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Otsuka Chemical, NY

FYI_1097_001310

Fax

To: Mr. Ed Gross **From:** Ken Tanigawa

Fax: 1-202-260-9555 **Pages:** 1

Co: EPA Information Management Div. **Date:** October 21, 1997

Re: Potassium Octatitanate **CC:** Dr. Masatoshi Taniguchi

Dear Mr. Gross:

First of all I would like to thank you for your assistance with this project as well as thank you for your patience with regards to our delayed response. We duly received your inquiry, unfortunately I was unable to reply since I was out of the office on business trips.

We received word from Dr. Taniguchi regarding your inquiry. Otsuka Chemical Company's answer to your question is that we are **not claiming CBI**. Therefore please proceed with the report you presently obtain.

Secondly, in the future Otsuka Chemical Company may be in a possible position to make a donation to EPA for your research on the environment and toxics. If this becomes feasible for us to do, could you please inform us of the standard procedure we need to follow in-order to make a financial contribution?

Thank you again for your time and effort concerning the above matters. Should you have any other questions, please do not hesitate to contact me.

Best regards,

Ken Tanigawa
Ken Tanigawa

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May 24, 1997

MR 124

Dr. Vanessa T. Du
U.S.EPA Office of Pollution
and Prevention-Toxic Substances
Health and Environment Review Division
401 M Street, SW
Washington, DC 20460



FYI-97-001310

RE: Safety Assessment: Potassium Octatitanate Fiber

Dear Dr. Du

Otsuka Chemical Co.,Ltd. is aware of the EPA's scientific program to study toxicity issues and establish risk assessment guidelines for fibrous materials. Even though Otsuka Chemical Co.,Ltd. realizes that potassium octatitanate is listed in the TSCA, we understand any manufacturers have to develop the latest safety and technical information and offer it to customers. Therefore we have been taking information from professional documents and articles in journals and doing own safety tests at public research laboratories. Otsuka Chemical Co.,Ltd. has decided to share the enclosed study data with you for the enhancement of EPA's occupational health and environmental toxicity information database according to your desire and suggestion. Otsuka Chemical Co.,Ltd. believes that the study data and the results of the enclosed pre-chronic toxicology test of inhaled potassium octatitanate fiber are useful for you and customers.



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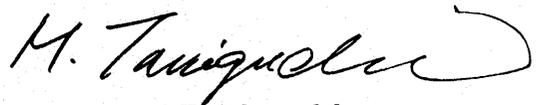
This experiment has done by Battelle Memorial Institutes and we have some scientific advisers. If you have any questions relating this data and experiment, please don't hesitate to contact me. I will make an arrangement to have somebody contact you immediately.

We have almost finished making the protocol of long term toxicity test and we are about to start the experiment at Battelle.

We wish to keep this document a secret from competitors, if possible. We hope you will inform and take our agreement before disclosure when someone wants to see this document, if possible.

Thank you for your attention and interest.

Sincerely Yours,



**Dr. Masatoshi Taniguchi
Senior Executive Director
Otsuka Chemical Co.,Ltd.
Chemical Division**

cc: Mr. Keijirou Tanigawa

**New York Office of Otsuka Chemical
747 Third Avenue, 26th Floor
New York, NY 10017
TEL. #212-826-4374**

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17, 1997

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OTSUKA CHEMICAL

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DR. Vanessa T. Vu

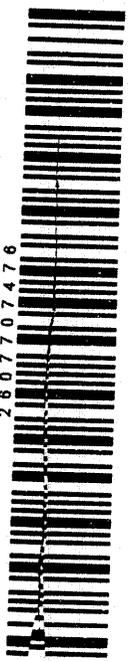
U.S. EPA Office of Pollution and Prevention- Toxic Substances

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Washington D.C. 20460

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Rev. Date 6/96
PART #1756
GFE 9/96

FYI-1097-001310

FINAL REPORT

on

**INHALATION TOXICITY STUDY OF FIBROUS AEROSOL IN RATS:
LIMIT TEST**

to

**Otsuka Chemical Company, Ltd.
Naruto Research Center
615 Hanamen, Satoura-cho, Naruto
Tokushima 772, Japan**

by

**Michael J. Brooker, Michael E. Placke, and
Arthur C. Peters**

June, 1992

**Study Initiation Date: December 18, 1991
Study Completion Date: July 6, 1992**

**BATTELLE
Columbus Operations
505 King Avenue
Columbus, Ohio 43201**

This study was conducted in compliance with EPA GLP Regulations 40 CFR, Part 792, the Japanese Ministry of International Trade and Industry and OECD (Organization for Economic Cooperation and Development). This study was conducted according to the study protocol and Battelle's Standard Operating Procedures and to the best of my knowledge the data presented accurately reflect the results of this study.



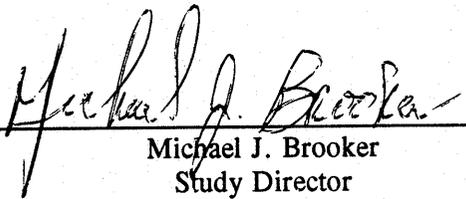
Michael J. Brooker
Study Director
Battelle Columbus Operations

FINAL REPORT

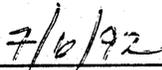
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**INHALATION TOXICITY STUDY OF FIBROUS AEROSOL IN RATS:
LIMIT TEST**

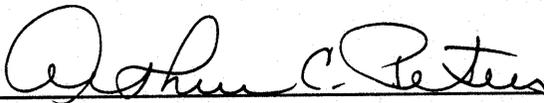
June, 1992



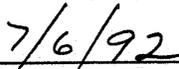
Michael J. Brooker
Study Director



Date



Arthur C. Peters
Manager, General Toxicology



Date

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to the study director and management as follows:

<u>Phase Inspected</u>	<u>Date Inspected/ Report to Study Director</u>	<u>Date of Report to Management</u>
Randomization/Identification	1-6-92	N/R
Body Weights	1-6-92	N/R
Animal Exposure	1-7-92	N/R
Filter Sampling	1-7-92	N/R
Clinical Observations	1-9-92	N/R
Fiber size and Count determinations	1-17-92	N/R
Study File Audit	1-20-92	N/R
Study File Audit	2-24-92	2/28/92
Draft Final Report Audit	3-19-92	3/24/92
Final Report	7-2-92	N/R

N/R = Not Required

To the best of my knowledge, the methods described were the methods followed and the data presented accurately represent data generated during the study.



 Quality Assurance Unit
 Health and Environment Group

7-6-92
Date

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SUMMARY

Five male and five female Fischer 344 rats were exposed to an aerosol of potassium octatitanate fibers. This was a single exposure, four hours in duration, with a gravimetrically determined mean total mass concentration of 2.0 mg/L. The fiber concentration of fibers > 5 micron in length was 5.3×10^5 fibers/cc for the first sample and 7.3×10^5 fibers/cc for the second sample. The concentration of fibers < 5 microns in length was 21.1×10^5 fibers/cc for the first sample and 21.7×10^5 fibers/cc for the second sample. All fibers measured were less than 3 microns in width.

All animals survived the exposure and were necropsied after a fourteen-day holding period. Gross necropsy results showed randomly distributed, minute, white, foci in all lobes of the lungs of nine of the ten animals exposed.

FINAL REPORT

on

INHALATION TOXICITY STUDY OF FIBROUS AEROSOL IN RATS: LIMIT TEST

to

Otsuka Chemical Company, Ltd.
Naruto Research Center
615 Hanamen, Satoura-cho, Naruto
Tokushima 772, Japan

June, 1992

INTRODUCTION

The purpose of this study was to evaluate the potential acute inhalation toxicity of potassium octatitanate fibers in rats following a single 4-hour inhalation exposure at an aerosol concentration of 5 mg/L, or the highest reasonably achievable concentration (Limit Test). During the development phase of the project, a mass aerosolized concentration of 2 mg/L was determined to be the highest reasonably achievable concentration and was considered appropriate for the purposes of this test.

The Study Protocol was prepared by Battelle and approved by the Sponsor's Project Monitor, Mr. Junji Imada for Otsuka Chemical Co., Ltd. Protocol amendments to the Study Protocol are presented in Appendix A. There were no protocol deviations. Michael J. Brooker was the Study Director; Dr. Arthur Peters served as the Program Manager for Battelle.

This study was conducted under Battelle Study Number SC910187.

MATERIALS AND METHODS

Test Article

The test article for this study was TISMO[®], a potassium octatitanate formulated as fibers, Lot No. 1F81L, which was received by Battelle on June 26, 1991. Five bags of material were received with a stated net weight of 10 kilograms per bag. Upon receipt at Battelle, the test article was repackaged into one-gallon plastic containers and was stored in a controlled access area at room temperature and humidity conditions, out of direct contact with light. The identity, purity and stability of the test article were the responsibility of the Sponsor.

Test System

A sufficient number of 5-week old Fischer 344 rats were obtained from Charles River Laboratory on December 27, 1991 to provide the required number of healthy animals for testing. The rat was the test system of choice since it has been used historically for this type of study and is considered an appropriate model for toxicity testing of materials having potential for human exposure. The animals were held for an 11 day quarantine period before study initiation during which time they were examined daily for ill health. The animals were individually housed in stainless steel wire cages and were given Purina Certified Rodent Chow (pellets) and water *ad libitum*. Water was from the City of Columbus. Feed and water were withheld during the 4-hour exposure period. There were no known food or water contaminants that interfered with the conduct of the study or interpretation of the results.

Animal room temperature and humidity ranges were 72 to 74 degrees Fahrenheit and 56 to 60 percent, respectively. Room light cycle was 12 hours light/12 hours dark each day. All housing and care conformed to current ILAR standards as published in the "Guide for the Care and Use of Laboratory Animals", NIH Publication no. 86-23.

The animals were individually identified by tail tattoo and numbered cage cards. On the day prior to exposure, the animals were assigned to the treatment group using a computerized randomization procedure.

During the quarantine period, blood for serology evaluation was collected from the orbital sinus of 5 rats/sex and sent to Microbiological Associates Inc, Rockville, MD. Tests for

antibodies to Kilham Rat Virus, pneumonia virus of mice, Sendai virus, sialodacryoadenitis virus/rat coronavirus and *Mycoplasma pulmonis* were conducted. Positive titers were not detected in any of the serum samples. The animals were visually inspected by a Battelle Staff Veterinarian before they were released from quarantine and found to be healthy and fit for study.

Biological Measurements

Animal Survival and Clinical Observations

Observations for toxicity/morbidity were conducted twice daily (mornings and afternoons) during the study. Abnormal clinical signs were recorded using the Xybion® system for each animal; rats observed to be normal were also documented.

Body Weight Determinations

Individual animal body weights were recorded using the Xybion® system at randomization (Day -1), and on Dose Days 1, 8 and 15 (study termination).

Necropsy Methods

Fourteen days after exposure, all animals were euthanatized by sodium pentobarbital overdose and a gross necropsy was performed on each animal. All gross lesions were preserved but histopathology was not completed on these tissues and no other tissues were collected.

Test Article Generation and Delivery System Description

The objective in designing the aerosol generation and delivery system was to provide a uniform concentration of respirable aerosol to the breathing zone of the animals. The test material aerosol generator was designed as a two-part system; the first was a mechanism to feed test material at a constant rate into an aerosol generator, and the second was a high-energy dispersion device to aerosolize the test material into a constant flow of entrainment air. The first component of the generation system was an Accurate Model 300 Dry Chemical Feeder (Accurate, Inc., Whitewater,

WI) which accurately delivered preset amounts of potassium octatitanate fibers into the aerosol generator. This feeder device employed a large capacity hopper with an auger type feed screw. This feeder directed a continuous stream of test compound past the inlet of a one inch Fox Coaxial Eductor (Fox Valve Development Corp., Dover, NJ), which aspirated the material by venturi effect, entraining it into the high pressure stream of air, then introduced it into a plenum chamber. Directly in line with this inlet stream inside the plenum was an impaction plate to break apart the agglomerates of test material that might remain after aerosolization.

The plenum chamber was supplied with High Efficiency Particulate (HEPA)/Charcoal filtered carrier air and the aerosol was allowed to expand in uniform distribution in the higher volume, lower velocity carrier air stream. Carrier air entered the plenum chamber near the bottom and the air flow pattern was from the bottom to the top. This allowed any remaining agglomerates or larger (highly non respirable fibers) to settle out at the bottom of the plenum.

From the plenum, the test atmosphere was directed to the exposure chamber through a stainless steel duct. The exposure chamber was approximately 0.5 cubic meters in volume with all animals exposed on a single plane within the chamber. Airflow through the exposure chamber was from top to bottom. A schematic diagram of the entire system is included as Figure 1. All tables and figures in this report are presented after the text, beginning on page 12.

Atmosphere Monitoring

Concentration Analysis

Exposure system aerosol concentrations were monitored by gravimetric technique. Gelman #66075, 25 mm, glass fiber filters (Gelman Sciences, Ann Arbor, MI) were placed into open-face filter holders and inserted into an exposure port. Calibrated air flow rates were regulated, using critical orifice meters, to sample a known volume of test atmosphere through the filters collecting the aerosolized fibers on the filter. Immediately after sampling, the filters were weighed and the mass concentration of the total aerosol was calculated from the accumulated mass and the sample volume.

Fiber Size Analysis

Fiber count and size measurements were determined twice during the four hour trial run and the four hour exposure. Fibers were collected on a 0.2 μm pore diameter Nuclepore filter (Nuclepore Corp., Pleasanton, CA) by drawing test atmosphere through a filter for approximately 30 seconds. Random samples of the filter were cut and fixed on double-sided tape using colloidal carbon. The samples were placed in a Polaron Sputter Counter and coated with gold. The filters were analyzed by Scanning Electron Microscopy (SEM) at 1930X magnification. Three polaroid photographs were taken; one of each mount. The photographs were converted to projection slides.

Slides were projected onto a Scriptel Graphics Tablet[®] and digitized to determine the size and number of fibers per field. SigmaScan[®] software was used to collect the data. All fibers in the field were counted to determine the number concentration of fibers (total fibers/Liter). The area of the slide was measured and compared to the total collection area of the filter to determine a percentage of the total filter field. The percentage of the total field was multiplied by the volume of air drawn through the filter sample to determine the volume of air passing through the measured area. Fiber size measurements were made on all fibers on the slide that could be individually identified (criteria were that both ends of the fiber must be visible). From these measurements, the aspect ratio for each fiber was determined. Fiber concentration was calculated by multiplying the percent of fibers in a length range by the total fiber count. The data from all three slides was summarized to provide a single data set for each filter.

Bulk fiber samples were prepared for analysis by placing small amounts of bulk material on 5 cm by 5 cm sticks of double sided tape. The samples were then placed in a Polaron Sputter Coater and coated with approximately 20 nm of Gold. All samples were analyzed in an ISI OS-130 Scanning Electron Microscope.

Bulk fiber size analysis was completed using the methods described above for filter analysis.

Temperature and Humidity Measurements

The chamber environment was controlled by controlling the exposure room environment. The test atmosphere was sampled in the exposure chamber during the actual exposure. A Cole

Parmer Tri-Sensor, thermo humidity measuring device, Model 37000-50 (Cole-Parmer, Chicago, IL) was used to determine the temperature and humidity.

Chamber Uniformity

Chamber uniformity was determined by gravimetric concentration sampling on the plane of the chamber where the animals were exposed. The sampling scheme included a reference location to determine temporal uniformity as well as four locations within the plane for spatial uniformity. The reference location was sampled before, during, and after other uniformity measurements to assess the contribution of within-port variability to the uniformity determinations. The total port variability (TPV) was estimated from the relative standard deviation of analyses from one measurement at each location, and represented the sum of variability between ports (spatial), and the variability within a port (temporal).

The within port variability (WPV) was estimated from the relative standard deviation of the concentration measured at the reference location. This variability included temporal fluctuations as generator output.

The between port variability (BPV) represented the variability associated with spatial variability of test article concentration within the exposure chamber. The BPV was calculated from the total port mean (M_t), total port standard deviation (S_t), and the within port standard deviation (S_w) by applying the following formula:

$$BPV = 100 \cdot (S_t^2 - S_w^2)^{1/2} / M_t$$

Because of random error, aggravated by the small sample size, the estimated WPV was occasionally greater than the estimated TPV. In these cases, the BPV cannot be calculated directly. Since the estimate of TPV is more reliable than the estimate of WPV due to its greater sample size, and since BPV, by definition, cannot exceed TPV, BPV is reported to be less than the TPV for those cases where it can not be calculated.

Statistical Methods and Data Management

In-life and postmortem data (necropsy findings) were collected using the Xybion Path/Tox System, (Xybion Medical Systems Corporation, Cedar Knolls, NJ). This real-time computer data capture system recorded body weights, daily clinical observations, and gross findings on-line with Battelle's VAX computer system. Data collected off-line were reviewed for accuracy of transcription.

Individual animal body weight data was collected weekly and summarized by sex. Clinical observations, and necropsy incidence data were not analyzed statistically since there were no group to group comparisons.

RESULTS

Atmosphere Characterization Results

Pre-Study Exposure Chamber Uniformity Data

Chamber uniformity measurements were completed during the prestudy validation of the inhalation system. The data show the between port variability (BPV) to be less than 4.89 percent. These data are summarized in Table 1. Because the estimate of within port variability (WPV) was greater than the estimate of total port variability (TPV) for the chamber, the BPV could not be calculated directly. The BPV is presented as being less than the TPV since, by definition, it cannot exceed this value. This prestudy analysis showed a completely uniform distribution of the atmosphere within the chamber.

Bulk Fiber Size Distribution

Bulk fiber measurements were completed during the development phase of the project to assure the fiber size distribution of test atmosphere generated was comparable to that of the bulk material. Approximately 62 percent of the fibers measured were less than five microns in length. All fibers measured were less than 3 microns in width with the greatest percentage of fibers between 0.25

and 0.75 microns. Results of fiber length and width measurements are represented graphically in Figure 2. Individual fiber measurements are listed in Appendix B.

Trial Exposure Data

Characterization of the test article atmosphere in the four-hour trial run consisted of chamber concentration analyses, fiber size distribution measurements, fiber count measurements and temperature and humidity measurements. The mean chamber test article concentration was 1.83 mg/L with a Relative Standard Deviation (RSD) of 8.34 percent. These data are summarized in Table 2. The mean fiber concentration was 2,778,041 fibers/cc for the first sample and 1,694,477 fibers/cc for the second sample. Fiber size distribution data are plotted in Figure 3 (Sample 1) and Figure 4 (Sample 2). Temperature and humidity measurements recorded were 19.1°C and 55.7 percent RH, respectively.

Four Hour Exposure Chamber Data

Characterization of the test article atmosphere in the four-hour exposure consisted of chamber concentration analyses, fiber size distribution measurements, fiber count measurements and temperature and humidity measurements. The mean chamber test article concentration was 2.01 mg/L with a Relative Standard Deviation (RSD) of 3.44 percent. These data are summarized in Table 3. The mean fiber concentration was 2,639,638 fibers/cc for the first sample and 2,905,946 fibers/cc for the second sample. Approximately 80 percent of the fibers measured in Sample 1 and 75 percent of the fibers measured in Sample 2 were less than five microns in length. The calculation of fibers $> 5 \mu\text{m}$ in length is shown at the bottom of Figure 5. All fibers measured were less than 3 microns in length with the greatest percentage between 0.25 microns and 0.75 microns. Fiber size distribution data are plotted in Figure 5 (Sample 1) and Figure 6 (Sample 2). Individual fiber measurements are listed in Appendix B. Temperature and humidity measurements recorded were 22.8°C and 42.6 percent RH, respectively.

Animal Survival

All animals survived the four hour exposure and the fourteen day observation period and were terminated in good physical condition on Study Day 15.

Clinical Observations

The individual animal morning (1st set/day) and afternoon (2nd set/day) clinical observations are provided in Appendix A. Abnormal findings were recorded for all animals after exposure and for 1 week post exposure. These findings included lethargic behavior, abnormal (labored) breathing, and ruffled fur. All animals recovered to a normal clinical condition by Study Day 8 and remained normal for the remainder of the observation period. Clinical observations are summarized in Table 4.

Body Weight Results

At randomization, the body weight of the males ranged from 133.1 to 138.0 grams and the females ranged from 101.2 to 104.5 grams. Mean body weights for the male animals were 141.5, 158.7, and 188.6 on study days 1, 8, and 15, respectively. Mean body weights for the female animals were 105.5, 114.7, and 125.3 grams on study days 1, 8, and 15, respectively. These data are summarized in Table 5. The individual animal body weight data are detailed in Appendix A. Mean body weight gain data and individual animal body weight gain data are shown in Tables 6 and Appendix A, respectively.

Necropsy Results

After a 14-day observation period, all ten rats were sacrificed and examined for gross lesions. Lesions attributed to exposure to test article consisted of white foci, randomly distributed among all lung lobes, and were found in all five males and in four of the five females. The foci were minute, and were suggestive of foreign body presence and an alveolar macrophage response to a particulate. The individual animal gross pathology observations are detailed in Table 7 and the incidence of gross pathology observations is detailed in Table 8. No other exposure or incidental lesions were noted.

DISCUSSION

A four hour whole body inhalation exposure to Tismo® Fibers at a concentration of 2.01 mg/L in Fisher 344 rats did not produce mortality. There was no weight loss in any of the animals exposed, rather animals continued to gain weight. Non-specific clinical signs of lethargy and ruffled fur and moderate dyspnea occurred after exposure and persisted for several days, disappearing within a week after exposure in all animals. The necropsy findings of minimal discoloration and white foci in the lungs of nine of ten animals exposed, is not unexpected and is typically seen at necropsy in the lungs of animals exposed to high levels of particulates, including nuisance or inert materials.

The comparison of the bulk test article fiber size distribution with the fiber size distribution in the exposure atmosphere shows very similar fibers in length and width. There is a slight enrichment of shorter fibers in the exposure atmosphere that is a naturally occurring process due to sedimentation of very large fibers and line loss from the transport of fibers via an airstream.

RECORD ARCHIVES

All records required to reconstruct the study and to demonstrate adherence to the protocol are maintained at Battelle Columbus Laboratories, 505 King Avenue, Columbus, OH. The records are identified using a unique eight digit SC Number (SC910187) assigned to the Study Protocol. The test article (potassium titanate) is identified by a unique Battelle Assigned Substance Number (873). Records will be maintained for five years following completion of the study. Battelle may then contact the Sponsor to determine the final disposition of these materials.

ACKNOWLEDGEMENTS

Members of Battelle's Toxicology, Pathology, Animal Resources, and Quality Assurance Sections whose signatures appear in the report or in the study records are acknowledged for their participation in the conduct of this study. The names of the principal contributors in this study are listed below.

Principal Contributors

Participant	Title	Department
M. J. Brooker, B.S.	Study Director	General Toxicology
J. E. Frye, A. A.	Inhalation Technician	General Toxicology
W. E. French	Animal Technician	Animal Resources
E. E. Vinci, B.S.	Quality Assurance Specialist	Quality Assurance
A. W. Singer, D.V.M., M.S., A.C.V.P., D.A.B.T.	Pathologist	Pathology
M. E. Placke, Ph.D.	Vice President	Pharmaceutical Development
A. C. Peters, D.V.M.	Manager	General Toxicology

TABLE 1.
INHALATION TOXICITY STUDY OF FIBROUS AEROSOL
IN RATS: LIMIT TEST CHAMBER UNIFORMITY

Location ¹	Concentration, mg/L
1 (REF)	2.06
2	1.86
3	1.83
1 (REF)	2.12
4	1.85
5	1.91
1 (REF)	1.80
Variability	(% RSD)
Total Port (TPV)	4.89
Within Port (WPV)	8.53
Between Port (BPV)	< 4.89

1. Sample locations within the exposure chamber on a single horizontal plane.

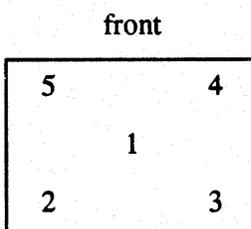


TABLE 2.
INHALATION TOXICITY STUDY OF FIBROUS AEROSOL IN
RATS: LIMIT TEST-FOUR HOUR TRIAL EXPOSURE DATA

Sample No.	Mass Concentration (mg/L)	Fiber Count (F/cc)	Temperature (°C)	Humidity (%RH)
1	1.71	2,778,041	19.1	55.7
2	1.71	1,694,477		
3	1.86			
4	2.03			
	$\bar{X} = 1.83$			
	RSD = 8.34%			

*Fiber count based on Samples A and B.

TABLE 3.
INHALATION TOXICITY STUDY OF FIBROUS AEROSOL IN
RATS: LIMIT TEST ANIMAL EXPOSURE DATA

Sample No.	Mass Concentration (mg/L)	Fiber Count (F/cc)*	Temperature (°C)	Humidity (%RH)
1	1.96	2,639,638	22.8	42.6
2	1.95	2,905,946		
3	2.02			
4	2.10			
	$\bar{X} = 2.01$			
	RSD = 3.44%			

*Fiber count based on Samples A and B.

TABLE 5.

BATTELLE COLUMBUS LABORATORIES TOXICOLOGY DEPARTMENT COLUMBUS, OHIO 43201 SPECIES: RAT/FISCHER 344		MEAN ANIMAL BODY WEIGHTS IN GMS STUDY NUMBER: SC910187 ABSOLUTE BODY WEIGHT GAINS REFERENCED TO STUDY DAY: 1 STUDY START DATE: 07-JAN-92		STUDY TYPE: ACUTE/INHALATION TOXICITY	
GROUP(S)		DAY OF STUDY			
		-1			15
1	(N) MEANS SDEVS	5 136.1 1.8	MALE ANIMALS 5 141.5 2.1	5 158.7 4.4	5 188.6 5.8
1	(N) MEANS SDEVS	5 102.5 1.2	FEMALE ANIMALS 5 105.5 2.1	5 114.7 4.3	5 125.3 3.1

* = MEAN VALUE OF GROUP WAS SIGNIFICANTLY DIFFERENT FROM THE CONTROL AT P = .05 USING DUNNETT'S TEST OF SIGNIFICANCE

TABLE 6.

BATTELLE COLUMBUS LABORATORIES TOXICOLOGY DEPARTMENT COLUMBUS, OHIO 43201 SPECIES: RAT/FISCHER 344		MEAN ANIMAL ABSOLUTE WEIGHT GAINS IN GMS STUDY NUMBER: SC910187		PRINTED: 06-FEB-92 PAGE: 1	
		ABSOLUTE BODY WEIGHT GAINS REFERENCED TO STUDY DAY: 1		STUDY TYPE: ACUTE/INHALATION TOXICITY	
		STUDY START DATE: 07-JAN-92			
GROUP(S)		8	15	DAY OF STUDY	
	(N) MEANS SDEVS	MALE ANIMALS			
1	5 17.2 3.1	5 47.1 4.5			
		FEMALE ANIMALS			
1	5 9.2 3.9	5 19.7 3.0			

* = MEAN VALUE OF GROUP WAS SIGNIFICANTLY DIFFERENT FROM THE CONTROL AT P = .05 USING DUNNETT'S TEST OF SIGNIFICANCE

TABLE 7

INDIVIDUAL ANIMAL GROSS PATHOLOGY OBSERVATIONS				SC910187
ANIMAL #/SEX	GROUP	DESIGN CONC.	ORGAN/GROSS OBSERVATION	SEVERITY
201/M	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
202/M	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
203/M	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
204/M	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
205/M	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
101/F	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
102/F	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
103/F	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
104/F	1	5 MG/L	LUNG DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1
105/F	1	5 MG/L	NO GROSS LESIONS FOUND DISCOLORATION COMMENT: PATCHY, WHITE, MULTIFOCAL	1

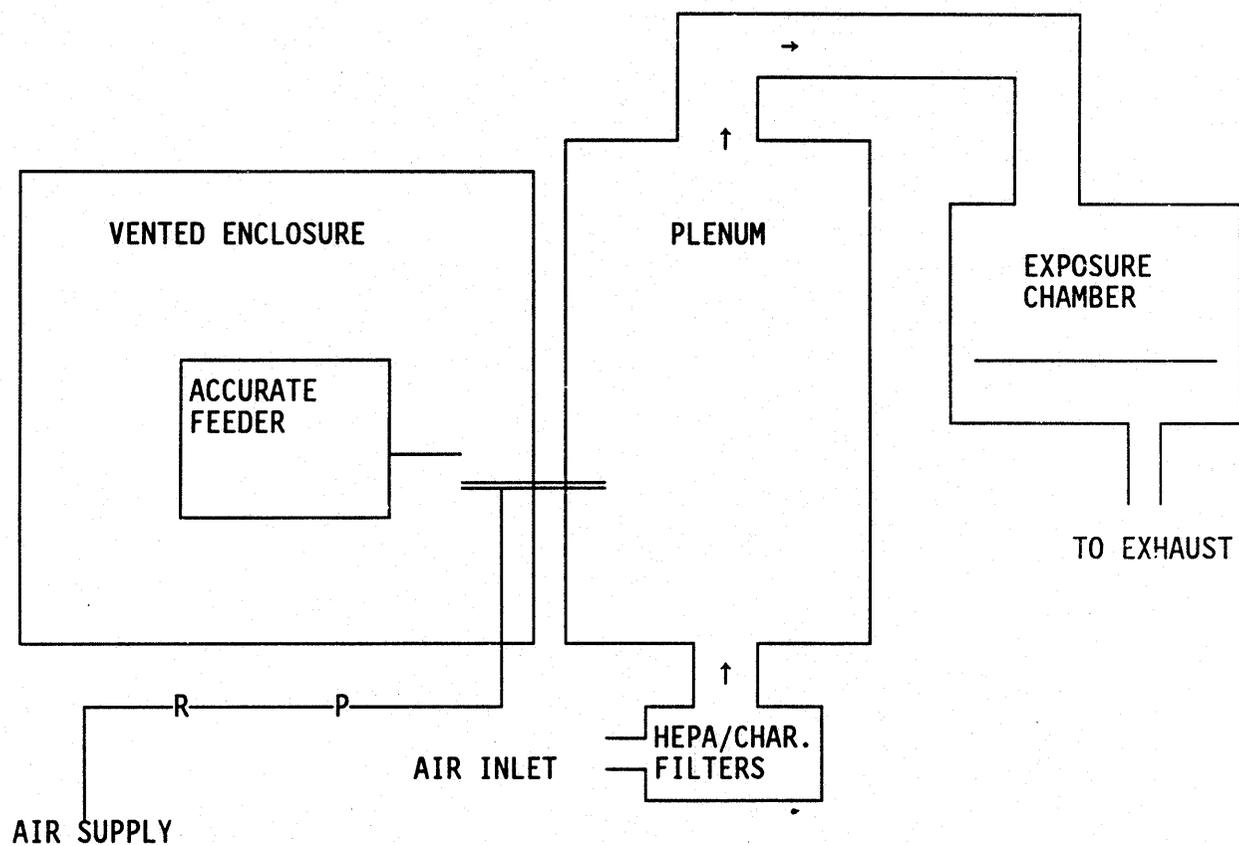
NOTE: *1* UNDER SEVERITY REFERS TO *MINIMAL* EFFECT OBSERVED.

TABLE 8

SC910187

INCIDENCE OF GROSS PATHOLOGY OBSERVATIONS

	MALES	FEMALES
ANIMAL SEX GROUP:	1	1
DESIGN CONCENTRATION:	5 MG/L	5 MG/L
NO. IN GROUP:	5	6
LUNG DISCOLORATION	5	4



== - Fox Eductor Valve

P - Pressure Gauge

R - Pressure Regulator

FIGURE 1. SCHEMATIC DIAGRAM OF FIBER GENERATION AND EXPOSURE SYSTEM

FIGURE 2. DISTRIBUTION OF POTASSIUM OCTATITANATE FIBERS - BULK FIBERS
PERCENT OF TOTAL FIBERS

Fiber Diameter (μm)	0-3	3-5	5-8	8-10	10-15	> 15
> 3	0.00	0.00	0.00	0.00	0.00	0.00
2-3	0.00	0.00	0.00	0.00	0.00	0.00
1-2	0.00	0.00	1.37	0.00	0.00	0.00
0.75-1	2.74	0.00	1.37	1.37	1.37	0.00
0.5-0.75	6.85	9.59	8.22	6.85	4.11	0.00
0.25-0.5	21.92	13.70	5.48	6.85	1.37	0.00
0-0.25	5.48	1.37	0.00	0.00	0.00	0.00

Fiber Length (μm)

**FIGURE 3. DISTRIBUTION OF POTASSIUM OCTATITANATE FIBERS
TRIAL EXPOSURE SAMPLE 1**

PERCENT OF TOTAL FIBERS

**Fiber
Diameter
(μm)**

> 3	0.00	0.00	0.00	0.00	0.00	0.00
2-3	0.00	0.57	0.00	0.00	0.00	0.00
1-2	1.70	1.14	1.70	0.00	1.70	0.00
0.75-1	6.25	2.27	3.41	0.57	0.57	0.00
0.5-0.75	17.05	11.93	10.23	1.14	2.27	0.57
0.25-0.5	16.48	4.55	6.82	1.14	1.7	0.00
0-0.25	5.11	0.57	0.57	0.00	0.00	0.00
	0-3	3-5	5-8	8-10	10-15	> 15

Fiber Length (μm)

**FIGURE 4. DISTRIBUTION OF POTASSIUM OCTATITANATE FIBERS
TRIAL EXPOSURE SAMPLE 2**

PERCENT OF TOTAL FIBERS

Fiber Diameter (μm)	0-3	3-5	5-8	8-10	10-15	> 15
> 3	0.00	0.00	0.00	0.00	0.00	0.00
2-3	0.00	0.73	0.00	0.00	0.00	0.00
1-2	4.38	2.19	1.46	1.46	0.00	0.00
0.75-1	7.30	1.46	2.19	0.73	2.19	0.00
0.5-0.75	22.63	7.30	3.65	0.73	1.46	1.46
0.25-0.5	22.63	9.49	0.73	1.46	0.73	0.73
0-0.25	1.46	1.46	0.00	0.00	0.00	0.00

Fiber Length (μm)

**FIGURE 5. DISTRIBUTION OF POTASSIUM OCTATITANATE FIBERS
ANIMAL EXPOSURE SAMPLE 1**

PERCENT OF TOTAL FIBERS¹

Fiber Diameter (μm)	0-3	3-5	5-8	8-10	10-15	> 15
> 3	0.00	0.00	0.00	0.00	0.00	0.00
2-3	0.00	0.00	0.00	0.00	0.00	0.00
1-2	2.13	0.00	0.53	0.00	0.00	0.53
0.75-1	4.26	2.13	1.60	1.06	0.53	0.53
0.5-0.75	19.15	6.91	7.45	1.06	1.06	0.53
0.25-0.5	30.32	6.91	1.60	0.53	1.06	0.53
0-0.25	6.91	1.06	1.60	0.00	0.00	0.00
	0-3	3-5	5-8	8-10	10-15	> 15

Fiber Length (μm)

¹Percent of Fibers > 5 μm =

Total Fibers [100-(percent fibers 0-3 in length + percent fibers 3-5 in length)].

Percent of Fibers > 5 μm = 2639638 (100-79.78) = 5.3 x 10⁵

**FIGURE 6. DISTRIBUTION OF POTASSIUM OCTATITANATE FIBERS
ANIMAL EXPOSURE SAMPLE 2**

PERCENT OF TOTAL FIBERS

Fiber Diameter (μm)	0-3	3-5	5-8	8-10	10-15	> 15
> 3	0.00	0.00	0.00	0.00	0.00	0.00
2-3	0.00	0.00	0.00	0.50	0.00	0.50
1-2	4.95	1.98	1.98	0.50	0.99	0.00
0.75-1	4.95	4.95	1.98	0.00	0.50	1.49
0.5-0.75	20.79	5.45	4.46	1.49	2.97	1.49
0.25-0.5	21.78	6.44	1.98	0.99	0.99	0.00
0-0.25	3.47	0.00	1.49	0.50	0.50	0.00

Fiber Length (μm)

APPENDIX A

Protocol Amendment

Battelle Study Number SC910187

INHALATION TOXICITY STUDY OF FIBROUS
AEROSOL IN RATS: LIMIT TEST

Amendment Number 1

December 18, 1991

Effective Date: December 18, 1991

1. Part to be Changed: Page 3, Section 2.0, Proposed in-life Starting and Completion Dates, is to be changed as follows:

Quarantine Start: Week of 12/23/91
Exposure: Week of 1/6/92
Final Scheduled Necropsies: Week of 1/20/92

Reason for change: The study schedule was not properly updated when the protocol was signed.

2. Part to be changed: Page 10, Section 10.0,C,4, Experimental Measurements, is to be changed as follows:

Animals will be weighed prior to necropsy and killed by Sodium Pentobarbital overdose.

Reason for change: Sodium pentobarbital is standard for inhalation studies unless specifically deemed inappropriate.

APPROVED BY:

Michael J. Booker
Study Director

Junji Imada
Sponsor's Representative

12/18/91
Date

Dec. 24, 1991
Date

Protocol Amendment

Battelle Study Number SC910187

INHALATION TOXICITY STUDY OF FIBROUS
AEROSOL IN RATS: LIMIT TEST

Amendment Number 2

July 2, 1992

Effective Date: July 2, 1992

1. Part to be Changed: Page 6, Section 7.0, Test Article, is to be changed as follows:

A. Test Article

- 1. Fibrous Aerosol: TISMO (Potassium Octatitanate)

Reason for change: The original designation of potassium titanate does not refer to the specific compound that was used for this work.

