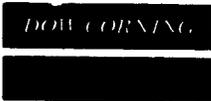


8EHQ-0799-13585



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July 7, 1999

1999 JUL 15 AM 11:14

CERT # Z 092 451 180

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

*pdcn 88960000065*

Re: Supplemental Submission to 8EHQ-0296-13585  
TSCA Section 8(e) Notification: 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, 16 March 1978), Dow Corning is submitting the following final report as a supplemental submission to our TSCA Section 8(e) Notification of February 13, 1996 (8EHQ-0296-13585). The information presented in this supplemental submission was obtained from an inhalation reproductive toxicity study in Sprague-Dawley rats with octamethylcyclotetrasiloxane (OMCTS) that we conducted as part of our Siloxane Research Program. This program 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 Salzburg Road  
Midland, Michigan 48686-0994

*Containing No PCB*

*RR 24387*

**Recently Completed Study:**

AN INHALATION REPRODUCTIVE TOXICITY STUDY OF  
OCTAMETHYLCYCLOTETRASIOXANE (D4) IN FEMALE RATS  
USING MULTIPLE AND SINGLE DAY EXPOSURE REGIMENS

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Dow Corning Corporation  
Midland, Michigan 48686-0994  
Phone: (517) 496-4000



89990000267

Dow Corning Corporation  
1999-10000-47049  
June 14, 1999

**Summary:**

In several earlier and previously reported single generation reproductive toxicity studies, rats were exposed to high concentrations of octamethylcyclotetrasiloxane (OMCTS) for a prolonged pre-mating interval (at least 28 consecutive days). Exposures continued during the mating period, throughout gestation, and (in some studies) during lactation. In these studies, decreased litter sizes, decreased numbers of uterine implantations, and reduced numbers of corpora lutea were seen; these findings were previously communicated to EPA.

In an ongoing, previously reported two generation inhalation reproductive toxicity study in male and female rats, a reduction in mean live litter size was seen in litters produced by both F<sub>0</sub> and F<sub>1</sub> animals. Mating and fertility indices were reduced among F<sub>1</sub> rats and some F<sub>1</sub> female rats displayed signs of prolonged diestrus. The decreased mating and fertility indices are likely due, at least in part, to the increased numbers of females in diestrus; it is well known that female rats in diestrus will not mate.

The study which is the subject of this supplemental submission was conducted to evaluate the temporal responsiveness of female rats to the reproductive effects of OMCTS. Female rats were exposed by whole-body inhalation for six hours daily to the 700 ppm of test article at different times during the pre-mating and post-mating phases of their reproductive cycles. Laparohysterectomies were performed on gestation day 8, and corpora lutea and uterine contents were examined.

Toxicity was expressed in some segments of the pre-mating phase by a reduced pregnancy rate and effects on mean body weight gains, reduced food consumption, and/or reduced numbers of mean corpora lutea and implantation sites, increased numbers of small implantation sites, and reduced mean uterine weight for different treatment regimens. In the post-mating phase, mean numbers of corpora lutea and implantation sites, pre-implantation losses, and numbers of small implantation sites were unaffected under all treatment regimens, with toxicity being expressed in a single group by reduced mean body weight gain and food consumption.

The effects we are reporting occurred only at exposure concentrations that greatly exceed typical occupational or consumer exposures. Consequently, we believe that the results of this study are not indicative of a substantial risk to human health or the environment. Nevertheless, we are reporting these findings to EPA to ensure our compliance with both the letter and the spirit of TSCA Section 8(e).

## **Background:**

### Previous Findings

Previously, Dow Corning informed EPA of preliminary data from several single generation inhalation reproductive toxicity studies conducted with OMCTS in rats using exposure concentrations up to 700 ppm (see submissions to 8EHQ-0296-13585). Final reports from these studies have been forwarded to EPA. In these studies, decreases in mean live litter size, decreased numbers of uterine implantation sites, and reduced numbers of corpora lutea were noted. No effects were seen on the number of days elapsed between pairing and mating, on mating indices, or on fertility indices. In studies where the estrous cycle was evaluated, no effects on estrous cyclicity were detected.

In other supplemental submissions to 8EHQ-0296-13585, Dow Corning has informed EPA of preliminary data from an ongoing two generation inhalation reproductive toxicity study in male and female rats in which a reduction in mean live litter size was seen in litters produced by both F<sub>0</sub> and F<sub>1</sub> animals. Mating and fertility indices were reduced among F<sub>1</sub> rats and some F<sub>1</sub> female rats displayed signs of prolonged diestrous. The decreased mating and fertility indices are likely due, at least in part, to the increased numbers of females in diestrous; it is well known that female rats in diestrous will not mate.

### New Findings

This study which is the subject of this supplemental submission was conducted to evaluate the temporal responsiveness of female rats to the reproductive effects of OMCTS. Female rats were exposed by whole-body inhalation for six hours daily to the 700 ppm of test article at different times during the pre-mating and post-mating phases of their reproductive cycles. Lapohysterectomies were performed on gestation day 8, and corpora lutea and uterine contents were examined. Results were as follows:

- For females that were exposed in a single six-hour exposure one day prior to mating, (Pre-mating Phase Group 2), the pregnancy rate was reduced compared to concurrent controls (Pre-mating Phase Group 1). Pre-implantation loss and mean uterine and ovarian weights were unaffected compared to concurrent controls. All ovaries had a normal complement of corpora lutea.
- For females that were exposed in a single six-hour exposure two days prior to mating, (Pre-mating Phase Group 3), in a single six-hour exposure three days prior to mating, (Pre-mating Phase Group 4), or in a single six-hour exposure four days prior to mating, (Pre-mating Phase Group 5), pregnancy rate, pre-implantation loss, and mean uterine and ovarian weights were unaffected compared to concurrent controls.

- For females that were exposed in daily six-hour exposures from three days prior to mating through one day prior to mating, (Pre-mating Phase Group 6), pregnancy rate, pre-implantation loss, and mean uterine and ovarian weights were unaffected compared to concurrent controls. Effects on mean body weight gains were observed.
- For females that were exposed in daily six-hour exposures starting three days prior to mating and continuing through a two-day mating interval and gestation through gestation day 3 (Pre-mating Phase Group 7), pregnancy rates and pre-implantation loss were unaffected compared to concurrent controls. The mean numbers of corpora lutea and implantation sites were reduced. An increased number of small implantation sites was observed. Mean uterine weights were reduced while mean ovarian weights were unaffected. Effects on mean body weight gains and reduced food consumption were observed.
- For females that were exposed in a single six-hour exposure on gestation day 0 (Post-mating Phase Group 2), in a single six-hour exposure on gestation day 1 (Post-mating Phase Group 3), or in a single six-hour exposure on gestation day 2 (Post-mating Phase Group 4), pregnancy rate, mean numbers of corpora lutea and implantation sites, pre-implantation loss, numbers of small implantation sites, and mean uterine and ovarian weights were unaffected compared to concurrent controls (Post-mating Phase Group 1)
- For females that were exposed in daily six-hour exposures from gestation day 0 through gestation day 2 (Post-mating Phase Group 5), pregnancy rate, mean numbers of corpora lutea and implantation sites, pre-implantation loss, numbers of small implantation sites, and mean uterine and ovarian weights were unaffected compared to concurrent controls. Reduced mean body weight gain and food consumption were observed.

In summary, toxicity was expressed in the pre-mating phase by a reduced pregnancy rate in Group 2, by effects on mean body weight gains in Group 6, and by effects on mean body weight gains, reduced food consumption, and reduced numbers of corpora lutea and implantation sites, increased numbers of small implantation sites, and reduced mean uterine weight in Group 7. In the post-mating phase, mean numbers of corpora lutea and implantation sites, pre-implantation losses, and numbers of small implantation sites were unaffected under all treatment regimens, with toxicity being expressed in a single group (Group 5) by reduced mean body weight gain and food consumption.

