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TITLE. **LONG-TERM HEALTH EFFECTS IN HAMSTERS AND RATS EXPOSED CHRONICALLY TO MAN-MADE VITREOUS FIBERS**

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LONG-TERM HEALTH EFFECTS IN HAMSTERS AND RATS EXPOSED CHRONICALLY TO MAN-MADE VITREOUS FIBERS*

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ABSTRACT

As part of a comprehensive inhalation study, groups of Osborne-Mendel rats and Syrian golden hamsters were exposed to several types of airborne man-made vitreous fibers. Exposure protocols were "nose-only" 6 h a day, 5 d a week for 24 m, with surviving animals maintained for the rest of their lives. Challenge aerosols consisted of 4 types of fibrous glass, 1 refractory ceramic fiber (RCF), and 1 mineral wool fiber. UICC crocidolite asbestos and clean air served as positive and negative controls for the inhalation groups. Groups of additional controls were unmanipulated caged animals, intraperitoneally (IP) injected animals, and intratracheally (IT) instilled animals.

Animals, after their deaths, were examined macroscopically and microscopically. Fiber lung burdens were significant for the inhalation exposures and related to the mean diameters of the fibrous challenge aerosols. The inhalation exposures with MMVF did not result in any adverse effects except for a mesothelioma of the lung in 1 hamster exposed to the RCF, not a statistically significant finding.

Consistent with other reported work, abdominal mesotheliomas were induced in the groups of hamsters and rats injected IP with 0.45-micron mean diameter fibrous glass, RCF, and crocidolite asbestos.

With IT instillations, primary lung tumors were found only in hamsters and rats receiving UICC crocidolite; no lung tumors occurred in animals instilled IT with 2 types of MMVF.

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I. Introduction

Exposure to airborne asbestos has been associated with degenerative pulmonary disease and tumor induction in humans (Wagner, 1965) and experimentally in rats (Wagner *et al*, 1974). As a result, concern has developed regarding the widespread use of man-made mineral fibers (MMMF), especially man-made vitreous fibers (MMVF), and their possible health effects A comprehensive review of the first published results of animal studies delineating the biological effects of exposure to MMMF and MMVF was presented by Kotin (1984) at a WHO/IARC Conference on the Biological Effects of Man Made Mineral Fibers in 1982. Kotin's paper describes long-term health effects in hamsters and rats exposed chronically to four different glass fibers, a refractory ceramic fiber (RCF), and a mineral wool fiber. UICC crocidolite asbestos served as a "positive" control material.

The study reported in this paper addressed the question of whether MMVF, when inhaled by laboratory animals, induce lung tumors

II. Materials and Methods

A. Fiber Preparation and Aerosol Generation

All 7 fibrous exposure aerosols were produced directly from unaltered bulk materials using our modified Timbrell-type generator and complementing plug-packing mold assembly (Ortiz, *et al*, 1977). This aerosol-generating device relies on the controlled feeding of a fibrous plug compact into a rotor/blade assembly for aerosol production. Fibers are either shaved or brushed from the end of the advancing plug, and a moving air stream exhausts fibers from the generator chamber (Fig. 1) This aerosol-production method was purposely selected to minimize possible external contamination of the aerosolized fibers. The fibrous plug-packing procedures in this study were simplified from those described previously (Ortiz, *et al*, 1977) to accommodate the task of reproducibly preparing many feed plugs of the different fiber types over an extended time as required for chronic inhalation exposures.

Two variations of the basic fiber preparation/packing/generation procedures were employed to accommodate aerosolizing two specific fiber types: bulk MMVF and premilled UICC crocidolite

MMVF were supplied by the Thermal Insulation Manufacturer's Association (TIMA). We are grateful to R E.G Rendall and V Timbrell of the British Medical Research Council, Penarth, Wales, for their kindness in supplying us with UICC crocidolite asbestos All MMVF were prepared for aerosolization by weighing dry, hand-pulled strands or fiber swatches from bulk insulation bats or bags These preweighed swatches of bulk fiber were pressed gently into a plug-packing mold, "wet" with 5 to 15 cm^3 of ethanol and hand compressed to a fixed dimension (Fig 2) "Wet" mold packing of these vitreous fibers produced more cohesive and uniform fibrous compacts for subsequent infusion into the generator shaving blades than did dry packing The steel mold with "wet" compressed plug was oven dried (80°C, overnight), cooled to room temperature, and transferred from the mold to the generator Plug transfer was accomplished by removing the mold end pieces, mating the mold to the generator, and hand pushing the plug from mold to generator using a fitted nylon plunger

This procedure for producing challenge aerosols directly from bulk fibers was selected specifically to minimize alteration of exposure fibers Bulk fibers were not preminced or premilled prior to loading the generators Bulk fibers containing organic

binders and or lubricants were generated with the binder lubricant in place. Every attempt was made to present each of the vitreous fiber aerosols to the animals in a state that people may experience during exposure to fibrous dusts produced from commercially available products during their manufacture or use. A prime objective was to expose experimental animals to significant concentrations of long, thin, "respirable" fiber singlets.

The refractory ceramic fiber (RCF) used in the study was prepared for aerosolization using similar "wet" infusion plug-packing procedures. However, the generation chamber was modified to better accommodate the extremely abrasive property of RCF exhibited in the processing/chopping of these fibers for aerosolization The stainless steel (SS) cavities of the generators were machined to a slightly larger diameter and retrofitted with hardened steel cylindrical sleeves prefabricated to provide the same internal diameter as the original chambers. This insert, which replaced the original SS chamber interior, ensured that the two primary generator surfaces most exposed to abrasive action were made of abrasive-resistant tungsten carbide cutting blades and hardened steel generator chamber walls. These generators performed without mishap for the entire 24-m exposure period Comparative energy dispersive x-ray (EDX) analysis of bulk materials and membrane-filter-collected aerosol materials indicated that external metal contamination originating from aerosol generation was insignificant.

Crocidolite exposure aerosols were produced using the same generating/packing approach with two significant modifications (1) the crocidolite infusion plug was dry packed into the mold and (2) the standard carbide-tipped rotor was replaced by a flexible, soft brush rotor. This brush rotor had no hard cutting edges but rather used only soft flexible sable hair for sweeping the premilled asbestos fibers from the infusion plug--an extremely gentle process, minimizing alteration and contamination of these premilled UICC standard fibers.

Regardless of the fiber type being generated, the output from our generator is influenced by the nature of the starting material, density of the plug compact, plug infusion rate, generator rotor speed and exhausting air-flow rate For the animal inhalation studies, plug infusion rates ranged from 5 to 200 microns per minute, total air flow rates from 40 to 70 l per minute, aerosol mass concentrations from 0.3 to 12 mg m^{-3}, and fiber numbers from 10 to 3000 cm^{-3} (Table I)

When the aerosols left the generation chamber, they were diluted with clean air and passed through a 10-mCi ^{85}Kr deionization source (Thermo Systems, St Paul MN) and into the top of our "nose-only" animal exposure chambers Air flow was from top to bottom and ranged from 40 to 70 l min^{-1}, depending on the fiber being aerosolized

B Aerosol Characteristics

1. Sampling Exposure Aerosols

To define and characterize each challenge aerosol, three parameters were monitored during the exposures (1) aerosol mass concentration, (2) fiber size and number, and (3) aerodynamic properties

Aerosol mass concentration was measured and fiber sizing/counting samples were collected using a "breathing zone" sampling concept, i.e., collection devices were placed in the polycarbonate animal restraining tubes so that airborne fibers were sampled at the locations in the chambers simulating the "breathing zone " of the exposure animals

2. Fiber Sizing - Length x Diameter Matrix

Fiber diameters and lengths were obtained for each of the six MMVF aerosols These data were determined from membrane filter (Nucleopore) aerosols collected at random 2- to 4-m intervals during the exposure interim. The samples were pooled, and 6 to 8 samples of each fiber were randomly selected and sized from scanning electron micrographs (SEM) taken at 500 to 2000x, adjusted to suit the collected fiber deposit

a 0 45-Micron MD Glass Fiber Exposure Aerosol

Dual dimension (l x d) sizing data indicate that this aerosol comprised individual fibers ranging from 0.2- to 0.6-micron diameter, with 94% being = or <20 microns long The count mean length (CML) was 7.5 ± 10 microns, and the count mean diameter (CMD) was 0.4 ± 0 3 microns. The mean aspect ratio for the 999 fibers sized was 15 The geometric mean fiber length (GML) was 4.7 microns, the geometric mean diameter (GMD) 0.4 microns, and the geometric mean aspect ratio (GMAR), 11. Thus, the fibers in this aerosol were relatively short and potentially highly "respirable" Sizing details for this fiber are summarized in Table II. At a concentration of 3,000 F cm^{-3}, there were approximately 530 F cm^{-3} longer than 10 microns with diameters = or <1 0 micron in this aerosol.

