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DCN: 89090000085

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08 DEC 22 AM 11:00

December 16, 2008

TSCA Confidential Business Information Center (7407M)
EPA East – Room 6428
Attn: Section 8(e)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

8EHQ 07 16778

Re: Supplemental TSCA Section 8(e) Submission; Results of a 2-Week Whole-Body Inhalation Toxicity Range-Finder Study of Trimethylsilanol in Albino Rats and 28-Day Whole-Body Repeated Dose Inhalation Toxicity Study with Reproductive/Developmental Screening of Trimethylsilanol in Albino Rats

Dear TSCA Section 8(e) Coordinator:

In accordance with the provisions of Section 8(e) of the Toxic Substances and Control Act (TSCA), as interpreted in the TSCA Section 8(e) Policy Statement and Guidance, Fed. Reg. 33129 (June 3, 2003) and other Agency guidance, the Silicones Environmental, Health and Safety Council (SEHSC)[1], on behalf of its member companies, is providing the following information as a supplemental submission to our March 9, 2007 TSCA Section 8(e) notification. Our initial March 9, 2007 notification reported the results of an Acute Inhalation Toxicity Study of Trimethylsilanol in Albino Rats. This supplemental submission provides the results of the subsequent 2-Week Whole-Body Inhalation Toxicity Range-Finder Study and 28-Day Whole-Body Repeated Does Inhalation Toxicity Study with Reproductive/Developmental Screening of Trimethylsilanol in Albino Rats. Neither SEHSC, nor any member company, has made a determination at this time that any significant risk of injury to human health or the environment is presented by these findings

Chemical Substance

1066-40-6 Trimethylsilanol

89090000085

Studies

A 2-Week Whole-Body Inhalation Toxicity Study of Trimethylsilanol in Albino Rats
WIL Research Laboratories, LCC; Study Reference Number: WIL-401008

A Whole-Body Inhalation Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Trimethylsilanol in Rats
WIL Research Laboratories, LCC; Study Reference Number: WIL-401009

[1] SEHSC is a not-for-profit trade association whose mission is to promote the safe use of silicones through product stewardship and environmental, health, and safety research. The Council is comprised of North American silicone chemical producers and importers.

Contains No CBI

CONTAINS NO CBI

MR315915

Summary

Results from the previously conducted and reported acute inhalation study (OECD 403 Guideline) with trimethylsilanol included treatment-related effects of ataxia, loss of coordination, immobility, non-responsiveness, and death in Crl:CD(SD) rats following single 4-hour exposure at concentrations above 1824 ppm. Subsequent studies with trimethylsilanol have included a 2-week whole-body inhalation range-finding study (OECD 412 Guideline) and a 28-day whole-body repeated dose inhalation toxicity study with reproductive/developmental screening (OECD 422 Guideline).

During the 2-week range finding study, exposure levels of 60, 300 and 600 ppm were evaluated via whole-body inhalation exposure conducted 5 days/week. CNS/behavior-related findings in Crl:CD(SD) rats including hypoactivity for males and impaired equilibrium for females were observed at the 600ppm exposure level. These findings were similar to those observed in the acute study at higher concentrations. These clinical signs were noted after removal of the animals from the inhalation chamber and had resolved prior to the following day's exposure. Two of 5 males in the 600ppm exposure group were considered hypoactive on three exposure days (day 3, 4, and 7). Impaired equilibrium was observed in 2 females in the 600 ppm exposure group on study days 3 and 4. This same effect was not present in any animals exposed to ≤ 300ppm. Macroscopic observations during this range-finding study included diffusely pale lungs in 1 of 5 males and 4 of 5 females at the 600ppm level. There were no other test article-related affects observed during the range-finding study.

A 28-day repeated dose study with reproductive/developmental screening was conducted after the range-finding study. The 28-day study utilized whole-body inhalation exposure 7 days/week at 60, 300, and 600 ppm trimethylsilanol using Crl:CD(SD) rats. There were no treatment related clinical signs, changes in body weight, food consumption, organ weights, macroscopic or microscopic observations, reproductive or behavior parameters evaluated. With identical concentrations, the CNS/behavioral related findings noted during the range-finding study were not reproduced. A no-observable-adverse-affect-level (NOAEL) of 600ppm was assigned for all parameters evaluated in the 28-day study.