**Actions:**

These findings from the aforementioned study will be communicated to appropriate internal and external audiences. Dow Corning is now considering

further studies to understand the potential relevance to humans for the reproductive effects seen in the earlier range-finding studies as well as in the ongoing two generation study. Dow Corning has submitted is also preparing an exposure assessment to provide support for an interim quantitative risk assessment which will be provided to the Agency on completion.

Dow Corning will notify EPA of any further relevant information that may be developed concerning this material.

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

If you have any questions with any of the aforementioned studies, please contact me at 517-496-4057 or at the address provided herein. If you require further general information regarding this supplemental submission, please contact Dr. Rhys G. Daniels, Senior Regulatory Compliance Specialist, Regulatory Compliance Group, HERA Americas, at 517-496-4222 or at the address provided herein.

Sincerely,



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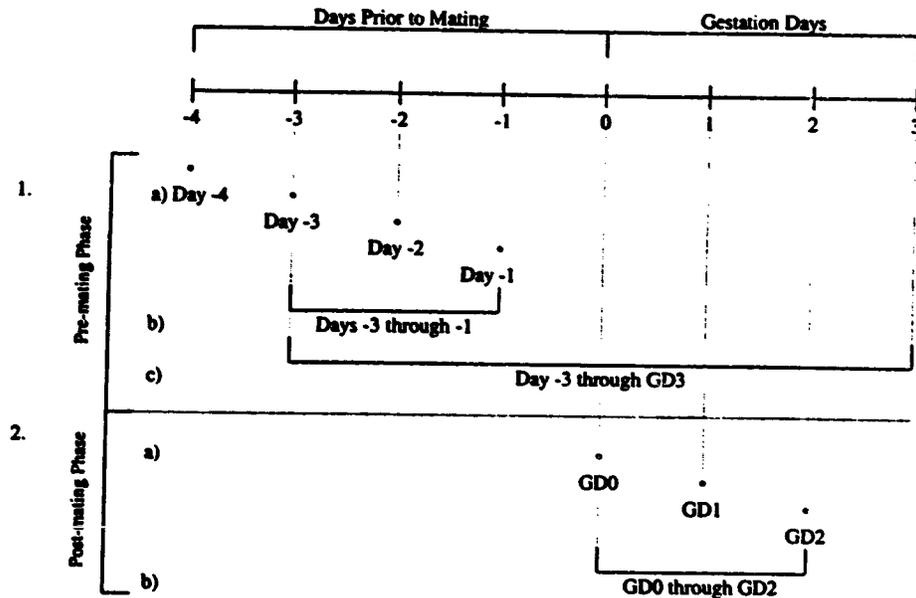
Michael P. Hill  
Executive Director of  
Environmental, Health and Safety  
(517) 496-4057

RGD99070

**An Inhalation Reproductive Toxicity  
Study of Octamethylcyclotetrasiloxane (D4) in  
Female Rats Using Multiple and Single Day Exposure Regimens**

**ABSTRACT**

This study was conducted to evaluate the temporal responsiveness of female rats to the reproductive effects of octamethylcyclotetrasiloxane (D4). Groups of female CrI:CD<sup>®</sup>(SD)IGS BR rats were exposed by whole-body inhalation of D4 vapor at an exposure level of 700 ppm for the following scheme:



**1. Pre-mating Phase:**

- a) a single six-hour exposure, one day prior to, two days prior to, three days prior to, or four days prior to mating (Groups 2-5; a total of 125 females exposed)
- b) daily six-hour exposures from three days prior to mating through one day prior to mating (Group 6; 125 females exposed)

- c) daily six-hour exposures starting three days prior to mating and continuing through a two-day mating interval and gestation through gestation day 3 (Group 7; 70 females exposed)

2. Post-mating Phase:

- a) a single six-hour exposure on gestation day 0, 1, or 2 (Groups 2-4, 25 females per group)
- b) daily six-hour exposures from gestation day 0 through gestation day 2 (Group 5, 25 females)

Separate concurrent control groups for both the Pre-mating and Post-mating Phases (Group 1; 25 females in each phase) were exposed to clean, filtered air on a regimen comparable to that used for Groups 2-5 (Pre-mating Phase) or Group 2 (Post-mating Phase).

For the Pre-mating Phase, females were exposed to either test article or filtered air and then paired with unexposed males of the same strain and source. Following positive evidence of mating, 22, 17, 22, 40, 23, 40, and 40 females were assigned to Groups 1, 2, 3, 4, 5, 6, and 7, respectively, based upon the pre-coital interval (one to five days for Group 1, one day for Group 2, two days for Group 3, three days for Group 4, four days for Group 5, one day for Group 6, and one or two days for Group 7). All remaining exposed females were euthanized and discarded. For the Post-mating Phase, females were mated with unexposed males prior to exposure. Following observation of positive evidence of mating, the females were consecutively assigned to Groups 1-5 in a block design until each group contained 25 mated females. On gestation day 8, uterine examinations were performed on all females from both phases. Ovaries and uteri were weighed; selected tissues were examined microscopically.

All females in the Pre-mating Phase and Post-mating Phase survived to the scheduled necropsy on gestation day 8. No exposure-related findings were noted in females from either phase at necropsy.

In the Pre-mating Phase, the pregnancy rate (no. pregnant/no. with evidence of mating) was reduced (64.7%) in the Group 2 females (exposed one day prior to mating; Day -1 group) compared to the concurrent controls (95.5%). Pregnancy rates were unaffected by exposure in the remainder of the Pre-mating Phase groups and in all Post-mating Phase groups.

In the Pre-mating Phase, mean body weight in the Group 6 females (Days -3 through -1 group) was reduced on gestation day 0. Mean body weight gains in this group were increased during gestation days 0-4 and 0-8. In Group 7 (the Day -3 through GD3 group), mean body weight gains were decreased and increased during gestation days 0-4 and 4-6, respectively. Food consumption in this group was reduced during gestation days 0-4 and 0-8. In the Post-mating Phase, mean body weight gain was reduced in Group 5 (the GD0 through GD2 group) during gestation days 0-4. Food consumption in this group was decreased during gestation days 0-4 and 0-8.

In the Pre-mating Phase, the mean numbers of corpora lutea and implantation sites were reduced in Group 7 (the Day -3 through GD3 group). Pre-implantation loss was unaffected by exposure under all Pre-mating Phase treatment regimens. An increased number of small implantation sites (less than 3.6 mm in diameter) was observed in Group 7 (the Day -3 through GD3 group). In the Post-mating Phase, mean numbers of corpora lutea and implantation sites, pre-implantation loss, and numbers of small implantation sites were unaffected by test article exposure under all treatment regimens.

In the Pre-mating Phase, mean uterine weights (absolute and relative to final body weight) were reduced in Group 7 (the Day -3 through GD3 group). The mean ovarian weights in this group were unaffected by exposure. Mean uterine and ovarian weights were unaffected by exposure in all other Pre-mating Phase groups and in all Post-mating Phase groups.

In the Pre-mating Phase, the ovaries from all females in Group 2 (the Day -1 group) had a normal complement of corpora lutea. All were consistent with the corpora lutea of pregnancy (11 pregnant rats) or pseudopregnancy (six non-pregnant

DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-K0000-47049  
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rats). In the Pre-mating Phase, some of the implantation sites that appeared small macroscopically also appeared small microscopically. However, this may have been due to sample sectioning. The cells of the decidual reaction were normal and the reaction was comparable to the larger normal sites.