b 3 1-Micron MD Glass Fiber Exposure Aerosol

Independent and complimentary fiber l x d matrix measurements of this aerosol demonstrated that 99% of the airborne fibers were >5 microns long with 77% >10 microns long Approximately 68% of these fibers were between 10 and 80 microns long with 30% between 20 and 50 microns in length The CML was 37 ± 48 microns, CMD, 1.4 ± 1.1 microns, and the mean aspect ratio 27. The GML was 24 microns, GMD, 1 2 microns, and the GMAR, 19. This aerosol was a long, (> 10 microns) fibered aerosol with most airborne fibers potentially "respirable" Sizing data for this fiber are summarized in Table III. In this aerosol, with a mean concentration of 100 F cm^{-3}, there were 30 F cm^{-3} longer than 10 microns with diameters = or <1.0 micron

c. 5.4-Micron MD Glass Fiber Exposure Aerosol

Diameter length sizing data for this material demonstrated that 74% of the aerosol was <2 microns in diameter with 67% between 10 and 80 microns long (Table IV) Approximately 77% of all airborne fibers were between 5 and 50 microns long with 97% <3.5 microns in diameter. The CML was 31 ± 33 microns, with the CMD 1 4 ± 0 9 microns The MAR was 24. The GML was 20 microns, the GMD 1 1 microns, and the GMAR 18 At concentrations of 100 F cm^{-3}, this aerosol contained 25 F cm^{-3} with lengths >10 microns and diameters = or <1 0 micron

d 6 1-Micron MD Glass Fiber Exposure Aerosol

Approximately 53% of the fibers in this aerosol had diameters <2 5 microns with 36% between 10 and 80 microns long Approximately 29% were between 5 and 50 microns long with 71% having diameters <3 5 microns More than 50% of all collected fibers were >80 microns long The CML was 114 ± 9 4 microns and the CMD 3 0 ± 2 2 microns The MAR was 49, the GML, 83 microns, and the GMD 3.0 microns The GMA was 36 Thus, this fibrous material produced an extremely long, coarse-diameter challenge aerosol (Table V) At a concentration of 25 F cm^{-3}, this aerosol had 5 F cm^{-3} longer than 10 microns with diameters = or <1.0 micron

e. 1.8-Micron MD Refractory Ceramic Fiber Exposure Aerosol

The CML was 35 ± 34 microns, the CMD, 1.1 ± 0.7 microns, the MAR, 36, the GML 25 microns, the GMD, 0.9 microns, and the GMAR was 26. Approximately 83% of this aerosol was >10 microns long and 86% <2.0 microns in diameter. The aerosol was long fibered (> 10 microns) with most fibers potentially "respirable" (<3.0-micron diameter and <80 microns long (Table VI). Assuming a concentration of 200 F cm^{-3}, this aerosol contained approximately 88 F cm^{-3} >10 microns long having diameters = or <1.0 microns.

f. 2.7-Micron MD Mineral Wool Exposure Aerosol

The CML was 40 ± 63 microns, the CMD 1.1 ± 0.9 microns, and the MAR 40. The GML was 4.5 microns, the GMD 0.9 microns, and the GMAR was 26. Approximately 75% of this aerosol was >10 microns long with 93% having diameters <2.5 microns. More than 70% of the fibers in this exposure aerosol were <1.6-micron diameter and between 6 and 80 microns long; thus, this was a relatively long-fibered, aerosol (Table VII). This aerosol, with a mean concentration of 200 F cm^{-3}, had approximately 76 F cm^{-3} longer than 10 microns with diameters = or <1.0 micron.

g. UICC Crocidolite Asbestos Exposure Aerosol

Our fiber-length data for this aerosol are in agreement with UICC data; i.e., both concur that it is a short-fibered material. UICC data indicate that approximately 97% of the premilled fibers were = or <5 microns long (Rendall, 1970). Our fiber-length measurements indicated that approximately 95% of UICC crocidolite fibers were = or <5 microns long (Table VIII). Our mean fiber length for this material was 3.1 ± 10.2 microns. The sizing data in Table VIII demonstrate that the UICC material was not significantly altered during aerosolization. At a concentration of 3,000 F cm^{-3}, there were approximately 90 F cm^{-3} >10 microns long.

C. Aerodynamic Characterization (Size-Selective Sampling)

1. Man-Made Vitreous Fibers

Supplementary aerosol characterization data for all MMVF were obtained using nylon cyclone samplers. These data complemented our sizing results in providing an estimate of the "respirable" mass fraction of these exposure aerosols. Cyclone sampler penetration data for the MMVF aerosols are summarized in Table IX. Each penetration value is the mean obtained from at least 9 separate sets of cyclone sampling runs. Each set of cyclone sampling runs consisted of the mean cyclone penetration value from three separate samplers operated simultaneously at various locations within the exposure chambers. All samplers were operated at 1.7 l min^{-1} for 30 minutes. Cyclone samplers used were the 10-mm nylon (Dorr-Oliver) models that are the standard for coal dust sampling and follow the American Conference of Governmental Industrial Hygienists (ACGIH) curve defining "respirable" dust (ATC, 1970). Previous experiments have demonstrated their utility for characterizing fibrous aerosol clouds (Ortiz, *et al*, 1979). The 0.45-micron MD glass fiber aerosol had the highest mass fraction penetrating the cyclones (81%) followed by the 1.8-micron MD RCF,(33%). The 3.1-micron MD glass fiber cyclone penetration value was 30%, the 6.1-micron MD glass fiber 19%, the 2.7-micron MD mineral wool 15%, and the 5.4-micron MD glass fiber 13%.

It is important to recognize that the data presented in Table IX are a function of measuring total aerosol mass penetrating the cyclones and not just fibrous mass.

Building insulations such as the 5.4- and the 6.1-micron MD fibrous glass and the 2.7-micron MD mineral wool are heterogeneous bulk products consisting of large fractions of both fibrous and nonfibrous materials. Hence, they result in heterogeneous aerosols. The problem is further complicated as more nonfibrous debris is produced from the chopping of coarse diameter fibers during aerosolization with this system. Aerosols generated from these coarse materials contain significant quantities of nonfibrous particulates, many capable of penetrating cyclone samplers. Thus, for these coarse, heterogeneous materials, cyclone penetration did not have a direct relationship to the predicted "respirable" fraction based on mean fiber diameter as observed with the more homogeneous fibrous materials like the 0.45-micron MD glass fiber, the 1.8-micron RCF, and the 3.1-micron MD glass fiber.

2. UICC Crocidolite Asbestos

Anderson impactor characterization of this aerosol demonstrated a mass median aerodynamic diameter (MMAD) of 2.5 ± 0.2 microns with a geometric standard deviation of 1.9 ± 0.1 microns. These impactor size parameters define a 65 ± 4% "respirable" mass fraction when related to the ACGIH respirable dust curve (ATC, 1970), and a 76 ± 5% "respirable" fraction when compared to the BMRC standard (Moss and Ettinger, 1970; BMRC, 1961; Hamilton and Walton, 1961).

3. Fibrous/NonFibrous Particulates

After the animal inhalation experiments were completed, we quantified the relative nonfibrous-to-fibrous particulate concentration in each of the exposure aerosols (Table X). The measurements demonstrate three important aspects regarding these "fibrous" challenge aerosols: (1) all exposure aerosols contained more nonfibers than fibers; (2) the nonfibrous content of four of the "fibrous" aerosols was significant when addressed in terms of total particle concentration (i.e., particles cm^{-3}); and (3) total particle count measurements of this nature could be important when assessing exposure aerosol effects as high concentrations of "respirable" particles are known to influence lung clearance kinetics.

