

**CODING FORMS FOR SRC INDEXING**

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<b>New Doc ID</b>	89960000133	<b>Old Doc ID</b>	8FHQ-0596-13585
<b>Date Produced</b>	04/18/96	<b>Date Received</b>	05/21/96
		<b>TSCA Section</b>	8E
<b>Submitting Organization</b>		DOW CORNING CORP	
<b>Contractor</b>		WIL RESEARCH LABORATORIES INC	
<b>Document Title</b>		SUPPORT: AN INHALATION RANGE-FINDING REPRODUCTIVE TOXICITY STUDY OF OCTAMETHYLCYCLOTETRASIOXANE (D4) IN RATS, WITH COVER LETTER DATED 5/10/96	
<b>Chemical Category</b>		OCTAMETHYLCYCLOTETRASIOXANE	

SUPP

8EHQ-0596-13585

PDCN: 88960000065

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May 10, 1996

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



8EHQ-96-13585  
SP001 05/21/96

Re: Supplemental Submission to 8EHQ-0296-13585  
TSCA Section 8(e) Notification of Substantial Risk  
Octamethylcyclotetrasiloxane

**ORIGINAL**

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 Corporation is submitting the following final report as a supplemental submission to our Notification of Substantial Risk of February 13, 1996 (8EHQ 0296-13585).

**Chemical Substance:**

556-67-2 Octamethylcyclotetrasiloxane

**Manufacturer:**

Dow Corning Corporation  
2200 West Salzburg Road  
Midland, Michigan 48686-0994



89960000133

**Submitted Study:**

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

Dow Corning Corporation  
1995-16000-40919  
April 18, 1996

**Contains No CBI**

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**Background:**

In a letter dated February 13, 1996, Dow Corning Corporation provided EPA with a notification of substantial risk under TSCA Section 8(e) concerning interim data obtained from preliminary range-finding inhalation reproductive toxicity studies in Sprague-Dawley rats with octamethylcyclotetrasiloxane (OMCTS, D<sub>4</sub>). These studies were conducted as part of our siloxane research program, a thirty million dollar voluntary health effects research program on six siloxane materials, a program which has been reviewed with EPA's Office of Pollution Prevention and Toxics on several occasions and which was the subject of a Memorandum of Understanding between Dow Corning and EPA which was signed April 9, 1996. Even though we did not believe that the results of these preliminary range-finding studies represented a substantial risk to human health or the environment, we nevertheless

DOW CORNING CORPORATION, MIDLAND, MICHIGAN 48686-0994

TELEPHONE: (517) 496-4000

RCD/999/WP

reported them to EPA to assure our compliance with the letter and spirit of TSCA Section 8(e). As promised in our initial notification, we are now providing EPA with a copy of the recently completed final report for the first of the two studies cited in our submission of February 13, 1996.

A copy of this final report also is being provided to the Agency via a separate cover letter in compliance with TSCA Section 8(d) (health and safety data reporting).

#### **Executive Summary:**

This screening study was designed to determine the potential adverse effects of OMCTS on male and female reproduction in Sprague-Dawley rats in a single generation. Both male and female rats (20/sex/exposure level) were exposed to OMCTS vapor via whole body inhalation at 70 and 700 ppm six (6) hours per day for twenty-eight (28) days prior to mating; a concurrent filtered air control followed a similar regimen. Exposure of both sexes continued through a two week mating phase until necropsy of the males. For females, exposure was continued after mating until gestation day 20. Exposure of the dams was discontinued from gestation day 21 through lactation day 4, and then resumed on lactation day 5 until weaning on postnatal day 21.

No significant clinical signs of toxicity were noted in male or female rats in the F<sub>0</sub> or F<sub>1</sub> generations exposed to OMCTS. Decreased body weight gain was seen in the 700 ppm F<sub>0</sub> males during the first three weeks of the pre-breeding period and in the 700 ppm F<sub>0</sub> females during week 0-1 prior to breeding and during gestation days 14-20. Decreased food consumption was observed in the 700 ppm F<sub>0</sub> females during week 0-1 prior to breeding.

Fertility indices, mating indices, days between pairing and coitus, gestation, and the process of parturition were not affected by exposure to OMCTS at 70 and 700 ppm. On necropsy of the F<sub>0</sub> males, of the F<sub>0</sub> females 25 days post-mating and 27 days following the conclusion of the breeding period, and of the F<sub>1</sub> pups, no internal findings related to exposure to OMCTS were observed at any exposure level. On necropsy of the F<sub>0</sub> females on lactation day 21, a reduction in the mean number of implantation sites in the 700 ppm group relative to the control group was noted; interpretation of these data was confounded by the atypically high values for this parameter in the control and 70 ppm groups. A reduction in mean litter size (11.6 pups/litter) was observed in the 700 ppm group only. While this reduction was statistically significant when compared to concurrent controls, the control group had an atypically high mean viable litter size (16.4 pups/litter compared to a historical range of 11.7 - 15.9 pups/litter). The mean viable litter size of 11.6 pups/litter in the 700 ppm exposure group, approximating the lower end of the historical control data for this strain of rat, coupled with the fact that all other reproductive performance indices were unaffected by exposure to OMCTS, confounds interpretation of these data.

We also observed an increased incidence of ejaculatory plugs during the premating exposure interval among the F<sub>0</sub> males in the 700 ppm group. The plugs may be the result of an excessive stereotypical grooming behavior. This finding was previously communicated in briefings to EPA representatives.

#### **Actions:**

In attempt to ascertain whether or not OMCTS could have an impact on mean viable litter size, a second inhalation reproductive study was undertaken. Although this study has not yet been completed, interim data from the study confirm the effect of 700 ppm OMCTS exposure on parental body weight gains and food consumption. Again, the

Inhibition of body weight gain was particularly evident in the OMCTS exposed females during gestation. An increased number of ejaculatory plugs again was noted during the pre-mating exposure interval from males in the 700 ppm group. The second study also confirmed the lack of effect of OMCTS exposure on overall reproductive performance (male or female fertility index, pregnancy rate, mating behavior, proportion of females with viable pups, or postcoital interval). Sperm counts and sperm production rates as well as morphologic assessment of testes, ovaries, and epididymides were unaffected by OMCTS exposure. A statistically significant reduction in mean viable litter size (8.7 pups/litter versus controls of 14.6 and 13.2 pups/litter) was observed as well as a statistically significant reduction in the mean number of implantation sites (10.8 versus controls of 14.6 and 15.8) in the 700 ppm OMCTS group. Additionally, pup viability was reduced on day 1 of lactation. No gross or visceral abnormalities were observed in either dams or pups exposed to OMCTS.

Dow Corning will notify EPA of any further pertinent information that may be developed concerning OMCTS and will provide EPA with a copy of the final report for the second study cited in our initial notification of substantial risk of February 13, 1996.

If you have any questions concerning the aforementioned studies, please contact Dr. Richard W. Mast, Scientific Director, Product Safety and Toxicology, at 517-496-8569. If you require further general information concerning this submission, please contact Dr. Rhys G. Daniels, Regulatory Compliance Specialist, Product Stewardship and Regulatory Compliance Department, at the address provided herein or by telephone at 517-496-4222.

