided by all papers having Y. Suzuki as co-author on this topic and some early papers by Sebastien (1980); the topic has been fully reviewed elsewhere'' [64,125].

42. ''Some panelists noted that translocation of fibers into the pleura does not necessarily imply causation of pleural disease, the mechanisms and site of action of these mechanisms being unknown [126]''. ERG [6] and Churg [86], discussing work by Suzuki, said that findings could be explained on the basis of normal macrophage clearance, contamination and entrapment within fibrotic areas.
43. Suzuki's first ''translocation'' report with Kohyama appeared in 1991 [60]. In 1997, his translocation work gained significant funding ($\sim$ 30% of his salary came from the Galiher De Robertis Plaintiff law firm in Honolulu) through a series of annual grants ($60,000–$70,000 p.a.) earmarked for Suzuki through the Ledesma Foundation in Hawaii [65].This Foundation received funds directly from the Galliher firm. Correspondence attached as exhibits to a general causation deposition taken on 10 May 05 of Suzuki documented communications between Suzuki and Galliher; between Galliher and the Ledesma Foundation and the CEO, CFO, Sr VP, and Landrigan (available from the author on request). Suzuki admitted [65] that he worked exclusively for Plaintiffs and about 35% of his time was spent doing medical legal consultations. He said Mt Sinai hospital in New York got a portion of any money he received from those consultations. Suzuki denied receiving any support from lawyers for his studies. He then indicated that he did not receive such funds since they went to the GCO of Mt Sinai and had to ''be approved by Mt Sinai's committee to do this type of work''. He then admitted that such funding came for nearly a decade and probably totaled ca $700,000. When asked why Galliher gave him the grants he correctly said: ''probably they liked my work''. He refused to say whether he received any other similar grants and simply said ''such information came to him from Mt Sinai but I never receive such information''. Suzuki also signed waivers to the GCO of Mt Sinai hospital indicating he had no significant financial interests to disclose (available on request from the author) and the Chairman of the Board of Mt Sinai, Stephen Peck wrote to the Trust Officer of the Ledesma foundation, Dr George Druger, that ''in accordance with IRS requirements, we confirm that no goods or services were provided in exchange for this contribution'' (available on request from the author). Parenthetically, Druger is a Plaintiff Expert used by the Galliher firm. Various additional correspondence acknowledges the contribution from the Ledesma Foundaton and/or the Galliher firm from various officials at Mt Sinai including Dr Nathan Kasc, Dr Kenneth Berns (CEO & President), Dr Stefanie Steel (Sr VP for Development), and Dr Kenneth Davis (Dean) (available on request from the author). Dr Phil Landrigan is acknowledged in some of these communications (available on request from the author).
44. Suzuki admitted that Boutin and others found amphibole in the pleura but quibbled on the point saying that it was really not the pleura but the sub-pleural area. Moreover, he admitted that his failure to find amphibole did not mean it was not there. Suzuki and Kohyama [127] concluded that translocated chrysotile fibers could produce mesothelioma even though they noted the appearance of ''intrapleural amosite'' in concentrations up to 2.2×10^6 f/g (lung dry weight). They also cite the findings of Le Bouffant et al. [71] and Sebastien et al. [62] of short fiber chrysotile in parietal pleura ignoring the fact that they had also demonstrated amphibole as well. In addition, they found crocidolite in the lungs of 7 of the 13 North American insulators in significant quantities (mean: 11×10^6 f/g (lung dry weight)). This was not surprising since Suzuki had admitted in testimony (Shaw pers. commun., 2006) that his sampling error rate periodically approached 100% so that in one sample he may not have found any fiber whilst in the very next he saw only crocidolite. Kohyama and Suzuki [127] also demonstrated amphibole in ''pleural plaque'' and also indicate that in at least one case, amosite fibers as long as 82 μm were noted. Suzuki's next ''translocation'' paper [128] was a joint publication with the ''National Occupational and Safety Health'' in South Africa. This examined the pleural and parenchymal fiber distribution in Baboons subjected to long term asbestos inhalation. Not surprisingly Suzuki once again examined ''mesotheliomatous'' tissue as a presumptive surrogate for parietal pleura and ''confirmed'' the presence of short fibers therein. Hiroshima et al. [128] revealed that ''amphibole fibers also have a capacity to translocate''. Suzuki and Yuen [66–68] discussed, as indicated above, the ongoing analysis of autopsy materials performed pursuant to the ''grants'' received from the Galliher firm. The area selected for analysis of ''pleura'' was the ''primary serosal tumor where the tumor was intimately associated with fibrosis and/or hyaline plaque''. Short thin