

8EHQ-1298-13585

2 DCN: 88961000065

December 3, 1997

TSCA Document Processing Center (7407)  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
Attn: TSCA Section 8(e) Coordinator  
401 M Street S.W.  
Washington, D.C. 20460

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Re: Supplemental Submission to 8EHQ-0796-13585 and 8EHQ-0197-13585  
TSCA Section 8(e) Notification of Substantial Risk  
Octamethylcyclotetrasiloxane

Dear Sir:

In accordance with the provisions of Section 8(e) of the Toxic Substances Control Act (TSCA), as interpreted in the Statement of Interpretation and Enforcement Policy (40 FR 11110, March 16, 1978), Dow Corning is submitting a pair of recently completed studies as a supplemental submission to our Notifications of Substantial Risk of July 2, 1996 (8EHQ-0796-13585), and December 20, 1996 (8EHQ-0197-13585).

The information described in this supplemental submission was generated in preliminary and repeat range-finding inhalation reproductive toxicity studies with octamethylcyclotetrasiloxane that we conducted as a part of our Siloxane Research Program, a voluntary health effects testing program which was the subject of a Memorandum of Understanding dated April 9, 1996 between Dow Corning and EPA.

**Chemical Substance:**

556-67-2 Octamethylcyclotetrasiloxane

**Manufacturer:**

Dow Corning Corporation  
2200 West Saizburg Road  
Midland, Michigan 486867-0994

Contains No CBI

**Recently Completed Studies:**

- (1) Dow Corning Study Number 8462



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Dow Corning Corporation  
Midland, Michigan 48686-0994  
Phone: (517) 495-4000



39930020083

**AN INHALATION RANGE-FINDING REPRODUCTIVE TOXICITY STUDY  
OF OCTAMETHYLCYCLOTETRAILOXANE (D4) IN MALE RATS**

Dow Corning Corporation  
1997-10000-43725  
October 28, 1997

(2) Dow Corning Study Number 8601

**AN INHALATION RANGE-FINDING REPRODUCTIVE TOXICITY STUDY  
OF OCTAMETHYLCYCLOTETRAILOXANE (D4) IN MALE RATS**

Dow Corning Corporation  
1997-10000-43726  
October 29, 1997

**Executive Summary:**

As a supplemental submission to our earlier TSCA Section 8(e) notifications of July 2, 1996 and December 20, 1996, Dow Corning is submitting the final reports for two range-finding inhalation reproductive toxicity studies in which male rats were exposed to octamethylcyclotetrasiloxane (OMCTS, D<sub>4</sub>). In the first study, slight decreases (statistically insignificant) in pup viability and statistically significant reductions in F<sub>1</sub> pup weights were noted in offspring of male rats from the high exposure group (700 ppm). A second study of similar design but of greater statistical power failed to detect any indication of adverse effects in pups sired by male similarly exposed to OMCTS. We conclude that OMCTS does not cause adverse effects in the offspring of male rats exposed to OMCTS.

**Background:**

In a letter dated July 2, 1996, Dow Corning notified EPA of results obtained in an ongoing preliminary range-finding inhalation reproductive toxicity study in male rats with octamethylcyclotetrasiloxane (OMCTS, D<sub>4</sub>), identified as Dow Corning Study Number 8462, in which decreased pup viability and reduced pup weights were seen in the offspring of male rats exposed by whole body vapor inhalation to 700 ppm OMCTS. In a letter dated December 2, 1997, Dow Corning notified EPA that a repeat range-finding inhalation reproductive toxicity study of greater statistical power, identified as Dow Corning Study Number 8601, failed to detect an indication of adverse effects in pups sired by male rats similarly exposed to OMCTS.

**Details of Study Findings:**

(1) Dow Corning Study Number 8462

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A study was conducted to identify adverse reproductive effects that might occur following whole body vapor inhalation exposure of male rats to OMCTS and to provide data useful for assisting in the selection of exposure concentrations for a two-generation reproductive toxicity study. Five groups of male CrI:CD<sup>1</sup>(SD)BR rats (N = 22) were exposed to 0 (control group, exposed to filtered air only), 70, 300, 500, and 700 ppm OMCTS six hours daily for at least 70 days prior to mating with female rats which had been exposed only to filtered air for at least 21 consecutive days prior to mating, all exposures continuing throughout the mating interval. Exposure of males ceased at the end of their mating period, at which time ten males from each group were euthanized and subject to sperm motility assessment and morphology evaluation. Exposure of females to filtered air continued through gestation until gestation day 20.

All F<sub>0</sub> females were allowed to deliver and rear their pups until lactation day 4. The surviving F<sub>0</sub> dams were necropsied following completion of F<sub>1</sub> pup necropsies. F<sub>0</sub> females which failed to deliver were necropsied on post-mating day 25 (evidence of mating) or 25 days following the breeding period (no evidence of mating). The surviving F<sub>0</sub> males were necropsied just prior to necropsy of the F<sub>0</sub> females.

Treatment-related clinical signs noted in the 700 ppm males one hour after exposure included red material around the nose. An exposure-related increase in the number of ejaculatory plugs produced by males was noted in all exposure groups. Mean body weights/body weight gains and food consumption was unaffected by exposure to the test article. No exposure-related macroscopic or microscopic findings were noted at scheduled necropsy of F<sub>0</sub> males. Mean absolute and relative liver, kidney, and thyroid glands were increased in the 700 ppm males and mean absolute and relative liver weights were increased in 500 ppm males at the week 12 necropsy. No effects on organ weight data were noted at the week 16 necropsy, indicating that the observed increases in organ weights were reversible. No adverse effects were noted in overall reproductive performance, gestation length, number of implantation sites, number of pups born, unaccounted for implantation sites, or on sperm motility or morphology.

Slight, statistically insignificant reductions in pup survival were noted in the 700 ppm group on PND 0 and during the birth to PND 4 interval. Interpretation of the significance of this finding was confounded by the fact that the decreases were due primarily to two litters with increased numbers of dead pups on PND 0. Mean pup body weights in the 700 ppm group on PND 1 and 4 were statistically significantly decreased compared to the concurrent control group, but were within the range of values in the test facility historical control data. Mean live litter sizes and pup sex ratios were unaffected by exposure at all concentrations tested. No

treatment-related signs were noted in the pups at any capture level. No exposure-related findings were noted on necropsy of F<sub>1</sub> pups.

(2) Dow Corning Study Number 8601

A repeat study was conducted to evaluate the reproducibility of the F<sub>1</sub> findings (a statistically insignificant decrease in pup viability and a statistically significant reduction in pup weights) noted in the earlier study (see above). This repeat study was designed with larger test animal groups at the upper end of the exposure concentrations to allow greater statistical power.

Three groups of male Sprague-Dawley rats (N = 40) were exposed to 0 (control group, exposed to filtered air only), 500, and 700 ppm OMCTS six hours daily for at least 70 days prior to mating with female rats which had been exposed only to filtered air for at least 21 consecutive days prior to mating. Exposures of F<sub>0</sub> males continued throughout the mating interval through study day 113. The males then entered a non-exposure recovery period lasting approximately five weeks. Exposure of females continued through the mating interval and through gestation and lactation until the day prior to necropsy, except that exposures were discontinued from gestation day 21 through lactation day 4.

All F<sub>0</sub> females were allowed to deliver and rear their pups to weaning on lactation day 21. F<sub>0</sub> females which failed to deliver were necropsied on post-mating day 25 (evidence of mating) or 25 days following the breeding period (no evidence of mating). The surviving F<sub>0</sub> males were necropsied at the conclusion of the non-exposure recovery period.

Treatment-related clinical signs noted in the 700 ppm males one hour after exposure included red material around the nose. An exposure-related increase in the number of ejaculatory plugs produced by males was noted in the 500 and 700 ppm group males. Mean body weight gains and food consumption were reduced in the 700 ppm group males during week 0-1. Body weight and food consumption data were unaffected at an exposure level of 500 ppm. No exposure-related macroscopic or microscopic findings were noted at scheduled necropsy of F<sub>0</sub> males. Absolute and relative organ weights were unaffected by test article exposure.

The mean live litter size, number of pups born and percentage of males per litter at birth were similar in the control and treated groups. Pup survival and mean pup weights were unaffected at both exposure levels throughout lactation. No exposure-related findings were noted at the necropsy of pups on PND 21.

**Actions:**

Copies of both studies also are being provided to EPA under TSCA Section 8(d) (health and safety data reporting).

For purposes of supplemental reporting under TSCA Section 8(e), the general INTERNAL designation on the attached health and safety studies is waived by Dow Corning.

Dow Corning will inform EPA of any pertinent information that may be developed concerning this material.

If you have any questions concerning this study, please contact me at the address provided herein. If you require further general information regarding this submission, please contact Dr. Rhys G. Daniels, Regulatory Compliance Specialist, Regulatory Compliance Department, at 517-496-4222 or at the address provided herein.