APPROVED BY:

Michael A. Becker
Study Director

Junji Imada
Sponsor's Representative

July 2, 1992
Date

July 3, 1992
Date

GROUP/ ANIMAL SUBGROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
	FINDING = BEHAVIOR / ABNORMAL BREATHING																
205 1/1	-	1	1	1	1	0	-	-	-	-	-	-	-	-	-		
	(I)	5	5	5	2	0	0	0	0	0	0	0	0	0	0		
							MALE ANIMALS										
101 1/1	-	1	1	1	-	-	-	-	-	-	-	-	-	-	-		
102 1/1	-	1	1	1	1	1	-	-	-	-	-	-	-	-	-		
103 1/1	-	1	1	1	1	-	-	-	-	-	-	-	-	-	-		
104 1/1	-	1	1	1	1	-	1	-	-	-	-	-	-	-	-		
105 1/1	-	1	1	1	1	1	-	-	-	-	-	-	-	-	-		
	(I)	5	5	5	3	1	2	0	0	0	0	0	0	0	0		
							FEMALE ANIMALS										

GROUP/ ANIMAL SUBGROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
	FINDING = BEHAVIOR / LETHARGIC																
201 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
202 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
203 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
204 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
205 1/1	-	1	-	-	-	0	0	0	0	0	0	0	0	0	0		
	(I)	5	0	0	0	0	0	0	0	0	0	0	0	0	0		
							MALE ANIMALS										
101 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
102 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
103 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
104 1/1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-		
105 1/1	-	1	-	-	-	0	0	0	0	0	0	0	0	0	0		
	(I)	5	0	0	0	0	0	0	0	0	0	0	0	0	0		
							FEMALE ANIMALS										

BATTELLE COLUMBUS LABORATORIES
 TOXICOLOGY DEPARTMENT
 SPECIES: RAT/FISCHER 344

INDIVIDUAL ANIMAL DATA FOR CLINICAL OBSERVATIONS (2ND SET/DAY)
 STUDY NUMBER: SC910187
 STUDY START DATE: 07-JAN-92

PRINTED: 08-FEB-92
 PAGE: 1
 STUDY TYPE: ACUTE/INHALATION TOXICITY

ANIMAL GROUP/ SUBGROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	FINDING = NORMAL / NO REMARKABLE FINDINGS													
	MALE							ANIMALS						
201 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
202 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
203 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
204 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
205 1/1	0	0	0	0	0	0	0	5	5	5	5	5	5	5
	FEMALE							ANIMALS						
101 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
102 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
103 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
104 1/1	-	-	-	-	-	-	-	1	1	1	1	1	1	1
105 1/1	0	0	0	0	0	0	0	5	5	5	5	5	5	5

ANIMAL GROUP/ SUBGROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	FINDING = GENERAL / RUFFLED FUR													
	MALE							ANIMALS						
201 1/1	-	-	-	1	1	1	1	-	-	-	-	-	-	-
202 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
203 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
204 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
205 1/1	0	0	4	5	5	5	5	0	0	0	0	0	0	0
	FEMALE							ANIMALS						
101 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
102 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
103 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
104 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
105 1/1	0	0	5	5	5	5	5	0	0	0	0	0	0	0

ANIMAL GROUP/ SUBGROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	FINDING = BEHAVIOR / ABNORMAL BREATHING													
	MALE							ANIMALS						
201 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
202 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
203 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
204 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
205 1/1	1	1	1	1	1	1	5	5	5	5	5	5	5	5
	FEMALE							ANIMALS						
101 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
102 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
103 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
104 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
105 1/1	0	0	5	5	5	5	5	0	0	0	0	0	0	0

ANIMAL GROUP/ SUBGROUP	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	FINDING = BEHAVIOR / ABNORMAL BREATHING													
	MALE							ANIMALS						
201 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
202 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
203 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
204 1/1	1	1	1	1	1	1	-	-	-	-	-	-	-	-
205 1/1	1	1	1	1	1	1	5	5	5	5	5	5	5	5
	FEMALE							ANIMALS						
101 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
102 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
103 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
104 1/1	-	-	1	1	1	1	1	-	-	-	-	-	-	-
105 1/1	0	0	5	5	5	5	5	0	0	0	0	0	0	0

ANIMAL SUBGROUP	GROUP/ FINDING = BEHAVIOR / ABNORMAL BREATHING	DAY													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
205	1/1 (I)	1	5	1	1	1	0	0	0	0	0	0	0	0	0
101	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
102	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
103	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
104	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
105	1/1 (I)	1	5	1	1	1	2	2	0	0	0	0	0	0	0

ANIMAL SUBGROUP	GROUP/ FINDING = BEHAVIOR / LETHARGIC	DAY													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
201	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
202	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
203	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
204	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
205	1/1 (I)	1	5	1	1	1	0	0	0	0	0	0	0	0	0
101	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
102	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
103	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
104	1/1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
105	1/1 (I)	1	5	1	1	1	0	0	0	0	0	0	0	0	0

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PAGE: 1

INDIVIDUAL ANIMAL BODY WEIGHTS IN GMS
STUDY NUMBER: SC910187
ABSOLUTE BODY WEIGHT GAINS REFERENCED TO STUDY DAY: 1
STUDY START DATE: 07-JAN-92
STUDY TYPE: ACUTE/INHALATION TOXICITY

BATTELLE COLUMBUS LABORATORIES
TOXICOLOGY DEPARTMENT
COLUMBUS, OHIO 43201
SPECIES: RAT/FISCHER 344

ANIMAL SUBGROUP	GROUP/ SUBGROUP	DAY OF STUDY		MEANS (N)	SDEVS
		1	8		
201	1/1	136.30	140.70	136.06	1.80
202	1/1	133.10	139.80	136.20	1.80
203	1/1	136.20	139.80	136.20	1.80
204	1/1	138.00	144.50	136.70	1.80
205	1/1	136.70	142.80	136.70	1.80
				136.06	1.80
				(N)	
				5	
				158.74	4.37
				183.40	5.76
				181.90	5.76
				189.70	5.76
				192.90	5.76
				195.00	5.76
				188.58	5.76
				183.40	5.76
				155.00	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76
				158.74	5.76
				141.52	5.76
				142.80	5.76
				144.50	5.76
				139.80	5.76
				139.80	5.76
				136.20	5.76
				133.10	5.76
				136.30	5.76
				140.70	5.76
				142.80	5.76
				144.50	5.76
				149.70	5.76
				155.80	5.76
				156.50	5.76
				161.10	5.76
				165.30	5.76

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PAGE: 1

INDIVIDUAL ANIMAL ABSOLUTE WEIGHT GAINS IN GMS
STUDY NUMBER: SC910187
ABSOLUTE BODY WEIGHT GAINS REFERENCED TO STUDY DAY: 1
STUDY START DATE: 07-JAN-92
STUDY TYPE: ACUTE/INHALATION TOXICITY

BATTELLE COLUMBUS LABORATORIES
TOXICOLOGY DEPARTMENT
COLUMBUS, OHIO 43201
SPECIES: RAT/FISCHER 344

ANIMAL	GROUP/ SUBGROUP	8	15
		MALE	ANIMALS
201	1/1	14.30	42.70
202	1/1	16.00	42.10
203	1/1	16.70	49.90
204	1/1	16.60	48.40
205	1/1	22.50	52.20
	(N)	5	5
	MEANS	17.22	47.06
	SDEVS	3.10	4.47
		FEMALE	ANIMALS
101	1/1	10.70	21.10
102	1/1	11.60	20.10
103	1/1	4.00	16.00
104	1/1	13.30	23.70
105	1/1	6.20	17.80
	(N)	5	5
	MEANS	9.16	19.74
	SDEVS	3.90	2.98

APPENDIX B

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
3.24	0.37	8.80
11.67	0.41	28.58
2.07	0.26	8.03
9.85	0.82	12.08
5.01	0.33	15.15
7.39	1.30	5.66
8.64	0.33	26.13
3.12	0.45	6.96
3.09	0.48	6.43
1.86	0.20	9.40
8.65	0.45	19.27
9.70	0.32	29.88
4.54	0.52	8.65
5.11	0.42	12.31
2.83	0.30	9.36
2.04	0.38	5.38
9.34	0.37	25.15
9.02	0.55	16.45
6.37	0.54	11.77
2.38	0.41	5.79
4.86	0.24	20.59
6.87	0.52	13.11
7.09	0.57	12.43
1.17	0.43	2.76
1.29	0.42	3.04
4.05	0.50	8.08
3.57	0.40	8.91
5.77	0.34	16.75
1.19	0.46	2.61
2.05	0.42	4.88
10.39	0.67	15.57
1.80	0.54	3.33
1.20	0.25	4.85
4.16	0.61	6.76
2.08	0.62	3.38
7.79	0.67	11.59
3.02	0.42	7.22
2.80	0.51	5.52
3.27	0.51	6.41
2.92	0.47	6.19
2.24	0.49	4.58
7.09	0.76	9.35
12.36	0.74	16.60
4.35	0.36	12.01
3.09	0.42	7.26
5.33	0.56	9.49
9.07	0.55	16.35
2.05	0.30	6.82
2.67	0.32	8.29
2.90	0.24	11.98
2.00	0.84	2.39
8.67	0.59	14.70

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
1.19	0.40	2.96
4.88	0.73	6.70
2.33	0.49	4.75
12.17	0.56	21.71
3.37	0.62	5.42
3.29	0.42	7.78
5.52	0.71	7.77
3.27	0.46	7.12
11.81	0.88	13.39
8.76	0.62	14.05
1.94	0.20	9.52
2.90	0.62	4.70
1.67	0.70	2.39
2.01	0.35	5.69
9.69	0.50	19.54
3.85	0.47	8.28
3.40	0.72	4.74
9.52	0.61	15.74
2.23	0.33	6.65
2.67	0.84	3.16
6.70	0.49	13.79

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
2.56	0.94	2.73
0.66	0.50	1.32
3.09	0.48	6.49
1.76	0.98	1.80
1.10	0.63	1.73
2.43	0.33	7.38
2.23	0.56	3.95
1.61	0.42	3.83
1.96	0.52	3.78
7.59	0.45	16.99
10.18	0.63	16.22
7.51	0.40	18.91
7.86	0.32	24.50
6.70	0.31	21.63
6.38	0.62	10.32
1.88	0.07	25.93
3.71	0.66	5.63
2.11	0.67	3.13
8.11	0.49	16.50
7.27	0.71	10.24
1.31	0.22	5.98
10.76	0.30	35.89
2.00	1.20	1.67
1.40	0.71	1.97
1.37	0.82	1.68
1.77	0.78	2.28
11.21	1.08	10.39
4.89	0.56	8.71
0.89	0.44	2.01
1.75	0.24	7.24
7.12	0.69	10.29
1.43	0.50	2.86
3.48	0.41	8.58
1.97	0.46	4.27
1.79	0.46	3.85
1.94	0.44	4.41
0.51	0.54	0.95
1.68	0.53	3.19
1.45	0.83	1.74
1.94	0.43	4.48
1.41	0.48	2.93
0.64	0.56	1.14
1.66	1.30	1.28
6.89	0.25	28.02
1.15	0.34	3.37
0.48	0.41	1.16
4.92	0.65	7.56
1.05	0.65	1.61
5.48	0.32	16.92
2.10	0.36	5.88
1.24	0.27	4.54
2.08	0.77	2.70

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
2.00	0.58	3.46
2.82	0.49	5.80
1.56	0.58	2.68
1.98	0.42	4.67
1.16	0.43	2.68
1.53	0.99	1.55
7.16	0.83	8.58
1.27	0.20	6.21
3.49	1.37	2.54
0.95	0.44	2.16
1.99	0.42	4.74
6.66	0.36	18.43
2.23	0.36	6.15
4.75	0.35	13.59
2.92	0.52	5.59
3.22	0.66	4.86
3.78	0.56	6.71
2.85	0.35	8.22
1.30	0.22	5.95
6.48	0.84	7.73
3.75	0.61	6.10
3.27	0.17	19.19
5.92	0.50	11.89
6.85	0.57	11.93
3.55	0.53	6.66
1.94	0.62	3.14
6.80	0.64	10.65
1.04	0.32	3.21
8.90	0.43	20.65
0.85	0.30	2.82
5.81	0.62	9.38
5.11	0.52	9.86
1.78	0.61	2.91
1.28	0.56	2.27
1.89	0.25	7.65
1.62	0.39	4.18
0.72	0.60	1.20
5.69	0.74	7.66
11.24	1.08	10.38
3.67	0.52	7.13
1.20	0.74	1.62
5.04	1.47	3.42
1.90	0.70	2.73
1.26	0.58	2.15
3.55	0.67	5.29
6.87	0.82	8.39
4.50	1.10	4.09
6.08	0.87	7.03
5.80	0.48	12.14
1.22	0.56	2.17
10.37	0.65	15.86
4.14	0.40	10.41

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
10.27	1.06	9.71
4.62	0.88	5.28
4.55	0.69	6.64
12.31	0.46	26.64
5.51	0.78	7.09
9.17	0.70	13.10
1.23	0.55	2.23
1.87	0.46	4.05
3.86	0.78	4.94
5.60	0.75	7.42
4.08	2.57	1.58
7.97	0.52	15.19
0.85	0.40	2.11
8.93	0.64	13.97
5.60	0.70	8.02
1.84	0.49	3.77
7.02	0.59	11.95
1.75	0.51	3.43
5.75	1.11	5.16
2.00	0.87	2.30
3.28	0.67	4.88
4.97	0.69	7.18
2.53	1.25	2.02
2.80	0.51	5.47
1.98	0.49	4.04
2.90	0.77	3.76
2.52	0.72	3.53
6.91	0.42	16.53
4.25	0.92	4.60
3.11	0.44	7.14
13.48	0.55	24.37
10.17	0.76	13.33
7.98	0.60	13.26
3.58	0.64	5.62
3.99	0.28	14.31
2.55	0.63	4.02
5.24	0.70	7.44
2.80	0.64	4.38
4.55	0.71	6.36
3.35	0.61	5.49
15.88	0.71	22.40
5.54	0.55	10.13
6.27	0.67	9.37
3.53	0.64	5.51
5.65	0.35	16.02
2.60	0.39	6.75
4.70	0.91	5.14
1.70	0.54	3.13
1.54	0.24	6.44
5.44	0.42	12.93
4.05	0.55	7.32
6.97	0.51	13.78

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
5.79	0.52	11.02
3.99	0.38	10.61
11.32	0.36	31.21
6.82	0.61	11.10
2.84	0.45	6.27
9.39	0.83	11.32
1.61	0.30	5.41
3.50	0.63	5.54
1.27	0.21	6.12
1.28	0.25	5.13
6.71	1.41	4.77
2.05	0.91	2.26
2.74	0.52	5.26
5.61	0.39	14.52
3.40	0.56	6.07
3.62	0.68	5.32
12.16	0.51	23.93
2.52	0.98	2.57
3.29	0.63	5.22
3.18	0.31	10.12

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
1.47	0.75	1.97
1.92	0.48	4.00
1.69	0.48	3.56
0.90	0.34	2.66
3.61	0.59	6.14
2.77	0.51	5.43
1.79	0.61	2.96
0.96	0.59	1.62
4.11	0.47	8.74
1.43	0.59	2.43
2.18	0.57	3.81
3.58	0.56	6.34
2.25	0.72	3.11
3.83	0.74	5.19
1.97	0.16	12.70
2.00	0.39	5.15
1.15	0.77	1.50
3.52	0.19	18.85
1.70	0.79	2.15
2.23	0.46	4.85
0.85	0.59	1.45
0.75	0.33	2.26
2.84	0.74	3.86
0.47	0.50	0.93
0.65	0.52	1.24
2.11	0.56	3.78
16.42	0.47	35.07
5.78	0.32	18.04
2.10	0.54	3.88
1.22	0.73	1.67
1.67	0.35	4.83
4.57	0.52	8.83
1.00	0.31	3.22
2.67	0.34	7.95
12.23	0.79	15.44
2.25	1.05	2.13
2.28	0.36	6.31
4.23	0.43	9.82
1.06	0.84	1.26
3.85	0.29	13.12
3.47	0.88	3.93
5.06	0.60	8.48
0.88	0.44	1.98
1.61	0.44	3.67
2.98	0.31	9.58
4.60	0.42	11.07
4.25	0.46	9.26
0.63	0.26	2.40
2.19	0.56	3.88
3.87	1.21	3.21
3.02	1.23	2.46
4.92	2.22	2.22

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
3.53	0.38	9.36
9.92	1.39	7.13
3.25	1.07	3.03
4.85	0.46	10.54
1.85	0.40	4.60
1.30	0.75	1.73
1.42	0.44	3.21
1.90	1.17	1.63
2.77	0.53	5.27
1.49	0.88	1.68
12.23	0.65	18.76
39.19	0.69	56.61
7.03	0.63	11.15
5.93	0.81	7.34
5.10	0.94	5.40
2.18	0.70	3.09
4.15	0.68	6.08
3.70	0.57	6.48
7.20	1.16	6.22
1.21	1.05	1.16
4.72	0.51	9.27
2.92	1.27	2.30
1.29	0.91	1.41
2.26	1.06	2.12
1.23	0.47	2.64
30.45	0.51	59.94
1.43	0.77	1.85
1.10	0.84	1.31
3.97	0.62	6.41
2.54	0.58	4.35
9.81	1.02	9.59
6.60	0.58	11.37
2.58	0.38	6.77
8.29	0.27	30.72
1.04	0.62	1.69
8.61	0.98	8.78
3.83	0.75	5.12
3.62	0.87	4.18
2.80	0.75	3.72
1.51	0.79	1.91
12.05	0.60	20.13
4.94	0.40	12.33
1.00	0.48	2.10
0.55	0.68	0.81
2.26	0.35	6.51
2.22	0.91	2.43
2.48	0.73	3.39
5.86	0.51	11.52
0.88	0.26	3.36
2.36	0.56	4.24
2.39	0.58	4.15
0.51	0.41	1.25

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
0.59	0.29	2.05
2.40	0.44	5.50
1.48	0.72	2.06
1.32	0.67	1.97
1.85	0.51	3.61
1.46	1.16	1.26
13.10	0.47	27.66
3.05	0.37	8.23
3.13	0.44	7.16
4.20	0.28	14.83
1.02	0.54	1.89
0.70	0.30	2.37
4.09	0.23	17.51
8.07	0.36	22.63
2.21	0.22	9.84
0.72	0.35	2.05
4.02	0.35	11.40
1.05	0.43	2.45
1.51	0.25	5.98
12.05	0.92	13.14
0.57	0.56	1.03
5.11	0.56	9.14
1.56	0.39	3.97
2.47	0.31	7.96
0.69	0.56	1.22
14.39	0.77	18.75
6.84	1.01	6.77
3.51	0.53	6.62
3.05	0.48	6.31
1.29	0.35	3.66
8.95	0.72	12.38
6.04	0.77	7.86
1.95	0.69	2.82

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
7.85	0.57	13.87
3.39	0.39	8.70
3.39	0.59	5.71
1.95	0.34	5.66
0.75	0.16	4.58
2.07	0.35	5.92
0.58	0.24	2.40
1.37	0.23	5.89
4.23	0.66	6.42
1.12	0.67	1.69
1.95	0.41	4.78
2.74	0.33	8.33
4.82	0.27	17.71
2.60	0.81	3.21
4.35	0.67	6.46
1.22	0.29	4.22
1.83	0.27	6.72
2.22	0.31	7.14
0.31	0.26	1.22
1.30	0.37	3.47
1.58	0.28	5.63
11.31	0.71	15.91
0.55	0.29	1.88
6.44	0.33	19.73
0.84	0.43	1.95
6.16	0.64	9.66
3.79	0.33	11.37
0.39	0.37	1.05
1.93	0.81	2.37
5.16	0.37	13.98
2.51	0.26	9.47
2.53	0.52	4.85
2.75	0.37	7.35
3.94	0.45	8.79
4.96	0.54	9.16
2.61	0.32	8.23
2.98	0.34	8.63
4.64	0.21	21.68
1.70	0.70	2.43
0.98	0.36	2.73
0.71	0.38	1.86
4.59	0.36	12.91
0.94	0.34	2.75
2.11	0.85	2.47
1.56	0.44	3.53
2.39	0.07	36.00
2.33	0.28	8.29
1.42	0.23	6.09
1.91	0.22	8.80
1.73	0.56	3.10
0.92	1.14	0.80
0.64	0.22	2.87

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
4.50	0.64	7.05
1.34	0.43	3.14
5.07	0.73	6.96
1.61	0.38	4.22
0.77	0.33	2.34
0.46	0.27	1.71
1.96	0.50	3.93
3.35	0.19	17.45
3.47	0.47	7.37
2.34	0.37	6.27
2.97	0.60	4.92
2.05	0.30	6.83
2.25	0.75	3.02
17.41	0.29	59.89
1.44	0.68	2.10
1.12	0.58	1.93
3.97	0.52	7.71
0.84	0.50	1.69
3.59	0.36	9.91
6.81	0.20	33.90
0.74	0.70	1.06
1.68	0.76	2.23
2.79	0.29	9.64
1.08	0.44	2.43
1.26	0.31	4.08
1.04	0.58	1.79
2.33	0.46	5.07
2.01	0.53	3.78
3.64	0.81	4.52
2.22	0.53	4.17
4.65	0.78	5.97
0.75	0.49	1.54
1.73	0.75	2.30
5.31	0.21	25.37
1.99	0.85	2.33
1.45	0.75	1.95
0.79	0.62	1.27
1.64	0.54	3.01
0.94	0.21	4.54
1.99	0.49	4.09
2.64	0.31	8.55
0.72	0.23	3.13
1.77	0.59	2.97
5.69	0.57	9.96
1.18	0.74	1.58
0.58	0.20	2.94
0.49	0.50	0.96
2.11	1.07	1.98
0.66	0.43	1.54
9.14	0.97	9.45
3.33	0.65	5.10
0.63	0.59	1.07

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
0.52	0.36	1.44
5.27	0.76	6.94
3.31	0.73	4.52
1.50	0.53	2.85
1.98	0.45	4.42
2.12	0.19	11.04
5.72	0.56	10.30
1.36	0.37	3.63
1.23	0.26	4.74
1.40	0.35	4.00
2.15	0.63	3.42
0.91	0.54	1.69
2.45	0.39	6.21
3.28	0.78	4.20
5.02	0.73	6.90
0.87	0.41	2.13
1.73	1.07	1.61
1.84	0.59	3.13
4.67	0.39	12.04
3.91	0.33	11.70
1.54	0.46	3.31
2.08	0.50	4.16
0.37	0.43	0.86
1.54	0.40	3.81
1.99	0.70	2.83
0.41	0.39	1.06
0.92	0.71	1.31
0.69	0.80	0.86
3.64	0.90	4.05
1.55	0.59	2.64
0.73	0.33	2.21
3.98	0.69	5.78
5.88	0.66	8.97
4.74	0.50	9.57
7.31	0.91	8.02
16.94	1.06	16.03
0.73	0.86	0.85
0.58	0.23	2.50
7.58	0.62	12.14
6.75	0.69	9.84
3.18	0.62	5.11
5.55	0.47	11.78
6.02	1.06	5.69
27.56	0.76	36.38
3.51	0.29	12.28
2.08	0.46	4.47
5.42	0.57	9.46
6.61	0.69	9.57
9.53	0.76	12.50
10.50	0.49	21.63
2.36	0.63	3.77
7.08	0.74	9.56

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
5.53	0.64	8.64
2.98	0.65	4.60
5.14	0.52	9.88
8.20	0.59	13.82
1.83	0.53	3.48
4.75	0.36	13.14
1.45	0.38	3.79
8.03	0.45	17.79
6.05	0.75	8.04
11.19	0.78	14.41
1.29	0.58	2.24
0.74	0.52	1.45
3.76	0.72	5.19
11.04	0.51	21.74
0.89	0.32	2.82
0.50	0.18	2.83
0.82	0.51	1.61
1.77	0.52	3.40
3.89	0.67	5.77
1.34	0.39	3.47
11.29	0.37	30.46
8.16	0.51	16.15
1.68	0.66	2.53
4.19	0.33	12.62
2.09	0.68	3.09
5.40	0.16	33.22
15.66	0.53	29.35
1.73	0.49	3.51
0.78	0.44	1.80
4.66	0.72	6.49
1.13	0.41	2.76
1.52	1.20	1.27

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
1.58	0.49	3.22
5.23	0.77	6.81
0.98	0.69	1.42
10.24	0.16	62.21
3.00	0.70	4.27
0.75	0.45	1.67
0.56	0.41	1.38
1.56	0.91	1.71
5.85	0.36	16.11
3.18	1.38	2.31
0.59	0.31	1.92
2.08	0.69	3.03
1.34	0.86	1.56
5.15	0.17	30.09
3.67	0.77	4.76
7.25	1.24	5.85
0.95	0.62	1.54
1.01	1.08	0.93
2.38	0.55	4.35
1.00	0.60	1.67
1.38	0.81	1.71
0.96	0.17	5.51
2.11	0.78	2.70
4.33	0.99	4.37
1.53	0.33	4.59
24.44	0.71	34.46
10.37	0.54	19.11
2.22	0.36	6.16
2.76	0.64	4.33
5.97	0.22	27.41
16.70	0.66	25.42
1.03	0.57	1.81
13.03	0.71	18.32
6.73	1.78	3.77
16.38	2.19	7.47
1.67	0.84	2.00
0.91	0.55	1.64
0.97	0.50	1.92
0.88	0.52	1.68
4.77	0.58	8.22
6.93	0.94	7.35
8.12	0.35	22.98
0.64	0.63	1.03
17.09	0.91	18.80
0.93	1.16	0.80
6.93	0.61	11.40
2.38	0.77	3.10
1.48	0.52	2.87
1.00	0.68	1.47
1.96	1.17	1.68
3.94	0.51	7.75
1.75	0.61	2.84

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
1.60	0.48	3.32
3.08	0.48	6.45
9.41	0.42	22.29
2.04	1.26	1.61
1.01	0.42	2.41
3.40	0.32	10.64
1.17	0.28	4.12
5.27	0.54	9.75
2.60	0.56	4.68
2.12	0.49	4.36
1.68	0.18	9.18
4.89	0.97	5.05
2.05	0.47	4.34
3.47	0.57	6.11
7.60	0.59	12.86
3.74	1.07	3.51
12.32	0.33	37.36
1.27	1.03	1.23
0.92	0.51	1.80
1.03	1.16	0.89
12.32	0.72	17.09
3.45	0.33	10.31
1.75	0.64	2.75
6.05	1.23	4.92
2.15	0.50	4.31
2.56	0.41	6.21
2.69	1.51	1.78
10.86	1.00	10.88
5.20	0.15	35.19
2.74	0.33	8.18
1.84	0.43	4.30
3.16	0.63	5.02
8.54	2.15	3.97
2.74	0.66	4.18
4.97	0.89	5.61
1.17	1.13	1.04
10.06	0.68	14.89
0.65	0.43	1.50
3.44	0.73	4.74
2.95	0.36	8.14
3.51	0.88	3.99
1.45	0.69	2.12
1.43	0.44	3.24
1.16	0.48	2.41
0.78	0.39	1.97
3.12	0.84	3.70
0.71	0.32	2.24
0.99	0.56	1.76
2.76	1.40	1.97
2.11	0.51	4.12
2.23	0.65	3.45
4.86	0.40	12.28

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
3.15	0.35	9.11
1.53	0.39	3.98
0.70	0.39	1.81
4.05	1.32	3.06
19.66	0.81	24.29
16.23	0.83	19.61
2.85	0.63	4.53
3.07	0.60	5.12
3.48	0.36	9.64
1.75	0.49	3.59
22.49	0.65	34.82
7.96	0.71	11.24
3.59	0.45	7.93
4.20	0.66	6.40
9.98	0.71	14.11
4.38	0.47	9.34
6.79	0.49	13.84
3.71	1.73	2.14
8.12	0.68	12.02
4.23	0.49	8.63
6.92	0.63	10.94
1.67	0.60	2.80
0.49	0.49	0.99
0.63	0.31	2.01
1.92	0.37	5.26
3.79	0.61	6.24
1.14	0.17	6.56
2.85	0.70	4.06
0.96	0.65	1.47
0.95	0.59	1.62
1.28	0.24	5.43
2.93	0.50	5.90
1.35	0.85	1.58
1.40	0.48	2.90
1.94	0.46	4.27
1.68	0.32	5.33
4.28	0.45	9.43
1.57	0.41	3.80
0.84	0.44	1.92
1.88	0.65	2.89
3.39	0.82	4.12
1.44	0.45	3.20
1.74	0.45	3.87
2.80	0.31	8.90
1.58	0.49	3.20
1.38	0.44	3.11
1.39	0.54	2.59
1.26	1.13	1.12
0.89	0.19	4.66
1.02	0.88	1.16
3.69	0.55	6.74
1.02	0.37	2.78

Raw Data Listing of Fiber Dimensions (um)

Length (um)	Width (um)	Aspect Ratio
0.99	0.19	5.16
0.77	0.47	1.64
1.32	0.54	2.43
0.69	0.65	1.06
3.43	0.40	8.48
5.11	0.59	8.65
3.19	0.39	8.09
9.16	0.11	82.69
10.12	0.62	16.42
0.74	0.46	1.60
1.59	0.26	6.21
2.26	0.70	3.25
1.99	0.70	2.83
2.03	0.69	2.95
2.39	0.85	2.82
2.22	0.73	3.06
0.67	0.59	1.13
5.55	0.57	9.66
3.85	0.63	6.14
1.44	0.56	2.55
13.80	1.14	12.16
0.65	0.43	1.50
6.02	0.72	8.36
2.26	0.80	2.80
3.12	0.72	4.33
10.09	1.10	9.15
5.67	0.37	15.42
3.28	0.79	4.15
3.23	0.91	3.54
6.33	0.79	8.05
1.78	0.59	3.04
1.13	0.54	2.09
2.07	0.60	3.43
2.96	0.70	4.22
9.79	1.14	8.61
7.76	0.74	10.42
1.74	0.20	8.72
6.17	0.76	8.11
10.06	0.31	32.19
6.35	0.40	15.75
4.72	0.36	13.02
8.27	0.69	11.97
2.14	0.43	4.93
3.79	0.86	4.40
14.61	0.73	20.07
5.02	1.53	3.28

FINAL REPORT

(Battelle Study Number SC920040)

on

**REPEATED-EXPOSURE INHALATION STUDY OF
A FIBROUS AEROSOL IN RATS**

to

Otsuka Chemical Company

March, 1995

by

**Michael E. Placke, MaryEllen Lynch,
Michael J. Brooker, and Allen W. Singer**

**BATTELLE
505 King Avenue
Columbus, Ohio 43201-2693**

This study was conducted in compliance with EPA's GLP regulations 40 CFR, Part 792. This study was conducted according to the study protocol and Battelle's Standard Operating Procedures. The data presented accurately reflect the results of the study.

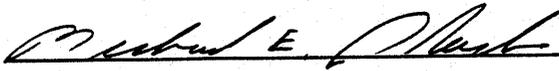

Michael E. Placke, Ph.D., D.A.B.T.
Study Director

FINAL REPORT

on

REPEATED-EXPOSURE INHALATION STUDY OF
A FIBROUS AEROSOL IN RATS

March, 1995



Michael E. Placke, Ph.D., D.A.B.T.
Study Director

3/30/95

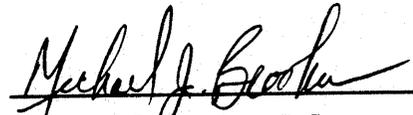
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March 30, 1995

Date



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3-30-95

Date

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to the study director and management as follows:

<u>Phase Inspected</u>	<u>Date Inspected/ Reported to Study Director</u>	<u>Date of Report to Management</u>
Body weights for randomization	8/13/92	8/28/92
Audit: Pre-exposure Data	8/13/92	9/21/92
Randomization/Identification	8/13-14/92	12/29/92
Exposure-Day 1, filter weights	8/19/92	8/28/92
Chamber sampling for mass concentration	8/20/92	8/28/92
Necropsy	8/31/92	12/30/92
Audit: Study file	9/18/92	12/28/92
Audit: Study file, draft report	12/29/92	12/30/92
Audit: Final Report	11/22/94	1/26/95


 Kathleen E. Reed 3-29-95
 Quality Assurance Unit Date
 Health Division

Battelle
 505 King Avenue
 Columbus, OH 43201-2693

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SUMMARY

The objective of this study was to evaluate the potential toxicity resulting from 10 days of repeated inhalation exposure to fibrous aerosols of the test article (potassium octatitanate fiber, trade name Tismo®) and to select concentrations for longer-term subchronic exposure studies. For this study, 30 male and 30 female Fisher 344 rats were exposed by whole-body inhalation to one of five aerosol concentrations of the test article or to air filters. Animals were exposed for 6 hours/day, 5 days/week, for a total of 10 exposures conducted within 14 days. Animals were necropsied the day following their last exposure. Twice daily clinical observations, body weight values, gross and microscopic observation and organ weight measurements were used to assess the toxic potential of exposure.

Results of the pre-exposure and in-life exposure monitoring indicated that the generation system performed as designed. There were no significant differences in fiber size distribution between the distribution plenum or any exposure chambers. Therefore, there was no aerosol concentration related difference in fiber size distribution between the chambers.

There were no deaths in this study. The most frequent abnormal clinical sign was thin appearance seen in all male and female animals in the high concentration (1,000 mg/m³) group beginning Day 5 and persisting until necropsy. The largest reduction in body weight gain occurred in animals exposed to the high concentration (1,000 mg/m³). Male and female rats exposed to 330 mg/m³ gained weight throughout the study but the rate of increase was significantly less than the air control animals. Animals in the 10 mg/m³, 30 mg/m³ and 100 mg/m³ exposure groups gained weight throughout the study at a rate similar to the air control group.

Animals in the 1,000 and 330 mg/m³ exposure groups showed significant increases, relative to controls, in all lung weight parameters. In addition, the kidney, liver and an occasional other organ in the 1,000 mg/m³ exposure group were significantly decreased in weight compared to the air control group. Also, the adrenal-to-body weight ratio was increased in animals exposed to 1,000 mg/m³.

Exposure-related macroscopic anatomical changes were observed in the lungs of all rats exposed to 1,000 mg/m³ and in one 330 mg/m³ male rat. The changes consisted of a pale discoloration of the normally pink surface. The pattern of discoloration was either diffuse (uniform) or patchy in distribution. The discoloration was considered to be due to the presence of large numbers of alveolar macrophages engorged with particulate fibers (based on microscopic evaluations).

There was evidence of test article exposure in all concentration groups. Microscopically there were increasing amounts of free fiber in lung tissue and fiber laden pulmonary alveolar macrophages within the lungs of animals associated with exposure to increasing concentrations. The number of fibers and total lung burden (based on microscopic evaluation) increased proportionately with increasing aerosol concentrations. The mere presence of these fibers and pulmonary macrophage response in the lower concentration groups was not necessarily considered a toxic response, but rather represented normal lung depression patterns and clearance mechanisms for inorganic materials. However, there was evidence in the form of acute/chronic inflammation, supporting toxic responses in rats exposed to 330 and 1,000 mg/m³ aerosol concentrations.

The histopathology of the lungs in the high concentration group indicated the animals were severely compromised. In addition to the proportional increase in free fibers and fiber laden pulmonary macrophages, there were additional inflammatory changes throughout the upper and lower airways of the respiratory tract of these animals. Many of the terminal bronchi were blocked by aggregations of organizing macrophages and proliferative inflammatory changes. These changes were often accompanied by mucinous exudates and organized granulomas that obstructed many of the airways. It appeared that if this lesion progressed, it would likely be life threatening. Similar acute inflammatory changes also ascended up the respiratory tract along the bronchi and extended into the nasal cavities, where an exudated rhinitis was observed in most animals.

Based on these findings, under the conditions used in this two-week study, a no-adverse-effect level was estimated to be approximately 30 mg/m³ for male rats (due to relative increases in lung:brain weight values) and 100 mg/m³ for female rats. Animals exposed to both higher concentrations of 330 and 1,000 mg/m³ showed concentration-dependent respiratory tract changes. In the case of the higher-exposure group, secondary-related systemic changes. Since fibers were readily observable in animals in even the lowest exposure group, albeit this was considered a pulmonary alveolar macrophage response, there was no absolute no-effect level determined in this study.

Based on the results of this range-finding study, it would be recommended that for future studies fiber aerosol concentrations not exceed a range of between 100 and 300 mg/m³ and concentrations less than 10 mg/m³ also be included in the study design. Since the test material is an inorganic based fiber and is not readily cleared from the respiratory tract, it is likely that significant lung burdens will occur even at lower concentrations with increasing duration of exposure. Future term studies should be designed to identify the concentration at which normal clearance mechanisms equal the deposition rate resulting from the inhalation of new fibers with repeated daily exposures.

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FINAL REPORT

(Battelle Study Number SC920040)

on the

Repeated-Exposure Inhalation Study of
A Fibrous Aerosol in Rats

to

Otsuka Chemical Company

by

Michael E. Placke, MaryEllen Lynch,
Michael J. Brooker, and Allen W. Singer

1.0 INTRODUCTION

The objective of this study was to evaluate the potential toxicity resulting from repeated inhalation of fibrous aerosols of the test article (6 hours/day, 5 days/week, for a total of 10 exposures conducted within 14 days). The study was also conducted to select concentrations for a subsequent 91-day subchronic inhalation toxicity study. The test article used in this study was potassium octatitanate fibers, trade name Tismo®. Otsuka Chemical Company was the Sponsor of the study. The study was conducted in conformance with the Environmental Protection Agency (TSCA) Guidelines for Toxicity of Chemicals (1990) and the study was listed on Battelle's Master Study Schedule. The Study Protocol and amendments to the protocol are contained in Appendix A.

The study was conducted at Battelle Columbus Operations under the direction of Dr. Michael E. Placke. The in-life portion of the study began on August 19, 1992 and ended on August 31, 1992. This study was initiated on May 20, 1992 with the signing of the protocol and completed on March 30, 1995 with signing of the final report.

2.0 EXPERIMENTAL DESIGN

For this inhalation toxicity study of Tismo® (Potassium Octatitanate), 30 male and 30 female Fischer 344 rats were used. Five aerosol concentration levels of the test article and an air control group were exposed by whole body inhalation as shown below. Animals were exposed for 6 hours/day, 5 days/week, for a total of 10 exposures conducted within 14 days. Animals were necropsied the day following their last exposure.

Group Number	Fiber Aerosol Concentrations (mg/m ³)	Male Animal Numbers	Female Animal Numbers
1	Air Control, 0	101-105	106-110
2	10	201-205	206-210
3	30	301-305	306-310
4	100	401-405	406-410
5	330	501-505	506-510
6	1000	601-605	606-610

The parameters used to assess toxicity during the in-life portion of the study included clinical observations (twice daily) and body weight measurements (Days 1, 5, 8 and at necropsy). Post-mortem studies included a complete necropsy with macroscopic tissue evaluations and organ weight measurements. Histopathologic assessments were performed on the entire respiratory tract of all study animals.

3.0 MATERIALS AND METHODS

3.1 Test and Control Articles

The test article used for this study was Potassium Octatitanate, trade name, Tismo®, Lot #1F81L. The test article was supplied by the Sponsor and was received at Battelle on June 26, 1991. Upon receipt, the test article was assigned Battelle Substance Control Number 873 and was stored at ambient room temperature. The test article had no stated expiration date.

The control article in this study was filtered, conditioned room air.

3.2 Test Article Identity, Purity, and Stability Analysis

The test article identity, purity, stability, and methods of synthesis were the responsibility of the Sponsor.

3.3 Experimental Animals

A total of 60 Fischer 344 rats (30 males and 30 females) were required for this study. A sufficient number of animals were obtained from Charles River's Kingston Laboratory, to provide the required number of healthy animals for testing. Animals were 4 to 6 weeks old at the time of receipt and no older than 6 to 8 weeks at the initiation of exposure. The rats were housed in Room 7C-401 during the quarantine and study periods. The rat was chosen as the test system because this species has been accepted for toxicity studies to assess the safety of chemicals to which humans may be exposed.

3.3.1 Animal Receipt and Quarantine

The animals were received at Battelle on August 4, 1992, approximately 15 days prior to the initiation of exposure. During the quarantine period, the rats were acclimated to the animal room and exposure chamber environmental conditions that were used for the study. Each animal was observed daily during quarantine for clinical signs of abnormality that would make it unfit for study. Healthy animals were released at the conclusion of quarantine following examination by the staff veterinarian.

At the time of receipt, sera were randomly collected from 5 rats/sex for evaluation of selected antibody titers. These animals were not used on study. Samples of sera were sent to and analyzed by Microbiological Associates, Bethesda, Maryland for measurement of antibody titers to Sendai (Send), Pneumonia Virus of Mice (PVM), Rat Coronavirus/Sialodacryoadenitis Virus (RCV/SDA), Kilham Rat Virus (KRV), and *Mycoplasma pulmonis* (MPUL). None of the sera samples tested had significant titers to the infectious agents listed above.

3.3.2 Animal Housing and Environmental Conditions

All animals were individually housed in stainless steel, wire-bottom cages within the inhalation exposure chambers. All housing and care practices conformed to the requirements stated in the NIH "Guide for Care and Use of Laboratory Animals", National Institutes of Health Publication No. 86-23.

