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To Whom It May Concern:

Rockwool International A/S (Rockwool®) is writing to share with the Agency on an FYI basis the final lung histopathology results of a long-term inhalation study in rats involving an experimental stone wool fiber. Rockwool® which is incorporated and headquartered in Denmark, is a manufacturer of stone wool (sometimes also called "rock wool"), and products made from stone wool. Stone wool products manufactured by Rockwool® or its subsidiaries are imported into the United States by unrelated entities. No corporate entity related to Rockwool® (including any parent or subsidiary corporation) that manufactures, processes or distributes Rockwool® products is incorporated or headquartered in the United States.

The experimental stone wool fiber that was the subject of the above-referenced bioassay was produced in Denmark as part of Rockwool®'s ongoing research and development program. This experimental fiber has a dissolution rate > 200 ng $cm^{-2}h^{-1}$ measured at pH 7.4, and at a flow/area ratio = 0.03 µm/s. For further experimental details on dissolution rate measurement, see enclosed article by Zoitos, et al. The experimental fiber has never been manufactured on a commercial scale or used in a commercial product offered for sale in the U.S. or elsewhere, and its composition is markedly different from the stone wool fibers contained in products currently or previously exported into the U.S. by Rockwool® and its subsidiaries. Additional information about the composition and manufacturing process for the subject experimental stone wool fiber is competitively sensitive and therefore proprietary.

The experimental stone wool was tested in a long-term inhalation study using a protocol similar to those used in previous tests on man-made vitreous fibers (MMVF), as described in the enclosed article by McConnell, et al. The study was conducted by Research & Consulting Company in Switzerland under contract to Rockwool®.

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The mean aerosol exposure concentrations were 305 respirable fibers (WHO)/cm^3, and 53 fibers longer than 20 µm per cm^3. At the interim sacrifices after 78 and 104 weeks of exposure, 4 out of 6 rats, and 3 out of 6 rats, respectively, had minimal fibrosis (Wagner grade 4) in the lungs. Wagner grade 4 fibrosis is considered to be an irreversible effect.

There was no significant increase in lung tumors compared with the air controls.

Although the study described above is not subject to mandatory reporting under Section 8(e) of the Toxic Substances Control Act (TSCA), EPA guidance under Section 8(e) encourages submissions on an FYI basis, particularly where the information is of potential regulatory or other interest in the U.S. Rockwool® products, as noted above, are exported into the United States. In addition, Roxul Inc., a Rockwool® subsidiary that is incorporated and headquartered in Canada, is a member of the North American Insulation Manufacturers Association (NAIMA). Through NAIMA, Rockwool® and Roxul have participated in other studies of the health effects of MMVF, including the submission of such joint NAIMA studies to EPA under TSCA Section 8(e). NAIMA has also been involved in various proceedings and discussions with U.S. regulatory agencies concerning the potential regulation of stone wool and other MMVF. For these and other reasons, Rockwool® is providing the above information on a voluntary FYI basis.

Should you wish further information on the results of this study, please contact me.

Sincerely,
Rockwool International A/S
Health & Safety

Ole Kamstrup
Vice-President

IN VITRO MEASUREMENT OF FIBER DISSOLUTION RATE RELEVANT TO BIOPERSISTENCE AT NEUTRAL pH: AN INTERLABORATORY ROUND ROBIN

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Measurements are presented of the dissolution rates in neutral pH simulated lung fluid (SLF) of several man-made vitreous fibers (MMVF) and crocidolite asbestos that were used recently in chronic rodent inhalation studies. The measured dissolution rate depended strongly on the fiber composition. The MMVF tested dissolved from 30 times to nearly 1000 times faster than the crocidolite asbestos. Measurements were made in flow-through equipment in four different laboratories in North America and Europe. The standard deviations of the measured values for each fiber were typically between 30 and 50% of the average value. It is believed that in order to be relevant to the dissolution of lung fibers in the extracellular fluid of the lung, the in vitro measurement must be performed at a flow rate high enough that corrosion products do not accumulate in sufficient concentration to affect the dissolution rate.

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There is intense interest today in the durability of fibers in the lung because of the role that dissolution plays in the clearance of fibers from the lung after they are inhaled. Upon inhalation into the deep lung, a fiber may reside in one of at least two "compartments." Long fibers (greater than about 15–20 μm in length) primarily reside in the extracellular environment and are subject to dissolution by lung fluid. Fibers shorter than this, and especially fibers less than about 10 μm, are subject to engulfment by macrophages. Once engulfed, these fibers are acted upon by the acidic environment within the macrophage and may be chemically attacked and/or transported out of the lung. Some researchers, working with refractory ceramic fiber (RCF), have observed that long fibers may have part of their length within a macrophage and the remainder in the extracellular environment, thus existing in two chemical environments at once (Morimoto et al., 1994). This article deals with in vitro measurement of fiber dissolution in neutral pH simulated lung fluid (SLF) as is relevant to biopersistence in the extracellular environment of the deep lung.

This article presents measurements made by different researchers in different laboratories of the dissolution rate at neutral pH of several types of man-made vitreous fibers (MMVF) (also known as synthetic vitreous fibers) and crocidolite asbestos that have been studied recently in inhalation experiments with rats (Hesterberg et al., 1993; Mast et al., 1995; McConnell et al., 1994; Hamilton et al., 1994; Musselman et al., 1994). These results show the type and approximate magnitude of differences expected when comparing measured fiber dissolution data from different sources. In addition, conditions under which the measurements should be made to be relevant to fibers in the extracellular fluid in the lung are discussed. The intent of this article is not to define the entire measurement protocol, but rather to identify those experimental variables that contribute most to the variations in the measured results and to suggest how these may be controlled.

GLASS CORROSION MECHANISMS

In order to interpret the behavior and causes of fiber dissolution in the lung, it is important to distinguish between the concepts of *solubility* and *dissolution rate*. Solubility refers to the concentration of a solute that exists in equilibrium with the parent solid. Dissolution is a more general term and simply describes the rate at which mass is removed per unit surface area of a solid. The clearance of long fibers from the lung does not appear to be related to the solubility of a fiber in lung fluid, since attempts to correlate particle clearance with solubility have failed (Kanapilly et al., 1973). It has since been recognized that the dissolution products of particles in the lung are so quickly removed (Kanapilly, 1977) that solubility plays no role and the

dissolution rate is a more relevant parameter (Scholze, 1988). A number of studies of the dissolution rate of various fibers under different conditions have been reported (Bauer et al., 1988; De Meringo et al., 1994; Forster, 1982; Klingholz & Steinkopf, 1982; Leineweber, 1982; Potter & Mattson, 1991; Scholze & Conradt, 1987). Mattson has suggested conditions under which such dissolution measurements should be performed so as to obtain self-consistent results relevant to biodurability (Mattson, 1994).

Silica-based MMVFs typically consist of a silica–alumina glass network with modifiers such as Ca, Mg, Na, etc. Corrosion of such glasses may occur by two processes (White, 1992). First, modifying cations may be removed from the glass by ion exchange with protons from solution. This process, known as selective dissolution, results in the formation of a silica-rich, modifier-depleted surface layer on the glass.

In addition, hydroxyl ions may directly attack the aluminosilicate structure of the glass, resulting in network dissolution. In general, these two processes proceed at different rates; however, if both proceed at the same rate, the glass is said to dissolve by congruent dissolution, in which all glass constituents are released to solution in the proportion in which they occur in the glass.

The rate of glass corrosion may be impacted by the accumulation of reaction products in solution. However, if the leachant solution is continuously replenished (a "flow-type" experiment) this effect can be minimized and fiber corrosion may be maintained at its maximum forward rate relative to the leachant. In such an experiment, the ratio of reaction product removal (proportional to leachant flow rate) to the fiber dissolution rate (proportional to fiber surface area) is the controlling parameter (White, 1992). If this ratio (denoted R/SA) is small, dissolution products such as hydroxyl ions may raise the solution pH, leading to increased dissolution of the silica network (Scholze, 1988). In addition, dissolution products may accumulate to near saturation levels in solution and decrease the dissolution rate (Chandier et al., 1986). Probably none of these effects occurs with fibers inhaled into the deep lung, since fibers there are typically dispersed from one another by many times their length and are separated from the bloodstream only by a submicrometer-thick membrane through which small ions diffuse rapidly. In this sense, fibers in the lung experience a high R/SA environment.

To measure lung-relevant, in vitro dissolution rates, one would ideally duplicate such a high-flow condition; however, such high flows are not easily achieved experimentally, and appropriate compromises must be considered. As previously stated, if the R/SA of a test is too low, corrosion products will accumulate in the solution surrounding the fiber to concentrations sufficient to impact the corrosion rate of

the fiber. Alternately, if the R/SA is too high, corrosion products in the leachate will be too dilute to measure accurately. This latter problem is exacerbated by the concentrated ion background presented by the SLF.

It is not possible a priori to recommend a minimum or maximum value of R/SA, since the degree to which this variable affects dissolution varies strongly depending on fiber type. In the course of this study, the dissolution of some fibers, such as MMVF-22, was observed to be very strongly dependent on R/SA, while the dissolution rate of other fibers, such as MMVF-11, was nearly independent of R/SA within the ranges studied here.

For these reasons, it is advisable to make measurements at several R/SA values in order to assess the dependence of k on R/SA. In cases where k depends strongly on R/SA, k values should be accepted only from higher ranges of R/SA, with ability to accurately measure ions in the leachate the only constraint on maximum R/SA.

KINETICS OF FIBER CORROSION: THEORY

The dissolution of thin fibers poses some challenge to the calculation of a rate constant. First, because the ratio of fiber surface area to fiber volume is so high, the fiber surface area presented to solution over the duration of the experiment cannot be considered constant over the reaction time. In addition, the diameters of fibers in a corrosion test may vary widely, and some fibers may corrode completely during the course of an experiment.

This problem may be solved provided that appropriate assumptions are made (Leineweber, 1982, Scholze, 1988). At a sufficiently high flow rate, the rate of mass loss of a fiber is proportional to its surface area, A:

$$\frac{dM}{dt} = -kA \tag{1}$$

where k is the dissolution rate constant, assumed to be independent of time and expressed in ng/cm² · h. For uniform diameter fibers dissolving congruently from an initial mass M_0 and initial diameter D_0, a solution to Eq. (1) is:

$$1 - \sqrt{\frac{M}{M_0}} = 2\,\frac{kt}{D_0\rho} \tag{2}$$

where ρ is the initial fiber density. A consequence of Eqs. (1) and (2) is that the diameter D of a fiber decreases at a constant rate according to:

$$D = D_0 - 2\frac{kt}{\rho} \tag{3}$$

The dissolution rate of a fiber may also be expressed as the dissolution velocity v, or the rate of decrease in radius:

$$v = \frac{k}{\rho} \tag{4}$$

conveniently expressed in nanometers per day (Scholze & Conradt, 1987).

These equations may be modified to account for a fiber diameter distribution within the sample, provided that no fibers dissolve completely during the corrosion process. In this case, Eq. (2) may be solved for the mass M of each fiber with initial diameter D_0. The total mass ΣM of all fibers is found to obey the equation:

$$\frac{\Sigma M}{\Sigma M_0} = 1 - 4\frac{vt\langle D\rangle}{\langle D^2\rangle} + 4\frac{v^2t^2}{\langle D^2\rangle} \tag{5}$$

where ΣM_0 is the total initial mass, $\langle D\rangle$ is the length-weighted arithmetic average initial fiber diameter, and $\langle D^2\rangle$ is the average value of the square of the initial diameters. This latter quantity is given in terms of the length-weighted, arithmetic standard deviation of the initial fiber diameter by $\langle D^2\rangle = \langle D\rangle^2 + \sigma^2$.

Equation (5) is a quadratic equation for v for a measured initial fiber diameter distribution and total fiber mass remaining at a given time. It may be solved for v at each time and k obtained by Eq. (4).

Alternatively, a "simulation-type" method may be used to evaluate k. In this type of calculation, the initial fiber diameter distribution may be used to calculate the mass loss of each fiber in the distribution at a given time using Eq. (2) based upon an assumed k. The resulting calculated mass loss may be compared to that measured in the experiment, and the estimate of k varied as needed until calculated mass loss agrees with that measured in the experiment. This form of calculation has the advantage of allowing for both a distribution of diameters and the disappearance of individual fibers.

These calculations are based upon an idealization of the fiber dissolution process that is not followed in detail for most inorganic fibers. In particular, the various oxide components seldom dissolve at the same rate. Instead, dissolution occurs through a combination of network and selective dissolution. This would be expected to occur in vivo as well as in the in vitro measurements reported here. In general, however, these equations are a reasonably good approximation to the mass loss of a collection of fibers, which allows the dissolution rate constant to be used to characterize a fiber composition.

METHOD

The dissolution rates of five MMVF and crocidolite asbestos fibers were measured in several different laboratories. The sized, shot-free samples measured were obtained from the Thermal Insulation Manufacturers Association (TIMA) fiber repository. The fiber repository is now maintained by the North American Insulation Manufacturers Association (NAIMA, Alexandria, VA) or, in the case of RCF-1, by the Refractory Ceramic Fiber Coalition (RCFC, Washington, DC). The compositions (Hesterberg et al., 1993; Mast et al., 1995; McConnell et al., 1994) of the fibers and their type (TIMA, 1991) and relevant properties are given in Table 1.

All of the methods used to obtain the results reported here involved the flow of simulated lung fluid at 37°C at a controlled rate through a mat of the fibers (Leineweber, 1982). The fluid for all measurements was similar to that reported by Gamble (1967) with pH buffers and complexing agents, but there were some differences in the compositions from laboratory to laboratory. These are summarized in Table 2. All studies were performed with a solution at nearly neutral pH to simulate the extracellular lung fluid to at least the degree that affects the fiber dissolution rate.

To obtain the results reported here, the effluent was analyzed for

TABLE 1. Fiber types, compositions, and density

Component	MMVF 10, glass wool	MMVF 11, glass wool	MMVF 21, rock wool	MMVF 22, slag wool	RCF 1, RCF
SiO_2	57.4	63.5	46.2	38.4	47.7
Al_2O_3	5.17	3.76	13.0	10.6	48.0
CaO	7.65	7.27	16.9	38.0	0.07
MgO	4.16	2.77	9.3	9.9	0.08
FeO			4.6		
Fe_2O_3	0.072	0.27	1.8	0.3	0.97
MnO			0.2	0.7	
Na_2O	15.5	15.27	2.6	0.4	0.54
K_2O	1.07	1.38	1.3	0.5	0.16
B_2O_3	8.53	4.5			
ZrO_2		0.018			0.11
TiO_2	0.01	0.062	3.0	0.5	2.05
SO_3	0.07	0.21	0.2	1.8	
F_2	0.68				
Density (g/cm³)	2.5	2.5	2.8	2.8	2.6

Note. Compositions are given in weight percent oxide.

TABLE 2. Solution compositions (mM) in four laboratories

Component	A	B	C	D: High Ca	D: Low Ca
Na	144.8	152.9	152.4	154.3	154.4
K		-	0.0003		-
Ca^{2+}	0.196	0.197	0.4082	1.74	0.20
Mg^{2+}		1.043	0.7867	1.04	1.04
Al^{3+}	10				
$H_2C(OH)_2CO_2H$ (glycolate)	6.05	1.57	-	1.57	1.57
H_2CO (formaldehyde)	12	12		12	12
CH_3OH (methanol)	4	4	--	4	4
Cl	126.4	122.2	110.7	115.4	118.5
H_4			1.19		
SiO_2^{2-}	1	0.556	0.5612	0.56	0.56
HCO_3	22	24.21	23.8	32.17	32.17
HPO_4^{2-}, H_2PO_4	1.7	1.042	1.056	1.00	1.00
CH_3CO_2 (acetate)			6.16		
$CH_3CH(OH)CO_2$ (lactate)		1.56		1.56	1.56
$CH_3CHOHCO_2$ (lactate)		1.56		1.56	1.56
$HOOCCH_2CH(OH)CH_2CO_2H^-$ (citrate)	0.7	0.520		0.520	0.520
$HO_2CCHOHCHOHCO_2H^-$ (tartrate)	-	0.782	-	0.782	0.782
Bubbled with 5% CO_2 using carrier of	N_2	N_2	N_2	N_2	N_2

Note. Key to laboratories: A, Unifrax; B, Rockwood International; C, Schuller Mountain Technical Center; D, Saint Gobain.

several of the fiber dissolution products. It was not possible to detect all of the dissolved glass components in solution (sodium, for example) because of the overwhelming presence of those elements in the SLF, which made accurate solution analysis impossible. In such cases, the quantity of an element dissolved was estimated from the measured amounts of elements expected to dissolve at a similar rate. For example, it has been observed (Lehuede et al., 1994) that the elements Na, K, Ca, Mg, and B are rapidly and nearly completely depleted during the corrosion of soluble glasses, while silica and alumina are dissolved more slowly. Thus in cases where Na and/or B losses were not measured for a fiber, their dissolution may be estimated by assuming that they are depleted in the same proportion as are Ca and Mg. Once individual element losses were determined, the overall weight loss of the fiber was computed by assuming that all cations dissolved from the glass as oxides.

An accurate estimate of the fiber diameter distribution is required to interpret the dissolution rate results and to obtain the dissolution constant. For MMVF 10, 11, 21, and 22, a representative sample of the fibers was measured by light microscopy and by scanning electron microscopy in four laboratories, and a composite of the results was

chosen for this purpose. The composite length-weighted diameter distribution was obtained by randomly sampling the fiber diameter data from each laboratory. The measured diameters were sampled in proportion to the measured length. A total of 90 diameter samples was drawn from each set to obtain 360 diameters for each fiber type. Table 3 gives some useful statistics about the composite distributions. In Table 3, the 5th percentile is the diameter below which falls 5% of the measured fiber length, in the same way that the median or 50th percentile is the diameter below which falls 50% of the fiber length. The values for RCF-1 reported in Table 3 are based on 200 fiber measurements made using light microscopy in the Unifrax laboratory. To insure adequate measurement statistics, it is recommended that a minimum of 200 fiber diameters (and preferably as many as 500–1000) be measured for fiber durability calculations (Koenig et al., 1993).

