ENVIRONMENTAL RESEARCH 46, 86–106 (1988)

REVIEW

Asbestos Exposure Indices[1]

MORTON LIPPMANN

Institute of Environmental Medicine, New York University Medical Center, Tuxedo, New York 10987

Received July 18, 1987

The ability of inhaled asbestos to produce asbestosis, lung cancer, and mesothelioma in both humans and animals is well established, and asbestos exposures in the occupational and general community environment are recognized as significant hazards. However, it has not been possible to establish realistic and credible dose–response relationships, primarily because of our inability to define which constituents of the aerosols produce or initiate the pathological responses. It is generally acknowledged that the responses are associated with the fibers rather than the nonfibrous silicate mineral of the same chemical composition. Available data from experimental studies in animals exposed by injection and inhalation to fibers of defined size distributions are reviewed, along with data from studies of fiber distributions in lungs of exposed humans in relation to the effects associated with the retained fibers. It is concluded that asbestosis is most closely related to the surface area of retained fibers, that mesothelioma is most closely associated with numbers of fibers longer than ~5 μm and thinner than ~0.1 μm, and that lung cancer is most closely associated with fibers longer than ~10 μm and thicker than ~0.15 μm. The implications of these conclusions on methods for fiber sampling and analyses are discussed.

INTRODUCTION

The evidence from both human epidemiology and experimental animal inhalation studies is clear and consistent. Inhaled asbestos fibers cause (1) asbestosis, a diffuse fibrosis in the nonciliated portion of the lung; (2) lung cancer; and (3) mesothelioma, a cancer of the pleura and peritoneum. However, the exposure–response relationships for these diseases are much less clear and consistent.

There are three different types of concentration indices which have been used for airborne asbestos. Initially, the most widely used index was the number of particles per unit volume of air, expressed in millions of particles per cubic foot (MPPCF), and determined from impinger samples analyzed by the old USPHS standard dust counting technique using a 10× objective lens. Since there was no discrimination between fibrous and non fibrous particles, and since fibers are a very variable fraction of the total dust in most cases, dust counting for occupational exposure evaluations was replaced by a technique which counts fibers only. At the time the fiber counting technique was first adopted in the UK, it was already clear that long fibers were of most concern. This, combined with the

[1] Presented at the Eighth Annual Scientific Meeting, Universities Occupational Safety and Health Educational Resource Center, New York, New York, April 2, 1987.

0013-9351/88 $3.00

practical limitation that fibers shorter than ~5 μm could not be reliably identified by light microscopy, led to the adoption of a counting procedure which uses a 45× phase-contrast objective to count the fibers collected on a membrane filter having a length/diameter (aspect) ratio >3 and longer than 5 μm (ACGIH-AIHA Aerosol Comm., 1975). The phase-contrast optical method (PCOM) is specified in the OSHA occupational health standard for asbestos. Table 1 summarizes recommended occupational exposure limits and standards used in the United States over the last 40 years.

The third type of concentration index is based on the mass concentration of asbestos, or on the mass concentration passing a pre-collector meeting the British Medical Research Council (BMRC) or American Conference of Governmental Industrial Hygienists (ACGIH) sampler acceptance criteria. Some of the recent animal inhalation studies report the chamber concentrations in terms of the "respirable" mass based on samples collected using samplers which meet the BMRC criteria.

Environmental exposures have been measured either in terms of fiber count or fiber mass. Fiber counts have been made using both phase-contrast optical and electron microscopy. The reported concentrations have differed according to the size distributions of the fibers, the resolving power of the microscope, and whether there was any discrimination in the analyses according to fiber type. The fiber mass index was developed by Selikoff *et al.* (1972) at Mt. Sinai School of Medicine. The fibers in the sample are mechanically reduced to fibrils, which are then identified and measured by electron microscopy. Mass concentrations in nanograms per cubic meter are calculated from the numbers of fibrils and their dimensions.

Use of these various exposure indices has sometimes led to the development of a site- or industry-specific exposure–response relationship for one or more of the asbestos-related diseases, but it has not been possible to develop any generic relationships. This demonstrates the inadequacy of our current indices of exposure.

TABLE 1

RECOMMENDED AIR CONCENTRATION LIMITS AND STANDARDS FOR ASBESTOS

Group	Year	Limit
ACGIH	1946	5×10^6 particles/ft^3
ACGIH	1968[a]	12 fibers[c]/ml or 2×10^6 particles/ft^3
ACGIH	1970,[a] 1974[b]	5 fibers/ml
OSHA	1972	5 fibers/ml
OSHA	1976	2 fibers/ml
NIOSH	1976	0.1 fiber/ml
ACGIH	1978,[a] 1980[b]	0.2 fiber/ml for crocidolite 0.5 fiber/ml for amosite 2.0 fiber/ml for chrysotile and other forms
OSHA	1986	0.2 fiber/ml

[a] Notice of Intent.
[b] Adopted as threshold limit value (TLV).
[c] All fiber limits based on phase-contrast optical determination at 400-450× magnification.

While our past failures are regrettable, we are now, at last, at a position where we can begin to identify which of the characteristics of airborne fibers affect the pathogenesis of the various diseases. When this information is more fully developed, we will be able to specify the fiber characteristics which should be measured in exposure evaluations to be used in hazard evaluations. Unfortunately, the reality is complex, and it now appears that the hazards of asbestos in relation to asbestosis, lung cancer, and mesothelioma each relate to different critical fiber characteristics. This paper provides a brief review of the fiber characteristics which affect deposition in the respiratory tract, translocation to distant sites of toxic action, retention at critical sites, observed associations between the characteristics of retained fibers and disease processes, recommendations on appropriate fiber size criteria to be used in sampling and analyzing airborne fibers for exposure assessments, and recommendations on sampling strategies.

REVIEW OF FIBER DEPOSITION AND RETENTION

Fiber Deposition in the Lung Conductive Tract

Mineral fibers become deposited in respiratory airways in five main ways: impaction, sedimentation, interception, electrostatic precipitation, and diffusion.

Impaction and sedimentation probabilities are governed by the aerodynamic diameter of the fibers, which, in the case of long mineral fibers, is close to three times their physical diameters (Timbrell, 1972; Stöber *et al.*, 1970). Most impaction occurs downstream of air jets in the larger airways, where the flow velocities are high, and the momentum of the fiber propels it out of the bending flow streamlines and onto relative small portions of the epithelial surfaces, especially at airway bifurcations. Sedimentation, on the other hand, is favored by low flow velocity, a long residence time in the lung airways, and small airway size.

Electrostatic precipitation occurs primarily by image forces, and depends on the ratio of electrical charge to aerodynamic drag. Little is known about the charge levels on fibers. Vincent (1985) has shown that asbestos fiber processing operations do generate fibrous aerosols with relatively high charge levels, and that these charge levels are sufficient to cause an enhancement of fiber deposition in the lungs.

Interception is favored by fiber length. The longer the fiber, the more likely it is that the position of a fiber end will cause it to touch a surface which the center of mass would have missed.

Single symmetrical fibers tend to become aligned with the flow axis as they move through an airway. On the other hand, fiber agglomerates or nonfibrous particles have more random orientations, which depend on their distribution of masses and drag forces. A fiber whose flow orientation differs from axial alignment has an enhanced probability of deposition by interception. Amphibole asbestos is comprised of straight fibers that tend to align with the flow axis. By contrast, the curly fibers of chrysotile often travel sideways through airways, and are more likely to be deposited on airway bifurcations (Timbrell, 1982).

A fiber's alignment is altered as it enters an airway bifurcation, and this loss of alignment with the flow at the entry of the daughter tube contributes to its depo-

sition by interception at or near the carinal edge. To the extent that a fiber is entrained in the secondary flow streams which form at bifurcations, its deposition probability by interception should be further enhanced.

Deposition in Nonciliated Airways and Effects at Deposition Sites

Deposition patterns within the nonciliated airways distal to the terminal bronchioles may be quite varied. Brody *et al.* (1981) have studied the deposition of chrysotile asbestos in lung peripheral airways. They exposed rats for 1 hr to 4.3 mg/m^3 of respirable chrysotile. The animals were killed in groups of 3 at 0, 5, and 24 hr and at 4 and 8 days after the end of the exposure. The pattern of asbestos fiber retention on the epithelial surfaces was examined by scanning electron microscopy of lung sections cut to reveal terminal bronchiolar surfaces and adjacent airspaces. The rat does not have recognizable respiratory bronchioles, and the airways distal to the terminal bronchioles are the alveolar ducts. In rats killed immediately after exposure, asbestos fibers were rarely seen in alveolar spaces or on alveolar duct surfaces, except at alveolar duct bifurcations. There were relatively high concentrations on bifurcations nearest the terminal bronchioles, and lesser concentrations on more distal duct bifurcations. In rats killed at 5 hr, the patterns were similar, but the concentrations were reduced. Similar deposition patterns were seen in rats exposed for 1 hr to an aerosol of crocidolite asbestos by Roggli *et al.* (1987).