In conclusion, toxicity was expressed in the Pre-mating Phase by a reduced pregnancy rate in Group 2 (the Day -1 group), by effects on mean body weight gains in Group 6 (the Days -3 through -1 group), and by effects on mean body weight gains, reduced food consumption, reduced numbers of corpora lutea and implantation sites, increased numbers of small implantation sites, and reduced mean uterine weight in Group 7 (the Day -3 through GD3 group). Toxicity was expressed in the Post-mating Phase by reduced mean body weight gain and food consumption in Group 5 (the GD0 through GD2 group).

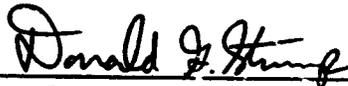
DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-10000-47049  
Security - INTERNAL

An Inhalation Reproductive Toxicity  
Study of Octamethylcyclotetrasiloxane (D4) in  
Female Rats Using Multiple and Single Day Exposure Regimens

**GLP COMPLIANCE STATEMENT**

This study 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 characterization of the test article and the compliance with Good Laboratory Practices for this aspect of the study were the responsibility of the Sponsor. The study was conducted in accordance with the protocol and protocol amendments as approved by the Sponsor, except as indicated in Appendix G.



Donald G. Stump, Ph.D.  
Study Director

6/14/99

Date

DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-10000-47049  
Security - INTERNAL

**An Inhalation Reproductive Toxicity  
Study of Octamethylcyclotetrasiloxane (D4) in  
Female Rats Using Multiple and Single Day Exposure Regimens**

**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>
11/3/97	Test Material Exposure	11/3/97	12/23/97
11/3, 4/97	Cohabitation/Confirmation	11/4/97	12/23/97
11/10/97	Cohabitation/Confirmation	11/11/97	12/23/97
11/11/97	Test Material Exposure	11/11/97	12/23/97
11/11/97	Animal Care and Equipment	11/11/97	12/23/97
11/12/97	Uterine and Macroscopic Examination	11/12/97	12/23/97
11/14/97	Animal Care and Equipment	11/14/97	12/23/97
11/19/97	Uterine Examinations	11/19/97	12/23/97
1/6, 7, 3/3/98	Study Records (I-1)	3/4/98	4/29/98
1/7, 3/3/98	Study Records (I-2)	3/4/98	4/29/98
1/8, 3/3/98	Study Records (I-3)	3/4/98	4/29/98
1/8, 2/9/98	Study Records (N-1)	2/10/98	3/27/98
1/9, 12, 15, 16, 20/98	Study Records (Ex-1, 2, 3, 4)	1/23/98	2/25/98
3/31, 4/1, 2, 6, 13/98	Draft Report (without Purity Analysis Appendix & Inhalation Appendix)	4/13/98	5/28/98
4/2, 13/98	Study Records (I-1) (Report of In-life Gross and Morphometric Evaluation on Rats)	4/13/98	5/28/98
4/3, 6, 13/98	Draft Report (Purity Analysis Appendix Only)	4/13/98	5/28/98
4/13, 14/98	Draft Report (Inhalation Appendix Only)	4/14/98	5/28/98
10/7-8/98	Study Records (H-1)	10/8/98	11/30/98
3/1-2/99	Draft Report (Appendix D)	3/2/99	4/29/99

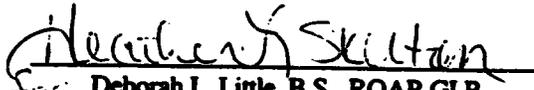
This study was conducted and inspected in accordance with the current Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States

DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-10000-47049  
Security - INTERNAL

Food and Drug Administration (21 CFR Part 58) and Environmental Protection Agency (40 CFR Parts 160 and 792), the Standard Operating Procedures of WIL Research Laboratories, Inc., and the Sponsor's protocol and protocol amendment(s). Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the Study Director. A status report is submitted to management monthly.

The raw data, retention and residual samples of the test article, the original protocol and amendments, and the original final report are stored in the Archives at WIL Research Laboratories, Inc.

  
For: Deborah L. Little, B.S., RQAP-GLP  
Manager II, Quality Assurance

6/14/99  
Date

DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-1000-47049  
Security - INTERNAL

An Inhalation Reproductive Toxicity  
Study of Octamethylcyclotetrasiloxane (D4) in  
Female Rats Using Multiple and Single Day Exposure Regimens

APPROVAL SIGNATURES

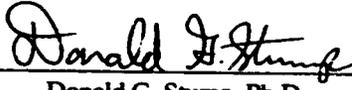
This report consists of pages 1 through 439 including Tables 1 through 39 and  
Appendices A through H.

  
\_\_\_\_\_  
Vincent L. Reynolds, Ph.D., D.A.B.T.  
Sponsor Representative

11-11-1999  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Patrick W. Langvardt, M.S.  
Sponsor Management

11 June 1999  
\_\_\_\_\_  
Date

  
\_\_\_\_\_  
Donald G. Stump, Ph.D.  
Assistant Director of Developmental,  
Reproductive and Neurotoxicology  
Study Director