Study Designs

2-Week Range Finder (OECD 412 Guideline):

The objective of this study was to evaluate potential subchronic toxic effects of trimethylsilanol following whole-body inhalation exposure in an effort to establish dose levels for subsequent longer term studies. Trimethylsilanol was administered via whole-body inhalation exposure, 6 hours/day, 5 days/week for 2 weeks (10 total exposures) to 3 groups of Crl:CD(SD) rats. Target exposure levels were 60, 300 and 600 ppm. Overall mean measured test article exposure concentrations were 60.9, 299.7 and 599.8 ppm for the 60, 300 and 600 ppm groups, respectively. A concurrent control group (Group 1) was exposed to filtered air on a comparable regimen. All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded approximately weekly. Complete necropsies were conducted on all animals on the day following the final exposure, and selected organs were weighed at the scheduled necropsy (study day 12). Selected tissues were collected and examined microscopically from all animals in the control and 600 ppm groups.

28-day with Reproductive/Developmental Screening (OECD 422 Guideline):

The objective of this study was to investigate the potential toxic effects of trimethylsilanol when administered via whole-body inhalation to rats for a minimum of 28 days and to evaluate the potential of the test article to affect male and female reproductive performance such as gonadal function, mating behavior, conception, parturition and early postnatal development.

Three groups of Crl:CD(SD) rats, each group consisting of 10 males, 10 toxicity phase females and 10 reproductive phase females, were exposed via whole-body inhalation to vapor atmospheres of the test article, trimethylsilanol, 6 hours/day, 7 days/week. Target exposure concentrations were 60, 300 and 600 parts per million (ppm). A concurrent control group was exposed to filtered air on a comparable regimen. Mean measured test article exposure concentrations were 0, 61, 303 and 602 ppm (males), 0, 61, 304 and 604 ppm (toxicity phase females) and 0, 61, 304 and 603 ppm (reproductive phase females) for the control, 60, 300 and 600 ppm groups, respectively. Exposures were initiated when the animals were approximately 11-12 weeks of age. F_0 males and reproductive phase females were exposed daily for 14 days prior to mating, throughout the mating period and continuing through the day prior to euthanasia (males) or gestation day 20 (reproductive phase females), for a total of 34 and 35-48 consecutive days, respectively. F_0 reproductive phase females with no evidence of mating or that failed to deliver were exposed through post-mating or post-cohabitation day 24 (the day prior to euthanasia) for a total of 52 consecutive days. F_0 toxicity phase females were exposed for 28 consecutive days.

All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. Functional observational battery (FOB) and locomotor activity data were recorded for all males and toxicity phase females prior to initiation of exposure for each sex (baseline evaluation) and near the end of exposure. F_0 males were euthanized following completion of the mating period and F_0 toxicity phase females were euthanized following 28 days of exposure. Clinical pathology evaluations (hematology and serum chemistry) were performed on all F_0 males and toxicity phase females at necropsy. Complete necropsies were conducted on all F_0 males and toxicity phase females, and selected organs were weighed; selected tissues in the control and high-exposure groups were examined microscopically. All F_0 reproductive phase females were allowed to deliver and rear their pups until lactation day 4. F_1 clinical observations and body weights were recorded on postnatal days (PND) 1 and 4. Reproductive phase dams and pups were euthanized and examined on lactation day/PND 4.

Results

2-week Range-Finder (OECD 412):

All animals survived to the scheduled necropsy. There were no test article-related effects on body weights, food consumption or organ weights. There were no test article-related microscopic findings. Test article-related clinical findings included CNS/behavior-related findings of hypoactivity for 2 of 5 males and impaired equilibrium for 2 of 5 females in the 600 ppm group within 1 hour following exposure. Exposure of male and female albino rats to 600 ppm of trimethylsilanol was not associated with significant macroscopic alterations in the tissues examined (brain, heart, kidneys, liver and lungs). Macroscopic findings included diffusely pale lungs observed in 1 of 5 males and

4 of 5 females exposed to 600 ppm of the test article. However, there were no microscopic correlates to this gross observation. In addition, multiple pinpoint or irregular dark red areas were observed in the lungs of 1 of 5 males in the control group and 1 of 5 males and 1 of 5 females exposed to 600 ppm of the test article. These areas microscopically corresponded to hemorrhage.

28-day with Reproductive/Developmental Screening (OECD 422 Guideline):

There were no remarkable clinical observations noted at any exposure level. Mean body weights, body weight gains and food consumption for males and females at all exposure levels were similar to the control group for all phases. There were no test article-related macroscopic findings or effects on organ weights. There were no test article-related effects on FOB parameters, including home cage, handling, open field, sensory, neuromuscular or physiological observations and no effects on locomotor activity patterns for males and toxicity phase females. No remarkable shifts in the pattern of habituation occurred during locomotor activity assessments in any of the test article-exposed groups when the F_0 males and toxicity phase females were evaluated prior to the initiation of exposure or during the study week 4 evaluation.