Calculation of the initial fiber surface area was made from the fiber diameter distribution, density, and mass of fiber used in a given measurement. Alternately, fiber specific surface area was measured directly using gas adsorption techniques.

The following is a description of the techniques used in each laboratory to measure the dissolution rates reported here.

Unifrax

The dissolution constants were measured in a flow-through durability test apparatus. SLF was pumped through a mat of fiber at the rate of 0.3 ml/min and R/SA was controlled by varying the quantity of fiber used. The leachate solutions were collected and analyzed for Al, Ca, Fe, Mg, and Si at 1, 2, and 4 wk. B, K, and Na were not measured but were assumed to have been removed from the fiber in the same proportion as Ca and Mg during a given time interval.

The dissolution rate constant k was determined by mathematically simulating the dissolution of each fiber in the distribution using an arbitrarily chosen value for k. As the fiber diameters dropped to zero, they were removed from the simulated distribution. The output of this

TABLE 3. Length-weighted fiber diameter statistics for the composite fiber diameter distributions

Code	MMVF 10	MMVF 11	MMVF 21	MMVF 22	RCF 1
Average	1.70	1.14	1.46	1.41	1.54
SD	0.79	0.79	0.87	0.68	0.88
Geometric mean	1.49	0.93	1.25	1.24	1.30
Geometric SD	1.746	1.926	1.796	1.684	1.825
Median	1.62	0.82	1.32	1.27	1.35
5th %ile	0.45	0.37	0.47	0.52	0.42
95th %ile	3.16	2.61	2.80	2.82	3.57

process, expressed as $1 - \sqrt{M/M_0}$, was compared with the value determined from solution analysis. The assumed k was then adjusted and the process repeated until a satisfactory agreement was obtained. This process was repeated for each measurement interval so that a value of k was provided at 1, 2, and 4 wk. A detailed description of this calculation is provided as an appendix. Only the 4-wk values are reported here.

Because of the extremely slow dissolution of crocidolite, the fiber surface area was considered unlikely to change significantly and dissolution rate was computed directly from Eq. (1) using gas adsorption fiber surface area measurements.

Rockwool International

The methods used by this laboratory have been previously reported (Christensen et al., 1994). For all fibers, Al, Ca, Mg, and Si were measured. In the calculation K_2O and Na_2O were treated as if they dissolved like CaO and MgO. Then all components were pooled as oxides and the total dissolution rate was calculated for each period, 1 day at the start and 1 wk at the end of the measurement. In case an oxide continued to be found higher than background level in the fluid, after it all should be gone from the fiber according to the mass balance, that oxide was neglected and set equal to zero thereafter. In some cases MgO was initially lower than the background level. This was probably the result of local precipitation, which later in the test would be redissolved and then would neutralize the initial negative dissolution rate for MgO. All results reported are the mean over the first 25 days. Components not measured (mainly Ti, Fe, and B) are expected to dissolve as the average of the four measured components plus K_2O and Na_2O. Weight determination of the residual fibers agreed with that calculated from the solution analysis to within about 11%.

Saint-Gobain

The procedures used here have been described previously (Thélohan & DeMeringo, 1994; DeMeringo et al., 1994). Two different solutions were used for the measurements, differing mainly in Ca content (Table 2). The low-Ca solution allows accurate measurement of Ca from the dissolving fibers in the effluent solution, in addition to Si, which was determined in both solutions. Aluminum was assumed to dissolve at the same rate as Si, whereas all the other components of the fiber were assumed to dissolve at the same rate as Ca. The measurement of Si allows one to follow the network dissolution of the glass, and the measurement of Ca allows one to follow the leaching and dissolution processes.

The calculation of k was done using Eq. (5) as described previously. It was calculated for each measurement interval and averaged

over 28 days of the experiment. The measurements were performed at several flow rates from 0.002 to 0.13 μm/s. In some cases, the measured dissolution constant did not appear to stabilize at even the highest flow rates.

Johns Manville Technical Center

The simulated lung fluid was formulated to the composition shown in Table 2. Dissolution was measured via the following sequence: determining the concentrations of elemental components dissolved in the leachate at each time interval, converting elemental concentrations to oxide concentrations and converting oxide concentrations to mass, and summing to yield a cumulative determination of the amount of material removed from the sample over time. All major components of the glass comprising each sample were measured with the exception of Na. The Na concentrations at each time point were determined by analyzing the Na content of the residual fiber after each experiment and extrapolating back to the initial time. The extrapolations were linear, assuming that Na underwent a rather rapid ion-exchange reaction with the fluid. The calculation of k was performed iteratively by first simulating the dissolution of each fiber in a representative collection of fibers with measured diameters, computing the mass loss for each fiber at each time using Eq. (2) and summing for the entire collection of fibers used in the simulation. This k value was then varied until a best fit via least squares was obtained with the experimental mass loss data.

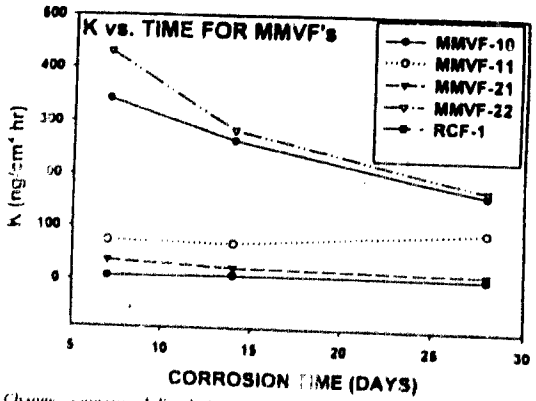

FIGURE 1. Change in measured dissolution rate as a function of study duration, measured in the Unifrax laboratory. Some fiber dissolution rates remain constant over the 28-day test, while others rates decrease by as much as a factor of 2.

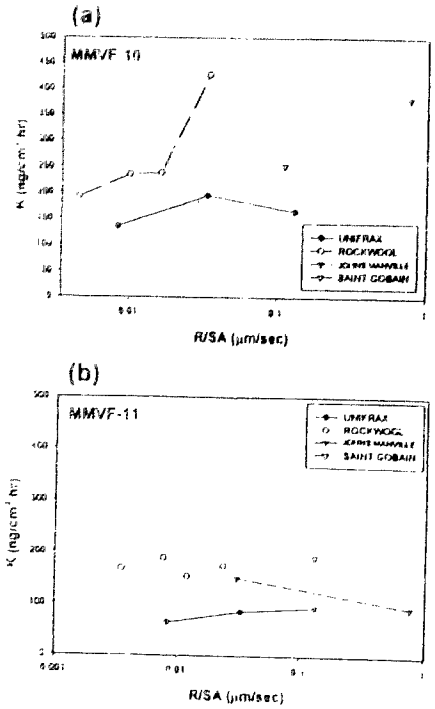

FIGURE 2. Dissolution rate of study fibers measured in participants' laboratories as a function of flow parameter, R/SA: (a) MMVF 10 and (b) MMVF 11

Continued on following page

RESULTS

Based on the results of each laboratory, a set of dissolution rate constants was obtained for the R/SA values studied. Figure 1 shows the behavior of fibers measured in the Unifrax lab at the highest R/SA as a function of time and indicates the degree to which k may vary over the duration of a test. Fibers MMVF 11 and 21 show little variation in k over the 28-day study, while k values for MMVF 10 and 22 decrease by as much as a factor of 2 during the same test interval. This information suggests that when comparing data it is important to compare measurements from similar test intervals.

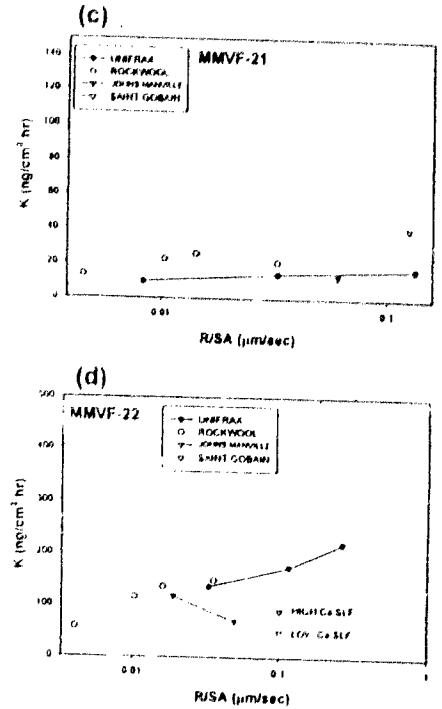

FIGURE 2. (*Continued*) Dissolution rate of study fibers measured in participants' laboratories as a function of flow parameter, R/SA: (c) MMVF 21 and (d) MMVF 22.

Figure 2 compares the measurement results from all participating labs, expressed as a function of R/SA. To assemble this graph, data were collected only from the first 20–30 days of the tests. This data shows considerable variation in the measured k; however, in spite of this variability, the k values group into well-defined ranges depending on fiber. It is also noted that, with the possible exception of MMVF 11, k increases with increasing R/SA. From these data it is not possible to identify a value of R/SA above which k becomes constant; however, 0.01 µm/s appears to be a reasonable minimum value of this parameter for the more nondurable fibers studied here.

Table 4 presents the average dissolution rate (obtained by taking an average of the average k as determined by each lab) and standard deviation of dissolution constants obtained in this study at or above 0.01 µm/s.

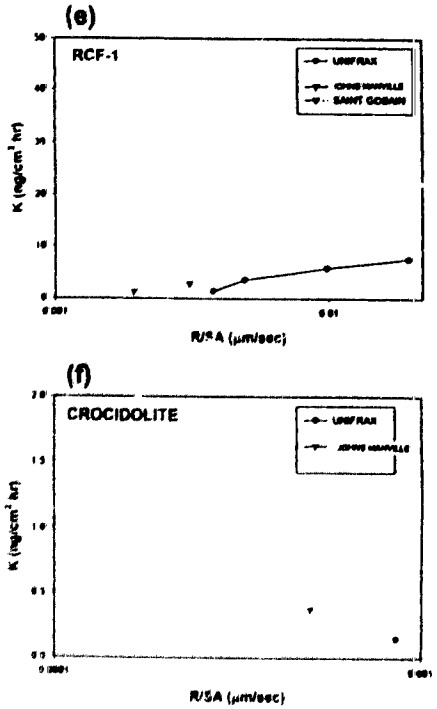

FIGURE 2. (Continued) Dissolution rate of study fibers measured in participants' laboratories as a function of flow parameter, R/SA: (e) RCF-1 and (f) crocidolite asbestos.

TABLE 4. Averaged dissolution rate values

Fiber	k (mg/cm²/h)	Standard deviation	Number of Labs in average
MMVF 10	259	75	4
MMVF 11	142	39	4
MMVF 21	23	11	4
MMVF 22	119	41	4
RCF-1	8		1
Crocidolite[a]	0.3	0.3	2

[a]Due to high durability of crocidolite, k was obtained at R/SA >0.01 μm/s

CONCLUSIONS

The results presented here have demonstrated that the in vitro dissolution rates of fibers can be measured by different laboratories to obtain consistent results under conditions thought to be relevant to the dissolution of fibers in the extracellular fluid in vivo. The standard deviations of the results for a single fiber are typically 30–50% of the average value from all laboratories, although individual values may differ from one another by a factor of four. In spite of this variability, clear differences may be seen in the dissolution rate of different fiber types.

The following are some of the most important parameters affecting the measured result: An accurate measurement of the length-weighted fiber diameter distribution is needed. All laboratories in this study used the same consensus fiber diameter distribution. The composition of the simulated lung fluid may affect the results, although results did not vary widely for a given fiber type for the variety of SLF compositions used in this study. For some fibers, the dissolution rate changes as the fiber dissolves, resulting in a k value that may change as much as a factor of two. To account for this, it is necessary to measure dissolution over an appreciable fraction of the fiber's lifetime, and to use only data from the same time interval for comparisons. As a general rule, this time interval should be at least long enough for 50% of the fiber mass to dissolve, up to a maximum of 40–60 days for more durable fibers. In addition, the method used to compute the dissolution rate constant from raw data must be a reasonably accurate representation of the physical chemistry of the dissolution process.

Finally, this study has demonstrated that dissolution is a sensitive function of the solution flow rate to fiber surface area ratio (R/SA). For in vitro fiber durability measurements to be relevant to in vivo dissolution, a sufficiently high flow rate must be maintained so that corrosion products do not accumulate and impact the dissolution process. These conditions can be ensured by measuring k at several values of R/SA and accepting only those at high enough R/SA that k is nearly independent of it. In cases where k does not plateau and continues to increase with R/SA, it is necessary to assess dissolution rate at as high an R/SA value as is experimentally feasible.

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APPENDIX: CALCULATION OF DISSOLUTION RATE BY SIMULATION METHOD

This appendix provides details of how the dissolution rate, k, may be determined by mathematically simulating the dissolution of a given fiber diameter distribution. Briefly stated, this method calculates the predicted mass loss based on a "best-guess" value for k. This predicted mass loss may then be compared with the measured value, and a "better-guess" k obtained. The process is iterative, ending once sufficient agreement is obtained. These steps may be easily adapted to any spreadsheet type software.

1. Calculate the value of (M/M_0) from experimental data $[(M/M_0)_{experiment}]$ for the desired time interval. Obtain the length-weighted diameter distribution and density for the fiber.
2. Establish a "best-guess" value for k.
3. For each fiber, D_0, in the diameter distribution, use Eq. (2) to calculate (M/M_0) $(= [1 - (2kt/D_0\rho)]^3)$ using fiber density and best-guess k. If the quantity $[1 - (2kt/D_0\rho)]$ is less than 0, then set $(M/M_0) = 0$. A value of zero for this quantity indicates that the fiber has completely dissolved and no longer contributes to the fiber mass.
4. Multiply the quantity from step 3 by D_0^3. This provides a weighting based on the volume (i.e., mass) of the individual fiber.
5. Sum all values from step 4 and divide this sum by ΣD_0^3 to normalize. This quantity is the value of (M/M_0) for the overall collection of fibers predicted by the simulation.
6. Compare the number from step 5 to (M/M_0) obtained from the experiment. If step 5 result is greater than $(M/M_0)_{experiment}$, reduce the guessed value of k. Increase the guessed k if the value in step 5 is less than $(M/M_0)_{experiment}$.
7. Continue this process until the value from step 5 equals $(M/M_0)_{experiment}$.

CHRONIC INHALATION STUDY OF SIZE-SEPARATED ROCK AND SLAG WOOL INSULATION FIBERS IN FISCHER 344/N RATS

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This study was designed to investigate the potential pathogenic effects in Fischer 344/N rats of two different types of man-made vitreous fibers (MMVF). Eight-week-old male rats were exposed in nose-only inhalation chambers, 6 h/day, 5 days/wk, for 24 mo to 3 concentrations (3, 1o, and 30 mg/m³) each of the two MMVFs: a basalt-based rock wool (stone wool), and a slag wool (blast furnace). Crocidolite asbestos (10 mg/m³) was used as a positive control. The experimental groups were compared to unexposed (chamber) controls. The MMVFs used in this study were size selected to be larger, respirable in rats. Interim sacrifices took place at 3- and 6-mo intervals to monitor the progression of pulmonary changes. Fibers were recovered from digested lung tissue for determination of changes in fiber number and morphology. Exposure to crocidolite asbestos was terminated after 10 mo because of increased morbidity/mortality. Exposure to rock and slag wool, while producing a dose-related nonspecific inflammatory response (rock and slag) and minimal focal pulmonary fibrosis (rock only), showed no evidence of carcinogenic activity in either the lung or pleura, in contrast to crocidolite asbestos, which induced neoplasms in both tissues. Since workplace airborne levels are several orders of magnitude lower than even the lowest exposure level to which the animals were exposed, these results suggest that these MMVFs do not pose a significant health risk to humans at a dose level found in the workplace, during installation or for the in-use product.

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Man-made vitreous fibers (MMVF) are fibrous, inorganic substances made primarily from rock, clay, slag, or sand. Major MMVF classes are comprised of glass wool, slag wool, rock wool (often called "stone" wool in Europe), refractory ceramic fiber (RCF), continuous filament, and special purpose fibers (TIMA, 1993). Slag and rock wool fibers are composed of calcium magnesium aluminum silicate glass. In the United States, from about 1975 to the present, slag from the blast furnace reduction of iron ore to pig iron has been the major raw material used to manufacture slag wool, accounting for 70–90% by weight of the raw material mix. Other components of the mix include phosphate smelter slag, and natural materials like silica gravel, shale, and limestone. Rock wool fibers are made from a charge consisting primarily of basaltic rocks with some additive such as limestone or dolomite.

Present production methods include the making of briquettes, which use a clay or cement to bind ground materials. The main purpose of the briquettes is to facilitate recycling of rock and slag wool waste and to adjust the chemical composition of the end-product.

Rock and slag wool products have excellent thermal, acoustical, and fire-resistant properties. They have applications in residential insulation batts, blankets, blowing wool, industrial and commercial low- and high-temperature insulation, fire protection, and acoustical wall panels and ceiling [illegible] production is used in acoustical [illegible] in press). Horticultural application [illegible] slag wool as a [illegible] or growing medium is a relatively and developing market in Europe and the United States (TIMA, 1993).