Sincerely,

  
Michael P. Hill  
Americas Vice President and Corporate Director  
Health and Environmental Sciences  
(517) 496-4057

RGD97259

**DOW CORNING CORPORATION  
HEALTH & ENVIRONMENTAL SCIENCES  
TECHNICAL REPORT**

**WIL Research Laboratories, Inc.  
1407 George Road  
Ashland, Ohio 44805**

**Report No.:** 1997-10000-43725

**Title:** An Inhalation Range-Finding Reproductive Toxicity Study of Octamethylcyclotetrasiloxane (D4) in Male Rats

**Study No.:** 8462

**External Testing Facility No.:** WIL-51042

**Test Article:** Octamethylcyclotetrasiloxane (D4)

**Study Director:** Joseph F. Holson, Ph.D.  
President, Director

**Author:** Lewis E. Kaufman, B.A.  
Group Supervisor of Technical Report Writing

**Sponsor:** Dow Corning Corporation

**Sponsor Study Monitor:** Vincent L. Reynolds, Ph.D., D.A.B.T.

**Test Facility:** WIL Research Laboratories, Inc.  
1407 George Road  
Ashland, Ohio 44805

**Study Completion Date:** October 28, 1997

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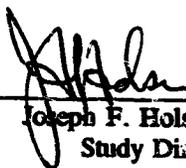
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External No. - WIL-51042

DC Report No. - 1997-10000-43725  
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An Inhalation Range-Finding Reproductive Toxicity  
Study of Octamethylcyclotetrasiloxane (D4) in Male Rats

**COMPLIANCE STATEMENT**

This study, designated WIL-51042, was conducted in compliance with the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States Food and Drug Administration (21 CFR Part 58) and Environmental Protection Agency (40 CFR Parts 160 and 792) and the Standard Operating Procedures of WIL Research Laboratories, Inc. The study was conducted in accordance with the protocol as approved by the Sponsor, except as indicated in Appendix G.



\_\_\_\_\_  
Joseph F. Holson, Ph.D.  
Study Director

10/28/97

\_\_\_\_\_  
Date

**An Inhalation Range-Finding Reproductive Toxicity  
Study of Octamethylcyclotetrasiloxane (D4) in Male Rats**

**I. SUMMARY**

This study was conducted 1) to identify adverse reproductive effects that might occur following whole body vapor inhalation exposure of male rats to octamethylcyclotetrasiloxane (D4), and 2) to provide data useful for assisting in the selection of exposure concentrations for a two-generation reproductive toxicity study.

Groups of male CrI:CD<sup>®</sup>(SD)BR rats (N=22) were exposed to test article six hours daily for at least 70 consecutive days prior to mating. Target test article concentrations were 70, 300, 500, and 700 ppm; the actual mean measured exposure concentrations were 71, 300, 492, and 694 ppm, respectively. A control group of identical design was exposed to filtered air on a comparable regimen. The female CrI:CD<sup>®</sup>(SD)BR rats used in this study were not exposed to test article, but were exposed to filtered air six hours daily for at least 21 consecutive days prior to mating. All exposures (both to test article and to filtered air) for both sexes continued throughout the mating interval. Exposure of the males ceased at the end of their mating period; exposure of the females to filtered air continued through gestation until gestation day 20. All animals were observed twice daily for appearance and behavior. Body weights were recorded weekly for both sexes prior to mating; maternal body weights were also recorded on gestation days 0, 7, 10, 14, and 20 as well as on lactation days 1 and 4. Food consumption was measured for corresponding intervals prior to mating, during gestation, and during lactation. All F<sub>0</sub> females were allowed to deliver and rear their pups until lactation day 4. The surviving F<sub>0</sub> dams were necropsied following completion of the F<sub>1</sub> pup necropsies. F<sub>0</sub> females which failed to deliver were necropsied on post-mating day 25 (evidence of mating) or 25 days following the breeding period.

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(no evidence of mating). The surviving F<sub>0</sub> males were necropsied after the breeding period (week 12; 10/group) or just prior to necropsy of the F<sub>0</sub> females (weeks 15-16; all remaining animals). The males that were necropsied during week 12 were exposed to D4 until one or two days prior to necropsy, whereas the males necropsied during week 16 had a one-month non-exposure period prior to necropsy. Selected organs from all F<sub>0</sub> males were weighed at the scheduled necropsies. For the F<sub>0</sub> males that were necropsied following completion of mating, selected tissues were examined microscopically (all groups), sperm motility was measured (all groups), and sperm morphology was evaluated (control, 500, and 700 ppm groups).

One male in the 700/0 ppm exposure group died on study day 97. This death was due to renal inflammation with septicemia, and was not attributed to the test article. One female in the 700/0 ppm exposure group was euthanized *in extremis* by order of the Study Director on lactation day 1 (study day 95). The moribundity of this female was not treatment-related since the females were not exposed to the test article. All other animals survived to the scheduled necropsies. Treatment-related clinical signs noted in the 700/0 ppm group males one hour following exposure included red material around the nose. Increased numbers of ejaculatory plugs were produced by the males in all exposure groups, primarily during the daily exposure period. This increase was exposure-related and became evident approximately two weeks after treatment initiated. Plug production then continued throughout the remainder of the study. The significance of this clinical sign is not known.

Reproductive parameters (days between pairing and coitus, mating indices, fertility indices, and duration of gestation and parturition) were not adversely affected by exposure to the test article at any exposure level. Fertility and mating indices were 90.9% or higher in all exposure groups, including the control group.

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Mean body weights and body weight gains in the 70/0, 300/0, 500/0, and 700/0 ppm groups were unaffected by exposure to the test article. Food consumption, evaluated as g/animal/day and g/kg/day, was unaffected by exposure throughout the study.

At the scheduled necropsies of the F<sub>0</sub> males, no exposure-related internal findings were noted. No treatment-related findings were noted at the microscopic examination of tissues collected during the necropsy immediately following completion of the breeding period (week 12 necropsy of F<sub>0</sub> males). Sperm motility was unaffected by exposure to the test article at any concentration. Sperm morphology in the 500/0 and 700/0 ppm groups was comparable to the control group; this parameter was not evaluated in the 70/0 and 300/0 ppm groups. Mean absolute and relative liver, kidney, and thyroid gland weights were increased in the 700/0 ppm group males at the week 12 necropsy. Mean absolute and relative liver weights were also increased in the 500/0 ppm group males at the week 12 necropsy. No effects on organ weight data were noted at the week 16 recovery necropsy, indicating that the increases in liver, kidney, and thyroid gland weights noted at the week 12 necropsy were reversible.

Slight, statistically insignificant reductions in pup survival (% per litter) were noted in the 700/0 ppm group on PND 0 and during the birth to PND 4 interval. However, interpretation of the significance of this finding was confounded by the fact that the decreases were primarily due to two litters with increased numbers of dead pups on PND 0. Mean pup body weights in the 700/0 ppm group on PND 1 and 4 were statistically significantly decreased compared to the concurrent control group, but were within the ranges of values in the WIL historical control data. Mean live litter sizes and pup sex ratios were unaffected by exposure at all concentrations tested. No treatment-related clinical signs were noted in the pups

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at any exposure level. At the scheduled necropsy of pups on PND 4 and the necropsies of pups that were found dead, no exposure-related findings were noted.

In conclusion, parental toxicity (males) was exhibited at an exposure level of 700/0 ppm by clinical signs and apparently reversible increases in liver, kidney, and thyroid gland weights. Slight parental toxicity was also observed at the 500/0 ppm concentration by apparently reversible increases in liver weights. No parental toxicity was apparent at concentrations of 70/0 and 300/0 ppm. No adverse effects on F<sub>1</sub> pups were observed at exposure levels of 70/0 and 300/0 ppm. At the 700/0 ppm exposure level, reduced pup survival (statistically insignificant) and reduced pup body weights (statistically significant) were observed during the postnatal period. However, interpretation of the significance of these findings is confounded by the facts that 1) the reduced pup survival was primarily due to two litters with increased PND 0 mortality, and 2) the pup weights were within the range of the WIL historical control data.

## II. OBJECTIVE

The purposes of this study were:

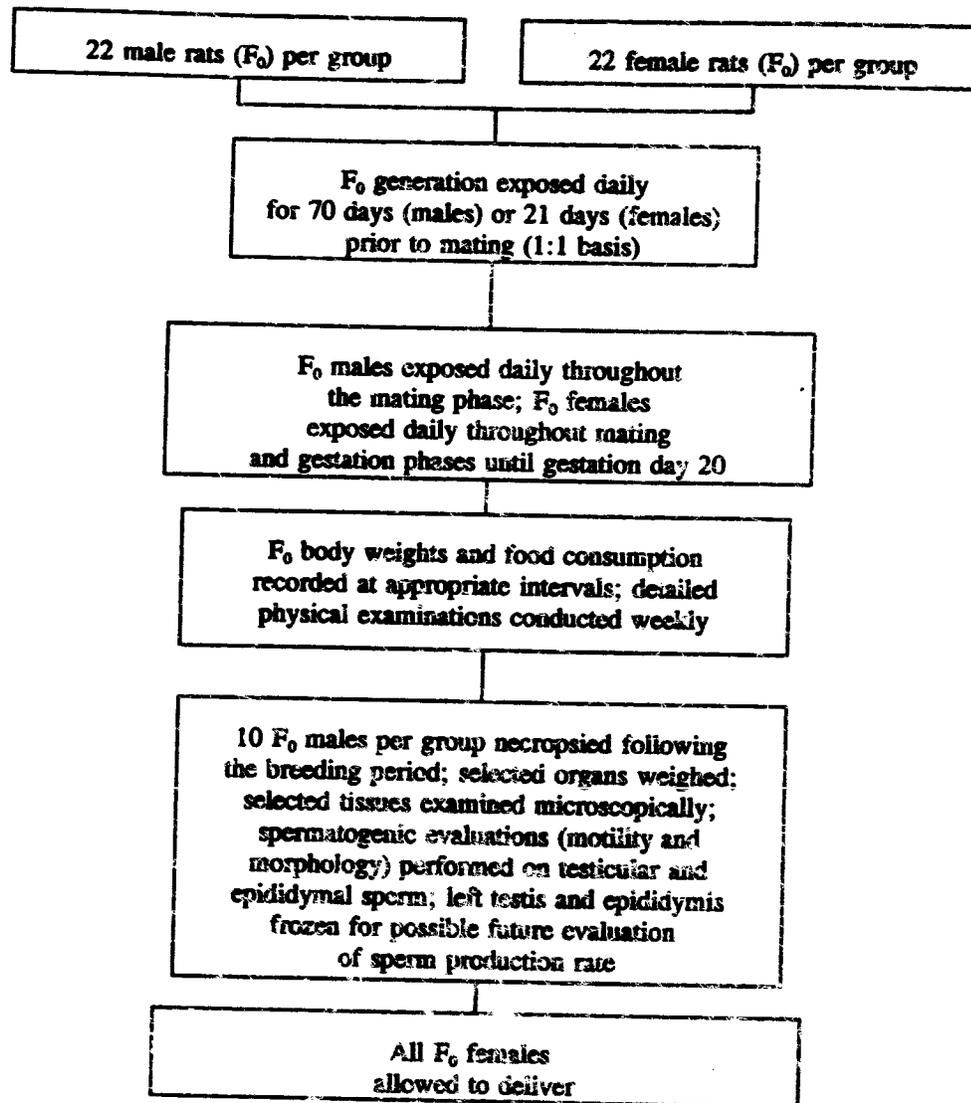
- 1) to identify adverse reproductive effects (if any) that might occur following whole-body vapor inhalation exposure of male rats to D4; and
- 2) to provide data useful for assisting in the selection of exposure concentrations for a definitive two-generation reproductive toxicity study.