Decreased mean eosinophil and lymphocyte counts were noted for the 600 ppm group F_0 males. In addition, increased mean alanine aminotransferase values were noted for F_0 males and toxicity phase females in the 600 ppm group when compared with the control group. Although these changes were considered test article-related, they were not considered adverse in the absence of correlating histopathology.

Mean mating, fertility and copulation/conception indices for all exposure concentrations were similar to the control group. Mean gestation lengths, postnatal survival and F_1 body weights were similar to the control group. There were no test article-related macroscopic findings observed for pups.

Actions

Enclosed please find copies of the final reports for these studies.

If you have any questions concerning this submission, please contact me at (703) 788-6570, kthomas@sehsc.com, or at the address provided herein.

Sincerely,

Karluss Thomas
Executive Director

FINAL REPORT

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STUDY TITLE

A 2-WEEK WHOLE-BODY INHALATION
TOXICITY STUDY OF TRIMETHYLSILANOL IN ALBINO RATS

STUDY NUMBER

WIL-401008

DATA REQUIREMENT

OECD Guideline Section 412

STUDY DIRECTOR

Jeffrey T. Weinberg, BS

STUDY INITIATION DATE

19 February 2007

STUDY COMPLETION DATE

16 July 2007

PERFORMING LABORATORY

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

SPONSOR

Silicones Environmental Health and Safety Council
2325 Dulles Corner Boulevard, Suite 500
Herndon, VA 20171

MR 315915

COMPLIANCE STATEMENT

This study, designated WIL-401008, was conducted in compliance with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C (97) 186/Final], 26 November 1997; the standard operating procedures of WIL Research Laboratories, LLC, and the protocol as approved by the sponsor.

The protocol was designed to be in general accordance with the OECD Guidelines for Testing Chemicals, Section 412.

Jeffrey T. Weinberg, BS
Project Specialist, Inhalation Toxicology
Study Director

16 July 2007
Date

TABLE OF CONTENTS

Page

Compliance Statement **2**
Table Of Contents **3**
Index Of Figures **5**
Index Of Tables **6**
Index Of Appendices **7**
1. Summary **8**
1.1. Objective 8
1.2. Study Design 8
1.3. Results 8
1.4. Conclusions 9
2. Introduction **10**
2.1. General Study Information 10
2.2. Key Study Dates 10
3. Study Design **11**
4. Experimental Procedures - Materials And Methods **12**
4.1. Test Article And Vehicle 12
4.1.1. Test Article Identification 12
4.1.2. Organization of Test Groups, Exposure Levels and Treatment Regimen 12
4.1.3. Exposure Methods 13
4.1.4. Exposure Atmosphere Generation And Monitoring 13
4.2. Test System 15
4.3. Animal Receipt And Acclimation/Pretest Period 15
4.4. Animal Housing 15
4.5. Diet, Drinking Water And Maintenance 16
4.6. Environmental Conditions 16
4.7. Assignment Of Animals To Treatment Groups 16

Page

5. Parameters Evaluated 18
5.1. Clinical Observations And Survival 18
5.2. Body Weights 18
5.3. Food Consumption 18
5.4. Anatomic Pathology 19
5.4.1. Macroscopic Examination 19
5.4.2. Organ Weights 19
5.4.3. Slide Preparation And Microscopic Examination 19
5.5. Statistical Methods 20
5.6. Data Retention 20
6. Results And Discussion 22
6.1. Clinical Observations And Survival 22
6.2. Body Weights 22
6.3. Food Consumption 22
6.4. Anatomic Pathology 23
6.4.1. Macroscopic Examination 23
6.4.2. Organ Weights 23
6.4.3. Microscopic Examination 23
6.4.4. Discussion Of Anatomic Pathology Findings 24
7. Conclusions 25
8. Key Study Personnel And Report Submission 26
9. Quality Assurance Unit Statement 28
9.1. Phases Inspected 28
9.2. Approval 30
10. References 31
11. Deviations From The Protocol 32

INDEX OF FIGURES

Page

1. Summary Of Body Weights (G) (Males) 34
2. Summary Of Body Weights (G) (Females) 35

INDEX OF TABLES

Page

1. Summary Of Survival And Disposition 37

2. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (Detailed Physical Examinations/Dispositions) 39

3. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (Prior To Exposure) 41

4. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (1 Hour Post-Exposure) 43

5. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (Daily Observations - Non-Exposure Days) 45