Sincerely,

  
\_\_\_\_\_  
Michael P. Hill  
U.S. Area Vice-President  
Corporate Director HES

**Contains No CMI**

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

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

Report No.: 1995-I0000-40919

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

Study No.: 8305

External Testing Facility No.: WIL-51035

Test Article: Octamethylcyclotetrasiloxane (D4)

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

Author: Ann S. Stump, Ph.D.  
Senior Report Writer

Sponsor: Dow Corning Corporation

Sponsor Representative: Waheed H. Siddiqui, Ph.D.  
Associate Toxicology Scientist

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

Study Completion Date: March 7, 1996

Security Statement: **DOW CORNING EXTERNAL INFORMATION**

This information has been approved for external  
distribution

Volume 1 of 2

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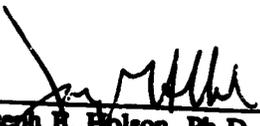
DC Study No. - 8305  
External No. - WIL-51035

DC Report No. - 1995-10000-40919  
Security - EXTERNAL

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

**COMPLIANCE STATEMENT**

This study, designated WIL-51035, 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.

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

3/7/96  
Date



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 External No. - WIL-51035

DC Report No. - 1995-I0000-40919  
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**QUALITY ASSURANCE UNIT STATEMENT**

<u>Date(s) of Inspection(s)</u>	<u>Phase Inspected</u>	<u>Date(s) Findings Reported to Study Director</u>	<u>Date(s) Findings Reported to Management</u>
10/18/94	Test Material Administration	10/18/94	11/28/94
12/8/94	Parturition Observations	12/8/94	1/25/95
12/9/94	Pup Test Material Exposure	12/28/94	1/25/95
4/6/95	Study Records (I-4)	4/6/95	5/30/95
4/11,17,18/95	Study Records (N-2)	4/18/95	5/30/95
2/1,2,3/28, 4/13,18-20, 5/9/95	Study Records (I-1)	5/9/95	6/26/95
4/11,18 & 5/9/95	Study Records (N-1)	5/9/95	6/26/95
4/6,11,17 & 5/9/95	Study Records (I-3)	5/10/95	6/26/95
4/7,10,11,17, 5/10/95	Study Records (I-2)	5/10/95	6/26/95
4/21,24-28 & 5/1,2,10, 11/95	Study Records (A-1)	5/11/95	6/26/95
5/1,2,11/95	Study Records (A-2)	5/11/95	6/26/95
4/21 & 5/11/95	Study Records (A-3)	5/11/95	6/25/95
4/21,24 & 5/11/95	Study Records (A-4)	5/11/95	6/26/95
4/24-26 & 5/11/95	Study Records (A-5)	5/11/95	6/26/95
4/24,25 & 5/2,11/95	Study Records (A-6/A-7)	5/11/95	6/26/95
5/1,2,11/95	Study Records (A-8)	5/11/95	6/26/95
4/28 & 5/1, 11/95	Study Records (A-9)	5/11/95	6/26/95
5/1,2 & 11/95	Study Records (A-10/A-11)	5/11/95	6/26/95
5/21-26,30,31 & 6/1,2,5,6/95	Draft Report (without Inhalation)	6/6/95	7/25/95
9/6-8, 11-14/95	Draft Report(Inhalation Appendix)	9/14/95	10/30/95

This study was conducted and inspected in accordance with the current Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States Food and Drug Administration and Environmental Protection Agency, the Standard Operating Procedures of WIL Research Laboratories, Inc., and the sponsor's protocol

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

**I. SUMMARY**

This screening study was designed to determine the potential adverse effects of Octamethylcyclotetrasiloxane (D4) on male and female reproduction in rats in a single generation. Two groups of 20 F<sub>0</sub> male and 20 F<sub>0</sub> female Sprague-Dawley Cri:CD®BR rats were exposed to the test article via whole body inhalation. Exposure levels were 70 and 700 ppm (parts per million). A control group of identical design was exposed to clean, filtered air on a comparable regimen. All F<sub>0</sub> animals were exposed for 6 hours per day for a minimum of 28 days prior to mating and through the day of necropsy. Exposure of the F<sub>0</sub> females was suspended from gestation day 21 through lactation day 4. 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 lactation days 1, 4, 7, 14 and 21. Food consumption was measured for corresponding intervals prior to mating, during gestation and during lactation. All of the F<sub>0</sub> females were allowed to deliver and rear their pups to weaning on lactation day 21 [postnatal day 21 (PND 21)]. The offspring were potentially exposed *in utero* (placental transfer), through suckling and/or dermal contact during lactation and via 6-hour exposures following weaning (PND 21) until euthanization on PND 28. The surviving F<sub>0</sub> dams were necropsied on lactation day 21. The F<sub>0</sub> males were necropsied after the breeding period. F<sub>0</sub> females which failed to deliver were necropsied on post-mating day 25 (evidence of mating) or 27 days following the breeding period (no evidence of mating).

Fertility indices, mating indices, days between pairing and coitus, gestation and the process of parturition were not adversely affected by test article exposure at exposure levels of 70 and 700 ppm.

One female in the control group was euthanized 27 days following the breeding period, and two females in the 700 ppm group were euthanized on post-mating day 25 because they did not deliver litters. All other F<sub>0</sub> males and females survived to their respective necropsies. Treatment-related clinical signs noted during the observations made one hour following exposure included dried red material around the nose and dried clear material around both eyes in males and females in the 700 ppm group. The incidences of these findings were much greater in the females than in the males. In addition, one or more ejaculatory plugs were found on the cage papers beneath the cages of male animals during the exposure period (from the sixteenth day of exposure until the day prior to euthanization). The incidence of this finding was increased in the 700 ppm group; the significance of the finding is not known. No increase in the incidences of ejaculatory plugs or clinical signs were observed at 70 ppm.

Inhibition of body weight gain was observed in the 700 ppm F<sub>0</sub> males (during the first three weeks of the pre-breeding period) and in the 700 ppm F<sub>0</sub> females (during week 0-1 prior to breeding and during gestation days 14-20).

In the 700 ppm F<sub>0</sub> males, food consumption was comparable to that in the control group throughout the study. In the 700 ppm F<sub>0</sub> females, reductions in food consumption were observed during week 0-1.

At the necropsy of the F<sub>0</sub> males and at the necropsies of the F<sub>0</sub> females 25 days post-mating and 27 days following the conclusion of the breeding period, no internal findings related to exposure to the test article were observed at any exposure level. In the necropsy of the F<sub>0</sub> females on lactation day 21, it was

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noted that the mean number of implantation sites was reduced in the 700 ppm group relative to the control group value (the interpretation of these data was confounded by the atypically high values for this parameter in the control and 70 ppm groups). Mean live litter size was reduced in the 700 ppm group relative to the control group value. The reduction was statistically significant, and the value was slightly below the range of the WIL historical control data (11.6 pups/litter as compared to a range of 11.7-15.9 pups/litter). No biologically significant effect of test article exposure was observed on the number of dead pups on PND 0 or on pup viability indices.  $F_1$  pup sex ratios were not adversely affected by exposure to the test article. No test article-related clinical signs were observed in the  $F_1$  pups. At the necropsies of surplus pups on PND 21 and selected pups on PND 28, no exposure-related internal findings were noted.

