

H-1

APPENDIX H CHEMICAL CHARACTERIZATION, ANALYSIS, AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF TALC	H-2
GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS	H-4
FIGURE H1 Infrared Absorption Spectrum of Talc	H-6
FIGURE H2 Fluid Bed Generator	H-7
FIGURE H3 Aerosol Dilution/Delivery System	H-8
FIGURE H4 Talc Chronic Exposure System	H-9
FIGURE H5 Talc Aerosol Filter Concentrations in the 6 mg/m ³ Rat Chamber	H-10
FIGURE H6 Talc Aerosol Filter Concentrations in the 18 mg/m ³ Rat Chamber	H-11
FIGURE H7 Talc Aerosol Filter Concentrations in the 6 mg/m ³ Mice Chamber	H-12
FIGURE H8 Talc Aerosol Filter Concentrations in the 18 mg/m ³ Mice Chamber	H-13
TABLE H1 Summary of Aerosol Size Measurements for the 6 and 18 mg/m ³ Rat Chambers	H-14
TABLE H2 Summary of Aerosol Size Measurements for the 6 and 18 mg/m ³ Mouse Chambers	H-15

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H-2

Talc, NTP TR 421

CHEMICAL CHARACTERIZATION, ANALYSIS, AND GENERATION OF CHAMBER CONCENTRATIONS

PROCUREMENT AND CHARACTERIZATION OF TALC

Talc was obtained from Walsh and Associates (North Kansas City, MO) in two lots (lot W101882 and lot B5415). Lot W101882 was used from the beginning of the 2-year studies through January 1986. Lot B5415 was used in the 2-year studies from 27 January 1986 to the end of the studies on 31 October 1986. The talc was extensively characterized by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO) and McCrone Associates (Norcross, GA). Reports on analyses performed in support of the talc studies are on file at the National Institute of Environmental Health Sciences.

The two lots of the chemical, a finely powdered white solid, were identified as talc by infrared spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra of talc (*Sadtler Standard Spectra*), as shown in Figure H1.

Lot W101882 was divided into three subbatches, which were analyzed separately. Each subbatch was characterized by elemental analyses, Karl Fischer water analysis, spark source mass spectrometry, and microscopic analyses. Microscopic analysis of each lot consisted of polarized light microscopy (PLM) and transmission electron microscopy (TEM). For PLM the sample was mounted in refractive index liquids and the optical parameters were determined. Dispersion staining has the advantage that small quantities of asbestos can easily be detected since the optical properties are interpreted from bright colors seen on a black background. The colors seen are the results of differences in refractive index dispersion for a liquid and a solid. TEM was performed by sonically dispersing approximately 0.1 g of talc in a solution of 0.001% methyl cellulose in particle-free water. A drop of the suspension was placed on a carbon coated 200-mesh copper grid, and 20 grid openings were examined. The detection limit was 0.1% by weight. No asbestos fibers were detected in any of the subbatches by polarized light microscopy or transmission electron microscopy.

Elemental analyses of hydrogen, magnesium, and silicon for all three subbatches of the lot were in agreement with the theoretical values for talc. The major impurities were 0.7% aluminum and 1.0% iron. Karl Fischer water analysis indicated approximately 0.2% absorbed water. Spark source mass spectrometry for the three subbatches also indicated approximately 0.1% phosphorus, 0.5% fluorine, and 0.05% calcium, while the remaining elemental impurities were less than 0.01%.

A special study was performed on this lot to determine if the sample met the American Society for Testing and Materials standard specifications for magnesium silicate. Results indicated that lot W101882 met the standard specifications.

Automated scanning electron microscopic analysis demonstrated that the talc was virtually free of silica. In the analysis a sample of talc is suspended in methylcellulose. Under computer control the particles are located, and maximum, minimum, and average diameters are determined; then a chemical analysis is performed. Of the 1,466 particles that were examined, 1 was identified as silica, 1,241 were talc, 136 were of tremolite type composition, 77 were mixed silicates, 1 was possibly zircon, and 10 were not identified. The single silica particle had an average diameter of 3.9 μm .

Lot B5415 was characterized by elemental analyses, Karl Fischer water analysis, spark source mass spectrometry, and microscopic analyses using the same methods described for lot W101882. Elemental analyses values were similar to results obtained for lot W101882. The major impurities present were 0.1% calcium, 0.5% aluminum, and 1% iron. Karl Fischer water analysis indicated 1.2% absorbed water. Spark source mass spectrometry also indicated 0.04% phosphorus, >0.5% aluminum, 0.03% sodium, 0.35% fluorine, and all other impurities were less than 0.03%. Microscopic analyses using PLM and TEM detected no asbestos fibers.

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H-3

Comparative purity analyses of the two lots used in these studies were conducted due to problems with the generation of inhalation concentrations. Four samples of talc were used, two samples each from lots W101882 and B5415. Samples A and B were from lot W101882, sample C was from lot B5415, and sample D was a frozen reference from lot B5415 that had been stored at MRI.