4. Elemental Composition

Elemental compositions of both bulk and collected aerosol materials were determined for each fiber using SEM EDX spectrometry (Table XI). Specific elements detected as compositional materials in the fibrous materials ranged from Na, atomic number 11, to Ce, atomic number 58. The 2.7-micron MD mineral wool fiber exhibited the greatest variety of constituent materials with nine different elements detected and the 1.8-micron MD RCF the fewest, having only two major elements (Al & Si) with a possible trace of Ti. All MMVF contained Al and Si and most, except the 1.8-micron MD RCF, contained Ca and K in significant quantities.

D. Inhalation Experiments

When they were approximately 100 d old, male Syrian hamsters and female Osborne-Mendel (OM) rats were exposed "nose-only," 6 h a day, 5 d a week for 24 m (Smith, *et al*, 1981) to aerosolized MMVF, crocidolite asbestos, or clean air (Table I). Unmanipulated cage control animals were also included as part of the study. In addition, groups of hamsters and rats were exposed to crocidolite for one m and other groups for 1 d (Table I). Approximately 1900 animals were used in the inhalation experiments.

Following the 24-m exposures, the remaining experimental and control animals were maintained for the rest of their lives

E. Intraperitoneal (IP) Injection Experiments

One-hundred-d-old male Syrian hamsters and female OM rats were injected IP with 25-mg fibers suspended in 0.5 ml physiological saline. Fibers injected were 0.45-micron MD glass, 1.8-micron MD RCF, and crocidolite asbestos. Fibers used for these injections were collected airborne materials from the respective inhalation exposure chambers. Thus, they reflected what the animals were exposed to in the inhalation experiments. All animals were then maintained for the duration of their lives

F. Intratracheal (IT) Instillation Experiments

Two mg of each fiber suspended in 0.2 ml of physiological saline were instilled IT to groups of hamsters and rats This suspension was instilled once a week for five weeks (10 mg total) (Smith, *et al*, 1974) under ether anesthesia. Fiber types instilled were 0.45-micron MD glass, 1.8-micron MD RCF, and crocidolite asbestos. Fibers used for these instillations were collected airborne materials from the respective inhalation exposure chambers, reflecting materials to which the animals in the inhalation groups were exposed. All animals were maintained for the duration of their lives.

G. Animals and Care

The hamsters were purchased from Engle Laboratory Animals (Hammond IN) and the rats from Camm Research Laboratory Animals (Wayne NJ) All animals were housed in class-100 laminar flow clean rooms (Hazleton Systems, Inc., Cornwell Heights PA), three hamsters or two rats to a polycarbonate cage containing low-dust aspen wood shavings. The cages were suspended on aluminum shelves and covered with spun polyester filters (DuPont #2 Spinbonded^{R} Polyester Filter, E.I. DuPont Co., Wilmington DE). Cages were cleaned and shavings changed twice a week. The hamsters were fed Teklad Hamster Diet^{R} and the rats Teklad Rat and Mouse Diet^{R} (Teklad Mills, Winfield IA). Chlorinated water was provided *ad libitum*, and the animal rooms were kept on a 12-h light-dark cycle.

Every animal was examined at least twice a day during the week and at least once a day on weekends. Those that appeared moribund were killed with an overdose of Nembutal^{R} injected IP Complete necropsies were performed as soon as possible The lungs of each animal were inflated via the trachea with 10% neutral buffered formalin and the trachea ligated Respiratory tracts, distal to the larynx, were removed *en bloc* and fixed in 10% neutral buffered formalin along with samples of other major organ systems and any tissues that had macroscopic lesions. Tissues were fixed for at least 72 h, processed by standard methods, sectioned at 5 microns, and routinely stained with hematoxylin and eosin. Additional selected histochemical stains were used when appropriate Pulmonary lesions were classified and graded according to the Wagner/WHO system (McConnell, *et al*, 1984, Wagner, *et al*, 1984)

Lungs from 1 to 6 animal(s), usually 3-6, of each specie in each inhalation exposure group were selected and the number of fibers deposited determined using Johnson *et al*'s (1984B) sodium hypochlorite digestion and SEM counting technique No lungs were used for dosimetry purposes if there were any macroscopic lesions present

III. Results and Discussion

A. Lifespans

The chamber control group of hamsters and all the groups of hamsters exposed to aerosolized MMVF, except the hamsters in the group inhaling the 3.1-micron MD glass fibers, lived significantly longer than their unmanipulated cage control counterparts (Table XII). The hamsters in the 3.1-micron MD glass fiber group lived longer than the unmanipulated cage controls, but the increase was not statistically significant. The shorter mean lifespan in that group of hamsters resulted from a few animals dying of causes unrelated to the exposure early into the 24-m exposure protocol. The group of OM rats exposed to the 3.1-micron MD glass fiber, in contrast, has the longest mean lifespan (803 d) of any group of animals in the study (Table XII).

Because a few hamsters in the first group exposed to the 6.1-micron MD glass fiber died unexpectedly, of causes unrelated to the exposures, we started a second group of hamsters to prevent any experimental bias in development of lesions in a lifespan study. The second group of hamsters had a mean lifespan (615 d) that was 39 d shorter than the first group but still significantly longer than the unmanipulated cage controls.

Two groups of hamsters exposed to airborne crocidolite had mean lifespans that were remarkably less than chamber controls (Table XII). The 24-m exposure group had a mean lifespan of 550 d and the 1-d group, 576 d. However, neither of these values varies significantly from the unmanipulated-cage-control mean lifespan for hamsters of 563 d, a high value but one nevertheless consistent with our previous experience (Thomas and Smith, 1979; Smith, *et al*, 1976).

Mean lifespans for the chamber control OM rats (754 d) were longer than for the unmanipulated cage controls (724 d), but the differences were not statistically significant. Two groups of OM rats exposed to MMVF (the 5.4-micron MD glass fiber, high level, and the 2.7-micron MD mineral wool fiber) had lifespans significantly shorter than either the chamber or unmanipulated cage control groups (Table XII). Groups of hamsters exposed to these same fibers had lifespans that did not vary significantly from their control counterparts. Thus, the differences in the rats' lifespans are probably of little or no biological significance. Little lifespan data are available for unmanipulated OM rats; however, the lifespans for all the groups of OM rats in the inhalation experiments are thought to be at least average and probably significantly longer than average (P. Hansen, 1986).

The hamsters and OM rats in the inhalation studies all had remarkably long mean and median lifespans, optimizing the probability that diseases with long latent periods, such as pulmonary fibrosis (asbestosis) or primary lung tumor induction, would be expressed in these experiments.

Hamsters injected IP with the 0.45-micron MD glass fiber had lifespans not statistically different from either physiological-saline-injected or unmanipulated cage controls (Table XIII). When the first group of 36 hamsters was injected with 25 mg RCF, 21 of them died within 30 d from acute hemorrhagic peritonitis and vascular collapse--the result of fluid extravasation into the abdominal cavity. To confirm this experience as a pattern, a second group of hamsters was likewise injected IP with 25 mg of the same RCF; 15 of 36 died within 30 d of acute hemorrhagic peritonitis. Lifespans for the hamsters surviving the initial 30 d were 462 d and 489 d, both statistically

significantly shorter than the values for either set of controls (Table XIII). Hamsters injected IP with 25 mg of UICC crocidolite had significantly reduced lifespans, the result of degenerative abdominal reactive tissue lesions and/or mesothelioma induction.

OM rats receiving the injected RCF or crocidolite IP had significantly reduced mean lifespans resulting from the induction of abdominal mesotheliomas (see below).

One group of hamsters receiving fibers IT with a reduced mean lifespan compared to controls was the one receiving five instillations of RCF (Table XIV), but none of these RCF-instilled hamsters developed primary lung tumors, and the incidence of pulmonary fibrosis in these animals was not elevated. None of the groups of OM rats instilled IT with fibers had significantly reduced lifespans compared to their control counterparts.

B. Pulmonary Lesions

In the inhalation experiments, groups of rats exposed to a fiber uniformly had higher Wagner/WHO lung lesion grades than groups of hamsters exposed to the same fiber (Table XV).

None of the inhalation exposure groups had mean lung lesion grades >4, the value where collagen deposition is first observed. Because lesion grades <4 do not have a fibrous component, they are thought to be potentially reversible. In this study, we were unable to detect any morphologic progression of pulmonary lesions exposed via inhalation to MMVF after the 24-m exposures were completed. There were relatively few pulmonary lesions, other than the presence of macrophages containing fibers. When they did occur, fibrotic lesions in animals exposed to MMVF, were peribronchiolar as also reported earlier by Kuschner and Wright (1976) after the instillation IT of fibrous glass in guinea pigs. Lee, *et al* (1981) likewise did not observe significant fibrosis induction in Sprague-Dawley rats, Syrian hamsters, or albino guinea pigs inhaling fibrous glass. The predominant lesion they did see, alveolar proteinosis, regressed after the inhalation exposures were completed.