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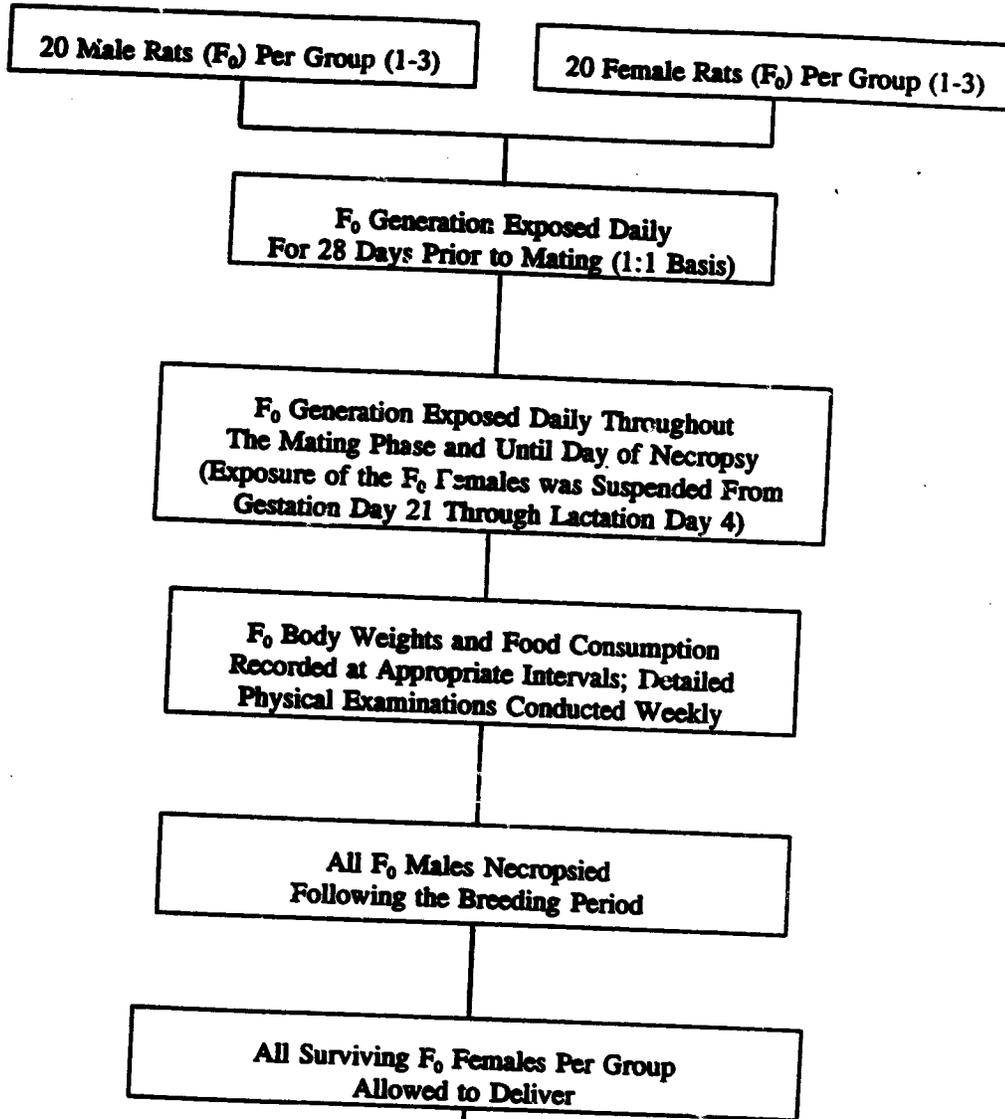
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## II. OBJECTIVE

The objective of the study was to determine appropriate exposure levels of the test article for a subsequent two-generation reproduction study, including an exposure concentration that produces toxicity but not excessive lethality (less than 10%) to the adult, and an exposure concentration that is a predicted NOAEL (no observed adverse effect level) for reproductive effects and effects on the offspring.

The selected route of administration was whole body inhalation exposure since this is an anticipated route of exposure for the general human population. The animal model, the Sprague-Dawley CrI:CD®BR rat, is recognized as appropriate for reproduction studies and was selected based on the availability of historical control data.

**III. STUDY DESIGN**

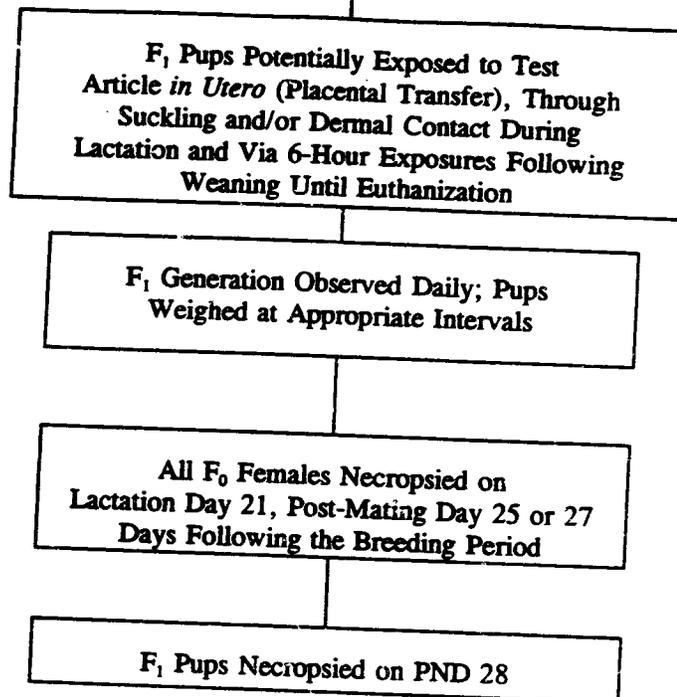


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III. STUDY DESIGN (continued)



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#### IV. EXPERIMENTAL PROCEDURES

##### A. STUDY SCHEDULE

Initiation of Exposure: October 18, 1994 (Week 0)  
F<sub>0</sub> Breeding: November 14-29, 1994 (Weeks 4-6)  
F<sub>0</sub> Male Necropsies: December 2, 1994 (Week 6)  
F<sub>0</sub> Lactation Day 21 Necropsies: December 27, 1994 - January 10, 1995 (Weeks 10-12)

Experimental Termination Date  
(Last necropsy of F<sub>1</sub> pups): January 17, 1995 (Week 13)

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 Rats."

##### B. TEST AND CONTROL ARTICLES

###### 1. TEST ARTICLE IDENTIFICATION

The test article, Octamethylcyclotetrasiloxane (D4), was received from Dow Corning Corporation, Auburn, Michigan, on August 23, 1994 and September 30, 1994, as follows:

<u>Label Identification</u>	<u>Quantity Received</u>	<u>Description</u>	<u>Date of Receipt</u>
1 <sup>a</sup> 244 Fluid Lot LL084721 002 Avoid Freezing Net Wt. 430 lb. (195.0 Kg)	1 Drum Total gross weight: 15130.3 g	Clear colorless liquid	8/23/94

<sup>a</sup> = This shipment was used for the pre-test validation for test atmosphere generation.

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<u>Label Identification</u>	<u>Quantity Received</u>	<u>Description</u>	<u>Date of Receipt</u>
2) <sup>b</sup> Dow Corning 2 + Fluid Lot LL084752 (Drum #1-008, #2-009, #3-010) Net. Wt. 430 lb. (195.0 Kg) Avoid freezing Caution - combustible	3 Drums Total gross weight: 649550.0 g Drum #1: 212850.0 g Drum #2: 225100.0 g Drum #3: 211900.0 g	Clear colorless liquid	9/30/94

<sup>b</sup> = This shipment was used for method development and for the exposures during the study.

Stability and purity data were the responsibility of the sponsor. The bulk test article from shipments 1) and 2) were 97.83% and 99.7% pure, respectively. The test article was stable when stored at room temperature. Reserve 4.56 and 1.05 g retention samples of the test article were taken from the first shipment and the first drum of the second shipment, respectively, on August 25, 1994 and October 5, 1994, respectively. The retention 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.