Commercial manufacture of rock and slag wool produces small amounts of airborne respirable fibers. Airborne manufacturing levels are generally <0.1 fibers/cm^3 (Cherrie et al., 1986; ILO, 1989; IPCS, 1988). Airborne levels (8-h time-weighted average) can vary from <0.1 to ~0.3 fibers/cm^3 during installation of the products (Lees et al., 1994; Schneider et al., 1993). Short-term exposures (e.g., 2-h) can be higher but are generally <1 fiber/cm^3 (Corn et al., 1992; Lees et al., 1993; Koenig & Axten, in press). Concern about the safety of workers/users of these materials stimulated the initiation of epidemiologic and experimental animal studies to detect and assess potential adverse health effects from breathing these fibers.

The present chronic inhalation studies of basalt-based rock wool and blast furnace-based slag wool are part of a series of investigations designed to determine the potential toxicity of fine fiber fractions obtained from commercial products representative of each of the major MMVF classes as well as chrysotile and long-fiber crocidolite asbestos. Results from chronic inhalation studies of fibrous glass, RCF, and chrysotile asbestos have been previously reported (Hesterberg et al., 1993; Mast et al., in press; McConnell et al., in press).

MATERIALS AND METHODS

Fiber Characteristics

The two types of fibers used in this study are fractions of a Rockwool International A/S (Denmark) basalt-based rock (stone) wool (MMVF 21) and

a USG Interiors (USA) slag wool (MMVF 22). Bulk fibers with chemical compositions typical of commercial insulation products were size separated to be largely rat respirable (<1.5 μm diameter and <80 μm length) and to have average dimensions (average aerosol geometric mean diameter <1.0 μm and length >15 μm) that are comparable to other MMVF inhalation studies recently completed (Mast et al., in press; Hesterberg et al., 1993). For each type of MMVF, approximately 4000 kg of bulk product was size separated using a water-based process (Hesterberg et al., 1993) to obtain 10 kg of stock fiber with the required dimensions to be used in the inhalation study. The rock wool bulk product was produced without the normal addition of oil or binder because it was not possible to size separate the latter. The chemical compositions of rock and slag wool are given in Table 1, while the physical characteristics of the stock fibers are presented in Table 2.

A previously characterized sample of crocidolite asbestos (Bureau of Mines, 1980) was used as a positive control. It was obtained from the National Institute of Environmental Health Sciences, Research Triangle Park, NC and was size separated in a similar manner to the MMVFs. However, because of its high fraction of long/thin fibers only ~50 kg of bulk material was needed to obtain 10 kg of fibers with the required dimensions.

Fiber Aerosol Exposure

Rats were exposed by nose-only inhalation to 3 dose levels (3, 16, or 30 mg/m^3 of rock or slag wool or 10 mg/m^3 for crocidolite asbestos) according to the method of Sachsse et al. (1976). The high dose (30 mg/m^3) was chosen to be comparable to studies of MMVFs (refractory ceramic fiber and glass insulation wool) previously studied in this laboratory (Hesterberg et al., 1993; Mast et al., in press). Exposure to crocidolite asbestos was stopped at 10 mo because of morbidity/mortality, and the rats were not placed in exposure tubes for the remainer of the study. The experimental groups were compared to unexposed (chamber) controls, which were also placed in exposure tubes in a similar manner to the exposed rats. Advanced techniques of nondestructive aerosolization were used to maximize the number of "rat-respirable fibers," that is, fibers of a size able to reach the alveolar region of the lung (Hesterberg et al., 1991). This system produces large numbers of unbroken fibers with lower levels of nonfibrous dust than in past inhalation studies. Temperature,

TABLE 1. Chemical Composition of Bulk Fiber Samples by Weight Percent

	SiO_2	Al_2O_3	CaO	MgO	FeO	Fe_2O_3	Na_2O	K_2O	TiO_2	MnO	S
Crocidolite	50.9		0.7	3.4	14.7	21.4	5.6	0.1		0.1	0.1
Rock wool	46.2	13.0	16.9	9.3	4.6	1.8	2.6	1.3	3.0	0.2	0.2
Slag wool	38.4	10.6	38.0	9.9		0.3	0.4	0.5	0.5	0.7	1.8

Note. Analyses: SiO_2, Al_2O_3, CaO, MgO by gravimetric and/or AA. FeO by ferrous iron titration with potassium dicromate, using a diphenylamine sulfonate indicator; Fe_2O_3, TiO_2, MnO by ICP; Na_2O, K_2O by AA; sulfur as SO_3 by LECO CS-344 furnace. Slag wool not analyzed for FeO due to low Fe content. All percent values rounded to the nearest 0.1%.

TABLE 2. [illegible] Selected Stock [illegible] Rock Wool Slag Wool [illegible] Fibers

[illegible]	Rock wool		
[illegible]			
Number	[illegible]		
Technique	SEM		
AMD ± SD	[illegible]		
GMD ± GSD	[illegible]		
MED ± SD	[illegible]		
Length (μm)			
Number	[illegible]		
Technique	Optical		
AMD ± SD	23.9 ± 21.7		
GMD ± GSD	16.1 ± [illegible] 2.2		
MED ± SD	19.1 ± [illegible]		
Fiber [illegible]	Rock wool low dose	Rock wool mid dose	Rock wool high dose
Diameter (μm)			
Number	9	9	9
Technique	SEM	SEM	SEM
AMD ± SD	[illegible]	[illegible] ± 0.60	1.14 ± 0.64
GMD ± GSD	[illegible]	0.90 ± 1.75	0.98 ± [illegible]
MED ± SD	[illegible]	0.92 ± 0.29	1.10 ± 0.07
Length (μm)			
Number	9	9	
Technique	Optical	Optical	Opt[illegible]
AMD ± SD	[illegible]	21.0 ± [illegible]	
GMD ± GSD	[illegible]	15.4 ± 2.3	
MED ± SD	[illegible]	[illegible]	
Concentrations			
mg/M³	[illegible] (n = 59)	[illegible] (n = 59)	[illegible] (n = 609)
Total fibers/cm³	44 ± 15 (n = 25)	[illegible] (n = [illegible])	[illegible] (n = 26)
WHO fibers/cm³	34 ± 13 (n = 63)	150 ± 51 (n = [illegible])	[illegible] (n = 64)
>10 μm fibers/cm³	26 ± 9 (n = [illegible])	74 ± 20 (n = 9)	[illegible] (n = 9)
>20 μm fibers/cm³	13 ± 5 (n = 10)	74 ± 20 (n = 9)	[illegible] (n = 9)
Aerosol particles			
Number	23	[illegible]	[illegible]
<1 μm part/cm³	9 ± 8	23 ± 27	18 [illegible]
1–3 μm part/cm³	2 ± 2	8 ± 6	[illegible]
>3 μm part/cm³	1 ± 1	4 ± 5	3 ± 3

Note. AMD, arithmetic mean diameter; SD, one standard deviation; GMD, geometric mean diameter; GSD, geometric standard deviation; MED, mediation; total fibers, fibers with length (μm) [illegible] (l/d) > 3; WHO, fibers with l/d > 3, length >5 μm, and diameters <3 μm; ND, not detected.

relative humidity, and oxygen concentrations were monitored continuously. The animals were confined separately in tubes that were positioned radially around the exposure chamber (Cannon et al., 1983). This nose-only system provides a positive pressure laminar flow to each animal individually, so that each is supplied fresh aerosol and the air exhaled by each animal does not contaminate the air of others in the chamber (Bernstein & Drew, 1980).

Slag wool			Crocidolite
4			4
SEM			SEM
1.21 ± 0.72			0.25 ± 0.12
1.03 ± 1.77			0.22 ± 1.60
1.01 ± 0.07			0.24 ± 0.01
4			4
Optical			SEM
22.4 ± 20.3		10.9 ± 12.00	
15.6 ± 2.4		7.2 ± 2.45	
15.8 ± 2.7		6.5 ± 0.10	
Slag wood low dose	Slag wool mid dose	Slag wool high dose	Crocidolite
9	9	9	4
SEM	SEM	SEM	SEM
0.96 ± 0.56	0.97 ± 0.54	1.02 ± 0.61	0.31 ± 0.12
0.84 ± 1.70	0.84 ± 1.69	0.87 ± 1.75	0.28 ± 1.54
0.82 ± 0.16	0.84 ± 0.12	0.85 ± 0.16	0.30 ± 0.03
9	9	9	4
Optical	Optical	Optical	Optical
16.5 ± 13.3	17.9 ± 14.8	20.5 ± 15.9	6.1 ± 6.3
12.3 ± 2.1	13.2 ± 2.2	15.2 ± 2.2	4.1 ± 2.3
12.1 ± 2.5	13.1 ± 1.2	15.5 ± 1.9	3.8 ± 0.8
3.1 ± 0.4 (n = 596)	16.1 ± 1.4 (n = 598)	29.9 ± 2.9 (n = 611)	10.0 ± 0.9 (n = 247)
33 ± 13 (n = 26)	158 ± 46 (n = 26)	245 ± 77 (n = 26)	4214 ± 2589 (n = 32)
30 ± 15 (n = 64)	131 ± 40 (n = 64)	213 ± 62 (n = 64)	1610 ± 989 (n = 32)
20 ± 8 (n = 9)	96 ± 28 (n = 9)	165 ± 51 (n = 9)	677 ± 416 (n = 4)
10 ± 4 (n = 9)	50 ± 14 (n = 9)	99 ± 31 (n = 9)	236 ± 145 (n = 4)
26	26	26	ND
7 ± 6	20 ± 17	24 ± 22	
5 ± 2	18 ± 8	18 ± 10	
7 ± 4	32 ± 17	38 ± 23	

Experimental Design

The study was conducted in rats, which are felt to be the most appropriate experimental animal for studying the potential toxic effects after inhalation of fibers (IPCS, 1988; McClellan et al., 1992). The study was conducted at the Research and Consulting Company, Geneva, Switzerland, using 6-wk-old weanling male F344/N rats (Charles River Laboratories, Raleigh, NC).

The rats were held for a 2-wk quarantine period, were individually identified using ear tattoos, and were randomly distributed to exposure groups using a computer-generated random algorithm. There were 140 rats in each of the rock and slag wool exposure groups, the positive control (crocidolite asbestos), and the unexposed chamber control groups. They were exposed for 6 h/day, 5 days/wk for up to 24 mo. When not being exposed, the rats were housed individually in polycarbonate cages containing hardwood bedding in Hazelton 2000 chambers (Lab Products) operated under negative pressure (−20 mm H_2O) with 20 air changes/h. Pelleted feed (Kliba 343, Klingentalmuehle AG, Switzerland) and filtered fresh water were supplied ad libitum during the nonexposure period.

Groups of 6 randomly selected rats, identified at the start of the study, from each exposure group were killed at 3, 6, 12, 18, and 24 mo for lung fiber burden measurements and to follow the progression of the pulmonary lesions. Additional groups of 3–6 rats from each group were removed from exposure at 3, 6, 12, and 18 mo and held until 24 mo (recovery groups), at which time they were sacrificed to ascertain whether there was progression or regression of lesions and to determine lung fiber burdens following cessation of exposure. Following the 24-mo exposure period, the animals were held for lifetime observation (until ~20% survived), which occurred at 28 mo. A lifetime observation period was chosen because previous studies of inhaled asbestos have shown that the induction of mesothelial neoplasms in rats usually occurs late in their lives (>24 mo) (Wagner et al., 1974).

Aerosol Monitoring and Characterization

Fiber mass concentrations were measured at least 4 times/wk during the 2-yr exposure period. Each aerosol sample from each fiber type and dose level, as well as the control aerosols, was collected on a Gellman membrane filter from one of the laminar flow ports in the exposure chamber to eliminate isokinetic sampling bias. Additionally, aerosol concentrations were monitored continuously during the exposure period using an RAS (GCA Corp.) light-scattering monitor to assure uniformity. The aerosols were analyzed for WHO fibers per cubic centimeter [defined as having a diameter <3 μm, a length >5 μm, and an aspect ratio >3 : 1 (WHO, 1985)] once each exposure week during wk 1–13 and every other week thereafter through exposure wk 104. In addition, the total numbers of fibers and nonfibrous particles were counted once each month on a filter for each fiber type and dose. Samples were collected on Millipore filters placed between glass slides, clarified, and counted by phase-contrast microscopy (PCM) at 400× magnification (Mountain Technical Center Industrial Hygiene Laboratory, Schuller International Co., Littleton, CO). At least 20 fields were counted on each slide. In addition, once each month total fibers (aspect ratio >3 : 1) and particles were counted to determine total fibers per cubic centimeter and particles per cubic centimeter.

Every 3 mo samples of rock and slag wool aerosols for each dose and crocidolite asbestos were collected on filters for determination of fiber length and diameter. The filter/fiber samples were stored in 100-ml glass bottles containing ~100 ml distilled water and 8 mg sodium azide. Due to their high solubility in water, the last seven slag wool filter samples were stored dry prior to counting. Each filter was removed and rinsed back into the original sample bottle. Suspensions were diluted to 250 ml with distilled water and homogenized by sonification. Aliquots were drawn and filtered onto 0.2-μm Nuclepore and 0.2-μm Millipore membranes. The Nuclepore membranes were dried, applied to scanning electron microscope (SEM) stubs, coated with gold, and counted by scanning electron microscopy (JEOL T-300 or JEOL 840 equipped with a Videoplan image analysis system). The Millipore membranes were placed between glass slides and clarified for optical measurement.

Diameters were measured at 5000× in a minimum of 20 fields or 200 fiber ends as outlined by WHO for counting MMVFs (WHO, 1985). Length distributions were determined at a lower magnification (1500–1850×) according to WHO guidelines (WHO, 1985) using a Leitz Orthoplan or Zeiss optical microscope equipped with a Videoplan image analysis system to allow visualization of entire fibers. A minimum of 20 fields or 200 fiber ends was counted, and the number of truncated ends was recorded. While 40% of the fibers in both the aerosol and recovered from the lungs had diameters less than 0.5 μm, the SEM measurements showed almost no fibers having diameters less than 0.1 μm. Fibers with diameters >0.01 μm can clearly be identified as lines using PCM at 2000×. While diameters obviously could not be accurately measured on fibers that small with PCM, the lengths could accurately be measured for all fibers in the distributions.

Crocidolite asbestos aerosol samples were prepared in the same way as rock wool, except only one aliquot was filtered onto an SEM stub. Diameter and length distributions were determined using SEM at 5000× and 2000×, respectively. Calculation of all arithmetic and geometric means, medians, standard deviations, and diameter and length classifications from raw data was done using the Videoplan computer system.

Lung Burden Analysis

At necropsy the accessory lobe of the lung from each animal was removed, weighed, and frozen. After thawing it was dehydrated in acetone and evaporated to constant weight. The dry lung tissue was then ashed in an LFE LTA 504 multiple chamber plasma unit. The ash from each lung was dispersed in distilled water, and aliquots of the suspension were filtered onto 25-mm diameter, 0.2-μm pore size Nuclepore membranes for SEM stub preparation and onto 25-mm diameter, 0.2-μm pore size Millipore membranes that were clarified for optical slides. Counting and measuring of the fibers were done in the same way as with the aerosol fiber samples.

Clinicopathology

The rats were observed daily for clinical signs, morbidity, and mortality throughout the study. They were individually examined and weighed once each week during the first 13 wk and at least once each month thereafter. Sick or moribund animals were exsanguinated following deep anesthesia by intraperitoneal injection of pentobarbital sodium, as were those remaining at the end of the study [28 mo (30 mo of age)].

A necropsy was performed on all animals, and a complete set of tissues (NTP, 1984) was obtained and fixed in 10% neutral buffered formalin, except for the lung. The lungs were removed, weighed, and examined under a dissecting microscope. The right accessory lobe was ligated, removed, weighed, and frozen at −20°C for lung fiber retention analysis. The remaining lung was perfused with Karnovski's fixative via the trachea at 30 cm H_2O pressure for 2 h. After fixation, a consistently uniform transverse (horizontal) section (2 mm diameter) of the left lung and right diaphragmatic lobe was obtained from each animal for routine histopathology. In addition sections were made from all grossly visible lesions in the lung. Replicate sections were routinely stained with hematoxylin and eosin (H&E) and Masson–Goldner's trichrome stain to highlight collagen.

Other tissues processed routinely for histopathological examination included three levels of the nasal cavity (anterior, midlevel, and posterior), larynx, trachea, bronchi, mediastinal and mesenteric lymph nodes, liver, spleen, kidneys, heart, and all grossly visible lesions.

The lungs were examined and classified histopathologically and given a Wagner score for inflammatory change and fibrosis (McConnell et al., 1984). In this system a grade of 1.0 is considered normal, grades 2–3 are evidence of focal cellular change, while grades 4–8 represent the former lesions plus increasing degrees of fibrosis (Table 3). In accordance with these guidelines,

TABLE 3. Pathology Grading Scale

	Grade	
		Cellular change
Normal	1	No lesion
Minimal	2	Macrophage response
Mild	3	Macrophage bronchiolization
		Fibrosis
Minimal	4	Fibrosis restricted to terminal bronchioles/proximal alveoli
Mild	5	Interlobular linking
Moderate	6	Early consolidation
Severe	7	Marked fibrosis/consolidation
	8	Complete obstruction of airways

Note. From McConnell et al. (1984).

proliferative lesions of the pulmonary parenchyma were designated as bronchoalveolar hyperplasia (BAH), pulmonary adenoma, or adenocarcinoma. Other types of lesions, including those in the pleura, were noted where appropriate and were recorded by the study pathologist (Chevalier) using on-line input into the PDS PATHDATA System (version 3.4.8, Experimental Pathology Services, Muttenz, Switzerland). Additionally, two other pathologists independently reviewed replicate sections of the lung and rendered diagnoses without knowledge of the study pathologist's diagnoses.