chrysotile was found to predominate in such tissues and this led them to conclude that these fibers contributed to the induction of mesothelioma. In fact, examination of Table 1 of Suzuki and Yuen [67] contradict their own proposal that short fiber chrysotile is the significant contributor since the data in Table 1 [67] clearly demonstrate the presence of long amphibole fibers. Data referable to these translocation lung burden studies [66–68] were replicated in the annual progress reports to the Ledesma foundation from 1998 to 2005 (available on request from the author). In virtually every case, chrysotile alone (usually short and thin) was reported in ''mesothelioma tissues'' as a surrogate for pleura. Thus, the finding of chrysotile alone in occupationally exposed shipyard workers with mesothelioma is totally inconsistent with their known exposures. This makes it abundantly clear that Suzuki's studies have produced no reliable information at all in terms of causation (see p. 2 of sixth year progress report, available on request from the author). It is hard to understand how Suzuki could justify a ''Request for Access to PHI (protected health information) of Decedents'' (let alone use their tissues for lung burden analysis) ''to clarify the question of which types of asbestos contribute to the induction of mesothelioma'' on the basis of such work. Sebastien et al. [62] also selected normal parietal pleura in addition to pleural plaque and found up to 99% amphibole in one of their 29 cases. Sebastien et al. [62] also examined normal pleura in 15 of the 29 cases. Amphibole was found in two (10 and 23%). Seventeen of the 29 cases examined pleural plaque and of these 9 had amphibole fibers (3, 3, 16, 10, 87, 20, 5, 1, 30, and 5%).
45. The panelists revisited fiber translocation issues when they discussed the role of fiber length, in causing pleural abnormalities. Unfortunately, less is known about this than what has been published, due to the exceptionally poor quality of what has been published, with two exceptions from a group based in Brussels and Marseilles. The few additional papers that have been published (in relation to human disease) have been for the most part based on static ''fiber burdens'' that purport to be in ''the pleura'' but which on careful reading are in fact in mesotheliomatous tissues and/or pleural plaques; the false assumptions are then made that ''short fibres'' – usually very short chrysotile fibres, averaging less than 0.2 μm in length – have ''translocated'' to the ''pleura'' from the lung. In fact the ''pleura'' was not studied, only tumor and plaque which by definition could not contain fibres except via specimen contamination or incorporation, most likely from adjacent lung [6]. Rogers et al. [129] and Case et al. [125] both reported contamination by short crocidolite fibers from Nucleopore^{TM} filter materials and in uncontrolled studies of this nature any material from air, fluids and paraffin in the pathology laboratory from which the specimens originally were referred to specimen preparation materials are suspect [64].
46. And Suzuki certainly believed the parietal pleural was the tissue of origin of mesothelioma [65].
47. The ASTDR EPA panelists [6] recognized many flaws in the work done by Suzuki and his colleagues. Case [64] in his premeeting comments said: ''two preliminary studies of fiber translocation, one in humans [69] and the other in goats, which were based on more robust methods using controls . . . found that 22.5% of fibers detected in the pleura were longer than 5 μm and that the pleural samples had far greater amounts of amphibole asbestos fibers than chrysotile asbestos fibers''. Case [64] commented further that ''It remains possible to do good studies of translocation of fibres, but for lung-to-pleura, in humans at least, only the two preliminary studies mentioned above have proved useful, and their results have been quite different: Boutin et al. [69] and Dumortier et al. [70] have found that ''the distribution of asbestos fibers in the pleura was heterogeneous and that they might concentrate in . . . ''black spots'' of the parietal pleura''. Using thoracoscopy in living patients from ''normal areas of the parietal pleura'' rather than plaques and tumor, and using controls, they showed that ''amphiboles outnumbered chrysotile in all samples'' and that of all fibres 22.5% were in fact greater than or equal to 5 microns in length; a proportion at least as great as that usually seen in lung tissue. The means of translocation remains unknown, although these findings strongly suggest lymphatic drainage paths. The pathogenic significance also remains unknown, although the authors emphasized their hypothesis that these fibres might contribute to plaque and mesothelioma genesis''.
48. This gave much weight to the most obvious of the 16 theories proposed by Hillerdal [81] on the manner in which pleural plaques arise.

49. Taskinenen [79] also remarks that ''Nagelschmidt [130] reports that he has seen material resembling asbestos in the parietal pleura but he has no explanation for this finding''.