The highest mean lung lesion grades in the inhalation groups occurred in the animals exposed to crocidolite for either 30 d or 24 m. The number of hamsters and rats in the 24-m crocidolite exposure group and hamsters in the 30-d crocidolite exposure group with fibrous pulmonary lesions--early stages of asbestosis--was statistically higher than either their chamber or unmanipulated cage control counterparts (Table XV), a finding consistent with other inhalation studies using rats and crocidolite (Wagner, *et al*, 1974) or chrysotile asbestos (Pinkerton, *et al*, 1984; Wagner, *et al*, 1980).

Bronchoalveolar metaplasia, possibly a preneoplastic event in the development of epithelial tumors, was significantly elevated only in those hamsters exposed to crocidolite for 24 m (Table XV); however, none of these animals developed primary lung tumors

One of 70 hamsters in the 1.8-micron MD RCF inhalation exposure group died after a 10-m exposure with a 2-cm-diameter spindle cell mesothelioma arising from the ventral posterior left lung. The tumor's presence was not statistically significant and would be readily dismissed if it were not for the association between fibers and mesothelioma induction (Wagner, 1986). Even with this relationship between fibers and mesotheliomas, the possibility exists that the tumor was a spontaneous event.

Interestingly, there was minimal stromal reaction or fibrosis in the lungs of this animal; RCF and ferruginous bodies were present in the pulmonary parenchyma and detectable with light microscopy. Though spontaneous mesotheliomas in the Syrian hamster are rare, one has been reported in an adult male (Fortner, 1961); whether it was a thoracic or abdominal tumor was not noted. Our findings are in contrast with those of Davis, *et al* (1984) who found that 8 of 48 Wistar rats exposed to aerosolized ceramic fibers (8.4 mg m^{-3}, 95 F cm^{-3}) for 12 m developed pulmonary neoplasms. Four of the tumors in the Davis study were epithelial. The other 4 tumors were thought to be malignant histiocytomas.

None of the other animals exposed to MMVF in our study developed primary lung tumors. One large peripherally located benign mucous-secreting bronchoalveolar tumor (BAT) was found in a chamber control hamster (Table XV). Three of 57 rats in the 24-m crocidolite inhalation exposure group developed lung tumors: 1 fibrous mesothelioma and 2 BATs. One rat in the 30-d crocidolite group had a borderline BAT and 1 rat in the 1-d crocidolite exposure group developed a hemangiosarcoma of the lung. Wagner, *et al* (1974) had much higher lung tumor incidence in the Wistar rats that they exposed to airborne UICC crocidolite asbestos: 6/43 animals were exposed for 1 d and 13/18 animals for 24 m, although only 4 of the total 45 tumors induced by crocidolite were mesotheliomas. The crocidolite-induced lung tumor incidence in our study might have been higher had we used a longer fiber as suggested by the the work of Wagner, *et al* (1984); Wagner's study delineated the effect of fiber size on the *in vivo* activity of UICC crocidolite asbestos.

C. Fiber Deposition and Recovery

Significant numbers of fibers were deposited in the lungs of all groups of animals inhaling MMVF and crocidolite (Table XVI). Fibers were detectable microscopically in virtually every section of lung from animals exposed after 2 m to the fine diameter fibers and after 3-4 m exposure to the larger diameter MMVF. No fibers were ever found microscopically in any of the controls. The fibers were almost always found phagocytized in parabronchial, parabronchiolar, or subpleural foci of syncytial macrophages in alveolar spaces. Acute inflammation was not a component of the response and the fibers in hamsters were often coated with ferruginous bodies. We did not see ferruginous bodies coating fibers in any of the sections of lungs from the OM rats, including those exposed to RCF, in contrast to Davis *et al*'s (1984) finding ferruginous bodies in their rats exposed to ceramic fibers. Morgan and Holmes have also reported (1984) that hamsters more readily than rats form pseudoasbestos (ferruginous) bodies coating fibers deposited in the respiratory tract.

Fibers with finer diameters and enhanced "respirabilities," were recovered in larger quantities from the the lung than fibers with larger diameters. Fiber recoveries were comparable to those reported from another laboratory using similar challenge aerosols (Johnson *et al*, 1984A, 1984B). Few short fibers were recovered from animals in any of the exposure groups; recovered, retained fibers were generally 8-10 microns long. With characteristic insight, Kuschner and Wright noted in 1976 that longer glass fibers were preferentially retained in pulmonary tissue, suggesting that short fibers were possibly removed selectively from the pulmonary milieu by alveolar macrophages. The glass fibers and the mineral wool fibers were highly etched as also reported by Johnson *et al* (1984A). In contrast, we were unable to discern any etching of the recovered RCFs.

D. Intraperitoneal Injection Experiments, Abdominal Lesions

Eight of 25 (32%) OM rats had abdominal mesotheliomas at necropsy (Table XVII), consistent with earlier intrathoracic implantation findings for glass fibers of this diameter (Stanton *et al*, 1977; Stanton and Wrench, 1972). Pott *et al* (1984) found fewer primary abdominal tumors when they injected the same diameter glass fiber IP, but they used much smaller doses (2 and 10 mg).

The incidences of abdominal tumors in hamsters (Table XVII) injected with RCF were 13% and 24% and the incidence of abdominal tumors in the OM rats was 19/23 (83%).

Forty percent of the hamsters and 80% of the OM rats injected with crocidolite had abdominal mesotheliomas at their deaths.

E. Intratracheal Instillation Experiments, Pulmonary Lesions

None of the animals instilled IT with the two types of MMVF developed primary lung tumors (Table XVIII). Six of the 22 OM rats instilled with RCF did have bronchiolar epithelial polypoid lesions, focal pedunculated proliferations of single layers of normal-appearing epithelium into the lumens of the peripheral bronchial tree. These were probably chronic inflammatory responses to the presence of very large quantities of foreign material deposited in the bronchial tree.

Seventy-four percent of the hamsters and 8% of the OM rats instilled IT with crocidolite developed primary lung tumors, all epithelial (Table XVIII). Thirteen of the 20 tumors (all BATs) in hamsters were benign, seven malignant. The two tumors in the rats were malignant BATs. Both the groups of hamsters and rats developed significant pulmonary fibrosis (Table XVIII) and they had lung lesion grades >4.

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This program benefited from numerous singularly talented, dedicated individuals To name them all would be almost impossible. We are especially indebted to the members of the TIMA Medical and Scientific Committee, without whose financial, moral, and scientific input none of this work would have been possible. Two members of that Committee who made especially invaluable contributions and whom we want to remember are Clifford L. Sheckler and James P. Leineweber.

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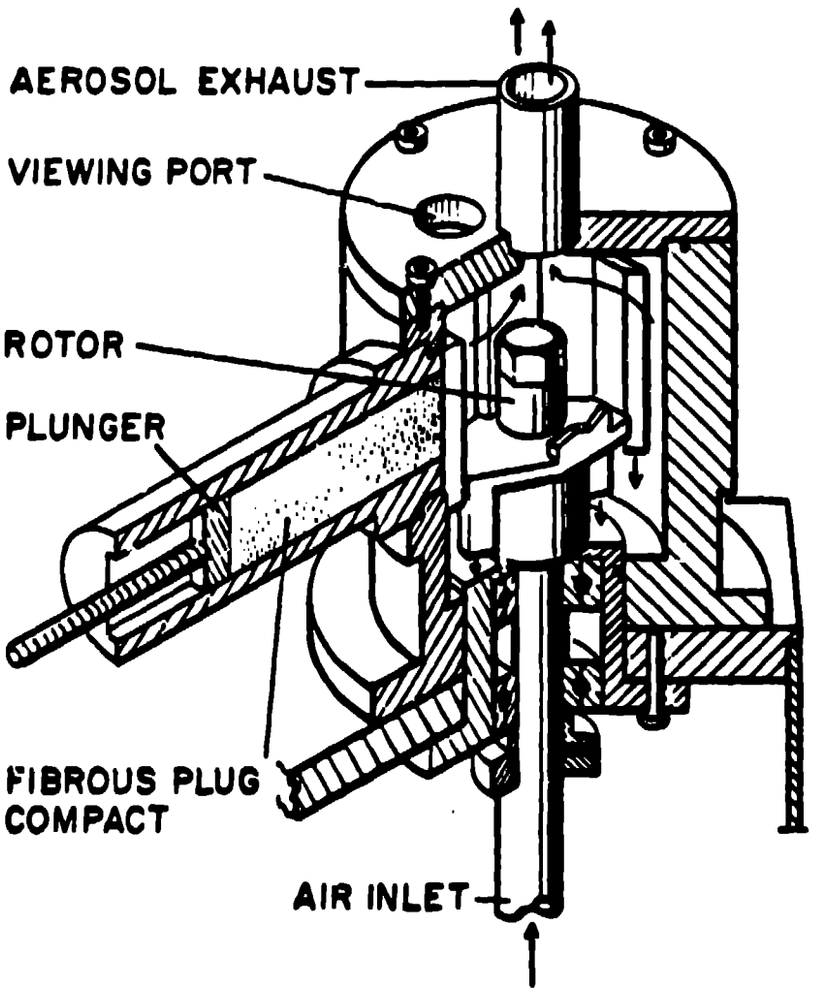
Figure Captions