The slides, individual animal data records, and pathology tables were evaluated by an independent quality assurance laboratory (Experimental Pathology Laboratories, Inc., Herndon, VA). The quality assessment pathologist reviewed all of the lung sections and selected all slides where a proliferative diagnosis had been made by any of the three above pathologists. These slides were in turn examined by a group of five independent pathologists (Pathology Working Group, PWG) with experience in rodent pulmonary pathology without knowledge of exposure group or previous rendered diagnoses. The PWG consensus diagnoses were considered by the study pathologist (J. Chevalier) when making his final diagnoses.

Statistics

Pairwise comparisons of tumor incidence between exposure groups were made using Fisher's exact test, and tests for trend were made using an exact algorithm for the Cochran–Armitage test. Only rats exposed to rock or slag wool for 12 mo or crocidolite for 10 mo were considered at risk for the induction of neoplasms, because this was the earliest time point a neoplastic finding was observed in this series of studies. All tests of significance were two-tailed, with no formal adjustment for multiple comparisons. Student's *t*-test was used for comparisons of the physical characteristics of the fibers.

RESULTS

Exposure Atmosphere and Fiber Characterization

The physical characteristics of the stock and aerosolized fibers are presented in Table 2. When rat respirable fibers (<1.5 μm diameter × <80 μm length) are considered, all of the MMVF aerosols were comparable in terms of fiber dimensions. The geometric mean length of aerosolized crocidolite was less than that of the stock material (7.9 ± 2.5 μm vs. 4.1 ± 2.3 μm).

The three airborne gravimetric concentrations (mg/m^3 ± SD) of rock wool averaged 3.06 ± 0.41, 16.14 ± 1.17, and 30.39 ± 2.79 and slag wool 3.06 ± 0.44, 16.11 ± 1.39, and 29.89 ± 2.87 during the 2 yr exposure (Table 2). These concentrations corresponded to average WHO fiber numbers (fibers/cm^3 ± SD) of 34 ± 13, 150 ± 51, and 243 ± 67 for rock, and 30 ± 15, 131 ± 40, and 213 ± 62 for slag (Table 2). The average numbers of rock wool fibers >10 μm in length were 26 ± 9, 122 ± 33, and 186 ± 51 and those >20 μm were 13 ± 5, 74 ± 20, and 114 ± 32. Slag wool fibers >10

μm in length numbered 20 ± 8, 96 ± 28, and 165 ± 51, and those >20 μm were 10 ± 4, 50 ± 14, and 99 ± 31. The aerosols also contained nonfibrous particulates. The crocidolite asbestos aerosol was 10.04 ± 0.90 mg/m³, which corresponded to an average of 1610 ± 989 WHO fibers/cm³. The average number of fibers/cm³ >10 μm in length was 677 ± 416, while the number of those >20 μm was 236 ± 145 (Table 2).

Lung Burden Analyses

Table 4 presents average lung retention values for WHO fibers and those longer than 10 or 20 μm in length per milligram of dry lung, while Table 5 shows them per lobe for various exposure and recovery periods. The average lung burdens of WHO fibers reached their peak at 1 yr and plateaued during the remainder of the study. WHO fiber levels were higher for rock wool than slag wool at various time points during the study, for example, at 53 wk 30 mg/m³ rock wool = 319,000 ± 53,000 (fibers >20 μm length = 55,000 ± 19,000) versus 30 mg/m³ slag wool = 225,000 ± 59,000 (fibers >20 μm length = 5000 ± 3000). The retention of both rock and slag wool fibers decreased rapidly after exposure ceased; for examples, with 18 mo exposure plus 6 mo of recovery, the average number of WHO fibers per milligram dry lung for slag wool was decreased from 230,000 to 47,000, compared to 283,000 to 116,000 for rock wool. The decrease was more dramatic for fibers >20 μm in length and more pronounced for slag wool, which decreased from 31,000 to 200, compared to rock wool, 62,000 to 14,000. A similar reduction in fibers was observed when evaluated per lobe of lung (Table 5).

The number of crocidolite asbestos fibers peaked at 10 mo (end of exposure) (WHO fibers = 1,250,000 ± 218,000; fibers >20 μm length = 55,000 ± 13,000 fibers/mg dry lung) but decreased at 24 mo (10 mo of exposure plus 14 mo of recovery) (WHO = 759,000 ± 50,000; fibers >20 μm length = 41,000 ± 12,000 fibers/mg dry lung). It is of particular interest that the number of fibers >20 μm in length did not decrease appreciably from the end of exposure (54,600) to the end of the study (28 mo, 40,600) (Table 4).

The electron microscopic examination used in the counting of the fibers also revealed ultrastructural changes in the MMVF fibers after retention in the lung (Figures 1–3). After 6 mo the rock wool fibers showed surface pitting and erosion, while in addition to these changes the slag wool fibers showed segmental dissolution and cross cleavage. In contrast, the crocidolite fibers showed no morphologic change, even after 24 mo in the lung.

Clinical Observations

No abnormal clinical signs were observed in any of the rock or slag wool exposure groups during the course of the study; body weight gain (Figure 4) and survival (Figure 5) were comparable to the unexposed controls. In contrast, the positive controls (crocidolite) failed to gain weight as rapidly beginning at 3 mo and were ~12% lighter than the unexposed con-

trols at 10 mo. Labored breathing and increased mortality were observed starting at 9 mo. Because of the decrease in survival, exposure of the crocidolite exposed rats was terminated at 10 mo. The rats then began to gain weight parallel to the other groups and mortality rates returned to near control levels, although survival was lower at the end of the study (28 mo).

Lung Weights

A highly significant ($p < .01$) increase in wet lung weights (Table 6) was observed in the crocidolite-exposed rats as early as 3 mo and continued through 18 mo, at which point they were ~43% heavier (2.02 g vs. 1.41 g) than the unexposed controls. The lungs of the 30 mg rock rats were heavier starting at 6 mo and were ~24% heavier (1.75 g vs. 1.41 g) than the controls at 18 mo; 16 mg group lungs weighed more at 12–18 mo when they were ~13% heavier (1.59 g vs. 1.41 g) than the air controls. No differences in lung weights (compared to controls) were found at any time point in the 3 mg rock groups or in any of the slag-wool-exposed rats. No lung weight differences were observed at 24 mo in any of the groups (compared to controls), probably due to the high incidence of pulmonary leukemia, which is common in older F344/N rats.

Pathology

Crocidolite Asbestos The crocidolite-exposed rats were the first experimental group to show macroscopic evidence of fiber related disease. At the first interim evaluation (3 mo) the lungs contained a few small (1–2 mm diameter) greyish-white foci scattered randomly over the surface of the lung, which tended to disappear upon inflation of the lung with fixative. The foci were more numerous and more apparent at 6 mo. Additionally, the lungs failed to collapse as rapidly as the unexposed controls after opening the thorax. At 10 mo (exposure was terminated due to morbidity/mortality) the lungs failed to collapse. The foci were still numerous, but slightly less apparent, at the next (12 mo) and subsequent sacrifice points, but the lungs still failed to collapse (Figure 6). No macroscopic evidence of pleural lesions was observed at any of the scheduled sacrifice points.

Microscopically, the first evidence (3 mo) of fiber-induced disease (crocidolite group) was restricted to the terminal bronchioles and proximal alveoli. It consisted of an influx of discrete (nonfoamy) pulmonary macrophages (present both on the epithelial lining and in the lumina), focal accumulations of macrophages (microgranulomas) in the walls of alveolar ducts, metaplasia of the alveolar duct epithelium from a squamous appearance to cuboidal (bronchiolization), and early collagen deposition (fibrosis) in the walls of the terminal bronchioles and proximal interalveolar septa, rating a classification of Wagner grade 4 (Table 7). The lesions progressed in severity during the exposure period (10 mo), but still were classified as Wagner grade 4 because of a lack of interlobular linking of the fibrotic lesion (Figure 7). Although there was a marked decrease in the number of macrophages

TABLE 4. Retentions of WHO Fibers and Fibers Longer than 10 or 20 μm per Milligram of Dry Lung Tissue for Crocidolite, Rocks, and Slag Wool Exposure Groups, and Air Controls for Various Exposure and Recovery Periods

Sacrifice exposure (wk)	13/13	26/26	44/44	53/44	78/44	104/44	124/44
Recovery periods (wk)	0	0	0	9	34	60	80
Crocidolite	$n = 6$	$n = 6$	$n = 6$	$n = 3$[a]	$n = 3$	$n = 3$	$n = 6$
WHO: Mean (1000's)	628.0	647.0	1250.0	1850.0	852.0	759.0	702.0
SD	78.2	254.0	218.0	407.0	133.0	50.1	96.7
>10 μm: Mean (1000's)	159.6	201.3	402.5	372.1	293.0	251.8	248.0
SD	41.6	61.8	75.6	103.7	84.0	63.4	42.3
>20 μm: Mean (1000's)	28.8	23.8	54.6	72.7	54.1	41.7	40.6
SD	12.9	10.2	12.6	46.0	10.2	12.2	17.7
Rock wool, 30 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	83.0	143.0					
SD	6.9	28.9					
>10 μm: Mean (1000's)	47.7	76.9					
SD	4.5	20.6					
>20 μm: Mean (1000's)	18.2	22.9					
SD	2.8	7.4					
Rock wool, 16 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	37.7	85.1					
SD	7.1	17.4					
>10 μm: Mean (1000's)	21.2	47.9					
SD	3.7	12.9					
>20 μm: Mean (1000's)	7.7	15.7					
SD	2.2	5.7					
Rock wool, 3 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	11.9	20.8					
SD	3.2	3.0					
>10 μm: Mean (1000's)	7.0	8.0					
SD	2.1	2.4					
>20 μm: Mean (1000's)	2.4	2.6					
SD	1.0	0.8					
Slag wool, 30 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	98.9	2.5					
SD	21.7	20.0					
>10 μm: Mean (1000's)	36.2	31.5					
SD	9.0	8.2					
>20 μm: Mean (1000's)	5.2	3.8					
SD	1.5	2.6					
Slag wool, 16 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	37.8	45.3					
SD	7.4	6.7					
>10 μm: Mean (1000's)	14.8	14.4					
SD	4.4	2.6					
>20 μm: Mean (1000's)	1.8	2.4					
SD	0.8	1.2					
Slag wool, 3 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	5.7	5.0					
SD	3.3	1.9					
>10 μm: Mean (1000's)	2.5	1.1					
SD	1.8	0.6					
>20 μm: Mean (1000's)	0.4	0.1					
SD	0.3	0.1					

53/53 0	78/78 0	104/104 0	124/104 20	104/78 20	104/53 51	104/26 78	104/13 91
						n = 3	n = 4
						418.0	297.0
						27.4	133.0
						144.6	101.2
						21.9	53.3
						22.2	19.2
						5.3	15.3
n = 6	n = 6	n = 6	n = 6	n = 3	n = 4	n = 4	n = 3
319.0	283.0	242.0	142.0	116.0	38.8	8.6	1.6
53.0	69.9	60.2	69.5	29.9	10.2	4.9	0.7
173.6	184.9	133.9	76.3	57.3	20.4	4.5	0.5
28.5	41.1	37.1	35.0	15.4	3.5	2.7	0.5
54.7	62.3	39.5	19.6	13.8	3.8	1.2	0.2
19.0	17.9	12.1	11.8	6.1	2.2	1.3	0.3
n = 6	n = 6	n = 6	n = 6	n = 3	n = 3	n = 3	n = 3
210.0	233.0	217.0	146.0	84.2	15.9	2.7	1.2
46.7	40.8	75.6	44.3	52.2	2.7	1.2	0.4
118.9	149.0	112.3	74.0	44.6	9.1	0.7	0.8
31.7	28.6	38.0	18.8	28.2	0.4	0.05	0.3
37.1	58.2	25.6	16.1	11.8	2.4	0.2	0.2
11.4	13.8	8.2	4.3	7.6	0.4	0.2	0.2
n = 6	n = 6	n = 6	n = 4	n = 4	n = 4	n = 3	n = 4
55.0	57.3	55.7	22.9	13.2	3.7	1.1	0.3
8.4	10.3	11.7	9.2	2.0	1.3	0.2	0.3
28.6	30.1	30.3	11.8	7.8	2.4	0.9	0.01
4.9	4.3	7.3	5.1	1.3	0.8	0.5	0.01
8.3	8.9	9.0	3.6	2.6	0.8	0.4	0.05
2.5	2.7	3.1	2.1	1.1	0.2	0.5	0.1
n = 6	n = 5	n = 6	n = 6	n = 5	n = 3	n = 4[b]	n = 3
225.0	230.0	177.0	108.0	47.1	20.8	31.7	1.4
58.8	26.9	37.3	24.5	12.9	4.8	59.3	2.1
54.1	107.2	63.0	15.2	4.9	0.5	10.6	0.4
23.2	12.1	17.6	10.2	4.1	0.9	21.2	0.7
5.0	31.0	11.0	0.7	0.2	0.2	1.2	0.2
3.1	11.4	3.8	1.0	0.3	0.4	2.4	0.4
n = 6	n = 6	n = 5	n = 6	n = 3	n = 2	n = 3	n = 3
144.0	154.0	96.7	43.1	22.0	6.4	0.1	0.5
14.6	17.9	33.1	16.6	3.6	0.5	0.1	0.9
45.6	74.7	29.8	5.9	3.3	ND	ND	ND
16.1	13.6	12.2	4.9	0.7	—	—	—
7.3	17.3	4.5	0.8	0.2	ND	ND	ND
6.1	2.3	2.5	1.4	0.2	—	—	—
n = 6	n = 6	n = 6	n = 6	n = 4	n = 4	n = 3	n = 3
22.8	30.6	44.4	11.3	1.9	0.2	ND	ND
6.0	6.1	19.1	5.7	1.7	0.3	—	—
7.2	11.3	14.2	0.2	0.2	ND	ND	ND
3.0	3.4	7.1	0.5	0.2	—	—	—
1.6	1.4	1.8	0.2	0.1	ND	ND	ND
0.5	1.0	1.6	0.5	0.1	—	—	—

(Table continues on next page)

TABLE 4. Retentions of WHO Fibers and Fibers Longer than 10 or 20 μm per Milligram of Dry Lung Tissue for Crocidolite, Rocks, and Slag Wool Exposure Groups, and Air Controls for Various Exposure and Recovery Periods (*Continued*)

Sacrifice.exposure (wk)	13.13	26.26	44.44	53.44	78.44	104.44	124.44
Recovery periods (wk)	0	0	0	3	34	60	80
Air controls	$n = 6$	$n = 6$					
WHO: Mean (1000's)	0.1	1.6					
SD	0.2	1.0					
>10 μm: Mean (1000's)	0.1	0.1					
SD	0.2	0.2					
>20 μm: Mean (1000's)	0.08	0.007					
SD	0.2	0.016					

Note: WHO fibers have aspect ratios >3 to 1, lengths >5 μm, and diameters less than 3 μm. ND: not detected.

[a]Technical difficulty in preparation of SEM sample for fiber counting and measurement.

[b]Outlier animal 1099 observed with fiber lung retention 3 orders of magnitude > than lung tissues from 3 other animals.

[c]Fiber (i.e.) estimated 30 s exposure to positive control (crocidolite) fibers.

TABLE 5. Retentions of WHO Fibers or Fibers Longer than 10 or 20 μm per Dry Lung Tissue for Crocidolite, Rock Wool, and Slag Wool Exposure Groups and Air Controls for Various Exposure and Recovery Periods

Sacrifice.exposure (wk)	13.13	26.26	44.44	53.44	78.44	104.44	124.44
Recovery periods (wk)	0	0	0	9	34	60	80
Crocidolite	$n = 6$	$n = 6$	$n = 6$	$n = 3^{b}$	$n = 3$	$n = 3$	$n = 6$
WHO: Mean (1000's)	11726	17176	64865	12438	23855	19670	22504
SD	1370	7617	11563	3521	1972	7658	5030
>10 μm: Mean (1000's)	20983	5311	21011	8095	8148	6769	8061
SD	860	1783	4856	875	1832	3867	2509
>20 μm: Mean (1000's)	531	633	2811	1522	1536	987	1321
SD	227	299	515	708	370	5	752
Rock wool, 30 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	1282	2826					
SD	136	552					
>10 μm: Mean (1000's)	738	1528					
SD	78	421					
>20 μm: Mean (1000's)	280	460					
SD	43	169					
Rock wool, 16 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	579	1673					
SD	137	299					
>10 μm: Mean (1000's)	342	940					
SD	75	230					
>20 μm: Mean (1000's)	120	307					
SD	40	10					
Rock wool, 3 mg/m³	$n = 6$	$n = 6$					
WHO: Mean (1000's)	151	334					
SD	31	59					
>10 μm: Mean (1000's)	100	128					
SD	25	42					
>20 μm: Mean (1000's)	35	42					
SD	12	13					

53/53 0	78/78 0	104/104 0	124/104 20	104/78 20	104/53 51	104/26 78	104/13 91
$n = 6$[c]	$n = 6$	$n = 6$	$n = 6$	$n = 4$	$n = 3$	$n = 3$	$n = 4$
5.7	2.1	0.6	0.4	0.3	1.0	0.1	0.1
3.6	1.3	0.8	0.7	0.5	1.1	0.1	0.1
2.2	1.2	0.02	0.1	ND	ND	0.1	ND
1.8	0.8	0.05	0.2	—	—	0.1	—
0.5	0.2	ND	ND	ND	0.03	0.008	ND
0.5	0.3	—	—	—	0.05	0.013	—

53/53 0	78/78 0	104/104 0	124/104 20	104/78 20	104/53 51	104/26 78	104/13 91
						$n = 3$	$n = 4$
						9400	8866
						2699	2984
						3153	2950
						197	932
						495	549
						140	373
$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 3$	$n = 4$	$n = 4$	$n = 3$
6648	6378	6724	3346	2424	775	260	38
1050	1762	2121	1657	502	231	66	14
3609	4173	3727	1815	1212	409	94	8
555	1117	1321	865	355	93	56	8
1161	1391	1094	467	310	77	25	3
373	370	404	295	153	47	24	4
$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 3$	$n = 3$	$n = 3$	$n = 3$
3280	4940	5137	3658	1895	383	45	33
788	1063	1715	762	1235	95	13	11
1860	3141	2679	1865	1004	219	13	25
535	661	933	269	668	38	2	15
588	1217	602	406	265	57	3	[illegible]
216	247	144	68	181	4	2	6
$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 4$	$n = 4$	$n = 3$	$n = 4$
772	1075	101	549	263	75	22	[illegible]
170	253	206	83	39	26	5	5
376	562	549	286	154	49	18	2
93	102	121	71	26	19	11	4
110	168	161	82	53	16	9	2
40	58	48	30	23	3	11	4

(Table continues on next page)

TABLE 5. Retentions of WHO Fibers and Fibers Longer than 10 or 20 μm per Dry Lung Lobe for Crocidolite, Rock Wool, and Slag Wool Exposure Groups and Air Controls for Various Exposure and Recovery Periods (*Continued*)

Sacrifice exposure (wk) Recovery periods (wk)	13/13 0	26/26 0	44/44 0	33/44 9	78/44 34	104/44 60	124/44 80
Slag wool, 30 mg/m³	n = 6	n = 6					
WHO, Mean (1000's)	1503	1569					
SD	397	211					
>10 μm, Mean (1000's)	548	536					
SD	149	176					
>20 μm, Mean (1000's)	78	67					
SD	26	43					
Slag wool, 10 mg/m³	n = 6	n = 6					
WHO, Mean (1000's)	537	745					
SD	109	98					
>10 μm, Mean (1000's)	202	237					
SD	60	39					
>20 μm, Mean (1000's)	25	39					
SD	10	20					
Slag wool, 3 mg/m³	n = 6	n = 6					
WHO, Mean (1000's)	76	74					
SD	42	31					
>10 μm, Mean (1000's)	33	17					
SD	23	11					
>20 μm, Mean (1000's)	6	2					
SD	4	1					
Air controls	n = 6	n = 6					
WHO, Mean (1000's)	1	9					
SD	2	16					
>10 μm, Mean (1000's)	1	2					
SD	2	3					
>20 μm, Mean (1000's)	1	0.1					
SD	2	0.3					

Note. WHO fibers have aspect ratios >3 to 1, lengths >5 μm, and diameters less than 3 μm. ND, not detected.