The environmental conditions of the animal study rooms conformed to the following: (1) the light/dark cycle was held at ~ 12 hours of light and dark each day during the study using fluorescent lighting, starting at ~ 6:00 a.m. each day, (2) the room temperature and relative humidity controls were set to 67 to 77°F and 40 ± 70 percent, respectively, and were monitored twice daily for conformance, and (3) fresh air was supplied to the room at a rate providing a minimum of ten changes of room air per hour. All animals were fed, *ad libitum*, certified Purina Rodent Chow in pelleted form (except during exposure). Contaminant analysis of each feed lot was supplied by the vendor and each analysis is maintained by the Battelle's Animal Resources Facility. Water was provided *ad libitum* (during exposure) via an automatic watering system monitored daily. The water source was the municipal city supply from the City of Columbus which conforms with the EPA water standards. There were no reported or known food or water contaminants that would interfere with the purpose or outcome of the study.

3.4 Animal Randomization and Identification

Animals were randomly assigned to each study group on Prestudy Day -5 by sex and body weight using a computer software program (Xybion Medical Systems®, Version 3.1) that employed an algorithm for selection of body weight, which provided for homogenous group mean body weights. During quarantine, animals were assigned prestudy cage numbers that were used to temporarily

identify the animals and record body weight data. Following randomization, the animals were identified by tail tattoo and cage card. A cross-reference file of prestudy and study identification numbers was maintained in the computer data base and study files. Animal numbers are listed in Section 2.0.

3.5 Clinical Observations

The animals were observed twice daily during the quarantine period and study period for mortality/moribundity (once in the morning and once in the afternoon). All animals were carefully examined twice daily for clinical signs of toxicity (in the morning and afternoon, at least 6 hours apart) during the 14-day exposure period. Any clinical evidence of toxicity was recorded in the Xybion® System.

3.6 Body Weight

Individual rat body weights were recorded once pretest (at randomization), on Day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, 1000.

3.7 Necropsy

All rats were necropsied the day following their final exposure. All rats were weighed prior to necropsy and were killed humanely by exsanguination after administration of pentobarbital anesthesia.

Each necropsy included examination of the external surface of the body; all orifices; liver, kidney, brain and heart; special attention given to the lungs and upper respiratory tract; collection of all tissues listed below.

The following tissues along with any identified gross lesions were preserved in 10 percent neutral buffered formalin solution, except eyes and testes, which were preserved in Bouin's solution. After the organ weight was determined, lungs were perfused with 10 percent formalin using a perfusion filling (25 cm hydrostatic pressure) device. The trachea was ligated after infusion to ensure penetration of fixative in airways and alveoli.

Adrenals
All gross lesions
Animal Identification
Aorta
Brain
Cecum
Colon
Duodenum
Esophagus
Eyes
Heart
Ileum
Jejunum
Kidneys
Liver
Lungs
Nasopharyngeal tissue

Ovaries
Pancreas
Peripheral Nerve
Pituitary
Rectum
Representative Lymph Node
Salivary glands
Spleen
Sternum/with Bone Marrow
Stomach
Testes
Thymus
Thyroid/Parathyroid
Trachea
Urinary Bladder
Uterus
Accessory genital organs (prostate, epididymis
and seminal vesicle, if present).

3.8 Organ Weight

Selected organs were weighed for all animals surviving to scheduled necropsy. Each of the following organs were removed, trimmed of extraneous tissue, and weighed:

Lungs	Adrenals
Liver	Brain
Kidneys	Testes or Ovaries

3.9 Histopathology

The tissues listed below were embedded in paraffin, sectioned at approximately 5 μ m, stained with hematoxylin and eosin (H&E), and submitted for light microscopic examination.

Lungs	Trachea (cross and longitudinal sections)
Nasal Cavity (four sections*)	Tracheobronchial lymph nodes
Nasopharynx	Thymic lymph nodes
Larynx (2 cross-sections)	Gross lesions

* The nasal cavity was prepared in four sections using the landmarks described by Young (Fundam. Appl. Toxicol., 1:309-312, 1981).

3.10 Data Management and Statistical Analysis

In-life and postmortem animal data were collected using the Xybion® Path/Tox Data System (Version 3.1), (Xybion Medical Systems Corporation, Cedar Knolls, NJ). This computer data capture system recorded body weights, clinical observations, and organ weights on-line with Battelle's VAX Computer System. Data collected off-line (i.e. gross necropsy observations and histopathologic findings) were key-entered into appropriate Xybion modules and reviewed for accuracy of transcription.

All quantitative in-life and postmortem pathology data were statistically analyzed. Normally distributed data (parametric) were analyzed for treatment effects by analysis of variance and pairwise comparisons made between groups using Dunnett's multiple-range t-test. Nonparametric data were analyzed by the Kruskal-Wallis Test and by the Mann-Whitney U Test for pairwise group comparisons.

3.11 Exposure System

3.11.1 Test Article Aerosol Generation and Delivery System

The purpose of the aerosol generation system was to provide a uniform concentration of fibrous aerosol, representative of the bulk test article, to the breathing zone of the animals. The test material aerosol generator was designed as a two-part system; the first was a mechanism to feed bulk test material at a constant rate into an aerosol generator, and the second was a high-energy dispersion device to aerosolize the test material and entrain the aerosol in a delivery airstream. The first component of the generation system was an Accurate Model 300 Dry Chemical Feeder (Accurate, Inc., Whitewater, WI) which accurately delivered preset amounts of potassium octatitanate into the aerosol generator. The Accurate feeder employed a large capacity hopper with an auger type feed screw. This feeder directed a continuous stream of test compound past the inlet of a Fox, one inch, Coaxial Eductor Valve (Fox Valve Development Corp., Dover, NJ), which aspirated the material, entrained it into a high pressure stream of air, then introduced it into a 1 m³ volume plenum chamber.

The plenum chamber was supplied with Hepa/Charcoal filtered carrier air and the aerosol was allowed to mix inside the plenum chamber. Additional carrier air entered the plenum chamber near the bottom of the chamber and the air flow pattern was from the bottom to the top. This allowed any agglomerates that formed to settle out of the airstream at the bottom of the plenum rather than being entrained in the air going to the delivery manifold.

From the plenum, the test atmosphere was ducted to the exposure chambers through stainless steel delivery manifold. The test article aerosol was introduced into the manifold and mixed with the manifold air to yield an aerosol concentration of $\sim 1000 \text{ mg/m}^3$. At the lower concentration exposure chambers, calibrated flowmeters removed appropriate aliquots of the manifold aerosol which was further mixed with HEPA filtered room air to obtain the proper chamber concentrations. The 1000 mg/m^3 chamber received manifold aerosol directly with no dilution. The air control chamber received HEPA filtered room air only.

The chambers used for this study were Hazleton H1000 and H2000 stainless steel and glass exposure chambers. The number of animals required for this study was such that all animals were housed on one level within each chamber. A schematic diagram of the entire system is included in Figure 1.

3.11.2 Exposure Atmosphere Concentration Analysis

Exposure system mass aerosol concentrations were measured by gravimetric filter analysis. Gelman, 25mm, glass fiber filters (Gelman Sciences, Ann Arbor, MI) were placed into open-face filter holders and inserted into exposure ports within the chambers. Air flow rates were regulated, using calibrated critical orifice meters, to sample a known volume of test atmosphere through the filters. Immediately after sampling, the filters were weighed and the mass concentration of the total aerosol was calculated from the accumulated mass and the sample volume.

3.11.3 Fiber Number and Size Analysis

Fiber number and size measurements were conducted during the prestudy validation of the system and again during the animal exposure phase to determine the approximate number of fibers per unit air and length and width of fibers in the exposure atmosphere. Fibers were collected on a Nucleopore filter by drawing test atmosphere through a filter for approximately 30 seconds. The

filters were examined by Scanning Electron Microscopy (SEM). SEM images of the fibrous material were generated and recorded on an automated image analysis system to compute the length, width and aspect ratio of the fiber. A TN-8500 Image Analysis System with a fiber size analysis program was used to perform the measurements. The fiber analysis software package was written and distributed by Noran (formerly Tracor-Northern) to be operated with the TN-8500 Image Analyzer and, more specifically, the Battelle owned TN8502 mainframe, a light optics station, and a JEOL Microprobe Analyzer (EPMA).

Selected samples were analyzed at 1000X and 2000X magnification with corresponding resolutions of 0.085 microns and 0.04 microns, respectively. The 2000X images were taken of the same total area as the 1000X analyses to determine if any smaller fibers existed in the same field examined at the lower magnification.

The fiber number (fibers/cc) was estimated by counting the number of fibers in the field or fields of view and multiplying the ratio of field size to the total collection area of the filter by the total amount of air drawn through the filter.

Fibers were also collected during the prestudy validation phase on double sided tape by inserting the tape into the chamber and allowing the fibers to adhere to the tape. This was done to verify the complete fiber size distribution was captured and represented on the filters.

3.11.4 Chamber Environmental Measurements

An inhalation chamber Environmental Acquisition System (EAS) was designed to measure and record inhalation chamber temperature, humidity, and exhaust flow rates. The EAS consisted of an IBM XT personal computer (PC), coupled with an A/D data acquisition board and associated instrumentation. A QuickBASIC program was written to log chamber environmental data, generate a hard copy of the data logged, and to store data to disk for subsequent statistical analysis. A PC SAS[®] summary program was written to summarize the daily data generated by the EAS.

The PC hardware interface to the EAS consisted of two Model T11 terminal panels and an ACPC-12-16 data acquisition and control board (Strawberry Tree Computers, Sunnyvale, CA).

Temperature in each chamber was monitored using 20-gauge type T thermocouple probes. Each thermocouple probe was located at the top of each chamber approximately 2 feet from the chamber inlet duct. The thermocouple probe exited each chamber at the top, and was connected to a quick disconnect type T thermocouple connector to facilitate easy removal of the probe during

chamber change-out activities. Type T 20-gauge polyvinyl-shielded duplex extension thermocouple wire (Omega Engineering, Stamford, CT) was used to connect the quick-disconnect coupling to the T11 terminal panels. The terminal panels contained isothermal plates and integral cold-junction sensors.

The dew point in each chamber was measured using an Optical Condensation Dew Point Hygrometer (General Eastern, Watertown, MA). The hygrometer output was logged by the EAS as an analog input channel. Sample lines were 0.25 inch O.D. polyethylene, and were installed near the top of each chamber in the vicinity of the thermocouple probes. Each sample line exited the chamber at the top. A Model ESC-10P Electronic Actuated Position Valve (Valco Instrument Co., Houston, TX) was remotely located in the exposure room, and each chamber's humidity sample line was connected to a valve port via 0.25 inch polyethylene tubing. The valve was equipped with a "select" and a "common" port. The common port allowed atmosphere from all chambers to be pulled through the sample lines, thereby allowing a fresh supply of chamber atmosphere in the sample lines at all times. The select port was used to direct a single chamber atmosphere into the inlet of the dew point hygrometer sensing volume. The flows through both the select and common ports were controlled by calibrated flowmeters; a flow rate of approximately 200 cc/min was maintained through the select port and approximately 2 L/min was drawn through the common port.

All chambers were operated at 15 air changes per hour (either 250 or 500 L/min for the Hazelton H1000 and H2000 chambers, respectively), with airflow rates controlled by calibrated orifice plate flowmeters located on each chamber's exhaust. The flowmeter's upstream and downstream pressure ports were connected to an EST-10P Electronic Actuated Position Valve (Valco Instruments Co., Houston, TX) via 0.125 inch ID Tygon® tubing. The valve's upstream and downstream exit ports were connected to a zero to 1.0 inch of water variable reluctance differential pressure transducer. A calibration of each flowmeter's flow rate versus transducer output was performed prior to exposures. The transducer output was logged by the EAS as an analog channel.

3.11.5 Chamber Uniformity

The uniformity of the chamber atmosphere was determined by gravimetric concentration sampling on the plane of each chamber where the animals were exposed. The sampling scheme included a reference to determine temporal uniformity as well as other locations within the plane of

spatial uniformity. The reference location was sampled before, during, and after other uniformity measurements to assess the contribution of within-port variability to the uniformity determinations. The total port variability (TPV) was estimated from the relative standard deviation of analyses from one measurement at each location, and represented the sum of variability between ports (spatial), and the variability within a port (temporal).

The within port variability (WPV) was estimated from the relative standard deviation of the concentration measured at the reference location. This variability included temporal fluctuations as generator output.

The between port variability (BPV) represented the variability associated with spatial variability of test article concentration within the exposure chamber. The BPV was estimated from the total port mean (M_t), total port standard deviation (S_t), and the within port standard deviation (S_w) by applying the following formula:

$$BPV = 100 \cdot (S_t^2 - S_w^2)^{1/2} / M_t$$

Because of random error, aggravated by the small sample size, the estimated WPV was occasionally greater than the estimated TPV. In these cases, the BPV can not be estimated directly. Since the estimate of TPV is more reliable than the estimate of WPV due to its greater sample size, and since BPV, by definition, cannot exceed TPV, BPV is reported to be less than the TPV for those cases where it can not be calculated. The air control chamber was not sampled for atmosphere uniformity.

4.0 RESULTS

4.1 Animal Survival

There were no unscheduled animal deaths in this study.

4.2 Clinical Observation Results

The incidence summary of pertinent abnormal clinical observations are presented in Table 1 (morning, preexposure) and Table 2 (afternoon, postexposure). Individual animal clinical observation records are contained in Appendix B.

The most frequent clinical sign of abnormality was thin appearance seen in all male and female animals in the high concentration group (1000 mg/m³). This sign appeared on Day 5 and continued through necropsy. Rough coat was observed in the high concentration group on Day 12 (afternoon) and was still present at necropsy. Labored respiration was observed in the high concentration males (3 rats) and females (2 rats) on Days 8 and 10, respectively.

All other animals exposed to test article or the air control were clinically normal throughout the study.

4.3 Body Weight Results

A summary of group mean animal body weights are presented in Table 3. Graphs of group mean body weight data are shown in Figures 2 and 3 for the males and females, respectively. Individual animal body weight values are contained in Appendix B.

The body weights of male control rats increased at an expected normal rate during the 13 days of the exposure period (25 percent from Day 1 to Day 13). Male rats in Groups 2 (10 mg/m³) and 3 (30 mg/m³) gained weight throughout the study at a rate similar to the air controls (27 to 28 percent from Day 1 to Day 13). The male rats in Groups 4 (100 mg/m³) and 5 (330 mg/m³) gained weight throughout the study, but the rates of increase were not as great as the air control animals (20 to 21 percent from Day 1 to Day 13). Male rats in Group 6 (1000 mg/m³) showed statistically significant differences ($p \leq 0.05$) in group mean body weight compared to the air controls on

Days 5, 8 and 13. This group of animals had body weight differences of 21, 17 and 29 percent less than air control animals on Days 5, 8 and 13, respectively.

The body weight of female control rats also increased at an expected normal rate during the 13 days of the exposure period (13 percent from Day 1 to Day 13). Female rats in Groups 2 (10 mg/m³), 3 (30 mg/m³) and 4 (100 mg/m³) gained weight throughout the study at a rate similar to the air controls (13 percent from Day 1 to Day 13). The female rats in Group 5 (330 mg/m³) gained weight throughout the study, but the rate of increase was not as great as the air control animals (11 percent from Day 1 to Day 13). Female rats in Group 5 (330 mg/m³) also had a statistically significant difference ($p \leq 0.05$) in group mean body weight on Day 13 compared to the air control group (7 percent). Female rats in Group 6 (1000 mg/m³) had statistically significant differences ($p \leq 0.05$) in group mean body weight compared to the air controls on Days 5, 8 and 13. This group of animals had body weight values that were 21, 16 and 28 percent less than air control animals on Days 5, 8 and 13, respectively.

4.4 Necropsy

All rats survived to the scheduled terminal necropsy. Exposure-related changes were observed in the lungs of all Group-6 (1000 mg/m³) rats, and in one Group-5 (330 mg/m³) male rat. These changes consisted of a pale discoloration of the normally pink lung surface. The discoloration was either diffuse (uniform) or patchy in distribution. The discoloration was considered to be due to the presence of large numbers of alveolar macrophages engorged with particulate fibers (based on microscopic evaluations).

The only other macroscopic alteration seen at necropsy was an incidental ovarian cyst, noted in one Group-4 female. Macroscopic tissue changes are summarized in Table 4, with individual animal data listed in Appendix C.

4.5 Organ Weight Results

Group mean absolute organ weight, organ-to-body weight, and organ-to-brain weight values are summarized in Tables 5, 6, and 7, respectively. Individual animal data are included in Appendix C.

There were no statistically significant differences in absolute organ weights, organ-to-body weights or organ-to-brain weight ratios in male and female rats from Groups 2 (10 mg/m³) or 3 (30 mg/m³).

The male rats in Group 4 (100 mg/m³) had significantly higher ($p \leq 0.05$) lung-to-brain weight ratios compared to air controls. No other significant differences were noted in this group.

The male and female rats in Group 5 (330 mg/m³) had significantly higher ($p \leq 0.05$) absolute lung weights, lung-to-body weights and lung-to-brain weight ratios compared to air control rats. Female rats in Group 5 also had a significantly higher ($p \leq 0.05$) brain-to-body weight ratio compared to the air control group. There were no other significant differences in this exposure group.

Male and female rats from Group 6 (1000 mg/m³) had significantly lower ($p \leq 0.05$) absolute organ weight, organ-to-body weight values and organ-to-brain weight ratios for the kidneys and testes compared to the air controls. Absolute liver weight and liver-to-brain weight ratios were significantly lower ($p \leq 0.05$) in male and female rats of this group. Absolute lung weight, lung-to-body weight and lung-to-brain weight ratios were significantly higher ($p \leq 0.05$) in male and female rats of this group. Male and female rats also had significantly higher ($p \leq 0.05$) adrenal-to-body weight ratios compared to the air control values. In addition, the brain-to-body weight ratios for male and female rats were significantly higher ($p \leq 0.05$) compared to the air control values. There were no significant differences in ovary weights compared to air controls.

4.6 Histopathology

The nasal cavity and turbinates, nasopharynx, trachea, larynx, lungs, respiratory lymph nodes (tracheobronchial and thymic/mediastinal) and gross lesions were trimmed from fixed tissues, processed, and examined by light microscopy. Microscopic lesions were graded when appropriate using a semiquantitative scale where 1 = minimal change, 2 = mild, 3 = moderate, and 4 = marked alteration (significant organ dysfunction possible). An incidence summary listing severity of lesions is presented in Table 8; individual animal microscopic lesions are tabulated in Appendix D. Representative photographs of lungs and lymph nodes are presented in Figures 4 through 11.

Lesions related to the fibrous aerosol exposure were found in the lungs of all rats (except the air controls), and in the respiratory lymph nodes and nasal cavity of rats exposed to higher levels of the aerosol, as shown in the table below.

**INCIDENCE OF MICROSCOPIC ALTERATIONS RELATED
TO EXPOSURE TO THE FIBROUS AEROSOL***

Sex	-----Males-----						-----Females-----					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Exposure Level (mg/m ³)	0	10	30	100	330	1000	0	10	30	100	330	1000
No. in Group	5	5	5	5	5	5	5	5	5	5	5	5
Lung:												
No. Examined	5	5	5	5	5	5	5	5	5	5	5	5
Particle-Laden Macrophages	0	5	5	5	5	5	0	5	5	5	5	5
Proliferative Bronchiolitis	0	0	0	0	0	5	0	0	0	0	0	5
Mucinous Exudate	0	0	0	0	0	5	0	0	0	0	0	5
Lymph Node(s):												
No. Examined	4	5	5	5	5	5	4	5	5	4	5	5
Particle-Laden Macrophages	0	0	0	3	5	5	0	0	0	4	5	5
Nasal Cavity:												
No. Examined	5	5	5	5	5	5	5	5	5	5	5	5
Exudate	0	0	0	0	0	5	0	0	0	0	0	4

*See Table 8 for severity code grades.

All rats exposed to the aerosol had fiber particles present within the cytoplasm of alveolar macrophages. In the hematoxylin and eosin-stained lung sections, the fibers appeared as elongated black spicules, approximately 1-10 microns in length. Occasional fiber spicules were seen free in alveoli, small airways, or tracheal or nasal sections; these free fibers were not diagnosed as a lesion. Fibers were also occasionally observed within the interstitial septae and at airway bifurcations. In an attempt to better quantify the macrophage response in the lungs, a more detailed grading scheme was used for this organ. A grade of 0.5 (trace or slight) was used to describe the lungs from the lowest

level exposure group. In these lungs, fiber-laden macrophages were somewhat randomly dispersed throughout the lung parenchyma with about 5-15 macrophages visible per high-power field (400-500X). There was only a slight increase in the presence of macrophages from control rats. Macrophages at this level usually contained only a few fibers each. In the lungs of rats exposed to 30 mg/m³ (graded "1"), a few more alveolar macrophages were present in each high-power field (approximately 10-20 phagocytes), and each macrophage tended to contain more particular material. In addition, the macrophages had a tendency to congregate around terminal airways.

At 100 mg/m³, still more macrophages were present per high-power field (approximately 15-25), with their cytoplasm often packed with fibers. These lung lesions were graded "2" (mildly affected). The lungs of rats exposed to 330 mg/m³ had 20-30 macrophages per high-power field, with aggregates of fiber-filled macrophages collecting near some terminal airways. These lesions were graded "3" or moderately affected.

Rats exposed to the high level (1000 mg/m³) had pulmonary macrophages which were densely packed with particles, and clumping of these cells in bronchioles and terminal airways created a striking black spotting of the lung slide subgrossly. Quantitatively, the severity of this change was coded as a "4" (marked).

Terminal bronchioles in lungs from these high-exposure-level rats were often blocked by luminal aggregates of fiber-laden macrophages or by an apparent proliferative inflammatory reaction to fibers which had been incorporated into the submucosa of the terminal airway. This latter change, diagnosed as "proliferative bronchiolitis", consisted of focal rounded projections of an expanded submucosa/interstitium, due to infiltrates of fiber-laden macrophages, lymphocytes and a few neutrophils. As the terminal airways became marginally obstructed, a mucinous exudate containing some fiber particles and cells was often present, and was separately diagnosed. Both the macrophage aggregates and the focal bronchiolitis could be interpreted to be an early "granulomatous" response; this term was not used, however, due to the paucity of multinucleated giant cells observed in this acute study.

In the respiratory lymph nodes, fiber-laden macrophages were observed at the higher-exposure levels. No significant tissue response was noted to their presence. Due to the minute nature of the bronchial and thymic lymph nodes, not all such nodes were captured on the microscopic slides for examination. In all but three rats, however, at least one of the two nodes was observed microscopically, and since they both drain respiratory tissues, it was not thought to be significant that full recovery of all lymph nodes was not achieved.

Rats from the high-exposure group also had an exudative rhinitis. The exudate consisted of proteinaceous material mixed with varying numbers of fiber-laden macrophages and neutrophils. Since the exudate was indicative of slight neutrophilic infiltration of upper turbinate or nasopharynx mucosa, such infiltrates, when observed, were not diagnosed separately.

Due to the apparent early obstruction of terminal bronchioles in rats exposed to 1000 mg/m^3 as evidenced by mucinous airway exudate (and possibly the nasal exudate), it is probable that rats could not survive continued exposure to this level of particulate for a prolonged period of time.

4.7 Exposure Results

4.7.1 Pre-Study Atmosphere Characterization Results

Chamber uniformity measurements were completed during the prestudy validation of the system. The data show that the between port variability (BPV) was less than 9 percent in all chambers. These data are summarized in Table 9. The prestudy analysis showed a uniform distribution of the atmosphere within the chamber.

Three consecutive six hour trial exposures were completed prior to the start of the study. Fiber aerosol concentrations were generally within 20 percent of target values, with the greatest variance from target at the lowest concentration (10 mg/m^3). Operation of the system was considered stable and suitable to conduct the range-finding animal exposures. The results of these trial exposures are presented in Tables 10, 11, and 12.

4.7.2 Bulk Fiber Size

Bulk fiber size measurements were conducted to assure that the fiber size distribution in the test atmosphere generated was comparable to the size of fibers in the bulk material. The bulk fiber size analysis was conducted at a magnification of 1000X, with an image analysis resolution of 0.085 microns. The mean fiber length in the bulk material was 3.16 microns and the mean width was 0.34 microns. The detailed bulk fiber (lot number 1F81L) length and width measurements are listed in Table 13.

4.7.3 Prestudy Aerosol Fiber Size and Count Analysis

Fibers were collected during the prestudy validation phase to determine the length and width distributions within the chamber atmosphere. Two filter samples were collected within each chamber and a sample was also collected from each chamber on double sided tape. The second filter sample was analyzed at both 1000X and 2000X magnification. The same total area was analyzed with both magnifications to determine if any significant number of submicron fibers were not detected at the 1000X magnification. The mean fiber length for the first filter sample at 1000X magnification was 2.98, 3.15, 2.51, 2.61, and 2.70 μm for the 10, 30, 100, 330, and 1000 mg/m^3 chambers, respectively. The mean fiber width for the first sample at 1000X magnification was 0.32, 0.34, 0.32, 0.32, and 0.38 μm for the 10, 30, 100, 330, and 1000 mg/m^3 chambers respectively. The second sample at 1000X magnification revealed similar lengths and width values. The mean fiber length was 3.01, 3.93, 3.53, 2.92, and 2.47 for the 10, 30, 100, 330, and 1000 mg/m^3 chambers, respectively, while the mean fiber width values ranged from 0.31 to 0.33 microns for the same samples. The second sample analyzed at 2000X over the same area resulted in very similar measurements. The mean length for the 2000X analysis was 1.90, 2.83, 2.53, 2.33, and 2.48 for the 10, 30, 100, 330, and 1000 mg/m^3 chambers, respectively. The mean fiber width values were only slightly smaller than those reported at 1000X magnification. These values ranged from 0.22 to 0.26 microns.

The mean fiber length and width values collected from the double sided tape (analyzed at 1000X) were very similar to the measurements taken from the filters. The mean fiber length for the tape samples was 3.43, 3.70, 4.30, 3.52, and 3.02 for the 10, 30, 100, 330, and 1000 mg/m^3 chambers, respectively. The mean fiber width values for these samples ranged from 0.31 to 0.44 microns. These data are detailed in Table 14.

Fiber count measurements were completed on the same filters and the number of fibers per cubic centimeter was calculated. The mean fiber counts determined from these samples were 17,000, 51,667, 273,667, 467,667, and 569,667 fibers per cubic centimeter for the 10, 30, 100, 330, and 1000 mg/m^3 chambers, respectively. These data are detailed in Table 15.

These pre-study data show that the aerosol generated had similar mean length and width values as the bulk material and that the fiber size distribution was equally distributed across the exposure chambers resulting in similar mean length and width values in all chambers (there was no concentration effect on fiber size distribution). The double sided tape measurements verified that all of the distribution was being collected on the filters and the comparison of two magnifications

revealed that there were few to no fibers in the small submicron size range. The mean fiber counts (fibers per cubic centimeter) values increased with the mass concentration values as would be expected as well, but not proportionally with incremented increases in mass concentration of the high levels. This was likely due to sampling and fiber counting system errors at the high mass concentrations.

4.7.4 Mass Concentration Data

The grand mean (mean of all samples collected) chamber mass concentrations were 10.2, 29.8, 114.1, 342.8, and 934.4 mg/m³ for the 10, 30, 100, 330, and 1000 mg/m³ chambers respectively. These data along with the corresponding relative standard deviations and percent of target values for the individual chambers are listed in Table 16. The daily mean chamber values and corresponding relative standard deviations are listed in Table 17.

4.7.5 Exposure Environmental Conditions

The grand mean chamber temperature, percent relative humidity, and air flow rates are listed in Table 18. All values were within the protocol specified range.

4.7.6 Animal Exposure Aerosol Fiber Size and Count Data

The mean fiber length for the first filter sample at 1000X magnification was 2.90, 3.38, 3.27, 2.57, and 2.91 μm for the 10, 30, 100, 330, and 1000 mg/m³ chambers, respectively. The mean fiber width for the first sample at 1000X magnification was 0.33, 0.47, 0.34, 0.36, and 0.32 μm for the 10, 30, 100, 330, and 1000 mg/m³ chambers, respectively.

The first filter sample analyzed at 2000X with the same total area revealed mean fiber lengths that ranged from 2.05 to 2.49 microns with mean fiber width values ranging from 0.22 to 0.38 microns. The second sample at 1000X magnification revealed similar lengths and width values. The mean fiber length was 2.24, 3.01, 3.43, 2.66, and 2.16 for the 10, 30, 100, 330, and 1000 mg/m³ chambers, respectively, while the mean fiber width values ranged from 0.31 to 0.43 microns for the same samples. These data are detailed in Table 19.

The mean fiber concentration values (fibers per cubic centimeter) collected from the samples are 60,549, 112,792, 340,228, 245,539, and 494,704 f/cc for the 10, 30, 100, 330, and 1000 mg/m³ chambers, respectively. The fiber count measurements are detailed in Table 20. All individual fiber data is listed by sample in Appendix E for the pre-study development as well as the exposure data. Additionally, Figures 12 through 17 graphically present the percent of total fibers by size category for each of the fiber concentration levels and the bulk material.

These results again, as with the pre-study results, show that a complete accounting of all fibers occurred at the 1000X magnification and that the fiber distribution was representative of the bulk material and equally distributed within all chambers.

5.0 DISCUSSION AND CONCLUSIONS

There were several objectives established for this study which included evaluating the performance of a multi-concentration aerosol exposure system and also to assess the biological effects in rats following inhalation exposure to fibrous aerosols of the test material, in order to characterize the potential toxicity of the inhaled compound and to select concentrations for longer-term definitive exposure studies.

The inhalation generation and delivery system was designed to deliver, to the breathing zone of the rats, aerosols of fibers generated from the bulk material, which had a size distribution roughly equivalent to that of the bulk material. The system was also configured to deliver mass aerosol concentrations over at least two orders of magnitude to multiple animal exposure chambers. The generation component was designed not to cause any physical or chemical alterations to the bulk fibers, rather only disperse the fibers into a large volume of air and then entrain those fibers into a delivery air stream which would carry the aerosol to the animal exposure chambers. Therefore, any significant changes in fiber size distribution, would likely be the result of sedimentation of larger fibers and not due to shearing, tearing, or other physical modifications of the fibers caused by the system.

The results of the preexposure and in-life exposure monitoring suggested that the generation system performed as designed. There were no recognizable differences in the fiber size distribution between the bulk material, the aerosol in the distribution plenum, or the test atmospheres in any of the five different concentration exposure chambers. These results suggested that virtually all of the material in the bulk test fiber was easily entrained and readily transported, as an aerosol, through the delivery and exposure system. Further, any sedimentation losses that occurred appeared to be distributed consistently over all fiber size distributions. Therefore, there were no measurable differences in fiber size between the distribution plenum or any of the exposure chambers. Therefore, there was no concentration related difference in fiber size among the chambers. Under these generation conditions, a concentration of approximately $1,000 \text{ mg/m}^3$ was considered the highest reasonable achievable value. This concentration was also considered (based on general experience of inhalation toxicity and aerosol generation of other fibers and inorganic dusts) to be a suitably high concentration value for the purposes of evaluating target organ toxicity.

There was biological evidence of test article exposure in all concentration groups. Microscopically there were increasing amounts of free fiber in lung tissue and fiber laden pulmonary

alveolar macrophages within the lungs of animals exposed to each of the different concentrations. The number of fibers and total lung burden (evaluated qualitatively) increased proportionately with increasing concentrations. The mere presence of these fibers in the lower concentration groups was not necessarily considered a toxic response, but rather represented normal lung clearance mechanisms for inorganic materials. However, there was evidence supporting characteristic toxic responses at the 330 and 1,000 mg/m³ concentrations.

At the 330 mg/m³ exposure level, male rats had a slightly slower rate of body weight gain than the control animals and other lower exposure groups. In addition, the lung weight parameters in both male and female animals in this group showed significant increases relative to controls suggesting that lung fiber burdens and associated inflammatory responses were causing increased tissue mass. This latter change is classically considered a lung-specific toxicity. Lastly, microscopically the lungs of animals exposed to 330 mg/m³ had moderate lesions responding to the fibers, characterized by aggregates of macrophages collecting in and around terminal airways.

The most significant toxic effects occurred in animals exposed to the 1,000 mg/m³. The mean body weight of both male and female animals differed significantly with those of controls and the magnitude of difference between the two respective groups increased throughout the two-week exposure period. The lung weight parameters of those animals were also significantly increased relative to controls; again suggesting a marked increase in tissue mass, likely the result of increased fiber lung burdens and associated inflammatory responses. In addition, there was evidence of a probable stress related effect in those rats, as the kidneys, livers, and occasionally other organs were delayed in development relative to those in the control animals, as evidenced by decreased organ weight parameters. The adrenal: body weight ratios were also increased in animals exposed to this high fiber aerosol concentration, suggesting that corticosteroid production and release were elevated, another good indicator of secondary stress related changes.

Most notably, the histopathology of the lungs in the high concentration group suggested that the animals were severely compromised. In addition to the proportional increase in free fibers and fiber laden pulmonary macrophages, there were additional inflammatory changes throughout the upper and lower airways of the respiratory track of these animals. Many of the terminal bronchi were blocked by aggregations of organizing macrophages and proliferative inflammatory changes. These changes were often accompanied by mucinous exudates and organized granulomas that obstructed many of the airways. It was the pathologist's opinion that if this lesion progressed, it would likely be life threatening. Similar acute inflammatory changes also ascended up the respiratory tract along the

bronchi and extended into the nasal cavities, where an exudated rhinitis was observed in most animals.

Based on these findings, under the conditions used in this two-week study, a no-toxic effect level was estimated to be approximately 30 mg/m³ for male rats (due to relative increases in lung:brain weight values) and 100 mg/m³ for female rats, where animals exposed to both higher concentrations of 330 and 1,000 mg/m³ showed concentration-dependent respiratory tract toxicity and in the case of the higher-exposure group, secondary-related systemic changes. Since fibers were readily observable in animals in even the lowest exposure group, albeit this was considered a normal pulmonary alveolar macrophage response, there was no absolute no-effect level determined for this study.

Based on the results of this range-finding study, it would be recommended that for longer-term studies fiber aerosol concentrations not exceed a range of between 100 and 300 mg/m³ and that lower concentrations less than 10 mg/m³ also be included in the study design. Since the test material is an inorganic based fiber and is not readily cleared from the respiratory tract, it is likely that significant lung burdens will occur even at lower concentrations with increasing duration of exposure. Longer term studies should be designed to identify the concentration at which normal clearance mechanisms can keep up with the inhalation of new fibers with repeated daily exposures.

6.0 SPECIMEN STORAGE AND RECORD ARCHIVES

Records of animal receipt, quarantine, animal exposure, body weights, and all other information pertinent to the conduct of this study are contained in labeled binders. These and all other raw data collected in this study will be maintained in Battelle's archives, along with a copy of the final report.

7.0 ACKNOWLEDGMENTS

Acknowledgments of principal contributors participating in the performance of this study is presented in the following list:

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**TABLE 1.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL IN
RATS: INCIDENCE SUMMARY OF CLINICAL OBSERVATIONS (PRE DAILY EXPOSURE)**

STUDY NUMBER: SC920040

STUDY START DATE: 19-AUG-92

EXPOSURE GROUP (mg/m ³)	DAY OF STUDY													
	1	2	3	4	5	6	7	8	9	10	11	12	13	
	FINDING = NORMAL / NO REMARKABLE FINDINGS													
0	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
10	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
30	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
100	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
330	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
1000	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
		FEMALE ANIMALS												
0	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
10	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
30	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
100	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
330	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5
1000	INCIDENCE (N)	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE 2.
 REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL IN
 RATS: INCIDENCE SUMMARY OF CLINICAL OBSERVATIONS (POST DAILY EXPOSURE) (Continued)

GROUP(S)	DAY OF STUDY												
	1	2	3	4	5	6	7	8	9	10	11	12	
	FINDING = RESPIRATION / LABORED RESPIRATION												
1	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
2	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
3	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
4	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
5	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
6	INCIDENCE (N)	-	5	5	5	5	5	3	5	5	5	5	5
		FEMALE ANIMALS											
1	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
2	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
3	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
4	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
5	INCIDENCE (N)	-	5	5	5	5	5	5	5	5	5	5	5
6	INCIDENCE (N)	-	5	5	5	5	5	5	5	2	5	5	5

TABLE 3.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL IN
RATS: GROUP MEAN BODY WEIGHTS (grams)

Male												
Exposure Group (mg/m ³)	1			5			8			13		
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.
Control	5	138.34	8.32	5	152.34	7.63	5	160.28	7.68	5	172.60	6.15
10	5	137.72	10.83	5	152.06	12.90	5	163.00	14.59	5	176.40	16.03
30	5	136.34	13.44	5	149.58	16.64	5	160.64	17.15	5	172.92	20.26
100	5	138.00	11.99	5	147.88	12.95	5	157.52	15.00	5	167.30	15.11
330	5	134.86	7.66	5	139.50	8.32	5	152.66	9.82	5	161.70	10.23
1000	5	139.42	8.09	5	120.38*	6.45	5	133.10*	5.94	5	122.34*	9.44
Female												
Exposure Group (mg/m ³)	1			5			8			13		
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.
Control	5	113.54	6.28	5	118.78	5.60	5	122.14	5.97	5	128.72	5.34
10	5	112.60	5.06	5	118.60	5.85	5	122.92	3.42	5	127.18	5.34
30	5	109.54	3.10	5	116.00	4.33	5	120.50	4.75	5	124.66	5.69
100	5	110.84	4.48	5	116.00	5.19	5	121.72	5.87	5	125.88	7.21
330	5	107.44	3.11	5	110.00	3.54	5	115.04	3.54	5	119.50*	1.94
1000	5	107.64	8.52	5	93.76*	5.52	5	102.10*	7.14	5	92.96*	4.67

* = Statistical significance at $p \leq 0.05$.

TABLE 4.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL IN
RATS: INCIDENCE SUMMARY OF MACROSCOPIC TISSUE OBSERVATIONS

	ANIMAL SEX:					
	MALES			FEMALES		
GROUP:	1	2	3	4	5	6
EXPOSURE LEVEL (MG/M3):	0	10	30	100	330	1000
NO. IN GROUP:	5	5	5	5	5	5
	1	2	3	4	5	6
	0	10	30	100	330	1000
	5	5	5	5	5	5
LUNG DISCOLORATION	0	0	0	0	1	5
OVARY CYST	0	0	0	0	1	0
ANIMAL NOTE	5	5	5	5	4	0
NO LESIONS FOUND AT NECROPSY	5	5	5	5	4	0

TABLE 5.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL
IN RATS: ABSOLUTE ORGAN WEIGHT VALUES (grams)

Exposure Group mg/m ³	Male												
	Adrenal			Kidney			Testes			Liver			
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	
Control	5	0.0365	0.0041	5	1.31	0.07	5	2.29	0.08	5	2.29	0.08	
10	5	0.0395	0.0064	5	1.35	0.14	5	2.33	0.17	5	2.33	0.17	
30	5	0.0416	0.0058	5	1.35	0.18	5	2.23	0.21	5	2.23	0.21	
100	5	0.0399	0.0029	5	1.31	0.13	5	2.20	0.30	5	2.20	0.30	
330	5	0.0400	0.0088	5	1.27	0.04	5	2.26	0.03	5	2.26	0.03	
1000	5	0.0437	0.0025	5	1.06*	0.04	5	2.01*	0.14	5	2.01*	0.14	
Exposure Group mg/m ³	Brain			Lung			Liver			Liver			
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	
	5	1.55	0.08	5	0.97	0.05	5	7.59	0.80	5	7.59	0.80	
	5	1.61	0.08	5	1.03	0.06	5	7.85	0.86	5	7.85	0.86	
	5	1.63	0.06	5	1.05	0.11	5	8.02	1.38	5	8.02	1.38	
	5	1.59	0.08	5	1.06	0.05	5	7.66	0.86	5	7.66	0.86	
	5	1.63	0.10	5	1.11*	0.08	5	7.50	0.82	5	7.50	0.82	
1000	5	1.57	0.05	5	1.34*	0.09	5	5.29*	0.75	5	5.29*	0.75	
Exposure Group mg/m ³	Female												
	Adrenal			Kidney			Ovary			Liver			
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	
	5	0.0491	0.0053	5	1.05	0.06	5	0.094	0.022	5	0.094	0.022	
	5	0.0438	0.0085	5	1.03	0.06	5	0.084	0.014	5	0.084	0.014	
	5	0.0477	0.0038	5	1.05	0.06	5	0.086	0.010	5	0.086	0.010	
	5	0.0464	0.0107	5	1.07	0.10	5	0.102	0.084	5	0.102	0.084	
	5	0.0455	0.0038	5	1.00	0.04	5	0.083	0.008	5	0.083	0.008	
	5	0.0543	0.0058	5	0.87*	0.03	5	0.068	0.015	5	0.068	0.015	
	Exposure Group mg/m ³	Brain			Lung			Liver			Liver		
		N	Mean	Std.	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.
		5	1.51	0.06	5	0.80	0.06	5	5.56	0.45	5	5.56	0.45
		5	1.51	0.09	5	0.81	0.04	5	5.41	0.15	5	5.41	0.15
5		1.52	0.06	5	0.85	0.09	5	5.40	0.51	5	5.40	0.51	
5		1.54	0.06	5	0.86	0.08	5	5.64	0.63	5	5.64	0.63	
5		1.53	0.03	5	0.94*	0.02	5	5.21	0.27	5	5.21	0.27	
1000	5	1.45	0.07	5	1.23*	0.07	5	4.03*	0.66	5	4.03*	0.66	

* = Statistical significance at $p \leq 0.05$.