The sudden enlargement in air path cross section at the junction of the terminal bronchiole and alveolar duct may play a role in the relatively high deposition efficiency at the first alveolar duct bifurcation. Little is known about the flow profiles in this region of the lung.

Johnson (1987) exposed rats to UICC crocidolite aerosols at 10 mg/m^3 for 6 hr/day, 5 days/week for periods ranging from 1 day to 12 months, and examined cells in structures distal to the terminal bronchioles. Alterations in the distribution of cells were seen within 3 months. Airway bifurcations were the initial sites where evidence of cell damage and collagen deposition was seen. By 12 months, there was substantial thickening of the epithelial lining of the bifurcations. Type II cell hyperplasia was evident without apparent damage to Type I cells.

For rats receiving inhalation exposures to chrysotile at 11 mg/m^3 for 7 hr/day, 5 days/week for 12 months, Pinkerton *et al.* (1986) found that fiber accumulation in the airways immediately distal to terminal bronchioles was inversely related to airway pathlength and, to an even greater extent, to the number of bifurcations along each conductive airway path. Fiber concentrations were much higher in the cranial regions of the left lung than in the costolateral, which had higher concentrations than the caudal. The differences increased with increasing fiber length, with the ratios increasing to 9:2:1 for fibers >20 μm in length. In addition, fiber burden within each region was strongly correlated with the degree of tissue injury present. The authors concluded that focal irregularities of pulmonary asbestosis of the type characteristic in exposed workers may be due to regional differences in the deposition and retention of asbestos fibers.

Fiber Clearance and Translocation

The fate of fibers deposited on surfaces within the lungs depends on both the

site of deposition and the characteristics of the fibers. Within the first day, fibers deposited in the tracheobronchial airways are carried proximally on the surface of the mucus to the larynx, to be swallowed and passed into the gastrointestinal tract. Some small fraction of the fibers on the tracheobronchial tree may be engulfed by epithelial cells. In any case, the time that fibers remain on the surface of the tracheobronchial region is too short for any significant change in the size or composition of the fiber to take place.

Fibers deposited in the nonciliated airspaces beyond the terminal bronchioles are more slowly cleared from their deposition sites by a variety of mechanisms and pathways. These can be classified into two broad categories: translocation and disintegration.

Translocation refers to the movement of the intact fiber along the epithelial surface to dust foci at the respiratory bronchioles, or onto the ciliated epithelium at the terminal bronchioles, or into and through the epithelium, with subsequent migration to interstitial storage sites or along lymphatic drainage pathways. Translocation may occur after ingestion of the fibers by alveolar macrophages, if the fibers are short enough to be fully ingested by the macrophages, or via movement of bare fibers.

Disintegration refers to a number of processes, including the subdivision of the fibers into shorter segments; partial dissolution of components of the matrix, creating a more porous fiber of relatively unchanged external size; or surface etching of the fibers, creating a change in the external dimensions of the fibers. For chrysotile, fibers can disintegrate into constituent fibrils. Thus, in turn, may facilitate dissolution and disintegration. The amphiboles exhibit much less dimensional change or loss of structural integrity during retention in the lung.

Bellmann *et al.* (1986) instilled suspensions of UICC crocidolite, UICC chrysotile A, and glass fibers in rat lungs, and examined the residual fibers after 1 day and 1, 6, 12, and 15 months. They reported that crocidolite fibers longer than 5 μm did not decrease in number for over 1 year. The number of chrysotile fibers >5 μm doubled, probably due to longitudinal splitting, while the number of >5 μm glass fibers was reduced with a half-time of 55 days, due to dissolution. All fibers <5 μm in length were cleared with half-times of 100–150 days. When the crocidolite fibers were pretreated in acid there was no change in retention. On the other hand, acid-treated chrysotile and glass fibers had much more rapid clearance, with half-times of 2 and 14 days, respectively.

In the inhalation study of Brody *et al.* (1981) with chrysotile, their examination of tissues by transmission electron microscopy revealed that fibers deposited on the bifurcations of the alveolar ducts were taken up, at least partially, by Type I epithelial cells during the 1-hr inhalation exposure. In the 5-hr period after exposure, significant amounts were cleared from the surfaces, and there was further uptake by both Type I epithelial cells and alveolar macrophages. Within 24 hr after the exposure, there was an influx of macrophages to the alveolar duct bifurcations. The observations provide a basis for fiber penetration of the surface epithelium which does not hypothesize movement within macrophages.

Roggli and Brody (1984) exposed rats for 1 hr to a chrysotile aerosol and showed that fiber clearance was associated with sequential dimensional changes in the retained fibers, with a tendency for long thin fibers to be retained within the

interstitium of the lung parenchyma. Roggli *et al.* (1987) subsequently performed essentially the same study with a crocidolite aerosol. For the crocidolite, there was a progressive increase in mean fiber length with increasing time postexposure, but the change was less pronounced than that for chrysotile. In addition, there was no change in fiber diameter with time for the crocidolite. In contrast, the longitudinal splitting of the chrysotile into fibrils had caused a marked reduction of diameter with time.

Accumulation of fibers in distal lung airways may, by itself, slow the clearance of fibers and other particles from the lung. Ferin and Leach (1976) exposed rats by inhalation to 10, 5, or 1 mg/m^3 of UICC amosite or Canadian chrysotile for periods ranging from 1 hr to 22 days. Exposures at 10 mg/m^3 for 1–3 hr, or for >11 days at 1 mg/m^3 suppressed the pulmonary clearance of TiO_2 particles.

REVIEW OF BIOLOGICAL EFFECTS OF SIZE-CLASSIFIED FIBERS

The pathological effects produced by fibers depend upon both the characteristics of the fibers and their persistence at sensitive sites. A number of carefully designed studies have been performed in which the size distributions of fiber suspensions have been well characterized as well as their persistence and/or effects.

King *et al.* (1946) instilled 100 mg of Rhodesian chrysotile into rabbit lungs at monthly intervals. One group received fibers microtomed to a length of 15 μm, and another group received fibers cut to 2.5 μm in length. At this huge dosage level, both groups showed foreign body reactions in the lungs. The long fiber produced a nodular reticulinosis, while the short fiber produced a diffuse interstitial reticulinosis.

Wright and Kuschner (1977) used short and long asbestos and manmade mineral fibers in intratracheal instillation studies in guinea pigs. With suspensions containing an appreciable number of fibers longer than ~10 μm, all of the materials produced lung fibrosis, although the yields varied with the materials used. However, with equal masses of short fibers of equivalent fiber diameters, none produced any fibrosis. The yields were lower for the long glass fibers than for the long asbestos, and this was attributed to their lesser durability within the lungs.

For fibers injected intraperitoneally (Davis, 1976; Pott *et al.*, 1976; Wagner *et al.*, 1976) or placed in a pledget against the lung pleura (Stanton and Wrench, 1972), a similar kind of fiber size and composition dependence was observed. The yield of mesotheliomas varied with both fiber diameter and length, and with dose, with very little response when long, thin fibers were not included. Asbestos fibers were more effective than glass in these studies also. At a dose of 2 mg of chrysotile, crocidolite, or glass fiber, Pott *et al.* (1976) found only slight degrees of fibrosis, but tumor yields of from 16 to 38% in rats. When the chrysotile was milled to the extent that 99.8% of the fibers were shorter than 5 μm, the dose required to produce a comparable tumor yield (32%) was 50 times greater (100mg).

Various hypotheses have been proposed to account for the pathological effects produced by asbestos. One was the contamination of the surface by trace metal and/or organic carcinogens. However, the studies of Stanton and Wrench (1972) found that surface contaminants played no role in mesothelioma yield, and con-

cluded that the carcinogenicity of asbestos and fibrous glass was primarily related to the structural shape of these fibrous materials rather than their surface properties.