6. Summary Of Body Weights [G] 47

7. Summary Of Body Weight Changes [G] 51

8. Summary Of Cumulative Body Weight Changes [G] 53

9. Summary Of Food Consumption [G/Animal/Day] 55

10. Summary Of Macroscopic Findings 57

11. Summary Of Organ Weights And Relative Organ Weights 60

12. Summary Of Microscopic Findings 69

INDEX OF APPENDICES

Page

A. Individual Animal Data 75

A1. Individual Survival And Disposition 76

A2. Individual Clinical Observations (Detailed Physical Examinations/Dispositions) 78

A3. Individual Clinical Observations (Prior To Exposure) 86

A4. Individual Clinical Observations (1 Hour Post-Exposure) 87

A5. Individual Body Weights [G] 88

A6. Individual Body Weight Changes [G] 96

A7. Individual Cumulative Body Weight Changes [G] 104

A8. Individual Food Consumption [G/Animal/Day] 112

A9. Individual Macroscopic And Microscopic Findings 120

A10. Individual Organ Weights And Final Body Weights [G] 161

A11. Individual Organ Weights Relative To Final Body Weights [G/100 G] 169

A12. Individual Organ Weights Relative To Brain Weights [G/100 G] 177

B. Certificate Of Analysis (Sponsor-Provided Data) 185

C. Exposure Atmosphere Generation Report (WIL Research Laboratories, LLC) 187

D. Pretest Clinical Observations 215

E. Animal Room Environmental Conditions 218

F. Unscheduled Clinical Observations 223

G. Pathology Report (WIL Research Laboratories, LLC) 225

H. Study Protocol 235

1. SUMMARY

1.1. OBJECTIVE

The objective of the study was to evaluate the potential toxic effects of the test article when administered via whole-body inhalation to rats for 2 weeks on a 5-day per week basis (minimum of 10 total exposures). Results of the study will be used to establish exposure levels for a subsequent 90-day study.

1.2. STUDY DESIGN

Trimethylsilanol was administered as a whole-body inhalation exposure 6 hours/day, 5 days/week for 2 weeks (10 total exposures) to 3 groups (Groups 2-4) of Crl:CD(SD) rats. Target exposure levels were 60, 300 and 600 ppm. A concurrent control group (Group 1) was exposed to filtered air on a comparable regimen. Each group consisted of 5 males and 5 females.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed weekly. Individual body weights and food consumption were recorded approximately weekly. Complete necropsies were conducted on all animals on the day following the final exposure, and selected organs were weighed at the scheduled necropsy (study day 12). Selected tissues were examined microscopically from all animals in the control and 600 ppm groups.

1.3. RESULTS

Overall mean measured test article exposure concentrations were 60.9, 299.7 and 599.8 ppm for the 60, 300 and 600 ppm groups, respectively.

All animals survived to the scheduled necropsy. There were no test article-related effects on body weights, food consumption or organ weights. There were no test article-related microscopic findings.

Test article-related clinical findings included CNS/behavior-related findings of hypoactivity for males and impaired equilibrium for females in the 600 ppm group within 1 hour following exposure.

Exposure of male and female albino rats to 600 ppm of trimethylsilanol was not associated with significant macroscopic alterations in the tissues examined (brain, heart, kidneys, liver and lungs). Macroscopic findings included diffusely pale lungs observed in 1 of 5 males and 4 of 5 females exposed to 600 ppm of the test article. However, there were no microscopic correlates to this gross observation. In addition, multiple pinpoint or irregular dark red areas were observed in the lungs of 1 of 5 males in the control group and 1 of 5 males and 1 of 5 females exposed to 600 ppm of the test article. These areas microscopically corresponded to hemorrhage.

1.4. CONCLUSIONS

Test article-related effects were limited to the 600 ppm exposure level and consisted of CNS/behavior-related clinical signs of hypoactivity (males) and impaired equilibrium (females) and macroscopic findings of diffusely pale lungs. Multiple pinpoint or irregular dark red areas in the lungs of 2 test article-treated animals and 1 control group animal were noted, but were not considered to be treatment-related. Based on the results of this study, the no-observed-effect level (NOEL) for trimethylsilanol administered to Crl:CD(SD) rats by inhalation for 2 consecutive weeks (minimum 10 total exposures) was 300 ppm.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This report presents the data from "A 2-Week Whole-Body Inhalation Toxicity Study of Trimethylsilanol in Albino Rats". Due to software spacing constraints, the study title appears as "A 2-Week Inhalation Study of Trimethylsilanol in Rats" on the report tables.