[1]Technical difficulty in preparation of SEM sample for fiber counting and measurement.

[2]Outlier (animal 1099) observed with fiber lung retention 3 orders of magnitude > than lung tissues from 3 other animals.

[3]Brief (i.e., estimated 30 s) exposure to positive control (crocidolite) fibers.

after exposure ceased, the other pulmonary changes noted earlier did not decrease in severity.

Primary lung neoplasms were recognized macroscopically as solitary whitish-gray, usually spherical nodules that varied from 3–4 mm to 15 mm in diameter. Microscopically, the neoplastic lesions consisted of a continuum of benign (bronchoalveolar adenoma) to malignant (bronchoalveolar carcinoma) changes of typical morphology to those seen in F344 rats (Boorman & Eustis, 1990). The primary growth pattern that differentiated "benign" from "malignant" change was the local invasion of bronchi or blood vessels. Squamous metaplasia was occasionally found in adenomas and carci-

53/53	78/78	104/104	124/104	104/78	104/53	104/26	104/13
0	0	0	20	20	51	78	91
$n = 6$	$n = 5$	$n = 6$	$n = 6$	$n = 5$	$n = 3$	$n = 4^b$	$n = 3$
2905	4411	4447	2482	930	469	725	29
578	635	725	1080	314	109	1380	38
696	2051	1579	394	93	10	245	8
252	274	405	401	71	18	489	14
65	594	292	18	4	4	27	4
38	226	138	28	6	7	55	7
$n = 6$	$n = 6$	$n = 5$	$n = 6$	$n = 3$	$n = 2$	$n = 3$	$n = 3$
1879	2879	1977	934	487	173	2	13
471	605	453	380	188	55	2	22
596	1389	593	137	80	ND	ND	ND
268	275	184	138	54	—	—	—
96	323	90	17	4	ND	ND	ND
86	62	41	29	4	—	—	—
$n = 6$	$n = 6$	$n = 6$	$n = 6$	$n = 4$	$n = 4$	$n = 3$	$n = 3$
321	502	766	228	30	3	ND	ND
99	88	308	112	25	5	—	—
101	182	248	25	2	ND	ND	ND
51	43	123	27	3	—	—	—
14	22	31	3	1	ND	ND	ND
7	13	26	8	2	—	—	—
$n = 6^c$	$n = 6$	$n = 6$	$n = 6$	$n = 4$	$n = 3$	$n = 3$	$n = 4$
82	35	11	10	4	29	1	2
51	21	16	16	8	31	2	2
31	20	0.3	5	ND	ND	1	ND
25	12	2.0	12	—	—	2	—
7	3	ND	ND	ND	1	0.1	ND
7	5	—	—	—	1	0.2	—

nomas. By the end of the study 16 of 106 (14.1%) of the crocidolite-exposed rats had a primary pulmonary neoplasm, compared with 2 of 126 (1.6%) ($p < .01$) of the unexposed controls (Table 8).

Minimal to mild focal fibrosis was noted in the visceral pleura in a majority of crocidolite exposed rats starting at 12 mo (Figure 8). It did not increase in severity during the remainder of the study, although a single well-developed mesothelioma was observed in one animal (Table 8). It covered a large portion of the lung and was of the typical papillary type.

Rock (Stone) Wool The first macroscopic evidence of treatment-related effects was at 12 mo when a few similarly appearing small (1–2 mm diame-

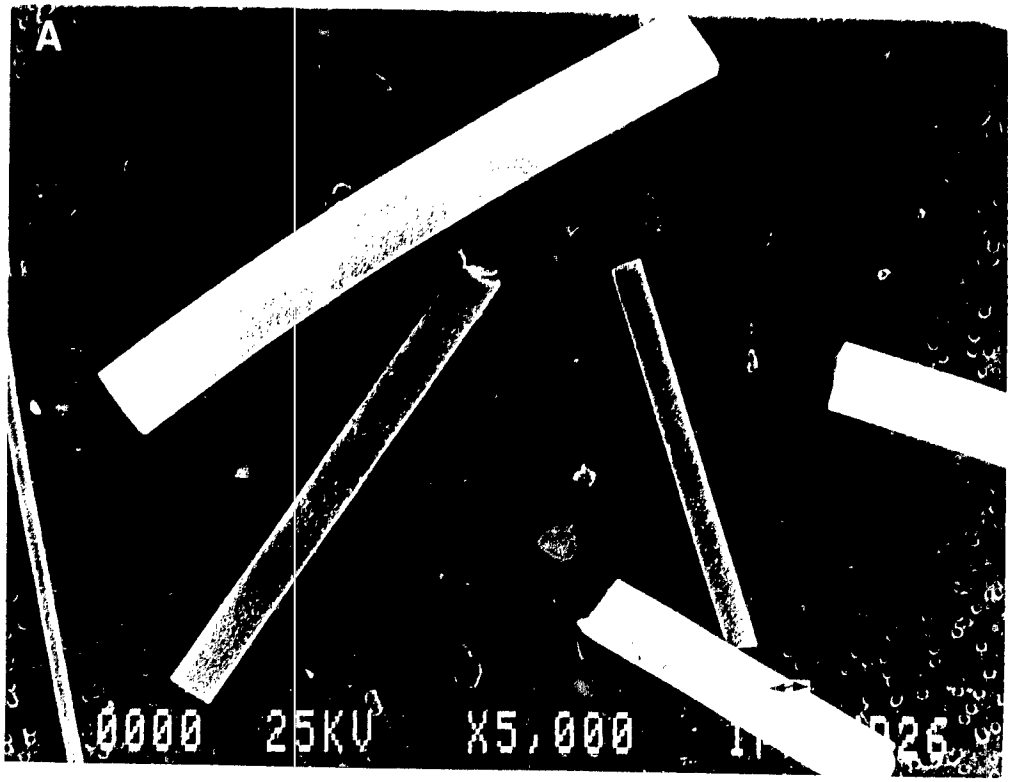

FIGURE 1. Scanning EM photomicrograph showing the appearance of the "sized" rock wool fibers (A) after aerosolization (×5000)

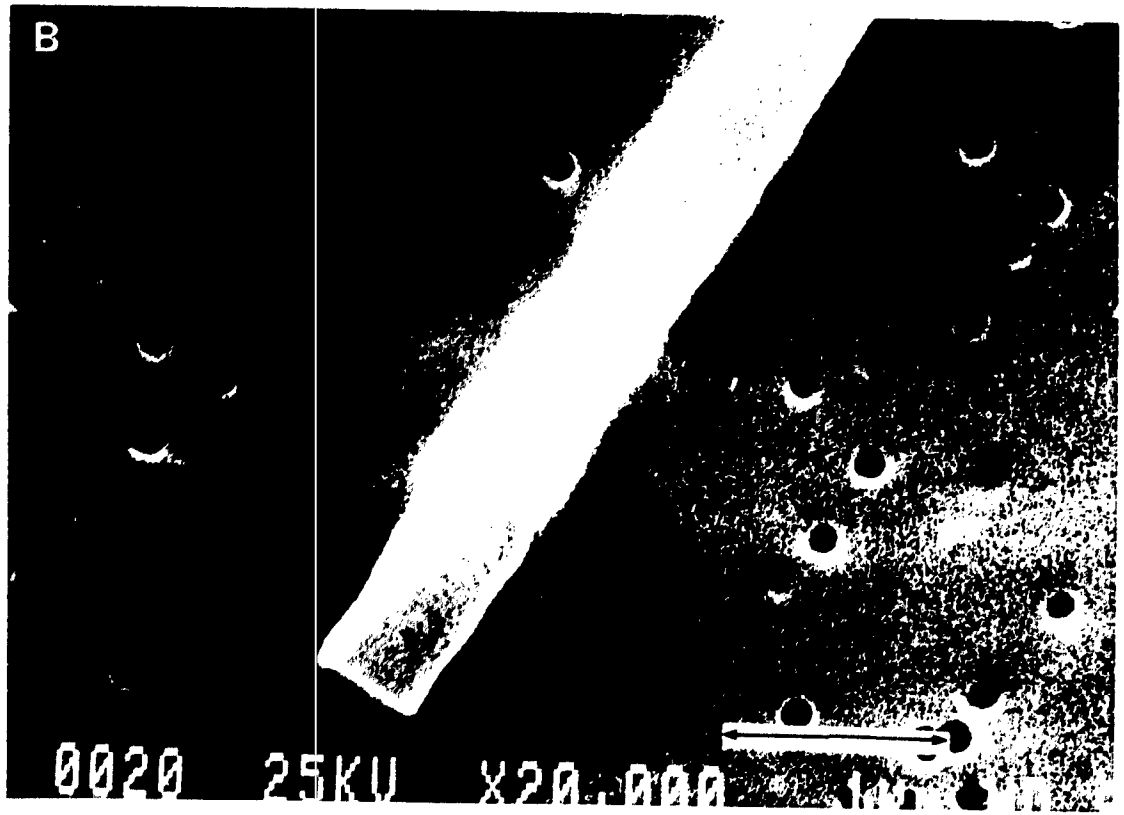

FIGURE 1. *(Continued)* Scanning EM photomicrograph showing the appearance of the "sized" rock wool fibers (B) after being in the lung for 6 mo (note surface etching), ×20,000.

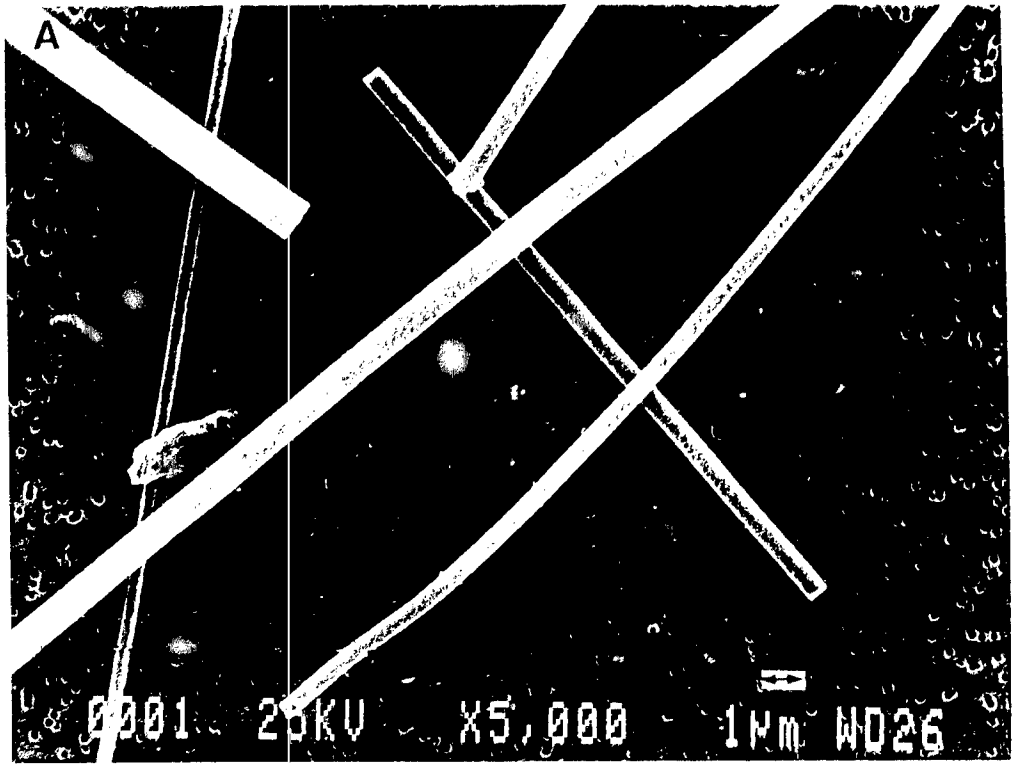

FIGURE 2. Scanning EM photomicrograph showing the appearance of the "sized" slag wool fibers (A) after aerosolization (×5000)

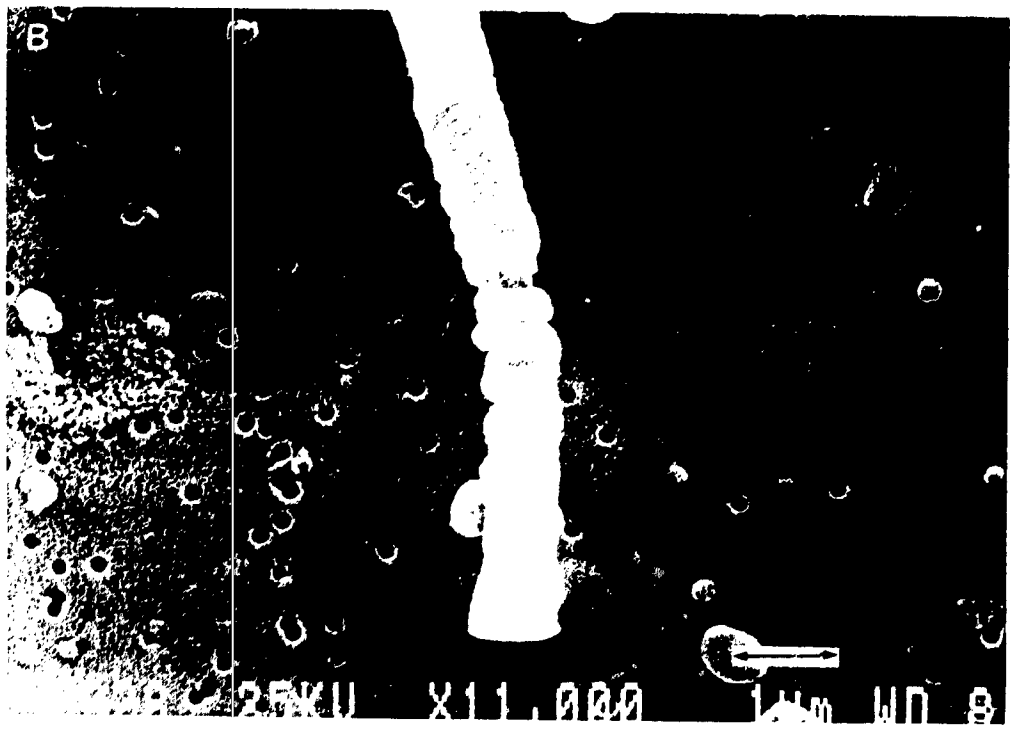

FIGURE 2. (*Continued*) Scanning EM photomicrograph showing the appearance of the "sized" slag wool fibers (B) after being in the lung for 6 mo (note severe damage to fibers), ×10,000.