TABLE 6.
 REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL
 IN RATS: PERCENT ORGAN TO BODY WEIGHT RATIO

Male												
Group	Adrenal			Kidney			Testes					
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.			
Control	5	0.0211	0.0026	5	0.761	0.036	5	1.33	0.05			
10	5	0.0224	0.0033	5	0.767	0.029	5	1.33	0.07			
30	5	0.0244	0.0045	5	0.777	0.028	5	1.30	0.07			
100	5	0.0240	0.0033	5	0.782	0.019	5	1.32	0.12			
330	5	0.0246	0.0046	5	0.790	0.034	5	1.40	0.10			
1000	5	0.0360*	0.0046	5	0.867*	0.048	5	1.64*	0.07			
Liver												
Group	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.			
Control	5	0.90	0.06	5	0.56	0.04	5	4.39	0.35			
10	5	0.92	0.07	5	0.58	0.02	5	4.45	0.26			
30	5	0.95	0.08	5	0.61	0.03	5	4.61	0.27			
100	5	0.95	0.05	5	0.63	0.05	5	4.58	0.28			
330	5	1.01	0.04	5	0.69*	0.02	5	4.63	0.22			
1000	5	1.29*	0.10	5	1.10*	0.10	5	4.32	0.45			
Female												
Group	Adrenal			Kidney			Ovary					
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.			
Control	5	0.0383	0.0053	5	0.818	0.032	5	0.0735	0.0190			
10	5	0.0345	0.0065	5	0.813	0.026	5	0.0664	0.0132			
30	5	0.0384	0.0046	5	0.842	0.033	5	0.0686	0.0083			
100	5	0.0366	0.0064	5	0.851	0.044	5	0.0789	0.0600			
330	5	0.0381	0.0033	5	0.833	0.028	5	0.0695	0.0076			
1000	5	0.0583*	0.0036	5	0.933*	0.029	5	0.0732	0.0125			
Liver												
Group	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.			
Control	5	1.18	0.04	5	0.62	0.03	5	4.32	0.25			
10	5	1.19	0.06	5	0.64	0.03	5	4.26	0.20			
30	5	1.22	0.04	5	0.68	0.05	5	4.33	0.23			
100	5	1.23	0.08	5	0.68	0.05	5	4.47	0.26			
330	5	1.28*	0.04	5	0.78*	0.02	5	4.36	0.25			
1000	5	1.57*	0.08	5	1.32*	0.04	5	4.31	0.52			

* = Statistical significance at $p \leq 0.05$.

TABLE 7.
 REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL
 IN RATS: PERCENT ORGAN TO BRAIN WEIGHT RATIO

Male											
Group	Adrenal			Kidney			Testes				
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.		
Control	5	2.35	0.23	5	84.66	5.51	5	147.80	4.88		
10	5	2.45	0.40	5	83.96	7.78	5	144.82	10.26		
30	5	2.55	0.37	5	82.20	8.61	5	136.66	9.84		
100	5	2.51	0.24	5	82.23	5.28	5	138.37	16.12		
330	5	2.44	0.46	5	78.12	3.80	5	138.83	8.77		
1000	5	2.78	0.17	5	67.29*	3.42	5	127.86*	10.51		
Liver											
Group	Lung			Kidney			Ovary				
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.		
Control	5	62.14	1.26	5	489.86	62.97	5	6.24	1.60		
10	5	63.87	3.19	5	487.23	49.20	5	5.59	1.02		
30	5	64.43	4.73	5	489.84	72.08	5	5.62	0.70		
100	5	66.38*	2.11	5	480.65	31.81	5	6.67	5.62		
330	5	68.14*	2.96	5	458.58	40.50	5	5.43	0.58		
1000	5	85.62*	7.39	5	335.83*	40.18	5	4.70	0.92		
Female											
Group	Adrenal			Kidney			Ovary				
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.		
Control	5	3.25	0.37	5	69.60	2.91	5	6.24	1.60		
10	5	2.91	0.53	5	68.55	3.92	5	5.59	1.02		
30	5	3.14	0.32	5	68.84	2.11	5	5.62	0.70		
100	5	3.01	0.72	5	69.39	5.86	5	6.67	5.62		
330	5	2.98	0.26	5	65.13	3.16	5	5.43	0.58		
1000	5	3.74	0.37	5	59.76*	3.92	5	4.70	0.92		
Liver											
Group	Lung			Kidney			Ovary				
	N	Mean	Std.	N	Mean	Std.	N	Mean	Std.		
Control	5	52.87	3.34	5	366.98	18.26	5	6.24	1.60		
10	5	53.57	0.94	5	359.38	25.80	5	5.59	1.02		
30	5	55.78	4.41	5	353.85	21.17	5	5.62	0.70		
100	5	55.74	5.75	5	364.74	38.68	5	6.67	5.62		
330	5	61.34*	2.30	5	341.04	16.53	5	5.43	0.58		
1000	5	84.67*	4.98	5	276.99*	42.09	5	4.70	0.92		

* = Statistical significance at $p \leq 0.05$.

TABLE 8.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE FIBROUS AEROSOL IN RATS: INCIDENCE AND SEVERITY SUMMARY OF MICROSCOPIC OBSERVATIONS

	ANIMAL SEX:						--- MALES ---						--- FEMALES ---					
	GROUP:	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5
EXPOSURE LEVEL (MG/M3):	0	10	30	100	330	1000	0	10	30	100	330	1000	0	10	30	100	330	1000
T I S S U E S W I T H F I N D I N G S	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
LARYNX	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
-LARYNGITIS, ACUTE	-->	5	5	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LUNGS	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
-EOSINOPHILIC CRYSTALS, ALVEOLAR	-->	4	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1>	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-PARTICLE-LADEN MACROPHAGES	-->	5	0	0	0	0	0	5	0	0	0	0	0	5	0	0	0	0
	0.5>	0	5	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
	1>	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	0	0
	2>	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5	0
	3>	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	5
	4>	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
-BRONCHIOLITIS, PROLIFERATIVE	-->	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	2>	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
	3>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
-MUCINOUS EXUDATE, BRONCHIOLES	-->	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	1>	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	2>	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
	3>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
LYMPH NODE, BRONCHIAL.....	4	5	3	3	5	5	5	5	5	5	5	5	5	5	5	5	5	5
-PARTICLE-LADEN MACROPHAGES	-->	4	5	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	1>	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
	2>	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0
	3>	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0

* OBSERVATION SEVERITY CODE GRADES:
 0.5 = TRACE OR SLIGHT; VERY SLIGHT DEGREE OF ALTERATION OBSERVED
 1 = MINIMAL DEGREE OF CHANGE PRESENT
 2 = MILD DEGREE OF CHANGE PRESENT
 3 = MODERATE, SIGNIFICANT AMOUNT OF ALTERATION WAS OBSERVED
 4 = MARKED; DEGREE OF ALTERATION IS SEVERE; ORGAN MAY BE FUNCTIONALLY AFFECTED
 P = LESION IS PRESENT, NOT GRADED FOR SEVERITY
 - = LESION NOT PRESENT
 NO DIAGNOSIS UNDER "NUMBER EXAMINED" = NORMAL

TABLE 8. (Continued)

T I S S U E S W I T H F I N D I N G S	A N I M A L S E X :					
	-- MALES --			-- FEMALES --		
GROUP:	1	2	3	4	5	6
EXPOSURE LEVEL (MG/M3):	0	10	30	100	330	1000
NO. IN GROUP:	5	5	5	5	5	5
L Y M P H N O D E , T H Y M I C	1	3	5	5	5	4
-PARTICLE-LADEN MACROPHAGES						
->	1	3	5	2	2	1
1>	0	0	0	3	0	0
2>	0	0	0	0	3	1
3>	0	0	0	0	0	2
N A S O P H A R Y N X	5	5	5	5	5	5
N O S E / N A S A L C A V I T Y	5	5	5	5	5	5
-SUPPURATIVE EXUDATE						
->	5	5	5	5	5	0
1>	0	0	0	0	0	1
2>	0	0	0	0	0	0
3>	0	0	0	0	0	4
4>	0	0	0	0	0	0
O V A R Y						
-CYST, PARAOVARIAN						
P>	0	0	0	0	0	0
T R A C H E A	5	5	5	5	5	5

* OBSERVATION SEVERITY CODE GRADES:

- 0.5 = TRACE OR SLIGHT; VERY SLIGHT DEGREE OF ALTERATION OBSERVED
- 1 = MINIMAL DEGREE OF CHANGE PRESENT
- 2 = MILD DEGREE OF CHANGE PRESENT
- 3 = MODERATE, SIGNIFICANT AMOUNT OF ALTERATION WAS OBSERVED
- 4 = MARKED; DEGREE OF ALTERATION IS SEVERE; ORGAN MAY BE FUNCTIONALLY AFFECTED

P = LESION IS PRESENT, NOT GRADED FOR SEVERITY
 - = LESION NOT PRESENT
 NO DIAGNOSIS UNDER "NUMBER EXAMINED" = NORMAL

TABLE 9.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE
FIBROUS AEROSOL IN RATS: PRE-EXPOSURE, CHAMBER MASS AEROSOL
CONCENTRATION UNIFORMITY

Location		Chamber				
		2 (10 mg/m ³)	3 (30 mg/m ³)	4 (100 mg/m ³)	5 (330 mg/m ³)	6 (1000 mg/m ³)
Middle		8.6	33.7	112	353	947
Front		10.0	33.0	113	370	1040
Middle		9.6	33.3	109	350	940
Back		8.7	32.7	111	343	1040
Middle		7.7	31.0	109	343	1047
	WPV ¹	11.0%	4.46%	1.57%	1.47%	6.12%
	TPV ²	8.58%	1.55%	0.89%	3.84%	5.32%
	BPV ³	< 8.58%	< 1.55%	< 0.89%	3.56%	< 5.32%

1. Relative Standard Deviation of all samples at the reference point (middle) is the Within Port Variability (WPV).
2. Relative Standard Deviation of all locations using the first reference point is the Total Port Variability (TPV).
3. BPV is the Between Port Variability = $100 \cdot (S_t^2 - S_w^2)^{1/2} / M_t$, where S_t and S_w are the absolute standard deviations from the total and within port samples, respectively, and M_t is the mean of analyses from one measurement at each port.

TABLE 10.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE
FIBROUS AEROSOL IN RATS: VALIDATION EXPOSURE #1

Hour	Chamber				
	2 (10 mg/m ³)	3 (30 mg/m ³)	4 (100 mg/m ³)	5 (330 mg/m ³)	6 (1000 mg/m ³)
1	12.6	33.7	129	404	1073
2	8.6	31.0	126	396	1053
3	7.7	28.7	111	328	947
4	5.1	24.0	79	328	953
5	8.4	26.0	101	356	940
6	7.1	31.0	NR ¹	340	980
\bar{X} mg/m ³	8.25	29.1	109.2	359	991
%RSD	30.0	12.3	18.6	9.40	5.83
% of Target	82.5	97.0	109	109	99.1

¹ Sample collected improperly, N=5 for calculations.

TABLE 11.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE
FIBROUS AEROSOL IN RATS: VALIDATION EXPOSURE #2

Hour	Chamber				
	2 (10 mg/m ³)	3 (30 mg/m ³)	4 (100 mg/m ³)	5 (330 mg/m ³)	6 (1000 mg/m ³)
1	9.0	25.0	96.0	296	973
2 ¹	5.3	24.7	99.0	296	807
3	5.0	24.7	91.7	287	767
4	3.3	21.7	93.0	296	767
5	3.0	31.0	154	404	833
6	1.3	14.7	123	280	713
\bar{X} mg/m ³	4.48	23.6	109	310	810
%RSD	59.1	22.5	22.5	15.0	11.0
% of Target	44.8	78.7	109	93.9	81.0

1. Problem developed approximately 100 minutes in run with exhaust filter loading up with material resulting in decreased chamber concentrations.

TABLE 12.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE
FIBROUS AEROSOL IN RATS: VALIDATION EXPOSURE #3

Hour	Chamber				
	2 (10 mg/m ³)	3 (30 mg/m ³)	4 (100 mg/m ³)	5 (330 mg/m ³)	6 (1000 mg/m ³)
1	7.9	26.0	88.0	296	1013
2	7.0	31.0	90.0	312	920
3	5.3	23.0	83.0	308	833
4	5.1	23.0	90.0	320	960
5	10.6	26.7	87.5	300	900
6	9.1	23.7	104	320	840
\bar{X} mg/m ³	7.5	25.6	90.4	309	911
%RSD	28.7	12.1	7.89	3.24	7.63
% of Target	75%	85.3	90.4	93.6	91.1

TABLE 13.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM
OCTATITANATE FIBROUS AEROSOL IN RATS: BULK FIBER
SIZE DISTRIBUTION LOT 1F81L

Fiber Length		
Size Range (μm)	Frequency	Percent of Total
0.085 ¹ -1	190	33.6
1-2	128	22.7
2-5	140	24.8
5-10	73	12.9
10-20	29	5.1
20-50	5	0.9
	Total 565	100.0
Fiber Width		
0.085-0.2	1	0.2
0.2-0.5	517	91.5
0.5-1.0	43	7.6
1.0-2.0	4	0.7
	Total 565	100.0

¹Lowest resolution on Image Analysis System at 1000X magnification.

Mean Fiber Length 3.16 μm

Mean Fiber Width 0.34 μm

TABLE 14.
REPEATED-EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE
FIBROUS AEROSOL IN RATS: PRE-ANIMAL EXPOSURE AEROSOL FIBER SIZE
DISTRIBUTION

Mean Fiber Length (μm)				
Aerosol Concentration	Filter 1 (1000X)	Filter 2 (1000X)	Filter 2 (2000X)	Tape (1000X)
10 mg/m ³	2.98	3.01	1.90 ¹	3.43
30 mg/m ³	3.15	3.93	2.83	3.70
100 mg/m ³	2.51	3.53	2.53	4.30
330 mg/m ³	2.61	2.92	2.33	3.52
1000 mg/m ³	2.70	2.47	2.48	3.02
Mean Values	2.79	3.17	2.41	3.59
Mean Fiber Width (μm)				
Aerosol Concentration	Filter 1 (1000X)	Filter 2 (1000X)	Filter 2 (2000X)	Tape (1000X)
10 mg/m ³	0.32	0.31	0.22	0.31
30 mg/m ³	0.34	0.33	0.25	0.33
100 mg/m ³	0.32	0.32	0.26	0.32
330 mg/m ³	0.32	0.33	0.26	0.34
1000 mg/m ³	0.38	0.32	0.25	0.44
Mean Values	0.34	0.32	0.25	0.35

¹Only nine fibers in sample.

TABLE 15.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM
OCTATITANATE FIBROUS AEROSOL IN RATS: PRE-ANIMAL EXPOSURE
AEROSOL FIBER CONCENTRATION

Chamber	Sample No.	Number of Fibers	Fibers/cc ¹	Mean (f/cc)
10 mg/m ³	5B	35	23,333	$\bar{X} = 17000$
	10B	16	10,667	
30 mg/m ³	4B	96	64,000	$\bar{X} = 51667$
	9B	59	39,333	
100 mg/m ³	3B	643	428,667	$\bar{X} = 273667$
	8B	178	118,667	
330 mg/m ³	2B	841	560,667	$\bar{X} = 467667$
	7B	562	374,667	
1000 mg/m ³	1B	650	433,333	$\bar{X} = 569667$
	6B	1059	706,000	

¹ Each filter was 25 mm in diameter with a collection area of 3.68 cm². Sample volume was 25 cc in all samples. Each filter had 3 fields of measurement for a total of 21941 μm² area.

$$f/cc = (\text{Number of fibers})/(\text{Sample Volume})$$

$$\text{Sample Volume} = (0.00021941 \text{ cm}^2/3.68 \text{ cm}^2)(25\text{cc}) = 0.0015\text{cc}$$

TABLE 16.
REPEATED-EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE
FIBROUS AEROSOL IN RATS: GRAND MEAN CONCENTRATION

Grand Mean Mass Aerosol Fiber Concentration (mg/m ³) by Chamber		
10 mg/m ³	Mean %RSD n % of Target	10.2 33.9 60 102
30 mg/m ³	Mean %RSD n % of Target	29.8 19.2 60 99.3
100 mg/m ³	Mean %RSD n % of Target	114.1 18.6 60 114
330 mg/m ³	Mean %RSD n % of Target	342.8 14.4 60 104
1000 mg/m ³	Mean %RSD n % of Target	934.4 9.8 60 93.4

TABLE 18.
REPEATED EXPOSURE INHALATION STUDY OF POTASSIUM OCTATITANATE
IN RATS: GRAND MEAN CHAMBER ENVIRONMENTAL DATA

Grand Mean Chamber Environmental Data				
Chamber		Temperature (°F)	%RH	Air Flow (L/min)
Air Control	Mean	74.4	52.8	505.3
	%RSD	0.8	8.1	0.9
	n	118	118	118
10 mg/m ³	Mean	73.9	56.6	500.7
	%RSD	1.0	8.9	2.2
	n	118	118	118
30 mg/m ³	Mean	73.3	59.0	491.8
	%RSD	1.1	8.8	1.1
	n	118	118	118
100 mg/m ³	Mean	72.9	59.2	492.2
	%RSD	1.2	8.5	1.6
	n	118	118	118
330 mg/m ³	Mean	72.6	58.7	242.0
	%RSD	1.4	11.2	4.1
	n	118	118	118
1000 mg/m ³	Mean	72.8	51.6	248.1
	%RSD	1.4	9.8	2.7
	n	118	118	118

FYI - 1097 - 001310

FINAL REPORT

SUBCHRONIC INHALATION

TOXICITY STUDY OF A

FIBROUS AEROSOL OF

TISMO® IN RATS

To

Otsuka Chemical Company

March, 1995

Volume I

FINAL REPORT

(Battelle Study Number SC920180)

on

**SUBCHRONIC INHALATION TOXICITY STUDY OF
A FIBROUS AEROSOL OF TISMO® IN RATS**

to

Otsuka Chemical Company

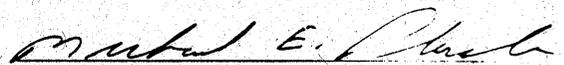
March, 1995

by

**Michael E. Placke, MaryEllen Lynch, Michael J. Brooker,
Michael J. Ryan, and John Yarrington**

**BATTELLE
505 King Avenue
Columbus, Ohio 43201-2693**

This study was conducted in compliance with EPA's GLP regulations 40 CFR, Part 792. This study was conducted according to the study protocol and Battelle's Standard Operating Procedure. The data presented accurately reflect the results of the study.

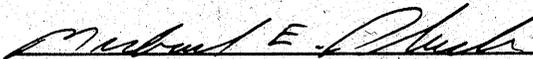


Michael E. Placke, Ph.D., DABT
Study Director

FINAL REPORT

on

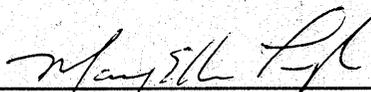
SUBCHRONIC INHALATION TOXICITY STUDY OF
A FIBROUS AEROSOL OF TISMO® IN RATS



Michael E. Placke, Ph.D., D.A.B.T.
Study Director

3/30/95

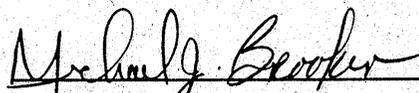
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MaryEllen Lynch, B.A., A.A. HT (ASCP)
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3-30-95

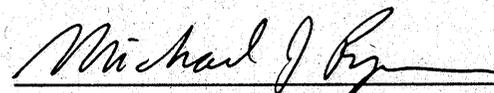
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Michael J. Brooker, B.S.
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March 30, 1995

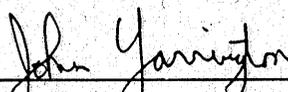
Date



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March 30, 1995

Date



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Study Pathologist

March-30-1995

Date

QUALITY ASSURANCE STATEMENT

This study was inspected by the Quality Assurance Unit and reports were submitted to the study director and management as follows:

<u>Phase Inspected</u>	<u>Date Inspected</u>	<u>Date Reported to Study Director</u>	<u>Date of Report to Management</u>
Serology Blood Collection, Room Inspection	10-23-92	10-30-92	10-30-92
Pre-Exposure Gravimetric Sampling	10-27-92	10-30-92	10-30-92
Randomization, Animal Identification	11-2-92	12-1-92	12-1-92
Gravimetric Sampling, Exposure	11-5-92	12-1-92	12-1-92
Clinical Observations, Body Weights	11-19-92	2-2-93	2-2-93
SEM Analysis	11-19-92	12-1-92	12-1-93
Exposure Room Inspection	12-4-92	12-4-92	12-8-92
Chamber Clean-out	12-21-92	1-4-93	1-4-93
Clinical Observation, Body Weights, Animal Exposure, Exposure Room Inspection, Gravimetric Sampling	1-14-93	2-2-93	2-2-93
Urine Collection	1-20-93	2-2-93	2-2-93
Urinalysis	1-21-93	2-2-93	2-2-93
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<u>Phase Inspected</u>	<u>Date Inspected</u>	<u>Date Reported to Study Director</u>	<u>Date of Report to Management</u>
Ti Analysis Lab Inspection	7-9-93	7-9-93	7-12-93
Ti Analysis Sample Preparation	7-12-93	8-2-93	8-2-93
Ti Analysis Sample Preparation	7-19-93	7-20-93	2-17-94
Ti Analysis ICP-AES	7-21-93	8-2-93	8-2-93
<u>Data Audits</u>			
Study File Audit	2-22-93	2-22-93	6-16-93
Study File Audit	6-7-93	6-7-93	6-16-93
Ti Analysis Method Validation Data	7-8-93	7-8-93	7-13-93
Ti Analysis Method Validation Data	7-13-93	7-13-93	2-17-94
Ti ICP-AES Data, Pathology and Histopathology Data, Draft Report Audit	8-4-93	8-4-93	8-6-93
Final Report Audit	1-26-95	1-26-95	3-29-95

Clara Gluckman 3-29-95
 Quality Assurance Unit Date
 Health Division

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SUMMARY

The objectives of this study were to characterize the potential toxic effects of potassium octatitanate fibers, trade name TISMO[®], as a result of inhaling a range of concentrations of fibers, identifying the specific effects on target organs, determining the potential reversal or progression of these effects, and estimating the lung burden and rate of lung clearance of the test material that was deposited in the lungs over the 13 week exposure period. The final objective was to provide a sufficient data base to assist in designing a possible future chronic study.

For this study, 140 male and 140 female Fischer 344 rats were exposed by whole-body inhalation to one of three aerosol concentrations of the test article (1, 10 and 100 mg/m³) and an air control group. Animals were exposed for 6 hours/day, 5 days/week, for 13 consecutive weeks, with subgroups of animals observed throughout a 13-week recovery period. There were a total of three subgroups of animals within each test concentration group; a base study group, a recovery group, and a lung clearance group. The base study and recovery groups consisted of 10 rats/sex/exposure concentration. Base study rats were necropsied the day following their last exposure. Recovery group animals were necropsied at the conclusion of the 13-week recovery period. The lung clearance group consisted of 15 rats/sex/exposure concentration. Five rats/sex/exposure concentration from the lung clearance subgroup were necropsied at three different intervals, (1) at the end of the 91-day exposure period, (2) after approximately 1.5 months following the end of exposure, and (3) after approximately 3 months following the end of exposures. Weekly clinical observations, body weight values, clinical pathology evaluations, organ weight measurements, and gross and microscopic observations were used to assess the toxic potential of exposure.

Results of the pre-exposure and in-life inhalation exposure system monitoring indicated that the generation system performed as designed. A uniform and stable atmosphere of test compound was produced and was delivered to the exposure chambers retaining similar physical and size dimensions as present in bulk material. The mean fiber length for all samples collected was 2.8, 2.7, and 2.8 microns for the 1, 10 and 100 mg/m³ chambers, respectively.

There were no unscheduled deaths in this study. There was evidence of test article concentration related reduction in body weight. The largest reduction in body weight gain occurred in male animals exposed to the high concentration (100 mg/m³). Clinical observations, clinical pathology and urinalysis determinations did not reveal any significant changes that would suggest test article-related effects.

Exposure-related macroscopic tissue changes, characterized as diffuse discoloration of the lungs following fixation, was observed in all high concentration group rats (100 mg/m³) at the interim

necropsy. All other macroscopic observations were considered to be spontaneous, incidental lesions that were not related to exposure.

Exposure-related microscopic anatomical changes were observed in the lungs of all rats. Rats at all concentrations of test article had particle-laden macrophages in the lungs and regional lymph nodes (bronchial and thymic). The amount of particles within the lungs was concentration-dependent, ranging from a very slight trace amount (severity grade 1) at 1 mg/m^3 to a moderate (severity grade 3)/severe (severity grade 4) distribution at 100 mg/m^3 . Additional lung responses included hyperplasia of type II pneumocytes and minimal fibrosis in groups exposed to concentrations of 10 mg/m^3 or greater.

Increased absolute lung weights were noted in the 1 mg/m^3 group males at interim necropsy (however, were similar to the controls at final necropsy), in the 10 mg/m^3 group males and females at interim and final necropsy, and in the 100 mg/m^3 group males and females at interim and final necropsy. Increased lung-to-body weight and lung-to-brain weight ratios were noted for both sexes in the 10 and 100 mg/m^3 groups at the interim and final necropsy. This demonstrated that the test compound deposited in the lungs of animals resulting in an increased mass.

The recovery group animals suggested that the lung and lymph node lesions were neither reversible nor progressive in nature after 13 weeks of recovery from the exposure to TISMO®. Lung clearance animals showed a well defined concentration relationship in total Titanium burden in the lung. However, the lung clearance data (Ti content) were less clear regarding the magnitude of fiber removal from the lung after either 6 or 12 weeks of recovery. These data were highly variable, which did not allow good quantification of fiber clearance. It is probable that some percentage of fibers became lodged within tissue spaces and were not removed by normal clearance mechanisms. This is typical of a fiber of this chemical makeup and size distribution. Histological evidence of fiber clearance was seen in recovery rats (macrophages containing phagocytized fibers accumulated at or near terminal airways). It was specifically noted that most fibers had apparently been removed from alveolar spaces and major airways.

Based on these findings, under the conditions used in this 13 week study, a no-toxic effect level was estimated to be 1 mg/m^3 for both male and female rats. Animals exposed to higher concentrations of 10 and 100 mg/m^3 showed significantly increased lung burdens of test article. Fibers were readily observed in animals in the lowest concentration group as well and the tissue responses observed were considered normal pulmonary alveolar macrophage responses. There was no absolute no effect level determined for this study.

FINAL REPORT

(Battelle Study Number SC920180)

on the

**Subchronic Inhalation Toxicity Study of
A Fibrous Aerosol of TISMO® in Rats**

to

Otsuka Chemical Company

by

**Michael E. Placke, MaryEllen Lynch, Michael J. Brooker,
Michael J. Ryan, and John Yarrington**

1.0 INTRODUCTION

The objectives of this study were to characterize the potential toxic effects resulting from inhalation of fibrous aerosols of the test article during a subchronic exposure period (6 hours/day, 5 days/week, for 13 consecutive weeks) and to assess the reversibility or progression of any toxic effects during a 13-week post-exposure recovery period. The study was designed to define the toxic effects associated with inhalation of the test article fibers, as a function of concentration, fiber size distribution, to identify the target organs and the potential for reversal or progression in each specific organ, to estimate lung burdens and clearance rates of the effect article fibers and to select concentrations for possible subsequent chronic toxicity and carcinogenicity studies. The test article used in this study was a potassium octatitanate fibers, trade name TISMO®. Otsuka Chemical Company was the Sponsor of the study. The study was conducted in conformance with the Environmental Protection Agency (TSCA) Guidelines for Toxicity of Chemicals (1990), 40 CFR 792 and the study was listed on Battelle's Master Study Schedule. The Study Protocol and amendments to the protocol are contained in Appendix A.

The study was conducted at Battelle Columbus Operations under the direction of Dr. Michael E. Placke, D.A.B.T. The in-life exposure portion of the study began on November 5, 1992 and ended on February 3, 1993. The in-life recovery portion began on February 4, 1993 and ended on May 7, 1993. This study was initiated on October 20, 1992 with the signing of the protocol and completed on March 30, 1995 with the signing of the final report.

2.0 EXPERIMENTAL DESIGN

For this inhalation toxicity study of TISMO® (Potassium Octatitanate), 140 male and 140 female Fischer 344 rats were used. Test groups of rats were exposed to one of three mass aerosol concentrations of the test article and an air control group were exposed by whole body inhalation as shown below. Animals were exposed for 6 hours/day, 5 days/week, for 13 consecutive weeks, with subgroups of animals observed throughout a 13-week recovery period. There were a total of three subgroups of animals within each test concentration group; a base study group, a recovery group, and a lung clearance group. The base study group and the recovery group consisted of 10 rats/sex/exposure concentration. Base study rats were necropsied the day following their last exposure. Recovery group animals were necropsied at the conclusion of the 13-week recovery period. The lung clearance group consisted of 15 rats/sex/exposure concentration. Five rats/sex/exposure concentration from the lung clearance subgroup were necropsied at three different intervals, 1) the end of the 91-day exposure period, 2) after approximately 1.5 months following the end of exposure, and 3) after approximately 3 months following the end of exposures. The study design is summarized in the table below.

Group Number	TISMO® Exposure Concentrations (mg/m ³)	Sex	Animal Study Numbers		
			Base Study	Recovery Group	Lung Clearance Groups
1	Air Control	M	101-110	111-120	121-135
		F	151-160	161-170	171-185
2	1	M	201-210	211-220	221-235
		F	251-260	261-270	271-285
3	10	M	301-310	311-320	321-335
		F	351-360	361-370	371-385
4	100	M	401-410	411-420	421-435
		F	451-460	461-470	471-485

The parameters used to assess toxicity during the in-life portion of the study included clinical observations (once weekly), body weight measurements (Day 1, weekly thereafter, and at necropsy).

clinical pathology (at necropsy) and urinalysis (Study Weeks 12 and 25). Postmortem studies included a complete necropsy with macroscopic tissue evaluations, organ weight measurements, and lung clearance studies. Histopathologic assessments were performed on all tissues from the base study and recovery animals in the high and air control concentration groups. Target organs were examined from the middle and low concentration groups.

3.0 MATERIALS AND METHODS

3.1 Test and Control Articles

The test article used for this study was Potassium Octatitanate, trade name, TISMO®, Lot #2D93J. The test article was supplied by the Sponsor and was received at Battelle on June 16, 1992. Upon receipt, the test article was assigned Battelle Substance Control Number 873A and was stored at ambient room temperature in the Animal Resources Compound Repository. The test article had no stated expiration date.

The control article in this study was filtered, conditioned room air.

3.2 Test Article Identity, Purity, and Stability Analysis

The test article identity, purity, stability, and methods of synthesis were the responsibility of the Sponsor.

3.3 Experimental Animals

A total of 280 Fischer 344 rats (140 males and 140 females) were required for this study. A sufficient number of animals were obtained from Charles River's Raleigh Laboratory, to provide the required number of healthy animals for testing. Animals were 4 to 6 weeks old at the time of receipt and no older than 6 to 8 weeks at the initiation of exposure. The rats were housed in Room 7C-419 during the quarantine period and in Room 7C-307 during the study period. The rat was chosen as the test system because this species has been accepted for toxicity studies to assess the safety of chemicals to which humans may be exposed.

3.3.1 Animal Receipt and Quarantine

The animals were received at Battelle on October 22, 1992, 14 days prior to the initiation of exposure. During the quarantine period, the rats were acclimated to the animal room and exposure chamber environmental conditions that were used for the study. Each animal was observed daily

during quarantine for clinical signs of abnormality that would make it unfit for study. Healthy animals were released at the conclusion of quarantine following examination by the staff veterinarian.

At the time of receipt, sera were randomly collected from 5 rats/sex for evaluation of selected antibody titers. These animals were not used on study. Samples of sera were sent to and analyzed by Microbiological Associates, Rockville, Maryland for measurement of antibody titers to Sendai Virus (Send), Pneumonia Virus of Mice (PVM), Rat Coronavirus/Sialodacryoadenitis Virus (RCV/SDA), Kilham Rat Virus (KRV), and Toolan's H-1 virus (H-1). None of the sera samples tested had significant titers to the infectious agents listed above.

3.3.2 Animal Housing and Environmental Conditions

All animals were individually housed in stainless steel, wire-bottom cages within the inhalation exposure chambers. All housing and care practices conformed to the requirements stated in the NIH "Guide for Care and Use of Laboratory Animals", National Institutes of Health Publication No. 86-23.

The environmental conditions of the animal study rooms conformed to the following: (1) the light/dark cycle was held at ~12 hours of light and dark each day during the study using fluorescent lighting, starting at ~6:00 a.m. each day, (2) the room temperature and relative humidity controls were set to 67 to 77°F and 40 ± 70 percent, respectively, and were monitored twice daily for conformance, and (3) fresh air was supplied to the room at a rate providing a minimum of ten changes of room air per hour. All animals were fed, *ad libitum*, certified Purina Rodent Chow in pelleted form (except during exposure). Contaminant analysis of each feed lot was supplied by the vendor and each analysis is maintained by the Battelle's Animal Resources Facility. Water was provided *ad libitum* (during exposure) via an automatic watering system monitored daily. The water source was the municipal city supply from the City of Columbus which conforms with the EPA water standards. There were no reported or known food or water contaminants that would interfere with the purpose or outcome of the study.

3.4 Animal Randomization and Identification

Animals were randomly assigned to each study group on Prestudy Day -3 by sex and body weight using a computer software program (Xybion Medical Systems®, Version 3.1) that employed an

algorithm for selection of body weight, which provided for homogenous group mean body weights. During quarantine, animals were assigned prestudy cage numbers that were used to temporarily identify the animals and record body weight data. Following randomization, the animals were identified by tail tattoo and metal cage tag. A cross-reference file of prestudy and study identification numbers was maintained in the computer data base and study files. Animal numbers are listed in Section 2.0.

3.5 Clinical Observations

The animals were observed twice daily during the quarantine period and study period for mortality/moribundity (once in the morning and once in the afternoon at least 6 hours apart). All animals were carefully examined once weekly for clinical signs of toxicity. Any clinical evidence of toxicity was recorded in the Xybion® System.

3.6 Body Weight

Individual rat body weights were recorded within 48 hours of receipt and again on the day of randomization. The individual body weights of the base study, recovery, and lung clearance group animals were recorded on Day 1, at weekly intervals thereafter, and at scheduled necropsy.

3.7 Clinical Pathology

Blood samples for clinical pathology determinations were obtained from all base study (10/sex/group) and recovery group rats (10/sex/group) at the time of their respective necropsies. Animals were anesthetized with propylene glycol-free sodium pentobarbital prior to sample collection. Blood was obtained from each rat via the vena cava. Blood samples for hematology were collected into tubes containing EDTA as the anticoagulant. Blood collected for serum chemistry analyses were collected into tubes without anticoagulant and the serum separated by centrifugation after clotting. The following clinical pathology evaluations were conducted:

- Hematology evaluations included:
 - Red blood cell count (RBC)
 - Hematocrit (HCT)

- Hemoglobin (HGB)
 - Mean corpuscular volume (MCV)
 - Mean corpuscular hemoglobin (MCH)
 - Mean corpuscular hemoglobin concentration (MCHC)
 - White blood cell count (WBC)
 - WBC differential count (Absolute and Relative)
 - Percent reticulocyte count (RETIC)
 - Platelet count (PLT)
 - Clotting time (Prothrombin times)
- Serum chemistry evaluations included:
 - Blood urea nitrogen (BUN)
 - Creatinine (CRE)
 - Glucose (GLU)
 - Total Protein (TP)
 - Albumin (ALB)
 - Albumin Globulin ratio (A:G)
 - Alanine aminotransferase (ALT)
 - Aspartate aminotransferase (AST)
 - Alkaline phosphatase (ALP)
 - Lactate dehydrogenase (LDH)
 - Electrolytes (Na, K, Phosphorus, Cl, Ca, Mg)
 - Total bile acids (BSLT)
 - Gamma glutamyl transpeptides (GGT)

3.8 Urinalysis

Urine was collected overnight (12-16 hour sample) from the base study rats during Week 12 and from the recovery group rats during Week 25. Urine was collected from animals individually housed in metabolism cages that facilitated separation of urine from possible contamination by fecal material. During the time of urine collection, animals had *ad libitum* access to feed and water. Preservation of the urine and retardation of evaporation was facilitated by collecting the urine sample in ice-chilled vessels. The following urinalysis evaluations were performed:

- Total Volume (VOL)
- Appearance (APPR)
- pH
- Specific gravity (SPG)
- Glucose (UGL)
- Creatinine (UCR)
- Urea Nitrogen (UUN)

- Protein (UPR)
- Ketones (KET)
- Urobilinogen (UBG)
- Occult blood (OB)
- Microscopic examination of urine sediment.

3.9 Necropsy

A complete gross necropsy was performed on all base study rats the day following their final exposure and all recovery group animals at the end of the 13-week recovery period. All rats were weighed prior to necropsy and were killed humanely by exsanguination after administration of pentobarbital anesthesia. Each necropsy included examination of the external surface of the body and examination of all required tissues.

The following tissues along with any identified gross lesions were preserved in 10 percent neutral buffered formalin solution, except eyes and testes, which were preserved in Bouin's fixative. After the organ weight was determined, lungs were perfused with 10 percent formalin using a gravity filling (25 cm hydrostatic pressure) device. The trachea was ligated after infusion to ensure trapping of fixative in airways and alveoli.

