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UNION CARBIDE

Calidria ASBESTOS

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July 13, 1976

Mr. R. H. Mereness
Executive Director
Asbestos Information Association/NA
Suite 402
1835 K Street, N.W.
Washington, D.C. 20006

Dear Bob:

Here's a copy of the latest paper out of Mt. Sinai re the biological effects of short fibers. It is suggested that it may be desirable to pass it on to Hans Weill for his comments. Although a specific request was not made to keep it confidential, it is a prepublication copy. It is probably not appropriate to give it general distribution at this time. A copy has also been sent to Ed Fenner for Dr. Kotin.

Very truly yours,

Harry

Harrison B. Rhodes
Technology Manager

HBR:dal
Enclosures

CC: Messrs. E. M. Fenner
J. L. Myers
W. C. Thurber

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MOUNT SINAI SCHOOL OF MEDICINE
of The City University of New York
FIFTH AVENUE AND 100TH STREET • NEW YORK N.Y. 10029

Department of Community Medicine

June 7, 1976

Dr. H.B. Rhodes
Area Manager, Marketing & Technology
Union Carbide Corporation
Mining & Metals Division
P.O. Box 579
Niagara Falls, N. Y. 14302

Dear Dr. Rhodes:

Enclosed is a prepublication copy of the paper describing our work using Calidria RG 144. The manuscript has been submitted for publication.

If you wish any further details on this work, please call me.

Yours truly,

Mary S. Wolff

Mary S. Wolff, Ph.D.
Environmental Sciences Laboratory

MSW:sl
Enc.

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Variation of Some Physical, Chemical and Biological Properties of Chrysotile Asbestos Subjected to Prolonged Milling*

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* The authors wish to acknowledge support under a grant from the National Institute of Environmental Health Sciences, ES 00028. One of us (MSW) wishes to acknowledge support under a post-doctoral fellowship from the NIEHS, ES 02565. The capable technical assistance of A. Tordini and T. Samo is gratefully acknowledged.

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Early standard for asbestos exposure in the United States

The first asbestos standard in the United States set a maximum allowable concentration of total dust particles in an environment where asbestos-containing materials were being handled or processed (Dreessen et al. 1938). Exposure levels were defined as total dust concentration in millions of particles per cubic foot of air (mppcf). On the basis of their study of asbestos textile mills, the authors suggested that concentrations of 5 mppcf were low enough to prevent asbestosis. In retrospect, the considerations used to justify the standard were extremely limited (Schall, 1965). Most of the workers examined in the 1938 study had been exposed to asbestos dust for less than ten years. Subsequent studies (e.g., Selikoff et al. 1965) established that occurrence of asbestosis usually follows a long clinical latent period, 20 years or more between onset of fiber exposure and appearance of radiological evidence of scarring. Also, questionable measurement techniques were used: impinger particle collection and optical microscopic analysis of dusts. Impinger collection was later found to be an incompletely efficient method (Holt, 1967), and because all particles (asbestos and other) were counted, the asbestos fiber concentration remained largely undetermined. Further, at that time the standard was directed only toward prevention of lung scarring, since neoplastic dangers of asbestos exposure had not yet been recognized.

The substitution of fiber count for total dust count came about in the United States through several later studies by the U.S. Public Health Service (Ayer et al. 1965; Lynch et al. 1970). In these studies, fiber

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counts* were compared with total dust particles in industries where processing, machinery and raw materials had not changed since 1938. Accordingly, in the mid 1960's, asbestos standards were "reset" to reflect the fact that some 10% of the total dust consisted of fibers. Thus, 5 mppcf corresponded roughly to 12f/ml. Still inherent in the new standard were major difficulties; neoplastic risk was not recognized and inadequate analytical methods continued to be used.

Present standard for asbestos exposure in the United States

The current United States federal standard for exposure to asbestos dust became effective on July 7, 1972 (Federal Register). This document established: atmospheric fiber concentration limits for an 8-hour time weighted average (per work day) and for short period maximum excursions (ceiling concentrations), expressed in f/ml; a uniform air sampling method and analytical technique for analysis; a defined explanation of particles to be enumerated (fibers 5μm -100μm in length).

The analytical method retained certain limitations e.g., only a portion of the workers exposure was characterized by the method -- those fibers longer than 5μm. The selection of fibers greater than 5μm in length was based upon methodology evaluation by the U.S. Public Health Service during a study of asbestos environments in the asbestos textile industries in the United States (Lynch et al. 1970). They were indebted to a British study by Addingly (1966), in which the 5μm fiber was selected as the lower limit for counting, mostly on a utilitarian basis and not because

*Fibers defined as objects with length-to-width ratio of at least 3:1.

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of biological considerations: the counting procedure, by optical microscopy was simplified, and the interobserver error for counting was reduced. Moreover, some existing theory of pathogenesis of asbestosis suggested that disintegration of large asbestos bodies, those necessarily nucleated on long fibers, were responsible for the process of collagen formation in asbestosis. Moreover, a variety of asbestos textile processes was assumed to produce dusts with similar size distribution characteristics, having a small concentration of fibers less than 5μm in length. Again, several important problems were sidestepped in promulgation of the 1972 standard. Disease considered was lung scarring, not malignant neoplasms; an unsubstantiated hypothesis that ("long" fibers are those most biologically active) was used to restrict fiber length enumeration; and a severe limitation of counting, by optical microscopy, was accepted.

Biological importance of small fibers

The study of small fibers and their biological activity is important for several reasons. In most of commercially available asbestos raw materials, small fibers constitute the numerical preponderance in all samples examined (Timbrell et al. 1970). Such small fibers tend to form stable aerosols with greater potential for inhalation. These small particles can penetrate beyond the terminal bronchioles and come to rest in alveolar spaces through impaction anddiffusion mechanisms (Timbrell, 1973). Although small in size, their relatively larger surface area provides greater particle-tissue interface. In addition, the number of such potential interfaces per cell, per organ, etc., is far greater than in materials with fibers of larger in size but fewer in number.

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From these theoretical considerations, a number of investigators, using both in vivo and in vitro test systems, have studied the biological effects of small asbestos fibers.

Animal studies using small fibers obtained from air-elutriated floats

During industrial processing of asbestos large fiber bundles are "opened" and comminuted. Very fine fibers ("superfine") tend not to settle and are collected in air-cleaning filters in a bag house. These "floats", i.e. fine asbestos fibers which retain fibrous integrity, have been used to investigate the biological activity of short asbestos fibers.

Wagner (1968) obtained a No. 7 (superfine) Canadian chrysotile fiber which he further fractionated by water sedimentation to free chrysotile from associated rock fragments. The final material contained fiber of which 92% was shorter than 6μm in length. Following intrapleural injection into Wistar rats, mesotheliomas developed in 61 of 96 animals, significantly more than observed for a similar group of rats exposed to crocidolite asbestos. In the same year, McIver reported using Canadian chrysotile floats for inhalation experiments with dogs. These materials produced marked epithelial hyperplasia, bronchial squamous cell metaplasia, progressive diffuse fibrosis, and two malignant neoplasms in 80 dogs exposed. In 1970 Wagner et al. again used a superfine Canadian chrysotile to induce mesotheliomas in Wistar rats. As before, the tumors appeared sooner and more frequently than in animals similarly exposed to crocidolite. In the same year, Timbrell et al. suggested that more mesotheliomas were produced in a specific geographical area of South Africa because of

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the finer characteristics of the dust, which produced a more stable aerosol and therefore greater dose-exposure to workmen. Wagner reiterated his belief that smaller asbestos fibers produced more mesotheliomas (Wagner, 1973; Wagner et al. 1973). These reports demonstrated that extremely short commercial asbestos fiber, undamaged during processing, induced significant disease in animals.