## 2. TEST ATMOSPHERE MONITORING

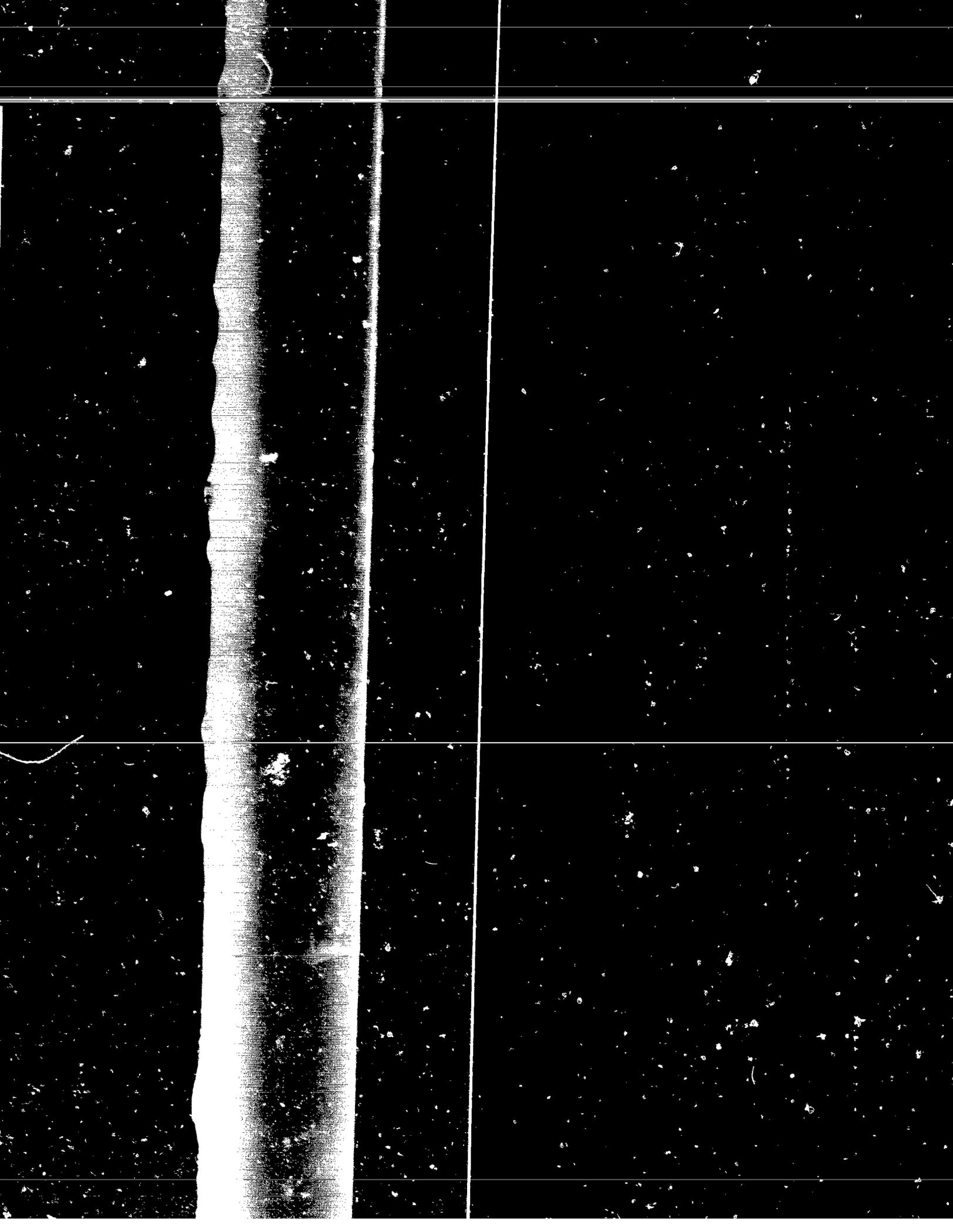
Exposure concentrations within the chamber were measured at least 12 times (approximately every 30 minutes) during each daily exposure period, by a validated gas chromatographic method. At least one standard was analyzed each day prior to exposure to confirm gas chromatographic calibration. Mass air flow, temperature, relative



humidity and oxygen content within the chamber were monitored continuously and were recorded at least every 30 minutes. Nominal chamber concentration was determined. Test atmosphere homogeneity data were generated during pre-study method development. The methodology and results of these analyses are presented in Appendix A.

3. EXPOSURE METHODS

Each group of animals was exposed in a 1.0 cubic meter stainless steel and glass whole body inhalation chamber. The chamber was operated under dynamic conditions to sustain air flows of at least 12 to 15 air changes per hour, ensuring an adequate oxygen content of 19% or above and an evenly distributed exposure atmosphere. The F<sub>0</sub> males were exposed to the test atmosphere for daily 6-hour exposures (seven days a week) for a minimum of 28 days prior to mating and continuing through the day of necropsy. The F<sub>0</sub> females were exposed to the test atmosphere following a similar schedule, but the exposure of the females was suspended from gestation day 21 through lactation day 4. This modification was necessary to minimize the likelihood of the F<sub>0</sub> maternal animals delivering their offspring during the exposure period and to prevent confounding the survival of the F<sub>1</sub> pups early in the postnatal period. The rats were removed from their home cages in the animal room and transported to the inhalation chambers for the 6-hour exposure period. The animals were exposed to the test article at approximately the same time each day. The animals were returned to their home cages following exposure. The litters remained in the maternity cages with nesting material while the maternal animals were



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transported to the inhalation chambers for the daily exposure. The F<sub>1</sub> pups were potentially exposed to the test article *in utero* (placental transfer), through suckling and/or dermal contact during lactation and via 6-hour exposures following weaning (PND 21) until euthanization on PND 28. Controls were set to maintain the temperature inside the exposure chamber at approximately 22°C ( $\pm$  2°C) and relative humidity between approximately 30% and 70%. Exposure methods and conditions are detailed in Appendix A.

Each chamber was dedicated to one dose group. In order to minimize any potential variation occurring due to positioning within the chamber, the cages were rotated around the available 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:

<u>Group Number</u>	<u>Group Name</u>	<u>Test Substance</u>	<u>Exposure</u>	<u>Number of Animals</u>	
			<u>Level</u> (ppm)	<u>Male</u>	<u>Female</u>
1	Control	Filtered Air	0	20	20
2	Low Dose	D4	70	20	20
3	High Dose	D4	700*	20	20

\* = The 700 ppm concentration is the maximum vapor concentration which can be generated without aerosolization.

### C. F<sub>0</sub> GENERATION

#### 1. ANIMAL RECEIPT AND ACCLIMATION

Seventy male and seventy female virgin Sprague-Dawley Cri:CD®BR rats were received from Charles River Breeding Laboratories, Inc., Portage, Michigan, on October 6, 1994. The males

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were approximately 60 days old and the females were approximately 75 days old upon receipt. Each animal was examined by the staff veterinarian upon receipt; the animals were initially weighed on October 7, 1994. All animals were uniquely identified by a metal eartag displaying the animal number and housed for an acclimation period of 12 days prior to initiation of dosing. During acclimation, individual body weights were recorded twice (following receipt and at randomization) and the animals were observed twice daily for mortality and moribundity.