Analyses performed included elemental analyses, microscopic analyses (PLM, TEM, determination of particle size distribution, and aspect ratios), X-ray diffraction, and thermogravimetric analysis (TGA). PLM and TEM analyses were performed on samples C and D. Analysis by PLM followed the procedures described earlier, TEM followed the same procedure described earlier except the talc was sonically dispersed in a solution of 90% (v/v) isopropanol in particle-free water. The determinations of particle size distribution and aspect ratios were performed on all four samples. Using TEM for both analyses, selected area diffraction (SAD) patterns were used to confirm that the particles being measured were talc. The particle size was taken as the average of two diameters 90° to each other and aspect ratios were taken as the ratio of the two diameters. Thermogravimetric analysis (TGA) was performed on samples A, B, and C on a DuPont 910 differential scanning calorimeter (DSC) with calcium oxalate monohydrate used as a calibrating standard, at an initial temperature of 50° C with a programmed maximum temperature of 1,100° C, at a rate of 20° C per minute.

Elemental analyses for hydrogen, magnesium, and silicon for all four samples were in agreement with theoretical values. Polarized light microscopy (PLM) and transmission electron microscopy (TEM) detected no asbestos fibers in any of the samples. The results for particle size distribution and aspect ratios indicated that there were only minor differences in particle size between the samples and more than 75% of the particles were in the 1.0 to 3.0 µm range. More than 90% of the talc particles had aspect ratios between 1 and 1.4, and less than 1% had ratios greater than 3:1. X-ray diffraction confirmed that all four samples were primarily talc with small quantities of chlorite and dolomite. Thermogravimetric analysis indicated that samples A, B, and C were similar. A main peak at 912° C in all three samples caused by the loss of chemically combined water was equal to a loss of 4.7% by weight. A minor peak at 590° C in all three samples may represent the loss of CO₂ from dolomite and amounted to a loss of 0.7% by weight which is equivalent to 1.5% dolomite.

Size Distribution Analysis of Talc Samples
(% of Total Particles Counted)

Size Range (µm)	Talc A	Talc B	Talc C	Talc D
0.5-1.0	5.88	2.97	12.50	1.94
1.0-1.5	15.69	9.90	19.23	11.65
1.5-2.0	26.47	26.73	24.04	26.21
2.0-2.5	20.59	17.82	21.15	23.30
2.5-3.0	11.76	18.81	10.58	8.74
3.0-3.5	5.88	12.87	4.81	7.77
3.5-4.0	3.92	5.94	2.88	5.83
4.0-4.5	2.94	1.98	1.92	4.85
4.5-5.0	2.94	0.99	0.96	3.88
5.0-5.5	1.96	0.99	0.96	2.91
5.5-6.0	1.96	0.99	0.96	1.94
6.0-6.5	-	-	-	0.97

The moisture content of the bulk chemical was reanalyzed every 4 months at the study laboratory by determining the weight loss following heating at 120°C for 16 hours. The results indicated that the moisture content of the talc was similar between the two lots and did not change during the 2-year studies. Bulk chemical stability studies were not performed on talc because the physical and chemical properties of talc indicate that it should be stable over a wide range of temperatures. The compound was stored in tightly sealed plastic bags at 25° C.

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H-4

Talc, NTP TR 421

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

Aerosol Generation System: Talc aerosol was generated from one 4-inch, fluid bed generator (FBG). Figure H2 shows the schematic of the FBG with the gravity feed and collecting pan collection systems. The FBG bed contained type 316 stainless steel powder (Hoeganaes Corporation, Riverton, NJ), consisting of irregularly shaped particles 125 to 180 μm in diameter. The stainless steel powder was cleaned prior to use. The cleaning system used a 4-inch FBG with dry, filtered air flowing through at a flow rate of 80 CFM. The high flow rate through the bed removed the finest stainless steel particles. The cleaning system was run for 24 hours to ensure that all the "fines" were removed.

Following cleaning of the bed material, talc was mixed with the stainless steel powder at approximately 1 to 2.5 g of talc per 500 g bed material. The concentration of talc in the bed material was one method used to adjust exposure concentrations in the chamber. During the time period of November 1985 to January 1986, when difficulty in maintaining target concentrations was experienced, higher loadings were used in an effort to maintain target concentrations.

For generation of the talc aerosol, fluidization of the bed material mixed with talc occurred when compressed air (≈ 200 Lpm) was injected into the bed through a porous metal distribution plate which supports the bed. The motion of the bed released the much smaller talc particles into the air; the larger, heavier stainless steel particles were retained in the bed. A Kr-85 discharger was placed above the bed to reduce the particle charges. The aerosolized talc particles were mixed with diluting air (≈ 200 Lpm) to achieve the desired concentrations and were then delivered to the exposure chambers (Figures H3 and H4). As the talc powder was removed from the bed, the bed material was continually drained from the FBG through an overflow port located at the side of the generator. As spent bed material was drained from the generator, fresh talc-containing bed material was constantly added into the generator from a hopper located above the generator.

Stainless steel multitiered whole-body exposure chambers (H2000, Lab Products, Inc.) were used to expose the rats in this study while the smaller H1000 chambers were used for the mice. Flow rates through the chambers were 12 ± 2 CFM. To reduce the spatial variation of aerosol concentration and to increase the uniformity of mixing, the aerosol was diluted using a dilutor prior to its introduction into the chamber. Also, animal cages were rotated once per week to reduce the variation of concentrations of talc aerosols that the rodents were exposed to during the 2-year studies.