Studies with small fibers prepared by laboratory manipulation

Most workers studying the biological activity of small asbestos fibers have themselves prepared small fibers in the laboratory by mechanical methods which differed in severity of manipulation. Other variables in these studies include animal model used (species) and route of administration.

Exposure to fibers comminuted by moderate methods

King et al. (1946) injected Rhodesian chrysotile fibers of different lengths intratracheally into rabbits. The small fibers were produced by microtoming (physically cutting) the chrysotile into appropriate lengths. Marked fibrosis was produced with 15μm long fibers and interstitial fibrosis was also produced with 2.5μm long fibers, under identical experimental conditions.

Holt et al. (1964) exposed rats by inhalation methods to Rhodesian chrysotile consisting of fibers nearly all less than 3μm in length. Fiber comminution was accomplished by centripetal hammer. No typical asbestos bodies were observed in the animals' lungs although progressive fibrosis was noted in all cases.

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The inhalation of similarly prepared Rhodesian chrysotile by guinea pigs was reported by Holt et al. in 1965 and 1966. For every asbestos body observed in the animal lung tissues, a vast number of small particles which were uncoated "packed" the cytoplasm of macrophages. Most of these fibers, too small to be seen by optical microscopy, were associated with diffuse fibrosis. They concluded that "very small dust particles are at least as lethal as long fibers". Short-term dusting (3-days) produced scarring, as did low concentrations of fibers (1966). Several years later, Gross et al. (1968), observed neoplasms in rats exposed to short fiber (centripetal hammer) by inhalation. Tumors developed in 31% of the animals, including adenocarcinoma, fibrosarcomas, squamous cell carcinomas, mesotheliomas, and a number of other tumors. Multifocal asbestosis was generally observed. Since the short chrysotile fiber was produced by milling, the author suggested that the malignant neoplasms arose from trace metal contamination produced by wearing of the mechanical mill. Several years later, both Wagner (1973) and Stanton (1973) produced evidence to refute this trace metal theory, and it has been abandoned (Gross and Harley, 1973).

Exposure to fibers prepared by severe methods (ball-milling)

Hilscher et al. (1970) observed that asbestos fiber, including chrysotile, milled for periods ranging from 48 hours to 16 weeks, produced no effect in laboratory animals. However, chrysotile fiber which was finely cut by microtoming, produced measurable fibrosis in animals. Pott et al. (1972) prepared chrysotile by manual grinding in an agate-mill, and in the material produced, 99% of the fibers were shorter than 3μm.

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Physical characterization of these materials suggested that the structure was not destroyed. Although the milled material required a longer latent period for tumor development, approximately 40% of the animals, in both long and short fiber groups, developed malignant tumors. These workers concluded that short fiber should not be considered unimportant in terms of carcinogenesis. Pott and Friedrich in 1972 again used agate-milled chrysotile. With unmilled fiber, fibrotic response was dose-related, but tumor development was about 45% for doses of 6, 25 and 100mg. Milled chrysotile (100mg) produced tumors in 13% of the rats, but no fibrosis. Latent period for fibrosis was not indicated in this report.

A number of workers have noted the possibility of structural degradation of chrysotile asbestos induced by prolonged grinding. Klosterkotter observed in 1968 that chrysotile milled 100 hours, which produced no fibrosis, appeared to be amorphous when viewed by electron microscopy. Davis (1972), who intrapleurally inoculated mice with milled chrysotile, observed fewer significant granulomatous lesions than with the long fiber. Stanton (1972) and Stanton et al. (1972) observed that ball-milled asbestos fibers were less carcinogenic than the unmilled larger samples.

Extensive studies by Vorwald et al. (1951) utilized inhalation, intratracheal, and intraperitoneal routes of administration to expose guinea pigs and five other animal species to a number of chrysotile preparations. A "long", a "short" and a "milled" fiber were tested. The long fiber consisted of 60% chrysotile whereas the milled fiber was crushed rock containing only 17% chrysotile. The occurrence of marked asbestosis

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as defined by peribronchiolar scar tissue, occurred only with long fiber. However, following inhalation, the short fiber induced slight to moderate fibrosis in guinea pigs after 16 months of continuous exposure and "fibrosis typical of asbestosis" was observed in animals allowed to recover after exposure for 12 months or more. These authors, while investigating the hypothesis that biological activity was inversely related to particle size, stated that "this again indicates that the biological activity of asbestos inhaled into the lung is not increased by a reduction of size of the fibers". However, peribronchiolar fibrosis, as we now recognize, may be related to long fibers, because they can accumulate in this anatomical constriction. Short fibers carry beyond the bronchioles and tend to produce the more classical septal fibrosis commonly observed in human asbestosis.

Most recently, Reeves et al. (1974) used 100 hour hammer-milled chrysotile for inhalation experiments with rabbits, rats, mice, gerbils and guinea pigs. Little or no fibrosis was observed, with a very low incidence of neoplasm (one lung cancer and one mesothelial tumor among only 69 rats); both responses were less marked than those with amosite and crocidolite. The authors attributed the reduced fibrotic and tumorigenic response to "destruction of the fibrous geometry of chrysotile". Indeed, their EM and x-ray data indicated greatly reduced crystallinity.

While several recent papers have concluded that long fibers produce more biological response than do short ones, there appear to be several possible interpretations of the data. For example, Stanton (1973)

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compared the size distribution of fiber samples with mesothelioma yield in animals. He listed several groups of materials on the basis of their mesothelioma production: greater than 50%; 40-25%; 0%. From the size distribution data, he concluded that long chrysotile fiber produced more mesotheliomas than short chrysotile fibers. However, for the fibers producing more than 50% mesothelioma, the data indicate that 27% were less than 5μm in length and only 15% greater than 40μm in length; in the 40-25% mesothelioma group, 8% of the fiber was less than 5μm in length and 85% of the fiber was greater than 40μm in length; in the group with no mesothelioma, 9% of the fibers were less than 5μm in length, and no fibers were greater than 40μm in length. Thus, the data can also suggest that perhaps the smaller fiber population (less than 5μm in length) may be responsible for the induction of mesotheliomas. Furthermore, the particles were counted by optical microscopy at 1000X, limiting resolution to about 1μm. Fibers smaller than 1μm were thus omitted.

A number of other reports, at about the same period suggested biological activity of small fibers. Suzuki and Churg (1969; 1972) reported intracellular response to chrysotile fibers significantly less than 1μm in length. A number of cells, in the hamster animal model, were observed to phagocytize and react with chrysotile fibrils. Even objects as small as several hundred angstrom units in width were observed to induce ferritin metabolism and formation of asbestos bodies. At the same time, Davis (1970) suggested that fibers greater than 5μm in length induced the formation of asbestos bodies through giant-cell mechanisms. However,

he remarked that most fibers remain uncoated and that the asbestos body may represent only one in one thousand such inhaled objects.

Bey and Harington (1971) utilizing harvested peritoneal macrophages from hamsters observed that ground UICC chrysotile produced cytotoxic effects in this cell line. No marked differences were observed in this shorter fiber population. Similarly, Davis (1964) reported the formation of asbestos bodies in animals in reaction to short chrysotile fiber. He also reported the survival of disproportionately more uncoated fibers than "asbestos bodies".

Conclusions concerning literature review

In a number of studies, short asbestos fiber has produced a variety of biological effects, in a number of animal species, with a range of routes of administration. Effects range from fibrosis to malignant neoplasms. However, many studies have failed to elicit biological response with small fibers. In most such cases, vigorous mechanical size reduction methods were used to produce short fiber suggesting the possibility that manipulation of the fibers during laboratory preparation may have so altered them as to change their character and biological potential.