Figure 1. Schematic representation of fibrous aerosol generator.

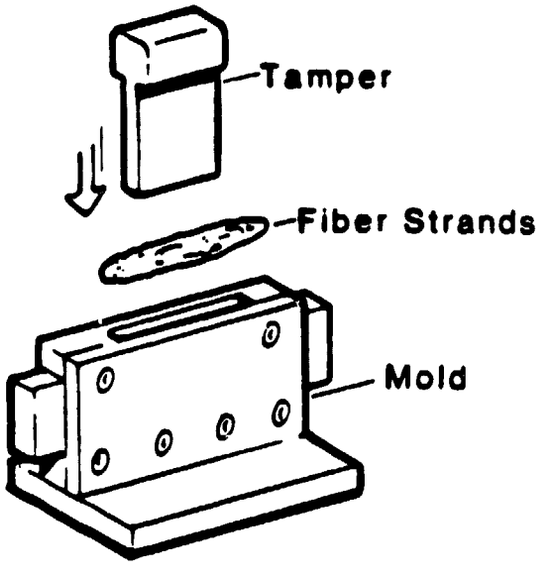
Figure 2. Schematic representation of fibrous plug preparation and loading of aerosol generator.

FIGURE 1

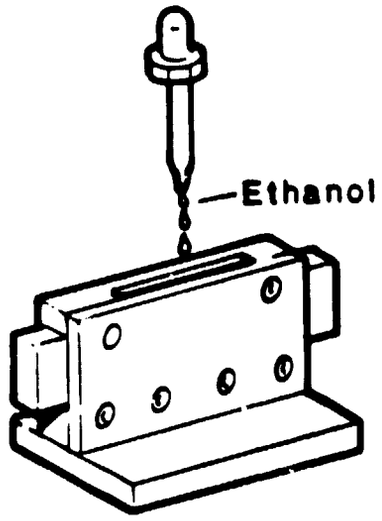


FIBROUS AEROSOL GENERATOR

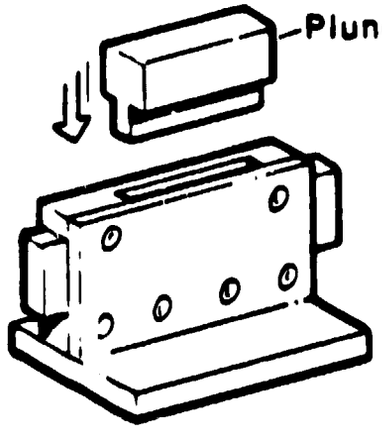
FIGURE 2



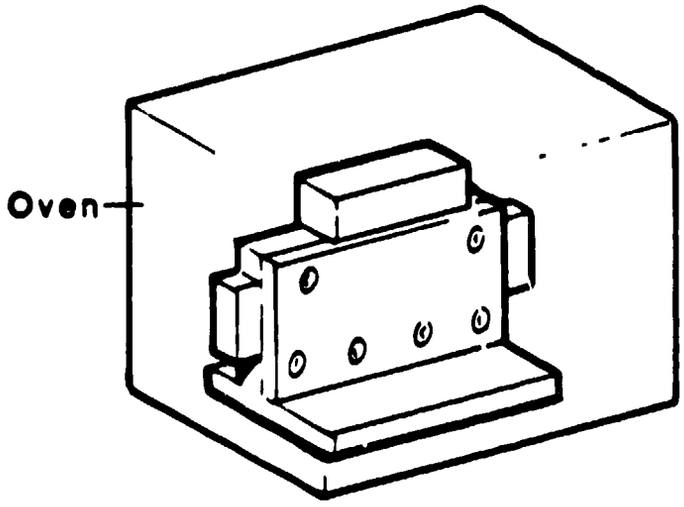
1. Dry Tamping of Preweighed Fiber Strands



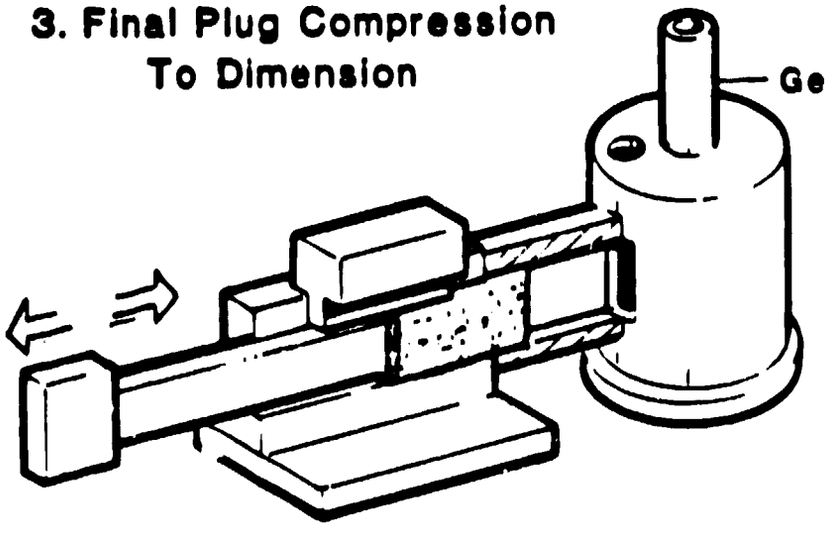
2. "Wetting" Tamped Strands with Ethanol (~10 cc)



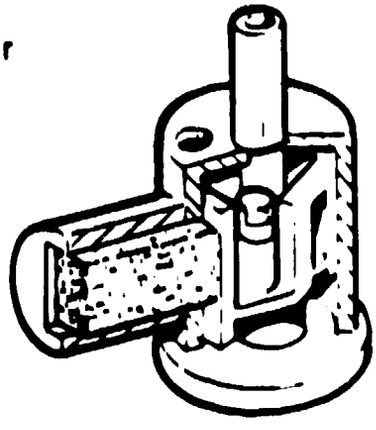
3. Final Plug Compression To Dimension



4. Oven Drying Overnight (80°c)



5. Plug Transfer Frcm Mold To Generator



6. Plug Loaded Generator

Table I

EXPERIMENTAL DESIGN - INHALATION EXPERIMENTS

FIBER	FIBERS cm^{-3}	MASS CONCENTRATIONS $mg\ m^{-3}$
0.45 μM MD Glass (No Binder)		
High Level	3000	3.0
Low Level	300	0.3
3.1 μM MD Glass (Silicone Lubricant)	100	10.0
5.4 μM MD Glass (Binder Coated)		
High Level	100	12.0
Low Level	10	1.2
6.1 μM MD Glass (Binder Coated)	25	9.0
1.8 μM MD Refractory Ceramic (No Binder)	200	12.0
2.7 μM MD Mineral Wool (No Binder)	200	10.0
UICC Crocidolite Asbestos		
24 - Month Exposure	3000	7.0
30 - Day Exposure	3000	7.0
1 - Day Exposure	3000	7.0
Chamber Controls (Clean Air)	-	-
Unmanipulated Cage Controls	-	-