50. As indicated in figure 6 of Taskinen [79].
51. Herbert [81] also noted the topographical coincidence of pleural plaques with such foci. She also states that the pathogenesis of pleural plaques, whilst incompletely understood, was still said by Thompson [131] to develop deep to any intact mesothelium suggesting that direct mesothelial damage did not play a part in their pathogenesis. Herbert [81] supports this with the observation that surface adhesions are absent in plaque formation and mesothelial hyperplasia is also not present. Herbert [81] further supports this notion by describing a ''recent experiment in which intra-pleural asbestos injections in rabbits produced pleural plaques which could be prevented by pre-treatment with nitrogen mustard that impaired macrophage mobility''. 'This study therefore suggests that plaque formation depends on mobilization of pleural' and sub-pleural macrophages in the region of the parietal pleura.
52. Boutin (pers. commun., 1996) also said that figures 16–18 in Brown [132] ''were the first pictures to give me the hypothesis that parietal or diaphragmatic pleura is the first tissue invaded by the mesothelioma''.
53. This observation serves to reinforce even further the notion of mesothelioma threshold described by Ilgren and Browne in 1991 [133] who said the genesis of mesothelioma was due to a critical accumulation of long thin amphibole fibers.
54. Suzuki wasted vast resources at his disposal not only in terms of human material (ca. 3000 cases from the general population and 1500 mesothelioma cases [65]) but also precious animal (rodent and primate) tissues [128]. Suzuki and his colleagues over the last 12 years (with regard to the materials provided as Exhibits to his 10 May 05 deposition) [65] have tailored their studies at the behest and request of the Galliher firm and others with an interest in demonstrating that short fiber chrysotile is a significant contributor to the induction of mesothelioma. This is part of the global anti-chrysotile campaign headed by various groups with which Suzuki is associated and which have been given continuous journal space by, amongst others, Mt Sinai's AJIM editor Phil Landrigan. It is therefore not surprising that Landrigan was kept appraised of the status of Suzuki's translocation study funding efforts.
55. Nolan et al. [35] wrote: ''reference specimen iii, often referred to as a naturally occurring short fiber, had a fiber length distribution indistinguishable from the other reference specimens''. According to Addison (pers. commun., 2000), the failure to find a difference from the other fiber types was due to differences in sample preparation, counting methods, and examination techniques, amongst others.

References

1 Morgan A: Acid leaching studies of chrysotile asbestos from mines in the Coalinga region of California and from Quebec and British Columbia: Ann Occup Hyg 1997;41(3): 249–268.
2 Ilgren E, Chatfield E: Coalinga fibre – a short, amphibole – free chrysotile. Part 1: evidence for lack of fibrogenic activity: Indoor Built Environ 1997;6:264–276.
3 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.
4 Langer A, Wolff M, Rohl A, Selikoff I: Variation of properties of chrysotile asbestos subjected to milling: J. Tox Environ. Hlth 1978;4:173–188.
5 Davis JMG: The role of clearance and dissolution in determining the durability or biopersistence of mineral fibers: Environ Health Perspect 1994;102(S5):113–117.
6 Eastern Research Group. Report on the expert panel on health effects of asbestos and synthetic vitreous fibers: the influence of fiber length. Prepared for the agency for toxic substances and disease registry division of Health Assessment and Consultation Atlanta, GA, 2003.
7 Bernstein DM, Hoskins JA: The health effects of chrysotile: Current perspective based upon recent data: Reg Tox Pharm 2006;45: 252–264.
8 Browne K: A threshold for asbestos related lung cancer; in Sluyser M (ed.): *Asbestos Related Cancer*. Chichester, UK, Ellis Horwood, 1991, pp. 218–40.
9 Berman W, Crump K, Chatfield E, Davis J, Jones A: The sizes, shapes and mineralogy of asbestos structures that induce lung tumors or mesotheliomas in AF/HAN rats following inhalation: Risk Anal 1995;15:181–195.
10 Berman W, Crump B: Methodology for conducting risk assessments at asbestos superfund sites. Parts 1 & 2. Methodology & technical background documents. Prepared for Kent Kitchingman, US EPA, Region 9, San Francisco, Under EPA Review, 1999.
11 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.
12 Langer A, Mackler A, Pooley F: Electron microscopic investigation of asbestos fibers: Environ Hlth Perspect 1974;9:63–80.
13 Langer A, Rohl A, Wolff M, Selikoff I: Asbestos, fibrous minerals and acicular cleavage fragments: nomenclature and biological properties; in Lemen R, Dement JM (eds): Dusts and Disease (occupational and environmental exposures to selected fibrous and particulate dusts). Research Triangle Park, NC, Pathotox Publishers, Inc., 1979, pp. 1–22.