We have begun a study of small fibers, which includes size reduction processing similar to that which produced "inactive" small fibers. These materials have been examined by a number of analytical techniques to determine whether or not alteration of innate surface or structural properties has taken place. In addition to this, in vitro test systems have

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been used to deduce any change in biological activity.

Materials used in study and their preparation

Chrysotile asbestos occurs naturally as compressed mats of short fibers, with a large proportion of accompanying fibrils, in several major serpentine deposits in the western United States. These deposits have been referred to in the mineralogical literature as the Coalinga-type. Samples were obtained from the New Idria, California deposit through the courtesy of the Union Carbide Corporation. This material, referred to as Calidria RG-144, was used for experimental purposes.

Impact-milling was accomplished using a Wig-L-bug amalgamator*, consisting of a 2.5cm length, 1cm diameter, steel cylinder, which could be attached to a vibrator operating at about 10 cycles per second. Portions of chrysotile (50mg) were placed in the cylinder chamber with a 10x13mm pellet and milled for time periods ranging from 60 to 3,600 seconds. These materials were then examined for change in crystal structure, surface chemistry and change in chemical-biological behavior as a function of duration of ball-milling.

A. Transmission microscopy (TEM) and selected area electron diffraction (SAED)

Aliquots ($\leq$1mg) of the material, as received, and of the milled samples, were dispersed in 0.1ml nitrocellulose (1% in amyl acetate) on a precleaned glass slide by gentle stirring. A second slide was placed over the first and the two slides pulled apart, producing a film on each. The film was floated on water, and carbon-coated, formvar-coated grids

*trade mark

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were placed on the film. The grids were lifted out with filter paper and dried on filter paper in a petri dish. They were examined by transmission electron microscope (JEOL JEM-120U) at 120kv. Photographs were obtained at direct magnifications ranging from 2,800 to 20,000 directly on the TEM screen. All counting, for size distribution characterization, was done directly on photographs with the aid of a Porton graticule. Two observers participated in this process, both of whom alternated counting and size recording every one-hundred counts. Some 1,000 objects were counted for each preparation. Interobserver measurements were made consistent by establishing counting criteria: a particle composed of at least two fibrils, the shorter fibril being contiguous with and comprising at least one-half the length of the first, was called a fiber; objects counted as fibers possessed a length:width aspect ratio of at least 3:1; objects with smaller aspect ratios, composed of many fibrils, were counted as clumps; single fibrils, of any aspect ratio, were called fibrils.

Progressive grinding of chrysotile produced marked reduction in both fiber and fibril length (Fig. 1; Table 1). In the untreated chrysotile, 77% of the fibrils were less than 0.5μm in length. In comparison, in the 3,600 second milled sample, virtually the entire fibril population was less than 0.5μm. Chrysotile fibers comprised 21% of the population in the untreated material, and were reduced to only 13% of the 3,600 second milled material. Lengths were less sharply reduced, with 95% of the fibers less than 5μm in length in the untreated material and over 99% of the fiber reduced below 0.5μm in length in the milled material (Table 1). Although not included in the table, clumps increased from 0.5% to almost 10% of the objects counted, from the untreated

material to the 3,600 second milled material.

In addition to these changes, the milled asbestos possessed unusual morphological features which more readily deformed under the electron beam. Although the fibril form in milled samples resembles the form observed in untreated material, the former possesses more flattened internal capillaries and sustains greater damage per unit time under the electron beam (compare Figs. 2A, 2C). Selected area electron diffraction patterns obtained on fibers of similar dimension in both populations, display only a small loss in intensity of reflections and a decrease in streaking of the (hko) reflections in the milled materials (compare Figs. 2B, 2D). The loss of streaking of the (hko) reflections may indicate a flattening in the ab plane, produced by breaking along the curved surface. These features suggested structural damage during comminution (Langer et al. 1974).

Electron microscopy confirms that mechanical comminution of chrysotile produces marked size reduction of both fibers and fibrils, reduces the overall fiber population to fibril form, induces the formation of clumps, and induces structural damage to the chrysotile fibril.

B. X-ray diffraction analysis

Using a Norelco x-ray diffractometer, aliquots of samples of equal weights were poured into an aluminum sample holder, flattened into place with a precleaned glass slide, and subjected to x-ray analysis in the 5°-60° 2θ range. Sample preparation induced preferred orientation of

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chrysotile fiber axes in the ab plane, parallel to the sample holder, which enhanced the first and second order reflections along the c-axis stacking, specifically the (002) and (004). These are normally the two most intense reflections of this mineral (Whittaker and Zussman, 1956).

Two important parameters of each peak reflection normally change upon size comminution, peak intensity and width. Peak width increase, referred to as line-broadening, is related mainly to particle size decrease and subsequent relaxation of the Bragg equation for reflection reinforcement (Azaroff and Buerger, 1958). Although extensively ball-milled, the chrysotile patterns show no line-broadening effects. This may be related to the inherent size distribution of the material, in that the bulk of the particles lie at or below the size limit required to produce enhanced broadening effects on comminution (Azaroff and Buerger, 1958). The factors inducing broadening, outlined in Scherrer equation, may also be absent in the present case since the majority of the particles are already in fibril form, and comminution mainly affects particle length. In this case, therefore, the orientation effects are such that the ab plane remains essentially unaffected.

We observed, however, a marked decrease in the total x-ray count under two major reflections (002), (004) (Table 2). Using a polar planimeter for measuring areas under these reflections, total x-ray photon count may be determined as a function of reflection area, within the 1% error (Rohl et al. 1976). Measured from a flattened baseline with no discernible satellite peaks, radial distribution analysis for incoherent

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scattering contribution to the baseline was not deemed necessary. The relative loss of x-ray photons was greatest from the most intense reflection.

Similar loss of x-ray crystallinity for chrysotile asbestos, upon grinding, was observed previously by Occella and Maddalon (1963). We corroborate this and now report that mechanical milling of chrysotile for time periods of only five minutes produces marked alteration of its structural integrity and decrease in its crystallinity.

C. Infrared spectroscopy (IR)

Untreated and manipulated samples of chrysotile were prepared for infrared spectroscopic examination by pelletization in KBr matrix (0.25% w/w) utilizing the standard solid preparation technique. Specimens were examined with a Perkin Elmer, Model 457 infrared spectrophotometer in the 200-400°cm^{-1} range (λ = 2.5-50.0μm). Selected portions of spectra for specimens ground for 60, 600 and 3,600 seconds are shown in Fig. 3.

Several important features may be noted in Fig. 3. The material milled for 60 seconds produces an IR spectrum consistent with untreated chrysotile (Brindley and Zussman, 1959). Unmilled Calidria asbestos gave similar spectra showing strong peaks at 3683 cm^{-1}, corresponding to the Mg-OH hydroxyl group, with a shoulder at 3640 cm^{-1} (probably the interlayer hydroxyl), and a triplet at 1082, 1020 and 955 cm^{-1}, possibly corresponding to Si-O, Si-O-Mg, Si-$\overset{H}{O}$-Mg) stretching frequencies (as suggested by IR spectra of quartz, nemalite, heated chrysotile and

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kaolin, the latter the aluminum analogue of serpentine).

The assignment of these three absorption bands between 1081 and 955 cm^{-1} is tentative because the stretching and vibrational modes are difficult to resolve and uniquely assign for this mineral. Two additional triplets, corresponding to the Mg-O bonds, appear centered at 605 cm^{-1} and 438 cm^{-1}, the latter with two shoulders at 480 and 407 cm^{-1}.