Adrenals
 All gross lesions
 Animal Identification
 Aorta
 Bone Marrow (evaluated only
 if determined necessary by
 the study pathologist)
 Brain
 Cecum
 Colon
 Duodenum
 Esophagus
 Eyes
 Heart
 Ileum
 Jejunum
 Kidneys
 Liver
 Lungs

Nasal Cavity (4 levels)
 Ovaries
 Pancreas
 Peripheral Nerve
 Pituitary
 Rectum
 Representative Lymph Node
 Salivary glands
 Spleen
 Sternum/with Bone Marrow
 Stomach
 Testes
 Thymus
 Thyroid/Parathyroid
 Trachea
 Urinary Bladder
 Uterus
 Accessory genital organs (prostate, epididymis
 and seminal vesicle, if present).

3.10 Organ Weight

Selected organs were weighed for all animals surviving to scheduled necropsy. Each of the following organs were removed, trimmed of extraneous tissue, and weighed:

Lungs	Adrenals (paired)
Liver	Brain
Kidneys	Testes or Ovaries

3.11 Histopathology

Histopathological examination were conducted on all tissues from the base study group animals from the high concentration exposure group and the air control group. Examinations were also conducted on tissues from the recovery group animals from the high concentration exposure group and the air control group. Target organs were examined from the middle and low concentration groups.

The lungs were sectioned to present a maximal section of the mainstem bronchi and the nose and nasal turbinates were prepared in four sections using the landmarks described by Young (Fundam. Appl. Toxicol., 1:309-312, 1981).

In addition to the first review of slides, a blind reevaluation of the target organ slides were performed.

3.12 Lung Clearance Studies

In order to estimate the lung burden of potassium octatitanate fibers and the rate of fiber clearance following the end of the 13-week exposure period, a subgroup of animals were used to measure the total lung content of titanium. Titanium (Ti) was used as the marker for fiber lung burdens. Five rats/sex/group from the lung clearance subgroup were necropsied at the following intervals; within 24 hours of their last exposure of the 91-day exposure period, near the end of the 6-week post-exposure recovery, and near the end of the 12-week post-exposure recovery period. The lungs from all rats were dissected and the fresh weights determined. After the fresh (wet) weights were taken, the lung was placed in a labelled megacassette and dried at approximately 47°C in a

forced air oven for a minimum of 24 hours and the dry weight recorded. All lung samples were then kept frozen (-20°C) until the analysis for titanium.

Each lung was then digested in an acid solution and formulated in an appropriate matrix for atomic absorption analysis of Ti. The concentration of Ti per unit mass of dry lung and wet lung was calculated along with the total lung burden of Ti. (Analytical method for titanium analysis is provided in Appendix B.)

3.13 Data Management and Statistical Analysis

In-life and postmortem animal data were collected using the Xybion® Path/Tox Data System (Version 3.1), (Xybion Medical Systems Corporation, Cedar Knolls, NJ). This computer data capture system recorded body weights, clinical observations, and organ weights on-line with Battelle's VAX Computer System. Data collected off-line (i.e. clinical pathology, urinalysis, and gross necropsy observations, histopathologic findings) were key-entered into appropriate Xybion modules and reviewed for accuracy of transcription.

All quantitative in-life and postmortem pathology data were statistically analyzed. Normally distributed data (parametric) were analyzed for treatment effects by analysis of variance and pairwise comparisons made between groups using Dunnett's t-test. Nonparametric data were analyzed by the Kruskal-Wallis Test and by the Mann-Whitney U Test for pairwise group comparisons.

3.14 Exposure System

3.14.1 Test Article Aerosol Generation and Delivery System

The purpose of the aerosol generation system was to provide a representative, uniform concentration of test article aerosol with a defined respirable fraction to the breathing zone of the animals. The test article aerosol generator was designed as a two-part system; the first was a mechanism to feed test article material at a constant rate into an aerosol generator, and the second was a high-energy dispersion device to aerosolize the test material. The first component of the generation system was an Accurate Model 302 Dry Chemical Feeder (Accurate, Inc., Whitewater, WI) which delivered preset amounts of potassium octatitanate bulk fiber into the aerosol generator.

This device employed a large capacity hopper with an auger type feed screw. The feeder directed a continuous stream of test compound past the inlet of a Fox, one inch, Coaxial Eductor Valve (Fox Valve Development Corp., Dover, NJ/East Hanover, NJ), which aspirated the material, entrained it into a high pressure stream of air, then introduced it into a plenum chamber.

The plenum chamber was supplied with Hepa/Charcoal filtered carrier air and the aerosol was allowed to mix inside the plenum chamber. Carrier air entered the plenum chamber near the bottom and the air flow pattern was from the bottom to the top. Any large fibers or agglomerates that formed settled and were deposited at the bottom of the plenum rather than being entrained in the air rising to the top of the delivery system.

The test atmosphere was ducted from the plenum to the exposure chambers through stainless steel delivery manifold. The test article aerosol was introduced into the manifold and mixed with the manifold air to yield a manifold aerosol concentration of 100 mg/m^3 . At the lower concentration exposure chambers, flowmeters removed appropriate aliquots of the manifold aerosol which was further mixed with HEPA filtered room air to obtain the proper chamber concentrations. The 100 mg/m^3 chamber received manifold aerosol directly with no dilution. The air control chamber received HEPA filtered room air only.

The chambers used for this study were Hazleton H2000 stainless steel and glass exposure chambers. The number of animals required for this study was such that animals were housed on three levels within the chamber. A schematic diagram of the entire system is included in Figure 1.

3.14.2 Exposure Atmosphere Concentration Analysis

Exposure system aerosol concentrations were monitored by gravimetric filter analysis. Gelman #66075, 25mm, glass fiber filters (Gelman Sciences, Ann Arbor, MI) were placed into open-face filter holders and inserted into exposure ports within the chambers. Calibrated air flow rates were regulated, using critical orifice meters, to sample a known volume of test atmosphere through the filters. Immediately after sampling, the filters were weighed and the mass concentration of the total aerosol was calculated from the accumulated mass and the sample volume.

3.14.3 Fiber Analysis

Fiber count and fiber size distribution measurements were completed during the pre-study validation of the system and weekly during the exposures to determine the length and width of fibers in the exposure atmosphere. Fibers were collected on Nucleopore 25 mm filters. The filters were analyzed by Scanning Electron Microscopy (SEM), light microscopy, and/or electron beam techniques. Images of the fibrous material were generated and used to compute the length, width and aspect ratio of the fiber. A TN-8500 Image Analysis System with a fiber analyzer program was used to perform the measurements. The fiber analysis software package was written and distributed by Noran (formerly Tracor-Northern) to be run with the TN-8500 Image Analyzer and, the Battelle TN8502 mainframe, a light optics station, and a JEOL Microprobe Analyzer (EPMA).

Samples were analyzed at 1000X magnification with a corresponding resolution of 0.085 microns.

The fiber concentration (fibers/cc) was calculated by dividing the number of fibers present in the field or fields of view by the product of the ratio of field size to the total collection area of the filter by the total volume of air sampled through the filter.

3.14.4 Chamber Environmental Measurements

An inhalation chamber Environmental Acquisition System (EAS) was designed to measure and record inhalation chamber temperature, humidity, and exhaust flow rates using an IBM XT personal computer (PC), coupled with an A/D data acquisition board and associated instrumentation. A QuickBASIC program was written to log chamber environmental data, generate hard copy of the data logged, and to store data to disk for subsequent statistical analysis. A PC-SAS® summary program was written to summarize the daily data generated by the EAS.

The PC hardware interface to the EAS consisted of a Model T11 terminal panel and an ACPC-12-16 data acquisition and control board (Strawberry Tree Computers, Sunnyvale, CA).

Temperature in each chamber was monitored using 20-gauge type T thermocouple probes. Each thermocouple probe was located at the top of each chamber approximately 2 feet from the chamber inlet duct. The thermocouple probe exited each chamber at the top, and was connected to a quick disconnect type T thermocouple connector to facilitate easy removal of the probe during chamber change-out activities. Type T 20-gauge polyvinyl-shielded duplex extension thermocouple

wire (Omega Engineering, Stamford, CT) was used to connect the quick-disconnect coupling to the T11 terminal panels. The terminal panels contained isothermal plates and integral cold-junction sensors.

The dew point in each chamber was monitored using an Optical Condensation Dew Point Hygrometer (General Eastern, Watertown, MA). The hygrometer output was logged by the EAS as an analog input channel. Sample lines were 0.25 inch O.D. polyethylene, and were installed near the top of each chamber in the vicinity of the thermocouple probes. Each sample line exited the chamber at the top. A Model ESC-10P Electronic Actuated Position Valve (Valco Instrument Co., Houston, TX) was remotely located in the exposure room, and each chamber's humidity sample line was connected to a valve port via 0.25 inch polyethylene tubing. The valve was equipped with a "select" and a "common" port. The common port allowed atmosphere from all chambers to be pulled through the sample lines, thereby allowing a fresh supply of chamber atmosphere in the sample lines at all times. The select port was used to direct a single chamber atmosphere into the inlet of the dew point hygrometer sensing volume. The flows through both the select and common ports were controlled by calibrated flowmeters; a flow rate of approximately 200 cc/min was maintained through the select port and approximately 2 L/min was drawn through the common port.

All chambers were operated at 15 air changes per hour (500 L/min), controlled by calibrated orifice plate flowmeters located on each chamber's exhaust. The flowmeter's upstream and downstream pressure ports were connected to an EST-10P Electronic Actuated Position Valve (Valco Instruments Co., Houston, TX) via 0.125 inch ID Tygon[®] tubing. The valve's upstream and downstream exit ports were connected to a zero to 5.0 inch of water variable reluctance differential pressure transducer (Battelle's Instrument Services). A calibration of each flowmeter's flow rate versus transducer output was performed prior to exposures. The transducer output was logged by the EAS as an analog input channel.

3.14.5 Chamber Atmosphere Uniformity

The uniformity of the chamber aerosol atmosphere was determined by gravimetric concentration sampling on the levels of each chamber where the animals were exposed. The sampling scheme included a reference to determine temporal uniformity as well as spatial uniformity. The determination of multiple locations within the exposure planes of the chamber. The reference location

was sampled before, during, and after other uniformity measurements to assess the contribution of within-port variability to the uniformity determinations.

The total port variability (TPV) was estimated from the relative standard deviation of analyses from one measurement at each location, and represented the sum of variability between ports (spatial), and the variability within a port (temporal).

The within port variability (WPV) was estimated from the relative standard deviation of the concentration measured at the reference location. This variability included temporal fluctuations as generator output.

The between port variability (BPV) represented the variability associated with spatial variability of test article concentration within the exposure chamber. The BPV was estimated from the total port mean (M_t), total port standard deviation (S_t), and the within port standard deviation (S_w) by applying the following formula:

$$BPV = 100 \cdot (S_t^2 - S_w^2)^{1/2} / M_t$$

Because of random error, aggravated by the small sample size, the estimated WPV was occasionally greater than the estimated TPV. In these cases, the BPV can not be estimated directly. Since the estimate of TPV is more reliable than the estimate of WPV due to its greater sample size, and since BPV, by definition, cannot exceed TPV, BPV is reported to be less than the TPV for those cases where it can not be calculated. The air control chamber was not sampled for atmosphere uniformity.

4.0 RESULTS

4.1 Animal Survival

There were no unscheduled animal deaths in this study.

4.2 Clinical Observations

The incidence summary of abnormal clinical observations are presented in Table 1. All other animals were observed as normal. Individual animal clinical observations are presented in Appendix C.

The most frequent clinical sign of abnormality was eye discharge (red and/or clear). Eye discharge persisted intermittently between female animals in all groups and the males in Group 2 (1 mg/m³) beginning on Day 50. The sign appeared first on Day 50 in Group 2 (1 mg/m³) males and females and continued to be observed sporadically until scheduled necropsy. The other groups had sporadic incidences of eye discharge. A second abnormality observed was opacity in the eye. This sign was observed in one female animal in Group 2 (1 mg/m³) from Day 120 until scheduled necropsy.

4.3 Body Weight

A summary of group mean animal body weights are presented in Tables 2 and 3 for males and females, respectively. Graphs of group mean body weight data are shown in Figures 2 and 3 for the males and females, respectively. Individual animal body weight values are contained in Appendix D.

There were no statistically significant differences in male group mean body weights in Group 2 (1 mg/m³) or Group 3 (10 mg/m³) compared to the air control group during the exposure or recovery periods. During the exposure period there were instances of statistically significant lower ($p \leq 0.05$) body weights in Group 4 (100 mg/m³) males compared to the air controls beginning on Day 8 and continuing until Day 85. There were several measurement periods on Days 29, 43, 64 and 71 where there were no statistical significance noted. During the recovery period, there were no statistically significant differences in any male group compared to the air control group.

There were no statistically significant differences in female group mean body weights in Group 2 (1 mg/m³) compared to the air control group during the exposure or recovery periods.

There were isolated incidences of statistically significant lower body weights in Group 4 (100 mg/m³) females on Day 36 (exposure) and statistically significant higher body weights in Group 3 (10 mg/m³) on Day 134 (recovery) compared to the air control group.

4.4 Clinical Pathology

Group mean clinical pathology data are presented in Tables 4 through 14. Individual animal results are included with Appendix E.

There were several instances in which statistical differences occurred in hematologic parameters between controls and one or more TISMO® exposed groups. In females, the 13-week platelet counts of the 10 and 100 mg/m³ groups were decreased, relative to controls, on Day 93 (end of exposures), and the decrease was statistically significant. At Day 184 (end of 13-week recovery), the 100 mg/m³ females still had a statistically significant decreases in platelet counts. Platelet counts were also decreased, relative to controls, in all treated groups of males at Day 92 (end of 13-week exposure) and in 1 and 100 mg/m³ males at Day 183 (end of 13-week recovery), but without statistical significance. The degree of platelet count decrease was minimal in clinical severity and would not be expected to produce any clinical signs. There were no significant differences in platelet counts among male groups. While the decreased platelet counts may have been a result of exposure, such an effect was of negligible toxicologic significance. All platelet values were near normal ranges for rats. Histopathologic examination of the bone marrow of exposed animals showed no evidence of treatment effects on platelet precursor number or morphology.

There were also small, but statistically significant decreases in mean blood hemoglobin, erythrocyte count and mean corpuscular volume at Day 93 (end of 13-week exposures) in the 100 mg/m³ females, mean corpuscular hemoglobin in the 100 mg/m³ males at Day 183 (end of 13-week recovery), and mean corpuscular volume in the 10 and 100 mg/m³ males at Day 183 (end of 13-week recovery). All RBC indices were near normal ranges for rats. As was noted for the platelet count data cited above, these data may represent an exposure related effect, but any effect would be of negligible clinical significance.

There were also some statistically significant changes in relative numbers of various types of leukocytes in peripheral blood at various times in female treated groups, but these seem of no particular significance in light of the fact that total white blood counts were quite steady throughout the study in control and treated rats.

There were several instances in which mean values of serum chemistry parameters in treated animals differed statistically from control values. Like the statistically significant differences cited above in the discussion of hematologic data, these data trends seem to represent changes of no clinical and probably little toxicologic significance. Values were near normal ranges for rats and there was a lack of clear concentration relationship. Statistically significant decreases in total protein and albumin were noticed in the 100 mg/m³ males at Day 92 (end of 13-week exposures), but this tendency was minimal and disappeared at Day 183 (end of 13-week recovery). The 1 and 10 mg/m³ males had elevated bile salt values, compared to controls, at Day 183 (end of 13-week recovery), but the mean values in the 100 mg/m³ males was lower than the means of either the 1 or 10 mg/m³ males. An incidental statistically significant increase in blood urea nitrogen was noted in 1 mg/m³ males on Day 183 (end of 13-week recovery). Serum alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) values were statistically different from controls in a number of treated groups at various times. The changes in ALP were very small and interpreted to be within the range of normal. The LDH values were consistently lowered, but LDH values are generally only considered to be a useful indice of organ damage when they are increased. A small but statistically significant increase in serum inorganic phosphate was noted at Day 92 (end of 13-week exposures) in the 100 mg/m³ males, but did not persist until Day 183 (end of 13-week recovery). These altered phosphorous levels were not accompanied by any clinical or anatomic pathology results indicating dysfunctional calcium/phosphorous metabolism or altered renal functions, which are the two most common disease states involving altered phosphorous levels.

4.5 Urinalysis

None of the results of urinalysis were statistically different between exposure groups nor did they indicate the presence of any toxic effects on renal function.

4.6 Necropsy

Necropsy observations are summarized for the interim necropsy conducted at the end of 13 weeks of exposure and the final necropsy completed at the conclusion of the 13-week recovery period in Tables 15 and 16, respectively. Macroscopic findings for individual rats are presented in Appendix F. An apparent exposure-related effect, characterized as diffuse discoloration of the lungs following fixation, was observed in all high concentration group (100 mg/m³) rats at the interim

necropsy. All other gross observations were considered to be spontaneous, incidental lesions that were not related to exposure.

4.7 Organ Weight Results

Group mean absolute organ weight values, organ-to-body weight ratios and organ-to-brain weight ratios are summarized in Tables 17 (male interim necropsy, after 13 weeks of exposure), 18 (male final necropsy, after 13-week recovery period), 19 (female interim necropsy, after 13 weeks of exposure), and 20 (female final necropsy, after 13-week recovery period). Individual animal data are included in Appendix C.

There were few statistically significant differences in absolute organ weight, organ-to-body weight or organ-to-brain weight ratios in male or female rats from Group 2 (1 mg/m³). The male absolute lung weights at the interim necropsy were significantly higher compared to the air control group. Absolute liver weight values were significantly higher in the Group 2 (1 mg/m³) final necropsy males compared to the air controls. In addition, female interim necropsy animals had significantly higher kidney-to-body weight values compared to the air control group.

Male and female rats in Group 3 (10 mg/m³) had significantly higher ($p \leq 0.05$) absolute lung weight, lung-to-body weight and lung-to-brain weight ratios at both the interim and final necropsy compared to the air controls. There were no other significant differences in this exposure group.

The male and female rats in Group 4 (100 mg/m³) had significantly higher ($p \leq 0.05$) absolute lung weight, lung-to-body weight and lung-to-brain weight ratios at both the interim and final necropsies compared to the air controls. Absolute brain weight values in females at the interim necropsy were significantly lower than the air control animals. Kidney-to-body weight values in females at the interim necropsy and absolute kidney weights at the final necropsy were significantly higher than the air control animals. No other significant differences occurred in this group.

4.8 Histopathology

Microscopic observations are reported as incidence summaries in Tables 21 (interim necropsy after 13 weeks of exposure) and 22 (final necropsy, after 13-week recovery period). All microscopic findings for individual rats are presented in Appendix H. Evaluation of target organs for individual rats read with knowledge of animal identification/exposure (coded) and without such information

(blinded) are presented in Appendix I. Representative photographs of lungs and lymph nodes are presented in Figures 4 through 13.

Compound-related effects at the end of 13-weeks of treatment ("Interim Sacrifice 1") were found in the following organs: lungs, bronchial lymph nodes, and thymic lymph nodes. The lungs in treated rats had minimal to slight thickening of walls of the alveolar ducts and septae characterized by increased numbers of particle-laden macrophages ($\geq 1 \text{ mg/m}^3$), hyperplasia of type II pneumocytes ($\geq 10 \text{ mg/m}^3$), and some fibrosis ($\geq 10 \text{ mg/m}^3$). The occurrence of particle-laden macrophages was nearly 100% in the affected dose groups while the severity was exposure-related, ranging from trace/minimal (severity grade 1) to moderate (severity grade 3)/severe (severity grade 4) involvement. The particles within the lungs appeared to be relatively inert because of the absence of any significant inflammatory response other than particulate phagocytosis and the multifocal distribution of generally minimal pneumocyte II hyperplasia. Many of the interstitial areas were thickened due to increased numbers of fibroblasts as seen with conventional hematoxylin and eosin (H & E) staining. To better characterize the nature of the interstitial thickenings, special trichrome-stained preparations were obtained. Although mature collagen fibrils were not readily apparent, the thickened areas stained positively for collagen with the special stain and thus, it was interpreted that some collagen formation (fibrosis) had occurred.

Lymphatic drainage of the particulate material was also apparent since the regional lymph nodes (bronchial and thymic) had particle-laden macrophages. Like the lesions in the lungs, the severity of the lymph node lesions was exposure-related. All other findings at the end of treatment were incidental in nature and not compound-related.

Rats examined microscopically at the end of the recovery phase had compound-induced lesions of the lungs and lymph nodes that were similar to those at the end of treatment. Lesions in recovery animals were of comparable severity to those necropsied at the termination of exposure with no new findings that suggested progressive changes. However, the distribution of the particulate material appeared to be more localized in macrophages near the alveolar ducts, suggesting some ongoing slow clearance. Thus, these lung and regional lymph node lesions were neither reversible nor progressive in nature after 13 weeks recovery from exposure to TISMO®.

Whether the tissues were evaluated "blinded" or with knowledge of exposure, there was little difference in severity grades. Thus, having access to exposure information did not bias the microscopic evaluation of the tissues.

Rats exposed to TISMO® at exposures of 1 to 100 mg/m^3 had particle-laden macrophages in the lungs and the regional lymph nodes (bronchial and thymic). The amount of the particles was

exposure-related, ranging from a very slight trace amount (severity grade 1) at 1 mg/m³ to a moderate/severe distribution at 100 mg/m³. Additional lung responses included slight hyperplasia of the type II pneumocytes and minimal fibrosis (≥ 10 mg/m³). The lung and lymph node lesions were neither reversible nor progressive in nature after 13 weeks recovery from exposure to TISMO®.

4.9 Lung Clearance Results

The wet and dry lung weight values are presented in Table 23 (males) and Table 24 (females). The total Titanium (Ti) burden, dry lung weight values, and Ti concentration per unit dry lung mass are presented in Table 25 (males) and Table 26 (females). The total titanium burdens are represented graphically over time in Figure 14 (males) and Figure 15 (females). There was a clear concentration dependent increase in Ti concentration and total Ti lung burden with greater exposure concentrations. Rats exposed to 1 mg/m³ did not have significantly different Ti levels compared to the background tissue levels measured in controls. However, rats exposed to 10 mg/m³ were 4 to 5 times greater than controls and the 100 mg/m³ rats had lung Ti levels a full order of magnitude greater than control rats.

There was only a nominal decrease or clearance of Ti from the lungs of exposed animals during the recovery period. The total Ti burdens remained relatively constant, while the dry lung mass increased (presumably due to the growth of the rats). This resulted in the concentration of Ti decreasing, but this was probably not due in any significant part of true clearance of Ti fibers from the lungs.

4.10 Exposure Results

4.10.1 Pre-Study Atmosphere Characterization Results

Chamber uniformity measurements were completed during the pre-study validation of the system. The data show the between port variability (BPV) less than 12 percent in all chambers. These data are summarized in Table 27. This pre-study analysis showed that there was a uniform distribution of the atmosphere within the chamber.

Three consecutive six hour trial runs were completed prior to the start of the study to confirm the system was suitable to produce consistent results on a daily basis. The results of these trial runs are presented in Tables 28, 29, and 30.

4.10.2 Bulk Fiber Distribution

Bulk fiber measurements were completed in earlier phases of work with this compound to assure that the test atmosphere generated was comparable to the bulk material. The bulk fiber analysis was completed at a magnification of 1000X and an image analysis resolution of 0.085 microns. The mean length of fiber was 3.61 microns and the mean width was 0.35 microns. The bulk fiber (lot number 2D93J) length and width measurements are detailed in Table 31, and Figure 16.

4.10.3 Pre-Study Fiber Size and Count Analysis

Fibers were collected during the pre-study validation phase to determine the length and width distributions within the chamber atmosphere. Two filter samples were collected within each chamber. The mean fiber length for the first filter sample at 1000X magnification was 3.75, 3.77, 3.06 for the 1, 10, and 100 mg/m³ chambers respectively. The mean fiber width for the first sample at 1000X magnification was 0.38, 0.42, and 0.47 for the 1, 10, and 100 mg/m³ chambers respectively. The second sample at 1000X magnification revealed similar lengths and width values. These data are detailed in Table 32.

Fiber count measurements were completed on the same filters and the number of fibers per cubic centimeter was calculated. The mean fiber counts determined from these samples were 3834, 11,555, and 244,000 fibers per cubic centimeter for the 1, 10, and 100 mg/m³ chambers, respectively. These data are detailed in Table 33.

These pre-study data indicate that the aerosol generated had equal mean length and width values as the bulk material and that the fiber size distribution was equally distributed throughout the exposure chambers resulting in similar mean length and width values in all chambers. The mean fiber concentration (fibers per cubic centimeter) values increased with the mass concentration values as would be expected as well.

4.10.4 Exposure Mass Concentration Data

The grand mean (mean of all samples collected) chamber mass concentrations were 1.1, 10.4, and 103.7 for the 1, 10, and 100 mg/m³ chambers respectively. All of these values are within 10% of targeted concentrations. Relative standard deviations were less than 20% indicating the concentrations were stable over time. These data for the individual chambers are listed in Table 34. Weekly mean concentration by chamber are listed in Table 35.

4.10.5 Exposure Environmental Conditions

The grand mean chamber temperature, percent relative humidity, and air flow rates are listed in Table 36. All values were within the protocol specified range.

4.10.6 Exposure Fiber Size and Count Data

The mean fiber length for all samples collected was 2.81, 2.69, and 2.79 microns for the 1, 10, and 100 mg/m³ chambers respectively. The mean fiber width for all samples collected was 0.48, 0.48, and 0.45 microns for the 1, 10, and 100 mg/m³ chambers respectively. The data are detailed in Table 37. Raw data of gravimetric filter analyses, fiber count measurement and fiber size distribution are contained in Appendix I.

The mean fiber concentration values (fibers per cubic centimeter) collected from the samples were 1707, 5875, and 112724 f/cc for the 1, 10, and 100 mg/m³ chambers, respectively. The fiber count measurements are detailed in Table 38.

The results again, as with the pre-study results, show the fiber distribution is representative of the bulk material and equally distributed within all chambers.

5.0 DISCUSSION AND CONCLUSIONS

There were several objectives for this study including characterizing the potential toxic effects of potassium octatitanate fibers, trade name TISMO®, as a result of inhaling a range of concentrations of fibers, identifying the specific effects on target organs, determining the potential reversal or progression of these effects, and estimating the rate of lung clearance of the test material deposited in the lungs over the 13 week exposure period. The final objective was to provide a sufficient data base to assist in designing a possible future chronic study.

The aerosol generation and exposure system performed as designed. A uniform and stable atmosphere of test compound was produced and was delivered to the exposure chambers retaining similar physical and size dimensions as present in bulk material. All exposures were conducted as planned and no protocol deviations of any significance occurred during any phase of the study.

Clinical observations of the animals, clinical pathology and urinalysis determinations did not reveal any significant changes that would suggest concentration or compound related toxic effects other than a slight but consistent decrease in the mean body weights of male rats exposed to the highest concentration (100 mg/m³). However, there was evidence of test article exposure in all concentration groups of animals. Rats at all concentrations of test article had particle-laden macrophages in the lungs and regional lymph nodes (bronchial and thymic). The amount of particles was concentration-dependent, ranging from a very slight trace amount (severity grade 1) at 1 mg/m³ to a moderate (severity grade 3)/severe (severity grade 4) distribution at 100 mg/m³. Additional lung responses included slight hyperplasia of type II pneumocytes and minimal fibrosis (≥ 10 mg/m³).

Additionally, increased absolute lung weights were noted in the 1 mg/m³ group males at interim necropsy (however, were similar to controls at final necropsy), in the 10 mg/m³ group males and females at interim and final necropsy, and in the 100 mg/m³ group males and females at interim and final necropsy. Increased lung-to-body weight and lung-to-brain weight ratios for both sexes in the 10 and 100 mg/m³ groups, demonstrates that the test compound deposited in the lungs of the animals resulting in an increased tissue mass.

The recovery group animals suggested that the lung and lymph node lesions were neither reversible nor progressive in nature after 13 weeks of recovery from the exposure to TISMO®. Lung clearance animals showed a well defined concentration relationship in total Titanium burden in the lung. However, the lung clearance data (Ti content) were less clear regarding the magnitude of fiber removal from the lung after either 6 or 12 weeks of recovery. These data were highly variable.

which did not allow good quantification of fiber clearance. It is probable that some percentage of fibers became lodged within tissue spaces and were not removed by normal clearance mechanisms. This is typical of a fiber of this chemical makeup and size distribution. Histological evidence of fiber clearance was seen in recovery rats (macrophages containing phagocytized fibers accumulated at or near terminal airways). It was specifically noted that most fibers had apparently been removed from alveolar spaces and major airways.

Based on these findings, under the conditions used in this 13 week study, a no-toxic effect level was estimated to be 1 mg/m^3 for both male and female rats. Animals exposed to higher concentrations of 10 and 100 mg/m^3 showed significantly increased lung burdens of test article. Since fibers were readily observed in animals in the lowest concentration group as well, the tissue responses observed were considered normal pulmonary alveolar macrophage responses. There was no absolute no effect level determined for this study.

6.0 SPECIMEN STORAGE AND RECORD ARCHIVES

Records of animal receipt, quarantine, animal exposure, body weights, and all other information pertinent to the conduct of this study are contained in labeled binders. All paraffin blocks, wet tissues and microscopic slides resulting from any portion of this study will be retained by Batteille until acceptance of the final report, when all materials will be returned to the Sponsor or his designated archival facility.

7.0 ACKNOWLEDGMENTS

Acknowledgments of principal contributors participating in the performance of this study is presented in the following list:

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TABLE 1.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF CLINICAL OBSERVATIONS (1ST SET/DAY)

GROUP(S)	DAY OF STUDY																					
	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141	
	FINDING = TOTAL BODY / LOCALIZED ABRASION(S)																					
	MALE											ANIMALS										
1	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
2	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
3	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
4	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
	FEMALE											ANIMALS										
1	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
2	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
3	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
4	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15

TABLE 1.
 SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
 INCIDENCE SUMMARY OF CLINICAL OBSERVATIONS (1ST SET/DAY) (Continued)

GROUP(S)	DAY OF STUDY																					
	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141	
	FINDING = EYES / RED OCULAR DISCHARGE																					
	MALE											ANIMALS										
1	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
2	INCIDENCE (N)	35	35	35	35	35	35	35	35	2	2	1	1	35	35	35	35	35	35	35	35	35
3	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
4	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
	FEMALE											ANIMALS										
1	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
2	INCIDENCE (N)	35	35	35	35	35	35	35	35	4	3	2	1	35	35	35	35	35	35	35	35	35
3	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
4	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35

TABLE 1.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF CLINICAL OBSERVATIONS (1ST SET/DAY) (Continued)

GROUP(S)	FINDING = EYES / CLEAR OCULAR DISCHARGE		DAY OF STUDY																					
	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141			
1	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
2	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
3	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
4	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
1	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
2	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
3	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
4	INCIDENCE (N)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	
		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
		35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	20	15	15	

M A L E A N I M A L S

F E M A L E A N I M A L S

TABLE 1.
 SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
 INCIDENCE SUMMARY OF CLINICAL OBSERVATIONS (1ST SET/DAY) (Continued)

GROUP(S)	FINDING = EYES / OPACITY																					
	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141	
	M A L E A N I M A L S																					
1	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
2	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
3	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
4	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
	F E M A L E A N I M A L S																					
1	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
2	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
3	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15
4	INCIDENCE (N)	35	35	35	35	35	35	35	35	35	35	35	35	35	35	20	20	20	20	20	15	15

TABLE 1.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF CLINICAL OBSERVATIONS (1ST SET/DAY) (Continued)

GROUP(S)	FINDING = EYES / OPACITY				DAY OF STUDY			
	148	155	162	169	176	183		
	M A L E A N I M A L S							
1	INCIDENCE (N)	15	15	15	15	10	10	10
2	INCIDENCE (N)	15	15	15	15	10	10	10
3	INCIDENCE (N)	15	15	15	15	10	10	10
4	INCIDENCE (N)	15	15	15	15	10	10	10
	F E M A L E A N I M A L S							
1	INCIDENCE (N)	15	15	15	15	10	10	10
2	INCIDENCE (N)	1	1	1	1	1	1	1
		15	15	15	15	10	10	10
3	INCIDENCE (N)	15	15	15	15	10	10	10
4	INCIDENCE (N)	15	15	15	15	10	10	10

TABLE 2.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN MALE BODY WEIGHTS (grams)

GROUP	DAY 1			DAY 8			DAY 15			DAY 22			DAY 29			DAY 36			
	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	
0 mg/m ³	35	157.44	6.99	35	176.79	8.34	35	191.02	10.08	35	202.59	11.45	35	213.95	12.68	35	224.52	13.97	
1 mg/m ³	35	156.51	7.26	35	172.72	9.41	35	186.94	11.58	35	200.10	13.31	35	214.45	15.36	35	224.85	16.35	
10 mg/m ³	35	157.54	7.31	35	173.97	8.24	35	190.42	10.05	35	202.20	12.72	35	214.57	14.39	35	224.77	15.04	
100 mg/m ³	35	156.89	6.46	35	171.37*	7.11	35	183.91*	8.28	35	195.18*	9.62	35	207.10	10.92	35	215.34*	11.97	
		DAY 43			DAY 50			DAY 57			DAY 64			DAY 71			DAY 78		
0 mg/m ³	35	232.31	15.29	35	241.16	16.44	35	255.34	16.46	35	263.05	16.82	35	270.01	17.18	35	278.26	18.18	
1 mg/m ³	35	233.96	18.51	35	241.54	19.98	35	257.83	20.87	35	266.31	21.44	35	275.58	22.64	35	281.44	22.75	
10 mg/m ³	35	232.87	16.02	35	241.16	17.19	35	256.73	16.99	35	266.01	16.41	35	272.78	17.13	35	280.08	17.39	
100 mg/m ³	35	223.91	13.30	35	230.89*	14.30	35	238.87*	15.29	35	253.40	15.25	35	260.16	16.42	35	266.40*	16.34	
		DAY 85			DAY 92			DAY 99			DAY 106			DAY 113			DAY 120		
0 mg/m ³	35	288.98	18.45	35	296.22	17.92	20	303.79	17.18	20	307.90	15.91	20	314.24	17.25	20	325.17	16.99	
1 mg/m ³	35	293.08	24.23	35	300.21	23.77	20	307.48	22.41	20	311.60	20.60	20	318.84	20.70	20	326.72	20.81	
10 mg/m ³	35	291.22	18.05	35	298.06	17.88	20	302.31	13.49	20	307.27	12.64	20	313.52	13.05	20	321.85	13.15	
100 mg/m ³	35	277.08*	17.20	35	285.48	17.18	20	291.28	16.17	20	298.39	16.59	20	305.18	15.83	20	312.67	15.17	
		DAY 127			DAY 134			DAY 141			DAY 148			DAY 155			DAY 162		
0 mg/m ³	20	334.12	16.77	15	336.10	16.62	15	339.18	18.44	15	343.01	17.03	15	350.48	17.29	15	349.43	16.34	
1 mg/m ³	20	336.95	21.97	15	341.44	21.49	15	344.17	21.61	15	351.14	20.60	15	358.05	19.73	15	358.75	21.40	
10 mg/m ³	20	331.81	14.32	15	336.99	15.49	15	337.04	14.26	15	344.52	13.36	15	350.75	14.82	15	351.67	14.23	
100 mg/m ³	20	321.45	15.17	15	329.03	17.07	15	332.58	15.99	15	338.60	15.37	15	346.33	15.66	15	346.89	15.14	
		DAY 169			DAY 176			DAY 183											
0 mg/m ³	15	354.77	17.19	10	352.64	17.58	10	360.91	20.73	10	369.91	20.73	10	377.75	21.24	10	385.67	21.46	
1 mg/m ³	15	363.01	21.10	10	366.13	22.13	10	377.75	21.24	10	385.67	21.46	10	394.52	22.13	10	403.37	22.74	
10 mg/m ³	15	358.04	13.54	10	359.23	16.66	10	367.80	16.46	10	376.65	16.46	10	385.50	16.46	10	394.15	16.46	
100 mg/m ³	15	353.55	16.15	10	355.01	16.73	10	364.90	17.74	10	373.75	17.74	10	382.60	17.74	10	391.45	17.74	

Days 1 - 92 = 13-week exposure period

Days 99 - 183 = 13-week recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 3.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN FEMALE BODY WEIGHTS (grams)

GROUP	DAY 1			DAY 8			DAY 15			DAY 22			DAY 29			DAY 36			
	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	
0 mg/m ³	35	119.62	4.02	35	126.50	4.09	35	132.82	4.34	35	138.72	5.03	35	144.93	5.13	35	148.68	5.22	
1 mg/m ³	35	117.81	3.69	35	125.74	4.09	35	131.39	4.87	35	136.79	4.78	35	143.14	5.22	35	146.07	5.59	
10 mg/m ³	35	118.13	4.35	35	126.29	4.56	35	132.15	4.75	35	137.66	5.55	35	144.88	6.54	35	147.67	6.52	
100 mg/m ³	35	118.44	4.18	35	125.01	4.53	35	130.35	4.77	35	136.67	5.05	35	142.49	5.96	35	145.05*	5.66	
		DAY 43			DAY 50			DAY 57			DAY 64			DAY 71			DAY 78		
0 mg/m ³	35	150.98	5.48	35	152.30	6.20	35	158.31	6.38	35	160.82	6.84	35	163.73	7.10	35	167.85	6.92	
1 mg/m ³	35	149.21	5.58	35	150.18	6.08	35	157.57	6.92	35	160.30	7.31	35	163.62	7.60	35	167.81	7.67	
10 mg/m ³	35	150.21	6.83	35	151.55	7.46	35	158.57	6.98	35	162.17	7.00	35	165.47	7.93	35	169.49	7.90	
100 mg/m ³	35	147.87	6.50	35	149.79	7.11	35	156.00	6.72	35	159.38	7.88	35	161.40	7.71	35	164.91	8.07	
		DAY 85			DAY 92			DAY 99			DAY 106			DAY 113			DAY 120		
0 mg/m ³	35	170.60	7.60	35	172.02	7.96	20	173.94	6.41	20	174.10	6.68	20	174.09	6.17	20	177.82	6.14	
1 mg/m ³	35	170.44	7.80	35	171.70	8.60	20	174.88	8.25	20	174.15	8.10	20	176.72	8.02	20	178.82	7.26	
10 mg/m ³	35	172.04	7.68	35	174.70	8.68	20	176.63	8.80	20	174.87	7.90	20	177.62	7.86	20	179.64	7.45	
100 mg/m ³	35	167.59	8.72	35	170.35	9.23	20	174.94	10.05	20	174.92	9.70	20	177.60	9.84	20	179.81	10.09	
		DAY 127			DAY 134			DAY 141			DAY 148			DAY 155			DAY 162		
0 mg/m ³	20	181.07	5.91	15	182.35	3.83	15	184.47	3.56	15	183.59	4.04	15	186.98	3.96	15	186.61	4.54	
1 mg/m ³	20	183.30	7.28	15	182.78	6.95	15	182.69	6.59	15	183.49	7.05	15	186.22	7.83	15	184.70	7.23	
10 mg/m ³	20	184.15	8.31	15	188.40*	7.37	15	185.82	7.67	15	185.35	7.68	15	188.34	7.73	15	187.93	7.88	
100 mg/m ³	20	184.74	9.60	15	185.42	10.16	15	184.94	10.36	15	185.18	9.83	15	187.97	11.2	15	187.86	10.28	
		DAY 169			DAY 176			DAY 183											
0 mg/m ³	15	189.35	3.14	10	189.50	1.99	10	193.75	4.38										
1 mg/m ³	15	188.05	7.90	10	188.53	6.89	10	194.25	6.58										
10 mg/m ³	15	193.37	8.58	10	189.92	6.58	10	196.26	8.49										
100 mg/m ³	15	190.77	9.22	10	192.67	8.72	10	199.15	7.89										