The route of administration was whole-body inhalation since this is a potential route of exposure for humans. A 70-day pre-mating exposure regimen was used in this study to allow exposure over an entire spermatogenic cycle before mating. This 70-day pre-mating exposure is recommended by the extant EPA draft guidelines for reproduction studies<sup>1</sup>. The animal model, the CrI:CD<sup>0</sup>(SD)BR rat, is recognized as appropriate for reproduction studies<sup>1</sup> and was selected based on the availability of historical control data (Appendices E, F) and because of its proven susceptibility to the effects of developmental and reproductive toxicants. The highest concentration tested (700 ppm) was selected because it is the highest vapor concentration of D4 that can be reliably produced without aerosol formation.

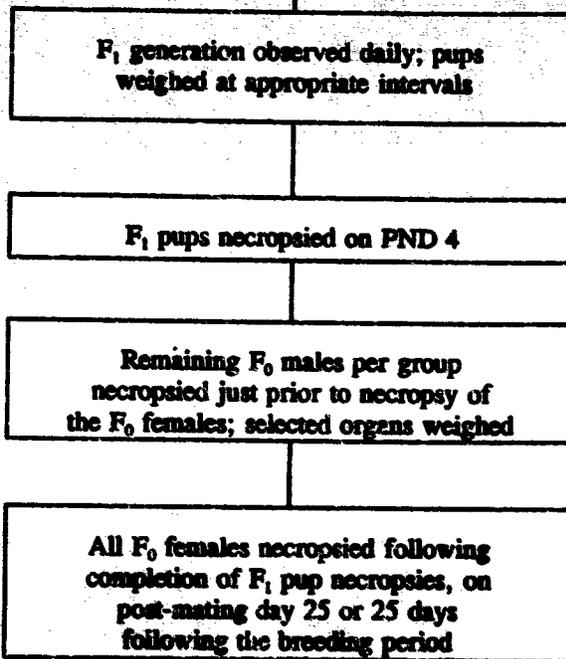
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### III. STUDY DESIGN



III. STUDY DESIGN (continued)



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#### IV. EXPERIMENTAL PROCEDURES

##### A. STUDY SCHEDULE

Study Initiation Date:	September 18, 1995
F <sub>0</sub> Male Exposures:	September 19 - December 12, 1995
F <sub>0</sub> Breeding:	November 27 - December 12, 1995
F <sub>0</sub> Male Week 12 Necropsies:	December 13-14, 1995
Necropsy of F <sub>1</sub> pups:	December 23, 1995 - January 4, 1996
F <sub>0</sub> Male Week 16 Recovery Necropsies:	January 8-10, 1996
Scheduled F <sub>0</sub> Female Necropsies:	January 12, 1996
Experimental Termination (Last day of test article purity determinations):	June 16, 1997

At the request of the Sponsor, Dr. Joseph F. Holson served as Study Director on this study due to his expertise in reproductive toxicology. Dr. Holson is the President, Director of WIL Research Laboratories, Inc.

Due to spacing constraints, the study title on the report tables was limited to "Inhalation RF Reproductive Toxicity Study of D4 in Male Rats."

##### B. TEST AND CONTROL ARTICLES

###### 1. TEST ARTICLE SYNTHESIS

The test article was synthesized by Dow Corning Corporation (Carrollton, Kentucky) in August, 1994. The methods used in the synthesis of the test article are summarized in the Material Documentation Management System document for the test article. A copy of this document is included in the study records maintained by the Sponsor for this study.

## 2. TEST ARTICLE IDENTIFICATION

The test article, octamethylcyclotetrasiloxane (D4), was received from Dow Corning Corporation, Auburn, Michigan, on September 30, 1994, as follows:

<u>Label Identification</u>	<u>Quantity Received</u>	<u>Description</u>
Dow Corning 244 Fluid Lot LL084732 (Drum #1-008, #2-009, #3-010) Net. Wt. 430 lb. (195.0 Kg) Avoid freezing Caution - combustible	3 Drums Total gross weight: 649850.0 g Drum #1: 212850.0 g <sup>a</sup> Drum #2: 225100.0 g <sup>b</sup> Drum #3: 211900.0 g <sup>c</sup>	Clear colorless liquid

- <sup>a</sup> = This drum was used for method development.
- <sup>b</sup> = This drum was used for method development and animal exposures.
- <sup>c</sup> = This drum was used for animal exposures.

Retention and residual samples of the test article were analyzed for purity by the Analytical Chemistry Department at WIL Research Laboratories, Inc. Details of the sample collection and their analyses are presented in Appendix A. This study was conducted concurrently with another study using the same lot of test article for the same Sponsor (WIL-51041<sup>b</sup>); therefore, references to WIL-51041 appear in Appendix A of this study. The test article purity was determined to be at least 99.59% D4. The test article was stable when stored at room temperature. Reserve 1.05, 22.7, and 50 g retention samples of the test article were taken from the first, second, and third drums, respectively, on October 5, 1994, September 15, 1995, and January 4, 1996, respectively. After completion of the exposure phase, residual

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samples of the test article were taken (9.26 g, 12.88 g, and 12.39 g for the first, second, and third drums, respectively). The retention and residual samples are stored in the Archives at WIL Research Laboratories, Inc., 1407 George Road, Ashland, Ohio 44805-9281. The test article has not been returned to the Sponsor, as it is being used on additional studies for this Sponsor.

3. TEST ATMOSPHERE MONITORING

Exposure concentrations within each chamber were measured 8-10 times (approximately every 30 minutes) during each daily exposure period by a validated gas chromatographic method. At least one standard was analyzed each day prior to exposure to confirm gas chromatographic calibration. Chamber ventilation rate, negative pressure, temperature, relative humidity, and oxygen content within the chambers were monitored continuously and were recorded approximately every 30 minutes. Nominal chamber concentration was determined daily for each chamber by weighing the amount of test article used during atmosphere generation, converting this mass to volume using standard gas laws, and dividing this test article volume by the total volume of air displaced through the chamber during the exposure. Total air volume was calculated by multiplying mean chamber ventilation rate (in liters per minute) by the exposure duration (in minutes), including the ventilation flow through the chamber and the compressed air flow through the J-tube. Test atmosphere homogeneity was validated during method development prior to the initiation of exposures. The methodology and results of these analyses are presented in Appendix B.

#### 4. EXPOSURE METHODS

All males in the 70/0, 300/0, 500/0, and 700/0 ppm groups were exposed in 1.0 cubic meter stainless-steel and glass whole-body inhalation chambers. The control group males and all females were exposed in a 2.0 cubic meter chamber, with the following exceptions. Several control group males were exposed to filtered air in a 0.5 cubic meter chamber during the first four days of the study. The use of chambers of different sizes was a result of space constraints in the exposure room; however, all D4-treated animals were exposed in 1.0 cubic meter chambers. The chambers were operated under dynamic conditions at air flows of approximately 12 to 15 air changes per hour, ensuring an adequate oxygen content of 19% or above and an evenly distributed exposure atmosphere. The  $F_0$  males were exposed to the test atmosphere for daily 6-hour exposures (seven days a week) for a minimum of 70 days prior to mating and continuing through the end of the breeding period. The  $F_0$  females were exposed to clean, filtered air for daily 6-hour exposures (seven days a week) for a minimum of 21 days prior to mating and continuing through gestation day 20. The control groups were exposed to clean, filtered air under conditions identical to those used for the groups exposed to the test article (with the exception of the size of the chamber used). The rats were removed from their home cages in the animal room at approximately the same time each day and were transported to the inhalation chambers for the 6-hour exposure period. The animals were returned to their home cages following exposure. Instrumentation was set to maintain the temperature inside the exposure chamber at approximately 20-26°C and

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relative humidity between approximately 30% and 70%. Exposure methods and conditions are detailed in Appendix B.

In order to minimize any potential variation occurring due to positioning within the chamber, the animals were rotated around the available cage rack positions within the chamber on a daily basis throughout the study, in accordance with the standard operating procedures at WIL Research Laboratories, Inc.

The following diagram presents the study group assignment:

Group Number	Group Name	Test Substance	Exposure Level (ppm)	Number of Animals	
				Male	Female*
1	Control	Filtered Air	0	22	22
2	Low Dose	D4	70	22	22
3	Intermediate Dose-1	D4	300	22	22
4	Intermediate Dose-2	D4	500	22	22
5	High Dose	D4	700	22	22

\* = The females in all groups were exposed to filtered air and were not exposed to test article.