## 2. ANIMAL HOUSING

Upon arrival and until pairing, all animals were individually housed in clean, wire-mesh cages suspended above cage-board. 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. Bred females were transferred to clean, individual plastic maternity cages with nesting material, ground corn cob bedding (Bed-O'Cobs®; The Andersons, Industrial Products Division, Maumee, OH 43537). The dams were housed in these cages through lactation day 21, the scheduled day of necropsy. Females which did not deliver were necropsied on *post coital* day 25. Females for which there was no evidence of mating were placed in clean, plastic maternity cages with nesting material upon completion of a 15-day mating period. Twenty-seven days following the conclusion of the mating period, females with no evidence of mating and which did not deliver were euthanized and necropsied. All animals were maintained 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 utilized 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 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

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  $72^{\circ} \pm 4^{\circ}\text{F}$  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. Temperature ranged from  $66^{\circ}\text{F}$  to  $76^{\circ}\text{F}$  and relative

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humidity ranged from 24% to 64% during the study period. Maximum deviations from the set temperature and humidity levels were 2°F and 6%, respectively. These deviations did not apparently 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. Eastern Standard Time) and air handling units were set to provide approximately 10 to 15 fresh air changes per hour.

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. At the discretion of the study director, animals judged to be in good health and meeting acceptable body weight requirements were selected for use in the computer randomization procedure. The individual body weights 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 experimental design consisted of two exposure groups and a control group, with 20 males and 20 females per group. At initiation of exposure, the males were approximately 72 days old and body weights ranged from 325 g to 380 g. The females were approximately 87 days old and body weights ranged from 227 g to 259 g. Several females were not within the protocol specified weight range (200-250 g). The upper limit of the weight range was extended to 260 grams to allow for the placement of enough female rats on the study. The female rats

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were 87 days old at the initiation of exposure, which was above the age range specified in the protocol (70-84 days). This slight deviation from the protocol had no apparent effect on the outcome of the study.

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. The configuration of the cages and the location of the chamber windows precluded observations during exposure for some animals. However, the positions of the animals in the inhalation chambers were rotated on a daily basis, allowing all animals to be observed periodically throughout the exposure period. All significant clinical findings were recorded at the post-exposure observations. The observations included, but were not limited to, 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 (stereotypies). Females which delivered were also

observed twice daily during the period of expected parturition and at parturition for dystocia.

b. BODY WEIGHTS

Individual F<sub>0</sub> male body weights were recorded on a weekly basis until euthanization, beginning with the initiation of exposure. Mean body weights were calculated for each of these periods. Corresponding weekly body weight changes were also calculated for each weekly interval.

Individual F<sub>0</sub> female body weights were recorded on a weekly basis, beginning with the initiation of exposure and continuing until evidence of copulation 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, 4, 7, 14 and 21. 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 and lactation days 1-21.

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, 4, 7, 14 and 21. Male food consumption was recorded weekly until

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euthanization. Food intake was calculated as g/animal/day and g/kg/day for the corresponding body weight change intervals.

7. BREEDING PROCEDURES

Before pairing on November 14, 1994 (week 4), male body weights ranged from 361 g to 465 g; female body weights ranged from 249 g to 314 g. The males were approximately 14 weeks old, and the females were approximately 16 weeks old. Animals were paired on a 1:1 basis within each exposure group after a minimum of 28 days of exposure to the test article. 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. Positive evidence of mating was confirmed by the presence of sperm in a vaginal smear or a copulatory plug. Each mating pair was examined daily. The day when evidence of mating was identified was termed day 0 of gestation. The animals were separated, and the female was housed in an individual plastic cage with nesting material. When evidence of copulation was not detected after 10 days of pairing, the female was placed with another male from the same exposure group for an additional five days. The second male always had positive evidence of mating detected with a previous female. When 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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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. FEMALES ALLOWED TO DELIVER

All females from each dose group were allowed to deliver naturally and rear their young to PND 21. 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 complete was designated PND 0. When parturition was judged complete, litters were sexed, 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. F. LITTER DATA

1. LITTER VIABILITY AND DEATHS

Each litter was examined daily for survival, and all 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. In addition, to facilitate differentiation

between litters and groups during exposure. Tattoos bearing the last two digits of the dam number were placed on the tails of the pups that were selected for exposure. This process was completed prior to the scheduled exposure period. A daily record of litter size was maintained. Intact offspring dying from PND 0 to 4 were necropsied using a modification of the Stuckhardt and Poppe<sup>2</sup> fresh dissection technique (including the heart and the brain examined by a mid-coronal slice). The carcasses were eviscerated and fixed in 100% ethyl alcohol. Following fixation, each fetus was macerated in potassium hydroxide and stained with Alizarin Red S by a method described by Dawson<sup>3</sup>. The carcasses were examined if a skeletal anomaly was suspected. Gross lesions were preserved in 10% neutral buffered formalin for possible future microscopic examination. Pups dying between PND 5 and 28 were necropsied, and tissues were saved for histopathological examination as deemed necessary by the gross findings.

2. LITTER REDUCTION

To reduce variability among the litters, eight pups per litter, four per sex when possible, were randomly selected on PND 4. Culled pups were weighed, euthanized and discarded.

3. CLINICAL OBSERVATIONS

Litters were examined daily for any adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1, 4, 7, 14, 21 and 28; only remarkable observations were reported. Pups were also observed for pharmacotoxic signs during exposure and within approximately one hour after completion of exposure. Abnormalities in nesting and nursing behavior, if present, were recorded.

4. BODY WEIGHTS

Pups were individually weighed on PND 1, 4, 7, 14, 21 and 28.

5. FOOD CONSUMPTION

Food consumption was not recorded between PND 21 and 28 because the weaned pups were housed together during this period.

6. SEX DETERMINATION

Pups were individually sexed on PND 0, 4 and 21.

7. SELECTION OF F<sub>1</sub> GENERATION

When litters reached an age of 21 days, a minimum of one male and one female per litter were selected. An additional male or female was selected from a litter, if necessary, to obtain 20 males and 20 females for each group. Selection was done randomly by computer generation [WIL Toxicology Data Management System (WTDMS™)].

8. CALCULATION OF LITTER PARAMETERS

Litter parameters were defined as follows:

$$\text{Live Litter Size} = \frac{\text{Total Viable Pups Day 0}}{\text{No. Litters With Viable Pups Day 0}}$$

$$\text{Viability Index (\%)} \text{ [Before Culling]} = \frac{\text{Pups Viable on Day 1 or 4 (before culling)}}{\text{Pups Viable on Day 0}} \times 100$$

$$\text{Viability Index (\%)} \text{ [After Culling]} = \frac{\text{Pups Viable on Day } n}{\text{Pups Viable on Day 4 (after culling)}} \times 100$$

where n = (7, 14 or 21)

9. PND 21 AND 28 NECROPSIES

The surplus F<sub>1</sub> pups were euthanized via caudal tail vein injection of sodium pentobarbital and necropsied on PND 21 with an emphasis on developmental morphology, with the following exceptions. Several F<sub>1</sub> pups were euthanized via intraperitoneal injection of Socumb® instead of

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intravenous injection by tail vein, as specified in the protocol. Tissues were preserved in 10% neutral buffered formalin for possible future histopathological examination only as deemed necessary by the gross findings. The carcasses of the pups were then discarded. On PND 28, all surviving selected F<sub>1</sub> pups were euthanized via caudal tail vein injection of sodium pentobarbital and necropsied. Tissues were preserved in 10% neutral buffered formalin for possible future histopathological evaluation only as deemed necessary by the gross findings.