Days 1 - 92 = 13-week exposure period
Days 99 - 183 = 13-week recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 4.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN MALE CELL COUNTS

GROUP	DAY	PLT			MCHC			MCH			MCV		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³	92	6	521.67	96.81	6	33.58	0.71	6	17.87	0.39	6	53.27	0.31
1 mg/m ³	92	6	464.17	63.90	6	33.73	0.49	6	17.90	0.30	6	53.05	0.46
10 mg/m ³	92	8	420.50	74.49	8	33.81	0.49	8	18.04	0.26	8	53.39	0.51
100 mg/m ³	92	7	421.29	80.34	7	33.51	0.53	7	18.11	0.49	7	54.03	0.78
			HCT			HGB			RBC			WBC	
0 mg/m ³	92	6	45.58	1.26	6	15.3	0.36	6	8.56	0.22	6	3.58	0.93
1 mg/m ³	92	6	43.78	1.55	6	14.77	0.39	6	8.25	0.27	6	3.97	0.32
10 mg/m ³	92	8	44.25	1.83	8	14.96	0.57	8	8.29	0.34	8	4.26	0.87
100 mg/m ³	92	7	41.41	5.51	7	13.87	1.79	7	7.68	1.08	7	3.69	0.96
			PLT			MCHC			MCH			MCV	
0 mg/m ³	183	9	544.11	88.11	9	33.89	0.93	9	17.49	0.49	9	51.61	0.47
1 mg/m ³	183	8	509.25	61.75	8	33.99	0.83	8	17.35	0.45	8	51.09	0.52
10 mg/m ³	183	10	547.10	54.93	10	33.83	0.85	10	17.15	0.42	10	50.70*	0.44
100 mg/m ³	183	8	481.25	90.41	8	33.21	0.82	8	16.66*	0.50	8	50.17*	0.78
			HCT			HGB			RBC			WBC	
0 mg/m ³	183	9	44.79	1.76	9	15.17	0.49	9	8.68	0.36	9	4.69	1.60
1 mg/m ³	183	8	45.48	2.49	8	15.44	0.57	8	8.90	0.45	8	4.38	0.30
10 mg/m ³	183	10	44.99	2.51	10	15.20	0.64	10	8.87	0.47	10	4.74	1.16
100 mg/m ³	183	8	43.98	2.26	8	14.60	0.57	8	8.77	0.43	8	4.72	1.50

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-week recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 5.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN FEMALE CELL COUNTS

GROUP	DAY	PLT			MCHC			HGB			HCT			MCH			MCV		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³	93	7	622.57	71.43	7	34.39	0.46	7	14.83	0.31	7	7.56	0.18	7	19.63	0.24	7	57.04	0.32
1 mg/m ³	93	9	565.67	55.13	9	34.19	0.97	9	14.01	2.13	9	7.19	1.23	9	19.58	0.7	9	57.28	0.63
10 mg/m ³	93	8	545.38*	61.57	8	34.39	0.47	8	14.63	0.32	8	7.41	0.19	8	19.71	0.23	8	57.38	0.33
100 mg/m ³	93	9	497.78*	34.69	9	34.16	0.43	9	14.30*	0.44	9	7.29*	0.24	9	19.61	0.24	9	57.42*	0.24
0 mg/m ³	93	7	43.13	1.03	7	14.83	0.31	7	14.83	0.31	7	7.56	0.18	7	19.63	0.24	7	57.04	0.32
1 mg/m ³	93	9	41.14	6.88	9	14.01	2.13	9	14.01	2.13	9	7.19	1.23	9	19.58	0.7	9	57.28	0.63
10 mg/m ³	93	8	42.54	1.21	8	14.63	0.32	8	14.63	0.32	8	7.41	0.19	8	19.71	0.23	8	57.38	0.33
100 mg/m ³	93	9	41.88	1.43	9	14.30*	0.44	9	14.30*	0.44	9	7.29*	0.24	9	19.61	0.24	9	57.42*	0.24
0 mg/m ³	184	10	577.4	77.26	10	33.90	0.36	10	33.90	0.36	10	18.87	0.28	10	18.87	0.28	10	55.66	0.53
1 mg/m ³	184	10	561.9	54.41	10	33.73	0.39	10	33.73	0.39	10	18.85	0.33	10	18.85	0.33	10	55.88	0.48
10 mg/m ³	184	10	524.1	55.65	10	34.03	0.63	10	34.03	0.63	10	19.03	0.37	10	19.03	0.37	10	55.89	0.46
100 mg/m ³	184	10	471.5*	62.59	10	33.84	0.67	10	33.84	0.67	10	18.97	0.45	10	18.97	0.45	10	56.06	0.51
0 mg/m ³	184	10	43.74	1.17	10	14.83	0.43	10	14.83	0.43	10	7.86	0.24	10	7.86	0.24	10	3.28	0.52
1 mg/m ³	184	10	44.60	1.71	10	15.04	0.58	10	15.04	0.58	10	7.98	0.31	10	7.98	0.31	10	3.25	0.37
10 mg/m ³	184	10	43.56	1.56	10	14.82	0.51	10	14.82	0.51	10	7.79	0.24	10	7.79	0.24	10	3.54	0.59
100 mg/m ³	184	10	42.57	1.19	10	14.40	0.43	10	14.40	0.43	10	7.59	0.19	10	7.59	0.19	10	3.05	0.37

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-week recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 6.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN MALE WBC DIFFERENTIAL COUNTS

GROUP	DAY	SEG			BAND			LYM			MON		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³	92	6	24.17	7.25	6	0	0	6	75.33	7.69	6	0.167	0.408
1 mg/m ³	92	6	21.17	7.55	6	0	0	6	76.67	7.17	6	0.833	0.983
10 mg/m ³	92	8	25.00	7.82	8	0	0	8	73.63	8.16	8	0.375	0.518
100 mg/m ³	92	7	29.71	6.37	7	0	0	7	69.29	6.70	7	0.571	0.787
		EOS			BAS			NRBC			RETIC		
0 mg/m ³	92	6	0.33	0.52	6	0	0	6	0.167	0.408	6	1.57	0.16
1 mg/m ³	92	6	1.33	1.21	6	0	0	6	0.333	0.516	6	1.62	0.17
10 mg/m ³	92	8	1.00	0.93	8	0	0	8	0.125	0.354	8	1.64	0.34
100 mg/m ³	92	7	0.43	0.79	7	0	0	7	0.571	0.535	7	2.24	0.85
		SEG			BAND			LYM			MON		
0 mg/m ³	183	9	29.11	12.56	9	0	0	9	69.78	12.89	9	0.333	0.707
1 mg/m ³	183	8	25.13	11.93	8	0	0	8	73.00	12.14	8	0.625	1.061
10 mg/m ³	183	10	27.90	5.80	10	0	0	10	70.40	5.70	10	0.700	0.823
100 mg/m ³	183	8	31.50	6.63	8	0	0	8	66.63	7.56	8	0.625	1.408
		EOS			BAS			NRBC			RETIC		
0 mg/m ³	183	9	0.78	0.83	9	0	0	9	0	0	9	1.90	0.43
1 mg/m ³	183	8	1.25	0.89	8	0	0	8	0	0	8	2.00	0.70
10 mg/m ³	183	10	1.00	1.05	10	0	0	10	0	0	10	2.01	0.36
100 mg/m ³	183	8	1.25	1.16	8	0	0	8	0	0	8	1.96	0.57

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-week recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 7.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN FEMALE WBC DIFFERENTIAL COUNTS

GROUP	DAY	SEG			BAND			LYM			MON		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	93	7	19.57	3.74	7	0	0	7	78.71	3.95	7	0.429	0.535
	93	9	22.44	4.98	9	0	0	9	76.22	5.04	9	0.444	0.527
	93	8	28.00*	5.32	8	0	0	8	71.13*	5.22	8	0.250	0.463
	93	9	29.22*	3.23	9	0	0	9	68.56*	3.00	9	0.778	0.667
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	93	7	1.29	0.76	7	0	0	7	0.429	1.134	7	1.39	0.37
	93	9	0.89	0.78	9	0	0	9	0.444	0.882	9	1.88	1.12
	93	8	0.63	0.92	8	0	0	8	0.125	0.354	8	1.71	0.34
	93	9	1.44	1.42	9	0	0	9	0.444	0.527	9	2.31*	0.57
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	184	10	22.10	5.28	10	0	0	10	76.70	6.04	10	0.700	0.820
	184	10	21.30	5.10	10	0	0	10	76.20	5.18	10	1.400	1.070
	184	10	28.80	7.90	10	0	0	10	68.90*	7.84	10	1.000	1.250
	184	10	34.10*	7.52	10	0	0	10	63.70*	7.96	10	0.700	0.820
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	184	10	0.50	0.85	10	0	0	10	0	0	10	2.02	0.64
	184	10	1.10	0.74	10	0	0	10	0	0	10	1.80	0.46
	184	10	1.30	0.95	10	0	0	10	0	0	10	1.97	0.58
	184	10	1.50*	0.71	10	0	0	10	0	0	10	2.61	0.43

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-week recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 8.
SUBCHRONIC INHALATION TOXICITY STUDY OF A
FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN PROTHROMBIN TIMES

GROUP	DAY	N	MEAN	SD
MALE				
0 mg/m ³	92	6	13.20	0.58
1 mg/m ³	92	3	13.00	1.56
10 mg/m ³	92	9	12.77	0.93
100 mg/m ³	92	5	13.92	0.75
FEMALE				
0 mg/m ³	183	7	14.13	1.37
1 mg/m ³	183	7	13.91	0.90
10 mg/m ³	183	8	14.32	0.46
100 mg/m ³	183	7	13.99	1.34
FEMALE				
0 mg/m ³	93	5	11.56	1.86
1 mg/m ³	93	7	11.89	1.27
10 mg/m ³	93	7	12.07	1.13
100 mg/m ³	93	6	11.88	1.34
FEMALE				
0 mg/m ³	184	10	12.40	0.95
1 mg/m ³	184	9	12.38	0.85
10 mg/m ³	184	8	12.54	0.94
100 mg/m ³	184	9	12.31	1.16

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
 Day 183 (males) or 184 (females) = Conclusion of 13-recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 9.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN MALE SERUM CHEMISTRY VALUES

GROUP	DAY	GLU			BUN			CRE			TP		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³	92	10	222.8	32.78	10	17.6	1.51	10	0.68	0.079	10	6.33	0.21
1 mg/m ³	92	10	225.0	46.63	10	18.8	4.57	10	0.74	0.207	10	6.26	0.26
10 mg/m ³	92	10	234.5	57.58	10	17.2	1.40	10	0.70	0.047	10	6.24	0.23
100 mg/m ³	92	10	263.3	63.52	10	18.2	5.05	10	0.69	0.074	10	5.92*	0.47
			ALB			GLOB			A/G RATIO			BLST	
0 mg/m ³	92	10	4.35	0.11	10	1.98	0.14	10	2.21	0.14	10	24.34	9.71
1 mg/m ³	92	10	4.29	0.17	10	1.97	0.15	10	2.19	0.16	10	30.62	9.72
10 mg/m ³	92	10	4.33	0.13	10	1.91	0.13	10	2.27	0.13	10	31.61	10.47
100 mg/m ³	92	10	4.11*	0.27	10	1.81	0.22	10	2.29	0.17	10	29.82	12.27
			GLU			BUN			CRE			TP	
0 mg/m ³	183	10	218.8	25.68	10	16.7	1.49	10	0.78	0.092	10	6.40	0.24
1 mg/m ³	183	10	208.5	24.27	10	19.1*	1.45	10	0.75	0.097	10	6.66	0.16
10 mg/m ³	183	10	222.9	29.03	10	17.7	1.06	10	0.77	0.095	10	6.59	0.27
100 mg/m ³	183	10	222.3	15.83	10	17.9	0.99	10	0.77	0.095	10	6.48	0.30
			ALB			GLOB			A/G RATIO			BLST	
0 mg/m ³	183	10	4.33	0.16	10	2.07	0.11	10	2.10	0.09	10	31.30	7.80
1 mg/m ³ *	183	10	4.48	0.15	10	2.18	0.08	10	2.06	0.11	10	49.58*	19.95
10 mg/m ³	183	10	4.43	0.14	10	2.16	0.15	10	2.06	0.11	10	46.34*	12.81
100 mg/m ³	183	10	4.37	0.21	10	2.11	0.12	10	2.08	0.09	10	37.65	8.32

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 10.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN FEMALE SERUM CHEMISTRY VALUES

GROUP	DAY	GLU			BUN			CRE			TP		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	93	10	194.1	22.70	10	17.4	1.90	10	0.74	0.171	10	6.07	0.25
	93	10	201.0	23.89	10	19.2	3.12	10	0.71	0.129	10	6.04	0.22
	93	10	190.6	24.77	10	18.3	2.21	10	0.75	0.158	10	6.18	0.25
	93	10	206.5	23.83	10	18.0	2.87	10	0.66	0.052	10	5.99	0.19
			ALB		GLOB		A/G RATIO		BLST				
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	93	10	4.20	0.15	10	1.87	0.15	10	2.26	0.16	10	26.82	9.84
	93	10	4.17	0.16	10	1.87	0.13	10	2.24	0.18	9	26.79	10.30
	93	10	4.30	0.16	10	1.88	0.15	10	2.30	0.19	9	27.58	12.22
	93	10	4.16	0.10	10	1.83	0.13	10	2.28	0.14	9	24.37	7.85
			GLU		BUN		CRE		TP				
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	184	10	191.5	18.85	10	19.7	2.67	10	0.70	0.149	10	6.14	0.22
	184	10	207.3	22.94	10	18.8	1.55	10	0.70	0.067	10	6.18	0.23
	184	10	196.5	19.51	10	19.6	2.55	10	0.79	0.247	10	6.22	0.20
	184	10	188.9	15.91	10	18.4	2.01	10	0.68	0.063	10	6.10	0.31
			ALB		GLOB		A/G RATIO		BLST				
0 mg/m ³ 1 mg/m ³ 10 mg/m ³ 100 mg/m ³	184	10	4.12	0.10	10	2.02	0.15	10	2.05	0.13	10	38.50	13.35
	184	10	4.17	0.15	10	2.01	0.10	10	2.08	0.07	10	50.07	21.83
	184	10	4.19	0.14	10	2.03	0.13	10	2.07	0.14	10	27.57	7.97
	184	10	4.13	0.17	10	1.97	0.18	10	2.11	0.14	10	41.74	21.80

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
 Day 183 (males) or 184 (females) = Conclusion of 13-recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 11.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN ENZYME VALUES

GROUP	DAY	MALE				FEMALE			
		N	MEAN	SD		N	MEAN	SD	
			AST	ALT	ALP	GGT	LDH		
0 mg/m ³	92	10	107.6	73.4	411.0	2.3	676.1	10	211.63
1 mg/m ³	92	10	141.8	100.0	393.5	2.0	697.0	10	218.00
10 mg/m ³	92	10	126.3	103.0	403.7	2.3	486.7*	10	107.24
100 mg/m ³	92	10	171.8	126.5	378.2	2.2	556.8	10	397.21
			AST	ALT	ALP	GGT	LDH		
0 mg/m ³	183	10	163.9	128.2	314.1	0	724.7	10	216.05
1 mg/m ³	183	10	246.5	194.9	356.9*	0	797.0	10	401.53
10 mg/m ³	183	10	170.1	144.8	345.0*	0	444.3*	10	128.04
100 mg/m ³	183	10	182.7	145.6	349.3*	0	455.5*	10	245.77
			AST	ALT	ALP	GGT	LDH		
0 mg/m ³	93	10	94.7	50.0	359.7	0	600.3	10	179.52
1 mg/m ³	93	10	152.1	70.1	380.8	0	625.6	10	219.42
10 mg/m ³	93	10	158.8	68.5	369.7	0	372.9*	9	80.74
100 mg/m ³	93	10	133.9	71.1	375.8	0	290.6*	9	97.15
			AST	ALT	ALP	GGT	LDH		
0 mg/m ³	184	10	123.0	90.2	377.0	0	415.2	10	113.33
1 mg/m ³	184	10	144.9	110.4	365.9	0.6	381.8	10	148.19
10 mg/m ³	184	10	130.5	92.7	348.2	0.3	256.4*	10	96.09
100 mg/m ³	184	10	137.6	96.0	366.7	0.9	227.6*	10	52.96

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 12.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN MALE ELECTROLYTE VALUES

GROUP	DAY	NA			K			CL		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³	92	10	146.7	1.06	10	5.14	1.21	10	102.4	2.41
1 mg/m ³	92	10	146.1	0.88	10	5.04	1.1	10	102.6	2.27
10 mg/m ³	92	10	146.5	1.08	10	5.61	1.75	10	101.8	2.78
100 mg/m ³	92	10	145.8	1.75	10	6.19	1.47	10	100.6	3.44
			CA			IP			MG	
0 mg/m ³	92	10	10.57	0.29	10	8.64	1.70	9	2.57	0.31
1 mg/m ³	92	10	10.64	0.30	10	8.54	0.92	10	2.53	0.38
10 mg/m ³	92	10	10.71	0.35	10	8.50	1.50	10	2.67	0.64
100 mg/m ³	92	10	10.81	0.42	10	10.27*	1.20	10	2.78	0.29
			NA			K			CL	
0 mg/m ³	183	10	144.5	0.71	10	4.64	0.96	10	94.4	1.26
1 mg/m ³	183	10	144.6	1.17	10	4.65	1.09	10	93.2	1.48
10 mg/m ³	183	10	144.9	1.52	10	4.97	1.43	10	92.9	1.52
100 mg/m ³	183	10	144.2	0.92	10	5.21	1.76	10	93.2	2.10
			CA			IP			MG	
0 mg/m ³	183	10	10.50	0.17	10	8.09	1.05	10	2.16	0.25
1 mg/m ³	183	10	10.72	0.27	10	8.29	1.12	10	2.21	0.33
10 mg/m ³	183	10	10.79	0.29	10	8.55	1.29	10	2.32	0.31
100 mg/m ³	183	10	10.70	0.36	10	8.43	1.24	10	2.20	0.44

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 13.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN FEMALE ELECTROLYTE VALUES

GROUP	DAY	NA			K			CL		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
0 mg/m ³	93	10	147.40	1.08	10	4.31	0.97	10	105.40	1.65
1 mg/m ³	93	10	147.40	0.70	10	4.74	0.73	10	106.30	2.11
10 mg/m ³	93	9	147.67	1.00	9	4.14	0.50	9	104.56	2.30
100 mg/m ³	93	9	147.89	0.78	9	4.74	0.73	9	105.22	2.05
		CA			IP			MG		
0 mg/m ³	93	10	10.24	0.39	10	7.76	2.15	10	2.37	0.31
1 mg/m ³	93	10	10.29	0.30	10	7.96	1.42	9	2.34	0.34
10 mg/m ³	93	9	10.30	0.22	9	8.22	1.88	9	2.41	0.25
100 mg/m ³	93	9	10.28	0.35	9	7.74	0.88	9	2.41	0.31
		NA			K			CL		
0 mg/m ³	184	10	145.80	0.79	10	4.26	0.75	10	98.40	1.78
1 mg/m ³	184	10	146.50	1.65	10	4.63	0.71	10	96.90	2.28
10 mg/m ³	184	10	146.10	0.88	10	4.22	0.78	10	98.00	1.76
100 mg/m ³	184	10	145.60	0.96	10	3.96	0.69	10	98.30	1.89
		CA			IP			MG		
0 mg/m ³	184	10	18.77	27.84	10	6.46	1.36	10	2.31	0.29
1 mg/m ³	184	10	10.13	0.21	10	7.52	1.83	10	2.47	0.31
10 mg/m ³	184	10	10.02	0.16	10	6.63	1.56	10	2.26	0.18
100 mg/m ³	184	10	9.93	0.20	10	6.03	1.21	10	2.10	0.27

KEY: Day 92 (males) or 93 (females) = Conclusion of 13-week exposure period
Day 183 (males) or 184 (females) = Conclusion of 13-recovery period

* = Statistically significant at $p \leq 0.05$.

TABLE 14.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN URINE VALUES

GROUP	DAY	MALE				FEMALE											
		N	MEAN	SD	UUN	N	MEAN	SD	UCR	N	MEAN	SD	SPG	N	MEAN	SD	VOL
0 mg/m ³	78	10	2400.0	579.49	10	81.94	19.83	10	1.04	0.01	10	6.66	2.31				
1 mg/m ³	78	10	2311.2	451.82	10	80.37	20.77	10	1.04	0.01	10	6.41	2.56				
10 mg/m ³	78	9	2607.0	374.74	9	91.17	19.97	9	1.05	0.01	9	6.47	1.58				
100 mg/m ³	78	10	2348.2	683.82	10	87.06	29.39	10	1.04	0.01	10	5.57	2.18				
0 mg/m ³	173	10	2205.3	552.30	10	107.72	29.08	10	1.05	0.01	10	6.39	3.14				
1 mg/m ³	173	10	2273.3	389.68	10	99.44	12.40	10	1.04	0.01	10	7.27	1.16				
10 mg/m ³	173	9	2076.7	718.24	9	93.50	33.34	9	1.04	0.01	9	11.22	12.37				
100 mg/m ³	173	10	2135.0	841.65	10	95.58	41.57	10	1.04	0.02	10	11.47	14.41				
0 mg/m ³	79	10	2010.4	375.38	10	56.06	10.48	10	1.03	0.01	10	7.04	2.26				
1 mg/m ³	79	10	2178.8	346.01	10	62.99	9.19	10	1.04	0.01	10	6.43	1.16				
10 mg/m ³	79	10	1875.0	554.02	10	54.29	12.99	10	1.03	0.01	10	7.56	2.57				
100 mg/m ³	79	9	2002.2	830.45	9	63.13	37.01	9	1.03	0.01	9	13.18	21.43				
0 mg/m ³	174	10	2050.0	726.81	10	63.13	15.51	10	1.04	0.01	10	8.16	2.79				
1 mg/m ³	174	10	1913.3	489.82	10	63.04	14.90	10	1.03	0.01	10	7.86	1.99				
10 mg/m ³	174	10	2054.5	440.18	10	67.26	13.30	10	1.04	0.01	10	7.33	2.18				
100 mg/m ³	174	10	1558.1	861.61	10	54.93	24.76	10	1.03	0.02	10	10.90	8.06				

KEY: Day 78 (males) or 79 (females) = Week 12 collection
Day 173 (males) or 174 (females) = Week 25 collection

* = Statistically significant at $p \leq 0.05$.

TABLE 15.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF GROSS NECROPSY OBSERVATIONS - INTERIM NECROPSY*

NOTE: CTLs = CONTROLS	ANIMAL SEX:	--- MALES ---		--- FEMALES ---	
		NO. IN GROUP:	TISSUE	NO. IN GROUP:	TISSUE
COMPOUND:		0	1	0	1
EXPOSURE (MG/M ³):		10	10	10	10
NO. IN GROUP:		10	10	10	10

ADRENAL					
SMALL		0	1	0	0
TOTAL:		0	1	0	0

LUNG					
FOCI		0	1	0	0
DISCOLORATION		1 ^a	0	1 ^a	10 ^b
TOTAL:		1	1	1	10

OVARY					
CYST		0	0	0	0
TOTAL:		0	0	0	0

SKIN					
CRUST		0	0	0	0
TOTAL:		0	0	0	0

TESTIS					
SMALL		0	0	0	1
TOTAL:		0	0	0	1

ANIMAL NOTE					
NO GROSS LESIONS		10 ^b	8	10 ^b	9 ^b
TAIL BROKEN		0	0	0	0
TOTAL:		10	8	10	9

* Necropsy was performed on 10 rats from the control group and 10 rats from the 10 mg/m³ group. Number does not include lung findings found after necropsy.

TABLE 16.
 SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
 INCIDENCE SUMMARY OF GROSS NECROPSY OBSERVATIONS - FINAL NECROPSY*

NOTE: CTLS = CONTROLS	ANIMAL SEX: COMPOUND: EXPOSURE (MG/M ³): NO. IN GROUP:	--- MALES ---			--- FEMALES ---		
		0	1	10	10	10	10
LIVER							
DEFORMITY		0	0	0	0	0	1
TOTAL:		0	0	0	0	0	1
OVARY							
CYST					1	2	0
ENLARGED					0	0	1
TOTAL:		0	0	0	1	2	1
ANIMAL NOTE							
NO GROSS LESIONS		10	10	10	10	8	9
TOTAL:		10	10	10	10	8	9

* The final necropsy was conducted after 13 weeks of recovery.

TABLE 18.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN MALE FINAL NECROPSY ABSOLUTE ORGAN WEIGHTS, ORGAN-TO-BODY
WEIGHTS, AND ORGAN-TO-BRAIN WEIGHT VALUES^a

GROUP	Adrenal		Kidney		Testis		Brain		Lung		Liver		
	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	N	MEAN	SD
ABSOLUTE													
0 mg/m ³	10	0.0441	10	2.18	10	3.04	10	1.81	10	1.36	10	12.27	0.76
1 mg/m ³	10	0.0456	10	2.30	10	3.04	10	1.89	10	1.52	10	13.15*	0.95
10 mg/m ³	10	0.0426	10	2.19	10	3.02	10	1.88	10	1.61*	10	12.68	0.66
100 mg/m ³	10	0.0446	10	2.22	10	3.01	10	1.89	10	2.01*	10	12.81	0.72
ORGAN-TO-BODY WEIGHT													
ABSOLUTE													
ORGAN-TO-BODY WEIGHT													
0 mg/m ³	10	0.0123	10	0.603	10	0.842	10	0.502	10	0.378	10	3.40	0.11
1 mg/m ³	10	0.0121	10	0.608	10	0.804	10	0.501	10	0.403	10	3.48	0.18
10 mg/m ³	10	0.0116	10	0.596	10	0.824	10	0.512	10	0.437*	10	3.45	0.09
100 mg/m ³	10	0.0122	10	0.609	10	0.826	10	0.520	10	0.553*	10	3.51	0.16
ORGAN-TO-BRAIN WEIGHT													
ORGAN-TO-BRAIN WEIGHT													
ABSOLUTE													
ORGAN-TO-BRAIN WEIGHT													
0 mg/m ³	10	2.47	10	122.00	10	170.10	10	23.5	10	76.12	10	686.91	94.24
1 mg/m ³	10	2.42	10	121.59	10	160.65	10	6.78	10	80.72	10	696.61	41.93
10 mg/m ³	10	2.26	10	116.58	10	160.81	10	4.61	10	85.41	10	673.98	30.50
100 mg/m ³	10	2.36	10	117.61	10	159.13	10	9.19	10	106.29*	10	677.64	48.97

a. The final necropsy was conducted after 13 weeks of recovery.

* = Statistically significant at $p \leq 0.05$.

TABLE 20.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GROUP MEAN FEMALE FINAL NECROPSY ABSOLUTE ORGAN WEIGHTS, ORGAN-TO-BODY
WEIGHTS, AND ORGAN-TO-BRAIN WEIGHT VALUES^a

GROUP	N	MEAN	SD	N	MEAN	SD	ABSOLUTE				N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
							Adrenal	Kidney	Ovary	Brain									
0 mg/m ³	10	0.0512	0.0034	10	1.30	0.06	10	0.114	0.080	10	1.73	0.05	10	0.94	0.06	10	6.37	0.30	
1 mg/m ³	10	0.0535	0.0057	10	1.27	0.09	10	0.099	0.019	10	1.73	0.03	10	1.01	0.08	10	6.45	0.40	
10 mg/m ³	10	0.0520	0.0025	10	1.31	0.06	10	0.094	0.011	10	1.75	0.05	10	1.19*	0.09	10	6.53	0.46	
100 mg/m ³	10	0.0513	0.0053	10	1.39*	0.08	10	0.096	0.011	10	1.75	0.04	10	1.35*	0.10	10	6.74	0.57	
ORGAN-TO-BODY WEIGHT																			
0 mg/m ³	10	0.0265	0.0023	10	0.671	0.035	10	0.0589	0.0420	10	0.892	0.030	10	0.487	0.030	10	3.29	0.13	
1 mg/m ³	10	0.0280	0.0029	10	0.667	0.031	10	0.0518	0.0109	10	0.904	0.025	10	0.529	0.040	10	3.37	0.14	
10 mg/m ³	10	0.0267	0.0017	10	0.672	0.045	10	0.0480	0.0051	10	0.901	0.049	10	0.610*	0.058	10	3.35	0.17	
100 mg/m ³	10	0.0258	0.0023	10	0.697	0.033	10	0.0485	0.0067	10	0.881	0.040	10	0.677*	0.045	10	3.38	0.20	
ORGAN-TO-BRAIN WEIGHT																			
0 mg/m ³	10	2.96	0.20	10	75.28	4.24	10	6.66	4.95	10	54.61	2.42	10	368.94	17.33				
1 mg/m ³	10	3.10	0.33	10	73.80	4.85	10	5.72	1.14	10	58.52	4.42	10	373.14	20.86				
10 mg/m ³	10	2.97	0.19	10	74.70	3.93	10	5.35	0.67	10	67.69*	5.17	10	372.49	28.95				
100 mg/m ³	10	2.93	0.31	10	79.26	5.33	10	5.49	0.65	10	76.94*	5.14	10	385.08	33.57				

a. The final necropsy was conducted after 13 weeks of recovery.

Statistically significant at p < 0.05

TABLE 21.

SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF MICROSCOPIC OBSERVATIONS (ALL FINDINGS) - INTERIM NECROPSY (Continued)

NOTES: ANIMALS = INTERIM SACRIFICE 1 CTLs = CONTROLS FROM GROUP(S): 1	ANIMALS AFFECTED	
	MALES	FEMALES
TISSUES WITH FINDINGS	0 1 10 100	0 1 10 100
NOSE/NASAL CAVITY	10 0 0 10	10 0 0 10
-INFLAMMATION-SUPPURATIVE, LEVEL 1	0 0 0 1	0 0 0 2
-VACUOLATION-GLANDULAR TISSUE	0 0 0 1	0 0 0 3
BONE MARROW	10 0 0 10	10 0 0 10
OVARY		
-CYST, PAROVARIAN		10 0 0 10
-MINERALIZATION		1 0 0 1
UTERUS		1 0 0 1
TESTIS	10 0 0 10	10 0 0 10
-DEGENERATION, TUBULES, UNILATERAL	0 0 0 1	
EPIDIDYMIS	10 0 0 10	
-DEGENERATION, SPERMATIC ELEMENTS, UNILATERAL	0 0 0 1	
AORTA	10 0 0 10	10 0 0 10
BONE	10 0 0 9	10 0 0 10
PERIPHERAL NERVE	9 0 0 10	9 0 0 9
PANCREAS	10 0 0 10	10 0 0 10
-ACTINUS, ATROPHY	2 0 0 0	0 0 0 1
-INFLAMMATION, CHRONIC ACTIVE, ISLETS	0 0 0 0	0 0 0 0
PROSTATE	10 0 0 10	
-CONCRETIONS	1 0 0 2	
-INFLAMMATION, LYMPHOCYTTIC	0 0 0 1	
THYMUS	10 0 0 10	10 0 0 10
SALIVARY GLAND	10 0 0 10	10 0 0 10
SEMINAL VESICLE	10 0 0 10	
BRAIN	10 0 0 10	10 0 0 10
-VACUOLATION, CEREBELLUM	0 0 0 0	0 0 0 0

TABLE 21.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF MICROSCOPIC OBSERVATIONS (ALL FINDINGS) - INTERIM NECROPSY (Continued)

T I S S U E S W I T H F I N D I N G S	ANIMALS AFFECTED	
	MALES	FEMALES
NOTES: ANIMALS = INTERIM SACRIFICE 1 CTLs = CONTROLS FROM GROUP(S): 1		
ANIMAL SEX:		
COMPOUND:		
EXPOSURE (MG/M ³):		
NO. IN GROUP:	0 1 10 100	0 1 10 100
NUMBER EXAMINED:	10 10 10 10	10 10 10 10
HEART	10 0 0 10	10 0 0 10
-INFLAMMATION, CHRONIC ACTIVE, MYOCARDIUM	4 0 0 3	0 0 0 5
-PIGMENTATION, HEMOSIDERIN	0 0 0 1	0 0 0 0
SPLEEN	10 0 0 10	10 0 0 10
-HYPERPLASIA, MESOTHELIIUM	0 0 0 1	0 0 0 0
THYROID	10 0 0 10	10 0 0 10
-CYST, ULTIMOBRANCHIAL	0 0 0 0	1 0 0 0
-CYST, FOLLICULAR	0 0 0 0	0 0 0 0
LUNG	10 10 10 10	10 10 10 10
-HYPERPLASIA, TYPE II PNEUMOCYTES	0 0 9 10	0 0 8 10
-PARTICLE-LADEN MACROPHAGES	0 10 10 10	0 10 10 10
-INFLAMMATION, CHRONIC ACTIVE	1 2 0 4	0 0 3 4
-MINERALIZATION, BLOOD VESSEL	0 0 1 0	2 4 0 0
-INTERSTITIAL THICKENING/FIBROSIS	0 0 9 10	0 0 8 10
LIVER	10 0 0 10	10 0 0 10
-INFLAMMATION, CHRONIC ACTIVE, BILE DUCTS	0 0 0 1	0 0 0 0
-MICROGRANULOMA	1 0 0 1	1 0 0 3
-VACUOLATION	0 0 0 0	0 0 0 0
-INFLAMMATION, SUPPURATIVE	0 0 0 0	0 0 0 0
LYM NODE, BRONCH	6 8 9 10	8 8 9 8
-PARTICLE-LADEN MACROPHAGES	0 0 5 8	0 0 6 6
-PIGMENTATION-BROWN/YELLOW	0 0 0 0	0 0 1 0
LYM NODE, THYMIC	9 9 10 6	7 7 7 10
-PARTICLE-LADEN MACROPHAGES	0 0 7 5	0 0 7 8
-CONGESTION	0 1 0 0	0 0 0 0
-PIGMENTATION-BROWN/YELLOW	0 2 1 0	0 3 0 0
EYE	10 0 0 10	10 0 0 10
LARYNX	10 0 0 10	10 0 0 10
-CORPORA AMYLACEA, SUBMUCOSA	5 0 0 4	1 0 0 3
-INFLAMMATION, CHRONIC ACTIVE, SUBMUCOSA	0 0 0 0	0 0 0 0
PARATHYROID	8 0 0 9	9 0 0 9

TABLE 21.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF MICROSCOPIC OBSERVATIONS (ALL FINDINGS) - INTERIM NECROPSY (Continued)

NOTES: ANIMALS = INTERIM SACRIFICE 1 CTLs = CONTROLS FROM GROUP(S): 1	ANIMAL SEX:	-- ANIMALS AFFECTED --	
		-- MALES --	-- FEMALES --
COMPOUND:	EXPOSURE (MG/M ³):	TISMO	
NO. IN GROUP:	FINDINGS	10	10
TISSUES WITH FINDINGS		0	0
SKIN	NUMBER EXAMINED:	10	10
-PARAKERATOSIS, TAIL		0	0
-INFLAMMATION-GRANULOMATOUS, DERMIS, TAIL		0	0

TABLE 22.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF MICROSCOPIC OBSERVATIONS (ALL FINDINGS) - FINAL NECROPSY

T I S S U E S W I T H F I N D I N G S	ANIMALS AFFECTED	
	MALES	FEMALES
NOTES: ANIMALS = FINAL SACRIFICE CTLs = CONTROLS FROM GROUP(S): 1		
ANIMAL SEX:		
COMPOUND:		
EXPOSURE (MG/M ³):		
NO. IN GROUP:	0 1 10 100	0 1 10 100
KIDNEY	10 0 0 10	10 0 0 10
-RENAL TUBULE, REGENERATION	4 0 0 5	0 0 0 1
-MINERALIZATION	8 0 0 5	5 0 0 3
-BROWN PIGMENTATION, TUBULES	0 0 0 0	0 0 0 1
-INFLAMMATION, CHRONIC ACTIVE	1 0 0 0	0 0 0 1
TRACHEA	10 0 0 10	10 0 0 10
-ECTASIA, LYMPHATICS	0 0 0 0	0 0 0 0
-INFLAMMATION, CHRONIC ACTIVE	0 0 0 0	0 0 0 0
ESOPHAGUS	10 0 0 10	10 0 0 10
STOMACH	10 0 0 10	10 0 0 10
-MINERALIZATION, MUSCULARIS	0 0 0 0	0 0 0 0
DUODENUM	10 0 0 10	10 0 0 10
JEJUNUM	10 0 0 10	10 0 0 10
ILEUM	9 0 0 10	10 0 0 10
CECUM	10 0 0 10	10 0 0 10
-INFLAMMATION, EOSINOPHILIC	1 0 0 0	0 0 0 0
-CONGESTION	0 0 0 0	1 0 0 0
COLON	10 0 0 10	10 0 0 10
-METAZOAN PARASITE	0 0 0 1	1 0 0 0
RECTUM	10 0 0 10	10 0 0 10
-METAZOAN PARASITE	0 0 0 1	0 0 0 0
URINARY BLADDER	10 0 0 10	10 0 0 9
ADRENAL GLAND	10 0 0 10	10 0 0 10
ALLENBURY ADIPONECTIC TISSUE	0 0 0 0	0 0 0 0
VALVULAR TISSUE	1 0 0 0	0 0 0 0
PERITONEAL FAT	10 0 0 10	10 0 0 10
CYST, PARS NERVOSA	0 0 0 0	0 0 0 0

TABLE 22.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF MICROSCOPIC OBSERVATIONS (ALL FINDINGS) - FINAL NECROPSY (Continued)

NOTES: ANIMALS = FINAL SACRIFICE CTLS = CONTROLS FROM GROUP(S): 1	ANIMAL SEX:		AFFECTED	
	MALES	FEMALES	MALES	FEMALES
T I S S U E S W I T H F I N D I N G S	0	1	100	100
EXPOSURE (MG/M ³):	10	10	10	10
NO. IN GROUP:	10	10	10	10
NOSE/NASAL CAVITY	10	0	0	0
-INFLAMMATION-SUPPURATIVE, LEVEL 1	0	0	0	0
-VACUOLATION-GLANDULAR TISSUE	0	0	0	0
BONE MARROW	10	0	0	9
OVARY				
-CYST, PAROVARIAN			10	0
-MINERALIZATION			1	0
UTERUS			0	0
TESTIS	10	0	0	8
-DEGENERATION, TUBULES, UNILATERAL	0	0	0	0
EPIDIDYMIS	10	0	0	8
-DEGENERATION, SPERMATIC ELEMENTS, UNILATERAL	0	0	0	0
AORTA	9	0	0	10
BONE	10	0	0	10
PERIPHERAL NERVE	10	0	0	10
PANCREAS	10	0	0	10
-ACINUS, ATROPHY	0	0	0	0
-INFLAMMATION, CHRONIC ACTIVE, ISLETS	0	0	0	0
PROSTATE	10	0	0	10
-CONCRETIONS	0	0	0	0
-INFLAMMATION, LYMPHOCYTIC	0	0	0	0
THYMUS	10	0	0	10
SALIVARY GLAND	10	0	0	10
SEMINAL VESICLE	10	0	0	10
BRAIN	10	0	0	10
-VACUOLATION, CEREBELLUM	0	0	0	0

TABLE 22.
SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
INCIDENCE SUMMARY OF MICROSCOPIC OBSERVATIONS (ALL FINDINGS) - FINAL NECROPSY (Continued)