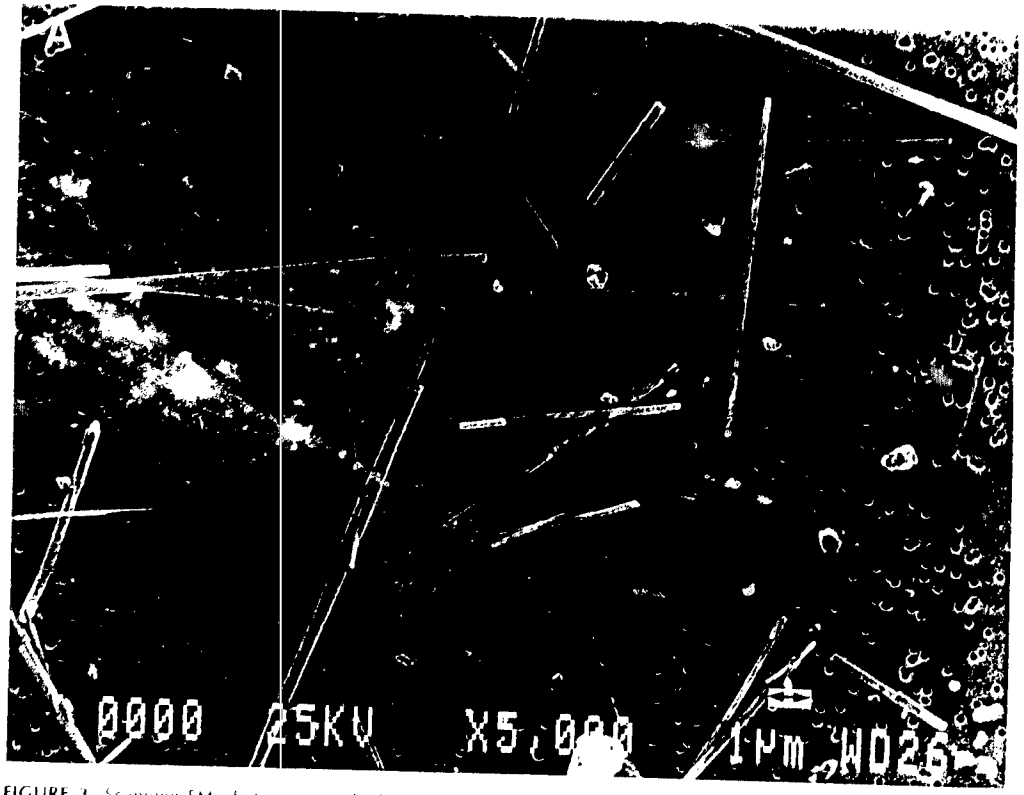

FIGURE 3. Scanning EM photomicrograph showing the appearance of the "sized" crocidolite asbestos fibers (A) after aerosolization

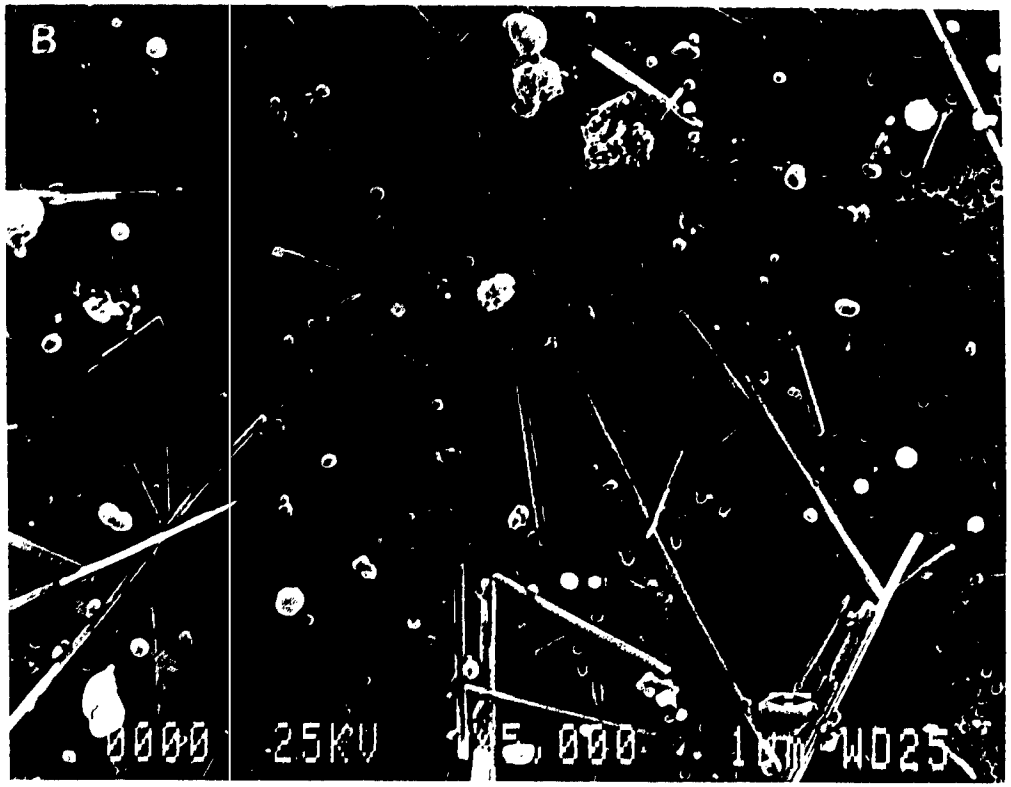

FIGURE 3. *(Continued)* Scanning EM photomicrograph showing the appearance of the "sized" crocidolite asbestos fibers (B) after being in the lung for 24 mo. ×5000.

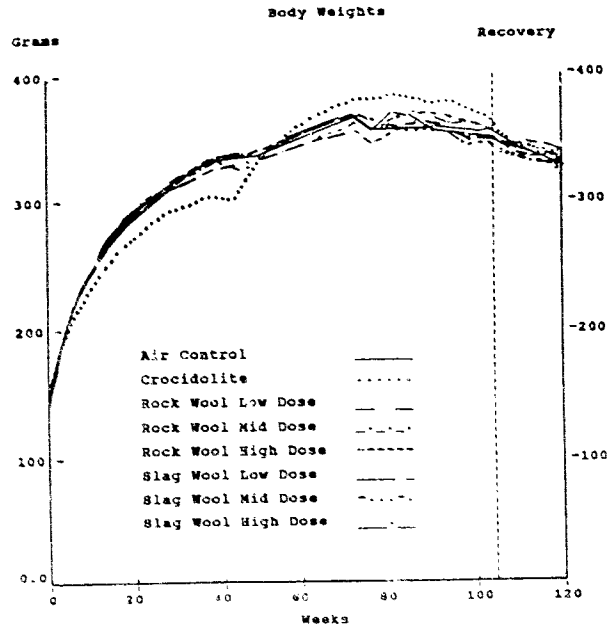

FIGURE 4. Graph showing body weight gain of male F344/N rats exposed to rock and slag wool and crocidolite asbestos.

ter) greyish-white foci were observed in 6 of 6 high- (30 mg) and 4 of 6 mid- (16 mg) dose rats; again, the foci tended to disappear upon inflation of the lung. Also, these lungs did not collapse in a normal fashion. These lesions were more apparent at 18 and 24 mo but of much less severity than in the crocidolite-exposed rats (Figure 9). No macroscopic evidence of pleural changes was noted at any point.

Microscopically, a dose-related increase in pulmonary macrophages and developing microgranulomas in the alveolar duct region were observed at 3 mo. Additionally, a mild amount of bronchiolization was noted in all high-dose rats. The pulmonary response had progressed at 6 mo with bronchiolization being found in the mid- as well as the high-dose group rats (Table 7). The lesions further progressed at 12 mo, with 2 of 6 rats in the high dose (30 mg) showing a minimal amount of fibrosis in the proximal portion of the alveolar duct region (Table 7). At 18 mo minimal (16 mg) and mild (30 mg) fibrosis was noted in all rats in the mid- and high-dose groups (Figure 10), although it was more apparent in the latter animals. No significant increase in severity was found at the end of the exposure period (24 mo) nor at the end of the study (28 mo). The number of pulmonary macrophages decreased dramatically during the nonexposure period. Fibers were noted in macrophages, in microgranulomas, and on surfaces of the

alveolar ducts and proximal alveoli throughout the study (Figure 11). Additional fibers and especially fiber fragments were observed in macrophages in the peribronchial lymphoid tissues. No treatment-related lesions were observed in the pleura.

Occasional bronchoalveolar neoplasms were found in all dose groups (5 of 114 low dose, 5 of 115 mid dose and 5 of 114 high dose), including the unexposed controls (2 of 126), but there was neither a statistically significant increase nor a dose-response relationship (Table 8). No mesotheliomas were observed.

Slag Wool No treatment-related macroscopic lesions were observed in the lungs or pleura of rats exposed to slag wool at any point during the study (Figure 12).

Microscopically, there was a dose- and time-related increase in pulmonary macrophages, microgranuloma formation, and bronchiolization (Table 7). However, there was no evidence of fibrosis at any point (Figure 13). Fibers and fiber fragments were readily found in many of the macrophages and microgranulomas. No treatment-related lesions were observed in the pleura. Occasional bronchoalveolar neoplasms were found: 2 of 116 low dose, 0 of 115 mid dose, and 3 of 115 high dose, compared to 2 of 126 unexposed controls (Table 8).

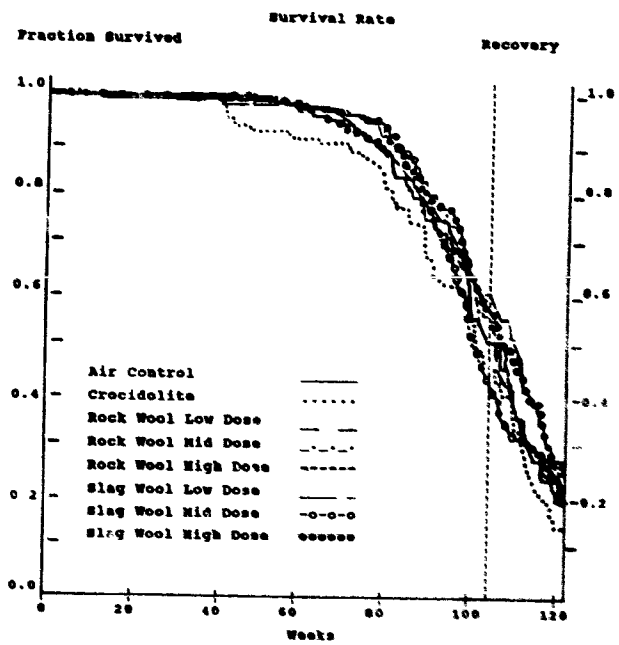

FIGURE 5. Kaplan-Meier survival curve for male F344/N rats exposed to rock and slag wool and crocidolite asbestos.

TABLE 6. Lung Weights from Rats Exposed to Rock or Slag Wool and Crocidolite Asbestos

	Control crocidolite			Rock wool		Slag wool		
Months	0 mg	10 mg	3 mg	16 mg	30 mg	3 mg	16 mg	30 mg
3	1.02 ± 0.12	1.37 ± 0.15[a]	1.12 ± 0.06	1.14 ± 0.12	1.18 ± 0.07[b]	1.06 ± 0.06	1.13 ± 0.05	1.18 ± 0.05[b]
6	1.20 ± 0.07	1.62 ± 0.08[a]	1.18 ± 0.06	1.31 ± 0.04	1.38 ± 0.09[a]	1.13 ± 0.07	1.20 ± 0.10	1.21 ± 0.06
12	1.30 ± 0.11	2.05 ± 0.27[a]	1.37 ± 0.07	1.51 ± 0.06[a]	1.64 ± 0.07[b]	1.30 ± 0.06	1.37 ± 0.09	1.42 ± 0.11
18	1.41 ± 0.06	2.02 ± 0.16[a]	1.42 ± 0.10	1.59 ± 0.08[b]	1.75 ± 0.11[b]	1.31 ± 0.11	1.45 ± 0.09	1.45 ± 0.04
24	1.66 ± 0.34	2.03 ± 0.50	1.57 ± 0.13	1.97 ± 0.24	2.05 ± 0.21	1.60 ± 0.18	1.71 ± 0.27	2.17 ± 0.77

[a]Significant at $p < .01$.
[b]Significant at $p < .05$.

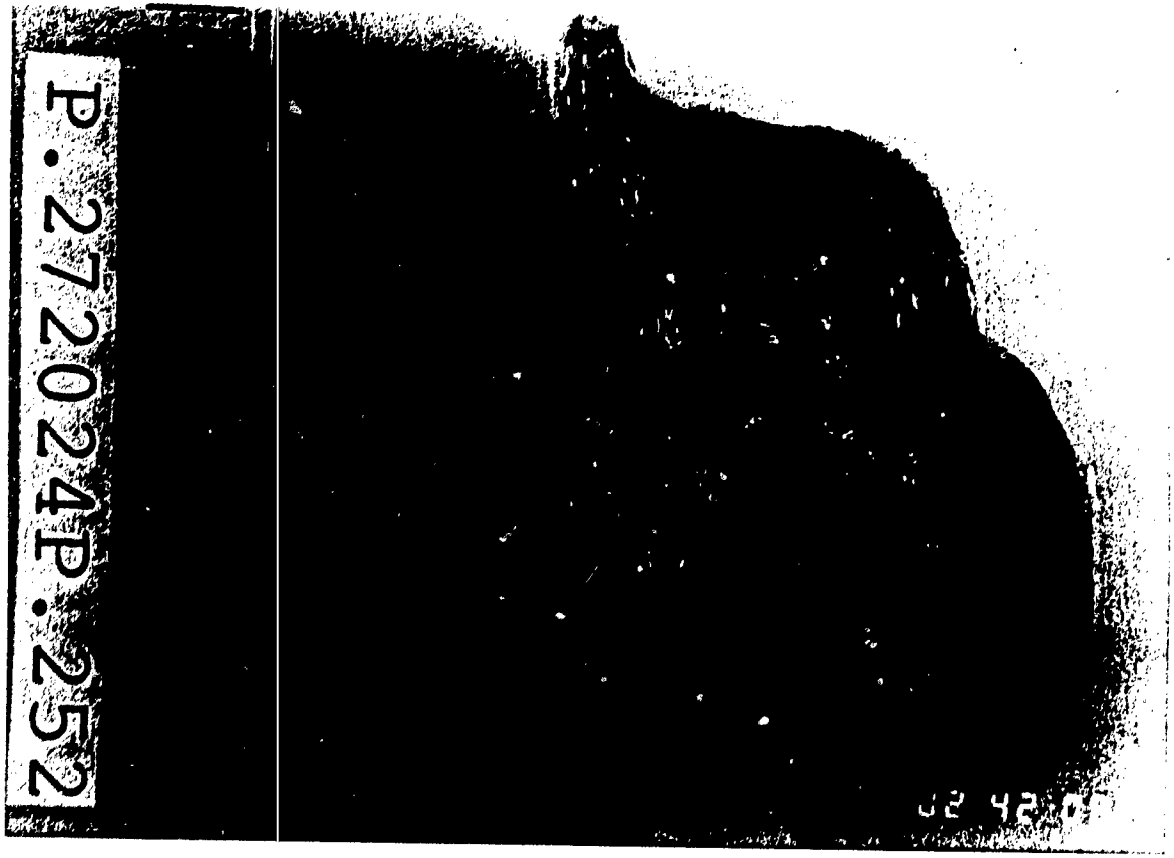

FIGURE 6. Macroscopic appearance of the lung of rat exposed to crocidolite asbestos for 10 mo and killed at 18 mo. Note multifocal white foci scattered over the surface of the lung and failure of the lung to collapse.

TABLE 7. Pulmonary Cellular Change and Fibrosis[1] in Male F344/N rats Exposed to Rock or Slag Wool and Crocidolite Asbestos Fibers for 3, 6, 9, 12, 15, 18, or 24 mo

Exposed/sacrificed	Control	Crocidolite	Rock wool 3 mg	Rock wool 16 mg	Rock wool 30 mg	Slag wool 3 mg	Slag wool 16 mg	Slag wool 30 mg
3/3	1.0	4.0	2.0	2.2	3.[illegible]	2.0	2.5	2.5
6/6	1.0	4.2	2.0	2.7	3.3	2.0	2.7	3.0
10/10	—	4.0[2]	—	—	—	—	—	—
12/12	1.0	4.0[2]	2.0	2.7	3.3	2.5	2.2	3.0
18/18	1.0	4.0[2]	2.5	4.0	4.0	2.7	2.5	2.7
24/24	1.0	4.0[2]	2.2	4.0	4.0	2.0	2.2	2.8
Recovery								
3/24	1.0	4.0	1.3	1.0	1.0	1.3	1.3	1.3
6/24	1.0	4.0	1.0	3.0	2.0	2.0	1.3	2.0
12/24	1.0	4.0[2]	1.3	3.3	4.0	1.5	2.0	2.5
18/24	1.0	—	2.0	4.0	4.0	2.2	2.3	2.2
24/28	1.0	4.0[2]	2.2	4.0	4.0	2.0	2.6	2.5

Note: — No animals sacrificed.
[1]Average Wagner scores (0 = 1–6) (McConnell et al., 1984).
[2]Crocidolite rats were exposed for only 10 mo.

Other lesions No treatment-related lesions were observed in the upper respiratory tract or in any other organ in the MMVF or crocidolite exposed rats. Many rats, including the unexposed control animals, showed evidence of mononuclear cell leukemia involvement of the lung (diffuse gray discoloration, paleness, and/or multifocal punctuate gray foci) at 24 mo, and especially so at the end of the study. An enlarged spleen and nodular liver confirmed this finding. This lesion was also a prominent feature in rats that died or were killed in a moribund condition during the course of the study.

DISCUSSION

Rock wool has been previously tested in two long-term inhalation studies in rats (La Bouffant et al., 1987; Wagner et al., 1984) and slag wool once each in rats (Smith et al., 1987) and hamsters (Smith et al., 1987). None of the inhalation studies identified an increase in either fibrosis or treatment-related increase in pulmonary neoplasms or mesotheliomas. Both MMVFs have also been studied by intracavitary instillation in rats (Minardi & Maltoni, 1989; Pott et al., 1987; Stanton et al., 1981; Wagner et al., 1984). While the results of the slag wool injection studies were uniformly negative, an experimental rock wool induced an increased incidence of abdominal mesotheliomas in some studies, but only after several instillations (3–5) and high levels (total 75 mg/animal) (Pott et al., 1987).