Aerosol Concentration Monitoring: Aerosol concentrations in each exposure chamber were monitored by taking filter samples for three, 2-hour periods during each 6-hour exposure day. The background concentration of total suspended particles in each control chamber was monitored each exposure day by taking one 6-hour filter sample. Overnight filter samples for total suspended particles were taken from the 18 mg/m^3 chambers once per month. All filter samples were taken at a flow rate of 3 L/minute. Each filter was weighed before and after the sample was taken, and the aerosol mass concentrations were calculated by dividing the mass increment (mg) by the volume sampled (m^3); the means and standard deviations for each chamber were calculated for each exposure day. Weekly mean exposure concentrations for the 2-year studies are presented in Figures H5 through H8. The concentrations during non-exposure hours in the 18 mg/m^3 chambers ranged from 0.02 to 1.1 mg/m^3 .

A RAM-S continuous aerosol monitor was used to monitor the stability of the aerosol concentrations and to determine the need to adjust the aerosol generation system during exposures. The RAM-S was used to monitor each chamber for at least 5 minutes at the beginning, middle, and end of the filter sampling period. A 2 L/minute flow rate through the RAM-S was achieved using an internal pump in the device. Both RAM-S and filter samples were taken at one point of the chambers above the animal cage. A Y-shaped probe was used, allowing simultaneous filter sampling and RAM-S aerosol mass monitor operation. The overall temporal variation in chamber concentrations in the 2-year studies were 33% and 27% relative standard deviation (RSD) for the mouse 6 and 18 mg/m^3 chambers. The variations were 31% and 36% RSD for the rat 6 and 18 mg/m^3 chambers. At least a portion of this variability may be ascribed to the period when talc generation problems were encountered (November 1985 through February 1986). In addition, a portion of the variability for the 18 mg/m^3 rat chamber

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H-5

may be ascribed to the time when higher concentrations were being generated (September through November, 1984).

During the period of November 5, 1985 through January 27, 1986 difficulties were experienced maintaining the required exposure levels of talc for the lifetime and 2-year exposure studies. Concentrations of aerosolized talc were significantly below target. Attempts were made to increase the flow of talc into the generator and raise the concentration; however, the talc-laden stainless steel bed material fed into the generator less freely than it had prior to November, 1985. There were no observable chemical changes in either the talc or the stainless steel bed material and no malfunctions in the generation system which could be pinpointed as the underlying cause for the poor flow characteristics of the bed material. On January 27, 1986, the generator was restarted with a new batch of talc. After a stabilization period of three weeks, the flow properties of the bed material showed significant improvement.

It was also observed during February, 1986 that when the ratio of talc to bed material was increased above 1.6 g talc per 500 g bed material, the bed began to show the poor flow properties characteristic of the previous batch of talc. When the bed loading was reduced below 1.6 g talc per 500 g bed material, the flow properties stabilized. This indicated that the bed has a maximum loading limit which must not be exceeded. By March 1986, the generator had stabilized and chamber target concentrations were achieved. The exact cause of these generation problems was never resolved.

In November, 1984 it was noticed that the RAM-S monitor indicated an off-scale reading (>10 V which is equivalent to 20 mg/m^3) for the 18 mg/m^3 rat chamber. Reasonable agreement was seen between RAM-S readings and filter samples in the other chambers. Investigations of this discrepancy indicated that the airflow through the critical orifice controlling flow through the filter was reduced. Evaluation of the previously collected pressure drop associated with this orifice and one having nearly identical nominal flow revealed that the flow to the sampling filter of the high level rat chamber dropped significantly on September 24, 1984. These data suggest that the sampling orifice had become partially clogged. In order to obtain a correction factor to recalculate the chamber concentration data, the filter pressure drop and exposure chamber pressure drop data were retrieved and used to determine the actual pressure drop across the sampling filter for the time period of September 24 through November 14, 1984. A group of 18 filters from different lots of the type used to sample the talc exposure chambers were tested to determine the pressure drop across them as a function of the flow through the filter. These data indicated that values for flow could be calculated from the pressure drop data. The relationship between pressure drop and filter flow rate was used to recalculate the sampling filter flow for each day. When the chamber sampling orifice flow rate was taken into account, the best estimate of the correction factor is 2.06. This factor has been used to multiply the originally recorded chamber concentrations for those dates. The corrected values are reported.

Aerosol size distribution was determined once a month for each chamber using a cascade impactor operated at a flow rate of 15 L/minute. Stainless steel disks coated with apiezon grease were used as impactor substrates and the amount of talc collected on each stage was determined by the difference in stage weight before and after the sample was taken. The mass medium aerodynamic diameter and the geometric standard deviation were calculated from the mass data, effective cutoff diameter of each stage, and impactor flow rate. The results are presented in Tables H1 and H2.