**B. NECROPSY EXAMINATIONS**

A complete necropsy examination was conducted on all F<sub>0</sub> animals surviving to the scheduled necropsies. When breeding was completed, all surviving F<sub>0</sub> males were necropsied on December 2, 1994. All surviving females with viable pups were necropsied on lactation day 21, and the numbers of previous implantation sites were recorded. Bred females which did not deliver a litter were necropsied on post-mating day 25 (evidence of mating) or 27 days following the conclusion of the breeding period (no evidence of mating). Female no. 31612 (control group) was negative for the presence of sperm at the end of the mating period. The scheduled day of necropsy for this animal was 25 days following the conclusion of the breeding period. However, the animal was inadvertently not sent to necropsy until 27 days following the conclusion of the breeding period, and was, therefore, exposed to filtered air for six hours on two additional days. This female was nongravid. This deviation from the protocol did not affect the outcome of the study. A detailed gross necropsy was performed on each of the bred females which did not deliver to determine pregnancy status with

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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>4</sup>. Surviving animals were euthanized with an injection of sodium pentobarbital via a caudal tail vein. 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 only as deemed necessary by the gross findings.

F. STATISTICAL METHODS

All analyses were conducted for a minimum significance level of 5% comparing each treated group 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. Data obtained from nongravid animals were excluded from statistical analysis following the mating period. The litter was used as the experimental unit. The numbers of animals (N) used to calculate the means are provided on the individual data tables. All statistical tests are performed by a Digital<sup>®</sup> MicroVAX<sup>®</sup> 3400 Computer (with appropriate programming) in this laboratory and are referenced on the report tables.

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STATISTICAL TEST

- Chi-square test<sup>1</sup> with Yates' correction factor
- ANOVA (two-tailed) with Dunnett's test<sup>2</sup>

PARAMETER

Pup Sex Ratios, Pup Survival Indices, Mean No. Stillborn and Dead Pups, Parental Fertility Indices

F<sub>0</sub> Body Weights and Weight Gains, Gestation and Lactation Body Weights and Weight Gains, Parental Food Consumption, Mean Litter Weights, Length of Gestation, Live Litter Sizes

**G. DATA RETENTION**

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

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

V. RESULTS

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

i. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 1, 3, 4

Individual Data: Appendices B, E

Female no. 31612 in the control group was euthanized on study day 69, 27 days following the breeding period, and female nos. 31667 and 31627 in the 700 ppm group were euthanized on study days 56 and 62, respectively, on post-mating day 25 because they did not deliver litters. All other F<sub>0</sub> females and males survived to the scheduled necropsies. Exposure-related clinical signs noted during the observations made one hour following exposure included dried red material around the nose and dried clear material around both eyes in males and females in the 700 ppm group. The incidences of both of these findings were much greater in the females than in the males in this group. Other clinical signs noted in the treated groups occurred infrequently and/or at a similar frequency in the control group.

During the F<sub>0</sub> phase of the study, an unusual observation was noted. One or more ejaculatory plugs were found on the cage paper beneath cages of male animals (a single ejaculatory plug was found on the cage paper beneath the cage of one female animal during the breeding period). Ejaculatory plugs are aggregated secretions of the coagulating gland of the male rodent which are formed post-ejaculation and functionally prevent leakage of seminal plasma from the vagina. These plugs were found regularly from the sixteenth day of exposure (November 2, 1994) until the day prior to euthanization (December 1, 1994). The incidence

of ejaculatory plugs in the 700 ppm group was increased relative to the control group values. Tabular presentations of the summary and individual data are presented in Appendix E. The significance of the presence of the ejaculatory plugs is not known.

2. REPRODUCTIVE PERFORMANCE

Summary Data: Table 2

Individual Data: Table 29

Historical Control Data: Appendices C, D

Reproductive performance was not adversely affected by exposure to the test article at exposure levels of 70 and 700 ppm. Fertility indices for males were 95.0%, 100% and 90.0% and for females were 95.0%, 100% and 95.0% in the control, 70 and 700 ppm groups, respectively. Male mating indices were 95.0%, 100% and 95.0% and female mating indices were 95.0%, 100% and 100% for the control, 70 and 700 ppm groups, respectively. Males which did not sire a litter numbered 1, 0 and 3 in the same respective exposure groups. Males that sired more than one litter numbered 0, 0 and 1 in the control, 70 and 700 ppm groups, respectively. In these same respective exposure groups, 0, 0 and 2 females had evidence of mating but did not deliver. Of the two females in the 700 ppm group that had evidence of mating but did not deliver, one was found to be gravid and the other was found to be nongravid at the *post mortem* examination. One female in the control group had no evidence of mating, did not deliver and was nongravid.

The mean numbers of days between pairing and coitus were 2.3, 2.5 and 3.5 in the control, 70 and 700 ppm groups, respectively, as compared to a mean value of 3.3 days and a range of 2.3 to 6.1 days in

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the WIL historical control data. The difference between the control and 700 ppm group values was not statistically significant (as determined by one-way analysis of variance), and was due to values of 10 and 14 days for female nos. 31627 and 31663, respectively, in the 700 ppm group. Therefore, this increase was not interpreted to be exposure-related.

### 3. BODY WEIGHTS

#### a. WEEKLY

Summary Data: Tables 5, 6

Individual Data: Tables 30, 31

The mean weekly body weight in the 700 ppm group F<sub>0</sub> males was comparable to the control group value for week 0. Mean weekly body weights in the 700 ppm group males were reduced relative to the control group values during weeks 1-6. The reductions during weeks 3-6 were statistically significant ( $p < 0.05$ ). Mean body weight gains were reduced in these animals during weeks 0-1 through 2-3. These reductions were statistically significant ( $p < 0.05$  or  $p < 0.01$ ) when compared to the control group. Mean body weight gains in the 700 ppm group males were comparable to the control group values during weeks 3-4 through 5-6.

The mean weekly body weight in the 700 ppm group females was comparable to the control group value for week 0. The mean weekly body weights in these animals were reduced relative to the control group values during weeks 1-4. The reductions during weeks 1-3 were statistically significant ( $p < 0.05$ ). A statistically significant ( $p < 0.01$ ) reduction was observed in the mean weekly body weight gain in these animals during week 0-1. The mean body

weight gains in these animals were comparable to the control group values during weeks 1-2 through 3-4.

Mean body weights and body weight gains in the 70 ppm group males and females were comparable to the control group values. The differences were slight and were not statistically significant.

b. GESTATION

Summary Data: Tables 7, 8

Individual Data: Tables 32, 33

The mean body weight in the 700 ppm group females was slightly reduced on gestation day 0, presumably as an extension of the effect on body weights observed during the pre-mating period. Mean body weights in these animals were similar to the control group values during gestation days 7, 10 and 14. On gestation day 20, mean body weight was reduced in these animals. The reduction was statistically significant ( $p < 0.01$ ) and was likely a result of a decrease in viable litter size in these females (please refer to section V.B.4). Mean gestation body weight gains in the 700 ppm group were comparable to the control group values during gestation days 0-7, 7-10 and 10-14. Statistically significant ( $p < 0.05$  or  $p < 0.01$ ) reductions in mean body weight gain were observed in these animals during gestation days 14-20 and when the entire gestation period (gestation days 0-20) was evaluated.

Mean body weights and body weight gains in the 70 ppm group were comparable to the control group values throughout the gestation period. The differences were slight and were not statistically significant.

c. LACTATION

Summary Data: Tables 9, 10

Individual Data: Tables 34, 35

Mean body weights and body weight gains in the 700 ppm group females were unaffected by test article exposure during the lactation period. The differences between the control and treated groups were slight and were not statistically significant.

4. FOOD CONSUMPTION

a. WEEKLY

Summary Data: Tables 11, 12

Individual Data: Tables 36, 37

Weekly food consumption, evaluated as g/animal/day and g/kg/day, in the 700 ppm group males was similar to that in the control group during weeks 0-1 through 3-4. The only statistically significant ( $p < 0.05$ ) difference was a reduction in food consumption, evaluated as g/animal/day, in these animals during weeks 2-3. However, the reduction was slight (2 g/animal/day) and was not observed when food consumption was evaluated as g/kg/day; therefore, the reduction was not considered to be related to exposure to the test article. During the interval following breeding and before euthanization (week 6-7), food consumption in these animals was comparable to that in the control group.