While the previously reported inhalation studies did not show evidence of overt pulmonary pathology in animals from breathing these types of fibers,

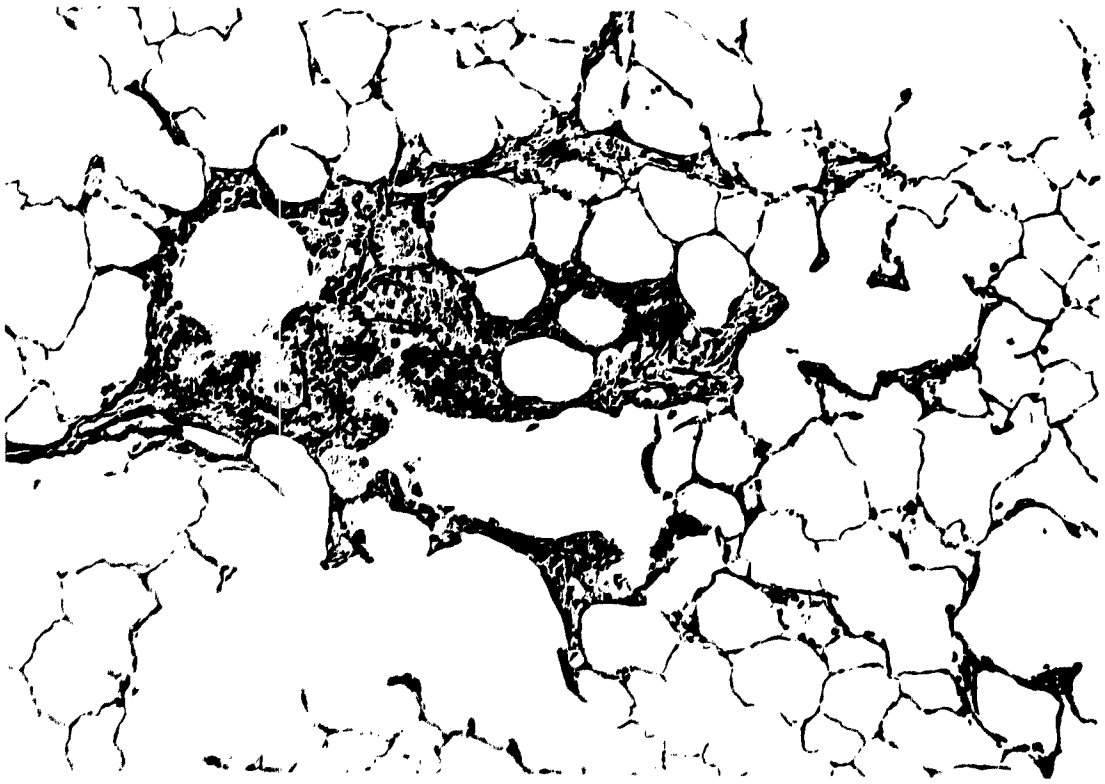

FIGURE 7. Light micrograph of the lung from a crocidolite exposed rat after 12 mo showing marked fibrosis in the bronchioalveolar region. Goldner's trichrome, ×200.

TABLE 8. Pulmonary Neoplasms in Male F344/N Rats Exposed to Rock or Slag Wool and Crocidolite Asbestos Fibers

Exposure group	At risk	Adenoma (%)	Carcinoma (%)	Total lung tumors (%)	Mesothelioma (%)
Controls	126	2 (1.6)	0	2 (1.6)	0
Crocidolite[a]	106	10 (9.4)	5 (5.7)	14	1 (0.9)
Rock wool					
3 mg/m³	114	4 (3.5)	1 (0.9)	5 (4.4)	0
16 mg/m³	115	4 (3.5)	1 (0.9)	5 (4.3)	0
30 mg/m³	114	4 (3.5)	1 (0.9)	5 (4.4)	0
Slag wool					
3 mg/m³	116	1 (0.9)	1 (0.9)	2 (1.7)	0
16 mg/m³	115	0	0	0	0
30 mg/m³[b]	115	2 (2.6)	1 (0.9)	3 (2.6)	0

Note. Number includes all rats exposed to MMVFs for 12 mo, crocidolite 10 mo; controls surviving at least 12 mo.

[a]Two rats not included in this table because they were not exposed for 10 mo, had either an adenoma or carcinoma.

[b]One rat not included in this table because it was not exposed for 12 mo, had an adenoma.

only one of these studies (Smith et al., 1987) used technically advanced nose-only exposures or a large number of "size-selected" fibers. Because of significant improvements in experimental inhalation including nose-only exposure and ability to generate aerosols without breaking fibers, the subject studies were undertaken using state-of-the-art technology. To insure exposure to the deep lung of environmentally relevant fibers (those that have been found in the workplace) and to stimulate any possible potential pathology, every effort was made to maximize the number of long/thin rat respirable fibers by size selection techniques. Additionally, the animals were exposed for a majority (>80%) of their potential life-span to levels that were at least 1000-fold higher than would be expected under typical manufacturing or user conditions. The highest dose of rock wool used in this study contained 243 and slag wool contained 213 WHO fibers/cm³ compared to workplace exposures that are generally less than 0.1 fibers/cm³ (Cherrie et al., 1986) and rarely greater than 1 fiber/cm³ (ILO, 1989).

The lung burdens observed in these rats were much greater than would be expected in humans exposed to these fibers during manufacture or use. In human lungs such fibers have not been found, although data are scarce (Karjalainen et al., 1993). For example, no rock/slag wool fibers were observed in lung tissues from 11 deceased rock/slag wool workers (McDonald et al., 1990). However, one might expect the levels in the lungs of rock/slag wool workers to be comparable to those in the glass wool insulation industry. In one study of mainly former glass fiber production workers (n = 121), only 26% had any MMVF in their lungs, and those that did only

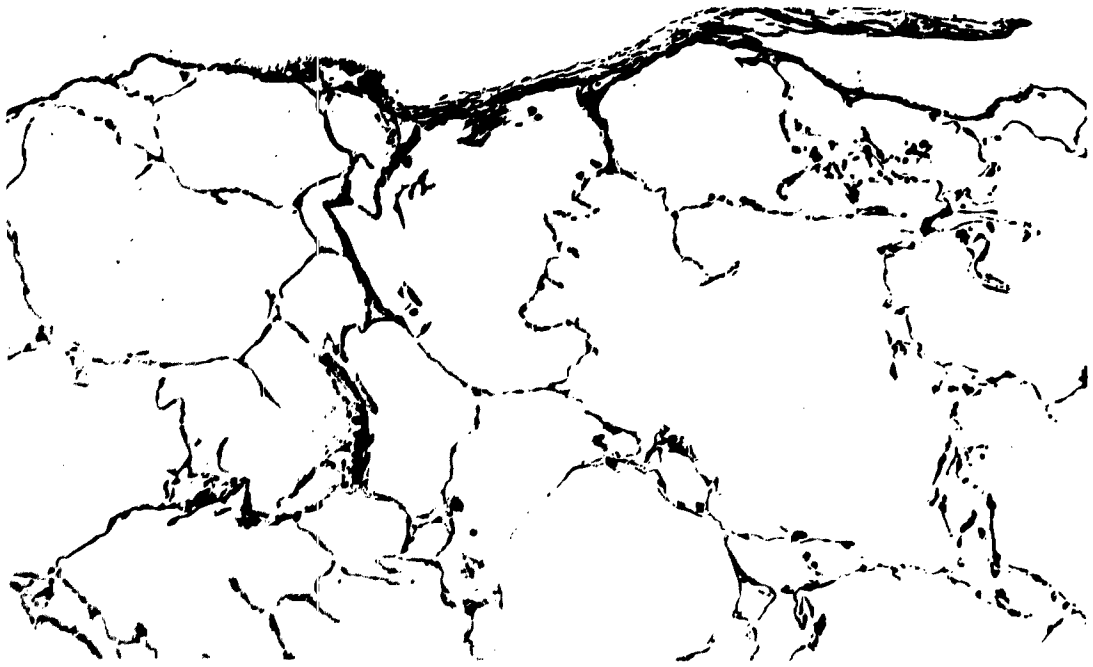

FIGURE 8. Light micrograph of the lung from a crocidolite exposed rat after 12 mo showing focal fibrosis of the pleura. Goldner's trichrome, ×200.

FIGURE 9. Macroscopic appearance of the lung of rat exposed to rock wool for 18 mo and killed at 18 mo. Note a few focal white foci scattered over the surface of the lung.

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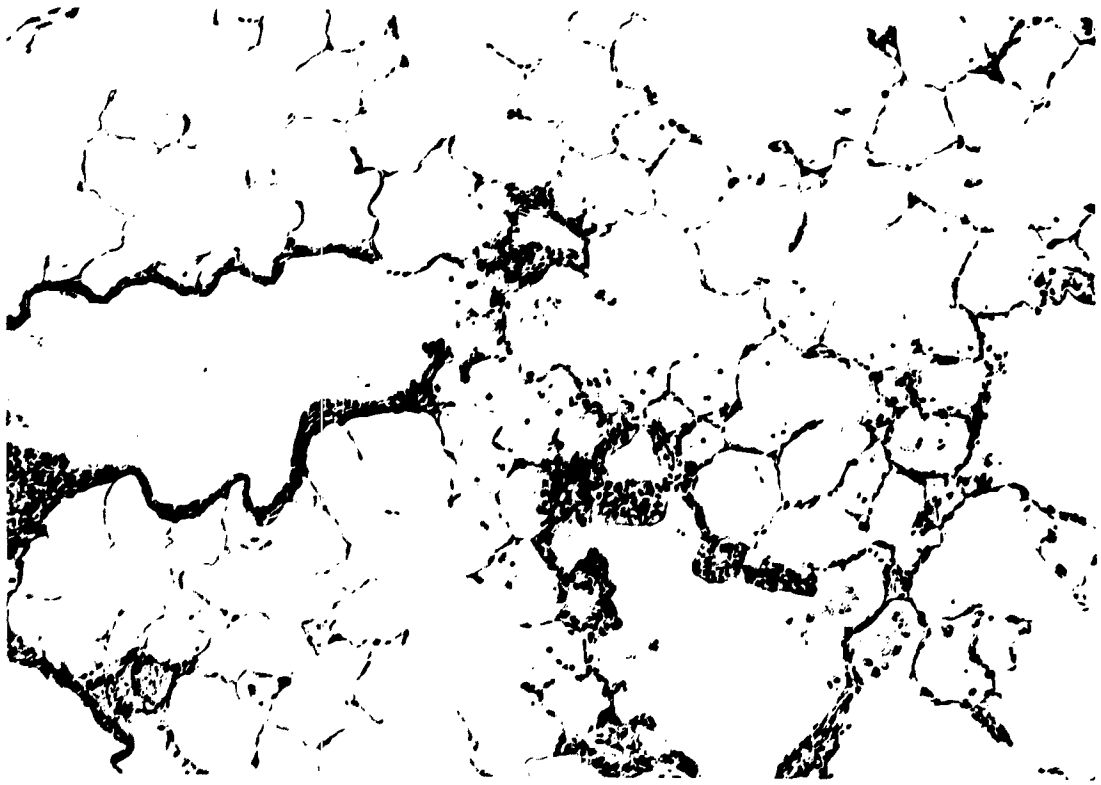

FIGURE 10. Light micrograph of lung from a rock-wool-exposed rat (30 mg/m³) after 18 mo showing macrophage infiltration, bronchiolization, and mild fibrosis in the wall of the proximal alveolar duct. Goldner's trichrome, ×200.

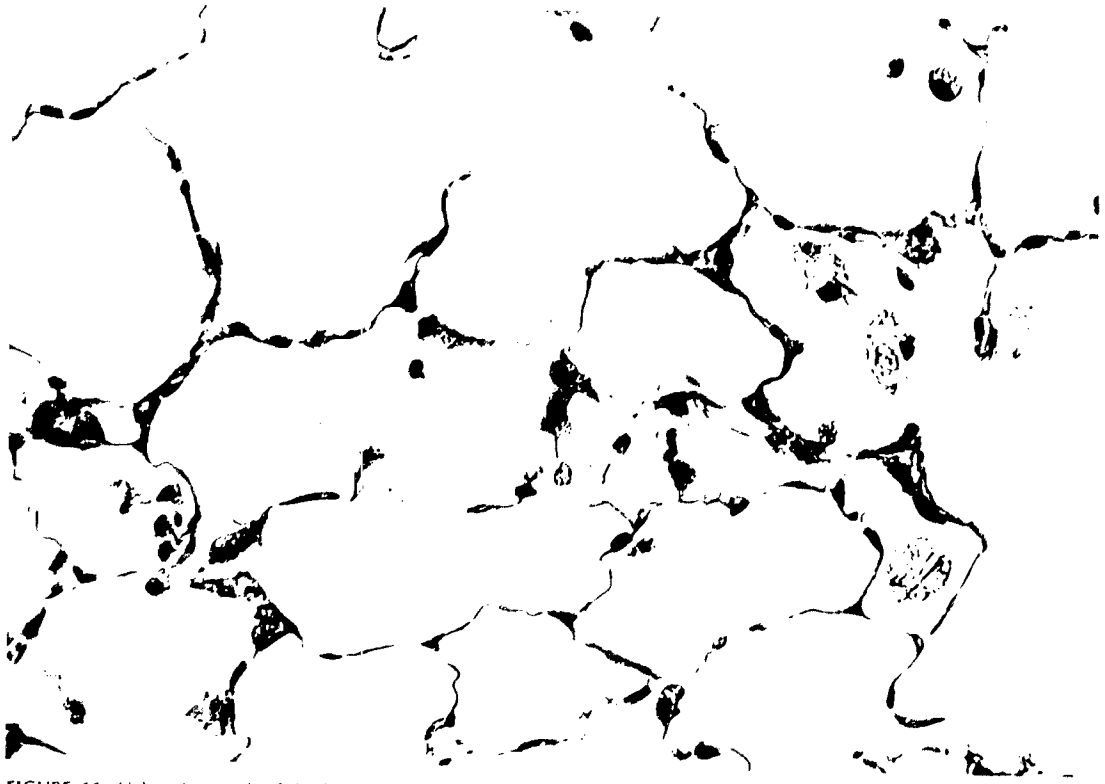

FIGURE 11. Light micrograph of the lung of a rock wool exposed rat showing fibers within macrophages and microgranulomas. Goldner's trichrome, ×600.

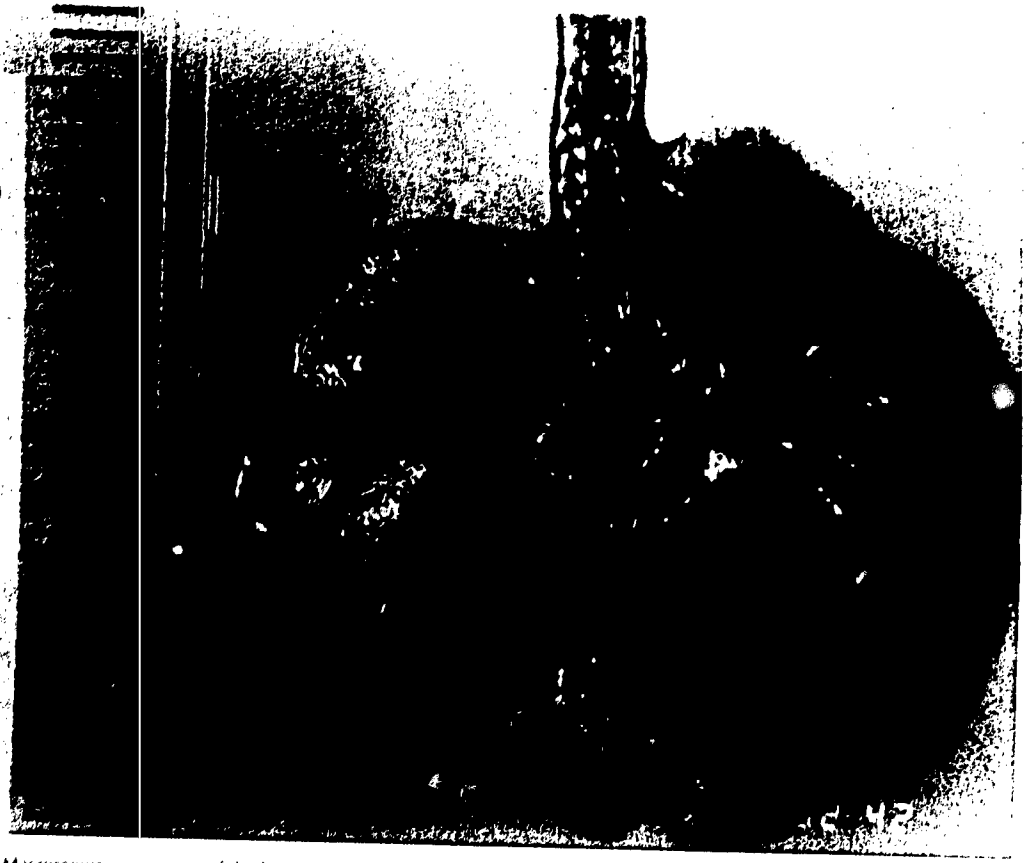

FIGURE 12. Macroscopic appearance of the lung of rat exposed to slag wool for 18 mo and killed at 18 mo. Only a single white focus is present