The relative potency of the various mineral forms of asbestos after inhalation exposure is still not firmly established. Crocidolite is generally considered the most hazardous because of its association with significant numbers of human mesotheliomas. The ACGIH threshold limit values (TLVs) for 1978 included new tentative limits for asbestos which, for the first time, were specific for the different fibers, i.e., 0.2 fibers/ml for crocidolite, 0.5 fibers/ml for amosite and tremolite, and 2 fibers/ml for chrysotile and other forms. These TLVs were adopted by ACGIH in 1980, with the exception that a specific recommendation on tremolite was not made, and it presumably was considered equivalent to chrysotile.

The relative toxicity of various asbestos minerals have been compared in a variety of experimental inhalation studies on small animals, but the results are in apparent conflict. Wagner (1963) reported more asbestosis with amosite than with chrysotile in guinea pigs, rats, and monkeys. Wagner *et al.* (1974), in studies involving inhalation exposures of rats for 1-day to 2-years duration, found that amosite and crocidolite were the least fibrogenic of five types of UICC asbestos, the others being Canadian chrysotile, Rhodesian chrysotile, and anthophyllite (see Table 2). Holt *et al.* (1965) found no differences in the fibrogenic potential of chrysotile, crocidolite, amosite, and anthophyllite. Davis *et al.* (1978) used UICC chrysotile A, amosite, and crocidolite in 12-month rat exposures, and at respirable mass concentrations comparable to those used by Wagner *et al.* (1974), found a similar pattern, i.e., chrysotile was the most fibrogenic, and amosite and crocidolite, the least. Hiett (1978) exposed guinea pigs by inhalation for 9 and 18 days and also found that chrysotile was more fibrogenic than amosite. Davis *et al.* (1986b) subsequently repeated the protocol with amosite with fiber length both shorter and longer than UICC amosite. The short amosite produced virtually no fibrosis, while the long amosite was more fibrogenic than chrysotile. The most fibrogenic asbestos was tremolite (see Table 2).

It is generally believed that amphibole fibers account for much of the mesothelioma incidence among exposed workers, even when they are predominantly exposed to chrysotile, since amphibole fibers are more persistant in lung tissue. Pooley (1976) examined postmortem lung tissue from 20 workers with asbestosis in the Canadian chrysotile mining industry, and found that amphibole and other fibers were present in 16 cases. In 7 of these, they were more numerous than chrysotile. In a later study of lung asbestos in chrysotile workers with mesothelioma, Churg *et al.* (1984) reported that the concentration ratio between cases and controls was 9.3 for temolite, but only 2.8 for chrysotile. In a Norwegian plant using 91.7% chrysotile, 3.1% amosite, 4.1% crocidolite, and 1.1% anthophylite, Gylseth *et al.* (1983) reported that the percentage of chrysotile in lung tissue ranged between 0 and 9%, while the corresponding numbers for the amphiboles were 76 to 99%.

In their review of the assessment of mineral fibers from human lung tissue, Davis *et al.* (1986a) attributed the high amphibole/chrysotile ratios to the dissolution of chrysotile within lung tissue. They suggested that the generally poor

TABLE 2
SUMMARY OF RESULTS OF MINERAL FIBER INHALATION STUDIES IN RATS

Reference	Fiber type	Fiber parameters: Resp. Conc. (mg/m^3)	d_m (μm)	Length—% > 5 μm	Length—% > 10 μm	No. rats	Number (%) of rats with tumors: Mesothelioma	Adenoma	Adeno-CA	Squam. CA	Interstitial fibrosis: Score[a]	%[b]
	UICC											
Wagner *et al.* (1974)	Amosite	10	NR	NR	NR	146	1(0.7)	19(13)	5(3.4)	6(4.1)	4.3	
	Anthophyllite	10	NR	NR	NR	145	2(1.4)	22(15)	8(5.5)	8(5.5)	6.2	
	Crocidolite	10	NR	NR	NR	141	4(2.8)	26(18)	7(5.0)	9(6.4)	4.8	
	Chrysotile											
	Canadian	10	NR	NR	NR	137	4(2.9)	20(15)	11(8.0)	6(4.4)	6.0	
	Rhodesian	10	NR	NR	NR	144	0	19(13)	19(13)	11(7.6)	5.8	
	UICC											
Davis *et al.* (1978)	Amosite	10	0.38	16	2.7	43	0	2(4.7)	0	0		2.6
	Crocidolite	10	0.38	12	4	40	0	1(2.5)	0	0		1.4
	Crocidolite	5	0.38	12	4	43	0	2(4.7)	0	0		0.8
	Chrysotile	10	0.42	30	16	40	0	7(18)	6(15)	2(5.0)		9.2
	Chrysotile	2	0.42	30	16	42	1(2.4)	6(14)	1(2.4)	1(2.4)		3.5
Davis *et al.* (1985)	Tremolite	10	0.25	28	7	39	2(5.1)	2(5.1)	8(21)	8(21)		14.5
Davis *et al.* (1986)	Amosite—short	10	0.32	1.7	0.1	42	1(2.4)	0	0	0		0.15
	Amosite—long	10	0.37	30	10	40	3(7.5)	3(7.5)	3(7.5)	4(10)		11.0
Wagner *et al.* (1985)	UICC crocidolite	10	0.30	53	12	28	0	0	0	1(3.6)		
	Erionite	10	0.22	44	7	28	27(96)	0	0	0		
Davis *et al.* (1987)	Chrysotile—short	10	0.17	5	0.7	40	1(2.5)	1(2.5)	6(15)	0		2.4
	Chrysotile—long	10	0.18	12	2	40	3(7.5)	8(20)	6(15)	5(13)		12.6

Note. NR, not reported.
[a] Relative scale where 1-nil; 2 = minimal; 4 = slight; 6 = moderate; 8 = severe—at 24 months.
[b] % of tissue with fibrosis—at 27–29 months.

correlation between dust counts and mesothelioma is likely to be due to the differences among the various asbestos types in the fraction that reaches the pleural surface. They suggest that this depends critically on the fiber dimensions, and note that amosite fibers need to be longer to produce pulmonary fibrosis and pulmonary tumors in experimental animals than to produce mesotheliomas after injection (Davis *et al.*, 1986b). Davis (1987) notes that both chrysotile and amphibole asbestos fibers inhaled by rats are plentiful in the most peripheral alveoli bordering on the pleura, but penetration of the external elastic lamina of the lung appears to be a rare event. On the other hand, erionite causes a very high incidence of mesotheliomas in humans exposed to low environmental concentrations (Baris *et al.*, 1987), and in 100% of rats exposed by inhalation (Wagner *et al.*, 1985). Davis (1987) reported that the erionite used by Wagner *et al.* (1985) had a general appearance and fiber size distribution very close to that of UICC crocidolite which produces a much smaller mesothelioma yield in rats exposed by inhalation. He attributed the difference to the enhanced ability of erionite to cross the pleural membrane.

Critical Fiber Parameters for Asbestosis

Asbestosis has been caused by respirable fibers of all of the commercially exploited kinds of asbestos. Within the respirable fraction, the fibers can differ in diameter and length distributions, and in retention times.

The influence of these variables on fibrosis in the human lung has been systematically explored and described by Timbrell *et al.* (1987) through analyses of both retained fibers and fibrosis in lung samples from exposed workers. He analyzed the fiber distributions in 0.5-g lung samples from several hundred workers by a technique known as Magnetic Alignment and Light Scattering (MALS) that he had previously developed (Timbrell, 1982). Optical microscopy was applied to measure the degree of fibrosis in paraffin sections prepared from adjacent samples of the same specimens. Wide intra- and intersubject variations were observed in fiber concentration and fibrosis score. The quantitative relationships between fibrosis and amphibole fibers in mineworkers' postmortem lung specimens were determined for the main sources of amosite (Transvaal, South Africa), anthophyllite (Paakkila, Finland), and crocidolite (NW Cape and Transvaal, South Africa, and Wittenoom, Australia). As illustrated in Fig. 1, the fibrosis-producing ability of the fibers was independent of amphibole type when normalized by the total surface area of long resident fibers per unit weight of lung tissue, presumably because the surface area determined the magnitude of the fiber–tissue interface. The wide range of the concentrations of retained fiber required to produce the same degree of fibrosis in the groups of mineworkers, when fiber quantity is expressed as number or total mass, stemmed from the large differences between the distribution in diameter and length of the airborne fibers.