14 Zoltai T: Asbestiform and acicular mineral fragments: Ann NY Acad Sci 1979;330: 621–643.
15 NRC – National Research Council: Asbestiform fibers. Non-occupational health risks, National Academy Press, 1984.
16 Hodgson A. Asbestos. UK, Anjelena Press, 1986.
17 Chatfield E: Testimony; in Conwed Corp. v Union Carbide Corp. 12 Sept 2002 (available from the author on request).
18 Ilgren E: Coalinga chrysotile: dissolution, concentration, regulation and general relevance: Indoor Built Environ 2008;17:42–57.
19 Pinkerton K, Brody A, McLaurin D, Adkins B, O'Connor R, Pratt P, Crapo JD: Characterization of three types of chrysotile asbestos after aerosolization: Environ Res 1983;31:32–53.
20 Campbell W, Huggins C, Wylie A: Chemical and physical characterization of amosite, chrysotile, and nonfibrous tremolite for oral ingestion studies by the National Institutes of Environmental Health Sciences, US Bur Mines Rep Invest #8452, 1980, p. 63.
21 Timbrell V: Human exposure to asbestos: dust controls and standards. The inhalation of fibrous dusts: Ann NY Acad Sci 1965;133: 255–273.
22 Muhle H, Pott F, Bellman B, Takenaka S, Ziem U: Inhalation and injection experiments in rats to test the carcinogenicity of MMMF: Ann Occup Hyg 1987;131: 755–764.
23 Longo W: Measurement of airborne chrysotile structure sizes from Calidria asbestos. Materials Analytical Services [MAS], Inc. Revision #2, Feb., 2002 (available from the author on request).
24 Longo W: Testimony; in Moore v ACandS et al. 19 Sept 2002 (available from the author on request).
25 Longo, W: Testimony; in Flowers v Aventis Cropscience USA et al. 5 Nov 2002 (available from the author on request).
26 Yamate G. Agaiwai S, Gibbons R: Methodology for the measurement of airborne asbestos in electron microscopy, US EPA Report No. 69-02-3266. US EPA, Wash., DC, 1984.
27 Bernstein DM, Chevalier J, Smith P: Comparison of Calidria chrysotile asbestos to pure tremolite: inhalation biopersistence and histopathology following short-term exposure: Inhal Tox 2003a;15:1387–1419.
28 Bernstein DM, Mast R, Anderson T, Hesterberg T, Musselman R, Kamstrup O, Hadley J: An experimental approach to the evaluation of the biopersistence of respirable synthetic fibers and minerals: Environ Health Perspect 1994;102(Suppl. 5):15–18.
29 Bernstein DM, Rogers R, Smith P: The biopersistence of Canadian chrysotile asbestos following inhalation: Inhal Tox 2003b;15: 1247–1274.
30 Bernstein DM, Rogers R, Smith P: The biopersistence of Brazilian chrysotile asbestos following inhalation: Inhal Tox 2004; 16:1–28.

31 Chatfield E: Testimony; in Conwed Corp. v Union Carbide Corp. 27 Oct 2003 (available from the author on request).
32 Rendall REG: The data sheets on the chemical and physical properties of the UICC standard reference samples. Pneumoconiosis. Proc Intl Conf, Johannesburg 1969, Published 1970, pp. 23– 27.
33 Timbrell V: Characteristics of the International Union against Cancer standard reference samples of asbestos. Pneumoconiosis. Proc. Intl. Conf., Johannesburg 1969, Shapiro HA (ed.), Oxford University Press, Cape Town, 1970, pp. 28–36.
34 Yeager H, Russo D, Yanez M, Gerardi D, Nolan R, Kagan E, Langer A: Cytotoxicity of a short-fiber chrysotile asbestos for human alveolar macrophages: Preliminary observations: Environ Res 1983;30,224–232.
35 Nolan R, Langer A. Addison J: Lung content analysis of cases occupationally exposed to chrysotile asbestos: Environ Health Perspect 1994;102(Suppl 5):245–250.
36 Langer A: Reduction of the biological potential of chrysotile asbestos arising from conditions of service on brake pads: Reg Tox Pharm 2003;38:71–77.
37 Wylie AG: Fibre length and aspect ratio of some selected asbestos samples: Ann NY Acad Sci 1979;330:605–610.
38 Siegrist HG, Wylie AG: Characterizing and discriminating the shape of asbestos particles: Environ Res 1980;23:348–361.
39 ASTECO: Fiber size determination and distribution of RG-244 Calidria chrysotile asbestos by TEM [3 Feb. 1993] RG-244 Fiber Size. Job No: 12515 ASTECO Inc.