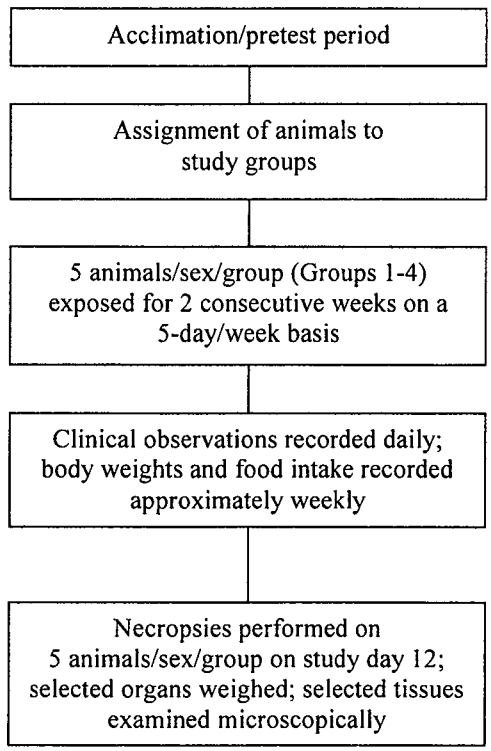
The following computer protocols were used for data collection during the study:

Computer Protocol	Type of Data Collected
WIL-401008	Main study data
WIL-401008P	Pretest data
WIL-401008U	Unscheduled clinical observations

2.2. KEY STUDY DATES

Date(s)	Event(s)
20 February 2007	Experimental starting date (animal receipt)
1 March 2007	Assignment to study groups
2 March 2007	Experimental start date (initiation of test article exposure; study week 0)
14 March 2007	Scheduled necropsy (study day 12)
2 May 2007	Experimental termination (completion) date (last histopathological examination)

3. STUDY DESIGN



4. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

4.1. TEST ARTICLE AND VEHICLE

4.1.1. TEST ARTICLE IDENTIFICATION

The test article, 31-OH-RM (Trimethylsilanol), was received from Shin-Etsu Silicones, Akron, Ohio, on 9 February 2007, as follows:

Identification	Quantity Received	Physical Description
Trimethylsilanol Lot no. 611001 Exp. date: 7 August 2007 CAS no. 1066-40-6 [WIL log no. 7340A]	5 buckets Total gross weight: 81494.0 g	Clear, colorless liquid

Data documenting the purity and stability of the test article are on file with the sponsor. A Certificate of Analysis for the test article was provided by the sponsor and is presented in Appendix B. The purity of the test article was 99.68%. The test article was stored at room temperature and was considered stable under this condition. A reserve sample of the test article (approximately 7 g) was collected on 21 February 2007, and stored in the Archives of WIL Research Laboratories, LLC.

4.1.2. ORGANIZATION OF TEST GROUPS, EXPOSURE LEVELS AND TREATMENT REGIMEN

The test article or filtered air (control group) was administered as a daily 6-hour (plus approximately 20 minutes) whole-body inhalation exposure. Exposures were conducted 5 days per week at approximately the same time each day for 2 consecutive weeks, through the day prior to the scheduled necropsy. The first day of exposure was study day 0, and the first week of exposure was study week 0.

The following table presents the study group assignment:

Group Number	Test Article	Target Exposure Level (ppm)	Number of Animals Males	Number of Animals Females
1	Filtered Air	0	5	5
2	Trimethylsilanol	60	5	5
3	Trimethylsilanol	300	5	5
4	Trimethylsilanol	600	5	5

The exposure levels were determined from the acute inhalation study (Weinberg, 2007). The selected route of administration for this study was whole-body inhalation exposure because this is a reliable and accurate method of evaluating toxicity and the possible route of human exposure. The number of animals selected for this study was the minimum number needed to meet the objectives of the study and meet testing guidelines.

4.1.3. EXPOSURE METHODS

Animal exposures were conducted as whole-body inhalation exposures in 1000-L stainless-steel and glass whole-body exposure chambers. One chamber was dedicated to each group for the duration of the study. The exposure period was 6 hours per day (plus the t_{99} equilibrium time) at approximately the same time each day, 5 days per week for 2 consecutive weeks (10 total exposures). Animals in each exposure group were caged individually. The cage batteries were rotated on a daily basis within the chambers to ensure similar exposure of all animals to the test article over the duration of the study. For each exposure, the animals were transferred from their home cages to whole-body exposure caging, transported to the exposure chamber room, exposed for the requisite duration and returned to their home cages. Food and water were withheld during each daily exposure period. Exposure methods and conditions are detailed in Appendix C.