NOTES: ANIMALS = FINAL SACRIFICE CTLs = CONTROLS FROM GROUP(S): 1	ANIMAL SEX:		AFFECTED			
	MALES	FEMALES	TISMO		FEMALES	
COMPOUND:	EXPOSURE (MG/W ³):	NO. IN GROUP:	NO. EXAMINED:	NO. AFFECTED:	NO. AFFECTED:	NO. AFFECTED:
TISSUES WITH FINDINGS						
HEART			10	0	0	0
-INFLAMMATION, CHRONIC ACTIVE, MYOCARDIUM			5	0	0	0
-PIGMENTATION, HEMOSIDERIN			0	0	0	0
SPLEEN			10	0	0	0
-HYPERPLASIA, MESOTHELIUM			0	0	0	0
THYROID			10	0	0	0
-CYST, ULTIMOBRANCHIAL			1	0	0	0
-CYST, FOLLICULAR			1	0	0	0
LUNG			10	10	10	10
-HYPERPLASIA, TYPE II PNEUMOCYTES			0	0	0	0
-PARTICLE-LADEN MACROPHAGES			0	10	10	10
-INFLAMMATION, CHRONIC ACTIVE			2	0	0	0
-MINERALIZATION, BLOOD VESSEL			6	1	0	0
-INTERSTITIAL THICKENING/FIBROSIS			0	0	10	10
LIVER			10	0	0	0
-INFLAMMATION, CHRONIC ACTIVE, BILE DUCTS			0	0	0	0
-MICROGRANULOMA			1	0	0	0
-VACUOLATION			4	0	0	0
-INFLAMMATION, SUPPURATIVE			1	0	0	0
LYM NODE, BRONCH			7	6	9	6
-PARTICLE-LADEN MACROPHAGES			0	2	9	5
-PIGMENTATION-BROWN/YELLOW			0	0	0	0
LYM NODE, THYMIC			10	9	10	8
-PARTICLE-LADEN MACROPHAGES			0	1	7	8
-CONGESTION			0	0	0	0
-PIGMENTATION-BROWN/YELLOW			1	1	2	0
EYE			10	0	0	9
LARYNX			10	0	0	10
-CORPORA ANYLACEA, SUBMUCOSA			0	0	0	0
-INFLAMMATION, CHRONIC ACTIVE, SUBMUCOSA			1	0	0	0
PARATHYROID			7	0	0	9

TABLE 23.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: MALE WET/DRY LUNG WEIGHTS

0-WK POST EXPOSURE NECROPSY			6-WK POST EXPOSURE NECROPSY			12-WK POST EXPOSURE NECROPSY		
ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)
AIR CONTROL GROUP								
121/M	892.1	202.1	126/M	1144.6	268.1	131/M	1205.5	331.7
122/M	610.2	134.2	127/M	1042.6	236.4	132/M	1236.1	297.2
123/M	1104.0	262.5	128/M	1127.4	251.2	133/M	1083.1	293.6
124/M	1202.0	272.9	129/M	1241.9	303.1	134/M	1239.6	299.9
125/M	863.2	202.3	130/M	1044.2	247.1	135/M	1250.3	291.8
MEAN	934.3	214.8	MEAN	1120.1	261.2	MEAN	1202.9	302.8
SD	230.4	55.8	SD	82.5	26.1	SD	69.0	16.4
1 MG/M3 GROUP								
221/M	1065.8	264.1	226/M	1287.8	295.7	231/M	1285.3	323.3
222/M	1036.6	232.6	227/M	1095.1	245.5	232/M	1237.7	288.5
223/M	1212.5	302.3	228/M	1092.3	242.8	233/M	1379.6	338.3
224/M	908.5	211.9	229/M	1194.0	283.9	234/M	1227.6	296.4
225/M	1020.9	230.9	230/M	1022.7	251.4	235/M	1169.3	288.8
MEAN	1045.9	248.4	MEAN	1138.4	263.9	MEAN	1259.9	307.1
SD	109.2	35.5	SD	103.4	24.2	SD	78.6	22.5

TABLE 23.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: MALE WET/DRY LUNG WEIGHTS (Continued)

0-WK POST EXPOSURE NECROPSY			6-WK POST EXPOSURE NECROPSY			12-WK POST EXPOSURE NECROPSY		
ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)
10 MG/M3 GROUP								
321/M	1204.1	307.1	326/M	1237.6	298.8	331/M	1415.8	343.9
322/M	1113.7	245.3	327/M	1337.7	327.7	332/M	1397.6	359.2
323/M	1410.5	357.2	328/M	1233.4	281.8	333/M	1377.5	337.1
324/M	1144.0	267.0	329/M	1370.9	324.7	334/M	1634.8	395.1
325/M	1378.1	306.8	330/M	1295.5	315.1	335/M	1430.6	359.3
MEAN	1250.1	296.7	MEAN	1295.0	309.6	MEAN	1451.3	358.9
SD	136.1	43.0	SD	60.6	19.2	SD	104.5	22.4
100 MG/M3 GROUP								
421/M	1562.1	380.3	426/M	1516.0	366.3	431/M	1818.9	453.5
422/M	1342.7	336.8	427/M	1365.7	332.7	432/M	1698.9	450.9
423/M	1269.2	334.3	428/M	1474.7	352.4	433/M	1549.2	407.6
424/M	1475.6	382.1	429/M	1699.0	422.6	434/M	1483.7	374.3
425/M	1393.3	322.2	430/M	1445.8	346.4	435/M	1652.7	438.6
MEAN	1404.6	354.3	MEAN	1500.2	364.1	MEAN	1640.7	425.0
SD	114.1	28.0	SD	124.0	34.9	SD	130.7	33.7

TABLE 24.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: FEMALE WET/DRY LUNG WEIGHTS

0-WK POST EXPOSURE NECROPSY			6-WK POST EXPOSURE NECROPSY			12-WK POST EXPOSURE NECROPSY		
ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)
AIR CONTROL GROUP								
171/F	697.3	147.6	176/F	862.1	188.7	181/F	909.7	208.6
172/F	774.1	168.2	177/F	802.7	175.5	182/F	849.1	199.6
173/F	745.3	161.5	178/F	756.7	166.4	183/F	903.0	209.5
174/F	691.1	159.0	179/F	813.0	173.0	184/F	871.9	204.2
175/F	748.5	168.9	180/F	882.1	191.0	185/F	858.5	205.3
MEAN	731.3	161.0	MEAN	823.3	178.9	MEAN	878.4	205.4
SD	35.7	8.6	SD	49.8	10.5	SD	26.8	3.9
1 MG/M3 GROUP								
271/F	722.6	167.8	276/F	814.4	180.6	281/F	828.5	199.1
272/F	763.7	169.3	277/F	869.7	195.1	282/F	859.0	201.5
273/F	698.6	160.8	278/F	863.2	187.7	283/F	900.2	206.9
274/F	883.8	186.1	279/F	885.8	214.6	284/F	840.1	184.6
275/F	799.6	182.6	280/F	824.5	181.2	285/F	955.1	217.6
MEAN	773.7	173.3	MEAN	851.5	191.8	MEAN	876.6	201.9
SD	72.7	10.6	SD	30.6	14.0	SD	51.7	12.0

TABLE 24.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: FEMALE WET/DRY LUNG WEIGHTS (Continued)

0-WK POST EXPOSURE NECROPSY			6-WK POST EXPOSURE NECROPSY			12-WK POST EXPOSURE NECROPSY		
ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)	ID	WET WEIGHT (mg)	DRY WEIGHT (mg)
10 MG/M3 GROUP								
371/F	1000.7	216.0	376/F	924.8	204.7	381/F	975.9	224.1
372/F	974.3	219.7	377/F	1006.0	230.4	382/F	1049.2	248.1
373/F	959.4	225.0	378/F	1041.4	239.2	383/F	979.8	230.3
374/F	957.2	220.0	379/F	969.1	225.4	384/F	1075.4	258.5
375/F	936.1	219.4	380/F	914.6	205.7	385/F	1131.4	267.6
MEAN	965.5	220.0	MEAN	971.2	221.1	MEAN	1042.3	245.7
SD	23.9	3.2	SD	53.6	15.3	SD	65.9	18.4
100 MG/M3 GROUP								
471/F	995.8	240.4	476/F	1062.6	260.6	481/F	1316.1	308.4
472/F	1185.7	281.4	477/F	1193.8	289.5	482/F	1085.2	264.4
473/F	1055.8	249.5	478/F	1225.7	285.2	483/F	1107.4	269.8
474/F	1049.8	249.7	479/F	1397.1	312.6	484/M	1117.4	262.3
475/F	1118.5	259.3	480/F	1124.5	258.4	485/F	1215.0	291.7
MEAN	1081.1	256.1	MEAN	1200.7	281.3	MEAN	1168.2	279.3
SD	72.9	15.7	SD	126.6	• 22.4	SD	96.5	20.0

TABLE 25.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: MALE LUNG CLEARANCE GROUP

0-WK POST EXPOSURE NECROPSY				6-WK POST EXPOSURE NECROPSY				12-WK POST EXPOSURE NECROPSY			
ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight	ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight	ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight
AIR CONTROL GROUP											
121	12.94	202.1	0.06	126	8.81	268.1	0.03	131	13.13	331.7	0.04
122	17.67	134.2	0.13	127	2.60	236.4	0.01	132	18.84	297.2	0.06
123	298.97	262.5	1.14	128	1.91	251.2	0.01	133	21.40	293.6	0.07
124	13.51	272.9	0.05	129	4.60	303.1	0.02	134	0.33	299.9	0.001
125	15.33	202.3	0.08	130	3.64	247.1	0.01	135	37.46	291.8	0.13
MEAN	71.68	214.8	0.29		4.31	261.2	0.02		18.23	302.8	0.06
STD	114	49.9	0.42		2.43	23.3	0.01		12.06	14.70	0.04
1 MG/M3 GROUP											
221	93.85	264.1	0.36	226	112.00	295.7	0.38	231	18.32	323.3	0.06
222	94.22	232.6	0.41	227	45.36	245.5	0.18	232	20.97	288.5	0.07
223	150.00	302.3	0.50	228	9.24	242.8	0.04	233	41.38	338.3	0.12
224	82.03	211.9	0.39	229	96.30	283.9	0.34	234	197.20	296.4	0.67
225	82.57	230.9	0.36	230	62.42	251.4	0.25	235	55.90	288.8	0.19
MEAN	100.53	248.4	0.40		65.06	263.9	0.24		66.75	307.1	0.22
STD	25.3	31.7	0.05		36.6	21.7	0.12		66.7	20.2	0.23

TABLE 25. (Continued)
 SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
 OF TISMO® IN RATS: MALE LUNG CLEARANCE GROUP

0-WK POST EXPOSURE NECROPSY				6-WK POST EXPOSURE NECROPSY				12-WK POST EXPOSURE NECROPSY			
ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight	ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight	ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight
10 MG/M3 GROUP											
321	357.5	307.1	1.16	326	653.1	298.8	2.19	331	660.3	343.9	1.92
322	240.7	245.3	0.98	327	186.9	327.7	0.57	332	615.0	359.2	1.71
323	916.6	357.2	2.57	328	545.8	281.8	1.94	333	481.8	337.1	1.43
324	204.4	267.0	0.77	329	894.1	324.7	2.75	334	514.7	395.1	1.30
325	332.8	306.8	1.08	330	807.2	315.1	2.56	335	649.8	359.3	1.81
MEAN	410.4	296.7	1.31		617.4	309.6	2.00		584.3	358.9	1.63
STD	259.3	38.5	0.65		247.0	172.0	0.77		72.6	20.1	0.23
100 MG/M3 GROUP											
421	2342.0	380.3	6.16	426	2016.0	366.3	5.50	431	2735.0	453.5	6.03
422	1836.0	336.8	5.45	427	308.2	332.7	0.93	432	2810.0	450.9	6.23
423	1716.0	334.3	5.13	428	1955.0	352.4	5.55	433	2456.0	407.6	6.03
424	1928.0	382.1	5.05	429	2601.0	422.6	6.15	434	1200.0	374.3	3.21
425	1763.0	322.2	5.47	430	1814.0	346.4	5.24	435	1841.0	438.6	4.20
MEAN	1917.0	351.1	5.45		1738.8	364.1	4.67		2208.4	425.0	5.14
STD	224.0	25.0	0.39		764.0	31.2	1.90		609.0	30.1	1.22

TABLE 26.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: FEMALE LUNG CLEARANCE GROUP

ID	IMMEDIATE NECROPSY			6-WK POST NECROPSY			12-WK POST NECROPSY		
	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/mg Dry Lung Weight
AIR CONTROL GROUP									
171	45.35	147.6	0.31	12.56	188.7	0.07	19.42	208.6	0.09
172	33.20	168.2	0.20	11.90	175.5	0.07	0.46	199.6	0.00
173	29.27	161.5	0.18	0.81	166.4	0.01	3.14	209.5	0.02
174	7.23	159.0	0.05	8.09	173.0	0.05	0.21	204.2	0.00
175	29.21	168.9	0.17	17.30	191.0	0.09	4.07	205.3	0.02
MEAN	28.85	161.0	0.18	10.13	178.9	0.06	5.46	205.4	0.03
STD	12.3	7.72	0.08	5.50	9.43	0.03	7.14	3.53	0.03
1 MG/M3 GROUP									
271	35.15	167.8	0.21	62.58	180.6	0.35	42.68	199.1	0.21
272	201.68	169.3	1.19	19.97	195.1	0.10	4.51	201.5	0.02
273	119.00	160.8	0.74	1691.00	187.7	9.01	7.24	206.9	0.03
274	35.34	186.1	0.19	19.19	214.6	0.09	9.49	184.6	0.05
275	42.43	182.6	0.23	25.60	181.2	0.14	13.40	217.6	0.06
MEAN	118.61	173.3	0.58	363.67	191.8	1.94	15.46	201.9	0.08
STD	66.40	12.5	0.34	664.0	12.5	3.54	13.9	10.8	0.07

TABLE 26. (Continued)
 SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
 OF TISMO® IN RATS: FEMALE LUNG CLEARANCE GROUP

IMMEDIATE NECROPSY				6-WK POST NECROPSY				12-WK POST NECROPSY			
ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/ mg Dry Lung Weight	ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/ mg Dry Lung Weight	ID	TOTAL Ti (µg)	DRY LUNG (mg)	µg Ti/ mg Dry Lung Weight
10 MG/M3 GROUP											
371	161.4	216.0	0.75	376	1253.0	204.7	6.12	381	80.2	224.1	0.36
372	291.0	219.7	1.32	377	914.0	230.4	3.97	382	58.00	248.1	0.23
373	273.4	225.0	1.22	378	1666.0	239.2	6.96	383	442.00	230.3	1.92
374	597.1	220.0	2.71	379	611.8	225.4	2.71	384	332.50	258.5	1.29
375	439.7	219.4	2.00	380	425.8	205.7	2.07	385	234.40	267.6	0.88
MEAN	352.5	220.0	1.60		974.1	221.2	4.37		229.42	245.7	0.93
STD	151.0	2.88	0.69		445.0	13.7	1.90		147.0	16.4	0.62
100 MG/M3 GROUP											
471	1542.0	240.4	6.41	476	1055.0	260.6	4.05	481	1942.0	308.4	6.30
472	1714.0	281.4	6.09	477	1721.0	289.5	5.94	482	876.5	264.4	3.32
473	1331.0	249.5	5.33	478	S/L	285.2	--	483	1475.0	269.8	5.47
474	1097.0	249.7	4.39	479	589.6	312.6	1.89	484	1807.0	262.3	6.89
475	1237.0	259.3	4.77	480	1856.0	258.4	7.18	485	1197.0	291.7	4.10
MEAN	1384.2	256.1	5.40		1044.3	281.3	4.77		1459.5	279.3	5.21
STD	291.0	14.0	0.76		695.0	20.1	2.00		391.0	17.9	1.33

S/L: Sample Lost

TABLE 27.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF
TISMO® IN RATS: PRE-STUDY SYSTEM MASS CONCENTRATION
UNIFORMITY

Location ¹		Chamber Conc.		
		2 (1 mg/m ³)	3 (10 mg/m ³)	4 (100 mg/m ³)
Reference		0.83	12.5	115.3
3F		1.00	13.0	111.3
3B		1.05	12.7	119.3
4F		1.10	12.5	128.0
4B		1.08	11.8	131.3
Reference		1.30	11.5	131.3
5F		1.23	10.6	122.0
5B		0.90	10.2	126.7
6F		1.10	11.7	131.3
6B		1.00	11.2	136.7
Reference		1.10	12.2	127.3
	WPV ²	21.9	4.25	6.68
	TPV ³	11.4	8.26	6.65
	BPV ⁴	<11.4	7.04	<6.65

1. Location is given by the shelf number and front or back of the chamber (reference is Shelf 3 in the middle).
2. Relative Standard Deviation of all samples at the reference point (middle) is the Within Port Variability (WPV).
3. Relative Standard Deviation of all locations using the first reference point is the Total Port Variability (TPV).
4. BPV is the Between Port Variability = $100 \cdot \left(S_t^2 - S_w^2 \right)^{1/2} / M_t$, where S_t and S_w are the absolute standard deviations from the total and within port samples, respectively, and M_t is the mean of analyses from one measurement at each port.

TABLE 28.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: VALIDATION RUN 1

Hour	Chamber Conc.		
	2 (1 mg/m ³)	3 (10 mg/m ³)	4 (100 mg/m ³)
1	1.88	15.9	137.0
2	1.25	9.5	86.5
3	1.05	9.4	83.3
4	1.25	10.2	89.3
5	0.83	11.3	99.3
6	1.30	11.0	96.7
\bar{X}	1.30	11.2	98.6
%RSD	27.8	21.5	19.9
% of Target	130	112	98.6

TABLE 29.
SUBCHRONIC INHALATION STUDY OF A FIBROUS
AEROSOL OF TISMO® IN RATS: VALIDATION RUN 2

Hour	Chamber Conc.		
	2 (1 mg/m ³)	3 (10 mg/m ³)	4 (100 mg/m ³)
1	1.13	10.0	99.3
2	1.13	8.9	89.3
3	0.88	7.8	68.7
4	1.05	11.3	118.0
5	1.35	12.5	119.0
6	1.52	13.0	118.0
\bar{X}	1.20	10.6	102
%RSD	19.2	19.3	19.9
% of Target	120	106	102

TABLE 30.
SUBCHRONIC INHALATION STUDY OF A FIBROUS
AEROSOL OF TISMO® IN RATS: VALIDATION RUN 3

Hour	Chamber Conc.		
	2 (1 mg/m ³)	3 (10 mg/m ³)	4 (100 mg/m ³)
1	1.50	15.7	164.0
2	1.45	13.2	132.0
3	1.16	10.8	95.3
4	1.22	11.2	92.0
5	1.40	12.1	92.0
6	1.03	11.1	94.7
\bar{X}	1.3	12.4	112
%RSD	14.3	15.1	26.8
% of Target	130%	124%	112

TABLE 31.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL
OF TISMO® IN RATS: BULK FIBER SIZE DISTRIBUTION
LOT# 2D93J

Fiber Length		
Size Range (μm)	Frequency	Percent of Total
0.085 ¹ -1	111	24.8
1-2	101	22.6
2-5	135	30.2
5-10	71	15.9
10-20	25	5.6
20-50	4	0.9
	Total 447	100.0
Fiber Width		
0.085-0.5	408	91.3
0.5-1.0	35	7.8
1.0-2.0	4	0.9
	Total 447	100.0

¹Lowest resolution on Image Analysis System at 1000X magnification.

TABLE 32.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF TISMO®
IN RATS: PRE-STUDY FIBER SIZE DISTRIBUTION

Mean Fiber Length		
Dose	Filter 1 (1000X)	Filter 2 (1000X)
1 mg/m ³	3.75	3.18
10 mg/m ³	3.77	3.24
100 mg/m ³	3.06	2.87
Mean Fiber Width		
Dose	Filter 1 (1000X)	Filter 2 (1000X)
1 mg/m ³	0.38	0.35
10 mg/m ³	0.42	0.35
100 mg/m ³	0.47	0.50

TABLE 33.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF TISMO® IN
RATS: PRE-STUDY FIBER CONCENTRATION

Chamber	Sample No.	Number of Fibers	Fibers/cc	Mean (F/cc)
1 mg/m ³	20	180	4615	3834
	21	177	3052	
10 mg/m ³	18	176	9263	11,555
	19	180	13846	
100 mg/m ³	16	103	206000	244,000
	17	141	282000	

TABLE 34.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
GRAND MEAN CONCENTRATION VALUES (mg/m³)

	1 mg/m³	10 mg/m³	100 mg/m³
MEAN	1.10	10.40	103.70
%RSD	16.20	15.90	18.20
N	385.00	385.00	385.00

TABLE 35.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
WEEKLY MEAN CONCENTRATION BY CHAMBER
EXPOSURE PERIOD: 05-NOV-1992 THROUGH 04-FEB-1993

Chamber	Exposure Week													75
	1	2	3	4	5	6	7	8	9	10	11	12	13	
2 - 1 mg/m ³	Mean	1.1	1.0	1.1	1.1	1.1	1.0	1.1	1.1	1.0	1.1	1.0	1.0	1.0
	%RSD	18.0	27.5	16.1	15.3	15.5	10.8	11.6	18.5	22.0	14.6	7.2	7.2	12.1
	n	30	30	30	24	30	30	30	24	30	31	30	30	36
3 - 10 mg/m ³	Mean	9.6	10.3	11.0	11.1	10.4	10.4	9.8	11.0	10.2	10.3	9.9	9.9	10.5
	%RSD	15.7	12.6	17.3	17.8	20.7	14.6	12.7	17.9	20.6	18.3	7.5	7.5	11.5
	n	30	30	30	24	30	30	30	24	30	31	30	30	36
4 - 100 mg/m ³	Mean	104.7	93.7	102.5	117.3	101.8	104.5	96.3	113.1	109.1	99.9	101.2	101.2	105.6
	%RSD	15.2	16.5	22.0	23.1	18.4	12.6	13.2	17.4	25.8	20.1	8.5	8.5	13.4
	n	30	30	30	24	30	30	30	24	30	31	30	30	36

TABLE 36.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
MEAN ENVIRONMENTAL DATA VALUES

	AIR CONTROL	1 mg/m³	10 mg/m³	100 mg/m³
TEMP.				
MEAN	72.9	72.4	71.5	72.0
%RSD	1.8	2.7	1.9	2.8
N	758.0	767.0	768.0	767.0
REL. HUM				
MEAN	53.7	55.5	56.5	53.6
%RSD	12.5	15.0	7.3	9.6
N	754.0	763.0	764.0	763.0
AIRFLOW				
MEAN	500.0	499.7	504.2	501.2
%RSD	0.7	0.5	0.7	0.8
N	758.0	767.0	768.0	767.0

TABLE 37.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF TISMO® IN RATS:
FIBER SIZE MEASUREMENTS (MICRONS)

DATE	1 mg/m ³		10 mg/m ³		100 mg/m ³	
	LENGTH	WIDTH	LENGTH	WIDTH	LENGTH	WIDTH
11/11/92	3.41	0.54	2.60	0.40	3.55	0.38
11/18/92	3.77	0.43	3.95	0.84	3.92	0.61
11/25/92	3.13	0.42	3.67	0.38	2.81	0.46
12/2/92	3.59	0.34	2.89	0.45	3.34	0.35
12/9/92	3.31	0.54	2.77	0.40	2.81	0.43
12/16/92	2.48	0.55	2.87	0.48	2.47	0.49
12/23/92	3.00	0.48	2.08	0.46	2.01	0.44
12/30/92	2.33	0.42	2.33	0.42	3.29	0.42
1/6/93	1.94	0.41	2.80	0.45	1.45	0.42
1/13/93	3.07	0.48	2.17	0.49	1.99	0.41
1/20/93	1.28	0.73	2.22	0.50	2.79	0.38
1/27/93	2.32	0.45	1.44	0.48	2.88	0.49
2/3/93	2.91	0.48	3.14	0.47	2.99	0.51
MEAN	2.81	0.48	2.69	0.48	2.79	0.45
STD DEV	0.71	0.10	0.67	0.12	0.68	0.07
%RSD	25.20	19.78	25.00	24.22	24.45	15.56

TABLE 38.
SUBCHRONIC INHALATION STUDY OF A FIBROUS AEROSOL OF TISMO®
IN RATS: FIBER CONCENTRATION MEASUREMENTS (F/CC)

DATE	1 mg/m³	10 mg/m³	100 mg/m³
11/11/92	1393	4324	137727
11/18/92	1105	817	40357
11/25/92	1746	2856	242143
12/2/92	2066	7046	48044
12/9/92	889	8168	253674
12/16/92	1333	8208	96089
12/23/93	1057	9769	121071
12/30/92	1333	5925	97370
1/6/93	857	2856	42661
1/13/93	1345	8968	73027
1/20/93	5381	10249	98971
1/27/93	1259	2105	112424
2/3/93	2426	5084	101849
MEAN	1707	5875	112724
STD. DEV	1191	3122	67104
%RSD	70	53	60

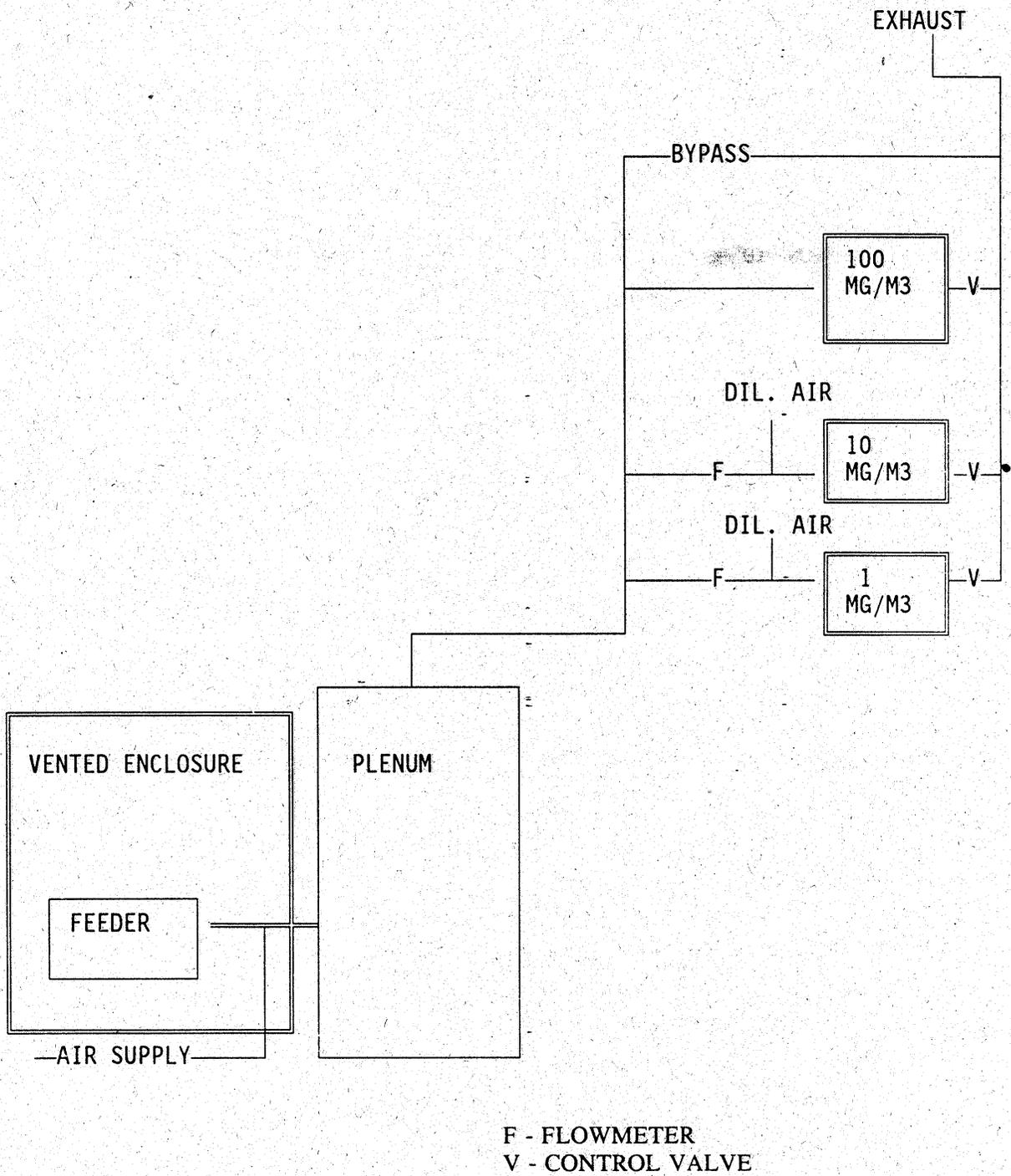


FIGURE 1. FIBER AEROSOL GENERATION AND EXPOSURE SYSTEM

GROUP MEAN BODY WEIGHTS OF MALES IN THE
SUBCHRONIC INHALATION TOXICITY STUDY OF
A FIBROUS AEROSOL OF TISMO IN RATS

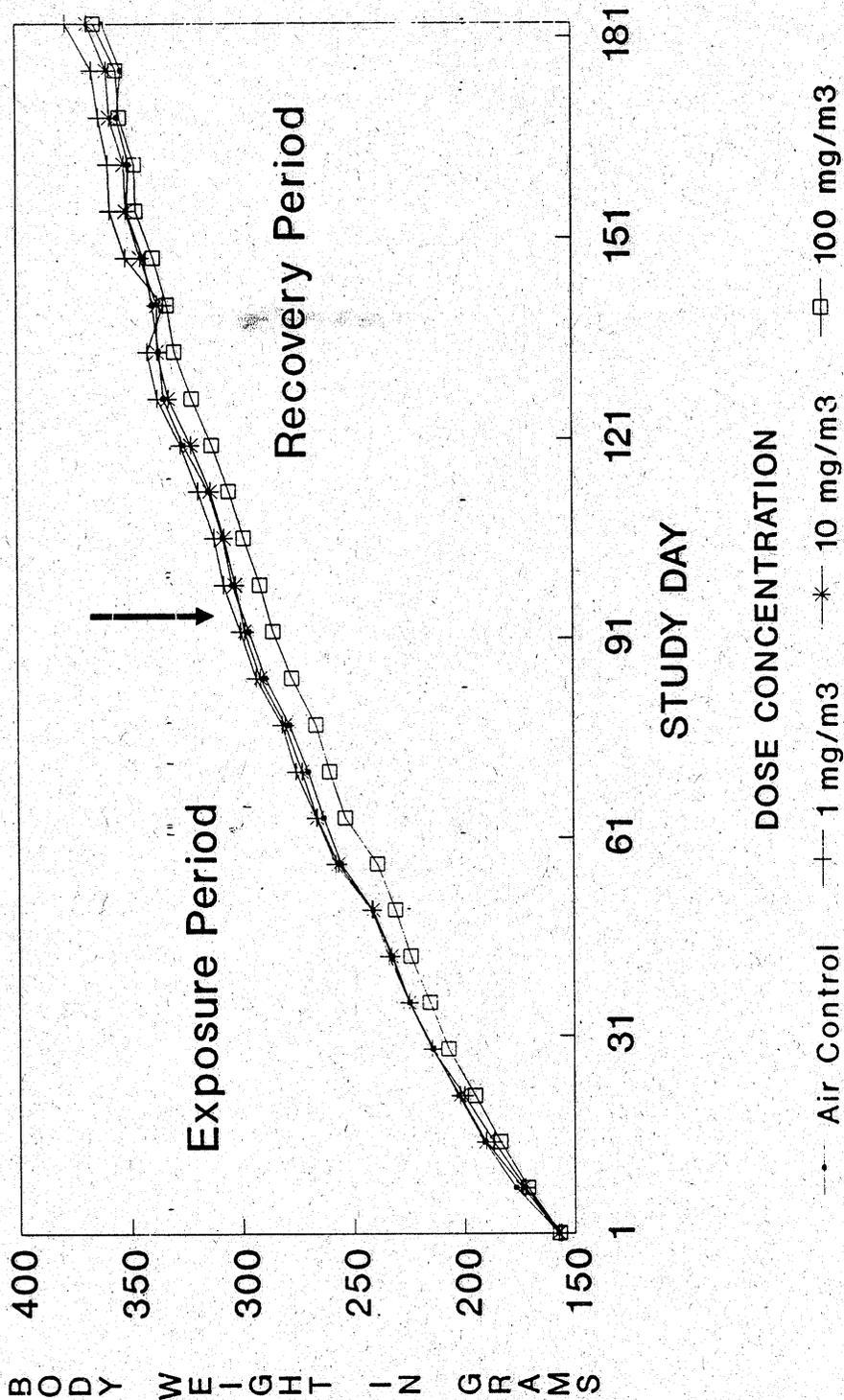


FIGURE 2. GROUP MEAN BODY WEIGHTS OF MALES IN THE SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL IN RATS

GROUP MEAN BODY WEIGHTS OF FEMALES FROM
THE SUBCHRONIC INHALATION TOXICITY STUDY
OF A FIBROUS AEROSOL OF TISMO IN RATS

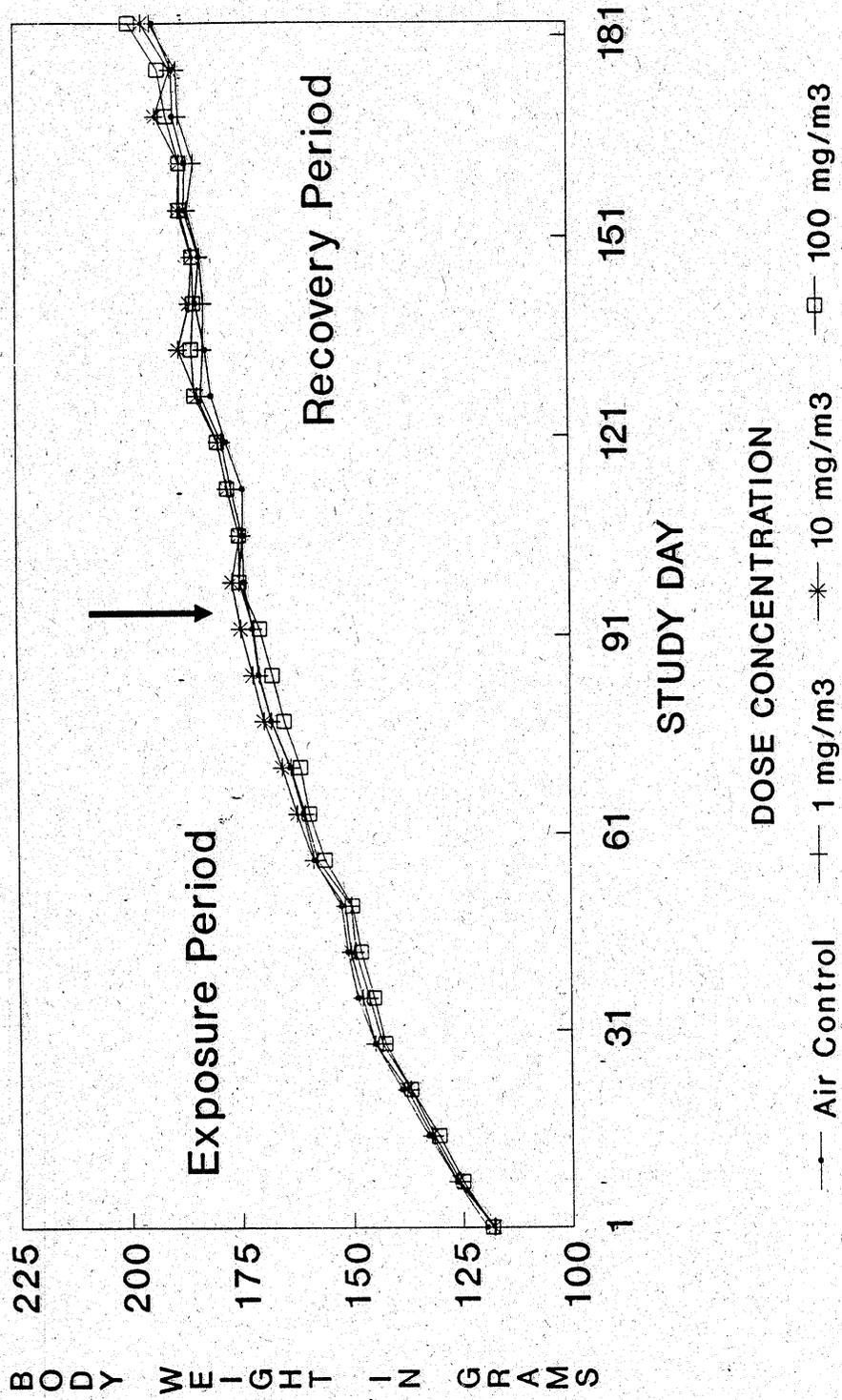


FIGURE 3. GROUP MEAN BODY WEIGHTS OF FEMALES IN THE SUBCHRONIC INHALATION TOXICITY STUDY OF A FIBROUS AEROSOL IN RATS

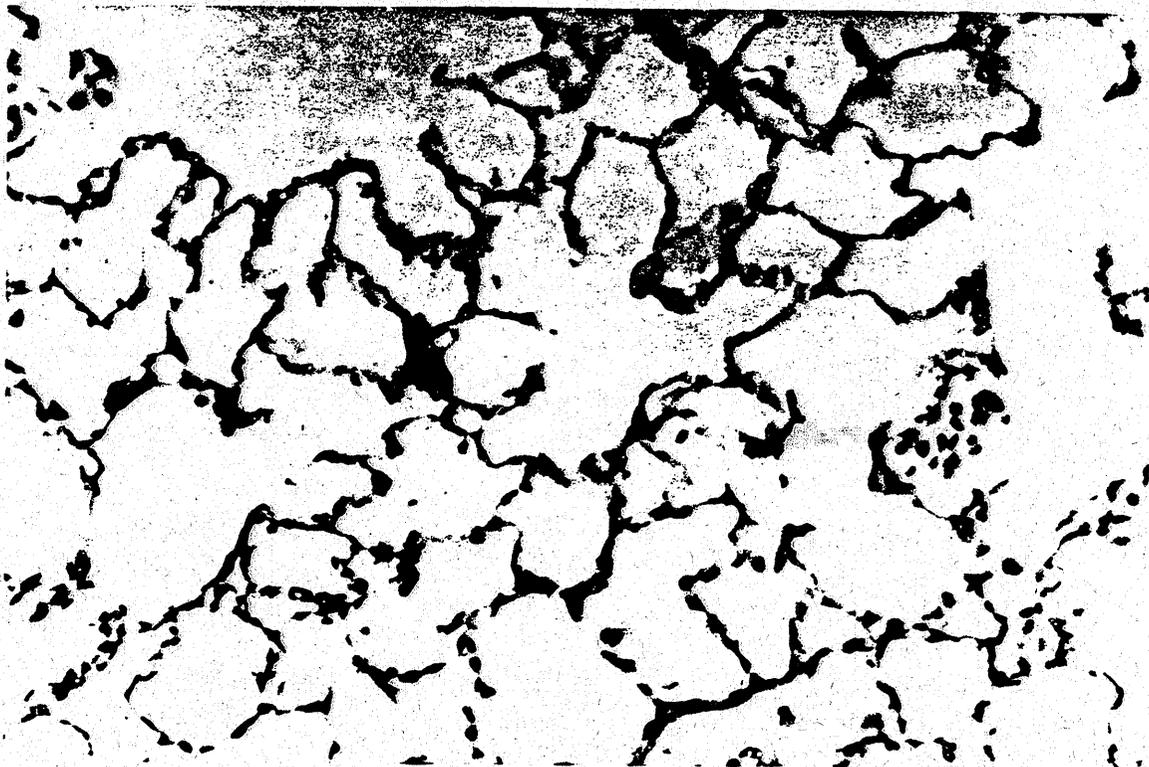


FIGURE 4. AIR CONTROL BASE-STUDY MALE RAT LUNG (#104)



FIGURE 5. 1 MG/M³ BASE-STUDY MALE RAT (#203). Note occasional particle-filled alveolar macrophages randomly scattered throughout alveoli.