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H-6

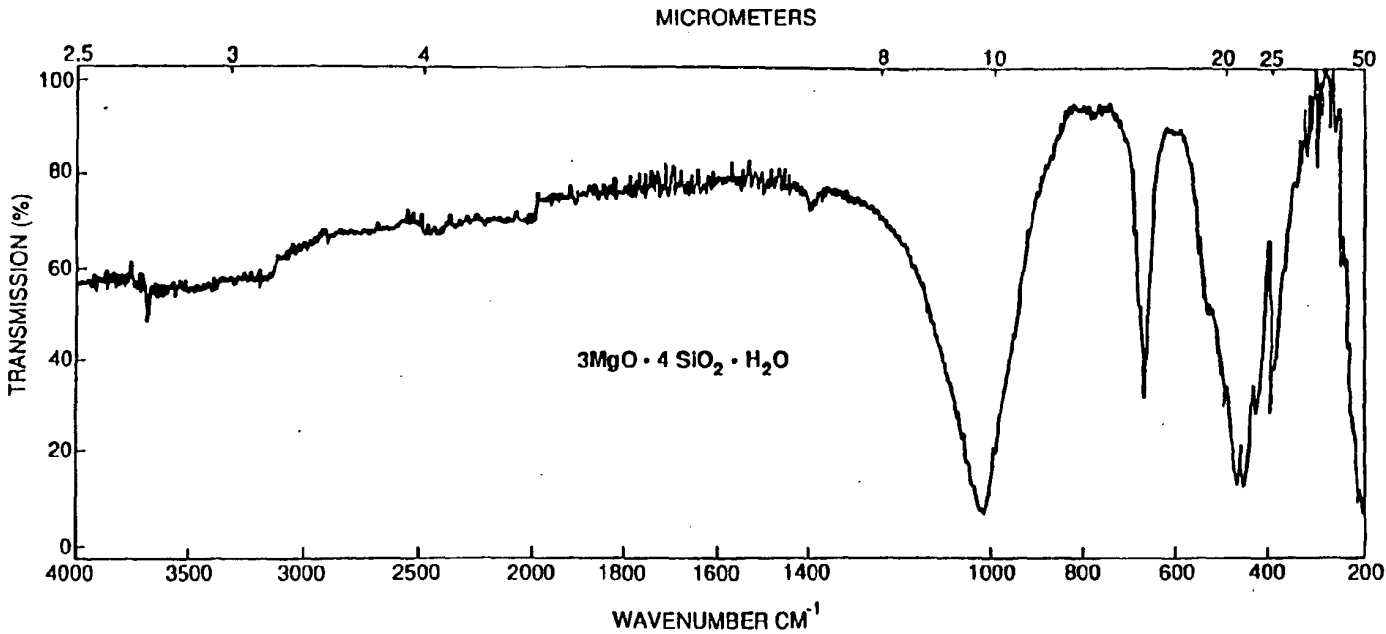


Figure H1
Infrared Absorption Spectrum of Talc

ABSCISSA EXPANSION <u>1</u> SUPPRESSION <u>-</u>		ORDINATE EXPANSION <u>1</u> %T 0-100 ABS <u>-</u>		SCAN TIME <u>24 min</u> RESPONSE <u>1</u> SLIT PROGRAM <u>6</u>		REP. SCAN <u>-</u> SINGLE BEAM <u>-</u> TIME DRIVE <u>-</u> PRE SAMPLE CHOP <u>-</u> OPERATOR <u>A. Clark</u> DATE <u>11/9/82</u>	
SAMPLE: Talc Lot W101882 Batch 02 Subbatch A		REMARKS <u>Trimmer comb</u> <u>in reference beam</u>		SOLVENT <u>-</u> CONCENTRATION <u>1% in KBr</u>		CELL PATH <u>-</u> REFERENCE <u>154N</u>	