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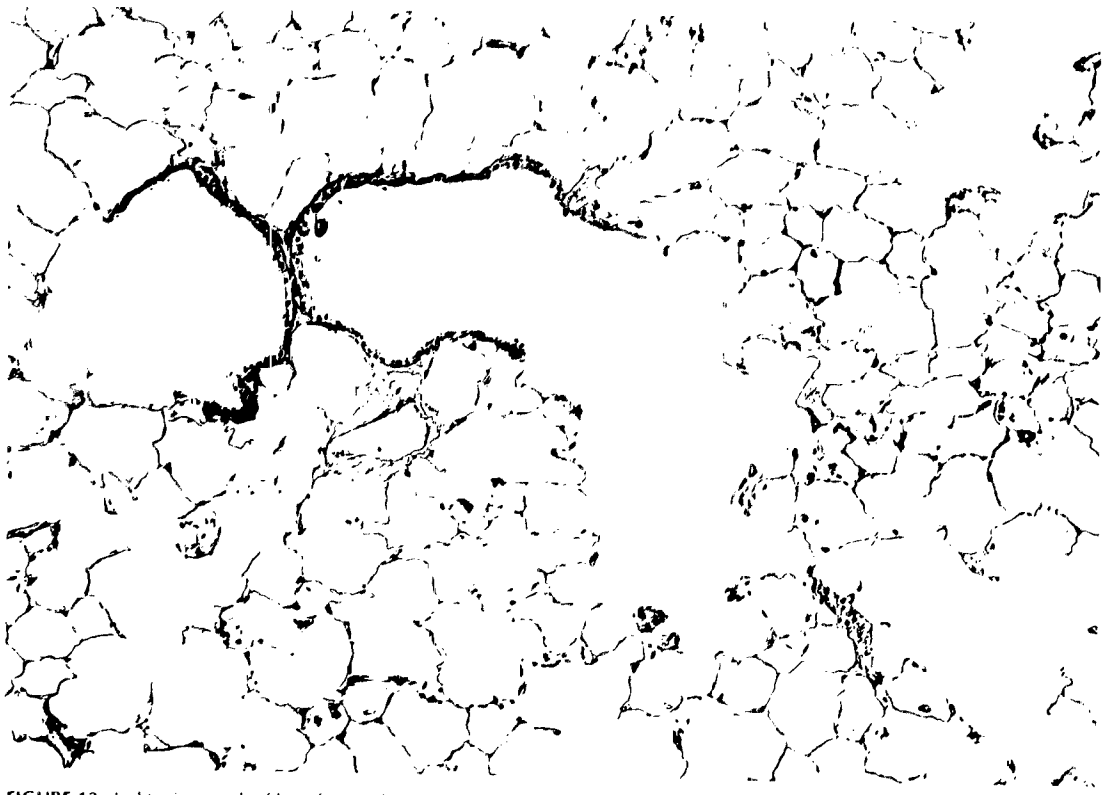

FIGURE 13. Light micrograph of lung from a slag-wool-exposed rat (30 mg/m^3) after 18 mo, showing macrophage infiltration and minimal bronchiolization in the proximal alveolar duct. Goldner's trichrome, ×200.

had ~70 WHO fibers/mg dry lung tissue (McDonald et al., 1990). A postmortem examination of 20 workers employed in a glass fiber factory revealed a lung burden of 95 fibers/mg dry lung (average diameter of 2.4 μm and length of 28 μm) (Gross et al., 1976). This compares to ~319,000 WHO and ~54,700 fibers >20 μm length/mg dry lung at 12 mo in the high-dose (30 mg/m^3) rock and ~225,000 WHO and 5,000 fibers >20 μm length in the slag wool rats. In summary, the rats in this study were exposed to and retained far greater numbers of fibers in their lungs than could occur under any conceivable human exposure condition.

Although the slag wool exposures of these rats were extremely high (213 WHO fibers/cm^3) compared to human exposure conditions (generally <1.0 WHO fibers/cm^3), there were no biologically significant adverse effects (fibrosis or tumorigenesis) in the lung or pleura of these rats. The fibers did, however, induce a prominent macrophage response, microgranulomas, and a mild localized metaplastic change (bronchiolization) of the epithelial lining of the proximal alveolar duct, as would be expected from the introduction of high levels of "inert" foreign particulates. Numerous fibers were observed in these macrophages, and fiber fragments were found in distant locations (peribronchial lymphoid tissues and bronchial lymph nodes), further indicating significant lung tissue exposure.

Rock wool also caused the previously described nonspecific particulate inflammatory response, but in addition produced dose-related fibrosis in the interalveolar interstitium. However, the fibrotic change was very restricted in location (terminal bronchioles and proximal alveoli), was of minimal (16 mg exposure) or mild (30 mg exposure) severity, and occurred only after an extended time (>12 months) with high lung burden levels of fibers >20 μm length, which are more prone to induce fibrosis (Davis, 1991). Nonfibrous particulates of inert dust (TiO_2) have also been shown to cause pulmonary fibrosis when the exposure is high (Lee et al., 1985). The degree of fibrosis with rock wool was of much less severity than that produced by crocidolite asbestos, although both were categorized as Wagner grade 4. In this respect the Wagner grading system can be misleading; the difference between grade 4 and grade 5 in terms of severity is great in that the latter grade requires that the fibrotic lesion extend between lobules. The Wagner grading system does not take into account the differences in severity of a specific lesion at a given location or the extent of that lesion in terms of the amount of the lung involved. For example, a lung containing a few widely scattered areas of fibrosis of minimal severity would be given the same score (Wagner grade 4) as one with widespread areas of moderate or severe fibrosis, as long as there was no interlobular linking of the fibrotic lesion.

The inflammatory response decreased markedly after exposure was terminated in both the rock- and slag-wool-exposed rats. The number of macrophages and microgranulomas, as well as the degree of bronchiolization, was much less apparent in rats that were removed from exposure at various times (3, 6, 12, or even 18 mo) and held until 24 mo. The macro-

phages that were still present usually contained fibers/fragments. This reversible change is best explained by the decrease in lung burden. In contrast, fibrosis did not regress and should be considered a permanent change.

However, just as importantly, examination of the "recovery" animals showed that pulmonary fibrosis did not progress after cessation of exposure to rook wool or even crocidolite asbestos. This observation is somewhat perplexing, especially with regard to crocidolite, where the decrease in lung burden was not nearly as evident as with rock wool. This same phenomenon has been observed in other rodent inhalation studies of fibrogenic materials such as chrysotile asbestos and refractory ceramic fibers (Mast et al., in press; McConnell et al., 1984; in press) The failure of interstitial fibrosis to progress after cessation of exposure suggests that continuing insult (exposure) may be necessary to fully manifest the fibrogenic potential of a given fiber. Nevertheless, recovery studies are a useful addition to an inhalation protocol for this very reason.

Wet lung weight was also an early and particularly sensitive indicator of fiber exposure. The lung weights from crocidolite exposed rats were significantly ($p < .01$) increased as early as 3 mo and were 43% heavier than the controls at 18 mo. While no significant increase in slag wool lung weights was observed during the course of the study, the lungs of the 30 mg rock-wool-exposed rats were marginally increased ($p < .05$) at 3 mo and increased in weight through 18 mo, when they were 24% heavier than the controls. The 16 mg rock wool lungs were marginally heavier starting at 12 mo and were 13% greater than the controls at 18 mo. These data suggests that the increased lung weights were due to more than an increase in collagen (fibrosis), since this was not observed in the rock wool exposed rats until 12 mo. It is speculated that pulmonary edema and inflammation may have been the cause of the early increase in lung weights.

The most reasonable explanation for the lack of a difference in lung weights at 24 mo is the high rate of mononuclear cell leukemia, which readily infiltrates the capillaries of the lung (Stephanski et al., 1990). Leukemia is a particular problem in aging (>21 mo) male F344/N rats, where the average incidence at 24 mo is 34% with a range up to 72% (Haseman, 1990). Therefore, lung weight data are less meaningful in this strain of rat after 18 mo of age.

It is of interest that the rock and slag wool fibers did not produce neoplasms or prominent fibrosis, even though those retained in the lung were of a size that would be expected to produce such lesions based on the Stanton hypothesis of fiber carcinogenesis (Stanton et al. 1981), that is, fibers of respirable diameter of long length (>8 μm). However, it is now known that a combination of criteria must be met before a fiber can induce cancer. In addition to dimension, such properties as persistence (related to in vivo durability) and exposure to the target tissue/cell are critical to the potential carcinogenic activity of a given fiber (McClellan et al., 1992; Spurny, 1993).

The marked disparity in biological activity between these MMVFs and crocidolite asbestos in this study is probably related to the MMVF's lack of

persistence in the lung, especially those fibers >20 μm in length, which are thought to be of greatest biological activity (Davis, 1991). One explanation as to why fibers >10 and especially those >20 μm in length are more pathogenic than shorter ones is because the latter are more readily phagocytized by pulmonary macrophages, which, in turn, remove them from the target tissue, either via the mucociliary escalator or via the lymphatics to regional lymphoid tissues.

There was a striking difference between the biopersistence of the long MMVFs and the long crocidolite fibers. While the aerosol concentrations of the MMVFs >20 μm length for the high-dose groups were within a factor of ~2 (crocidolite 236, rock wool 114 and slag wool 99 fibers/cm^3), the numbers of these fibers retained in the lung were dramatically different. At 3 mo the number of fibers >20 μm length/mg dry lung roughly reflected the inhalation exposures for crocidolite (~29,000) and rock wool (~18,000), although slag wool was considerably less (~5000). However, in the 3-mo-exposed rats that were held unexposed for another 21 mo, the retention levels of both MMVFs had decreased to background levels compared to crocidolite, which was still ~19,000 fibers >20 μm length/mg dry lung. At 10 mo (end of exposure) the number of crocidolite fibers >20 μm length/mg dry lung was ~54,600, compared to ~54,700 for rock wool and ~5000 for slag wool after 12 mo of exposure. When the 10-mo-exposed rats were allowed to recover for 14 mo, the >20 μm length fiber levels in the crocidolite exposed lungs had only decreased to ~41,100 (25% reduction). This compared to ~3800 (93% reduction) for rock wool and ~200 (96% reduction) for slag wool fiber retentions in animals exposed for 12 mo and allowed to recover for 12 mo.

The results from the "recovery" animals showed that the lung burdens of rock and slag wool fibers decreased rapidly after exposure ceased; there was a ~90% reduction of WHO fibers with both types of materials after an exposure of 12 mo followed by recovery for an additional 12 mo. This has also been observed for two glass wool compositions using a similar inhalation protocol (Hesterberg et al., 1993). Hesterberg et al. (1993) found an approximately 60% reduction of WHO fibers after 12 mo of exposure to both types of glass followed by 12 mo recovery. More importantly, the reduction of fibers >20 μm length decreased ~90% (10,400 to 900 and 20,200 to 2,700 fibers/mg dry lung) (RCC, 1993). This and the studies under discussion illustrate that it is not sufficient to consider only the removal rate of WHO fibers; it is also important to enumerate the removal rate of long fibers (>20 μm in length) because they have the most biological activity (Davis, 1991). However, delayed lung clearance can occur if the normal clearance mechanisms are overloaded or if the fiber is inherently durable (Muhle et al., 1990).

The differential persistence in the lung of the MMVFs compared to crocidolite asbestos may be explained by observations made during electron microscopic counting procedures employed for lung burden determinations. It was observed that the physical nature of the slag wool fibers, and to a lesser extent of the rock wool fibers, was highly altered after only a few

months in the lung. In comparison, the crocidolite asbestos fibers appeared essentially the same in the lung as in the stock material. It is reasonable to expect that such a physically altered fiber would be more prone to breakage into shorter fibers, which would, in turn, be cleared more easily from the lung, thereby reducing the effective dose. It should be noted that MMVFs are vitreous in nature and therefore break crosswise, as compared to asbestos, which tends to split longitudinally.

The above observations are supported by the results of a recent study (Musselman et al., in press) of lung burdens after short-term exposure to various MMVFs, including the same rock and slag wool and crocidolite asbestos samples used in this study, as well as the same strain of male rat, exposure level, and inhalation exposure system. The results of the study also showed that rock wool and slag wool fibers were more readily removed from the lung than crocidolite. Lung burden levels of rock and slag wool fibers were comparable to unexposed control values within 12 mo after exposure for 6 h/day for 5 days to 30 mg/m^3 (rock wool 212 and slag wool 288 WHO fibers/cm^3). In contrast, crocidolite asbestos lung burdens did not decrease after the initial airway removal.

Additionally, in vitro studies of the dissolution of MMVFs have shown that rock and slag wool fibers are less durable than asbestos in fluids that mimic those found in the lung (Potter & Mattson, 1992; Scholze & Conradt, 1987). Different types of MMVFs can have very different in vitro dissolution properties at pH 4.5 (inside a pulmonary macrophage) and at pH 7.4 (extracellular fluids of the lung) (Carr, 1973; Oberdorster, 1991). Due to the important role of macrophages in the clearance of fibers from the lung, newer in vitro studies have been developed to investigate fiber dissolution at pH 4.5 (Christensen et al., 1994). Generally, a typical glass wool dissolves or disentegrates more rapidly at pH 7.4 and less at pH 4.5; the opposite is true for rock wool, while the tested slag wool dissolves/disintegrates at a high rate at both pH levels. It is therefore not surprising that a slag wool like that used in this study tends to be more biodegradable than these rock wools or glass wools. This may be due to the high calcium content in combination with a relatively low amount of silica, resulting in a weak glass network. Other fibers of high calcium content, such as wollastonite (a natural calcium metabisilicate fiber), have also shown a similar high dissolution rate in lung tissues after inhalation (McConnell et al., 1991) and after intratracheal injection (Bellmann et al., 1987).

A further possible explanation for the noncarcinogenic effects of these MMVFs may be related to the low degree of pulmonary fibrosis observed in this study. It has been suggested that there is a relationship between pulmonary fibrosis and lung cancer in humans (Roggli et al., 1992) An increased incidence of lung cancer was not found in asbestos workers (even after high exposures) who did not show concomitant pneumoconiosis (asbestosis) (Sluis-Cremer & Bezuidenhout, 1989). It has even been suggested that when asbestosis is absent in a patient with lung cancer, an alternate etiology (such as a history of cigarette smoking) should be sought, notwithstanding a history of asbestos exposure (Churg, 1993).

A similar relationship between fibrosis and lung cancer has been observed in rodents following inhalation of various types of natural mineral fibers and MMVFs (Davis & Cowie, 1990). While a cause/effect relationship has not been established, (i.e., fibrosis in itself causes cancer), all fibers showing carcinogenic activity in the lung of rodents are also fibrogenic in the same experiment. However, just as importantly, the observation of fibrosis (at least in animals) does not automatically mean that a given fiber will show carcinogenic activity in a given experiment. For example, recent studies of a kaolin-based refractory ceramic fiber (RCF) in rats (using a similar protocol and in the same laboratory as in this study) failed to show carcinogenic activity in the lung in spite of the presence of pulmonary fibrosis (Mast et al, in press). This study is of particular importance in this regard because this RCF has been shown to cause pulmonary neoplasms at higher doses under similar experimental conditions (Mast et al., in press). A plausible explanation for the lack of lung tumors lies in the observation that fibrosis occurred later at the noncarcinogenic levels of RCF (12 mo vs. 3 mo). Note that fibrosis was also present at 3 mo with crocidolite asbestos, which was carcinogenic in this study. Perhaps fibrosis needs to be present for most of an animal's life to see the induction of lung neoplasms.

Finally, an attempt was made to evaluate the relative sensitivity of rats and humans to fiber-induced pulmonary disease by comparing the relative potency (dose/effect) of crocidolite asbestos in these rats to what has been reported in humans. However, comparisons are difficult because asbestos exposures are often mixed (chrysotile, amosite, crocidolite, anthophyllite, etc. are found in the same individual), data concerning the asbestos content of human lungs are not extensive, and there are confounding factors in humans such as cigarette smoking, life-style, exposures to other pulmonary irritants, unknown length of time since last exposed, etc. Comparisons are also complicated because investigators use different methods for tissue preparation, counting and measuring fibers, etc.

Nevertheless, rough comparisons can be made. For example, the average asbestos lung content in a study of 54 deceased men (primary cases of death: mesothelioma 43%, lung cancer 37%, asbestosis 17%, other causes 4%) with known asbestos exposure was 316,000 fibers/mg dry lung (DL) (Langer & Nolan, 1989). Sixteen insulation workers were included in the study with an average concentration of asbestos fibers in their lungs of 357,000 fibers/mg DL. The average lung burdens (fibers/mg DL) by type of asbestos were 48,000 ± 78,000 (amosite), 299,000 ± 1,100,000 (chrysotile), and, 5000 ± 2000 (crocidolite).

Similarly, the average asbestos lung burden for 13 insulation workers with fatal disease (3 asbestosis, 3 lung cancer, and 7 mesothelioma) was 227,000 ± 131,000 fibers/mg DL (Kohyama & Suzuki, 1991). The average lung burdens (fibers/mg DL) by type of asbestos were 150,000 ± 103,000 (amosite), 63,000 ± 54,000 (chrysotile), 11,000 ± 23,000 (crocidolite), 2000 ± 1000 (anthophyllite), and 2000 ± 3000 (tremolite).

These fiber concentrations for total asbestos are roughly comparable to

the average retention of crocidolite fibers observed in rat study, that is, 702,000 ± 97,000 WHO fibers/mg DL for rats exposed for 44 wk and held until the end of the study (124 wk) (Table 4). Note that all of these rats had pulmonary fibrosis (asbestosis), a 14% incidence of lung tumors, and 1 mesothelioma. Therefore, while only rough comparisons can be made, the available evidence suggests that rats and humans react in a similar fashion (quantitatively as well as qualitatively) to exposure to crocidolite asbestos, and by inference) to MMVFs.

In conclusion, these studies demonstrate that the inhalation of high levels of rock and slag wools, while causing nonspecific inflammatory lesions (rock and slag wool) and fibrosis with high lung burden of long fibers (rock wool only) in the lungs of exposed rats, showed no evidence of carcinogenic activity. These results suggest that respirable fractions of these MMVFs should not pose a significant health risk to humans at the relatively low airborne levels found in the workplace.

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