While the main focus of the Timbrell *et al.* (1987) study was on amphibole asbestos, they also reported results for three Wittenoom workers whose dominant exposure was to chrysotile asbestos. For these workers, chrysotile produced a similar degree of fibrosis to Wittenoom crocidolite for equal fiber mass concentrations in the lungs. Long residence in the tissue had almost completely dispersed

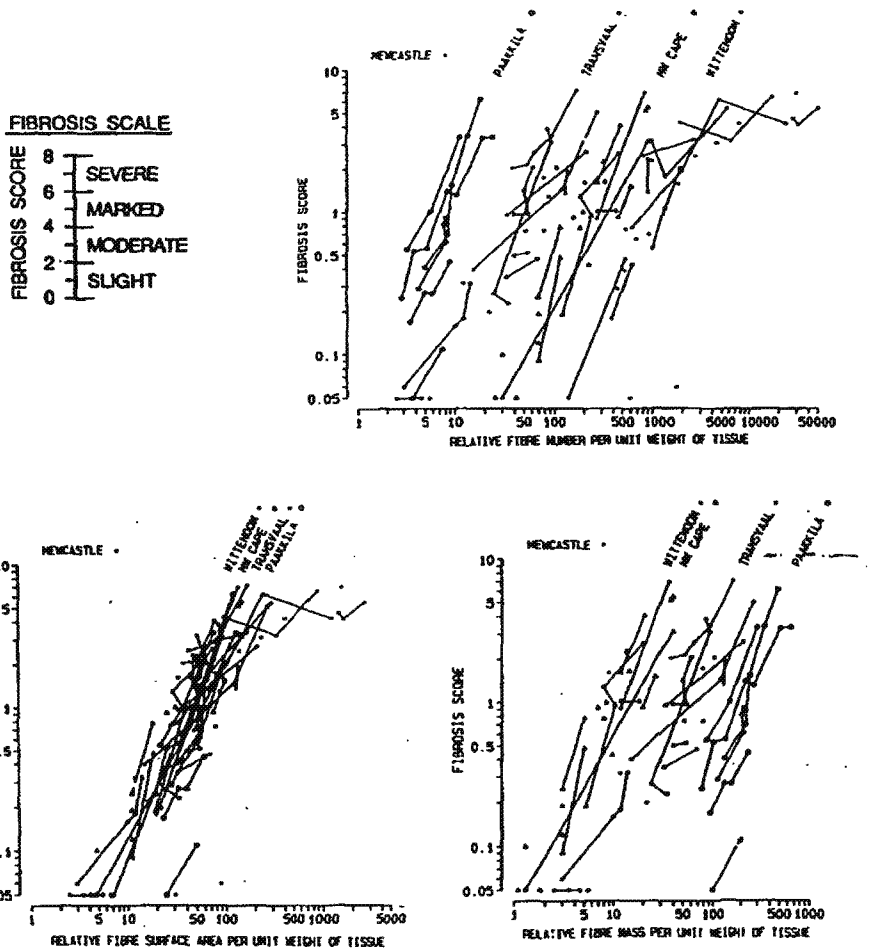

FIG. 1. Relationships between fibrosis scale and relative concentrations of fibers per unit weight of dry lung tissue. The lines connect data points from the same subject. The relative fiber surface area normalizes the data better than either the relative fiber number concentration or the fiber mass concentration (illustrations courtesy Dr. Vernon Timbrell).

the chrysotile fibers into fibrils, to give them a ratio of total surface area to mass resembling that of the particularly fine Wittenoom fibers. The result indicates that the fibrogenicity of the retained chrysotile per unit of surface area within the lungs was similar to that of the amphiboles.

Timbrell *et al.* (1987) also reported that amphibole mineworkers with a given fiber mass concentration in their lungs showed much higher degrees of fibrosis than goldminers with roughly the same mass concentration of retained quartz grains. The amphibole and quartz produced about the same fibrogenicity per unit

of surface area, but the smaller diameters and higher area/mass ratio of the amphibole fibers endowed them with the greater surface area, and thereby the superior fibrosis-producing capability.

Knowledge of the interrelationships between retained fibers and fibrosis is critical in understanding the pathogenesis of the disease, but is inadequate, by itself, in evaluating exposures to airborne fibers. This was recognized by Timbrell (1983, 1984), who developed a mathematical model relating fiber deposition and retention based on MALS analysis of lung samples. Specifically, he used samples from a woman at Paakkila who worked at a job which gave her exposure to an amphibole (anthophyllite) at high concentrations of fibers with a range of diameters and lengths sufficiently wide to encompass the size limits of respirable fibers. Her lungs contained 1.3 mg fiber/g of dry tissue, and she had asbestosis. One lung sample contained a fiber distribution matching expected deposition (Fig. 2—left panel). Timbrell speculated that severe fibrosis in the tissue in this sample had blocked the macrophage mediated clearance. Another sample from the same lung yielded a retention pattern closely matching those found in other Paakkila workers with small fiber burdens and virtually no short fibers. He assumed that the latter represents long-term retention in the normal lung. From the differences in reten-

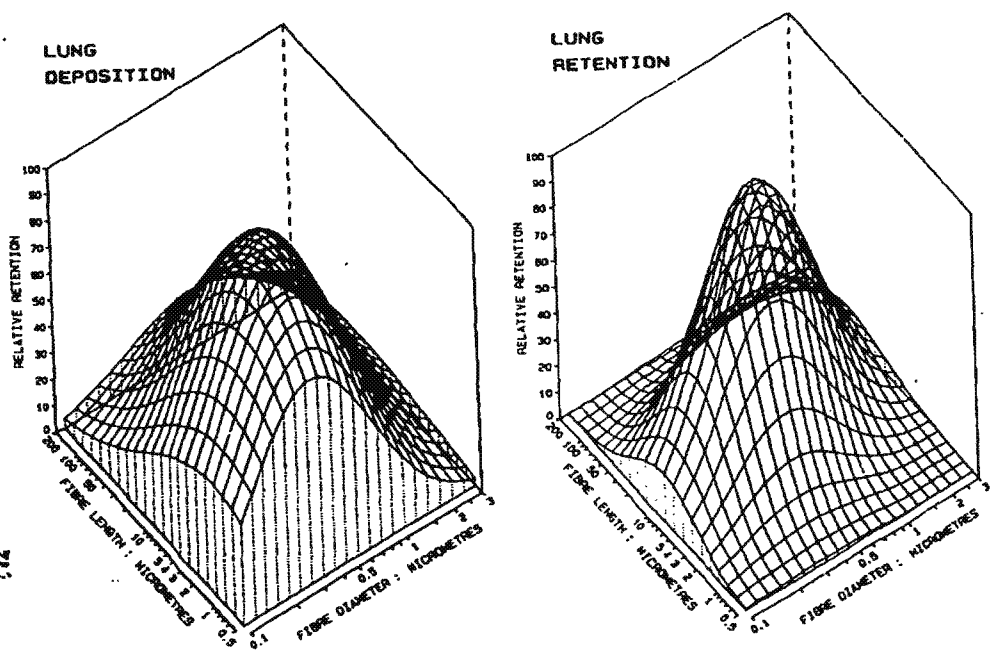

FIG . 2. The left panel shows the distribution of fiber lengths and diameters from a lung sample obtained from an anthophyllite worker exposed to fibers having a broad range of diameters and lengths. This sample had a high fiber burden and little or no clearance. Other samples from the same lung had much lower fiber burdens, indicating normal clearance. The panel on the right shows the difference or lung retention distribution (illustrations courtesy Dr. Vernon Timbrell).

tion, he developed a model for the retention of fibers as a function of length and diameter (Fig. 2—right panel). As shown in this panel, fiber retention rises rapidly with fiber lengths between 2 and 5 μm, and peaks at ~10 μm. Fiber retention also rises rapidly with fiber diameters between 0.15 and 0.3 μm, peaks at ~0.5 μm, and drops rapidly between 0.8 and 2 μm. The utility of the model was demonstrated by applying it to predict the lung retention of Cape crocidolite and Transvaal amosite workers on the basis of the measured length and diameter distributions of airborne fibers. The predicted lung distribution did, in fact, closely match those measured in lung samples from a Cape worker (Timbrell, 1984) and, as shown in Fig. 3, from a Transvaal worker (Timbrell, 1983). Thus, fibrosis is most closely related to the surface area of fibers with diameters between 0.15 and 2 μm, and lengths greater than ~2 μm. The work of King *et al.* (1946), showing that chrysotile with lengths = 2.5 μm produced interstitial fibrosis in rabbits following multiple intratracheal instillations, is consistent with the retention shown in Fig. 2 and a critical fiber length of ~2 μm.