40 Egilman D, Roberts M: Controlled use of asbestos: Intl J Occup Environ Hlth 2004;10:99–103.
41 Ilgren E, Chatfield E: Coalinga fibre – a short, amphibole – free chrysotile. Part 2: evidence for lack of tumorigenic activity: Indoor Built Environ 1998;7:18–31.
42 Ilgren E, Chatfield E: Coalinga fibre – a short, amphibole – free chrysotile. Part 3: lack of biopersistence: Indoor Built Environ. 1998;7:98–105.
43 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.
44 Ilgren, E: Testimony; in Conwed v Union Carbide 13 Aug 98. (available from the author on request).
45 Vorwald AJ, Durkan TM, Pratt PC: Experimental studies of asbestosis: AMA Arch Ind Hyg Occup Med 1951;3:1–43.
46 Elmes P: Conflicts in evidence on the health effects of mineral fibres; in Liddell D, Miller K (eds): Mineral Fibres in Health and Disease. Boca Raton, CRC press, 1991, pp. 321–337.
47 Langer A: Testimony; in Conwed v Union Carbide Corp. 2002 (available from the author on request).
48 Wylie AG: Relationship between the growth habit of asbestos and the dimensions of asbestos fibers. Mining Eng 1988;5:1036–1039.
49 Ilgren E: Coalinga fibre - a short, amphibole - free chrysotile. Part 6, lack of amphibole asbestos contamination: Indoor Built Environ 2004;13:325–341.
50 Chwastiak S: Properties of Colloidal chrysotile asbestos from the Coalinga region of California. 19 Dec 1968. Project No. 458-N11, File No. 19 (available from the author on request).
51 Martin C, Phillips V: The texture of chrysotile asbestos in relationship to mechanical properties: Mat Sci Eng 1977;30:81–87.
52 Atkinson MAL, O''Sullivan M, Zuber S, Dodson RF: evaluation of the size and type of free particulates collected from unused asbestos-containing brake components as related to potential for respirability: Am J Indust Med 2004;46:545–553.
53 Dodson RF, Williams MG, Corn CJ, Brollo A, Bianchi C: Asbestos content of lung tissue, lymph nodes and pleural plaques from former shipyard workers: Am Rev Respir Dis 1990;142:843–847.
54 Dodson RF, Williams MG, Corn CJ, Brollo A, Bianchi C: A comparison of asbestos burden in lung parenchyma, lymph nodes, and plaques: Ann NY Acad Sci 1991;643:53–60.
55 Dodson RF, O'Sullivan M, Corn C, McLarty JW, Hammar SP: Analysis of asbestos fiber burden in lung tissue from mesothelioma patients: Ultrastructural Patholog 1997;21: 321–336.
56 Dodson RF, Huang J, Bruce JR: Asbestos content in the lymph nodes of non-occupationally exposed individuals: Am J Indust Med 2000;37:169–174.
57 Dodson RF, Atkinson MAL, Levin JL: Asbestos fiber length as related to potential pathogenicity: A critical review: Am J Indust Med 2003;44:291–297.
58 Mancuso T: Relative risk of mesothelioma among railroad workers: Am J Indust Med 1988;13:639–657.
59 Lemen RA: Asbestos in brakes: exposure and risk of disease: Am J Indust Med 2004; 45:229–237.
60 Kohyama N, Suzuki Y: Analysis of asbestos fibers in lung parenchyma, pleural plaques, and mesothelioma tissues of North American insulation workers: Ann N Y Acad Sci 1991; 643:27–52.
61 Case BW, Sebastien P: Environmental and occupational exposures to chrysotile asbestos: a comparative micro-analytic study: Arch Environ. Hlth 1987;42:185–191.
62 Sebastien P, Janson X, Gaudichet A, Hirsch A, Bignon J: Asbestos retention in human respiratory tissues: comparative measurements in lung parenchyma and in parietal pleura; in Wagner JC (ed): Biological Effects of Mineral Fibres (effets biologiques des fibres minérales). Vol. 1, International Agency for Research on Cancer, Lyon, Scientific Publications No. 30, 1980, pp. 237–246.
63 Gibbs AR, Stephens M, Griffiths DM, Blight BJ, Pooley FD: Fibre distribution in the lungs and pleura of subjects with asbestos related diffuse pleural fibrosis: Br J Indust Med 1991;48:762–770.
64 Case BW: Pre-meeting comments; in: Eastern Research Group Report on the Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length. Prepared for the Agency for Toxic Substances and Disease Registry Division of Health Assessment and Consultation Atlanta, GA, 2003.