TABLE II

FIBER DIMENSIONS

FIBROUS GLASS (0.45 μm MEAN DIAMETER) EXPOSURE AEROSOL

DIAMETER/LENGTH MATRIX

Diameter (μm)	Length (μm) 0 - 2	3 - 4	5 - 10	11 - 15	16 - 20	21 - 25	26 - 30	31 - 40	> 40	Σ
0.1	11	7	0	0	0	0	0	0	0	18
0.2	64	69	50	6	0	0	0	1	0	190
0.3	37	47	35	3	2	1	0	0	0	125
0.4	32	63	58	14	6	3	0	0	0	176
0.5	11	49	77	17	6	5	0	3	0	168
0.6	2	34	52	21	3	3	3	3	0	121
0.7	0	5	39	13	4	2	3	3	1	70
0.8	0	4	24	10	5	3	0	6	3	55
0.9	0	1	7	2	3	1	0	3	1	18
1.0	0	0	18	7	2	2	0	1	2	32
1.2	0	2	8	3	0	0	0	1	2	16
1.5	0	0	2	0	2	0	1	1	4	10
Σ	157	218	370	96	33	20	7	22	13	999
PER CENT	16	28	37	10	3	2	1	2	1	
CUMULATIVE PER CENT	16	44	81	91	94	96	1	99	100	

ML = 7.5 ± 10 μm
MD = 0.4 ± 0.3 μm
AR = 15
Longest = 126 μm

GML = 4.9 μm
GMD = 0.4 μm
GMAR = 11.3

TABLE III

FIBER DIMENSIONS
FIBROUS GLASS "BLOWING WOOL" (3.1 μM MEAN DIAMETER) EXPOSURE AEROSOL
Diameter/Length Matrix
Length (μm)

D (μm) ↓	<5	6 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 80	81 - 100	>100	Σ
< 0.1	0	0	0	0	0	0	0	0	0	0
0.2-0.4	0	10	13	2	0	1	1	0	0	27
0.5-0.7	1	13	12	9	1	0	0	0	0	36
0.8-1.0	3	52	47	35	9	9	10	3	2	170
1.1-1.3	0	24	17	14	11	4	4	2	2	78
1.4-1.6	0	7	26	13	8	2	14	4	3	79
1.7-1.9	0	0	7	4	4	2	3	1	2	23
2.0-2.2	0	2	6	6	6	0	4	3	7	34
2.3-2.5	0	0	2	2	0	0	3	1	0	8
2.6-2.8	0	0	0	2	3	0	4	2	3	14
2.9-3.1	0	0	1	0	0	0	3	0	5	9
3.2-3.5	0	0	0	0	3	0	2	1	2	8
> 3.5	0	0	1	2	2	1	7	1	7	21
Σ	4	108	134	89	47	19	55	18	33	507
Per cent	1	21	26	18	9	4	11	4	6	
Cumulative Per cent	1	22	48	66	75	79	90	94	100	

ML: 37 ± 48 μm
MD: 1.4 ± 1.1 μm
AR: 27
Longest: 505 μm

GML: 24 μm
GMD: 1.2 μm
GMAR: 19

TABLE IV

FIBER DIMENSIONS

FLAME ATTENUATED FIBROUS GLASS (5.4 μM MEAN DIAMETER) EXPOSURE AEROSOL

Diameter/Length Matrix

Length (μm)

(μm) ↓	<5	6 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 80	81 - 100	>100	Σ
0.1	0	0	0	0	0	0	0	0	0	0
2-0.4	10	21	16	1	0	1	1	0	0	50
5-0.7	6	17	18	1	1	1	1	0	0	45
8-1.0	3	14	27	3	3	0	7	1	0	58
1-1.3	2	7	10	4	3	2	4	0	0	32
4-1.6	0	4	17	8	5	3	4	2	0	43
7-1.9	0	0	3	2	5	1	2	0	3	16
0-2.2	0	0	11	6	3	5	7	2	4	38
3-2.5	0	0	4	3	1	0	0	2	3	13
6-2.8	0	1	0	4	0	5	1	0	3	14
9-3.1	0	0	0	0	0	1	1	1	0	3
2-3.5	0	0	1	1	0	0	1	2	2	7
3.5	0	0	1	2	2	1	2	1	2	11
Σ	21	64	108	35	23	20	31	11	17	330
cent	6	19	34	11	7	6	9	3	5	
ulative r cent	6	25	59	70	77	83	92	95	100	

:	31 ± 33 μm	GML:	20 μm
:	1.4 ± 0.9 μm	GMD:	1.1 μm
:	24	GMAR:	18
gest:	198 μm		

TABLE V

FIBER DIMENSIONS

FIBROUS GLASS (6.1 μm MEAN DIAMETER) EXPOSURE AEROSOL

Diameter/Length Matrix

Length (μm)

D (μm) ↓	<5	6 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 80	81 - 100	>100	Σ
< 0.1	0	0	0	0	0	0	0	0	0	0
0.2-0.4	0	1	3	2	2	1	2	0	1	12
0.5-0.7	0	2	2	1	2	1	2	1	0	11
0.8-1.0	0	2	11	16	15	9	13	2	9	77
1.1-1.3	0	2	3	0	1	1	1	2	3	13
1.4-1.6	0	0	1	4	7	6	16	6	14	54
1.7-1.9	0	0	0	1	1	1	0	1	0	4
2.0-2.2	0	0	1	3	2	6	14	4	12	42
2.3-2.5	0	0	0	4	2	1	4	6	13	30
2.6-2.8	0	0	0	0	0	0	0	0	2	2
2.9-3.1	0	0	1	3	2	0	12	7	31	56
3.2-3.5	0	0	0	0	2	2	5	4	13	26
> 3.5	0	0	0	3	2	5	17	12	93	132
Σ	0	7	22	37	38	33	86	45	191	459
Per cent	0	1	5	8	8	7	19	10	42	
Cumulative Per cent	0	1	6	14	22	29	48	58	100	

GML: 114 ± 94 μm
GMD: 3.0 ± 2.2 μm
GMAR: 49
Longest: 510 μm

GML: 83 μm
GMD: 3.0 μm
GMAR: 36

TABLE VI

FIBER DIMENSIONS

REFRACTORY/CERAMIC (1.8 μm MEAN DIAMETER) EXPOSURE AEROSOL

DIAMETER/LENGTH MATRIX

	Length (μm)									
D(μm)	≤5	6-10	11-20	21-30	31-40	41-50	51-80	81-100	≥100	Σ
<0.1	0	0	0	0	0	0	0	0	0	0
0.2-0.4	5	19	27	12	3	4	0	1	0	71
0.5-0.7	2	18	19	9	8	2	5	2	1	66
0.8-1.0	1	17	33	19	16	11	15	0	4	116
1.1-1.3	0	8	17	15	11	5	7	2	3	68
1.4-1.6	0	5	16	5	6	9	6	2	5	54
1.7-1.9	0	2	7	4	0	0	1	4	1	19
2.0-2.2	0	0	10	6	4	5	5	1	7	38
2.3-2.5	0	0	4	1	2	0	4	1	0	12
2.6-2.8	0	1	0	2	1	2	1	0	1	8
2.9-3.1	0	0	0	1	1	0	0	0	1	3
3.2-3.5	0	0	0	0	0	0	0	0	1	1
> 3.5	0	0	0	1	0	0	1	1	0	3
Σ	8	70	133	75	52	38	45	14	24	459
Per Cent	2	15	30	16	11	8	10	3	5	
Cumulative Per Cent	2	17	47	63	74	82	92	95	100	

GML: 35 ± 34 μm
GMD: 1.1 ± 0.7 μm
MAR: 36
Longest: 230 μm

GML: 25 μm
GMD: 0.9 μm
GMAR: 26

TABLE VII

FIBER DIMENSIONS
MINERAL WOOL (2.7 μm MEAN DIAMETER) EXPOSURE AEROSOL
Diameter/Length Matrix
Length (μm)

) (μm) ↓	<5	6 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 80	81 - 100	>100	Σ
:0.1	0	1	2	6	3	1	3	0	0	16
).2-0.4	12	39	19	0	0	0	0	0	0	70
.5-0.7	8	32	40	13	4	7	4	1	2	111
.8-1.0	1	12	24	12	14	3	5	1	6	78
.1-1.3	0	5	16	6	1	2	3	2	8	43
.4-1.6	0	2	12	10	6	4	6	2	7	49
.7-1.9	0	0	5	2	2	2	1	1	4	17
.0-2.2	0	0	3	4	3	4	6	1	5	26
3-2.5	0	0	2	1	0	1	1	1	1	7
6-2.8	0	0	0	1	2	0	0	0	1	4
9-3.1	0	0	1	0	1	2	0	1	3	8
2-3.5	0	0	0	1	1	1	0	2	3	8
.5	0	0	1	1	0	1	1	1	5	10
Σ	21	91	125	57	37	28	30	13	45	447
cent	5	20	28	13	8	6	7	3	10	
mulative cent	5	25	53	66	74	80	87	90	100	