### C. F<sub>2</sub> GENERATION

#### 1. ANIMAL RECEIPT AND ACCLIMATION

One hundred twenty-five male and one hundred twenty-five female virgin Sprague-Dawley Crj:CD®BR rats were received from Charles River Laboratories, Inc., Portage, Michigan, on September 7 and October 26, 1995, respectively. The males were 31 days old and the females were 72 days old upon receipt. Each animal was examined by a staff veterinarian upon receipt; the males and females were initially weighed on September 8 and October 27, 1995, respectively. All animals were uniquely identified by a Monel metal ear tag displaying the

animal number and housed (as described in Section IV.C.2.) for an acclimation period of 12 days prior to initiation of exposure. During acclimation, individual body weights were recorded twice (following receipt and at randomization) and the animals were observed twice daily for mortality and morbidity.

## 2. ANIMAL HOUSING

Upon arrival and until pairing, all animals were individually housed in clean, wire-mesh cages suspended above cage-board which was changed at least three times each week, with the following exception. The males were housed three per cage for at least three days following receipt. The females were not triple-housed upon receipt because of their advanced age (72 days old at receipt). This deviation from the protocol had no effect on the outcome of the study because only the males were treated with test article. The animals were paired for mating in the home cage of the male. Following positive identification of mating (Section IV.C.7.), the males were housed in individual suspended wire-mesh cages until necropsy. Breed females were housed individually in clean, plastic maternity cages with nesting material consisting of ground corn cob bedding (Bed-O-Cobs<sup>®</sup>; The Andersons, Industrial Products Division, Maumee, OER 43537). The dams were housed in these cages through the scheduled day of necropsy. Females that did not deliver were necropsied on post-mating day 25. The females for which there was no evidence of mating (70/0 and 700/0 ppm groups) were placed in clean, plastic maternity cages with nesting material upon completion of a 15-day mating period. Twenty-five days following the conclusion of the mating period, the females with no evidence of mating which did not deliver (70/0 ppm group) was euthanized and necropsied.

All animals were maintained by the animal husbandry staff in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals". The animal facilities at WIL Research Laboratories, Inc. are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

3. DIET, DRINKING WATER, AND MAINTENANCE

The basal ration used in this study was Purina® Certified Rodent Chow® #5002, in meal form; the lot numbers used were recorded. The diet used at WIL Research Laboratories, Inc. is a certified feed with appropriate analyses performed and provided by the manufacturer. Municipal water supplying the facility is sampled and analyzed for contaminants according to WIL Standard Operating Procedures. The results of these analyses are maintained at WIL Research Laboratories, Inc. Contaminants were not present in feed or water at levels which would interfere with the objectives of this study. Drinking water, delivered by an automatic watering system, and the basal diet were provided *ad libitum* throughout the acclimation period and during the study, with the following exception. Food and water were withheld during each daily exposure period.

4. ENVIRONMENTAL CONDITIONS (NON-EXPOSURE PERIODS)

Except during exposure, all animals were housed throughout the acclimation period and during the study in an environmentally-controlled room. Controls were set to maintain a temperature of  $72^{\circ} \pm 4^{\circ}\text{F}$  ( $22 \pm 2^{\circ}\text{C}$ ) and a relative humidity between 30% and 70%. Room temperature and relative humidity were recorded twice daily (once in the morning and once in the afternoon) throughout the study period, with the following exception. Room temperature and relative humidity were

recorded once daily on the first two days of the study (September 19 and 20, 1995). This deviation had no apparent effect on the outcome of the study. Temperature ranged from 70°F to 73°F (21.1°C to 22.8°C) and relative humidity ranged from 19% to 64% (relative humidities of 12% and 13% were recorded on December 10, 1995 and January 4, 1996, respectively) during the study period. Deviations from the set humidity levels were generally slight and/or transient, and did not affect the outcome of the study. Light timers were calibrated to provide a 12-hour light/12-hour dark photoperiod (6:00 a.m. to 6:00 p.m.) and air handling units were set to provide approximately 10 to 15 fresh air changes per hour. The light cycle was extended by 35 minutes on December 2, 1995, due to an interruption of the exposure period on this day. This event had no adverse effect on the outcome of the study.

#### 5. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

At the conclusion of the acclimation period, all available animals were weighed and examined in detail for physical abnormalities. Animals judged to be in good health and meeting acceptable body weight requirements were selected for use in the computer randomization procedure. At this time, the individual body weights and corresponding animal identification numbers were entered into the WIL Toxicology Data Management System (WTDMS™). A printout containing the animal numbers, corresponding body weights, and individual group assignment was generated based on body weight stratification randomized in a block design. The animals were then arranged into groups according to the printout. The males were assigned to four test article exposure groups and one control group of 22 animals each. The females were assigned to five control groups of 22 animals each (one group of untreated females

was dedicated to each of the control and treated male groups for the 1:1 mating process). At randomization (one day prior to exposure initiation), the males were 42 days old and body weights ranged from 160 g to 229 g. The females were 84 days old and body weights ranged from 216 g to 311 g at initiation of exposure (week 7). One of the females and most of the males were not within the protocol-specified weight ranges (200-300 g for males and 200-310 g for females). It was necessary to include these animals to allow for the placement of enough male and female rats on the study. Mean body weights were comparable across all exposure groups at the start of the study, and the low-body-weight males were approximately equally distributed across all exposure groups. The original protocol-specified body weight range at initiation of exposures for females (200-300 g) was expanded to 200-310 g to allow for randomization of a sufficient number of females based on body weights.

6. OBSERVATIONS

a. CLINICAL OBSERVATIONS AND SURVIVAL

The animals were observed twice daily for appearance, behavior, moribundity, and mortality. Detailed physical examinations were recorded weekly throughout the study period for the males and females. Males and females were also observed for pharmacotoxic signs during exposure and within approximately one hour after completion of exposure. No significant observations were noted in rats of either sex during the exposure period; therefore, no table summarizing these data is included in this report. The configuration of the cages and the location of the chamber windows precluded observations during exposure for some animals. All significant clinical findings were noted at the post-exposure observations. The

examinations included, but were not limited to, evaluations for changes in the skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system function, somatomotor activity, and behavior patterns. Special attention was paid to the degree of salivation and lacrimation, presence or absence of urination and defecation (including polyuria and diarrhea), pupil size, degree of palpebral closure, presence of convulsions, tremors or abnormal movements, presence of posture and gait abnormalities and the presence of any unusual or abnormal behaviors and any repetitive actions (stereotypics). Particular attention was given to the presence or absence of ejaculatory plugs. Females which delivered were also observed for dystocia twice daily during the period of expected parturition and at parturition.

b. BODY WEIGHTS

Individual F, male body weights were recorded on a weekly basis, beginning with the initiation of exposure and continuing until euthanization. Mean body weights were calculated for each weekly period. Corresponding weekly body weight changes were also calculated for each weekly interval.

Individual F, female body weights were recorded on a weekly basis, beginning with the initiation of exposure to filtered air and continuing until evidence of mating was observed. Mean body weights were calculated for each of these weeks. Mean body weight changes were calculated for each weekly interval. Once evidence of mating was observed, female body weights were measured on gestation days 0, 7, 10, 14, and 20, and on lactation days 1 and 4. Mean body weights were calculated for each of these days. Mean

body weight changes were calculated for each corresponding gestation or lactation interval and for gestation days 0-20.

When body weights were unavailable for a given interval, the appropriate interval was footnoted as "NA" (Not Applicable) on the individual tables.

c. FOOD CONSUMPTION

Individual F<sub>0</sub> male and female food consumption was measured weekly until the initiation of the mating period. Food intake was not recorded during the mating period. Once evidence of mating was observed, individual female food consumption was measured on gestation days 0, 7, 10, 14, and 20, and on lactation days 1 and 4. Male food consumption was recorded weekly until euthanization. Food intake was calculated as g/animal/day and g/kg/day for the corresponding body weight change intervals.

When food consumption could not be measured for a given interval (due to spillage, weighing error, obvious erroneous value, breeding period, etc.), the appropriate interval was footnoted as "NA" (Not Applicable) on the individual tables. Apparent discrepancies in the number of animals per group on summary food consumption tables are due to unavailable food intake values for individual females at given intervals.

7. BREEDING PROCEDURES

Before pairing on November 27, 1995, male body weights (week 9) ranged from 373 g to 574 g; female body weights ranged from 226 g to 317 g. The males were approximately 16 weeks old, and the females were approximately 15 weeks old. Animals were paired on a 1:1 basis within each exposure group after a minimum of 70 days of exposure to

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the test article (males) or 21 days of exposure to filtered air (females). A breeding record containing the male and female identification numbers and the date of cohabitation was prepared. The females were housed in the home cage of the male. Each mating pair was examined daily. Positive evidence of mating was confirmed by the presence of a copulatory plug or the presence of sperm in a vaginal smear. The day when evidence of mating was detected was termed gestation day 0. The animals were separated, and the female was housed individually in a plastic cage with nesting material. If evidence of mating was not detected after 10 days of pairing, the female was placed with another male from the same group for an additional five days. The second male always had positive evidence of mating detected with a previous female. If evidence of mating was not apparent after 15 days, the female was placed in a plastic cage with nesting material.

Pre-coital intervals were calculated according to the following method: Rats paired over a 12-hour dark cycle were considered to have been paired for "one" day.

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Mating and fertility indices were calculated as follows:

Female Mating Index (%) =	$\frac{\text{No. of Females with Evidence of Mating}}{\text{Total No. of Females Used for Mating}}$	X 100
Male Mating Index (%) =	$\frac{\text{No. of Males with Evidence of Mating}}{\text{Total No. of Males Used for Mating}}$	X 100
Female Fertility Index (%) =	$\frac{\text{No. of Females with Confirmed Pregnancy}}{\text{Total No. of Females Used for Mating}}$	X 100
Male Fertility Index (%) =	$\frac{\text{No. of Males Siring at Least 1 Litter}}{\text{Total No. of Males Used for Mating}}$	X 100

#### 8. GESTATION AND PARTURITION

All females were allowed to deliver naturally and rear their young to PND 4. During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. The day on which delivery was judged complete was designated lactation day 0. When parturition was judged complete, litters were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. Individual gestation lengths were calculated using the date delivery initiated.