6/14/99  
\_\_\_\_\_  
Date

\*

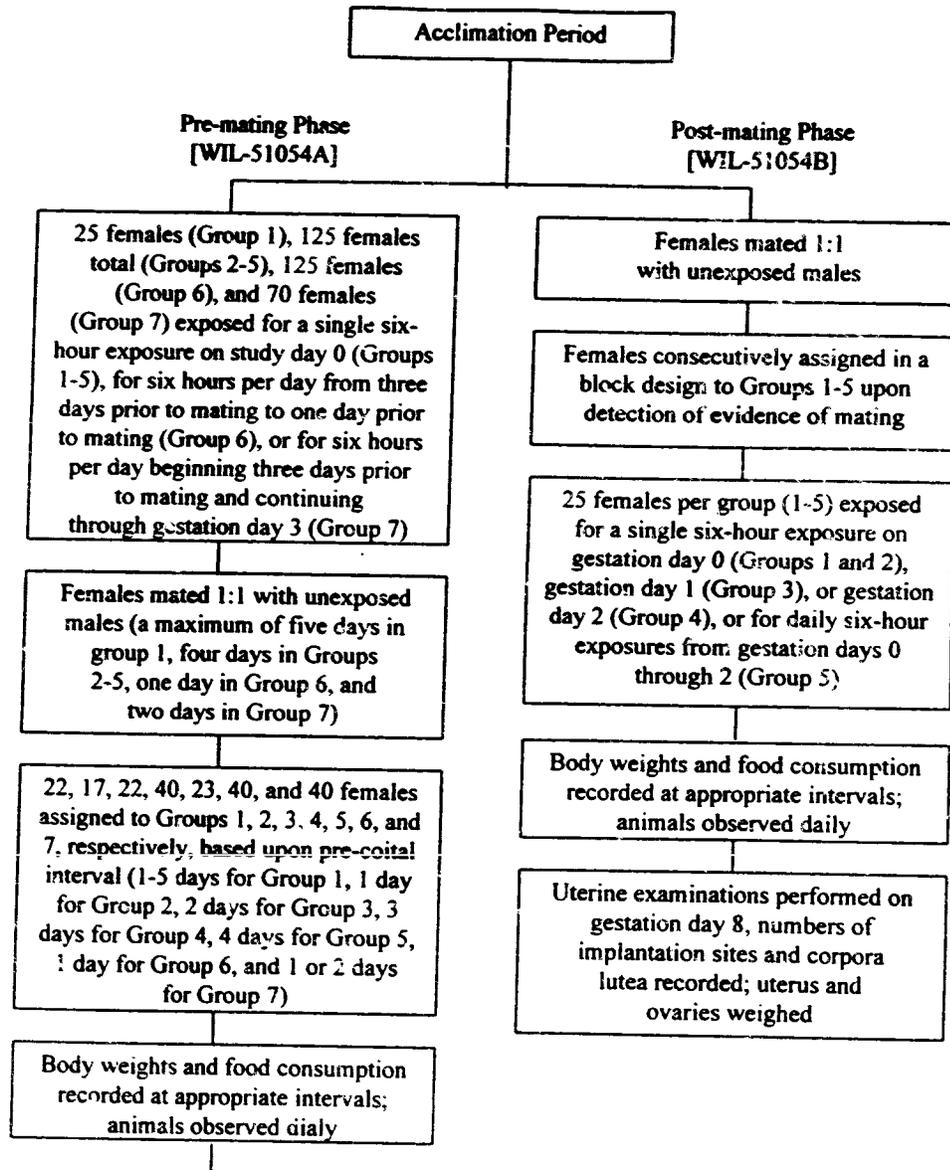
I. OBJECTIVE

The purpose of this study was to evaluate the relative temporal responsiveness of female rats to the reproductive effects of the test article. The 700 ppm exposure level was selected because it was the highest vapor concentration that could be reliably produced without aerosol formation. Female Sprague-Dawley CrI:CD<sup>®</sup>(SD)IGS BR rats were exposed by whole-body inhalation to test article vapor for six hours per day for the following regimens:

1. Pre-mating Phase:
  - a) a single six-hour exposure, one day, two days, three days, or four days prior to mating
  - b) daily six-hour exposures from three days prior to mating through one day prior to mating
  - c) daily six-hour exposures starting three days prior to mating and continuing through a two-day mating interval and gestation through gestation day 3
2. Post-mating Phase
  - a) a single six-hour exposure on gestation day 0, 1, or 2
  - b) daily six-hour exposures from gestation day 0 through gestation day 2

The route of administration was whole-body inhalation since this is a potential exposure pathway for humans. The test system was the Sprague-Dawley CrI:CD<sup>®</sup>(SD)IGS BR rat. The Sprague-Dawley rat is recognized as appropriate for reproduction studies<sup>1</sup> and was selected based on the availability of historical control data and because of its proven susceptibility to the effects of reproductive and developmental toxicants.

## II. STUDY DESIGN



DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-I0000-47049  
Security - INTERNAL

II. STUDY DESIGN (continued)

Uterine examinations performed  
on gestation day 8; numbers of  
implantation sites and corpora  
lutea recorded; uterus and  
ovaries weighed

III. EXPERIMENTAL PROCEDURES

A. STUDY SCHEDULE

Pre-mating Phase:

Exposure Period:

- Group 1: November 3, 1997
- Groups 2-5: November 3, 1997
- Group 6: November 7, 1997 - November 9, 1997
- Group 7: November 7, 1997 - November 14, 1997

Allotted Breeding Interval:

- Group 1: November 3, 1997 - November 8, 1997
- Groups 2-5: November 3, 1997 - November 7, 1997
- Group 6: November 9, 1997 - November 10, 1997
- Group 7: November 9, 1997 - November 11, 1997

Gestation Day 8 Uterine Examinations:

- Group 1: November 12, 1997 - November 15, 1997
- Group 2: November 12, 1997
- Group 3: November 13, 1997
- Group 4: November 14, 1997
- Group 5: November 15, 1997
- Group 6: November 18, 1997
- Group 7: November 18, 1997 - November 19, 1997

Histopathologic Examinations Completed (All Groups): December 8, 1998

Post-mating Phase

Breeding Interval (All Groups): November 10, 1997 - November 14, 1997

Exposure Period:

- Group 1: November 11, 1997 - November 14, 1997
- Group 2: November 11, 1997 - November 14, 1997
- Group 3: November 12, 1997 - November 15, 1997
- Group 4: November 13, 1997 - November 16, 1997
- Group 5: November 11, 1997 - November 16, 1997

Gestation Day 8 Uterine Examinations (All Groups):

November 19, 1997 - November 22, 1997

Final test article purity determinations: February 3, 1998

Histopathologic Examinations Completed (All Groups): December 8, 1998

For computer entry purposes, maternal and embryonic data in the Pre-mating Phase were designated WIL-51054A. Data generated during the exposure period were designated WIL-51054A1 (Group 1), WIL-51054A2 (Groups 2-5), WIL-51054A3 (Group 6), and WIL-51054A4 (Group 7). Maternal and embryonic data in the Post-mating Phase were designated WIL-51054B.

In the Pre-mating Phase, the groups exposed to test article as a single six-hour exposure one day, two days, three days, or four days prior to mating are presented in the report text as the Day -1, Day -2, Day -3, and Day -4 groups, respectively. The group exposed to test article daily for three days prior to mating through one day prior to mating is presented as the Days -3 through -1 group, and the group exposed beginning 3 days prior to mating and continuing through gestation day 3 is presented as the Day -3 through GD3 group in the report text. In the Post-mating phase, the groups exposed to test article as a single six-hour exposure on gestation day 0, 1, or 2 are presented as the GD0, GD1, and GD2 groups, respectively, in the report text. The group exposed to test article daily from gestation day 0 through gestation day 2 is presented as the GD0 through GD2 group.

For reporting purposes, study group names are presented on the report tables as follows:

Pre-mating Phase		Post-mating Phase	
<u>Group Number</u>	<u>Group Name</u>	<u>Group Number</u>	<u>Group Name</u>
1	0 ppm	1	0 ppm
2	700 ppm; Day -1	2	700 ppm; GD0
3	700 ppm; Day -2	3	700 ppm; GD1
4	700 ppm; Day -3	4	700 ppm; GD2
5	700 ppm; Day -4	5	700 ppm; GD0-GD2
6	700 ppm; Days -3 to -1		
7	700 ppm; Day -3 to GD3		

Due to spacing constraints, the study title is presented on the report tables as "Inhalation Reproductive Toxicity Study of D4 in Female 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, Midland, Michigan, as follows:

<u>Identification</u>	<u>Quantity Received</u>	<u>Description</u>	<u>Date of Receipt</u>
Octamethylcyclotetrasiloxane (supplied as Dow Corning <sup>®</sup> 244 Fluid) Lot # LL084732 [WIL Log No. 2700E]	4 Drums Total gross weight: 825.5 kg Drum #1: 206.4 kg Drum #2: 207.5 kg Drum #3: 204.6 kg Drum #4: 207.0 kg*	Clear colorless liquid	4/17/97
Octamethylcyclotetrasiloxane (supplied as Dow Corning <sup>®</sup> 244 Fluid) Lot # LL084732 [WIL Log No. 2700F]	2 Drums Total gross weight: 416.8 kg Drum #1: 209.6 kg* Drum #2: 207.2 kg	Clear colorless liquid	11/3/97

\* = These drums were used for exposures during the study.

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. The test article purity was determined to be at least 99.78% octamethylcyclotetrasiloxane. The test article was stable when stored at room temperature. Retention samples of the test article were taken from

DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-I0000-47049  
Security - INTERNAL

drum no. 4 of the first shipment and drum no. 1 of the second shipment on October 11, 1997 and November 11, 1997, respectively. After completion of the exposure phase, residual samples of the test article were taken from the same respective drums. The retention and residual samples are stored at room temperature 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 the chamber were measured 10 times (approximately every 35 minutes) during each exposure period by a gas chromatographic method established during the method development phase of this study. At least one standard was analyzed each day prior to exposure to confirm gas chromatographic calibration. Chamber ventilation rate (airflow), temperature, and relative humidity within the chamber were monitored continuously and were recorded approximately every 35 minutes. Oxygen content within the chamber was measured during pre-study method development and was determined to be at least 19%.