Critical Fiber Parameters for Mesothelioma

A National Research Council study (NRC, 1984) summarized mortality data for

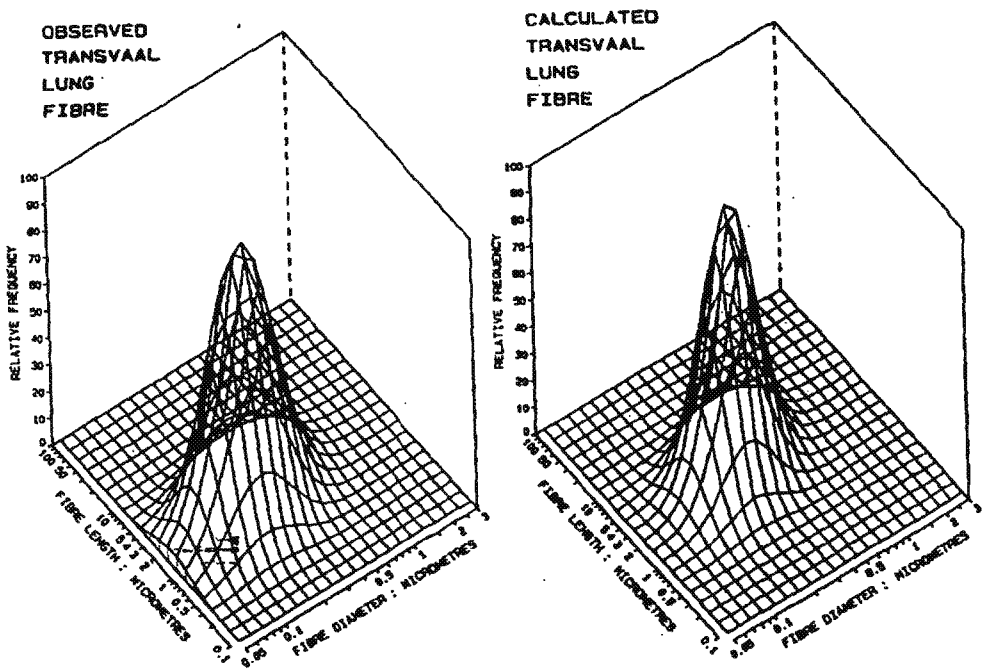

FIG. 3. Distributions of fiber lengths and diameters of amosite asbestos in the lungs of a Transvaal worker. The predicted distribution at the left is based on the lengths and diameters of the airborne fibers, and on the lung retention as a function of length and diameter from the right panel of Fig. 2. This corresponds closely to the distribution in the right panel, which was measured in samples from worker's lung (illustrations courtesy Dr. Vernon Timbrell).

mesothelioma and lung cancer in asbestos-exposed occupational cohorts. In 20 studies in which there was an excess in respiratory cancer and/or mesothelioma, the percentage of the excess which was due to mesothelioma varied from 0 to 100%, with a mean (±SD) of 38 ± 29%. The only study with 0% was that of Meurman *et al.* (1974, 1979), who reported 44 observed lung cancers (vs 22 expected) in a population of 1045 workers exposed to anthophyllite in Finland. By contrast, in several occupational cohorts the mesotheliomas accounted for more that 70% of the total. These included (1) the study of Newhouse *et al.* (1982) of 7474 British workers exposed to mixed asbestos among whom there were 8 mesotheliomas and only 3 more than the 140 expected lung cancers; (2) the study of Rossiter and Coles (1980) of 6076 British shipyard workers exposed to mixed asbestos among whom there were 31 mesotheliomas, and 13 fewer lung cancers than the expected number of 101; (3) the study of Jones *et al.* (1980) of 578 British female workers exposed to crocidolite among whom there were 17 mesotheliomas and 6 lung cancers more than the 6 expected; and (4) the study of Newhouse *et al.* (1982) of 3708 British female workers exposed to mixed asbestos among whom there were 2 mesotheliomas and 5 fewer lung cancers that the 11 expected.

Populations exposed to mineral fiber who are not occupationally exposed may have very high incidences of mesothelioma. The most extreme case is the study of Baris *et al.* (1987) of people living in four villages in Central Cappadocia in Turkey. Three villages (Karain, Sarihidir, and Tuzkoy) were exposed to erionite, a fibrous zeolite, while the fourth (Karlik) lacked this exposure and served as a control. There were 141 deaths during the study period in the four villages, including 33 mesotheliomas, 17 lung cancers, 1 cancer of the larynx, 8 cancers of other sites, and 13 cancers not specified. Thus, there were 72 cancers out of 141 deaths, with at least 33 of them due to mesothelioma. The age- and sex-specific mortality rates per 1000 persons-years from mesothelioma and respiratory cancer for the four villages were 20.2, 13.5, 5.2, and 0 for males from Karain, Sarihidir, Tuzkoy, and Karlik, respectively. The corresponding rates for females were 10.9, 3.9, 4.9, and 0. Sebastien *et al.* (1984) examined ferruginous bodies in the sputum of residents of Karain, Tuzkoy, and Karlik. They found that the content of ferruginous bodies increased with age in Karain and Tuzkoy, while only one of 19 specimens from Karlik had any.

Mesotheliomas among nonoccupationally exposed people living near crocidolite mining and milling regions in South Africa and Western Australia have been known to occur for some time (Wagner and Pooley, 1986). More recently, mesothelioma in nonoccupationally exposed residents of Cyprus has been attributed to tremolite by McConnochie *et al.* (1987).

Timbrell (1983) and Timbrell *et al.* (1987) and Harington (1981) have noted that animal inoculation experiments have been interpreted as suggesting a fairly high value of diameter, e.g., 1.5 μm (Stanton *et al.*, 1977), 1 μm (Pott *et al.*, 1976; WHO, 1986), and 0.25 μm (Wagner and Pooley, 1986), below which a fibrous material, so long as it is durable in lung fluids, can produce mesothelioma. In their view, these diameter limits are too high for human fiber-induced mesothelioma. If fibers with diameters >0.5 μm produced mesothelioma, then Paakkila, where the dust clouds contained on the order of 50 fibers/ml (PCOM) and a high proportion

of fibers in the 0.5- to 3-μm diameter range, should have produced many mesotheliomas as well as excesses in fibrosis and lung cancer. As noted earlier, an average of 38% of the excess lung cancer plus mesothelioma in working populations exposed to asbestos was expressed as mesothelima. Despite the very high exposures of the Paakkila population, no mesotheliomas were observed. Timbrell's (1983) examination of the size distributions and mesothelioma incidence at Paakkila and other asbestos mines world-wide led him to conclude that a good correlation was obtained if the threshold diameter was reduced to 0.1 μm. The mesotheliomas which Paakkila fiber has produced in animals were, most likely, due to the use of excessive doses, 10,000 times that observed in man. Paakkila asbestos contains only 1% of fibers with diameters below 0.1 μm, but with such a large dose this represents an enormous absolute number. Harington (1981) noted that the data for the northwest Cape in South Africa, where numerous mesotheliomas have been reported, and for the northeastern Transvaal, where mesotheliomas are rare, are consistent with a low fiber diameter limit. In the NW Cape, about 60% of the fibers have diameters <0.1 μm, while for the Transvaal, only about 1% have diameters < 0.1μm, comparable to Paakkila.

Timbrell (1983) also noted that the length distributions at Paakkila and the NW Cape point to a need to reduce the 10-μm threshold in Stanton's criteria. Paakkila had a high percentage of fibers longer than 10 μm, while the NW Cape had virtually none. And yet the NW Cape has been the major source of mesothelioma. Attributing potential carcinogenicity to shorter fibers by lowering the length threshold brings the estimated levels of significant fibers into closer line with the observed mesothelioma rates.

Combining the findings of Timbrell with the results of experiments reported by Davis *et al.* (1986b) leads to the conclusion that the critical fibers for mesothelioma induction have lengths between 5 and 10 μm. Davis *et al.* reported that intraperitoneal injections of short amosite (1.7% > 5 μm) produced only 1 mesothelioma among 24 rats (after 837 days), while UICC amosite (11% > 5 μm, 2.5% > 10 μm) produced 30 mesotheliomas among 32 rats, and long amosite (30% > 5 μm, 10% > 10 μm) produced 20 mesotheliomas among 21 rats. Thus, fibers shorter than 5 μm appear to be ineffective, while an appreciable fraction longer than 10 μm appears to be unnecessary.