65 Suzuki Y: Deposition testimony; in Asbestos Litigation, Cause No. 2004-039648. Harris County, Texas, 10 May'05 (available from the author on request).
66 Suzuki Y, Yuen SR: Asbestos tissue burden study on human malignant mesothelioma: Indust Health 2001;39(2):150–160.
67 Suzuki Y, Yuen S: Asbestos fibers contributing to the induction of human malignant mesothelioma: Ann. N Y Acad Sci 2002;982:160–176.
68 Suzuki Y, Yuen S: Erratum to "Short, thin asbestos fibers contribute to the development of human malignant mesothelioma": pathological evidence: Intl J Hyg Environ Hlth 2005;208:439–444.
69 Boutin C, Dumortier P, Rey F, Viallat JR, De Vuyst P: Black spots concentrate oncogenic asbestos fibers in the parietal pleura. Thoracoscopic and mineralogic study: Am J Respir Crit Care Med 1996;153(1):444–449.
70 Dumortier P, Rey F, Viallat JR, Broucke I, Boutin C, De Vuyst P: Chrysotile and tremolite asbestos fibers in the lungs and parietal pleura of Corsican goats: Occup Env Med 2002;59:643–646.
71 Le Bouffant L, Martin JC, Durif S, Daniel H: Structure and Composition of Pleural Plaques. Biological Effects of Asbestos. IARC– Lyon, France, IARC Scientific Publications No.8, 1973, pp. 249–257.
72 Wang NS: The preformed stroma connecting the pleural cavity and the lymphatics in the parietal pleura: Am Rev Respir Dis 1975; 111:12–20.
73 Holt P: Translocation of inhaled dust to the pleura: Environ Res 1983;31:212–220.
74 Beattie J, Knox JF. Studies of mineral content and particle size distribution in the lungs of asbestos textile workers; in Davis CN (ed): Inhaled Particles and Vapours, Oxford, Pergamon, 1961, pp. 419–432.
75 Hourihane D: The pathology of mesotheliomata and an analysis of their association with asbestos exposure: Thorax 1964;19, 268–278.
76 Hillerdal G: Pleural Plaques, Doctoral thesis, Uppsala Univ., Sweden, 1980.
77 Holt PF: Dust elimination from pulmonary alveoli: Environ Res 1980;23:224–227.
78 Leak LV, Rahil K: Permeability of the diaphragmatic mesothelium: the ultrastructure of stomata: Am J Anal 1978;151:557–594.
79 Taskinen E, Ahlamn K, Wukeri M: A current hypothesis of the lymphatic transport of inspired dust to the parietal pleura: Chest 1973;64:193–202.
80 Selikoff IJ: The occurrence of pleural calcification among asbestos insulation workers: Ann NY Acad Sci 1965;132:351–367.
81 Herbert A: Pathogenesis of pleurisy, pleural fibrosis and mesothelial proliferation: Thorax 1986;41:176–189.
82 Bignon J., Monchaux G, Hirsch. A, Sebastien P, Lafuma J: Human and experimental data on translocation of asbestos fibers through the respiratory system: Ann N Y Acad Sci 1979;330:745–749.
83 Bignon J, Sebastien P, Gaudichet A, Bonnaud G: in Gravatt CC, Lafleur PD, Heinrich JFK (eds): Workshop on Asbestos: Definitions and

Measurement Methods. Washington DC, NBS Special Publication 506, 1978; pp. 95–119.
84 Sebastien P, Fondimare A, Bignon J, Monchaux G, Desbordes J, Bonnaud G: Topographic distribution of asbestos fibres in human lungs in relation to occupational and non-occupational exposure; in Walton WH, McGovern B (eds): Oxford, Inhaled Particles IV. Pergamon, 1977, pp. 435–446.
85 Churg A: Non-asbestos pulmonary mineral fibers in the general population: Environ Res 1983;31:189–200.
86 Churg A: The pathogenesis of pleural plaques: Indoor Built Environ 1997;6:73–78.
87 Morgan A, Holmes A: The distribution and characteristics of asbestos fibers in the lungs of Finnish Anthophyllite mine-workers: Environ Res1984;33(1):62–75.
88 Wright GW, Kuschner M: The influence of varying lengths of glass and asbestos fibres on tissue response in guinea pigs; in Walton WH, McGovern B (eds): Inhaled Particles IV, Part 2. Oxford, Pergamon, 1977, pp. 455–474.