#### D. E. LITTER DATA

##### 1. LITTER VIABILITY AND DEATHS

Each litter was examined daily for survival, and all pup deaths were recorded. All pups were individually identified by the application of tattoo markings on the digits (AIMS® Identification Systems, Piscataway, New Jersey 08854) on PND 0. A daily record of litter size was

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maintained. Stillborn pups and pups found dead were necropsied using a modification of the Stuckhardt and Poppe<sup>4</sup> fresh dissection technique (including examination of the heart and mid-coronal slices of the brain). One pup (no. 43983-01 in the 300/0 ppm group) euthanized on PND 4 with a skeletal anomaly was eviscerated and fixed in 100% ethyl alcohol. Following fixation, this pup was macerated in potassium hydroxide and stained with Alizarin Red S by a method similar to that described by Dawson<sup>5</sup>. The carcass was then examined. In addition, the head of pup no. 43990-17 in the 500/0 ppm group (noted with unilateral microphthalmia) was preserved in 99% glycerin.

2. CLINICAL OBSERVATIONS

Pups were examined daily for any changes in appearance or behavior. Each pup received a detailed physical examination on FND 1 and 4; only remarkable observations were reported. Abnormalities in nesting and nursing behavior were recorded. Observations were made as described for adult animals in Section IV.C.6.a.

3. BODY WEIGHTS

Pups were individually weighed on PND 1 and 4.

4. SEX DETERMINATION

Pup sexes were individually determined on PND 0 and 4.

### 5. CALCULATION OF LITTER PARAMETERS

Litter parameters were defined as follows:

$$\begin{aligned} \text{Live Litter Size} &= \frac{\text{Total Viable Pups on PND 0}}{\text{No. Litters With Viable Pups on PND 0}} \\ \text{Postnatal Survival Between Birth and PND 0 or PND 4} &= \frac{\Sigma (\text{Viable Pups Per Litter on PND 0 or PND 4/No. of Pups Born Per Litter}) \times 100}{\text{No. of Litters Per Group}} \\ \text{Postnatal Survival for All Other Intervals (\% Per Litter)} &= \frac{\Sigma (\text{Viable Pups Per Litter at End of Interval N/Viable Pups Per Litter at Start of Interval N}) \times 100}{\text{No. of Litters Per Group}} \end{aligned}$$

Where N := PND 0-1 or 1-4

### 6. PND 4 NECROPSY

On PND 4, all remaining F<sub>1</sub> pups were euthanized via an intrathoracic injection of sodium pentobarbital and necropsied. The necropsy included an examination of the external surface, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, and the thoracic, abdominal, and pelvic cavities including the viscera. Tissues were preserved in 10% neutral buffered formalin for possible future histopathological evaluation only as deemed necessary by the gross findings.

### E. NECROPSY EXAMINATIONS

A complete necropsy was conducted on all F<sub>0</sub> animals surviving to the scheduled necropsies. Ten F<sub>0</sub> males/group were necropsied on December 13-14, 1995 (week 12), following the completion of breeding. All surviving remaining F<sub>0</sub> males were necropsied during week 16 just prior to necropsy of the F<sub>0</sub> females, instead of following the scheduled female necropsy as specified in the protocol. This deviation had no apparent effect on the outcome of the study. The males that were necropsied during week 12 were exposed to D4 until one or two days prior to necropsy, whereas the males necropsied during

week 16 had a one-month non-exposure period prior to necropsy. All surviving females with viable pups were necropsied following completion of the F<sub>1</sub> pup necropsies, and the numbers of former implantation sites were recorded. Bred females with evidence of mating but which did not deliver a litter were necropsied on post-mating day 25. The female with no evidence of mating that did not deliver a litter was necropsied 25 days following the breeding period. A detailed gross necropsy was performed on each of these females to determine pregnancy status with specific emphasis placed on anatomical or pathological findings which may have interfered with pregnancy. Uteri without macroscopic evidence of implantation were opened and placed in 10% ammonium sulfide solution for detection of implantation sites as described by Salewski<sup>2</sup>, with the following exceptions. The uteri of three females (nos. 43956, 44069, and 44035 in the 300/0, 500/0, and 700/0 ppm groups, respectively) euthanized on post-mating day 25 were examined for implantation sites, but no documentation of ammonium sulfide treatment was recorded. Because no necropsy findings were noted for any of the females euthanized on post-mating day 25, this deviation had no effect on the outcome of the study. Surviving males were euthanized with an injection of sodium pentobarbital via a lateral tail vein. Surviving females were euthanized via carbon dioxide inhalation. The necropsy of all F<sub>0</sub> parental animals included an examination of the external surface, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, and the thoracic, abdominal, and pelvic cavities including the viscera. At the time of necropsy of the F<sub>0</sub> males, the following tissues and organs were collected and preserved in 10% neutral buffered formalin (except as noted):

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Adrenals (2)	Pancreas
Aorta	Penis
Bone with marrow (sternum)	Peripheral nerve (sciatic)
Brain (forebrain, midbrain, hindbrain)	Pituitary
Eyes with optic nerve (2)	Prepuce
Gastrointestinal tract	Preputial gland
Esophagus	Prostate
Stomach	Salivary gland [submaxillary(2)]
Duodenum	Seminal vesicles
Jejunum	Skeletal muscle (vastus medialis)
Ileum	Skin
Cecum	Spinal cord (cervical, midthoracic, and lumbar)
Colon	Spleen
Rectum	Testes with epididymides*
Heart (atria, ventricles, and septum)	Thymus (if present)
Kidneys (2)	Thyroid gland [both lobes with parathyroids, if present (2)]
Liver (sections of two lobes)	Trachea
Lungs [including bronchi, fixed by inflation with fixative (2)]	Urinary bladder
Lymph nodes (mesenteric and mandibular)	All gross lesions and masses

\* = The left testis and epididymis were frozen (after weighing) for possible future evaluation of sperm production rate. The right testis and epididymis were fixed in Bouin's solution.

#### F. SPERMATOGENIC ENDPOINT EVALUATIONS

Immediately upon euthanization, the reproductive tract of each male necropsied following the completion of mating (week 12) was exposed via a ventral mid-line incision. The right testis and epididymis were excised and weighed separately. An incision was made in the distal region of the right cauda epididymis. The entire right cauda epididymis was then placed in 40 ml of Dulbecco's phosphate buffered saline (maintained at approximately 37°C) with 10 mg/ml Bovine Serum Albumin. After a ten minute incubation period, a sample was transferred to a 100- $\mu$ m cannula for determination of sperm

motility. Because sperm motility can be affected by temperature shock, all cannulas and diluents were pre-warmed in an incubator, and motility determinations were performed under constant temperature (approximately 37°C) using the Hamilton-Thorne HTM-IVOS Version 10 computer-assisted sperm analysis (CASA) system. Analysis of at least 200 motile and nonmotile spermatozoa per animal was performed by the analyzer.

After samples for the sperm motility evaluations were removed, a second sperm sample was obtained from the same 40-ml suspension from which the motility sample was drawn. Duplicate smears of samples of epididymal sperm (cauda) were prepared on microscope slides. The slides were allowed to air dry and were stained with Weigert's-iron hematoxylin, counter-stained with 1% eosin-phloxine, and cover-slipped. Abnormal forms of sperm (double heads, double tails, microcephalic, or megacephalic, etc.) from a differential count of 200 spermatozoa per animal, if possible, from the control, 500, and 700 ppm groups were recorded using the classification system of Linder *et al.*

#### G. ORGAN WEIGHTS

The following organs from all F<sub>1</sub> males euthanized at the scheduled necropsies (following completion of mating or just prior to necropsy of the F<sub>1</sub> females) were weighed:

Adrenals  
Brain  
Epididymides\* (total and cauda)  
Heart  
Kidneys  
Liver  
Lungs  
Pituitary

Prostate  
Seminal vesicles with coagulating glands (with accessory fluids)  
Testes\*  
Spleen  
Thymus  
Thyroid

\* - These paired organs were weighed separately.

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Paired organs (with the exceptions noted above) were weighed together. Ratios of organ to final body weight and organ to brain weight were calculated.

#### H. HISTOLOGICAL PROCEDURES AND MICROSCOPIC EXAMINATION

After fixation, specified tissues were trimmed as described by Thompson<sup>8</sup>. Trimmed specimens were placed in appropriately labeled and numbered cassettes. The fixed tissue samples were processed into paraffin blocks. The labeled paraffin blocks were sectioned at 5-8 microns, and the paraffin ribbons of the sectioned tissue were placed on clean glass microscope slides labeled with the appropriate study, animal, group, and cassette numbers. Upon completion of staining (AFIP Manual of Histologic Staining Methods<sup>9</sup>) with hematoxylin and eosin or with hematoxylin and PAS light green (right testis and epididymis), coverslips were placed on the slides. Microscopic tissue evaluations were then performed on the following tissues from the F<sub>0</sub> male that died prior to scheduled necropsy and the 10 F<sub>0</sub> males per group necropsied following the completion of mating:

Adrenals	Preputial gland
Brain-hypothalamus	Prostate
Epididymis (right)	Spleen
Liver	Testis (right)
Penis	Thymus
Pituitary	All gross internal lesions
Prepuce	

For clarification, the following terms/designations were used on the histopathology summary tables in addition to grades of "minimal", "mild", "moderate", and "severe":

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- "0" = Appears when a specific finding is not observed for any animal in a group
- "None" = Appears when grading is not applicable
- "Present" = Appears when a specific finding is observed but not graded

Microscopic examinations were conducted by Dr. Robert R. Dahlgren, Director of Pathology and Veterinary Medicine, WIL Research Laboratories, Inc.