Chrysotile milled for 600 and 3,600 seconds shows a progressive broadening and a shift in the 1020 cm^{-1} peak (Si-O-Mg) to about 1005 cm^{-1}. The shift to smaller wave number reflects a general decrease in both strength (increasing bond length) and a corresponding change in vibrational energy requirement. The shoulder at 480 cm^{-1} on progressive milling, broadens and disappears. The OH absorption peaks (3683, 3640 cm^{-1}) remain essentially unchanged. On the basis of observable change in the infrared absorption modes, it can be concluded that mechanical milling alters some of the bonding modes in chrysotile, especially the surface brucite layers, but significant dehydroxylation does not occur.

D. Electron spin resonance (ESR)

ESR spectra was obtained on solid samples or as sonicated suspensions (10 mg/ml) in carbon tetrachloride, using a Varian ESR-3 instrument.

With all samples, a strong, broad, resonance was observed centered

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at g=1.61 with a width of about 850 gauss.(fig.4) Hyperfine splitting (ΔH=100 gauss), corresponding to paramagnetic ion substitution in the crystalline lattice, was evident in the untreated and heat desiccated (150°C) samples. Progressive milling of asbestos fiber caused a loss of hyperfine resonance in the ESR, consistent with distortion of the crystal lattice. Reduced particle size may also be partly responsible for the loss of hyperfine splitting, since the resonance characteristics are dependent on orientation effects as well. The width of the major peak remains similar in the milled samples, but the resonance appears broadened due to a reduced intensity and loss of fine structure. The ESR spectrum of Calidria fiber probably reflects superposition of two resonances, attributed to ionic substitution in the magnesia layers and defects in the silica layer. In a chrysotile sample from another geological deposit, with differing trace metal chemistries, the observed resonances (a complex sextet at g=2.00 and singlet at g=1.47) are well separated.

A similar broadened appearance and loss of hyperfine splitting, with retention of the major broad resonance, is observed with Calidria chrysotile heated at temperatures below 450°C. Heating in the 600°-800°C region results in a shift of the broad resonance to g=2.006. It is generally accepted that the initial effects of heating are dehydroxylation of the brucite layer (200-400°C) followed by recoordination of the anhydrous magnesium silicate, and its subsequent recrystallization to forsterite (about 600-820°C). Thus, loss of ESR hyperfine structure points to structural disruption of the magnesium-containing "brucite" layer in chrysotile.

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E. Hemolytic activity and antagonism

Aliquots of unaltered and manipulated chrysotile were prepared and

tested for erythrocyte membrane activity with the hemolytic test system as outlined in Schnitzer and Pundsack (1970). This system for testing the membrane activity of asbestos minerals has developed over the last ten years so that the different fiber types may be compared in well defined systems (Macnab and Harington, 1967; Schlipkoter, 1968; Secchi and Rezzonico, 1968). In most of these test systems, mature sheep erythrocytes, in plasma-free suspensions, were tested with known quantities of asbestos fibers and their hemolytic potency determined by optical measurement of released hemoglobin. In our study, mature sheep erythrocytes were also used to test the hemolytic potency of known quantities of fiber (Table 4).

Using an equivalent mass of chrysotile, a marked decrease in the hemolytic potency is observed after 300 seconds of mechanical milling. This was demonstrated by a comparison of a 1mg/ml concentration hemolysis level, and the concentration of chrysotile required to produce 50% hemolysis (Table 4). Mechanical milling of chrysotile for a time period of 3,600 seconds produces a material which is weakly hemolytic, requiring almost 19 times more fiber to produce a 50% hemolytic level than unaltered material.

Asbestos hemolysis may be antagonized by a number of polymers, some of which have been described previously (Schnitzer and Bunescu, 1970). Carboxymethyl cellulose (CMC) is a potent antihemolytic agent in the chrysotile hemolytic system. It has been proposed that the polyanion complex becomes bound to the chrysotile fiber through interaction with the hydrogens on the surface hydroxyl groups (Schnitzer and Bunescu, 1970). Other polymers, e.g., polyvinyl-

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pyridine-N-oxide (PVP-N-O) do not appear to interact with these same groups so that for an antihemolytic response equal to that of CMC in the chrysotile test system, PVPNO must be used at 100X the concentration of CMC (Table 4). In our test systems, CMC was tested in the range of 4μg ± /ml concentrations, whereas PVPNO was tested at 1250μg ± /ml concentrations. With CMC at 4μm/ml, PVPNO at 1250μg/ml, and asbestos at 1mg/ml, varying antagonist response was observed. The antihemolytic effectiveness of CMC decreased in the samples that were progressively milled, reflecting the reduced hemolytic potential of the milled asbestos. The hemolytic antagonism was almost eliminated in the 3,600 second milled material (Table 4). The same effect was observed for the PVPNO. Both phenomena suggest that the brucite layer of chrysotile is progressively disordered on mechanical milling, altering the effects and the ability of polymers to adsorb to its surface.

F. Organic-free radical adsorption

Diphenypicrylhydrazyl (DPPH), a stable organic-free radical, is strongly adsrobed to chrysotile asbestos, a phenomenon attributable to strong interaction for the polar nitro groups and nitrogen groups. (M. Wolff, unpublished data). The adsorption is twofold, an irreversible chemical adsorption resulting in reduction of DPPH to the hydrazine, and a multilayer physisorption which may involve vertical or horizontal stacking of the molecule. Chemisorption probably occurs via the hydrogen atom from the "phenolic" hydrogens of the brucite layer.

Chemisorption potential was measured by titration of 50mg samples of

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asbestos in carbon tetrachloride with DPPH (10^{-3}M in CCl_4), until excess DPPH could be detected visually by its purple color in the supernatant. Results are given in Table 4. Physisorption was measured by determining the adsorption isotherm. Asbestos samples of 50mg were mixed with aliquots of varying concentrations of DPPH (10^{-4}-10^{-2}M in CCl_4) overnight in a shaker. The DPPH remaining in the supernatant was determined spectochemically (530nm). The isotherm was determined by regression analysis of millimoles adsorbed per gram asbestos vs. equilibrium concentration of DPPH. The values, given in Table 4, are calculated by extrapolating the adsorption isotherm to 10^{-2}M. Chemisorption was increased by ball-milling the chrysotile for 60 seconds, reflecting the increased surface area available for adsorption. However, milling for longer time periods, up to 1-hour, significantly reduced the chemisorption capacity of chrysotile. A similar reduced potential for physisorption was observed on prolonged milling.

Both of these processes can be attributed to interactions at the surface of the chrysotile fiber. Thus, short milling produces fiber with a greater surface area relatively unchanged in terms of chemistry. In this state, chemisorption is increased. Prolonged milling appears to degrade the surface brucite layer and disrupt the uniform proton distribution (hydrogens on the brucite). Both adsorption processes are dependent on interaction of the outer hydrogens and are thereby reduced by the structural degradation which follows prolonged mechanical milling.

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Summary and Conclusion

An asbestos fiber standard, establishing a threshold limit value (TLV) for fiber exposure to workmen engaged in handling asbestos-containing dusts in the United States will be 2 f/ml on July 1, 1976. The fibers counted, by light optical methods, will be only those greater than 5μm in length. Over 3,000 uses of asbestos exist in the United States, which indicates that a range of materials, processing operations, and fabrication plants are involved in supplying society with these products. Size distribution characteristics of the aerosols generated in these work areas must therefore range greatly. In addition to this, the exploitation of new types of asbestos deposits, primarily those in the western United States and Canada, have introduced a material which consists almost entirely of short fiber. The need to establish the biological potential of short fiber is therefore critical.