Nominal chamber concentrations were 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).

Test atmosphere homogeneity data were generated during pre-study method development. The methodology and results of these analyses are presented in Appendix B.

#### 4. EXPOSURE METHODS

Each group of animals was exposed in a 2.0-m<sup>3</sup> stainless-steel-and-glass whole-body inhalation chamber. The chambers were operated under dynamic conditions at a slight negative pressure (approximately 0.5 inch of water) at air flows of at least 12 to 15 air changes per hour. All females were exposed to the test atmosphere at a target exposure concentration of 700 ppm. The Pre-mating Phase females were exposed to test article for a single six-hour exposure on study day 0 (Groups 2-5), for six hours per day from three days prior to mating (beginning on study day 4) through one day prior to mating (Group 6), or for six hours per day for three days prior to mating (beginning on study day 4) and continuing throughout mating (a maximum of two days for mating) and gestation through gestation day 3 (Group 7). The starting of exposures for Groups 6 and 7 on study day 4 was a deviation from the protocol, but was necessitated by logistical concerns; this had no effect on the scientific integrity, validity or outcome of the study. The Post-mating Phase females were exposed to test article for a single six-hour exposure on gestation day 0, 1, or 2 (Groups 2-4) or for six hours per day from gestation days 0 through 2 (Group 5). Each phase included a concurrent control group (Group 1); these females were exposed to clean, filtered air for a single six-hour exposure on study day 0 (Pre-mating Phase) or gestation day 0 (Post-mating Phase). The males served as sires for the induction of pregnancy and were not exposed to clean, filtered air or the test article. The female rats were removed from their home cages in the animal room, placed in exposure caging, and transported to the inhalation chambers for the six-hour exposure period. The animals were exposed to test article at approximately the same time each day. The animals were returned to their home cages following exposure. Instrumentation was set to maintain the temperature inside the exposure

chamber at 20-26°C and relative humidity between 30% and 50%.  
Exposure methods and conditions are detailed in Appendix B.

Each chamber was dedicated to one exposure group. For groups that were exposed on more than one day, 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. This procedure was followed in order to minimize any potential variation occurring due to positioning within the chamber.

The following diagram presents the study group assignment:

Pre-mating Phase (WIL-51054A)

<u>Group Number</u>	<u>Group Name</u>	<u>Test Article</u>	<u>Exposure Level (ppm)</u>	<u>Number of Females Exposed</u>	<u>Number of Females Assigned to Group</u>
1	Control	Filtered Air	0	25	22
2	Single Exposure (-1)	D4	700	a	17
3	Single Exposure (-2)	D4	700	a	22
4	Single Exposure (-3)	D4	700	a	40
5	Single Exposure (-4)	D4	700	a	23
6	Pre-mating (-3 through -1)	D4	700	125	40
7	Pre-mating and Post-mating (-3 through GD3)	D4	700	70	40

a = A total of 125 females were exposed prior to placement in these groups; the number of females assigned to each group was determined by the numbers of days required for positive evidence of mating. For example: Seventeen females had evidence of mating after one day and were assigned to Group 2; 22 females had evidence of mating after two days and were assigned to Group 3; etc.

Post-mating Phase (WIL-51054B)

<u>Group Number</u>	<u>Group Name</u>	<u>Test Article</u>	<u>Exposure Level (ppm)</u>	<u>Number of Females<sup>b</sup></u>
1	Control	Filtered Air	0	25
2	GD0 Exposure	D4	700	25
3	GD1 Exposure	D4	700	25
4	GD2 Exposure	D4	700	25
5	GD0 through GD2 Exposure	D4	700	25

b = A total of 155 females were paired with unexposed males prior to group assignments. Upon detection of evidence of mating, the female rats were consecutively assigned in a block design to Groups 1-5 until each group contained 25 females.

C. ANIMAL RECEIPT AND ACCLIMATION

Two hundred forty-six male and 535 virgin female CrI:CD<sup>®</sup>(SD)IGS BR rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina, on October 14, 1997. The males were approximately 56 days old and the females were approximately 70 days old upon receipt. The animals were initially weighed on the day following receipt. Upon receipt, each animal was examined by a staff veterinarian for any abnormal clinical signs and external lesions. Twenty-six males were excluded from possible placement on study because of the presence of small testes (22 rats), soft testes (two rats), an enlarged testis (one rat), or a hernia (one rat). The decision to exclude males with testicular changes was made based upon findings noted at the gross examination and/or a subsequent morphometric evaluation.

All animals were uniquely identified on the day following receipt by a Monel<sup>®</sup> metal ear tag displaying the animal number. Individual cage cards were affixed to each cage and displayed the animal number, group number, study number, exposure level, sex, and the dates of animal arrival and initiation of exposure. The animals were housed in their home cages for an acclimation period of at least 20 days prior to the initiation of exposure (females in the



Pre-mating Phase) or breeding (females in the Post-mating Phase). During acclimation, individual body weights were recorded twice (following receipt and prior to exposure or at randomization), and the animals were observed twice daily for mortality and moribundity.

**D. ANIMAL HOUSING**

Upon arrival, all animals were housed individually in clean wire-mesh cages suspended above cage-board. The sizes of the suspended wire-mesh cages used to house the animals were approximately 6 3/4" W x 9 5/8" D x 7 1/8" H or 10 3/16" W x 10 1/16" D x 7" H. The animals were paired for mating in the home cage of the male. Following positive identification of mating (Section III.G.), the females were returned to individual suspended wire-mesh cages; nesting material was not required as the females were euthanized prior to the date of expected parturition. Females for which there was no evidence of mating were euthanized and discarded. All animals were maintained in accordance with the "Guide for the Care and Use of Laboratory Animals."<sup>2</sup> The animal facilities at WIL Research Laboratories, Inc. are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

**E. DIET, DRINKING WATER, AND MAINTENANCE**

The basal ration used in this study was PMI Nutrition International, Inc. Certified Rodent LabDiet<sup>®</sup> 5002, in meal form; the lot numbers used were recorded in the study records. The diet used at WIL Research Laboratories, Inc. is a certified feed with heavy metal and pesticide 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. The results of these analyses were reviewed by the Study Director, and it was determined that contaminants were not present in feed or water at levels that 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 periods and during the study, with the following exception. Food and water were withheld from the female rats during each daily exposure period.

F. ENVIRONMENTAL CONDITIONS

Except during exposure, all animals were housed throughout the acclimation period and during the study in an environmentally controlled room. Instrumentation was set to maintain a temperature of  $72 \pm 4^\circ\text{F}$  ( $22.2 \pm 2.2^\circ\text{C}$ ) and a relative humidity between 40% 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 in the nonexposure animal room ranged from  $70.1^\circ\text{F}$  to  $74.1^\circ\text{F}$  ( $21.2^\circ\text{C}$  to  $23.4^\circ\text{C}$ ) and relative humidity ranged from 37.8% to 51.6% during the study period. The occasional slight deviations from the target humidity levels were not expected to 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.

G. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS AND BREEDING PROCEDURES

1. PRE-MATING PHASE

a. RANDOMIZATION

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 assigned at random as follows: 25 females in Group 1, 125 females total in Groups 2-5, 125 females in Group 6 and 70 females in Group 7. At exposure initiation, the females in Groups 1-5 were approximately 90 days old, and the females in Groups 6 and 7 were

approximately 94 days old. Body weights at exposure initiation ranged from 222 g to 318 g.

b. BREEDING PROCEDURES

Following a single six-hour exposure to either clean, filtered air (Group 1) or test article (Groups 2-5) or three consecutive days of exposure to test article for six hours daily (Groups 6 and 7), females in the Pre-mating Phase were paired on a 1:1 basis with unexposed male rats of the same strain and source. 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 sperm in a vaginal smear or a copulatory plug. The day when evidence of mating was identified was termed gestation day 0. The animals were separated, and the female was housed in an individual suspended wire-mesh cage.

A maximum of five days was allowed for mating in Group 1. For Groups 2-5, a maximum of four days was allowed for mating; females with evidence of mating one day following exposure were assigned to Group 2, females with evidence of mating two days following exposure were assigned to Group 3, females with evidence of mating three days following exposure were assigned to Group 4, and females with evidence of mating four days following exposure were assigned to Group 5. For Groups 6 and 7, a maximum of one and two days, respectively, were allowed for mating. For all groups, females that had not shown evidence of mating in the allotted time were euthanized via carbon dioxide inhalation and discarded. All females with evidence of mating were allowed to continue on the study, with the following exception. Once 40 females had been assigned to a given study group,

any additional females with evidence of mating were euthanized and discarded. This deviation from the protocol was not expected to have an impact on the integrity of the data or the outcome of the study.

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.

## 2. POST-MATING PHASE

At the conclusion of the acclimation period, all females 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 (a minimum of 220 g) were placed in a suspended wire-mesh cage with a nonexposed male for breeding. A breeding record containing the male and female identification numbers and the dates of cohabitation was prepared. The selected females were approximately 98 days old when paired for breeding.

Positive evidence of mating was confirmed by the presence of a copulatory plug or the presence of sperm in a vaginal smear. Each mating pair was examined daily. The day on which evidence of mating was identified was termed gestation day 0, and the animals were separated.

The experimental design of the Post-mating Phase consisted of four exposure groups and one control group, with 25 females in each group. The bred females were consecutively assigned in a block design to groups containing 25 rats each by the following randomization procedure. The first mated female and the appropriate gestation day 0 designation were recorded and the female was assigned to Group 1, the second mated female was assigned to Group 2, and the third to Group 3, etc. This process was continued daily until 25 females were placed into each group. Body weight

values for the Post-mating Phase females ranged from 229 g to 336 g on gestation day 0.

## H. OBSERVATIONS

### 1. CLINICAL OBSERVATIONS AND SURVIVAL

Clinical observations were made by the same trained technician, whenever possible. The animals were observed twice daily for appearance, behavior, moribundity, and mortality. Detailed physical examinations were to be recorded weekly throughout the study for all females. Although not required by the protocol, each female received a daily clinical observation beginning on gestation day 0 instead of the protocol-specified weekly examination. This deviation was not expected to have an impact on the integrity of the data or the outcome of the study. Females were also observed for pharmacotoxic signs during exposure and within approximately one hour after completion of exposure. The observations included, but were not limited to, examinations 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 (stereotypies). No significant clinical signs were noted during or one hour following the exposure periods; therefore, no tables for these observations were included in this report. The configuration of the cages and the location of the chamber windows precluded observations during exposure for some females.

For the Pre-mating Phase females, clinical observations recorded prior to assignment of the females to study groups and dispositions for females not placed on study are presented in Appendix C.

2. BODY WEIGHTS

Individual body weights were measured on gestation days 0, 4, and 8 for all females. Group mean body weights were calculated for each of these days. Individual and group mean body weight changes were calculated for each corresponding gestation interval and for gestation days 0-8.

3. FOOD CONSUMPTION

Individual food consumption was measured on gestation days 0, 4, and 8 for all females. 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), the interval was footnoted appropriately on the individual tables.

I. GESTATION DAY 8 UTERINE EXAMINATION

All females were euthanized by intravenous injection of sodium pentobarbital via a tail vein on gestation day 8. The thoracic, abdominal, and pelvic cavities were opened by a ventral midline incision, and the contents were examined. In all instances, the *post mortem* findings were correlated with the *ante mortem* comments, and any gross abnormalities were recorded. The uterus of each dam was excised, and the number of corpora lutea on each ovary and the total number of implantation sites were recorded. The individual uterine distribution of implantation sites was documented using the following procedure. All implantation sites were numbered in consecutive order beginning with the left distal to the left proximal uterine horn, noting the position of the cervix, and continuing from the right proximal to the right distal uterine horn. The diameters of implantation sites that appeared to be small in

size based upon visual inspection were subsequently measured using calipers. For comparison, all implantation sites were measured for five control females with implantation site counts of 17 to 19 sites/dam. The five control females with 17 to 19 implantation sites were selected at the discretion of the study director to provide a representative estimate for the diameter of a normal gestation day 8 implant. Of the measured implantation sites, those that were less than 3.6 mm in diameter were considered, at the discretion of the study director, to be small compared to the mean size of 4.5 mm in diameter measured in five control group animals.

Uteri with no macroscopic evidence of implantation were excised, opened, and subsequently placed in 10% ammonium sulfide for detection of early implantation loss as described by Salewski.<sup>3</sup>

The uterus and ovaries from all females were weighed and preserved in 10% neutral buffered formalin for histopathological evaluation or possible histopathological examination. Other maternal tissues were preserved in 10% neutral buffered formalin for possible histopathological examination only as indicated by the gross findings. The carcass of each dam and the products of conception were then discarded.

After fixation, protocol-specified tissues were trimmed as described by Thompson.<sup>4</sup> Trimmed specimens were placed in appropriately labeled and numbered cassettes. The tissue samples were processed and then embedded in paraffin blocks. The labeled paraffin blocks were sectioned at five to eight microns. The tissue sections were placed on clean glass microscope slides and labeled with the appropriate study, animal, group and cassette number. Upon completion of the staining with hematoxylin and eosin (AFIP Manual of Histologic Staining Methods<sup>5</sup>), coverslips were placed on the slides.

Microscopic examinations were performed on all small implantation sites detected in this study and on representative implantation sites present in uteri

from control group females. The ovaries from dams treated on Day -1 (*i.e.*, proestrus) along with ovaries from their concurrent control group were also evaluated.

Microscopic examinations were conducted by Robert F. McConnell, D.V.M., P.A., D.A.C.V.P., Consulting Pathology Services, Flemington, New Jersey (Appendix D).

J. STATISTICAL METHODS

All analyses were conducted for a minimum significance level of 5% comparing each treated group to the concurrent 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 numbers of animals (N) used to calculate the means are provided on the individual data tables. Based on the recommendations of the Food and Drug Administration Guideline on Detection of Toxicity to Reproduction for Medicinal Products,<sup>6</sup> Holson *et al.*,<sup>7</sup> and Nelson and Holson,<sup>8</sup> the litter was used as the experimental unit. Data obtained from nonpregnant animals were excluded from statistical analysis. All statistical tests were performed by a Digital<sup>®</sup> MicroVAX<sup>®</sup> 3400 Computer (with appropriate programming) in this laboratory and are referenced on the report tables. The following statistical tests were done:

DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-10000-47049  
Security - INTERNAL

STATISTICAL TEST

- ANOVA<sup>9</sup> (two-tailed) with  
Dunnnett's test<sup>10</sup>

- Kruskal-Wallis test<sup>9</sup> with  
Mann-Whitney U test<sup>9</sup>

PARAMETER

Maternal gestation body weights  
and body weight gains  
Maternal gestation food  
consumption

Implantation sites

Corpora lutea

Organ weights (absolute and  
relative to final body weights)

Litter proportions of intrauterine  
data (considering the litter,  
rather than the embryo, as the  
experimental unit)

IV. RESULTS AND DISCUSSION

A. CHAMBER CONCENTRATIONS

Summary Data: Appendix B

Females in the Pre-mating Phase and the Post-mating Phase were exposed to a target test article concentration of 700 ppm. The actual measured mean exposure concentration in both phases was 700 ( $\pm 2.4$ ) ppm.