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257 of 283

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H-7

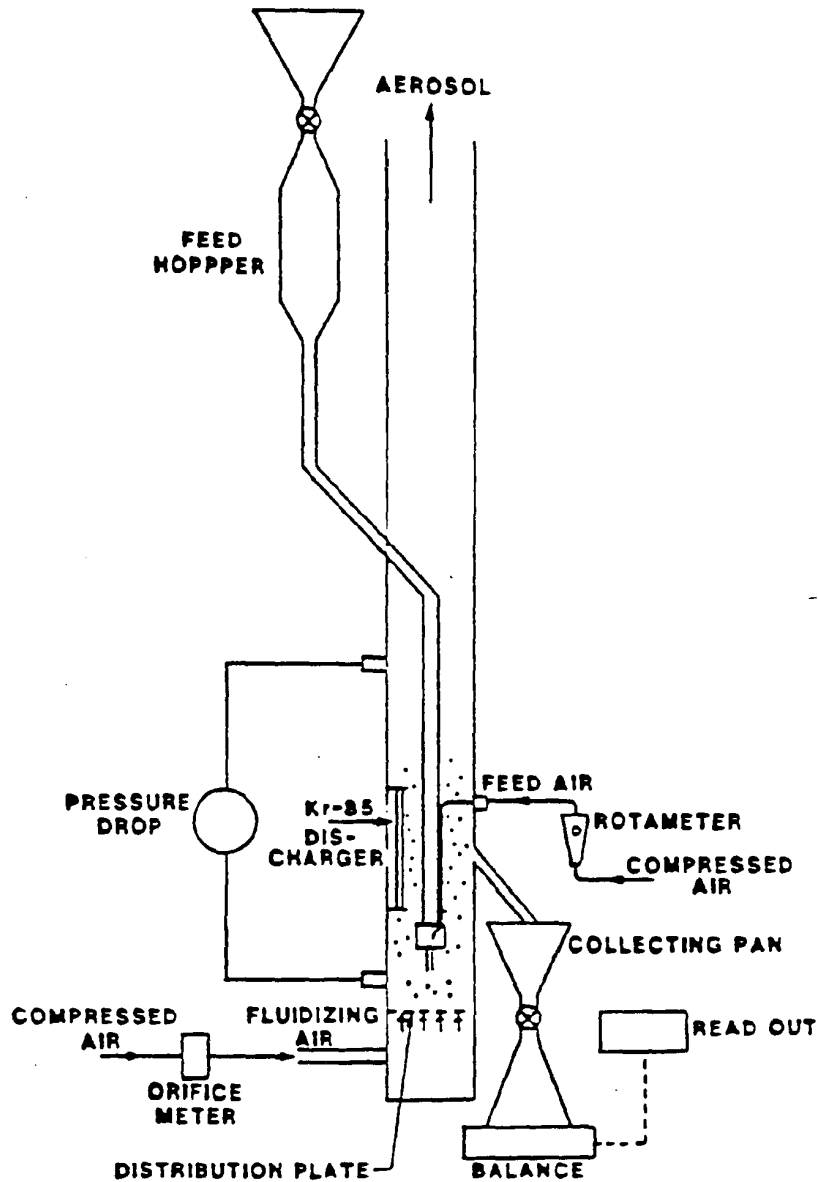


FIGURE H2
Fluid Bed Generator

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H-8

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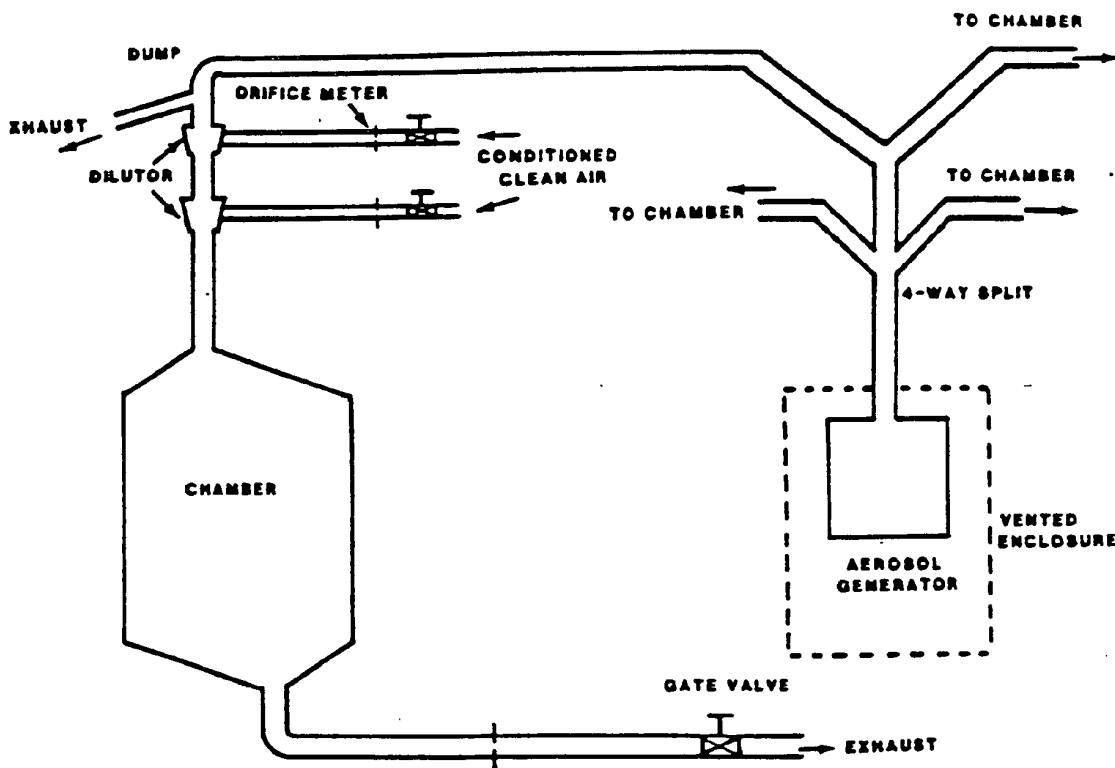