89 Rey F, Viallat JR, Tarisse P, Boutin C: Pleural migration of asbestos fibres after tracheal injection in rats: Eur Respir Rev 1993;3:145–147.
90 Kanasawa K: Exchanges through the pleura. Cells and particles; in Chrétien J, Bignon J, Hirsch A (eds): The pleura in health and disease. New York, Marcel Dekker Inc., 1985, pp. 195–231.
91 Pereira AS, Grande NR: Particle clearance from the canine pleural space into thoracic lymph nodes: an experimental study: Lymphology 1992;25:120–130.
92 Takada, K: Mesothelial damage and growth: experimental mechanisms: Am J Path 1991;132:12–23.
93 Moalli PA, MacDonald JL, Goodglick LA, Kane AB: Acute injury and regeneration of the mesothelium in response to asbestos fibers: Am J Pathol 1987;128:126–445.
94 Mitchev K, Dumortier P, De Vuyst P: "Black Spots" and hyaline pleural plaques on the parietal pleura of 150 urban necropsy cases: Am J Surg Path 2002;26:1198–1206.
95 Davis J, Beckett S, Bolton R, Donaldson K: The effects of intermittent high asbestos exposure (peak dose levels) on the lungs of rats: Br J Exp Path 1980;61:272–280.
96 Davis J, Beckett S, Bolton R, Collings P, Middleton A: Mass and number of fibers in the pathogenesis of asbestos related lung disease in rats: Br J Cancer 1978;37: 667–674.
97 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: Brit J Exp Path 1986;67:113–129.
98 Pott F, Dolgner R, Spurny K: Similarities and dissimilarities between asbestos fibres and man–made mineral fibres with regard to their carcinogenicity in man. Proc Intl Symp Prevention of Occupational Cancer, Helsinki 21–24 April, 1981, International Labor Office Geneva, Occupational Safety and Health Series 1982; 46: 118–123.
99 Lippman M: Asbestos exposure indices: Environ Res 1988;46:86–106.
100 Longo W: Testimony in Langved v Igley Co et al., 2004 (available from the author on request).
101 Berman W, Chatfield E: Environmental Asbestos Assessment Manual. Superfund Method for the Determination of Asbestos in Ambient Air. Part 1: Measurement Methods Document. Interim Version. EPA/ 540/2-90/005b, 1990a.
102 Berman, W, Chatfield, E: Environmental Asbestos Assessment Manual. Superfund Method for the Determination of Asbestos in Ambient Air. Part 2: Technical Background Document. Interim Version. EPA/540/2-90/005b, 1990b.
103 Davis JMG, Beckett ST, Bolton RE, Donaldson K: The effects of intermittent high asbestos exposure (peak dose levels) on the lungs of rats: Br J Exp Path 1980;61:272–280.
104 Zoltai T: Site Visit Report as Consultant to Minnesota Dept of Health (6 Jan 75) (available from the author on request).
105 McConnell, EE, Rutter HA, Ulland BM, Moore JA: Chronic effects of dietary exposure to amosite asbestos and tremolite in F344 rats: Environ Hlth Perspect 1983; 53:27–44.
106 McConnell EE, Shefner AM, Rust JH, Moore JA: Chronic effects of dietary exposure to amosite and chrysotile asbestos in Syrian golden hamsters: Environ Hlth Perspect 1983;53:11–25.
107 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. 1984 Annex 45: pp. 234–242.
108 IITRI Proj. C06570/C06591 - Investigation of feasibility of XRD thin layer methods for quantitative analysis - [C1], 1984.
109 Timbrell V: Producing a fibre cloud for animal inhalation experiments, Discussion # 2nd Internat. Conf. Bio. Effects Asbestos – Dresden – April 1968.
110 Egilman D: Testimony; in Lustgarten v A W Chesterton et al. 26 Mar 2004 (available from the author on request).
111 Donnet J, Cosme P: New results on the proton attack of macrofibers of chrysotile. 3rd. Intl. Asbetos Conf., Quebec, 1975.
112 Le Bouffant L: Physics and chemistry of asbestos dust; in Wagner JC (ed): Biological Effects of Mineral Fibres (Effets biologiques des fibres minérales). Vol. 1, International Agency for Research on Cancer, Lyon, Scientific Publications No.30, 1980, pp. 15–33.
113 Sebastien P, Janson X, Bonnaud G, Riba G, Masse R, Bignon J: Translocation of asbestos fibers through respiratory tract and gastrointestinal tract according to fiber type and size; in Lemen R, Dement JM (eds): Dusts and Disease (Occupational and Environmental Exposures to Selected Fibrous and Particulate Dusts Pathotox Publishers Inc., 1979, pp. 65–77.