B. PRE-MATING PHASE

1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 1, 2

Individual Data: Table 21

All females survived to the scheduled euthanizations. There were no exposure-related clinical observations. Clinical signs noted in the exposed group females occurred infrequently, were observed at a similar frequency in the control group, and/or were findings commonly observed in laboratory rats.

2. PREGNANCY STATUS

Summary Data: Table 1

Individual Data: Tables 22A, 22B, 22C, 22D

Historical Control Data: Appendices E, F

In the control, Day -1, Day -2, Day -3, Day -4, Days -3 through -1, and Day -3 through GD3 groups, 95.5%, 64.7%, 100%, 100%, 95.7%, 97.5%, and 90.0% of the females with evidence of mating, respectively, were gravid; in the WIL historical control data, the pregnancy rate is 91.3% (3585 females gravid/3926 females with evidence of mating; see page 374 in Appendix F). The reduced pregnancy rate in the females exposed one day prior to mating (Day -1 group) was considered to be a result of test article exposure.

### 3. BODY WEIGHTS

Summary Data: Tables 3, 4

Individual Data: Tables 23, 24

Mean body weights and body weight gains in the Day -1, Day -2, Day -3, and Day -4 groups were unaffected by test article exposure throughout the gestation period. A statistically significant ( $p < 0.05$ ) reduction in mean body weight was observed in the Day -2 group on gestation day 8, and a statistically significant ( $p < 0.05$ ) reduction in mean body weight gain was observed in this group during gestation days 4-8. The reductions in mean body weight and body weight gain in the Day -2 group were attributed to biological variation.

The mean body weight in the Days -3 through -1 group was reduced relative to the control group value on gestation day 0; the difference was statistically significant ( $p < 0.05$ ). Mean body weights in this group were similar to the control group values for the remainder of the study (gestation days 4 and 8). The body weight reduction was attributed to test article exposure. Mean body weight gains in the Days -3 through -1 group were increased relative to the control group values during gestation days 0-4 and when the entire gestation period (gestation days 0-8) was evaluated; the differences were statistically significant ( $p < 0.01$ ) and were attributed to test article exposure.

In the Day -3 through GD3 group, mean body weight gains were decreased and increased relative to the control group values during gestation days 0-4 and 4-8, respectively; the differences were statistically significant ( $p < 0.01$ ) and were attributed to test article exposure. When the entire gestation period (gestation days 0-8) was evaluated, mean body weight gain in this group was comparable to that in the control group.

4. FOOD CONSUMPTION

Summary Data: Tables 5, 6

Individual Data: Tables 25, 26

Food consumption during gestation was unaffected by test article exposure in the Day -1, Day -2, Day -3, Day -4, and Days -3 through -1 groups.

Food consumption, evaluated as g/animal/day and g/kg/day, was reduced in the Day -3 through GD3 group relative to the control group values during gestation days 0-4 and when the entire gestation period (gestation days 0-8) was evaluated; the differences were statistically significant ( $p < 0.01$ ) and were attributed to test article exposure.

5. GESTATION DAY 8 UTERINE EXAMINATION

Summary Data: Tables 7, 7A

Individual Data: Tables 27, 27A, 28

Historical Control Data: Appendix F

In the Day -1, Day -2, Day -3, and Day -4 groups, the numbers of corpora lutea and implantation sites were similar to the control group values; none of the differences was statistically significant.

In the Days -3 through -1 group, the mean number of corpora lutea (14.9) was slightly reduced (not statistically significant) relative to the concurrent control group value (16.0). However, the value was within the WIL historical control data range, and the decrease could not be definitively attributed to test article exposure.

The mean numbers of implantation sites and corpora lutea were reduced in the Day -3 through GD3 group relative to the control group values; the difference in the mean number of corpora lutea was statistically significant ( $p < 0.01$ ) and below the range in the WIL historical control data (13.9 corpora lutea in the Day -3 through GD3 group versus a range of 14.4

to 20.5 corpora lutea in the historical control data). The numerical decreases in implantation sites and corpora lutea were attributed to test article exposure.

Pre-implantation loss was not affected by exposure under any of the treatment regimens; differences between the control and exposed groups were slight and were not statistically significant.

In the control, Day -1, Day -2, Day -3, Day -4, Days -3 through -1, and Day -3 through GD3 groups, the numbers of small implantation sites (numbers of females affected) were 1(1), 1(1), 2(2), 0(0), 1(1), 1(1), and 22(10), respectively. The increased number of small implantation sites in the Day -3 through GD<sup>3</sup> group was considered to be related to test article exposure.

Uteri without macroscopic evidence of implantation were found to be nongravid (ammonium sulfide negative).

6. DAM NECROPSIES

Summary Data: Table 8

Individual Data: Table 28

All females survived to the scheduled necropsy on gestation day 8. With the exception of uterine findings described in section IV.B.5, no exposure-related internal findings were noted at the scheduled necropsy. Sporadic internal findings in the exposed groups were noted infrequently.

7. ORGAN WEIGHTS

Summary Data: Tables 9, 10

Individual Data: Tables 29, 30

Mean uterine weights (absolute and relative to final body weights) in the Day -1 group were slightly decreased relative to the control group values; the differences were not statistically significant. The toxicological significance of these decreases and their relationship to treatment are

uncertain in the absence of any remarkable differences in the mean number of implantation sites and the number of dams with small implantation sites in this group.

Mean uterine weights (absolute and relative to final body weight) in the Day -3 through GD3 group were reduced relative to the control group values (the absolute uterine weight was decreased 20%, and the relative uterine weight was decreased 21%); the differences were statistically significant ( $p < 0.01$ ). The decreases may have been due, at least in part, to decreased numbers of implantation sites and increased numbers of small implantation sites.

No other exposure-related effects on organ weight data were observed. A statistically significant ( $p < 0.01$ ) increase was observed in the mean relative ovarian weight in the Day -2 group relative to the control group value; however, the increase was attributed to a reduced mean final body weight in this group and was not considered to be an effect of exposure.

#### 8. MICROSCOPIC EXAMINATION

##### Appendix D

The ovaries from all females in the Day -1 group had a normal complement of corpora lutea. All were consistent with the corpora lutea of pregnancy (11 pregnant rats) or pseudopregnancy (6 non-pregnant rats). Ovaries from females in other groups were not examined microscopically. Some of the implantation sites that appeared small macroscopically also appeared small microscopically. However, this may have been due to sample sectioning. The cells of the decidual reaction of the small sites appeared histologically normal, and the reaction was comparable to the larger sites.

**C. POST-MATING PHASE**

**1. CLINICAL OBSERVATIONS AND SURVIVAL**

Summary Data: Tables 11, 12

Individual Data: Table 31

All females survived to the scheduled euthanizations. There were no exposure-related clinical observations. Clinical signs noted in the exposed group females occurred infrequently and/or were observed at a similar frequency in the control group.

**2. PREGNANCY STATUS**

Summary Data: Table 11

Historical Control Data: Appendices E, F

In the control, GD0, GD1, GD2, and GD0 through GD2 groups, 96.0%, 92.0%, 92.0%, 88.0%, and 88.0% of the females, respectively, were gravid. Slight differences between the pregnancy rates in the control and exposed females were not considered to be related to exposure.