A number of studies of human lung tissues, demonstrate that small particles predominate in the fiber burden of such tissues, and may be implicated in human disease (Pooley et al. 1969; Langer et al. 1971; 1973; Miller et al. 1975; Fischbein et al. 1976). In addition to this, while some animal studies over the past 30 years have supported the human observations, in other experimental efforts, small fibers appeared to have different degrees of activity.

Those studies using short fibers obtained by less vigorous methods, e.g., air-elutriation and water fractionation, induced significant responses in animals. In those studies where fibers were reduced in size by

A00139

vigorous mechanical methods, either no or significantly reduced responses were observed. Comminution undoubtedly altered the crystal and chemical properties of the material. It appears that the less mechanically degraded the surface of the fiber, the greater the biological response.

Previous investigators have reported reduced crystallinity (Occella and Maddalon, 1963; Reeves et al. 1974) and modified crystallinity, and reactivity (Robock and Klosterkotter, 1971) in milled fiber.

Our data support and amplify these conclusions. The crystallinity of chrysotile was reduced in proportion to the length of milling time. The nature of the altered crystallinity appears to be confined to shifts in interlayer bonding between the brucite and silica sheets, and changes in hydroxyl configuration (but not bond strength). Supporting these conclusions are the following data:

a. Reduced total x-ray photons, of selected reflections, without line broadening;
b. Increased susceptibility to damage by electron bombardment;
c. Changes in SAED patterns, decreased streaking of (hko) reflections, suggesting relaxation of fibril curvature;
d. Retention of intensity and location of OH absorption bands; shift and broadening of absorptions assigned to interlayer Si-O and Mg-O bonds with retention of all other significant Si and Mg bond absorptions;
e. Loss of hyperfine splitting and intensity in the ESR spectrum attributed to coordination changes in the brucite layer.

A00130

Changes in surface chemistry accompany reduction in crystallinity. The ability to reduce DPPH to the hydrazine and to physisorb DPPH is lowered with milled fiber, and these are both surface, stereochemically controlled, phenomena. Hemolytic potency, often considered a surface-related event, is also markedly decreased in proportion to milling time. This is not related to exposure of the silica layer in that neither hemolytic potency and antagonism by PVPNO are increased with milling time, but rather reduced. The diminished surface activity of chrysotile suggests a surface structure with distorted proton and/or magnesium ion orientation. Membrane activity in cells(both erythrocyte and macrophages) is reduced, and this interaction may be the initial biological response. After phagocytosis, fibrotic and cytotoxic response may follow slow degradation of the chrysotile intracellularly, to expose the silica surface, which stimulates collagen formation. Therefore a reduced response, or longer time period to produce response is expected for milled fiber in comparison with unmilled fiber. Indeed, this has been observed (Reeves et al. 1974; Pott, 1972, Klosterkotter, 1968; Hilscher, 1970). This theory intimates, furthermore, that fibrotic response from silica exposure would occur much faster than with chrysotile exposure, and epidemiological evidence supports this theory; there is, for example, no "acute asbestosis" to mimic acute or accelerated silicosis (Ziskind et al. 1976).

Theoretical and experimental consideration has been given to interference of asbestos with cellular oxidation-reduction (redox) equilibria processes, the experimental evidence consisting of oxygen consumption in relation

A00137

to both mineral treated macrophages and fibrotic response in animals. (Bey and Harington, 1971; Robock and Klosterkotter, 1971; Chvapil and Peng, 1975). Flowers (1975) has noted the critical role of $Fe^{2+} \leftrightharpoons Fe^{3+}$ equilibria in determining fragile cellular energy dynamics and enzymatic transformations. Participation of chrysotile asbestos in redox activity in biological systems may be predicted from chemical-physical data:

1. The ESR spectrum and the luminescence spectrum of chrysotile.
2. Reduction potential for TTC (Robock and Klosterkotter, 1971) and DPPH; oxidation potential for other organics (M. Wolff, unpublished data).

Whether these properties are related to fibrosis or carcinogenesis is purely speculative, but Allison has noted that "crocidolite, the blue colour of which is due to charge transfer, has little cytotoxicity". Indeed, most minerals possess distinct ESR spectra as well. (Robock and Klosterkotter, 1971).

The hypothesis that particle shape is the major etiological mechanism in fibrotic or carcinogenic responses, appears, in terms of these considerations, to be somewhat oversimplified, except as morphology reflects more fundamental structural and surface physical-chemical properties.

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Table 1

Calidria Chrysotile: Size Distribution upon Comminution as Determined by Electron Microscopy

Length of Fibers in Microns (Percent)

	≤ 0.1	0.11-0.5	0.51-1.0	1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0	5.0-10.0	> 10.0	Number Counted
Untreated	3.7	39.7	22.0	18.7	7.9	1.4	1.9	3.7	0.9	214
60 second mill	0.5	22.0	34.0	27.0	13.6	1.1	--	0.3	--	191
300 second mill	0.4	53.4	32.7	13.2	0.4	--	--	--	--	251
1,200 second mill	8.6	79.1	12.4	0.9	--	--	--	--	--	162
3,600 second mill	16.3	82.9	0.8	--	--	--	--	--	--	123

Length of Fibrils in Tenths-of Microns (Percent)

	≤ 0.1	0.11-0.5	0.51-1.0	1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0	5.1-10.0	> 10.1	Number Counted
Untreated	24.8	51.8	9.9	5.8	3.3	1.9	1.3	0.8	0.6	800
60 second mill	15.7	67.5	12.5	4.0	0.4	--	--	--	--	779
300 second mill	24.7	69.0	5.3	0.9	--	--	--	--	--	962
1,200 second mill	68.0	31.7	0.3	--	--	--	--	--	--	829
3,600 second mill	74.8	25.1	0.1	--	--	--	--	--	--	858

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Table 2

Change in Total X-ray Count (Peak Area) Under Selected Reflections with Progressive Ball-Milling*

	Peak Area (in^2) 7.35Å (002) I/I=100	Peak Area (in^2) 3.66Å (004) I/I=80
Untreated	8.36	6.99
60 seconds	7.80	6.96
300 seconds	7.38	6.01
1200 seconds	4.92	5.72
3600 seconds	3.50	5.69

* Repeated for mass-equivalents (packing change). Instrumental setting: Target: Cuk α nickel filter; graphite monochromator; $1^{\circ}2\theta$/minute; 4°-0.006-1° slit settings; target: operated at 45 kv/20ma.

A00140

Table 3

Variation of Hemolytic Activity with Fiber Manipulation

Cylinder milling, time, sec.	Percent hemolysis at 1mg/ml	Chrysotile concentration at 50% hemolysis	Percent hemolysis in the presence of antagonists	
		mg/ml	CMC, 4μg/ml	PVPNO, 1250μg/ml
Untreated	80 ± 5(1)	0.4	21 (78)	25 (75)
60	75 ± 6	0.4	38 (62)	19 (81)
300	52 ± 1	1.0	29 (71)	10 (90)
600	23 ± 1	2.6	19 (81)	6 (94)
1200	12 ± 1	4.4	11 (91)	5 (95)
3600	12	7.5	6 (94)	--

(1) Standard deviations determined by ten repeat determinations.