:
:
:
GEST:

40 ± 63
1.1 ± 0.9
40
710 μm

GML: 22 μm
GMD: 0.9 μm
GMAR: 26

TABLE VIII

UICC CROCIDOLITE - EXPOSURE AEROSOL
COMPARATIVE FIBER LENGTH DISTRIBUTION: UICC*/LOS ALAMOS
(PER CENT BY COUNT IN GIVEN SIZE RANGE μm)

	0.2 - 0.5	0.5 - 1.0	1 - 2	2 - 5	5 - 10	10 - 25	> 25	No. of Fibers Sized
UICC DATA* (TEM) BULK SAMPLE	28.5	35.8	22.5	10.3	2.3	0.6	0.1	--
LOS ALAMOS DATA AEROSOL SAMPLE (SEM)	0	29	44	20	4	1	2	345
LOS ALAMOS DATA BULK SAMPLE (SEM)	0	25	49	22	2	1	2	336

* DATA SHEETS ON THE CHEMICAL AND PHYSICAL PROPERTIES OF THE UICC REFERENCE SAMPLES R.E.G. RENDALL, N.R.I.O.D., JOHANNESBURGH, SOUTH AFRICA

TABLE IX

CYCLONE* SAMPLER PENETRATION DATA

Fibrous Aerosol Material	Challenge Aerosol Mass Conc (mg/m^3) (Mean)	(Std Dev)	Cyclone Penetration ("Respirable Mass Fraction") (%w) (Mean)	(Std Dev)	No. of Cyclone Samples
Fibrous Glass (0.45 μm mean diameter) (MD)	2.4	0.5	81.0	3.7	48
Refractory/Ceramic Fibers (1.8 μm MD)	10.8	3.4	34.5	7.1	39
"Blowing Wool" Bldg. Insulation (3.1 μm MD)	4.4	1.7	30.1	5.7	51
Binder Coated Bldg Insulation (6.1 μm MD)	7.0	3.0	18.7	8.5	27
Mineral Wool Fibers (2.7 μm MD)	7.8	3.1	15.2	7.7	27
Fibrous Glass Building Insulation (5.4 μm MD)	9.9	3.8	12.7	4.8	30
					222

* Cyclone Samplers: 10-mm nylon (Dorr-Oliver) operated at 1.7 L/min

TABLE X

MMVF EXPOSURE AEROSOLS
NONFIBROUS/FIBROUS PARTICULATE CONCENTRATIONS

FIBER DESIGNATION BY MEAN DIAMETER (uM)	TOTAL PARTICLES COUNTED NON-FIBERS	FIBERS	PARTICLE RATIO NON-FIBERS/FIBERS
0.45	465	125	4:1
1.8	3491	106	33:1
2.7	1821	66	28:1
3.1	416	71	6:1
5.4	2034	53	38:1
6.1	2620	84	31:1

TABLE XI

ELEMENTAL COMPOSITION OF FIBROUS EXPOSURE AEROSOLS

Fiber Designation By Mean Diameter (μm)	Na	Mg	Al	Si	S	Cl	K	Ca	Ti	Mn	Fe	Zn	Ba	Ce
0.45	X		X	X			X	X				X	X	X
1.8			X	X					X					
2.7		X	X	X	X		X	X	X	X	X			
3.1	X		X	X			X	X			X			
5.4	X	X	X	X	X	X	X	X						
6.1	X	X	X	X	X	X	X	X			X			
Crocidolite				X				X			X			

00781

Table XII

LIFESPANS (DAYS) FOR ANIMALS EXPOSED TO AEROSOLIZED FIBERS

EXPOSURE GROUP	MEAN LIFESPAN	MEDIAN LIFESPAN
0.45 μM MD Glass		
High Level (3000 F cm^{-3})		
Syrian Hamsters	665 ± 15	670
CM Rats	774 ± 29	834
Low Level (300 F cm^{-3})		
Syrian Hamsters	665 ± 18	707
CM Rats	756 ± 23	771
3.1 μM MD Glass (100 F cm^{-3})		
Syrian Hamsters	594 ± 24	661
CM Rats	803 ± 25	814
5.4 μM MD Glass		
High Level (100 F cm^{-3})		
Syrian Hamsters	628 ± 22	653
CM Rats	660 ± 30	681
Low Level (10 F cm^{-3})		
Syrian Hamsters	680 ± 19	722
CM Rats	726 ± 22	730
6.1 μM MD glass (25 F cm^{-3})		
Syrian Hamsters		
1st Group	654 ± 23	700
2nd Group	615 ± 24	627
CM Rats	702 ± 27	747
1.8 μM MD Refractory Ceramic (200 F cm^{-3})		
Syrian Hamsters	670 ± 20	698
CM Rats	703 ± 25	740
2.7 μM MD Mineral Wool (200 F cm^{-3})		
Syrian Hamsters	666 ± 21	725
CM Rats	677 ± 28	692

Table XII Continued

UICC Crocidolite Asbestos (3000 F cm^{-3})		
24-Month Exposure		
Syrian Hamsters	550 ± 20	537
CM Rats	763 ± 21	782
30-Day Exposure		
Syrian Hamsters	670 ± 21	680
CM Rats	784 ± 19	798
1-Day Exposure		
Syrian Hamsters	576 ± 26	524
CM Rats	694 ± 29	701
Chamber Controls (Clean Air)		
Syrian Hamsters	664 ± 16	682
CM Rats	754 ± 19	773
Unmanipulated Cage Controls		
Syrian Hamsters	563 ± 16	553
CM Rats	724 ± 16	741

Table XIII

LIFESPANS (DAYS) FOR ANIMALS INJECTED INTRAPERITONEALLY WITH FIBERS (25mg)

EXPOSURE GROUP	MEAN LIFESPAN	MEDIAN LIFESPAN
0.45 μM MD Glass		
OM Rats	593 ± 34	594
1.8 μM MD Refractory Ceramic		
Syrian Hamsters[a]		
1st Group	462 ± 14	480
2nd Group	489 ± 38	449
OM Rats	480 ± 32	445
UICC Crocidolite Asbestos		
Syrian Hamsters	385 ± 50	509
OM Rats	580 ± 29	582
Saline Controls		
Syrian Hamsters	560 ± 31	541
OM Rats	744 ± 28	747
Unmanipulated Cage Controls		
Syrian Hamsters	503 ± 16	553
OM Rats	724 ± 16	741

[a] Those animals surviving initial episode of hemorrhagic peritonitis and death.

Table XIV

LIFESPANS (DAYS) FOR ANIMALS INSTILLED INTRATRACHEALLY
WITH FIBERS (10 mg Total)

EXPOSURE GROUP	MEAN LIFESPAN	MEDIAN LIFESPAN
0.45 μM MD Glass		
OM Rats	783 ± 31	789
1.8 μM MD Refractory Ceramic		
Syrian Hamsters	446 ± 29	479
Om Rats	698 ± 35	736
UICC Crocidolite Asbestos		
Syrian Hamsters	594 ± 50	657
OM Rats	639 ± 56	663
Saline Controls		
Syrian Hamsters	567 ± 32	569
OM Rats	688 ± 34	696
Unmanipulated Cage Controls		
Syrian Hamsters	563 ± 16	553
OM Rats	724 ± 16	741