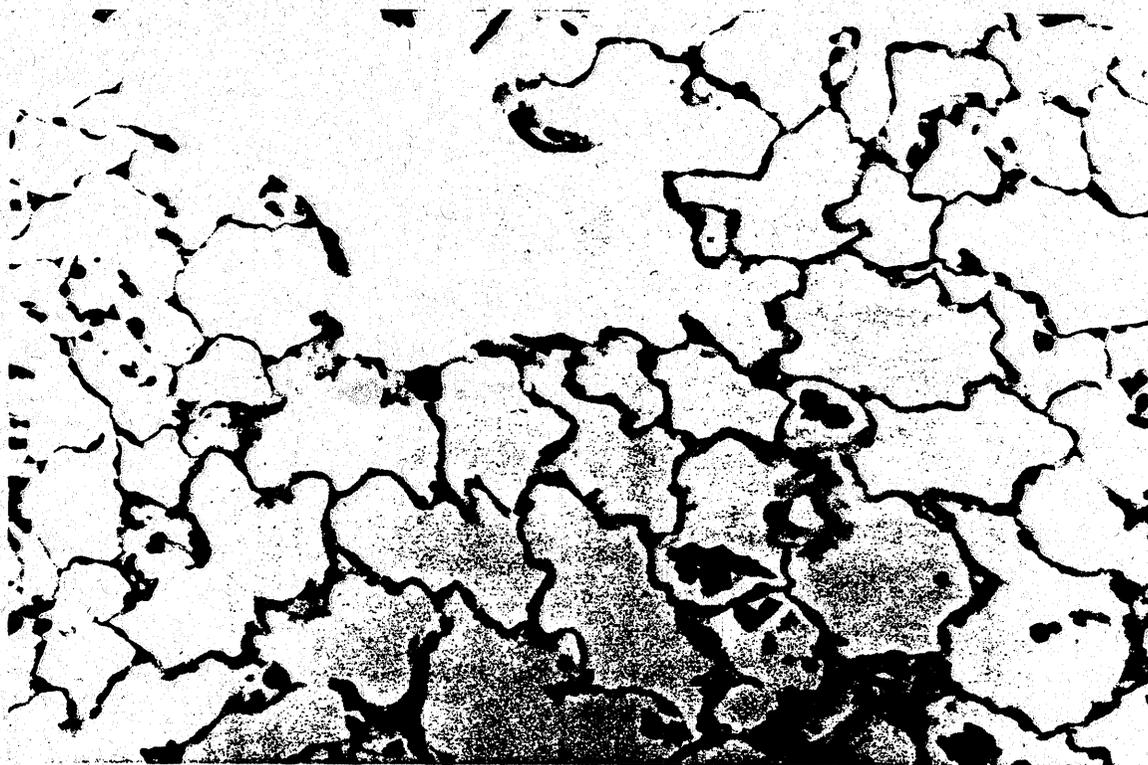


FIGURE 6. 1 MG/M³ RECOVERY MALE RAT (#213). Particle-filled alveolar macrophages are less likely to be randomly distributed, and are clumping near alveolar ducts.

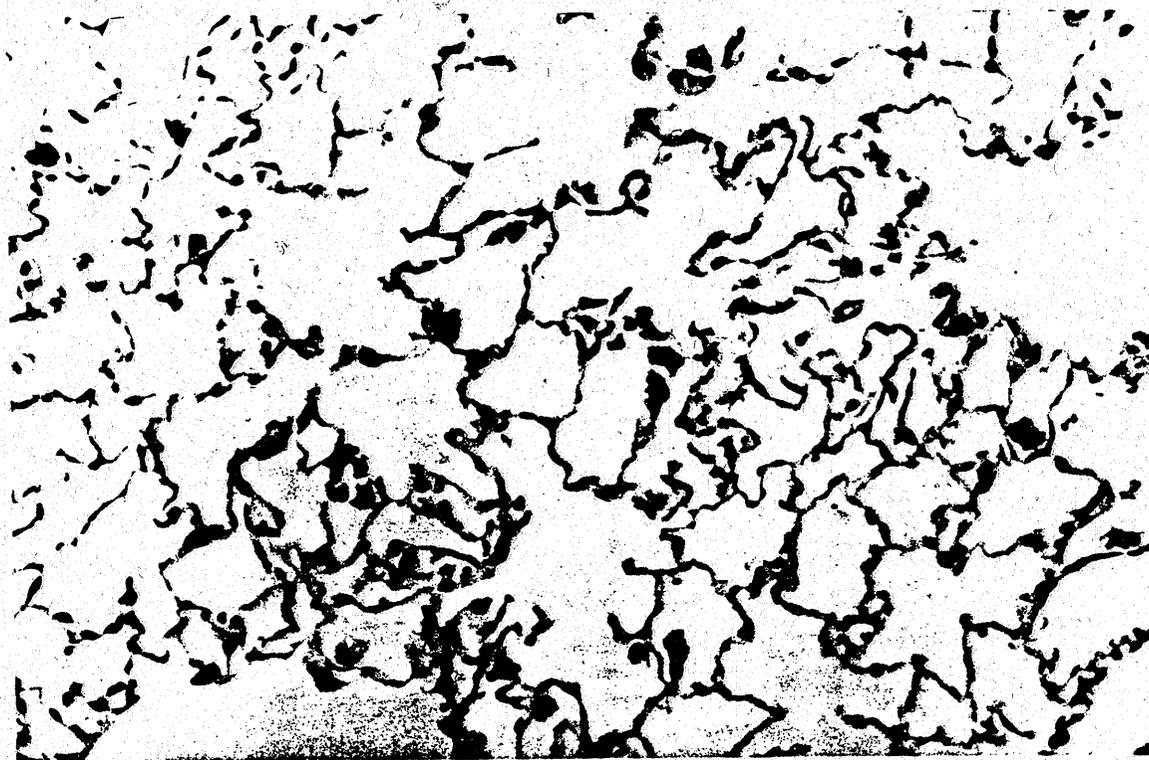


FIGURE 7. 10 MG/M³ BASE-STUDY MALE RAT (#307). Particle-filled macrophages are randomly distributed. Increased numbers of macrophages are present, relative to #203.



FIGURE 8. 10 MG/M³ RECOVERY MALE RAT (#320). Particle-filled macrophages are again clumping near alveolar ducts.

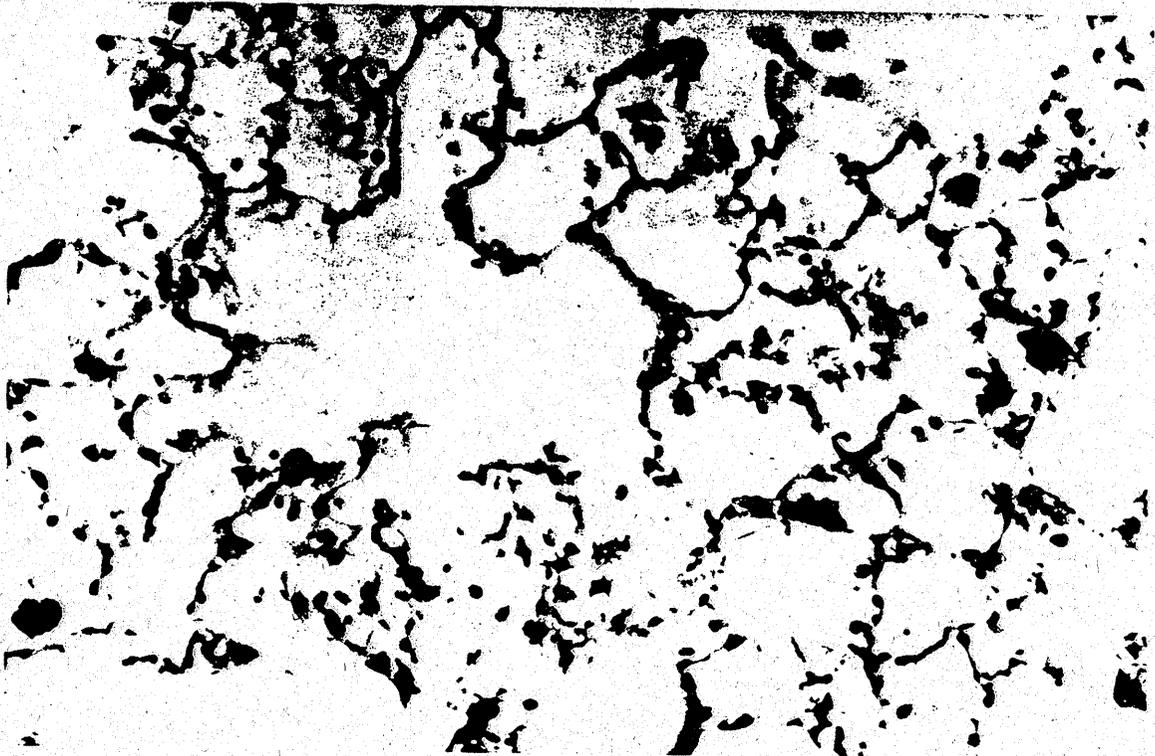


FIGURE 9. 100 MG/M³ BASE-STUDY MALE RAT (#410). Macrophages packed with fibers are scattered throughout the lung section. Rare fibers are also seen with alveolar septae.

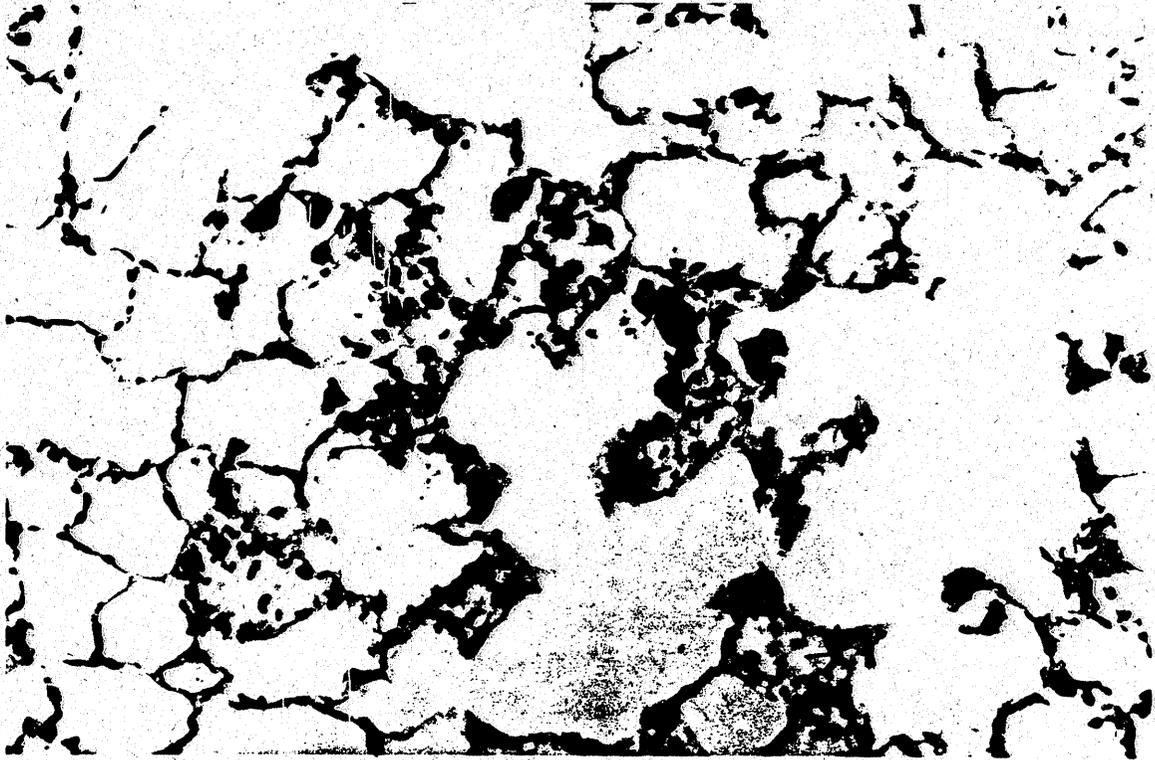


FIGURE 10. 100 MG/M³ RECOVERY MALE RAT (#413). Particle-filled macrophages are again clumping near alveolar ducts. A few fibers can be detected within the alveolar septae, which are marginally thickened by the fibers and macrophages.

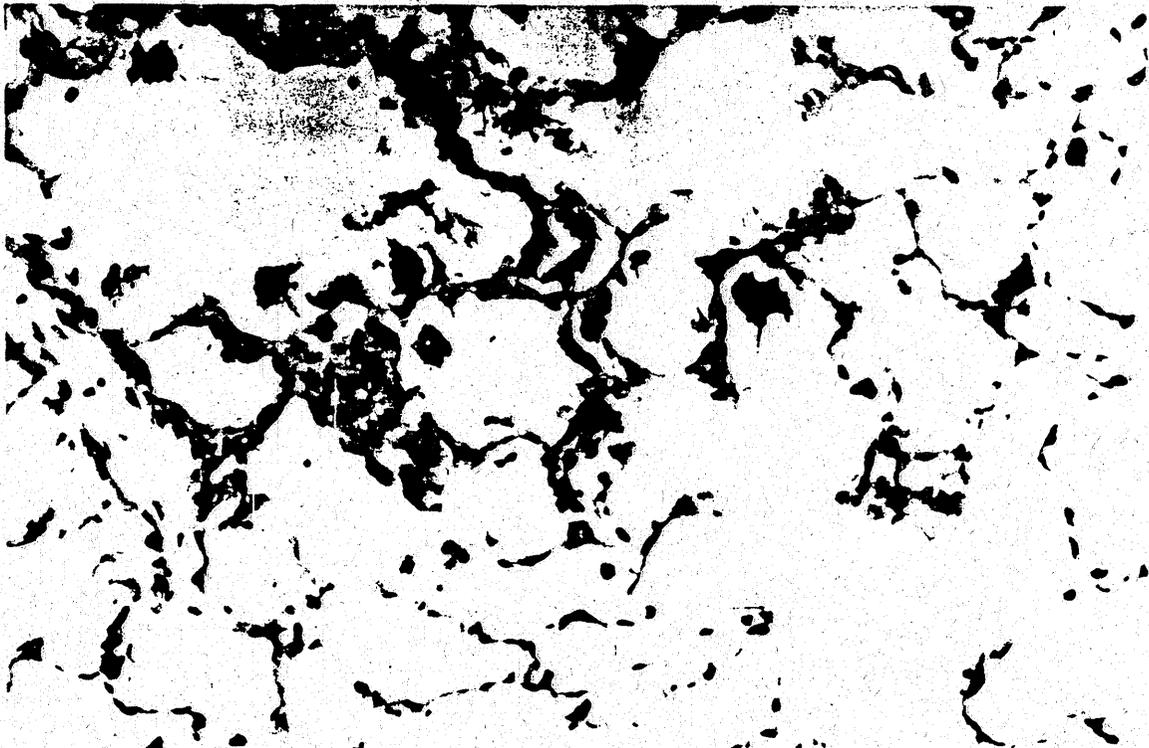


FIGURE 11. 100 MG/M³ BASE-STUDY FEMALE RAT (#460). Particle-filled macrophages are disseminated throughout the lung section, similar to the males of the same exposure level.

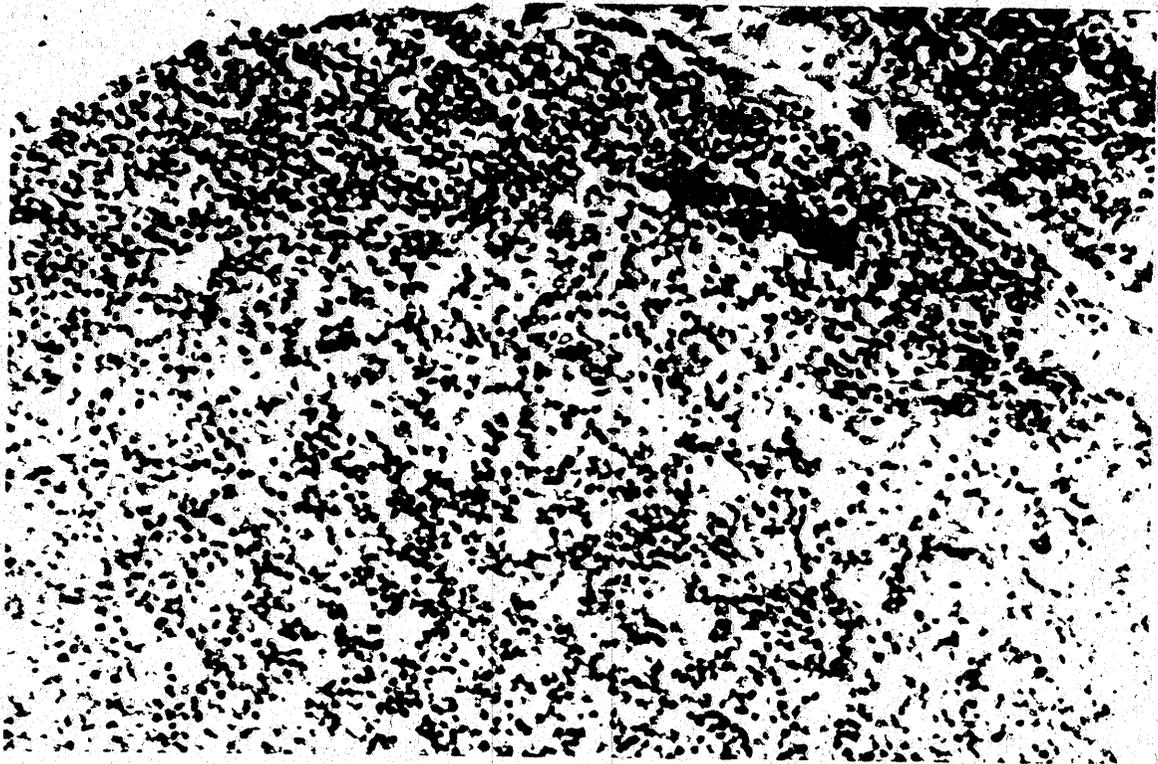


FIGURE 12. 100 MG/M³ BASE-STUDY MALE RAT (#410). Mediastinal lymph node; fiber-packed alveolar macrophages have migrated into the cortical sinuses.



FIGURE 13. 100 MG/M³ RECOVERY MALE RAT (#413). Mediastinal lymph node; particle-laden macrophages are found within the same general areas of the nodes of recovery rats.

MALE LUNG CLEARANCE ANIMALS

TOTAL TITANIUM (mcg)

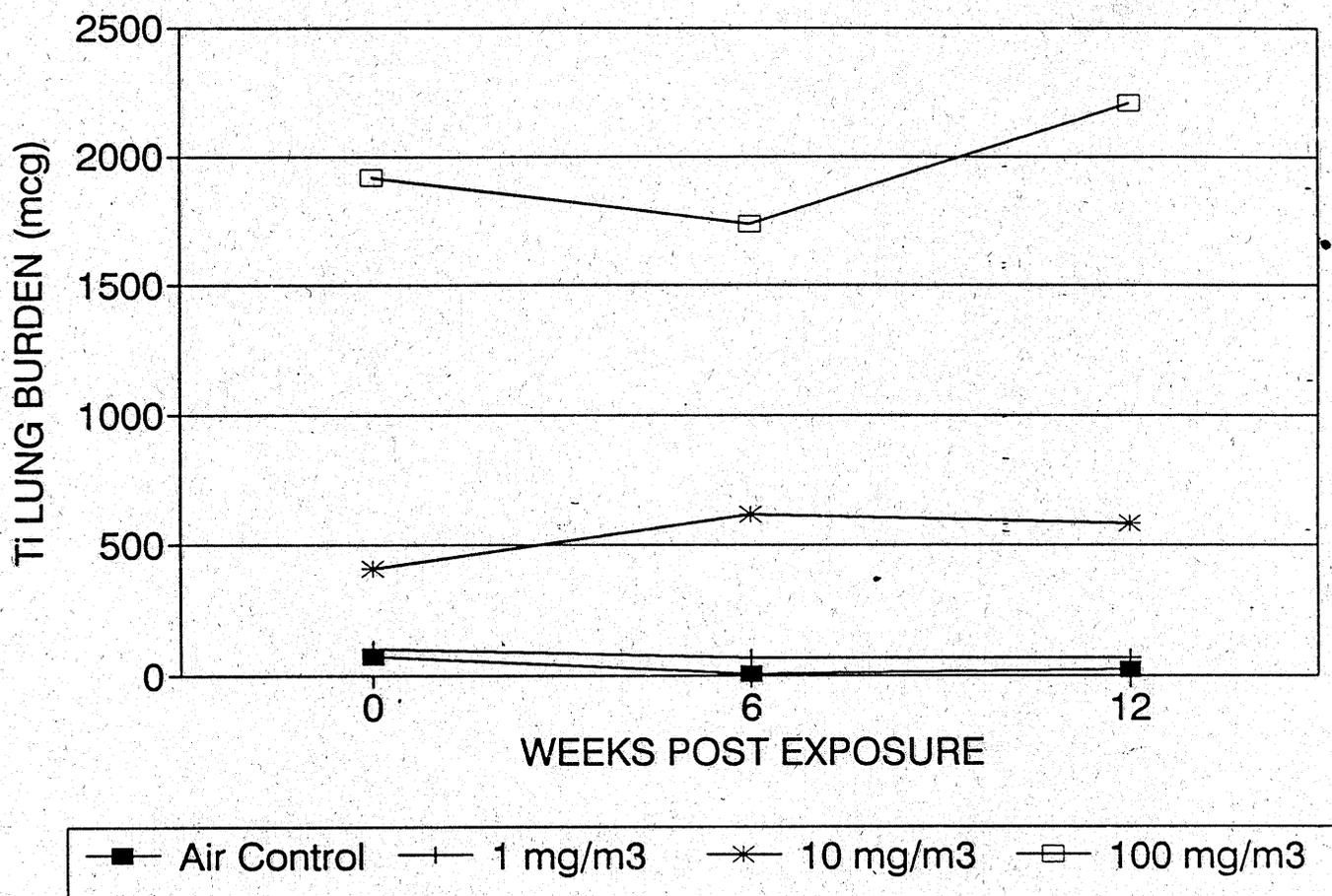


FIGURE 14. TITANIUM BURDEN IN MALE RATS

FEMALE LUNG CLEARANCE ANIMALS

TOTAL TITANIUM (mcg)

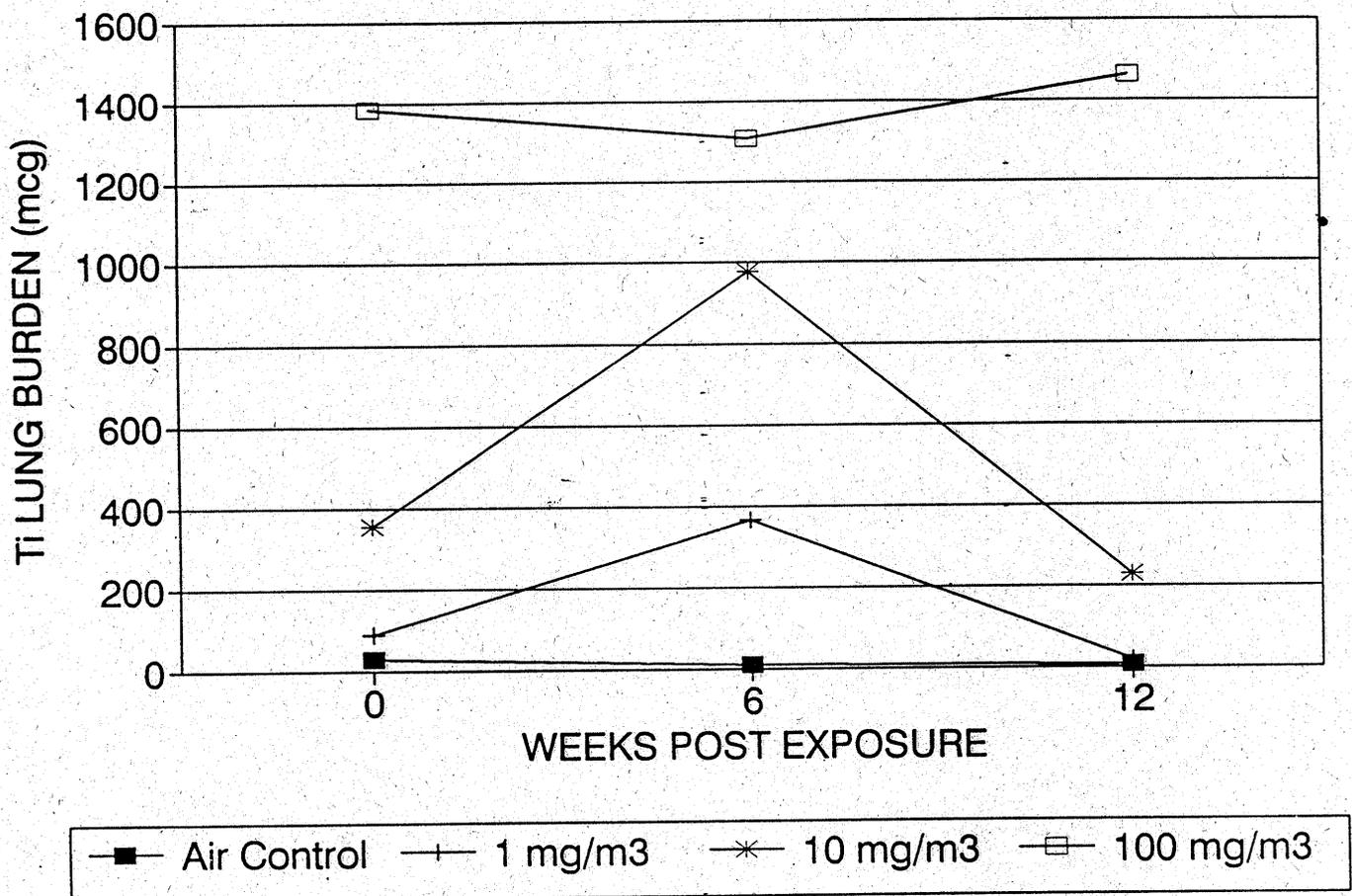


FIGURE 15. TITANIUM BURDEN IN FEMALE RATS

PERCENT OF TOTAL FIBERS¹
(LOT #2D93J)

Fiber Diameter (μm)						
1-2	0.00	0.22	0.22	0.22	0.22	0.00
0.5-1.0	2.01	2.01	2.01	0.89	0.22	0.67
0.2-0.5	22.8	20.4	28.0	14.8	5.1	0.2
0-0.2	0.00	0.00	0.00	0.00	0.00	0.00
	0-1	1-2	2-5	5-10	10-20	20-50
	Fiber Length (μm)					

¹Mean Fiber Length = $3.61\mu\text{m}$, Mean Fiber Width = $0.35\mu\text{m}$
 STD DEV = 4.2 STD DEV = 0.14

FIGURE 16. DISTRIBUTION OF POTASSIUM OCTATITANATE FIBERS - BULK FIBERS



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Review of Final Report: "Inhalation Toxicity Study of Fibrous Aerosol in Rats"
(Single-dose, 14-day study on Potassium Octatitanate fibers). FYI-1097-001310,
Submitted by Otsuka Chemical Company, Ltd., October 21, 1997.

FROM: David Lai, Ph.D. *David Lai (11/20/97)*
Toxicologist
Existing Chemicals Assessment Branch
Risk Assessment Division (7403)

TO: Vanessa Vu, Director
Risk Assessment Division (7403)

THRU: Lois Dicker, Chief
Existing Chemicals Assessment Branch
Risk Assessment Division (7403)

The attached is my review of the subject study. Please contact me should you
have any questions regarding this review.

Attachment



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I. CONCLUSIONS

The purpose of this study was to evaluate the potential acute inhalation toxicity of potassium octatitanate fibers (trade name "TISMO") in rats. Five male and 5 female Fischer 344 rats were exposed to an aerosol of potassium octatitanate fibers at a mean total mass concentration of 2.0 mg/L for 4 hours. There was no mortality, no weight loss, no non-reversible clinical signs in the exposed animals examined after a 14-day observation period. The necropsy findings of minimal discoloration and white foci in the lungs of 9 of 10 animals exposed is typically seen at necropsy in the lungs of rats exposed to high levels of particulates.

There are no apparent limitations and deficiencies in the design and conduct of the study. A mass aerosolized concentration of 2 mg/L was determined in a trial exposure study to be the highest achievable concentration. It should be noted, however, that although the samples tested were all < 3 microns in width and were thus respirable, they were primarily short fibers with 75-80% <5 microns in length.

II. BASIS FOR CONCLUSIONS

1. Study Design: The purpose of this study was to evaluate the potential acute inhalation toxicity of potassium octatitanate fibers (trade name "TISMO") in rats. Five male and 5 female Fischer 344 rats were exposed to an aerosol of potassium octatitanate fibers at a mean total mass concentration of 2.0 mg/L for 4 hours. Approximately 75-80% of the fibers were <5 microns in length; all fibers measured were <3 microns in width with the greatest % between 0.25 and 0.7 microns. The fiber concentration of fibers <5 microns in length was 21.4×10^5 fiber/cc and that of fibers >5 microns was 6.3×10^5 fibers/cc. Fourteen days after exposure, all animals were sacrificed and a gross necropsy was performed on each animal.

2. Results: All animals survived the four hour exposure and the fourteen observation period. There was no weight loss in any of the animals exposed. Clinical signs of lethargy and ruffled fur and moderate dyspnea occurred in the first week after exposure but disappeared in the second week. Gross necropsy results showed randomly distributed, minute, white foci in all lobes of the lungs of 9 of the 10 animals exposed, suggestive of foreign body exposure and an alveolar macrophage response to high levels of particulates.

3. Discussions: There are no apparent limitations and deficiencies in the design and conduct of the study. A mass aerosolized concentration of 2 mg/L was determined in a trial exposure study to be the highest achievable concentration. It should be noted, however, that although the samples tested were all < 3 microns in width and were thus respirable, they were primarily short fibers with 75-80% <5 microns in length.



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I. CONCLUSIONS

The purpose of this study was to evaluate the potential toxicity resulting from 10 days of repeated inhalation exposure to fibrous aerosols of potassium octatitanate fibers (trade name "TISMO") in rats. The study was also conducted to select concentrations for a subsequent 90-day subchronic inhalation toxicity study. In this study, groups of 5 male and 5 female Fischer 344 rats were exposed by whole-body inhalation to an aerosol concentration (0, 10, 30, 100, 330, or 1,000 mg/m³) of potassium octatitanate fibers, 6 hours/day, 5 days/week for a total of 10 exposures conducted within 14 days. The mean fiber length ranged from 2.57-3.38 microns and the mean fiber width ranged from 0.32-0.47 microns for the test fibers. The mean fiber concentrations collected from the samples were 60 x 10³, 113 x 10³, 340 x 10³, 246 x 10³ and 495 x 10³ fibers/cc for the 10, 30, 100, 330 and 1,000 mg/m³ chambers, respectively. All animals were necropsied on day 15 for macroscopic tissue evaluations, organ weight measurements and histopathologic examination on the entire respiratory tract.

There are no apparent limitations and deficiencies in the design and conduct of the study. Based on the findings in this 14-day study, a no observed adverse effect level (NOAEL) was estimated to be approximately 30 mg/m³ for male rats (due to relative increases in lung:brain weight values) and 100 mg/m³ for female rats, where animals exposed to 330 and 1,000 mg/m³ concentrations showed significant body and organ weight changes, and concentration-dependent respiratory tract toxicity.

II. BASIS FOR CONCLUSIONS

1. Study Design: The purpose of this study was to evaluate the potential toxicity resulting from 10 days of repeated inhalation exposure to fibrous aerosols of potassium octatitanate fibers (trade name "TISMO") in rats. The study was also conducted to select concentrations for a subsequent 90-day subchronic inhalation toxicity study. In this study, groups of 5 male and 5 female Fischer 344 rats were exposed by whole-body inhalation to an aerosol concentration (0, 10, 30, 100, 330, or 1,000 mg/m³) of potassium octatitanate fibers, 6 hours/day, 5 days/week for a total of 10 exposures conducted within 14 days. The mean fiber length ranged from 2.57-3.38 microns and the mean fiber width ranged from 0.32-0.47 microns for the test fibers. The mean fiber concentrations collected from the samples were 60 x 10³, 113 x 10³, 340 x 10³, 246 x 10³ and 495 x 10³ fibers/cc for the 10, 30, 100, 330 and 1,000 mg/m³ chambers, respectively. All animals were necropsied on day 15 for macroscopic tissue evaluations, organ weight measurements and histopathologic examination on the entire respiratory tract.

2. Results: There were no unscheduled animal deaths in this study. Significant weight loss was observed in the two high dose groups (330 and 1,000 mg/m³). The most frequent clinical signs were thin rough coat and labored respiration in the high concentration group (1,000 mg/m³). All other animals exposed to the test fibers or the air control were clinically normal throughout the study. The male rats exposed to 100 mg/m³ had significant higher lung to brain weight ratios compared to air controls. Animals in the two high dose groups (330 and 1,000 mg/m³) showed significant increase in absolute lung weights, lung to body weights and lung to brain weight ratios compared to air control rats. Also, the adrenal to body weight ratio was increased in animals exposed to the highest concentration (1,000 mg/m³). In addition, the liver and kidney in the 1,000 mg/m³ exposure group were significantly decreased in weight compared to the air control group.

Gross necropsy results showed a pale discoloration of the normal pink lungs in all animals exposed to the highest concentration (1,000 mg/m³), and in 1 of the 5 male rats in the 330 mg/m³ group. The discoloration was considered to be due to the presence of large numbers of alveolar macrophages in response to high levels of fibrous particulates.

Microscopically, there was evidence of inflammatory changes throughout the upper and lower respiratory tract of rats exposed to 330 and 1,000 mg/m³ aerosol concentrations. These changes often accompanied by mucinous exudates, exudated rhinitis and granulomas that obstructed many of the airways.

3. Discussions: There are no apparent limitations and deficiencies in the design and conduct of the study. Based on the findings in this 14-day study, a no observed adverse effect level (NOAEL) was estimated to be approximately 30 mg/m³ for male rats (due to relative increases in lung to brain weight ratios) and 100 mg/m³ for female rats, where animals exposed to 330 and 1,000 mg/m³ concentrations showed concentration-dependent respiratory tract toxicity.



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TO: Vanessa Vu, Director
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I. CONCLUSIONS

The objectives of this 90-day inhalation toxicity in rats were to: (i) characterize the potential toxicity of potassium octatitanate fibers (trade name "TISMO"), (ii) identify the specific effects on target organs, (iii) determine the potential reversal or progression of these effects, (iv) estimate the rate of lung clearance of the test material deposited in the lungs over the 13-week exposure period, and (v) to select concentrations for a subsequent chronic inhalation toxicity study. These objectives were met.

There are no apparent limitations and deficiencies in the design and conduct of the study. Based on the findings in this 90-day study, a no observed adverse effect level (NOAEL) was estimated to be 1 mg/m³ for both male and female rats. Animals exposed to concentrations of 10 and 100 mg/m³ showed significant increased lung burdens of test fibers (increased absolute lung weights, lung-to-body weight and lung-to-brain weight ratios, particle-laden macrophages) and toxic responses of the lungs (hyperplasia of type II pneumocytes and fibrosis).

The recovery groups studies suggested that the lung and lymph node lesions were neither reversible nor progressive in nature after 13-week of recovery from the exposure of TISMO. Lung clearance animals showed a well defined concentration relationship in total titanium burden in the lung. Histologic evidence of fiber clearance was seen in recovery rats. However, the lung clearance data were highly variable and did not allow good quantitation of fiber clearance

II. BASIS FOR CONCLUSIONS

1. Study Design:

In this study, groups of 10 male and 10 female Fischer 344 rats were exposed by whole-body inhalation to one of three aerosol concentrations (1, 10, and 100 mg/m³) of potassium octatitanate fibers or to filtered air (control), 6 hours/day, 5 days/week for a 13 weeks. In addition, there were three subgroups of rats within each test concentration group: a based study subgroup (10 rats/sex/concentration), a recovery subgroup (10 rats/sex/concentration), and a lung clearance subgroup (15 rats/sex/concentration). Based study subgroups were necropsied the day following their last exposure. Recovery subgroups were necropsied at the conclusion of the 13-week recovery period. Five rats/sex/concentration from the lung clearance subgroups were necropsied at three different intervals: 1) the end of the 91-day exposure period, 2) after approximately 1.5 months following the end of exposure, and 3) after approximately 3 months following the end of exposures.

The mean fiber lengths of all samples collected were 2.81, 2.69, and 2.79 microns for the 1, 10, and 100 mg/m³ chambers, respectively. The mean fiber width for all samples collected were 0.48, 0.48, and 0.45 microns for the 1, 10, and 100 mg/m³ chambers, respectively. The mean fiber concentrations collected from the samples were 1707, 5875, and 112,724 fibers/cc for the 1, 10, and 100 mg/m³ chambers, respectively.

Weekly clinical observations, body weight values, clinical pathology evaluations, organ weight measurements, and gross and microscopic observations were the parameters used to assess toxicity.

2. Results: There were no unscheduled animal deaths in this study. Significant weight loss was only observed in the high dose male group (100 mg/m³). Increased absolute lung weights, lung-to-body weight and lung-to-brain weight ratios were noted for both sexes in the 10 and 100 mg/m³ groups at the interim and final necropsy.

Exposure-related macroscopic tissue changes, characterized as diffuse discoloration of the lungs, was observed in all high-dose rats (100 mg/m³). Exposure-related microscopic changes were observed in the lungs of all rats. Rats at all dose groups had particle-laden macrophages in the lungs and regional lymph nodes (bronchial and thymic). The amount of particles within the lungs was dose-dependent, ranging from a very slight trace amount (severity grade 1) at 1 mg/m³ to a moderate (severity grade 3)/severe (severity grade 4) distribution at 100 mg/m³. Additional lung responses included hyperplasia of type II pneumocytes and fibrosis in groups exposed to concentrations of 10 mg/m³ and 100 mg/m³.

The recovery groups studies suggested that the lung and lymph node lesions were neither reversible nor progressive in nature after 13-week of recovery from the exposure of TISMO. Lung clearance animals showed a well defined concentration relationship in total titanium burden in the lung. Histologic evidence of fiber clearance was seen in recovery rats. However, the lung clearance data were highly variable and did not allow good quantitation of fiber clearance. Rats examined microscopically at the end of the recovery phase had compound-induced lesions of the lungs and lymph nodes that were similar to those at the end of treatment.

3. Discussions: There are no apparent limitations and deficiencies in the design and conduct of the study. Based on the findings in this 90-day study, a no observed adverse effect level (NOAEL) was estimated to be 1 mg/m³ for both male and female rats. Animals exposed to concentrations of 10 and 100 mg/m³ showed significant increased lung burdens of test fibers (increased absolute lung weights, lung-to-body weight and lung-to-brain weight ratios, particle-laden macrophages) and toxic responses of the lungs (hyperplasia of type II pneumocytes and fibrosis).