In the 700 ppm group females, food consumption, evaluated as g/animal/day and g/kg/day, was reduced during week 0-1; this reduction was statistically significant ( $p < 0.01$ ), and was considered to be related to exposure to the test article. Food consumption in

the 700 ppm group females was comparable to that in the control group during the remainder of the pre-breeding period (weeks 1-2 through 3-4).

Food consumption, evaluated as g/animal/day and g/kg/day, in the 70 ppm group males and females was unaffected by exposure to the test article throughout the pre-breeding period. The only statistically significant ( $p < 0.05$ ) difference between the control and treated group values was an increase in food consumption, evaluated as g/kg/day, in the 70 ppm group males during week 0-1. Food consumption, evaluated as g/animal/day, was unaffected by exposure to the test article in the 70 ppm males during the interval between the end of the breeding period and euthanization.

b. **GESTATION**

Summary Data: Tables 13, 14

Individual Data: Tables 38, 39

Food consumption, evaluated as g/animal/day and g/kg/day, was unaffected by test article exposure in the 700 ppm group throughout gestation. A statistically significant ( $p < 0.01$ ) increase in food consumption, evaluated as g/animal/day and g/kg/day, was observed during gestation days 10-14 in the 700 ppm group. A statistically significant ( $p < 0.01$ ) increase in food consumption, evaluated as g/kg/day, was observed in the 700 ppm group when the entire gestation period (gestation days 0-20) was evaluated.

Food consumption, evaluated as g/animal/day and g/kg/day, in the 70 ppm group was comparable to that in the control group

throughout the gestation period. The differences were slight and were not statistically significant.

c. LACTATION

Summary Data: Tables 15, 16

Individual Data: Tables 41, 42

Food consumption, evaluated as g/animal/day and g/kg/day, in the 70 and 700 ppm groups was slightly reduced throughout the lactation period (lactation days 1-4, 4-7, 7-14, 14-21 and 1-21). The reduction in the 700 ppm group during lactation days 7-14 was statistically significant ( $p < 0.05$ ) when food consumption was evaluated as g/animal/day. Because the reductions were slight, they were not interpreted to be exposure-related.

5. GESTATION LENGTH AND PARTURITION

Summary Data: Table 17

Individual Data: Table 42

Historical Control Data: Appendices C, D

The mean durations of gestation were comparable between the treated groups and the control group. No differences were statistically significant. The mean gestation durations in the 70 and 700 ppm groups were 21.8 days and 22.0 days, respectively, compared to mean gestation durations of 21.6 days in the concurrent control group and 21.9 days in the WIL reproductive historical control data.

One animal (no. 31664) in the 700 ppm group had signs of dystocia; this animal exhibited an extended duration of parturition. Other signs noted in this animal at parturition included paleness in color, red vaginal

discharge and labored respiration. No other signs of dystocia at parturition were observed for animals in this study.

**B. E. LITTER DATA**

**1. LIVE BIRTH AND VIABILITY INDICES**

Summary Data: Table 18

Individual Data: Tables 43, 44

Historical Control Data: Appendices C, D

The numbers of dead pups on lactation day 0 in the 70 and 700 ppm groups were increased relative to the control group value (8 dead pups in each of the treated groups, as compared to 1 dead pup in the control group). The increases were statistically significant ( $p < 0.05$  for the 70 ppm group and  $p < 0.01$  for the 700 ppm group). However, in each case, five of the pups found dead were from one litter (nos. 31621 and 31645 in the 70 and 700 ppm groups, respectively); therefore, the increases were not considered to be related to test article exposure.

Viability indices on lactation days 1, 4 (before and after culling), 7, 14 and 21 remained at or above 97.7% in the control and 70 ppm groups. Viability indices for the 700 ppm group were slightly reduced relative to the control group values on lactation days 1 and 4 (before culling); the reduction on lactation day 1 was statistically significant ( $p < 0.05$ ). However, this value (97.1%) was within the range in the WIL reproductive historical control data (83.6%-100%), and was not considered to be a result of exposure to the test article. Viability indices for the 700 ppm group remained at 100% on lactation days 4 (after culling), 7, 14 and 21. Between lactation days 21 and 28, 0, 2 and 3 pups in the control, 70 and 700 ppm groups were found dead. The

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differences in survival index between the control and treated groups were not statistically significant.

2. SEX RATIOS

Summary Data: Table 18

Individual Data: Table 43

Pup sex ratios were not adversely affected by exposure to the test article. The differences between the control and treated groups were not statistically significant.

3. GENERAL PHYSICAL CONDITION AND MORTALITIES

Summary Data: Tables 19, 20, 21

Individual Data: Tables 43, 44, 45, 46

Pups which were found dead during the lactation period numbered 7, 14 and 12 in the control, 70 and 700 ppm groups, respectively. In these same groups, 0, 4 and 4 pups, respectively, were missing and presumed to be cannibalized. The general physical condition of the F<sub>1</sub> pups during lactation was similar in all study groups, including the control group. The clinical signs that were noted occurred infrequently and/or at a similar frequency in the control group.

In the necropsy of the pups that were found dead, one pup (no. 31674-07) in the 700 ppm group had submeningeal hemorrhage. One pup each in the 70 and 700 ppm groups (nos. 31622-14 and 31664-03, respectively) had red contents in the stomach; pup no. 31622-14 in the 70 ppm group also had red intestinal contents.

4. LIVE LITTER SIZE AND PUP BODY WEIGHTS

Summary Data: Tables 18, 23

Individual Data: Tables 43, 44, 49, 50

Historical Control Data: Appendices C, D

The mean live litter size was reduced in the 700 ppm group relative to the control group value; the reduction was statistically significant ( $p < 0.01$ ), and was slightly below the range of the WIL historical control data (11.6 pups/litter as compared to a range of 11.7-15.9 pups/litter). The mean live litter size in the 70 ppm group was comparable to the control group value; the difference was slight and was not statistically significant.

No adverse effects of the test article were observed on mean pup weights on lactation days 1, 4 (before and after culling), 7, 14, 21 and 28 at exposure levels of 70 and 700 ppm. The only statistically significant ( $p < 0.01$ ) differences from the control group were increased mean pup weights in the 700 ppm group on lactation days 1 and 4 (before and after culling).

5. PND 21 AND 28 NECROPSIES

Summary Data: Tables 21, 22

Individual Data: Tables 47, 48

At the necropsy of surplus pups on PND 21, no exposure-related findings were noted. In the control, 70 and 700 ppm groups, 1, 1 and 2 pups, respectively, had dark red lungs. An abnormality was observed in pup no. 31649-02 in the 70 ppm group; the tail of this pup was absent. No other remarkable findings were noted.

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At the necropsy of selected pups on PND 28, 2, 5 and 8 pups in the control, 70 and 700 ppm groups, respectively, had dark red lungs, and one pup (no. 31650-02) in the 700 ppm group had multiple, irregularly-shaped dark red areas on the lungs. One animal (no. 31630-13) in the control group had an accessory spleen. No other remarkable findings were noted at any exposure level.

C. NECROPSY EXAMINATIONS

1. F. FEMALES-POST-MATING DAY 25

Summary Data: Table 24

Individual Data: Table 51

Two females (nos. 31627 and 31667) in the 700 ppm group had evidence of mating but did not deliver and were necropsied on post-mating day 25. Female no. 31627 was nonpregnant. Female no. 31667 was pregnant and was observed to have three early resorptions *in utero*. No other internal findings were noted at the necropsy of these animals.