FIGURE H3
Aerosol Dilution/Delivery System

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H-9

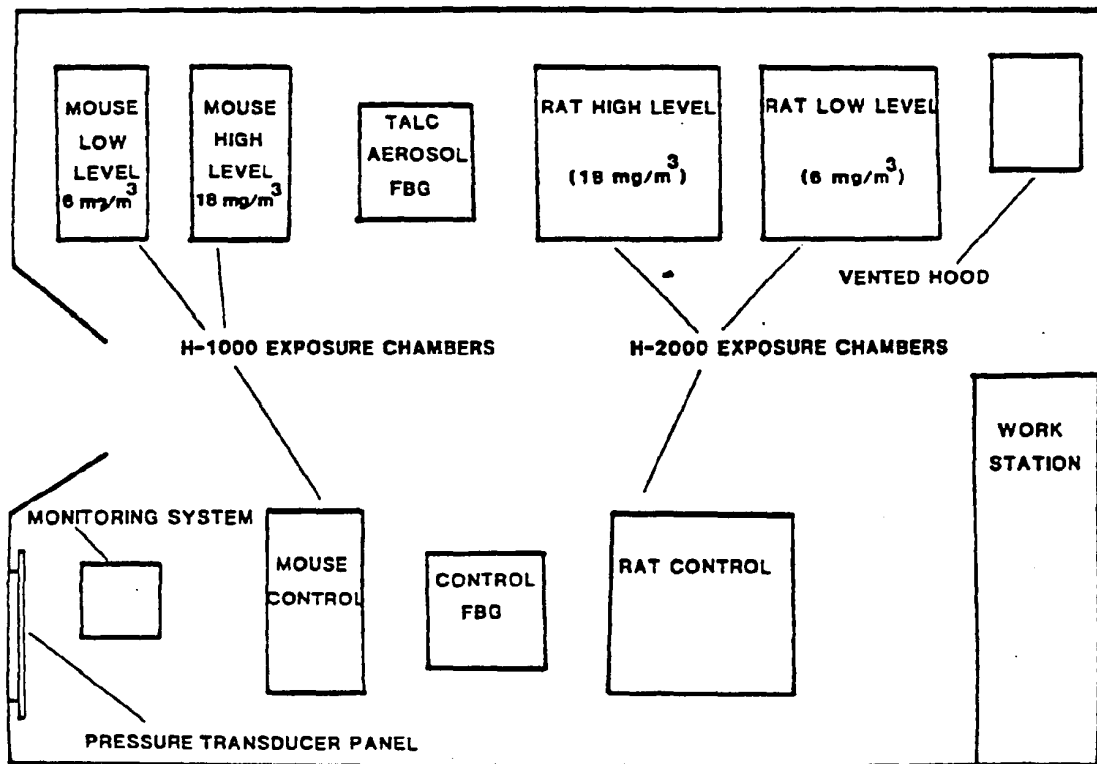


FIGURE H4
Talc Chronic Exposure System

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H-10

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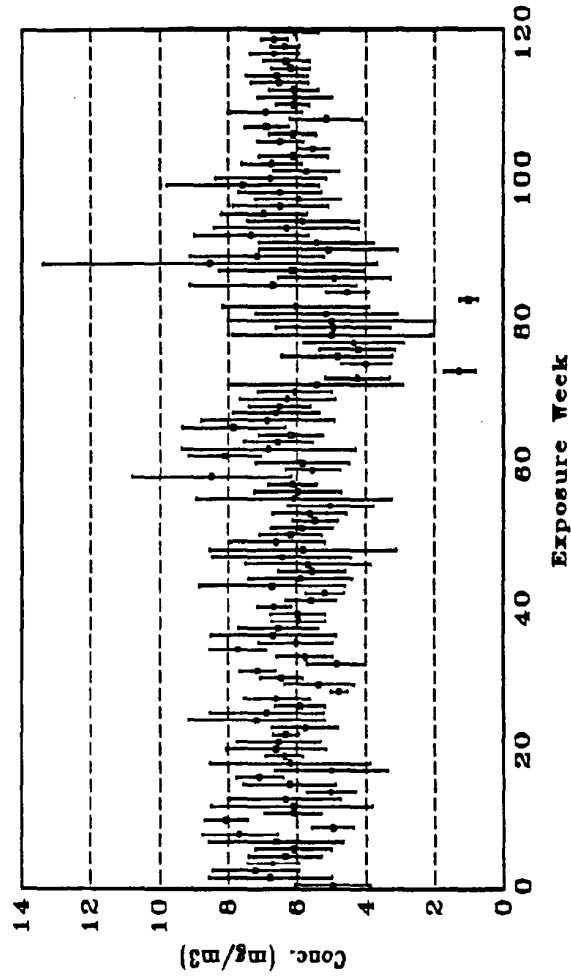


FIGURE H5
Talc Aerosol Filter Concentrations in the 6 mg/m³ Rat Chamber

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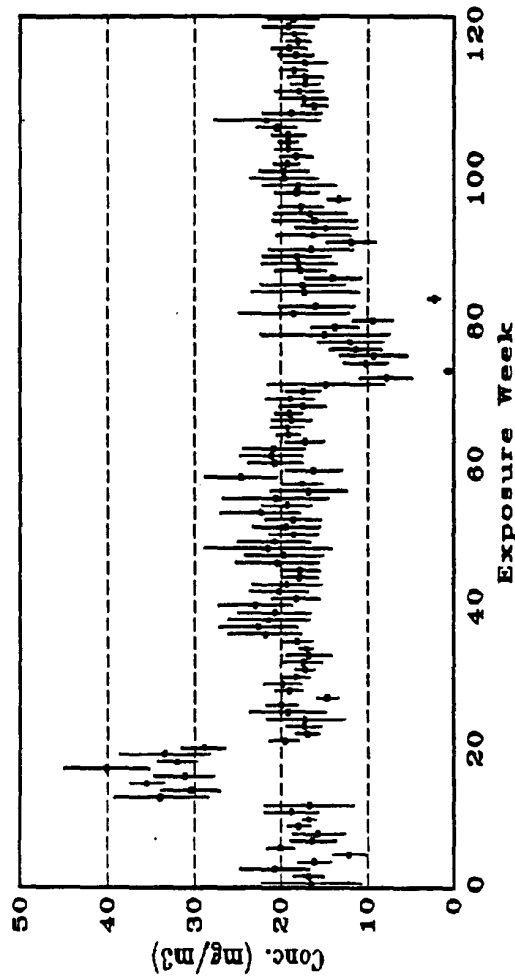