4.1.4. EXPOSURE ATMOSPHERE GENERATION AND MONITORING

Trimethylsilanol was generated for exposure as a vapor using a bubbler-type vaporization system located within a heated Plexiglas® containment box. Nitrogen flowed into the

inlet stem of the gas washing bottle containing an appropriate quantity of test article and bubbled through a fritted disc at the bottom of the bottle in order to create vapors of the test article. The concentrated vapors were piped to the heated chamber inlet where the concentration was reduced by mixing with chamber ventilation air. The control group was exposed to clean, filtered air. A detailed description of the generation methods is presented in Appendix C.

The exposure period was adjusted for calculated t_{99} equilibrium time (approximately 20 minutes) for this study. The exposure was initiated after the animals were placed in the exposure chamber and generation of the test article vapor had been ongoing for approximately 20 minutes. The exposure period was terminated after 6 hours when the test article vapor generation was stopped.

Temperature, relative humidity, chamber ventilation rate and negative pressure within the exposure chambers were monitored continuously and recorded approximately every 37 minutes. The daily mean chamber temperature ranged from 20°C to 24°C, with the following exception. During the animal exposure phase, the daily mean temperature for Chamber 4 (600 ppm group) ranged from 24°C to 26°C, where 8 daily mean temperatures were out of the protocol-specified range. Although the temperature in Chamber 4 was slightly above the protocol-specified range, 24°C to 26°C is within the normal temperature range for whole-body exposure chambers. The daily mean chamber relative humidity ranged from 30% to 70%. Chamber environmental conditions are summarized in Appendix C.

Actual exposure concentrations of the test article atmospheres in the exposure chamber were determined at approximately 37-minute intervals by standard gas chromatograph (GC) methods. Samples were collected from the approximate animal-breathing zone of the inhalation exposure chambers. The overall mean analyzed concentrations were 60.9, 299.7 and 599.8 ppm for the 60, 300 and 600 ppm groups, respectively.

4.2. TEST SYSTEM

Crl:CD(SD) rats from Charles River Laboratories, Inc., Raleigh, North Carolina were used as the test system on this study. This species and strain of animal is recognized as appropriate for inhalation studies. The Sprague-Dawley rat was used because it is a widely used strain for which significant historical control data are available.

4.3. ANIMAL RECEIPT AND ACCLIMATION/PRETEST PERIOD

Twenty-five male and 25 female Crl:CD(SD) rats were received in good health on 20 February 2007, from Charles River Laboratories, Inc., Raleigh, North Carolina. The animals were approximately 48 days old at receipt. Each animal was examined by a qualified technician on the day of receipt and weighed 3 days later. Each animal was uniquely identified by a Monel® metal eartag displaying the permanent identification number. All animals were housed for a 10-day acclimation/pretest period. During this period, each animal was observed twice daily for mortality and changes in general appearance or behavior.

Pretest data collection began on 23 February 2007. Individual body weights were recorded and detailed physical examinations were performed periodically during the pretest period. Food consumption data were also recorded for pretest animals prior to the initiation of exposure. Pretest clinical observations are presented in Appendix D.

4.4. ANIMAL HOUSING

Upon arrival, all animals were housed individually in clean, stainless steel, wire-mesh cages suspended above cage-board. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The animal facilities at WIL Research Laboratories, LLC are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

4.5. DIET, DRINKING WATER AND MAINTENANCE

The basal diet used in this study, PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002 (meal), is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research Laboratories, LLC. Reverse osmosis-treated (on-site) drinking water, delivered by an automatic watering system, and the basal diet were provided ad libitum throughout the study, except during exposure periods. Municipal water supplying the facility was sampled for contaminants according to the standard operating procedures. The results of the diet and water analyses are maintained at WIL Research Laboratories, LLC. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study.

4.6. ENVIRONMENTAL CONDITIONS

All animals were housed throughout the acclimation period and during the study in an environmentally controlled room. The room temperature and humidity controls in the animal room were set to maintain daily averages of 71 ± 5°F (22 ± 3°C) and 50 ± 20% relative humidity. Room temperature and relative humidity were controlled and monitored using the Metasys® DDC Electronic Environmental control system. These data were recorded approximately hourly and are summarized in Appendix E. Actual mean daily temperature ranged from 70.5°F to 70.7°F (21.4°C to 21.5°C) and mean daily relative humidity ranged from 34.7% to 38.9% during the study. Fluorescent lighting provided illumination for a 12-hour light (0600 hours to 1800 hours)/12-hour dark photoperiod. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

4.7. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

On 1 March 2007 (the day prior to the initiation of exposure), all available rats were weighed and examined in detail for physical abnormalities. These data were collected using the WIL Toxicology Data Management System (WTDMS™) and reviewed by the study director. The animals judged suitable for assignment to the study were selected for

use in a computerized randomization procedure. A printout containing the animal numbers, corresponding body weights and individual group assignments was generated based on body weight stratification in a block design. The animals were then arranged into groups according to the printout. Individual body weights at randomization were within ± 20% of the mean for each sex. Each group consisted of 5 males and 5 females. The selected animals were approximately 8 weeks old at the initiation of exposure; individual body weights ranged from 266 g to 310 g for males and from 181 g to 221 g for females.