Critical Fiber Parameters for Lung Cancer

Excess incidence of lung cancer has been reported for workers exposed to amphiboles (amosite, anthophyllite, and crocidolite), to chrysotile, and to mixtures of these fibers (NRC, 1984), but these studies have been uninformative with respect to the fiber parameters affecting the incidence. The series of rat inhalation studies performed by Davis *et al.* (1978, 1985, 1986, 1987), which have also produced lung cancers, have provided the most relevant evidence on the importance of fiber length on carcinogenicity in the lung.

The Wagner *et al.* (1974) study found that the yield of squamous cell carcinoma and adenocarcinoma was greatest with Rhodesian chrysotile, with decreasing yields for Canadian chrysotile, crocidolite, anthopyllite, and amosite, respective-

ly. As shown in Table 2, Davis *et al.* (1978) reported 2 squamous cell carcinomas, 6 adenocarcinomas, and 7 adenomas in 40 rats exposed to 10 mg/m^3 of respirable chrysotile. In 42 rats exposed to 2 mg/m^3 of chrysotile, there were 6 adenomas, 1 adenocarcinoma, and 1 squamous cell carcinoma. There were also adenomas in the groups exposed to amosite at 10 mg/m^3 (2) and to crocidolite (1 at 10 mg/m^3, 2 at 5 mg/m^3). Davis *et al.* (1978) attempted to examine the influence of fiber number concentration in relation to mass concentration in their inhalation studies. Their five exposure groups included three at the same respirable mass concentration of 10 mg/m^3, one each with chrysotile, crocidolite, and amosite. Of these, the amosite produced the lowest number concentration of fibers >5 μm in length. This fiber count was then matched with crocidolite (5 mg/m^3 respirable mass) and chrysotile (2 mg/m^3 respirable mass). In attempting to explain the greater fibrogenic and carcinogenic responses in the chrysotile-exposed animals than the crocidolite-or amosite-exposed groups, they suggested it might have been due, at least in part, to the greater number of >20-μm-long fibers in the chrysotile aerosol. The ratio of >20 to >5-μm-long fibers in the chrysotile was 0.185, compared to 0.040 for crocidolite, and 0.011 for amosite. The diameter distributions of all three types of asbestos was similar, with a median diameter of ~0.4 μm.

The importance of fiber length was further demonstrated by Davis *et al.* (1986b) in inhalation studies with amosite aerosols both shorter and longer than the UICC amosite studied earlier with the same protocols. Both aerosols had median diameters between 0.3 and 0.4 μm. The short fiber amosite (1.7% > 5 μm) produced no malignant cancers in 42 rats, while the long fiber amosite (30% > 5 μm, 10% > 10 μm) produced 3 adenocarcinomas, 4 squamous carcinomas and 1 undifferentiated carcinoma in 40 rats. In terms of adenomas, the frequency was 3/40, 2/43, 0/42, and 1/81 for the long, UICC, short, and control groups, respectively, Davis *et al.* (1985) also studied tremolite asbestos using the same protocols. Its length distribution was similar to those of the chrysotile in the 1978 study and the long amosite in the 1986 study (i.e., 28% > 5 μm, 7% > 10 μm), but its median diameter was lower, i.e., 0.25 μm. It produced 2 adenomas, 8 adenocarcinomas, and 8 squamous carcinomas in 39 rats. Davis (1987) reported on a study comparing the carcinogenic effects of "long" and "short" chrysotile at 10 mg/m^3. Unfortunately, the discrimination between "long" and "short" fibers was less successful than that achieved for amosite. The fiber counts (PCOM) for the fibers >10 μm in length for the "long" and "short" chrysotile were 1930 and 330 fibers/ml, whereas for the amosite they were 1110 and 12 fibers/ml respectively. Despite the much more rapid clearance of the chrysotile from the lungs, the tumor yields were higher. For the "long" fiber, there were 22 tumors for the chrysotile vs 13 for the amosite. For the "short" fiber, there were 7 vs 0. Davis (1987) concluded that fibers <5 μm in length may be innocuous, since the tumors produced by the "short" chrysotile are explicable by the presence of 330 fibers/ml longer that 10 μm.

In another recent study, Wagner *et al.* (1985) exposed rats by inhalation to 10 mg/m^3 of respirable dust composed of either UICC crocidolite (52.7% > 5 μm, 11.6% > 10 μm, median diameter of 0.30 μm) or Oregon erionite (44% > 5 μm,

7.4% > 10 μm, median diameter of 0.22 μm). The UICC crocidolite produced 1 squamous carcinoma in 28 rats (but no mesotheliomas), while the erionite produced no carcinomas in 28 rats, but did produce 27 mesotheliomas.

In summary, Table 2 shows that 10 mg/m^3 of short amosite (~0.1% > 10 μm), UICC amosite (~2.5% > 10 μm), UICC crocidolite (~3% > 10 μm), and Oregon erionite (7.4% > 10 μm) failed to produce malignant lung cancers, while 10 mg/m^3 of UICC chrysotile, long amosite, and tremolite (all with ≥ 10% > 10 μm) all produced malignant lung tumors. While there was no clear-cut influence of fiber diameter on tumor yield, the results suggest that carcinogenesis incidence increases with both fiber length and diameter. Since Timbrell (1983) has shown that fiber retention in the lungs peaks between 0.3 and 0.8 μm in diameter, it is likely that the thinner fibers, which are more readily translocated to the pleura and peritoneium, play relatively little role in lung carcinogenesis. Therefore, it appears that the risk of lung cancer is associated with long fibers, especially those with diameters between ~0.3 and 0.8 μm, and that substantial numbers of fibers >10 μm in length are needed.

One reason that short fibers may be less damaging could be the fact that they can be fully ingested by macrophages (Beck *et al.*, 1971), and can therefore be more rapidly cleared from the lung. The fibrogenic response to long fibers could result from the release of tissue digesting enzymes from alveolar macrophages whose membranes are pierced by the fibers they are attempting to engulf (Allison, 1977). The fibers may also cause direct physical injury to the alveolar membrane. A positive association between asbestosis and lung tumors has been demonstrated by Wagner *et al.* (1974). The induction of fibrosis would impair clearance of deposited fibers, increasing the persistence of fibers in the lung.

The preceding implies that short fibers will have a low order of toxicity within the lung, comparable to that of nonfibrous silicate minerals. Within this concept, the critical fiber length would most likely be on the order of the diameter of an alveolar macrophage, i.e., about 10 to 15 μm. This line of reasoning leads to the same conclusion reached on the basis of the incidence of lung cancer in rats exposed to fibrous aerosols, i.e., that the hazard is related to the number of fibers longer than ~10 μm deposited and retained in the lungs. The Timbrell (1983) model predicts alveolar retention of deposited fibers approaching 100% for 10-μm-long fibers in the 0.3- to 0.8-μm-diameter range. Airborne fibers longer than ~100 μm may be much less harzardous than those in the 10- to 100-μm range because they do not penetrate deeply into the airways as interception increases with fiber length.

DISCUSSION

The various hazards associated with the inhalation of mineral fibers, i.e., asbestosis, mesothelioma, and lung cancer, are all associated with fibers with lengths which exceed critical values. However, it now appears that the critical length is different for each disease, i.e., 2 μm for asbestosis, 5 μm for mesothelioma, and 10 μm for lung cancer. There are also different critical values of fiber diameter for the different diseases. For asbestosis and lung cancer, which are related to fibers retained in the lungs, only fibers with diameters >0.15 μm need

to be considered. On the other hand, for mesothelioma which is initiated by fibers which migrate from the lungs to the pleura and peritoneum, the hazard has been related to fibers with diameters <0.1 μm. (See Table 3.)

While all durable fibers of sufficient length can produce fibrosis and cancer, as documented in various animal studies, it appears that factors other than fiber size can influence the extent of the response. For example, inhaled erionite appears to be much more potent for mesothelioma in both humans and animals because of its greater ability to penetrate the pleural surface. On the other hand, the animal and human data appear to differ on the ability of inhaled chrysotile to induce mesothelioma. Animal data indicate that chrysotile produces as much or more mesothelioma than the amphiboles, while human data more often implicate amphiboles, even when the predominant exposures are to chrysotile.