FIGURE H6
Talc Aerosol Filter Concentrations in the 18 mg/m³ Rat Chamber

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H-12

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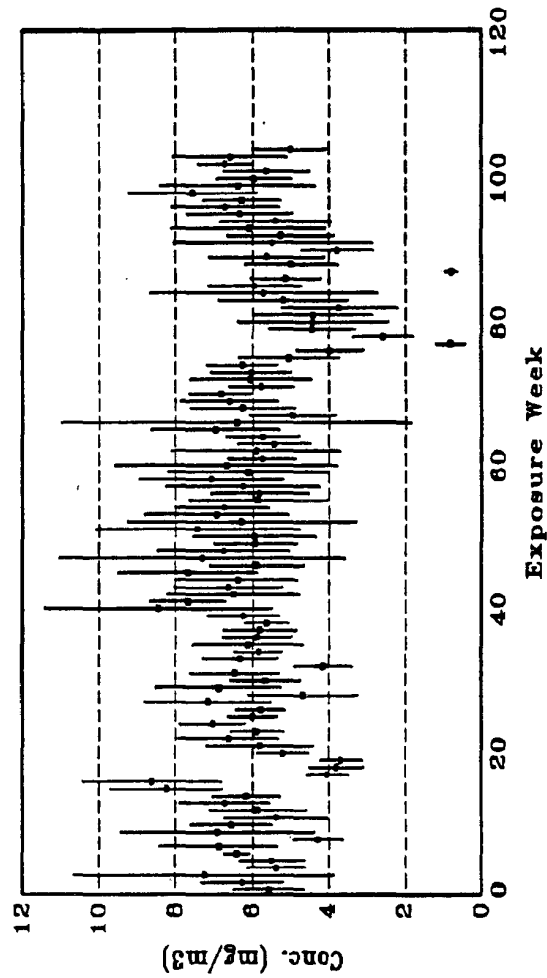


FIGURE H7
Talc Aerosol Filter Concentrations in the 6 mg/m³ Mice Chamber

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H-13

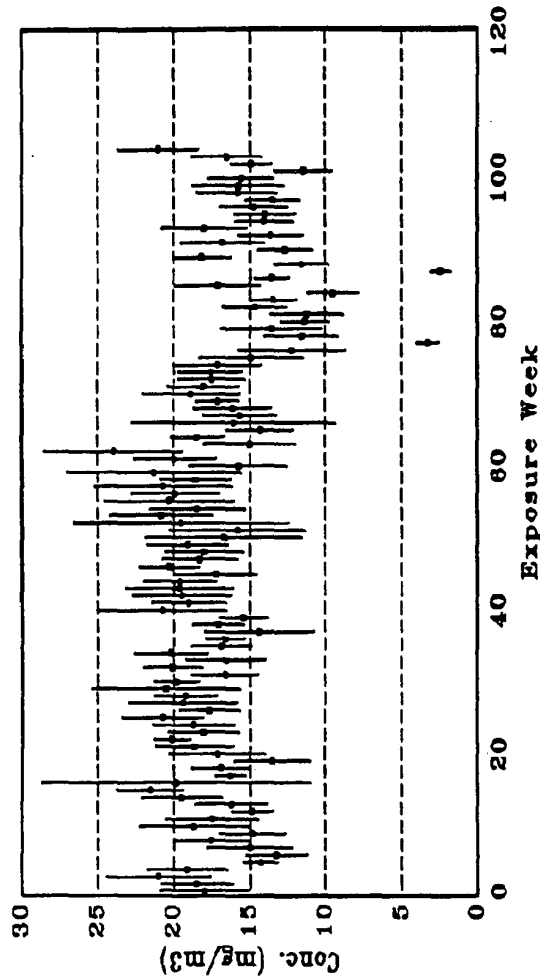


FIGURE H8
Talc Aerosol Filter Concentrations in the 18 mg/m³ Mice Chamber

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H-14

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TABLE H1
Summary of Aerosol Size Measurements for the 6 and 18 mg/m³ Rat Chambers