5. PARAMETERS EVALUATED

5.1. CLINICAL OBSERVATIONS AND SURVIVAL

All animals were observed twice daily, once in the morning and once in the afternoon, for mortality and moribundity.

Clinical examinations were performed twice daily, prior to exposure and within 1 hour following exposure (designated as 1 hour post-exposure for report presentation purposes). On non-exposure days, the animals were observed once daily. All significant findings were recorded. Detailed physical examinations were conducted on all animals weekly, beginning 1 week prior to test article exposure and prior to the scheduled necropsy. Observations prior to exposure were not performed on days when detailed physical examinations were conducted. A separate computer protocol was used to record any observations noted outside of the above-specified intervals. These unscheduled clinical observations are presented in Appendix F.

5.2. BODY WEIGHTS

Individual body weights were recorded at least weekly, beginning 1 week prior to test article exposure (study day -7). Mean body weights and mean body weight changes were calculated for the corresponding intervals. Final body weights were recorded prior to the scheduled necropsy.

5.3. FOOD CONSUMPTION

Individual food consumption was recorded at intervals of 6 days (study day -7 to -1), 7 days (study day 0 to 7) and 5 days (study day 7 to 11) over the pretest and test article exposure periods, respectively. The shorter interval during the second study week was due to the scheduled necropsy on study day 12. Food intake was calculated as g/animal/day for the corresponding body weight intervals.

5.4. ANATOMIC PATHOLOGY

5.4.1. MACROSCOPIC EXAMINATION

A complete necropsy was conducted on all animals. Animals were euthanized by isoflurane anesthesia and exsanguinated. The necropsies included, but were not limited to, examination of the external surface, all orifices, and the cranial, thoracic, abdominal and pelvic cavities, including viscera. Clinical findings that were confirmed macroscopically were designated CEO (correlates with externally observed) on the individual macroscopic data tables. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted):

- Brain
  - Cerebrum Level 1
  - Cerebrum Level 2
  - Cerebellum with medulla/pons
- Heart
- Kidneys (2)
- Liver (sections of 2 lobes)
- Lungs (including bronchi, fixed by inflation with fixative)
- Gross lesions (when possible)

5.4.2. ORGAN WEIGHTS

The following organs were weighed from all animals at the scheduled necropsy:

- Adrenals
- Brain
- Kidneys
- Liver
- Lungs
- Testes

Paired organs were weighed together. Organ-to-final-body-weight and organ-to-brain-weight ratios were calculated.

5.4.3. SLIDE PREPARATION AND MICROSCOPIC EXAMINATION

After fixation, protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with hematoxylin and eosin.

Microscopic examination was performed on all tissues listed in Section 5.4.1. from all animals in the control and 600 ppm groups at the scheduled necropsy. Missing tissues were identified as not found at necropsy, lost at necropsy, lost during processing or other designations as appropriate. Tissues may appear on the report tables as not examined due to the tissue not being in the plane of section, not present at trimming, etc. Microscopic examination was performed by Gopakumar Gopalakrishnan, BVSc, MS, WIL Research Laboratories, LLC (Appendix G).

A formal histopathology peer review was not conducted as part of this study.

5.5. STATISTICAL METHODS

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance level of 5%, comparing each test article-exposed group to the control group by sex. Each mean was presented with the standard deviation (S.D.), standard error (S.E.) and the number of animals (N) used to calculate the mean. In addition, percent difference from control is presented for body weights, food consumption and organ weights. Due to the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ by ±1 in the last significant figure.

Body weight, body weight change, food consumption and organ weight data were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-exposed groups to the control group.

5.6. DATA RETENTION

The sponsor has title to all documentation records, raw data, specimens, slides or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are

retained in the Archives at WIL Research Laboratories, LLC as specified in the study protocol.

Reserve samples of the test article, pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC in compliance with regulatory requirements.