A00141

Table 4

Adsorption of Diphenylpicrylhydrazyl (DPPH) to Chrysotile (Calidria)

Time of milling, second	Chemisorption, μmoles DPPH/gm asbestos	Physisorption, μmoles DPPH/gm asbestos
Untreated	4.3 ± 1.5	283 ± 2
60	6.6 ± 0.8	288 ± 2
300	6.9 ± 0.6	
600	7.9 ± 0.6	
1200	7.0 ± 0.7	
3600	3.4 ± 0.7	192 ± 6

A00142

References

Addingly, C.G. (1966) Ann. Occup. Hyg. 9,73

Allison, A.C. (1973) IARC 89,93

Ayer, H.E., Lynch, J.R. & Fanney, J.H. (1965) Ann. N.Y. Acad. Sci. 132,274

Azaroff, L.V. & Fanney, J.H. (1958) McGraw Hill Co., N.Y., 342

Bey, E. & Harington, J.S. (1971) J. Exp. Med. 133, 1149

Brindley, G.W. & Zussman, J. (1959) Amer. Mineral 44, 185

Chvapil, M. & Peng, Y.M. (1975) Arch. Environ. Health 30, 528

Davis, J.M.G. (1964) Brit. J. Exp. Path. 45, 634

Davis, J.M.G. (1970) Exp. & Molec. Path. 13, 346

Davis, J.M.G. (1972) Brit. J. Exp. Path. 53, 190

Dreessen, W.C., Dalla-Valle, J.M., Edwards, T.I., Miller, J.W., Sayers, R.R., Eason, H.F. & Trice, M.F. (1938) Public Health Bull. No. 241

Federal Register (1972) 37(110):11318, Wed. June 7, 1972

Fischbein, A.S., Langer, A.M., Suzuki, Y. & Selikoff, I.J. (1976) In: Proc. 3rd Intl. Symp. on Det. & Prev. Cancer, New York 4/26-5/1

Flowers, E. (1974) Am. Ind. Hyg. Ass. J. 35, 724

Gross, P.(1967) Arch. Environ. Health 15:343

Gross, P.(1968) In: Proc. Intl. Konf. über die Biologischen Wirkungen des Asbestos, Eds. Holstein, Anspach, Deut. Zent. Institut fur Arbeits-med. DDR. 22-25 Apr 1968, 244

Gross, P. and Harley, R.A. (1973) Arch. Environ. Health 27, 240

Gross, P., Harley, A., Swinburne, L.M., Davis, J.M.G. & Greene, W.B. (1974) Arch. Environ. Health 29, 341

Harington, J.S. (1973) In: Biolog. Effects Asbestos, IARC Conf. Lyon, Eds. Bogovski et al, 304

Hilscher, W. (1970) Naturwissenschaften 57, 356

Holt, P. (1957) Ed. Arnold, Pub. London, 268

Holt, P., Mills, J. & Young, D.R. (1964) J. Path. & Bacteriol. 87, 15

Holt, P., Mills, J. & Young, D.R. (1965) Ann. N.Y. Acad. Sci. 132, 87

A00143

King, E.J., Clegg, J.W. & Rae, V.M. (1946) Thorax 1, 188

Langer, A.M., Selikoff, I.J. & Sastre, A. (1971) Arch. Environ. Health 22, 348

Langer, A.M., et al., (1973) J. Occup. Med. 15, 287

Langer, A.M., Mackler, A.D. & Pooley, F.D. (1974) Env. Health Persp. 9, 63

Lynch, J.R., Ayer, H.E. & Johnson, D.L. (1970) Am. Ind. Hyg. Assoc. J. 12, 498

McIver, F.A. (1968) In: Proc. INTL. Konf. über die Biologischen Wirkungen des Asbestos, Eds. Holstein, Anspach, Deut. Zent. Institut fur Arbeitsmed. DDR 22-25 April 1968

Macnab, G. & Harington, J.S.(1967) Nature 214, 522

Miller, A., Langer, A.M., Teirstein, A.S. & Selikoff, I.J. (1975) New Eng. J. Med. 292, 91

Occella, E. & Maddalon, G. (1963) Med. Lavoro 54, 628

Pooley , F.D. et al. (1970) In: Proc. Intl. Conf. Pneumoconiosis, Johan., Ed. H.A. Shapiro, Oxford University, 108

Pooley, F.D. (1972) Brit. J. Indust. Med. 29, 146

Pott, F., Huth, F. & Friedrichs, K.H. (1972) Zb. Bakt. Hyg., Abt. Orig., 155, 463

Reeves, A.L., Puro, H.E. & Smith, R. (1974) Environ. Res. 8, 178

Robock, K. & Kosterkotter, W. (1971) Biophysikalisch untersuchungen zur wirkung von asbesten. Int. Pneumoconiosis Conf. Bucharest. Apimondia Publishing House

Rohl, A.N. & Langer, A.M. (1974) Env. Health Persp. 9, 95

Schall, F.L. (1965) Ann. N.Y. Acad. Sci. 132, 316

Schlipkoter, H.W. (1968) In: Proc. Intl. Konf. über die Biologischen Wirkungen des Asbestos, Eds. Holstein, Anspach, Deut. Zent. Institut fur Arbeitsmed. DDR. 22-25 April 1968

Schnitzer, R.J. & Bunescu, G. (1970) Arch. Environ. Health 20,481

Schnitzer, R.J. & Pundsack, F.L. (1970) Environ. Res. 3, 1

Secchi, G.C. & Rezzonico, A. (1968) Med. Lavoro 59, 1

Selikoff, I.J., Churg, J. & Hammond, E.C. (1965) Ann. N.Y. Acad. Sci. 132, 139

A00144

UCC 025692

Stanton, M., Blackwell, R. & Miller, E. (1972) Am. Ind. Hyg. Assoc. J. 30, 236

Stanton, M. (1973) In: Biolog. Effects Asbestos, IARC Conf. Lyon, Eds. Bogovski et al., 304

Suzuki, Y. & Churg, J. (1969) Am. J. Path. 55, 79

Timbrell, V., Pooley, F.D. & Wagner, J.C. (1970) In: Proc. Intl. Conf. Pneumoconiosis, Johan., Ed. H.A. Shapiro, Oxford University, 120

Timbrell, V. (1973) In: Biolog. Effects Asbestos, IARC Conf. Lyon, Eds. Bogovski et al. 295

Vorwald, A.J., Durkan, T.M. & Pratt, P.C. (1951) Arch. Indust. Hyg. & Occup. Med. 3, 1

Wagner, J.C. (1968) In: Proc. Intl. Konf. über die Biologischen Wirkungen des Asbestos, Eds. Holstein, Anspach, Deut. Zent. Institut fur Arbeitsmed. DDR 22-25 April 1968