6 mg/m ³			18 mg/m ³		
Date	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation	Date	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
9 July 1984	2.3	2.6	25 June 1984	3.6	2.0
6 August 1984	2.6	1.7	1 August 1984	3.0	1.8
4 September	2.8	1.8	27 August 1984	3.2	1.9
3 October 1984	2.6	1.8	26 September 1984	2.9	1.8
31 October 1984	2.9	1.8	24 October 1984	3.2	1.9
27 November 1984	2.5	1.8	20 November 1984	3.0	1.9
4 January 1985	2.6	1.8	24 December 1984	2.8	1.8
25 January 1985	2.5	1.7	14 January 1985	2.9	1.8
25 February 1985	2.6	1.8	19 February 1985	2.8	1.8
19 March 1985	2.8	1.8	15 March 1985	3.1	2.0
22 April 1985	2.9	1.7	12 April 1985	3.1	1.8
13 June 1985	3.0	1.9	8 May 1985	2.9	1.9
9 July 1985	2.8	1.8	10 June 1985	3.0	1.9
9 August 1985	2.7	1.9	5 July 1985	3.5	1.8
3 September 1985	2.7	1.5	1 August 1985	3.1	1.9
30 September 1985	2.3	1.3	26 August 1985	2.9	1.9
28 October 1985	2.6	1.4	23 September 1985	2.6	1.6
2 December 1985	3.1	1.7	21 October 1985	2.7	1.5
18 December 1985	3.0	1.7	25 November 1985	4.0	2.1
3 January 1986	1.8	2.8	17 December 1985	3.3	1.9
8 January 1986	3.6	1.9	30 December 1985	3.7	1.8
13 January 1986	3.1	1.8	3 January 1986	4.0	2.2
24 February 1986	2.9	2.2	8 January 1986	3.8	1.9
24 March 1986	3.4	1.9	18 February 1986	3.2	2.1
22 April 1986	3.2	2.3	17 March 1986	3.6	1.9
23 May 1986	2.4	1.9	14 April 1986	4.0	2.0
23 May 1986	2.9	1.9	19 May 1986	3.2	1.8
27 May 1986	2.3	1.9	2 June 1986	3.2	2.1
16 June 1986	2.7	2.7	17 June 1986	3.3	1.9
30 June 1986	2.2	2.4	15 July 1986	3.4	2.0
28 July 1986	2.5	2.3	11 August 1986	3.1	1.9
25 August 1986	2.1	2.5	9 September 1986	2.9	1.9
22 September 1986	2.5	2.0	6 October 1986	2.7	2.3
20 October 1986	2.7	2.3			
Mean ± standard deviation	2.7 ± 0.4	1.9 ± 0.4		3.2 ± 0.4	1.9 ± 0.2

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H-15

TABLE H2
Summary of Aerosol Size Measurements for the 6 and 18 mg/m³ Mouse Chambers

Date	6 mg/m ³		Date	18 mg/m ³	
	Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation		Mass Median Aerodynamic Diameter (μm)	Geometric Standard Deviation
18 June 1984	3.9	1.8	25 June 1984	3.6	2.0
16 July 1984	3.4	1.9	23 July 1984	3.7	1.9
14 August 1984	3.5	1.8	20 August 1984	3.5	1.8
18 September 1984	3.3	1.8	10 September 1984	3.9	2.0
10 October 1984	3.1	1.9	17 October 1984	3.8	1.9
7 November 1984	3.3	1.8	19 November 1984	3.5	1.7
4 December 1984	3.0	1.8	12 December 1984	3.3	1.9
7 January 1985	3.4	1.6	7 January 1985	3.4	1.8
4 February 1985	3.2	1.8	8 February 1985	3.6	1.9
1 March 1985	2.9	1.9	7 March 1985	3.6	1.9
29 March 1985	3.1	1.8	5 April 1985	3.5	1.9
23 April 1985	3.6	1.8	2 May 1985	3.6	1.8
22 May 1985	3.1	2.0	29 May 1985	3.5	2.2
21 June 1985	3.3	1.8	26 June 1985	3.7	2.0
23 July 1985	3.4	1.8	29 July 1985	3.5	1.9
15 August 1985	3.5	1.8	20 August 1985	3.8	1.9
9 September 1985	2.6	1.3	16 September 1985	3.3	1.8
7 October 1985	2.7	1.5	14 October 1985	2.8	1.7
4 November 1985	2.5	1.5	12 November 1985	4.1	2.1
9 December 1985	3.4	1.6	16 December 1985	3.8	2.0
19 December 1985	3.6	2.0	3 January 1986	3.6	1.9
3 January 1986	3.9	2.0	8 January 1986	5.0	2.0
8 January 1986	4.0	2.1	10 February 1986	3.3	2.4
20 January 1986	3.7	1.8	13 March 1986	3.1	2.5
3 March 1986	3.0	2.1	7 April 1986	3.4	2.0
31 March 1986	2.9	2.1	5 May 1986	3.3	2.2
28 April 1986	3.2	4.7			
Mean ± standard deviation	3.3 ± 0.4	1.9 ± 0.6		3.6 ± 0.4	2.0 ± 0.2

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H-16

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**APPENDIX I
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NIH-07 RAT AND MOUSE RATION**

TABLE I1	Ingredients of NIH-07 Rat and Mouse Ration	I-2
TABLE I2	Vitamins and Minerals in NIH-07 Rat and Mouse Ration	I-2
TABLE I3	Nutrient Composition of NIH-07 Rat and Mouse Ration	I-3
TABLE I4	Contaminant Levels in NIH-07 Rat and Mouse Ration	I-4

I-2

Talc, NTP TR 421

TABLE II
Ingredients of NIH-07 Rat and Mouse Ration^a

Ingredients ^b	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

^a NCI, 1976; NIH, 1978

^b Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE I2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration^a

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D ₃	4,600,000 IU	D-activated animal sterol
K ₃	2.8 g	Menadione
<i>d</i> - α -Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
<i>d</i> -Pantothenic acid	18.0 g	<i>d</i> -Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B ₁₂	4,000 μ g	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	<i>d</i> -Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

^a Per ton (2,000 lb) of finished product

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