#### I. STATISTICAL METHODS

All analyses were conducted for a minimum significance level of 5% comparing the treated groups to the control group; all means are presented with standard deviations (S.D.). All tests for significance at the 5% probability level were two-tailed for the group comparisons. The litter was used as the experimental unit. Data obtained from non-gravid animals were excluded from statistical analyses following the mating period. Statistical analyses were not conducted on weekly female body weight and food consumption data after one or more animals had entered the gestational phase. The numbers of animals (N) used to calculate the means are provided on the individual data tables. Apparent discrepancies in the number of animals per group between gestation and lactation body weight/food consumption tables are due to females that did not have evidence of mating (are not on gestation tables) but delivered litters (are on lactation tables). All statistical tests were performed by a Digital® MicroVAX® 3400 Computer (with appropriate programming) in this laboratory and are referenced on the report tables.

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<u>STATISTICAL TEST</u>	<u>PARAMETER</u>
- Chi-square test <sup>10</sup> with Yates' correction factor	Parental Mating/Fertility Indices
- ANOVA (two-tailed) with Dunnett's test <sup>10</sup>	F <sub>0</sub> Weekly, Gestational, and Lactational Body Weight and Food Consumption Data F <sub>0</sub> Organ Weights F <sub>0</sub> Gestation Lengths Numbers of Pups Born Numbers of Implantation Sites Numbers of Unaccounted Sites Live Litter Sizes Pup Body Weights
- Kruskal-Wallis test <sup>10</sup>	F <sub>0</sub> Sperm Motility F <sub>0</sub> Sperm Morphology Pup Sexes at Birth (% males per litter) Postnatal Survival
- Kolmogorov-Smirnov test <sup>10</sup> (one-tailed test)	Histopathological Findings

The original protocol specified the use of pup-based statistics (Chi-square test with Yates' correction factor<sup>10</sup>) for the evaluation of postnatal survival. Based on the recommendations of the Food and Drug Administration Guidelines on Detection of Toxicity to Reproduction for Medicinal Products<sup>11</sup>, Holson *et al*<sup>12</sup>, and Nelson and Holson<sup>13</sup>, the protocol was amended (Protocol Amendment VII) to replace these statistics with litter-based statistics (mean litter proportions), in which the litter is the experimental and statistical unit. For these analyses, the Kruskal-Wallis<sup>10</sup> test was used.

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#### **I. DATA RETENTION**

The Sponsor has title to all documentation records, raw data, specimens, or other work product generated in this study. All work product, including raw paper data and specimens, will be retained in the Archives at WIL Research Laboratories, Inc. until notification from the Sponsor regarding final disposition of the said work product.

Raw data in magnetic form, retention and residual samples of the test article, the original protocol and amendments, and the original final report will be retained at WIL Research Laboratories, Inc., 1407 George Road, Ashland, Ohio 44805-9281, in compliance with regulatory requirements.

V. RESULTS

A. CHAMBER CONCENTRATIONS

Summary Data: Appendix B

The F<sub>0</sub> males were exposed to target test article concentrations of 70, 300, 500, and 700 ppm. The actual measured mean exposure concentrations were 71, 300, 492, and 694 ppm, respectively.

B. F<sub>0</sub> GENERATION

1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 1, 3, 3A, 3B, 4, 5

Individual Data: Appendices C, D

One male (no. 42119) in the 700/0 ppm exposure group died after the mating interval on study day 97. Clinical findings noted for this animal on the day prior to death or the day of death included hypoactivity, unkempt appearance, prostration, shallow respiration, hypothermia (body cool to touch), decreased urination, mucoid feces, soft stool, red material around the nose, clear matting around the mouth, and brown matting on the anogenital area. Based on prior extensive experience with this species and strain of rat at similar exposure levels of this test article under essentially identical study conditions, this death was considered to be spontaneous in origin. Consequently, this death was not attributed to the test article. One female (no. 44000) in the 700/0 ppm exposure group was euthanized *in extremis* by order of the Study Director on lactation day 1 (study day 95). Clinical findings noted for this animal prior to euthanization included rocking, lurching or swaying, hypoactivity, absent righting reflex, prostration, hypothermia, pale extremities and eyes, labored respiration, shallow respiration, lacrimation, and red matting on

the urogenital area. All other animals survived to the scheduled necropsies.

The only exposure-related clinical finding was red material around the nose noted one hour following exposure in the 700/0 ppm group males. Red material around the nose was noted in all male groups, including the control group. However, the incidence of this finding in the 700/0 ppm group was slightly increased and appeared to be exposure-related. Other clinical signs in the treated groups were noted infrequently or at similar frequencies in the control group.

During the F<sub>0</sub> exposure phase of the study, ejaculatory plugs were often found on the cage paper beneath cages of male animals following exposure. Plugs were also found under the male cages during periods of non-exposure; these were lower in number than those found following the exposure interval. These ejaculatory plugs were first noted for the majority of the animals between September 29 and October 10, 1995 (between the eleventh and twenty-second days of exposure) and continued to be observed through the end of the exposure period. The incidence of ejaculatory plugs in the treated groups was increased relative to the control group. Microscopic evaluation (100x magnification) of randomly selected ejaculatory plugs collected between November 2, 1995, and December 10, 1995 revealed the presence of sperm in these plugs. The significance of the ejaculatory plugs is unknown.

## 2. REPRODUCTIVE PERFORMANCE

Summary Data: Table 2

Individual Data: Table 42

Historical Control Data: Appendices E, F

Reproductive performance was not adversely affected by exposure of males to the test article at any exposure concentration. The mean numbers of days between pairing and coitus in the 70/0, 300/0, 500/0, and 700/0 ppm groups were comparable to the control group value. Male mating indices in these same exposure groups were 100.0%, 90.9%, 100.0%, 100.0%, and 95.5%, respectively, and female mating indices were 100.0%, 95.5%, 100.0%, 100.0%, and 100.0%, respectively. Fertility indices for males and females were 100.0%, 90.9%, 95.5%, 95.5%, and 95.5% in the control, 70/0, 300/0, 500/0, and 700/0 ppm groups, respectively. No statistically significant differences were noted.

Males that did not sire a litter numbered 0, 2, 1, 1, and 1 in the control, 70/0, 300/0, 500/0, and 700/0 ppm groups, respectively. In these same respective exposure groups, 0, 1, 1, 1, and 1 females had evidence of mating but did not deliver. Female nos. 44026 and 44062 in the 70/0 ppm group had no evidence of mating when paired for 10 days with male nos. 42130 and 42149, respectively. After being re-paired with male nos. 42171 and 42157, respectively, female no. 44026 had evidence of mating but female no. 44062 did not. Neither of these females delivered litters and both were found to be nongravid at the *post mortem* examinations. In the 700/0 ppm group, two females (nos. 44003 and 44006) had no evidence of mating when paired for 10 days with male nos. 42088 and 42095, respectively, or when re-paired for five days with male nos. 42100 and 42118, respectively. Both of these females delivered litters

that were attributed to the first two males based on the timing of parturition. Another female (no. 44029) in the 700/0 ppm group had no evidence of mating with male no. 42129, but had evidence of mating when re-paired with male no. 42140. This female delivered a litter that was attributed to male no. 42140 based on the timing of parturition.

### 3. BODY WEIGHTS

#### a. WEEKLY

Summary Data: Tables 6, 7

Individual Data: Tables 43, 44

Mean body weights and body weight gains in the 70/0, 300/0, 500/0, and 700/0 ppm group males were comparable to the control group values throughout the study, with the following exceptions. Reduced body weight gains during week 6-7 and increased body weight gains during week 7-8 in the 70/0, 300/0, 500/0, and 700/0 ppm groups were usually statistically significant ( $p < 0.01$ ). However, these changes were observed in all treated groups and no exposure relationship was apparent; therefore, these changes during weeks 6-7 and 7-8 were not attributed to treatment. In addition, mean body weight gain in the 300/0 ppm group males during week 13-14 was increased and statistically significant ( $p < 0.05$ ). However, similar changes were not observed at the higher exposure levels, and this increase was not interpreted to be treatment-related. In the 700/0 ppm group males, transient reductions in mean body weights (week 7) and body weight gains (weeks 2-3 and 11-12) were statistically significant ( $p < 0.05$  or  $p < 0.01$ ). Due to the transient nature of these reductions, they were not considered to be adverse effects of treatment. No other differences from the control group were statistically significant.

Mean weekly body weights and body weight gains in the females were similar across all study groups. The only statistically significant difference from the control group was an increased ( $p < 0.05$ ) mean body weight gain in the 300/0 ppm group females during week 7-8 (i.e., after approximately one week of exposure to filtered air). Because of the transient nature of this increased body weight gain, and because the females were not exposed to test article, this finding was deemed to have no relevance to the study outcome.

b. GESTATION

Summary Data: Tables 8, 9

Individual Data: Tables 45, 46

Mean body weights and body weight gains during gestation were comparable among all female study groups. No statistically significant differences were observed.

c. LACTATION

Summary Data: Tables 10, 11

Individual Data: Tables 47, 48

Mean body weights and body weight gains during lactation were comparable among all female study groups. No statistically significant differences were observed.

4. FOOD CONSUMPTION

a. WEEKLY

Summary Data: Tables 12, 13

Individual Data: Tables 49, 50

Food consumption, evaluated as g/animal/day and g/kg/day, in the 70/0, 300/0, 500/0, and 700/0 ppm group males was unaffected by exposure to the test article. During weeks 1-2 through 6-7, reduced

g/animal/day food consumption values in these groups were often statistically significant ( $p < 0.01$  or  $p < 0.05$ ) when compared to the control group values. However, these reductions were slight (2-3 grams) and did not occur in a treatment-related manner; therefore, no relationship to treatment was evident. Grams/kg/day food consumption values in these groups were occasionally slightly reduced and statistically significant ( $p < 0.01$  or  $p < 0.05$ ) during weeks 2-3, 3-4, and 5-6 or slightly increased and statistically significant ( $p < 0.01$  or  $p < 0.05$ ) during weeks 7-8, 9-10, 12-13, and 13-14. No other differences from the control group were statistically significant.