Wagner, J.C. (1973) Cancer Res. 59, 37

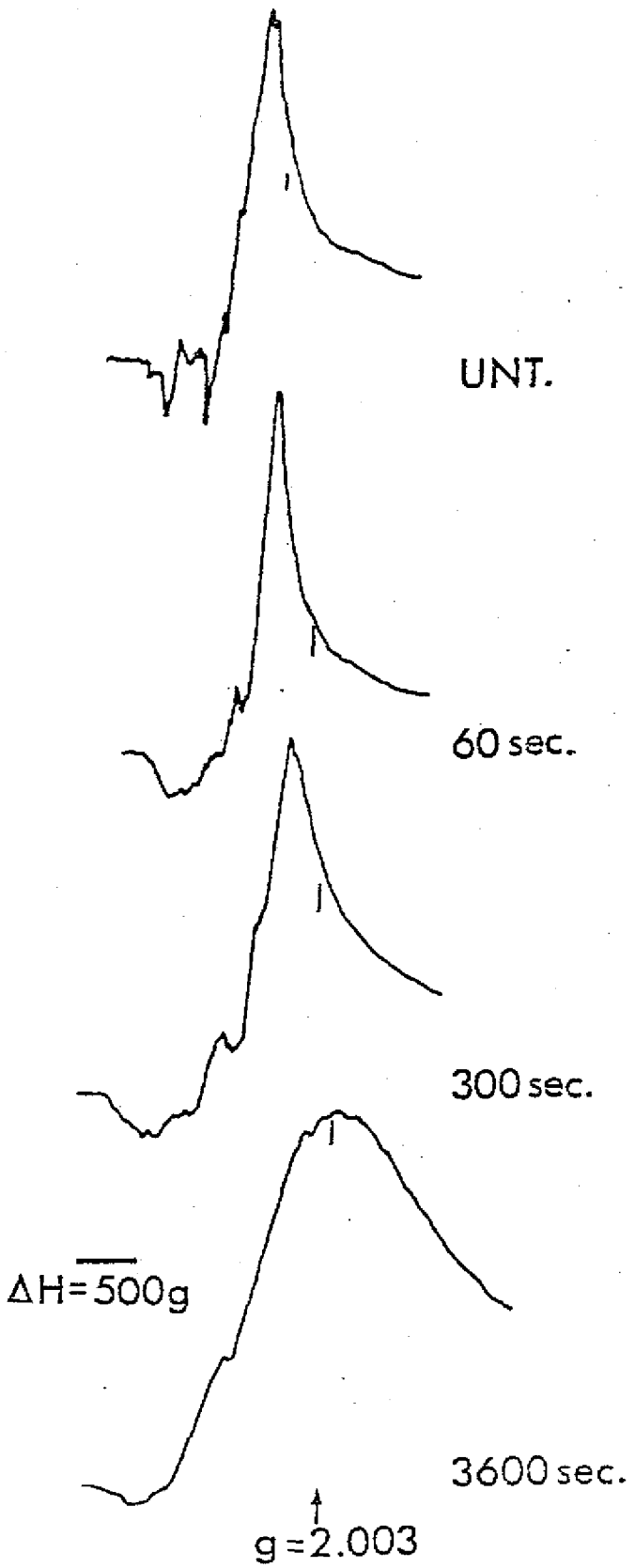
Wagner, J.C. & Berry, G. (1973) In: Biolog. Effects Asbestos. IARC Conf. Lyon, Eds. Bogovski et al., 285

Wagner, J.C., Berry, G. & Timbrell, V. (1970) In: Proc. Intl. Conf. Pneumoconiosis, Johan., Ed. H.A. Shapiro, Oxford University, 119

Whittaker, E.J.W. & Zussman, J. (1956) Mineral Mag. 31, 107

Ziskind, M., Jones, R.N., Weil, H. (1976) Amer. Rev. Resp. Dis. 113, 643

AC0145



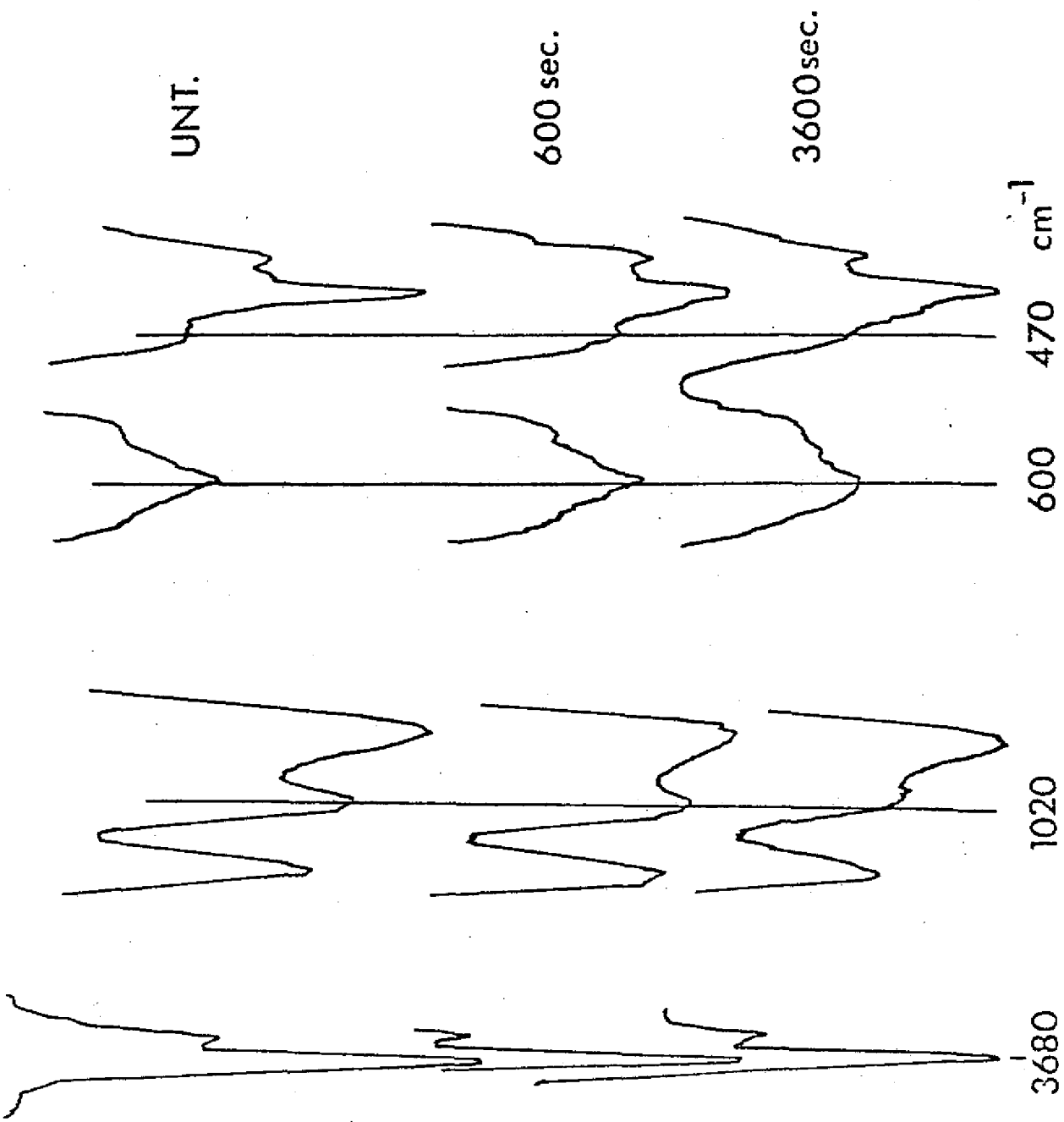
A00140

Fig. 4: Esr spectra of successively milled Calidria chrysotile show progressive loss of hyperfine structure, attributed to crystalline changes in the brucite layer. Resonances are all centered at g=1.61.

A00147

Fig. 3: Infrared spectra of Calidria asbestos, untreated and after milling times of 600 and 3600 seconds show the major absorption bands unchanged. Bands at 1020 and 470 cm^{-1}, tentatively assigned to interlayer Si-O-Mg bands, are broadened and sluted.