Examination of the fiber content of the lungs of asbestos workers and animals exposed by inhalation shows that chrysotile is cleared much more rapidly than the amphiboles. It breaks down within the lungs both by disaggregation into fibrils, and by dissolution. The differences between the responses in animals and humans may be in relative persistence, i.e., time of persistence of the long fibers in the lung relative to the time interval between exposure and the expression of the disease. In other words, the long fibers may be retained in the lung for a longer fraction of the lifespan in the rat.

While all durable fibers in the right size range can cause the asbestos-related diseases, they may have different potencies and need different concentration limits, in the manner of the ACGIH TLVs. The remainder of this discussion addresses the indices of exposure, but not the concentration limits for the fibers which fall within the indices. The concentration limits warrant separate and further discussion.

Exposure Indices

While the current occupational exposure index based on phase-contrast optical measurement of fibers with an aspect ratio >3 and a length >5 μm was a reasonable choice when it was made, it is now apparent that it cannot provide an adequate index for any of the several asbestos hazards.

A better index of the asbestosis hazard for the amphiboles is the total surface of fibers with diameters between 0.15 and 2 μm, and lengths greater than about 2 μm. This cannot be determined by optical microscopy, and electron microscopy

TABLE 3
SUMMARY OF RECOMMENDATIONS ON ASBESTOS EXPOSURE INDICES

Disease	Relevant exposure index
Asbestosis	Surface area of fibers with: Length >2 μm; diameter >0.15 μm
Mesothelioma	Number of fibers with: Length >5 μm; diameter <0.1 μm
Lung cancer	Number of fibers with: Length >10 μm; diameter >0.15 μm

is impractical for routine exposure assessments. The only practical surface analysis method currently available for membrane filter samples is MALS (Timbrell, 1982).

The applicability of this new index of asbestosis hazard for chrysotile is less well established than for the amphiboles. The long amphibole fibers clear very slowly from the lungs and do not dissolve, so the ratio of inhaled fiber surface area to lung retained fiber surface area remains relatively constant. The chrysotile fibers dissolve and clear more rapidly, reducing the ratio of retained fiber surface to airborne surface. On the other hand, the fibers split longitudinally within the lung, increasing the surface area of retained fibers. Another factor contributing to the uncertainty of the applicability of this index for chrysotile is that the calibration of MALS for chrysotile is less well established than for the amphiboles. These issues need to be addressed in further experimental studies.

A better index of mesothelioma hazard is the number of fibers longer than 5 μm and thinner than 0.1 μm. Since fibers with diameters less than 0.1 μm cannot be resolved by optical microscopy, analyses of relevant fiber counts must be done by electron microscopy or MALS. The differences in fiber retention between chrysotile and the amphiboles may necessitate different concentration limits for the different fiber types.

A tentative proposal for a better index of lung cancer hazard is the number of fibers longer than 10 μm which are retained within the lungs. Lung retention rises rapidly for diameters greater that about 0.15 μm. Thus, the relevant fibers have diameters >0.15 μm and lengths >10 μm. The current phase-contrast optical method of analysis of membrane filter samples is recommended for fibers with diameters between 0.25 and 3 μm (WHO, 1986). Since lung retention of fibers with diameters between 0.15 and 0.25 μm is relatively low (Fig. 2), PCOM analysis may provide an adequate index of hazard if the length limit is adjusted to 10 μm. Alternatively, analyses can be done by scanning electron microscope or MALS. Once again, the differences in fiber retention between chrysotile and the amphiboles may necessitate different concentration limits for the different fiber types.

Even if there were convenient and economical methods of sampling and analysis available for each of the three different asbestos hazards, it would undoubtedly be impractical to make three different kinds of exposure assessments at each potential exposure of concern. One option is to use the MALS analysis method, since the fiber size distributions data it generates can be used to determine quantitative values for each of the three separate indices. Another option is to do a limited amount of detailed analyses of fiber size distribution initially by electron microscopy to determine if one or more of the potential hazards can be considered to be de minimus. For example, if there is negligable potential for exposure to fibers longer than 5 μm, there would be virtually no risk of either mesothelioma or lung cancer. For most nonoccupational exposures, there is virtually no risk of asbestosis, since prolonged exposure to high dust concentrations are needed to produce evidence of this disease.

If there were appreciable concentrations of fibers >5 μm in length, but with essentially all having fiber diameters larger than ~0.1 μm, there would be virtually

no risk for mesothelioma. In this case, subsequent sampling could focus on the risk for lung cancer and asbestosis.

In essence, a rational preliminary examination would determine whether there was a controlling hazard at each potential asbestos exposure of concern, i.e., whether the fiber size distribution made it likely that the risk for asbestosis, mesothelioma, or lung cancer would dominate the others for that kind of exposure. If there was a dominant hazard, then only one kind of sampling and analysis protocol would be needed to demonstrate that the risk for all three hazards was under control. If two or more of the risks were approximately of equal concern, then monitoring should be done for each.

ACKNOWLEDGMENTS

This study was supported by Grant ES 00881 from the National Institute of Environmental Health Sciences (NIEHS). It was performed as part of a Center Program supported by NIEHS (Grant ES 00260) and the National Cancer Institute (Grant CA 13343). Special acknowledgement is due to Dr. Vernon Timbrell for providing the illustrations and his review of the manuscript. Review comments from Dr. Arthur C. Upton and Dr. David R. Hemenway are also gratefully acknowledged.

REFERENCES

ACGIH-AIHA Aerosol Hazards Evaluation Committee (1975). Recommended procedures for sampling and counting asbestos fibers. *Amer. Ind. Hyg. Assoc. J.* 36, 83–90.

Allison, A. C. (1977). Mechanisms of macrophage damage in relation to the pathogenesis of some lung diseases. *In* "Respiratory Defense Mechanisms" (J. D. Brain, D. F. Proctor and L. M. Reid, Eds.), Part II, pp. 1075–1102. Dekker, New York.

Baris, I., Simonato, L., Artvinli, M., Pooley, F., Saracci, R., Skidmore, J., and Wagner, C. (1987). Epidemiological and environmental evidence of the health effects of exposure to erionite fibers: A four-year study in the Cappadocian region of Turkey. *Int. J. Cancer* 39, 10–17.

Beck, E. G., Bruch, J., Friedricks, K. H., Hilscher, W., and Pott, F. (1971). Fibrous silicates in animal experiments and cell-culture in morphological cell and tissue reactions according to different physical chemical influences. *In* "Inhaled Particles III" (W. H. Walton, Ed.), pp. 477–486. Unwin Bros., Old Woking.

Bellmann, B., König, H., Muhle, H., and Pott, F. (1986). Chemical durability of asbestos and of man-made mineral fibres *in vivo*. *J. Aerosol Sci.* 17, 341–345.

Brody, A. R., Hill, L. H., Adkins, B., Jr., and O'Connor, R. W. (1981). Chrysotile asbestos inhalation in rats: Deposition pattern and reaction of alveolar epithelium and pulmonary macrophages. *Amer. Rev. Respir. Dis.* 123, 670–679.

Churg, A., Wiggs, B., Depaoli, L., Kampe, B., and Stevens, B. (1984). Lung asbestos content in chrysotile workers with mesothelioma. *Amer. Rev. Resp. Dis.* 130, 1042–1045.

Davis, J. M. G. (1976). Pathological aspects of the injection of fiber into the pleural and peritoneal cavities of rats and mice. *In* "Occupational Exposure to Fibrous Glass," pp. 141–199. HEW Publ. No. (NIOSH) 76–151.

Davis, J. M. G., Beckett, S. T., Bolton, R. E., Collings, P., and Middleton, A. P. (1978). Mass and number of fibers in the pathogenesis of asbestos-related lung disease in rats. *Brit. J. Cancer* 37, 673–688.

Davis, J. M. G., Addison, J., Bolton, R. E., Donaldson, K., Jones, A. D., and Miller, B. G. (1985). Inhalation studies on the effects of tremolite and brucite dust in rats. *Carcinogenesis* 6, 667–674.

Davis, J. M. G., Gylseth, B., and Morgan, A. (1986a). Assessment of mineral fibers from human lung tissue. *Thorax* 41, 167–175.