114 Vialliat J, Raybaud F, Passarel M, Boutin C: Pleural migration of chrysotile fibers after intratracheal injection in rats: Arch Environ Health 1986;41:282–286.
115 Final Report (FRAPR) on the Peer Consultation Workshop to Discuss a Proposed Protocol to Assess Asbestos-Related Risk, Prepared for U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, Washington, DC 20460: EPA Contract No. 68-C-98-148, Work Assignment 2003-05 Prepared by: Eastern Research Group, Inc. May 30, 2003.
116 Lockey J: Pre-meeting comments: in Eastern Research Group Report on the Expert Panel on Health Effects of Asbestos and Synthetic Vitreous Fibers: The Influence of Fiber Length. Prepared for the Agency for Toxic Substances and Disease Registry Division of Health Assessment and Consultation Atlanta, GA, 2003.
117 Case BW, Kuhar M, Harrigan M, Dufresne A: Lung fiber content of American children aged 8–15 years; preliminary findings; in Dodgson J, Mc Callum, RI (eds): Inhaled Particles VII, Oxford, Elsevier, Ann Occup Hyg 1994; 38(Supp 1): 639–645.
118 Morinaga K, Kohyama N, Yokoyama K, Yasui Y, Hara I, Sasaki M, Suzuki, Y, Sera Y: Asbestos fibre content of lungs with mesotheliomas in Osaka, Japan: A preliminary report; in Bignon J, Peto J, Saracci R (eds): Non-occupational Exposure to Mineral Fibres. Lyon, IARC Scientific Publications 90, International Agency for Research on Cancer, 1989, pp. 438–443.
119 Doll R: Concluding remarks; in Bignon J, Peto J, Saracci R (eds): Non-occupational Exposure to Mineral Fibres, Lyon, IARC Scientific Publications 90, International Agency for Research on Cancer, 1989, pp. 438–443.
120 Churg A, Wright JL, Depaoli L, Wiggs B: Mineralogic correlates of fibrosis in chrysotile miners and millers: Am Rev Respir Dis 1989; 139(4):891–896.
121 Churg A, Wright JL, Vedal S: Fiber burden and patterns of asbestos related diseases in chrysotile miners and millers: Am Rev Resp Dis 1993;148:25–31.
122 Sebastien P, McDonald JC, McDonald AD, Case B, Harley R: Respiratory cancer in chrysotile textile and mining industries: exposure inferences from lung analysis: Br J Indust Med 1989;46:180–187.
123 Case BW, Sebastien P: Fibre levels in lung and correlation with air samples; in Bignon J, Peto J, Saracci R (eds): Non-occupational Exposure to Mineral Fibres. Lyon, IARC, 1989, pp. 207–218.
124 Case BW: Health effects of tremolite. Now and in the future: Ann NY Acad Sci 1991;643:491–504.
125 Case BW. Biological indicators of chrysotile exposure. Ann Occup Hyg 1994;38(4):503–18, 410-411.
126 Kane AB, Bofetta P, Saracci R, Wilbourn JD (eds). Mechanisms of Fibre Carcinogenesis. IARC Sci Pub No 140, Lyon, International Agency for Research on Cancer, 1996.

127 Suzuki Y, Kohyama N: Translocation of inhaled asbestos fibers from the lung to other tissues: Am J Indust Med 1991; 19(6):701–4.
128 Hiroshima K, Murai Y, Suzuki Y, Goldstein B, Webster I: Characterization of asbestos fibers in lungs and mesotheliomatous tissues of baboons following long term inhalation: Amer J Indust Med 1993;23:883–901.
129 Rogers AL, Leigh J, Berry G, Ferguson DA, Mulder HB, Ackad M, Morgan GG: Dose-response relationship between airborne and lung asbestos fibre type, length, and concentration, and the relative risk of mesothelioma, Ann Occup Hyg 1994;38(S1):631–638.
130 Nagelschmidt G: Some observations of the dust content and composition in lungs with asbestosis made during work on coal miners pneumoconiosis: Ann NY Acad Sci 1965; 132:64–76.
131 Thomson JG: Asbestos and the urban dweller: Ann. NY Acad Sci 1965;132:196–214.
132 Brown J: Atlas of Video Assisted Thoracic Surgery. Oxford, UK, WB Saunders, 1977, Chapter 18, pp. 171–183.
133 Ilgren E, Browne K: Asbestos-related mesothelioma: Evidence for a threshold in humans and animals: Reg Tox Pharm 1991;13:116–132.