Table XV

PULMONARY LESIONS IN ANIMALS EXPOSED TO AEROSOLIZED FIBERS

EXPOSURE GROUP	LESION GRADE	BRONCHO-ALVEOLAR METAPLASIA	FIBROSIS	PRIMARY TUMORS
0.45 μM MD Glass				
High Level (3000 F cm^{-3})				
Syrian Hamsters	2.3 ± 0.1	6/69 (9%)	4/69 (6%)	0/69
OM Rats	2.3 ± 0.1	4/57 (7%)	4/57 (7%)	0/57
Low Level (300 cm^{-3})				
Syrian Hamsters	2.1 ± 0.1	2/70 (3%)	3/70 (4%)	0/70
OM Rats	2.3 ± 0.1	4/57 (7%)	3/57 (5%)	0/57
3.1 μM MD Glass (100 F cm^{-3})				
Syrian Hamsters	2.1 ± 0.1	3/60 (5%)	2/60 (3%)	0/60
OM Rats	2.3 ± 0.1	1/52 (2%)	7/52 (8%)	0/52
5.4 μM MD Glass				
High Level (100 F cm^{-3})				
Syrian Hamsters	1.6 ± 0.1	1/66 (2%)	0/66	0/66
OM Rats	2.1 ± 0.1	1/57 (2%)	4/57 (7%)	0/57
Low Level (10 F cm^{-3})				
Syrian Hamsters	1.8 ± 0.1	7/65 (11%)	1/65 (2%)	0/65
OM Rats	2.4 ± 0.1	0/61	5/61 (8%)	0/61
6.1 μM MD Glass (25 F cm^{-3})				
Syrian Hamsters				
1st Group	1.9 ± 0.1	4/61 (7%)	0/61	0/61
2nd Group	1.8 ± 0.1	0/38	0/38	0/38
OM Rats	2.3 ± 0.1	1/58 (2%)	3/58 (5%)	0/58
1.8 μM MD Refractory Ceramic (200 F cm^{-3})				
Syrian Hamsters	2.1 ± 0.1	2/69 (3%)	1/70 (1%)	1/70 (1%)[a]
OM Rats	3.0 ± 0.1	1/55 (2%)	12/55 (22%)	0/55
2.7 μM MD Mineral Wool (200 F cm^{-3})				
Syrian Hamsters	2.4 ± 0.1	2/69 (3%)	1/69 (1%)	0/69
Om Rats	2.8 ± 0.1	0/55	9/55 (16%)	0/55

Table XV Continued

UICC Crocidolite Asbestos (3000 F cm^{-3})				
24-Month Exposure				
Syrian Hamsters	2.8 ± 0.1	11/58 (19%)[s]	14/58 (24%)[s]	0/58
OM Rats	3.7 ± 0.2	4/57 (8%)	30/57 (53%)[s]	3/57 (5%)[b]
30-Day Exposure				
Syrian Hamsters	2.8 ± 0.1	4/64 (6%)	14/64 (22%)[s]	0/64
OM Rats	3.1 ± 0.1	5/60 (8%)	22/61(36%)	1/61 (2%)[c]
1-Day Exposure				
Syrian Hamsters	1.9 ± 0.1	3/47 (6%)	1/47 (2%)	0/47
OM Rats	2.2 ± 0.1	5/59 (8%)	6/59 (10%)	1/59 (2%)[d]
Chamber Controls (Clean Air)				
Syrian Hamsters	1.8 ± 0.1	3/57 (5%)	4/58 (7%)	1/58 (2%)[e]
OM Rats	2.2 ± 0.1	5/59 (8%)	6/59 (10%)	0/59
Unmanipulated Cage Controls				
Syrian Hamsters	1.7 ± 0.1	4/112 (4%)	3/112 (3%)	0/112
OM Rats	2.4 ± 0.1	3/125 (3%)	17/125(14%)	0/125

[a] Mesothelioma, [b] 1 Mesothelioma, 2 Bronchoalveolar Tumors [c] Borderline Bronchoalveolar Tumor [d] Hemangoisarcoma [e] Bronchoalveolar Tumor [s] Statistically significant compared to chamber or cage controls

Table XVI

FIBER RECOVERY, INHALATION GROUPS

EXPOSURE GROUP	FIBERS mg^{-1} LUNG (DRY WEIGHT)
0.45 μM MD Glas	
High Level (3000 F cm-3)	
Syrian Hamsters	$0.96 \pm 0.33 \times 10^6$
CM Rats	$1.87 \pm 0.64 \times 10^6$
Low Level (300 F cm^{-3})	
Syrian Hamsters	$0.52 \pm 0.16 \times 10^5$
CM Rats	1.23×10^5
3.1 μM MD Glass (100 F cm^{-3})	
Syrian Hamsters	$1.15 \pm 0.32 \times 10^4$
CM Rats	$2.85 \pm 1.23 \times 10^4$
5.4 μM MD glass	
High Level (100 F cm^{-3})	
Syrian Hamsters	$2.35 \pm 1.54 \times 10^3$
CM Rats	$1.00 \pm 0.48 \times 10^3$
Low Level (10 F cm^{-3})	
Syrian Hamsters	$0.73 \pm 0.17 \times 10^2$
CM Rats	1.11×10^3
6.1 μM MD Glass (25 F cm^{-3})	
Syrian Hamsters	$5.05 \pm 1.05 \times 10^2$
CM Rats	$5.72 \pm 2.43 \times 10^2$
1.8 μM MD Refactory ceramic (200 F cm^{-3})	
Syrian Hamsters	$0.86 \pm 0.45 \times 10^4$
CM Rats	$2.18 \pm 0.99 \times 10^4$
2.7 μM MD Mineral Wool (200 F cm^{-3})	
Syrian Hamsters	$3.07 \pm 1.32 \times 10^3$
CM Rats	$3.08 \pm 1.75 \times 10^3$
UICC Crocidolite (3000 F cm^{-3})	
24-Month Exposure	
Syrian Hamsters	$7.31 \pm 0.78 \times 10^5$
CM Rats	$3.87 \pm 1.53 \times 10^5$
Chamber Controls (Clean Air)	
Syrian Hamsters	<10
CM Rats	<10

Table XVI Continued

Unimanipulated Cage Controls	
Syrian Hamsters	<10
CM Hamsters	<10

Table XVII

ABDOMINAL LESIONS IN ANIMALS INJECTED INTRAPERITONEALLY WITH FIBERS (25mg)

EXPOSURE GROUP	ABDOMINAL REACTIVE TISSUE/ FIBROSIS IN (NON TUMOR BEARING ANIMALS)	ABDOMINAL MESOTHELIOMAS
0.45 μM MD Glass		
OM Rats	13/17 (76%)	8/25 (32%)
1.8 μM MD Refractory Ceramic		
Syrian Hamsters[a]		
1st Group	13/13 (100%)	2/15 (13%)
2nd Group	16/16 (100%)	5/21 (24%)
OM Rats	4/4 (100%)	19/23 (83%)[b]
UICC Crocidolite Asbestos		
Syrian Hamsters	13/17 (76%)	8/25 (40%)
OM Rats	3/5 (60%)	20/25 (80%)
Saline Controls		
Syrian Hamsters	0/0	0/25
OM Rats	0/0	0/25
Unmanipulated Cage Controls		
Syrian Hamsters	0/0	0/112
OM Rats	0/0	0/125

[a] Those Animals surving initial episode of hemorrhagic peritonitis and death

[b] Includes one Fibrosarcoma

Table XVIII

PULMONARY LESIONS IN ANIMALS INSTILLED INTRATRACHEALLY WITH FIBERS

EXPOSURE GROUP	LESION GRADE	BRONCHO-ALVEOLAR METAPLASIA (IN NON TUMOR BEARING ANIMALS)	FIBROSIS	PRIMARY TUMORS
0.45 μM MD Glass				
OM Rats	3.2 ± 0.1	3/22 (14%)	7/22 (32%)[s]	0/22
1.8 μM MD Refractory Ceramic				
Sryian Hamsters	3.0 ± 0.1	0/25	4/25 (16%)	0/25
OM Rats	3.0 ± 0.2	6/22 (27%)[s,a]	2/22 (9%)	0/22
UICC Crocidolite Asbestos				
Syrian Hamsters	4.7 ± 0.2	0/7	23/27 (85%)[s]	20/27 (74%) [s,b]
OM Rats	4.3 ± 0.1	3/23 (13%)	24/25 (94%)[s]	2/25 (8%) [s,b]
Saline Controls				
Syrian Hamsters	1.7 ± 0.1	2/24 (8%)	0/24	0/24
OM Rats	2.0 ± 0.2	0/25	1/25 (4%)	0/25
Unmanipulated Cage Controls				
Syrian Hamsters	1.7 ± 0.1	4/112 (4%)	3/112 (3%)	0/112
OM Rats	2.4 ± 0.2	3/125 (3%)	17/125 (14%)	0/125

[a] Bronchiolar polypoid lesions

[b] Bronchoalveolar Tumors

[s] Statistically significant compared to controls