AC8148



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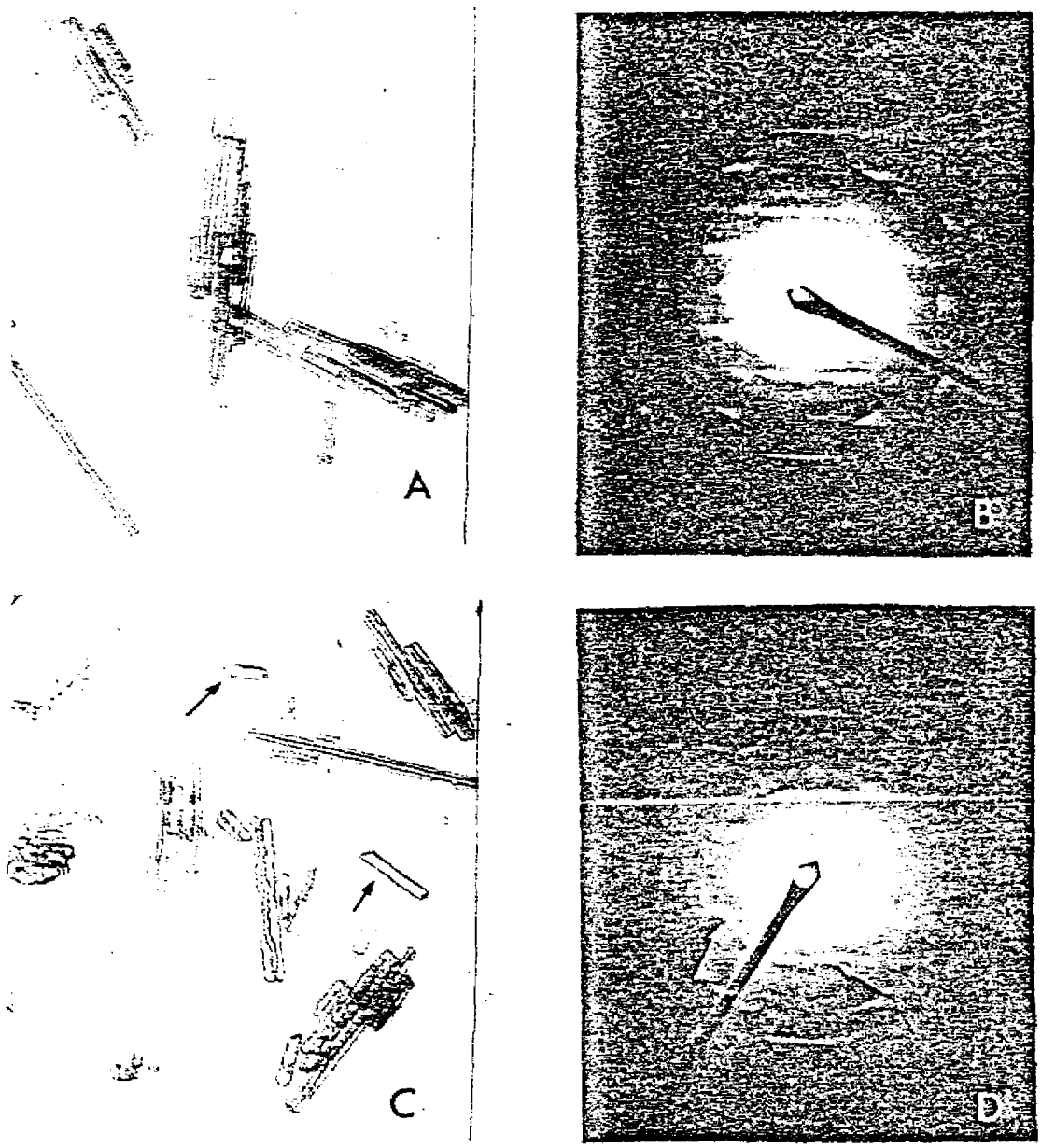
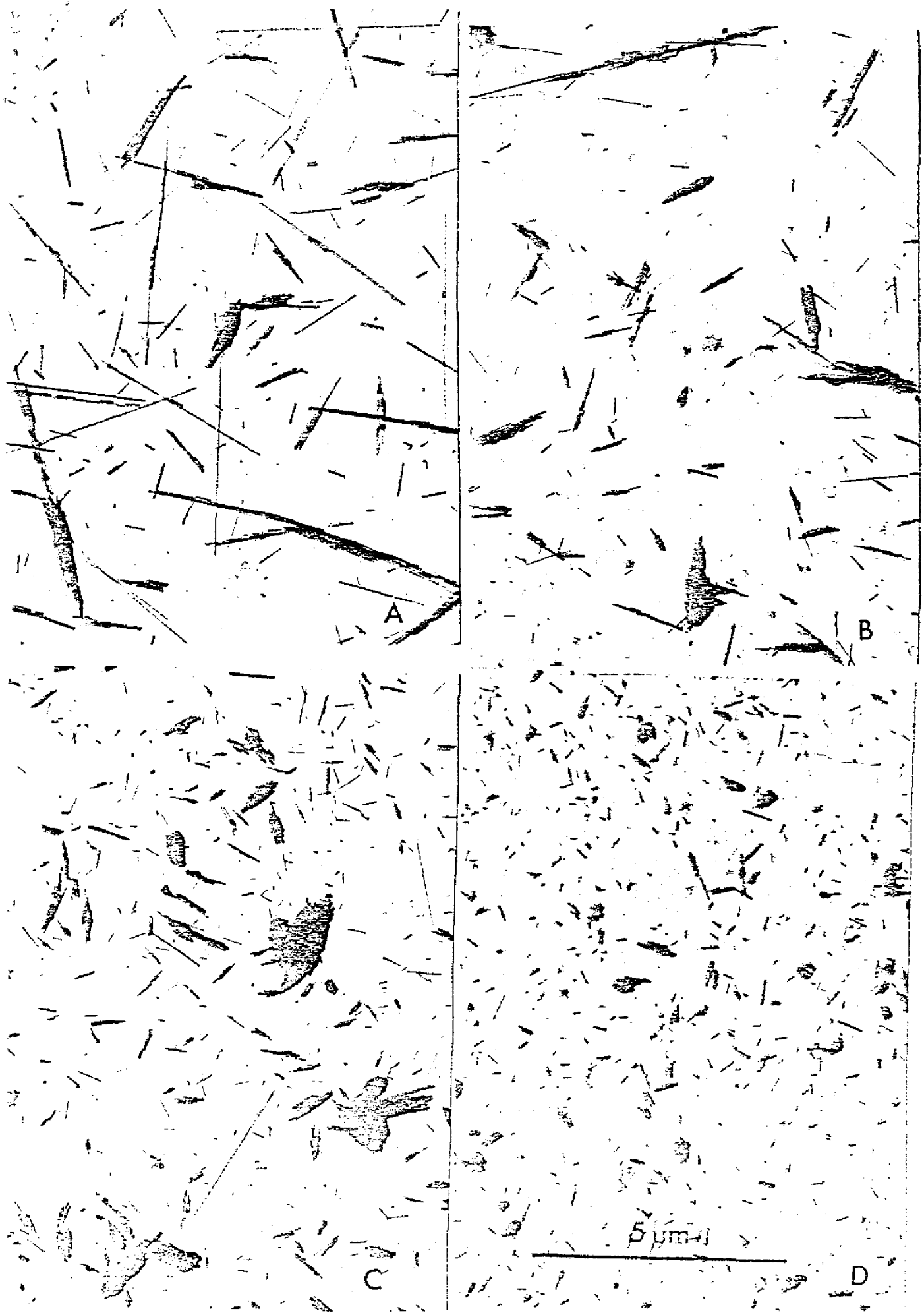


Fig. 2 Electronphotomicrograph of chrysotile asbestos milled for 60 seconds (A) and 1200 seconds (C) and their selected area diffraction patterns. (66,300X magnification). In A, fibrils are rectilinear with intact central capillary and morphology. In C., electron beam damage is evident, some fibrils having lost the classic morphology (arrows). The selected area diffraction D pattern for C shows less streaking, indicating a loss of outer fibril curvature.

AC0150

Fig. 1: Size distribution of fibers in milled chrysotile samples becomes progressively smaller. Electronphotomicrographs represent fiber unmilled (A), milled for 60 (B), 300 (C), and 3,600 (D) seconds.

A00151



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