2. F. FEMALE-27 DAYS FOLLOWING THE BREEDING PERIOD

Summary Data: Table 25

Individual Data: Table 52

One control group female (no. 31612) had no evidence of mating and was necropsied 27 days following the conclusion of the breeding period. This animal was nonpregnant and was internally normal.

3. E. FEMALES-LACTATION DAY 21

Summary Data: Tables 26, 27

Individual Data: Tables 53, 54

At the scheduled necropsy on lactation day 21, no exposure-related findings were noted at any exposure level. In the control, 70 and 700 ppm groups, 1, 1 and 2 females, respectively, had enlarged cervical lymph nodes. One female (no. 31617) in the 700 ppm group had dark red areas on the lungs. One female each in the 70 and 700 ppm groups had a nodule in the abdominal cavity, and one female (no. 31551) in the 70 ppm group had a nodule in the stomach. One female each in the control and 70 ppm groups had one or more cysts on the spleen. All other females examined at the necropsy on lactation day 21 were internally normal.

The mean number of implantation sites and the number of pups born were reduced, and the number of sites unaccounted for were increased in the 700 ppm group relative to the control group values. The differences were statistically significant ( $p < 0.01$ ) and were interpreted to be exposure-related. The mean number of implantation sites in the 700 ppm group (13.5 sites) was within the range of the WIL reproductive historical control data (11.0-16.9 sites). The interpretation of these data was confounded by the fact that the mean values in the control and 70 ppm groups (17.2 and 17.0 sites, respectively) were above the range in the historical control data. The mean number of sites unaccounted for (1.7 sites) was within the range of the historical control data (0.0-2.3 sites).

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4. E. MALES-SCHEDULED NECROPSY

Summary Data: Table 28

Individual Data: Table 55

At the scheduled necropsy of the males, no exposure-related findings were noted at any exposure level. One male (no. 31551) in the 700 ppm group had a diaphragmatic hernia, and two males in the control group had hemorrhagic thymus glands. All other animals were internally normal.

VI. DISCUSSION AND CONCLUSIONS

Fertility, mating, days between pairing and coitus, gestation and parturition were not adversely affected by exposure to the test article at exposure levels of 70 and 700 ppm. Fertility indices were 90.0% or higher and mating indices were 95.0% or higher for F<sub>0</sub> males and females in all groups, including the control group. One female in the 700 ppm group exhibited signs of dystocia (extended duration of parturition).

One female in the control group was euthanized 27 days following the conclusion of the breeding period, and two females in the 700 ppm group were euthanized on post-mating day 25 because they did not deliver litters. All other F<sub>0</sub> females and males survived to their respective necropsies. Exposure-related clinical signs noted during the observations made one hour following exposure included dried red material around the nose and dried clear material around both eyes in males and females in the 700 ppm group. The incidences of these findings were much higher in the females than in the males. In addition, one or more ejaculatory plugs were found on the cage papers beneath the cages of male animals during the exposure period. The incidence of this finding was increased in the 700 ppm group; the significance of the finding is not known.

Mean weekly body weights in the 700 ppm group males were reduced relative to the control group values during weeks 1-6. The reductions during weeks 3-6 were statistically significant. Statistically significant reductions in mean body weight gain were observed in these animals during weeks 0-1 through 2-3. Mean body weight gains in the 700 ppm group males were comparable to the control group values during weeks 3-4 through 5-6. In the 700 ppm group females, mean body weights were reduced relative to the control group values during weeks 1-4; the reductions during weeks 1-3 were statistically significant.

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Mean body weight gain was reduced in these animals during week 0-1 and comparable to the control group values during weeks 1-2 through 3-4. A slightly reduced mean body weight was observed in the 700 ppm group on gestation day 0. Mean body weights in the 700 ppm group females were similar to the control group values during gestation days 7, 10 and 14. On gestation day 20, the mean body weight was reduced in these animals; the reduction was statistically significant. Mean body weight gain in these animals was similar to that in the control group during gestation days 0-7, 7-10 and 10-14. Statistically significant reductions in mean body weight gain were observed in these animals during gestation days 14-20 and when the entire gestation period (gestation days 0-20) was evaluated. The reductions in mean body weight and body weight gain at the end of the gestation period may have been due, in part, to the reduced litter size in these animals. In the 70 ppm group, mean body weights and body weight gains were unaffected during the pre-breeding period and gestation. Mean body weights and body weight gains in the 70 and 700 ppm groups during lactation were similar to the control group values.

Food consumption, evaluated as g/animal/day and g/kg/day, in the 700 ppm group males was unaffected by exposure to the test article. In the 700 ppm group females, a reduction in food consumption, evaluated as g/animal/day and g/kg/day, was observed during week 0-1. The reduction was statistically significant and was considered to be related to test article exposure. Food consumption in these animals was comparable to that in the control group during the remainder of the pre-breeding period (weeks 1-2 through 3-4) and during gestation. Food consumption, evaluated as g/animal/day and g/kg/day, in the 70 and 700 ppm groups was unaffected by exposure to the test article throughout lactation.

At the necropsy of the F<sub>0</sub> males and at the necropsies of the females 25 days post-mating and 27 days following the conclusion of the breeding period, no internal findings related to exposure to the test article were observed at any exposure level. In the necropsy of the F<sub>0</sub> females on lactation day 21, it was noted that the mean number of implantation sites was reduced in the 700 ppm group relative to the control group value; the reduction was statistically significant. The interpretation of these data was confounded by the atypically high values for this parameter in the control and 70 ppm groups. A statistically significant increase was observed in the number of implantation sites that were unaccounted for in the 700 ppm group.

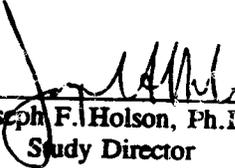
Mean live litter size was reduced in the 700 ppm group relative to the control group value. The reduction was statistically significant, and the value was slightly below the range of the WIL historical control data (11.6 pups/litter as compared to a range of 11.7-15.9 pups/litter). Mean pup body weights in the 700 ppm group were increased on lactation days 1 and 4, and comparable to the control group values thereafter. No test article-related effects were observed on the number of dead pups on PND 0 or on pup viability throughout lactation. No test article-related clinical signs were noted in the pups in the 70 and 700 ppm groups. F<sub>1</sub> pup sex ratios were unaffected by exposure to the test article at any exposure level. At the necropsy of selected pups on PND 28, no exposure-related internal findings were noted.

In conclusion, parental toxicity was demonstrated at an exposure level of 700 ppm by clinical signs and by inhibition of body weight gain (in F<sub>0</sub> males during the first three weeks of the pre-breeding period, and in F<sub>0</sub> females during the first week of the pre-breeding period and during gestation days 14-20) and food consumption (in F<sub>0</sub> females during week 0-1). Parental toxicity was not observed

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at an exposure level of 70 ppm. A potential effect on reproduction in the F<sub>0</sub> generation was exhibited in the 700 ppm group by a reduction in the mean live litter size. Postnatal toxicity was not observed at exposure levels of 70 and 700 ppm. Toxicity in the 700 ppm group was further demonstrated by a reduction in the number of implantation sites. In the absence of significant findings in the other reproductive parameters assessed in this study, it is difficult to ascribe a meaningful interpretation to this finding. Another study was therefore designed to determine whether this effect was reproducible at 700 ppm.

  
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3/7/96  
Date