Davis, J. M. G., Addison, J., Bolton, R. E., Donaldson, K., Jones, A. D., and Smith, T. (1986b). The pathogenicity of long versus short fiber samples of amosite asbestos administered to rats by inhalation and intraperitoneal injection. *Brit. J. Exp. Pathol.* 67, 415–430.

Davis, J. M. G. (1987). Experimental data relating to the importance of fibre type, size, deposition, dissolution and migration. *In* "Proceedings of 1987 Mineral Fiber Symposium, Lyon, France, Sept. 1987."

Ferin, J., and Leach, L. J. (1976). The effect of amosite and chrysotile asbestos on the clearance of TiO_2 particles from the lung. *Environ. Res.* 12, 250–254.

Gylseth, B., Mowe, B., and Wannag, A. (1983). Fiber type and concentration in the lungs of workers in an asbestos cement factory. *Brit. J. Ind. Med.* 40, 375–379.

Harington, J. S. (1981). Fiber carcinogenesis: Epidemiologic observations and the Stanton hypothesis. *J. Natl. Cancer Inst.* 67, 977–989.

Hiett, D. M. (1978). Experimental asbestosis: An investigation of functional and pathological disturbances. *Brit. J. Ind. Med.* 35, 129–145.

Holt, P. F., Mills, J., and Young, K. K. (1965). Experimental asbestosis with four types of fibers. *Ann. N.Y. Acad. Sci.* 132, 87–97.

Johnson, N. F. (1987). Asbestos-induced changes in rat lung parenchyma. *J. Toxicol. Environ. Health* 21, 193–203.

Jones, J. S. P., Pooley, F. D., Sawle, G. W., Madeley, R. J., Smith, P. G., Berry, G., Wignall, B. K., and Aggarwal, A. (1980). The consequences of exposure to asbestos dust in a wartime gas-mask factory. *In* "Biological Effects of Mineral Fibres," (J. C. Wagner, Ed.), Vol. 2, pp. 637–653. IARC Scientific Publ. No. 30. International Agency for Research on Cancer, Lyon.

King, E. J., Clegg, J. W., and Rae, V. M. (1946). The effect of asbestos, and of asbestos and aluminum, on the lung of rabbits. *Thorax* 1, 188–197.

McConnochie, K., Simonato, L., Mavrides, P., Christofides, P., Pooley, F. D., and Wagner, J. C. (1987). Mesothelioma in Cyprus: The role of tremolite. *Thorax* 42, 342–347.

Meurman, L. O., Kiviluoto, R., and Hakama, M.(1974). Mortality and morbidity among the working population of anthophyllite asbestos miners in Finland. *Brit. J. Ind. Med.* 31, 105–112.

Meurman, L. O., Kiviluoto, R., and Hakama, M. (1979). Combined effects of asbestos exposure and tobacco smoking on Finnish anthophyllite miners and millers. *Ann. N.Y. Acad. Sci.* 330, 491–495.

Newhouse, M. L., Berry, G., and Skidmore, J. W. (1982). A mortality study of workers manufacturing friction materials with chrysotile asbestos. *Ann. Occup. Hyg.* 26, 899–909.

NRC (1984). "Asbesiform Fibers—Nonoccupational Health Risks." National Academy Press, Washington.

Pinkerton, K. E., Plopper, C. G., Mercer, R. R., Roggli, V. L., Patra, A. L., Brody, A. R. and Crapo, J. D. (1986). Airway branching patterns influence asbestos fiber location and the extent of tissue injury in the pulmonary parenchyma. *Lab. Invest.* 55, 688–695.

Pooley, F. D. (1976). An examination of the fibrous mineral content of asbestos lung tissue from the Canadian chrysotile mining industry. *Environ. Res.* 12, 281–298.

Pott, F., Friedrichs, K. H., and Huth, F. (1976). Results of animal experiments concerning the carcinogenic effect of fibrous dusts and their penetration with regard to the carcinogenesis in humans. *Zbl. Bakt. Hyg.* 162, 467–505.

Pott, F., Huth, F., and Friedrichs, K. H. (1976). Results of animal carcinogenesis studies after application of fibrous glass and their implications regarding human exposure. *In* "Occupational Exposure to Fibrous Glass," pp. 183–191. HEW Publ. No. (NIOSH) 76–151.

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

Roggli, V. L., George, M. H., and Brody, A. R. (1987). Clearance and dimensional changes of crocidolite asbestos fibers isolated from lungs of rats following short-term exposure. *Environ. Res.* 42, 94–105.

Rossiter, C. F., and Coles, R. M. (1980). H.M. Dockyard, Devonport: 1947 mortality study. "Biological Effects of Mineral Fibres" (J. C. Wagner, Ed.), pp.713–721. IARC Scientific Publ. No. 30. International Agency for Research on Cancer, Lyon.

Sebastien, P., Bignon, J., Baris, Y. I., Awad, L., and Petit, G. (1984). Ferruginous bodies in sputum as an indication of exposure to airborne mineral fibers in the mesothelioma villages of Cappadocia. *Arch. Environ. Health* 39, 18–23.

Selikoff, I. J., Nicholson, W. J., and Langer, A. M. (1972). Asbestos air pollution. *Arch. Environ. Health* 25, 1–13.

Stanton, M. F., Layard, M., Tegeris, A., Miller, E., May, M., and Kent, E. (1977). Carcinogenicity of fibrous glass: Pleural response in the rat in relation to fiber dimension. *J. Natl. Cancer Inst.* 58, 587–603.

Stanton, M. F., and Wrench, C. (1972). Mechanisms of mesothelioma induction with asbestos and fibrous glass. *J. Natl. Cancer Inst.* 48, 797–821.

Stöber, W., Flachsbart, H., and Hochrainer, D. (1970). Der aerodynamische Durchmesser von Latexaggregaten und Asbestfasern. *Staub Reinhalt. Luft* 30, 277–285.

Timbrell, V. (1972). An aerosol spectrometer and its applications. *In* "Assessment of Airborne Particles" (T. T. Mercer, P. E. Morrow and W. Stober, Eds.), pp. 290–330. Thomas, Springfield, IL.

Timbrell, V. (1982). Deposition and retention of fibers in the human lung. *Ann. Occup. Hyg.* 26, 347–369.

Timbrell, V.(1983). Fibers and carcinogenesis. *J. Occup. Health Soc. of Australia* 3, 3–12.

Timbrell, V. (1984). Pulmonary deposition and retention of South African amphibole fibers: Identification of asbestosis-related measure of fiber concentration. *In* "Proceedings of VIth International Pneumoconiosis Conference, Bochum, 1983," Vol. 2, pp. 998–1008. ILO.

Timbrell, V., Ashcroft, T., Goldstein, B., Heyworth, F., Meurman, L. O., Rendall, R. E. G., Reynolds, J. A., Shilkin, K. B., and Whitaker, D. (1987). Relationships between retained amphibole fibers and fibrosis in human lung tissue specimens. *In* "Inhaled Particles VI" (W. H. Walton, Ed.). Pergamon, Oxford.

Vincent, J. H. (1985). On the practical significance of electrostatic lung deposition of isometric and fibrous aerosols. *J. Aerosol Sci.* 16, 511–519.

Wagner, J. C. (1963). Asbestos in experimental animals. *Brit. J. Ind. Med.* 20, 1–12.

Wagner, J. C., Berry, G., and Skidmore, J. W. (1976). Studies of the carcinogenic effects of fiber glass of different diameters following intrapleural innoculation in experimental animals. *In* "Occupational Exposure to Fibrous Glass," pp. 193–197. HEW Publ. No. (NIOSH) 76-151.

Wagner, J. C., Berry, G., Skidmore, J. W., and Timbrell, V. (1974). The effects of the inhalation of asbestos in rats. *Brit. J. Cancer* 29, 252–269.

Wagner, J. C., and Pooley, F. D. (1986). Mineral fibers and mesothelioma. *Thorax* 41, 161–166.

Wagner, J. C., Skidmore, J. W., Hill, R. J., and Griffiths, D. M. (1985). Erionite exposure and mesotheliomas in rats. *Brit. J. Cancer* 51, 727–730.

WHO (1986). "Asbestos and Other Natural Mineral Fibers." Environmental Health Criteria 53, World Health Organization, Geneva.

Wright, G. W., and Kuschner, M. (1977). The influence of varying lengths of glass and asbestos fibers on tissue response in guinea pigs. *In* "Inhaled Particles IV" (W. H. Walton, Ed.), Part 2, pp. 455–472. Pergamon, New York.