Mean weekly food consumption (g/animal/day and g/kg/day) values in the females prior to breeding (weeks 7-8 to 9-10) were comparable among all groups. No statistically significant differences were observed.

b. **GESTATION**

Summary Data: Tables 14, 15

Individual Data: Tables 51, 52

Mean food consumption, evaluated as g/animal/day and g/kg/day, during gestation was comparable among all female study groups. No statistically significant differences were observed.

c. **LACTATION**

Summary Data: Tables 16, 17

Individual Data: Tables 53, 54

Mean food consumption, evaluated as g/animal/day and g/kg/day, during lactation was comparable among all female study groups. No statistically significant differences were observed.

## 5. GESTATION LENGTH AND PARTURITION

Summary Data: Table 18

Individual Data: Table 55

Historical Control Data: Appendices E, F

The mean duration of gestation was comparable in all groups. No statistically significant differences were observed.

### C. E. LITTER DATA

#### 1. PND 0 LITTER DATA AND POSTNATAL SURVIVAL

Summary Data: Table 19

Individual Data: Tables 56, 57

The mean numbers of pups born per litter, live litter sizes on PND 0, and pup sex ratios (percentages of males per litter at birth) were unaffected by exposure of F<sub>0</sub> males to test article in all groups. Differences between the control and exposed groups were slight and were not statistically significant.

The numbers of pups found dead on PND 0 were 2, 3, 2, 8, and 17 in the control, 70/0, 300/0, 500/0, and 700/0 ppm groups, respectively. When initially analyzed using pup-based statistics, the increase at 700/0 ppm was statistically significant ( $p < 0.01$ ). However, these pup-based statistics were determined to be inappropriate<sup>11,12,13</sup>. The findings from the pup-based statistical analysis are not presented in this report; however, they are included in the raw data maintained at WIL Research Laboratories, Inc. for this study. Upon application of the appropriate litter-based statistics, it was found that postnatal pup survival (percent per litter) was not statistically significantly affected by exposure of the F<sub>0</sub> males to test article at any exposure level from birth to PND 0, PND 0 to 1, PND 1 to 4, and birth to PND 4. Pup survival in the 700/0 ppm group

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on PND 0 and during the birth to PND 4 interval (95.2% and 93.5%, respectively) was slightly reduced relative to the control group values (99.5% and 97.4%, respectively); the differences were not statistically significant. Interpretation of the biological significance of this finding was confounded by the fact that the decreases were primarily due to only two litters (nos. 43962 and 44023) in which 7/17 and 5/17 pups, respectively, were found dead on PND 0. All other differences between the control and exposed groups were slight and were not statistically significant.

## 2. GENERAL PHYSICAL CONDITION AND MORTALITIES

Summary Data: Tables 20, 21

Individual Data: Tables 56, 57, 58, 59

Pups which were found dead (PND 0-4) numbered 4, 4, 5, 9, and 20 in the control, 70/0, 300/0, 500/0, and 700/0 ppm groups, respectively. In addition, in these same groups, 5, 4, 5, 0, and 5 pups, respectively, were missing and presumed cannibalized; for purposes of statistical analyses, missing pups were treated as dead. The general physical condition of the F<sub>1</sub> pups during lactation was similar in all exposure groups, with the following exception. Thirteen pups from litter no. 44000 in the 700/0 ppm group were euthanized on PND 1 because the dam was euthanized moribund on the same day. These pups were observed with hypothermia (body cool to touch) prior to euthanization. The loss of these pups on PND 1 was attributed to the moribund condition of the dam, and therefore cannot be interpreted to be an adverse effect of test article exposure in the male rat. Other clinical signs noted in the 70/0, 300/0, 500/0, and 700/0 ppm groups occurred infrequently and/or at a similar frequency in the control group.

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At the necropsy of the pups that were found dead, no treatment-related findings were noted in animals in the 70/0, 300/0, 500/0, and 700/0 ppm groups. With the exception of the presence or absence of milk in the stomach, remarkable internal findings were as follows. One pup (no. 44000-05) in the 700/0 ppm group had a right renal papilla that was not developed. Another pup (no. 44023-02) in this group had dark red contents in the stomach, duodenum, and jejunum.

### 3. PUP BODY WEIGHTS

Summary Data: Table 23

Individual Data: Tables 61, 62

Historical Control Data: Appendices E, F

Mean pup weights on PND 1 were 7.4, 7.1, 7.1, 7.2, and 6.3 grams in the control, 70/0, 300/0, 500/0, and 700/0 ppm groups, respectively. In the same respective exposure groups, mean pup weights on PND 4 were 10.6, 10.0, 10.0, 10.6, and 9.7 grams, respectively. The decreases in the 700/0 ppm group on PND 1 and 4 relative to the control group values were statistically significant ( $p < 0.01$  and  $p < 0.05$ , respectively). However, interpretation of the significance of these weight differences was confounded by the fact that the values observed in the 700/0 ppm group for both days were within the ranges of values noted in the WIL historical control data. The WIL reproductive historical control data base consists of data from 85 individual data sets generated between 1982 and 1994. No other statistically significant differences between the control and treated groups were noted.

#### 4. PND 4 NECROPSY

Summary Data: Table 22

Individual Data: Table 60

At the scheduled necropsy of pups on PND 4, no treatment-related findings were noted. In the 700 ppm group, pup nos. 43976-02, -08, and -14 had a reddened testis, a white area on the liver, and a red area in one eye, respectively. Another pup (no. 44045-12) in the 700 ppm group had a necrotic area in the spleen. Pup no. 43983-01 in the 3000 ppm group had one malformation (filamentous tail). Another malformation (unilateral microphthalmia) was observed for pup no. 43990-17 in the 5000 ppm group. Pup no. 44008-08 in the 5000 ppm group had a red area in both eyes. A hemorrhagic eye was noted for another pup (no. 44074-02) in the 5000 ppm group. In the 7000 ppm group, pup no. 44013-07 had a major blood vessel variation (the right subclavian and right carotid arteries arose independently from the aortic arch). Another pup (no. 44018-17) in the 7000 ppm group had a hemorrhagic ring around one iris. None of these findings were attributable to test article exposure.

At the PND 4 pup necropsies, 4, 11, 14, 9, and 37 pups in the control, 700, 3000, 5000, and 7000 ppm groups, respectively, did not have milk in the stomach. However, the significance of this observation could not be determined due to the unusual study design for the necropsy phase. The atypical experimental design used in this study resulted in necropsies for the F<sub>0</sub> dams that occurred approximately two weeks following the scheduled PND 4 pup necropsies. Logistical procedures involved in the timing of the F<sub>0</sub> female necropsies resulted in the F<sub>1</sub> neonates being without access to maternal milk for several hours prior to necropsy (the 7000 ppm group pups were without milk the longest because they were

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necropsied last). The timing of F<sub>0</sub> female necropsies and routine determination of the presence or absence of milk in the stomach (required by WIL SOP) at PND 4 were not endpoints essential to the objective of this study, in which only paternal animals were exposed to the test article. Furthermore, in a similar study with this test article, in which both F<sub>0</sub> males and F<sub>0</sub> females were directly exposed to D4 (WIL-51039<sup>14</sup>), the reverse trend was exhibited. Both control groups in that study had several pups without milk in the stomach at PND 4, whereas in the 700 ppm group there were no pups with an absence of milk in the stomach at PND 4. Therefore, this finding in the present study was interpreted to be an artifact of the study design, and was not related to exposure of the F<sub>0</sub> males to D4.

#### D. NECROPSY EXAMINATIONS

##### 1. F<sub>0</sub> UNSCHEDULED DEATHS

Summary Data: Table 24

Individual Data: Table 63

One male (no. 42119) in the 700/0 ppm exposure group died on study day 97. Internal findings noted at the *post mortem* examination of this animal included kidneys with dilated pelves, calculi, and multiple white areas, a distended urinary bladder with dark green contents, multiple calculi, and reddened mucosa, a distended ureter, and reddened and enlarged iliac lymph nodes. One female (no. 44000) in the 700/0 ppm group was euthanized *in extremis* on lactation day 1 (study day 95). The moribundity of this female was not treatment-related since the females were not exposed to the test article. Macroscopic internal findings noted for this animal at the *post mortem* examination included reddened kidneys, dark red contents in the stomach, placenta which were retained, and clear fluid contents in the abdominal and thoracic cavities.

2. FEMALES - POST-MATING DAY 25

Summary Data: Table 25

Individual Data: Table 64

One female in each of the 70/0, 300/0, 500/0 and 700/0 ppm groups had evidence of mating but did not deliver and were necropsied on post-mating day 25. All of these animals were nongravid; no internal findings were noted at the necropsies of these animals.

3. FEMALES - 25 DAYS FOLLOWING BREEDING PERIOD

Summary Data: Table 26

Individual Data: Table 65

One female (no. 44062) in the 70/0 ppm group had no evidence of mating and was necropsied 25 days following the conclusion of the breeding period. This female was nongravid and had a cystic ovary (filled with red fluid) and green purulent material which filled the uterus and vagina.

4. FEMALES - SCHEDULED NECROPSY

Summary Data: Tables 27, 28

Individual Data: Tables 66, 67

At the scheduled necropsy of the F<sub>1</sub> females, remarkable internal findings were as follows. One control group female (no. 43996) had an accessory spleen. Female no. 44004 in the 70/0 ppm group had a white nodule in the mesentery. A cystic spleen was noted for one female (no. 44098) in the 300/0 ppm group. No other internal findings were noted at the scheduled necropsy.