**3. BODY WEIGHTS**

Summary Data: Tables 13, 14

Individual Data: Tables 32, 33

Mean body weight gain in the GD0 through GD2 group during gestation days 0-4 was reduced relative to the control group value; the difference was statistically significant ( $p < 0.01$ ). The reduction was considered to be related to exposure. No other effects of exposure were observed on mean body weights and body weight gains.

**4. FOOD CONSUMPTION**

Summary Data: Tables 15, 16

Individual Data: Tables 34, 35

Food consumption, evaluated as g/animal/day and g/kg/day, in the GD0 through GD2 group was reduced relative to the control group values

during gestation days 0-4 and 0-8; the differences were statistically significant ( $p < 0.01$ ). The reductions were attributed to test article exposure.

No other effects of test article exposure were observed on food consumption in the Post-mating Phase groups. Food consumption ( $g/kg/day$ ) in the GD0 group was reduced relative to the control group value during gestation days 0-4; the difference was statistically significant ( $p < 0.05$ ). However, because a similar reduction was not observed when food consumption was evaluated as  $g/animal/day$ , the reduced  $g/kg/day$  value in this group was not attributed to exposure. Food consumption, evaluated as  $g/animal/day$  and/or  $g/kg/day$ , in the GD1 group was reduced during gestation days 0-4 and 0-8; the differences from the control group values were generally statistically significant ( $p < 0.05$  or  $p < 0.01$ ). However, the differences from the control group values were slight and were not considered to be related to test article exposure.

#### 5. GESTATION DAY 8 UTERINE EXAMINATION

Summary Data: Tables 17, 17A

Individual Data: Tables 36, 36A, 37

Historical Control Data: Appendix F

Mean numbers of corpora lutea and implantation sites and pre-implantation loss (% per litter) were unaffected by exposure under all exposure regimens in the Post-mating Phase. Values in the exposed groups were similar to the control group values; no statistically significant differences were noted.

In the control, GD0, GD1, GD2, and GD0 through GD2 groups, the numbers of small implantation sites (numbers of females affected) were 2(2), 0(0), 0(0), 0(0), and 1(1), respectively. No effect of test article exposure was observed.

6. DAM NECROPSIES

Summary Data: Table 18

Individual Data: Table 37

All females survived to the scheduled necropsy on gestation day 8. No exposure-related internal findings were noted. Nongravid uteri were found to be ammonium sulfide negative. Sporadic internal findings in the exposed groups were noted infrequently.

7. ORGAN WEIGHTS

Summary Data: Tables 19, 20

Individual Data: Tables 38, 39

Mean ovarian and uterine weights (absolute and relative to final body weight) in the exposed groups were unaffected by exposure to the test article. No statistically significant differences from the control group were noted.

8. MICROSCOPIC EXAMINATION

Appendix D

None of the implantation sites that appeared small macroscopically also appeared small microscopically. The cells of the decidual reaction of the small sites (based on macroscopic examination) appeared histologically normal, and the reaction was comparable to the larger sites.

V. DISCUSSION AND CONCLUSIONS

All females in the Pre-mating Phase and Post-mating Phase survived to the scheduled necropsy on gestation day 8. No exposure-related clinical observations or internal findings (exclusive of the reproductive tract) were noted in females from either phase.

In the Pre-mating Phase, the pregnancy rate (no. gravid/no. with evidence of mating) in the females exposed one day prior to mating was reduced relative to the control group value (64.7% in the Day -1 group, as compared to 95.5% in the control group). Pregnancy rates were unaffected by exposure in the other Pre-mating Phase groups. In the Post-mating Phase, pregnancy rates were unaffected by test article exposure.

In the Pre-mating Phase, mean body weight in the Days -3 through -1 group was reduced (statistically significant) on gestation day 0. Mean body weights in this group were similar to the control group values on gestation days 4 and 8. Mean body weight gains in the Days -3 through -1 group were increased (statistically significant) during gestation days 0-4 and 0-8. In the Day -3 through GD3 group, mean body weight gains were decreased and increased during gestation days 0-4 and 4-8, respectively; the differences from the control group were statistically significant. Food consumption in this group was reduced (statistically significant) during gestation days 0-4 and 0-8. The aforementioned differences in mean body weight and food consumption data were attributed to test article exposure. Food consumption in all other groups and mean body weights and body weight gains in the Day -1, Day -2, Day -3, and Day -4 groups were unaffected by test article exposure.

In the Post-mating Phase, mean body weight gain was reduced (statistically significant) in the GD0 through GD2 group during gestation days 0-4. Food consumption in this group was decreased (generally statistically significant) during gestation days 0-4 and 0-8. The decreases in mean body weight gain and food

consumption in the GD0 through GD2 group were attributed to test article exposure. Mean body weights, body weight gains, and food consumption were unaffected in all other exposed groups in the Post-mating Phase.

In the Pre-mating Phase, the mean numbers of corpora lutea (statistically significant) and implantation sites (not statistically significant) were reduced in the Day -3 through GD3 group. In the Day -1, Day -2, Day -3, Day -4, and Days -3 to -1 groups, the mean numbers of corpora lutea and implantation sites were unaffected by exposure. Pre-implantation loss was unaffected by exposure under all Pre-mating Phase treatment regimens. An increased number of small implantation sites was observed in the Day -3 through GD3 group. The numbers of small implantation sites in the other exposed groups were similar to the control group value. In the Post-mating Phase, mean numbers of corpora lutea and implantation sites, pre-implantation loss, and numbers of small implantation sites were unaffected by test article exposure under all treatment regimens.

In the Pre-mating Phase, mean uterine weights (absolute and relative to final body weight) were reduced (statistically significant) in the Day -3 through GD3 group. The reductions in mean uterine weights were consistent with the reduced number of total implantation sites and the increased number of small implantation sites in this group. The mean absolute and relative ovarian weights in this group were unaffected by exposure. Mean uterine and ovarian weights were unaffected by exposure in all other Pre-mating Phase groups and in all Post-mating Phase groups.

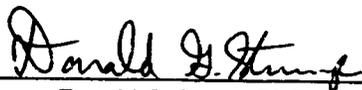
In the Pre-mating Phase, the ovaries from all females in the Day -1 group had a normal complement of corpora lutea. All were consistent with the corpora lutea of pregnancy (11 rats) or pseudopregnancy (6 non-pregnant rats). In the Pre-mating Phase, some of the implantation sites that appeared small macroscopically also appeared small microscopically. However, this may have been due to sample

DC Study No. - 8864  
External No. - WIL-51054

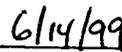
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sectioning. The cells of the decidual reaction of the small sites appeared histologically normal, and the reaction was comparable to the larger sites.

In conclusion, maternal and reproductive toxicity was expressed in the Pre-mating Phase by a reduced pregnancy rate in the Day -1 group, by effects on mean body weight gains in the Days -3 through -1 group, and by effects on mean body weight gains, reduced food consumption, reduced numbers of corpora lutea and implantation sites, increased numbers of small implantation sites, and reduced mean uterine weight in the Day -3 through GD3 group. Although a reduced pregnancy rate was observed in the Day -1 group, no permanent impairment in pregnancy rates was observed. The transient nature of this effect in the Day -1 group was demonstrated by the lack of effect on pregnancy rates of the animals exposed in the Day -4, Day -3, and Day -2 groups. Maternal toxicity was expressed in the Post-mating Phase by reduced mean body weight gain and food consumption in the GD0 through GD2 group.



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Study Director



Date

DC Study No. - 8864  
External No. - WIL-51054

DC Report No. - 1999-10000-47049  
Security - INTERNAL

VI DATA RETENTION

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

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

VII. REFERENCES

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