The mean numbers of uterine implantation sites, pups born, and implantation sites unaccounted for by offspring were comparable among

all study groups. No statistically significant differences from the control group were observed.

5. F<sub>0</sub> MALES - WEEK 12 NECROPSY

Summary Data: Table 29

Individual Data: Table 68

At the week 12 necropsy of F<sub>0</sub> males, no treatment-related findings were noted at any exposure level. Two males in each of the control, 70/0, and 500/0 ppm groups had reddened and/or enlarged mandibular lymph nodes. One male (no. 42098) in the control group had dilated renal pelvis and enlarged renal lymph nodes. Male no. 42130 in the 70/0 ppm group had a small left epididymis, a reddened Peyer's patch in the colon, and an edematous left testis. Another animal (no. 42102) in the 70/0 ppm group had a small left epididymis, a soft left testis, and an enlarged right testis. Male no. 42060 in the 70/0 ppm group had a dilated renal pelvis and a distended ureter. In the 700/0 ppm group, male no. 42070 had a depressed area in one kidney. All other males were internally unremarkable at the week 12 necropsy.

6. F<sub>0</sub> MALES - WEEK 16 RECOVERY NECROPSY

Summary Data: Table 30

Individual Data: Table 69

At the week 16 recovery necropsy of the F<sub>0</sub> males, no treatment-related findings were noted at any exposure level. Two, two, one, three, and two males in the control, 70/0, 300/0, 500/0, and 700/0 ppm groups, respectively, had reddened and/or enlarged mandibular lymph nodes. Two males in the control group had reddened and enlarged mediastinal lymph nodes. One male (no. 42174) in the control group had yellow salivary glands and a green ampullary gland. Male no. 42136 in the 70/0 ppm

group had a reddened pituitary gland, a hemorrhagic thymus gland, and a small coagulating gland. Another male (no. 42084) in the 700 ppm group had an enlarged right testis. One male (no. 42116) in the 300 ppm group had a cystic spleen. A diverticulum in the jejunum was noted for male no. 42164 in the 300 ppm group. Male no. 42120 in the 500 ppm group had dark red lungs. One male (no. 42118) in the 700 ppm group was observed with a small right epididymis and testis. All other males were internally unremarkable at the week 16 recovery necropsy.

#### **E. SPERMATOGENIC ENDPOINT EVALUATIONS**

Summary Data: Tables 39, 40, 41

Individual Data: Tables 76, 77, 78

Test article exposure at concentrations of 70 ppm, 300 ppm, 500 ppm, and 700 ppm for approximately twelve weeks (week 12 necropsy) had no apparent effects on spermatogenic endpoints. Sperm motility means (mean percentage of motile sperm) in these groups were comparable to the values observed in the control group. Sperm morphology means in the 500 ppm and 700 ppm groups were also comparable to the control group values. No statistically significant differences were observed.

#### **F. ORGAN WEIGHTS**

##### **1. F<sub>1</sub> MALES - WEEK 12 NECROPSY**

Summary Data: Tables 31, 33, 35

Individual Data: Tables 70, 72, 74

At the scheduled week 12 necropsy of F<sub>1</sub> males, mean liver weights (absolute and relative to final body and brain weights) in the 500 ppm and 700 ppm group males were increased by 9-14% and 16-21%, respectively. The differences from the control group were usually

statistically significant at  $p < 0.01$  or  $p < 0.05$ . Mean absolute and relative (to final body and brain weights) kidney and thyroid weights in the 700/0 ppm group males were also increased (by 21-26% and 29-40%, respectively) relative to the control group values; the differences were generally statistically significant at  $p < 0.01$  or  $p < 0.05$ .

No other exposure-related effects on organ weight data were noted at the week 12 necropsy of  $F_0$  males. Mean kidney weight relative to final body weight in the 500/0 ppm group males was increased (significant at  $p < 0.05$ ) in comparison to the control group value. However, the mean absolute kidney weight and kidney weight relative to brain weight in the 500/0 ppm group were unaffected, and all values were comparable to the values noted for the 70/0 and 300/0 ppm groups; therefore, no adverse effect on the kidney was apparent in the 500/0 ppm group. No other organ weight differences between the control and treated groups were statistically significant.

## 2. $F_0$ MALES - WEEK 16 RECOVERY NECROPSY

Summary Data: Tables 32, 34, 36

Individual Data: Tables 71, 73, 75

No effect of the test article was noted on mean absolute or relative organ weights in the 70/0, 300/0, 500/0, and 700/0 ppm group males at the scheduled week 16 recovery necropsy. Mean absolute brain weights in the 300/0, 500/0, and 700/0 ppm groups were lower than the control group value (statistically significant at  $p < 0.05$ ). However, the differences were slight (5-6%), no clear exposure-relationship was observed, and mean brain weights relative to final body weights in these groups were comparable to the control group values; therefore, these slight reductions in mean absolute brain weights were not attributed to treatment. Mean left

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testis weight relative to brain weight in the 3000 ppm group was significantly higher ( $p < 0.01$ ) than the control group value. However, no exposure-related response was observed at the higher concentrations, and this increase was attributed to normal biological variation. No other statistically significant differences between the treated and control group values were noted.

#### G. MICROSCOPIC EXAMINATION

##### 1. F. MALES - UNSCHEDULED DEATH

Summary Data: Table 37

Individual Data: Table 63

One male (no. 42119) in the 7000 ppm exposure group died on study day 97. The cause of death was renal inflammation with septicemia. Other microscopic findings noted for this male were suppurative pyelonephritis, acute hemorrhage along the penile urethra, suppurative inflammation of the prostate, spleen, and iliac lymph node, interstitial edema in the right testis, and an atrophied and necrotic thymus gland.

##### 2. F. MALES - WEEK 12 NECROPSY

Summary Data: Table 38

Individual Data: Table 68

Microscopic examination of gross lesions noted at the scheduled week 12 necropsy did not reveal any effects of test article administration.

No microscopic lesions attributed to test article administration were observed in any tissues upon histopathological examination. The lesions observed in the treated males were observed at a similar frequency to those in the control group, were present in only a limited number of animals, and/or were not observed in an exposure-related manner.

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No microscopic examination was conducted at the week 16 (recovery) necropsy because results of the week 12 (cessation of exposure) microscopic examination were unremarkable.

## VI. DISCUSSION AND CONCLUSIONS

One male in the 700/0 ppm group died on study day 97. The cause of death was renal inflammation with septicemia. Based on prior extensive experience with this species and strain of rat at similar exposure levels of this test article under essentially identical study conditions, this death was considered to be spontaneous in origin. Consequently, this death was not attributed to the test article. One female in the 700/0 ppm group was euthanized *in extremis* by order of the Study Director on lactation day 1 (study day 95). All other animals survived to the scheduled necropsies. Treatment-related clinical signs noted in the 700/0 ppm group males one hour following exposure included red material around the nose. Exposure-related increases in the incidence of ejaculatory plugs detected following the daily exposure interval were observed in all exposure groups beginning approximately two weeks after initiation of treatment and continuing throughout the treatment period. The significance of the ejaculatory plugs is unknown.

Reproductive parameters (days between pairing and coitus, mating indices, fertility indices, and duration of gestation and parturition) were not adversely affected by exposure to the test article in any exposure group. Fertility and mating indices were 90.9% or higher in all exposure groups, including the control groups.

Mean body weights and body weight gains in the 70/0, 300/0, 500/0, and 700/0 ppm group males were unaffected by exposure to the test article. Food consumption, evaluated as g/animal/day and g/kg/day, was unaffected by exposure throughout the study.

At the scheduled necropsies of the F<sub>0</sub> males, no exposure-related internal findings were noted. No treatment-related microscopic findings were noted at the necropsy of F<sub>0</sub> males following completion of the breeding period (week 12 necropsy). Sperm motility was unaffected by exposure to the test article at all concentrations evaluated. Sperm morphology means in the 500/0 and 700/0 ppm

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groups were comparable to the control group values; this parameter was not evaluated for the 70/0 and 300/0 ppm groups. Mean absolute and relative liver, kidney, and thyroid gland weights were increased in the 700/0 ppm group males at the week 12 necropsy. Mean absolute and relative liver weights were also increased in the 500/0 ppm group males at the week 12 necropsy. No adverse effects on organ weight data were noted at the week 16 recovery necropsy, indicating that the increases in liver, kidney, and thyroid gland weights noted at the week 12 necropsy were reversible.

Slight, statistically insignificant reductions in pup survival (% per litter) were noted in the 700/0 ppm group on PND 0 and during the birth to PND 4 interval. However, interpretation of the significance of this finding was confounded by the fact that the decreases were primarily due to only two litters with increased PND 0 mortality. Mean pup body weights in the 700/0 ppm group on PND 1 and 4 were statistically significantly decreased compared to the concurrent control group, but were within the ranges of values in the WIL historical control data. Mean live litter sizes and pup sex ratios were unaffected by exposure of F<sub>0</sub> males at all concentrations tested. No adverse clinical signs were noted in the pups at any exposure level. At the scheduled necropsy of pups on PND 4 and at the necropsies of pups that were found dead, no exposure-related findings were noted.

In conclusion, parental toxicity (males) was exhibited at an exposure level of 700/0 ppm by clinical signs and reversible increases in liver, kidney, and thyroid gland weights. Slight parental toxicity was also observed at the 500/0 ppm concentration by reversible increases in liver weights. No parental toxicity was evident at concentrations of 70/0 and 300/0 ppm. No adverse effects on F<sub>1</sub> pups were observed at exposure levels of 70/0 and 300/0 ppm. At the 700/0 ppm exposure level, reduced pup survival (statistically insignificant) and reduced pup body weights (statistically significant) were observed during the postnatal period.