6. RESULTS AND DISCUSSION

6.1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 1, 2, 3, 4, 5

Individual Data: Appendix A - Tables A1, A2, A3, A4; Appendix F

All animals survived to the scheduled necropsy. Test article-related clinical findings included CNS/behavior-related signs of hypoactivity for males and impaired equilibrium for females in the 600 ppm group. Hypoactivity was noted for 2 males (male nos. 56163 and 56172) in the 600 ppm group at 1 hour post-exposure on study days 3, 4 and 7. Impaired equilibrium was observed for female nos. 56205 and 56209 (600 ppm group) at 1 hour following exposure on study days 3 and 4, respectively. All other clinical findings in the test article-exposed groups were noted with similar incidence in the control group, were limited to single animals, were not noted in a dose-related manner and/or were common findings for laboratory rats of this age and strain.

6.2. BODY WEIGHTS

Summary Data: Tables 6, 7, 8; Figures 1, 2

Individual Data: Appendix A - Tables A5, A6, A7

Body weights were unaffected by test article exposure. There were no statistically significant differences when the control and test article-exposed groups were compared.

6.3. FOOD CONSUMPTION

Summary Data: Table 9

Individual Data: Appendix A - Table A8

Food consumption was unaffected by test article exposure. There were no statistically significant differences when the control and test article-exposed groups were compared.

6.4. ANATOMIC PATHOLOGY

6.4.1. MACROSCOPIC EXAMINATION

Summary Data: Table 10

Individual Data: Appendix A - Table A9

Pathology Report: Appendix G

Multiple pinpoint or irregular dark red areas were observed in the lungs of 1/5 males in the control group and 1/5 males and 1/5 females exposed to 600 ppm of trimethylsilanol. These areas microscopically corresponded to hemorrhage. Although there was a slightly increased incidence of this finding in the 600 ppm treatment level, it was not considered to be treatment-related. Diffusely pale lungs were observed in 1/5 males and 4/5 females exposed to 600 ppm of the test article. However, there were no microscopic correlates to this gross observation.

6.4.2. ORGAN WEIGHTS

Summary Data: Table 11

Individual Data: Appendix A - Tables A10, A11, A12

Pathology Report: Appendix G

There were no test article-related effects on organ weights.

6.4.3. MICROSCOPIC EXAMINATION

Summary Data: Table 12

Individual Data: Appendix A - Table A9

Pathology Report: Appendix G

No test article-related histologic alterations were present in the tissue sections examined.

Minimal subpleural and/or perivascular infiltrates composed of variable proportions of macrophages, eosinophils and lymphocytes sometimes associated with minimal hemorrhage and alveolar histiocytosis present in the control group animals (4/5 females and 4/5 males) and the 600 ppm exposure group animals (5/5 females and 5/5 males)

were considered unrelated to exposure to the test article. Minimal intra-alveolar acicular eosinophilic crystalline material present in the lungs of 1 male rat was also interpreted to be an incidental finding due to lack of dose association.

Remaining histologic changes were considered to be incidental findings, manifestations of spontaneous diseases, or related to some aspect of experimental manipulation other than exposure to the test article. There was no test article-related alteration in the incidence, severity or histologic character of those incidental and spontaneous tissue alterations.

6.4.4. Discussion Of Anatomic Pathology Findings

Although mononuclear cell infiltrates and occasional inflammatory lesions were noted in the lungs of rats exposed to the test article at 600 ppm, similar changes were also present in the control group animals with no significant variation in incidence or severity. Moreover, the morphologic appearance and anatomic distribution of the lesions were not typical for an inhaled toxicant but rather reminiscent of an infectious etiology. The pulmonary changes share some histologic features with those reported with "rat respiratory syndrome" (Elwell et al., 1997; Slaoui et al., 1998). The minimal inflammatory lesions in the lungs of control and test article-exposed animals observed in this study did not interfere with observation or discrimination of test article-related findings in the lungs.

7. CONCLUSIONS

Test article-related effects were limited to the 600 ppm exposure level and consisted of CNS/behavior-related clinical signs of hypoactivity (males) and impaired equilibrium (females) and macroscopic findings of diffusely pale lungs. Multiple pinpoint or irregular dark red areas in the lungs of 2 test article-treated animals and 1 control group animal were noted, but were not considered to be treatment-related. Based on the results of this study, the no-observed-effect level (NOEL) for trimethylsilanol administered to Crl:CD(SD) rats by inhalation for 2 consecutive weeks (minimum 10 total exposures) was 300 ppm.

8. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Jeffrey T. Weinberg, BS — 16 July 2007 Date
Project Specialist, Inhalation Toxicology
Study Director

Report Prepared By:

Kathy L. Tyson, BS — 16 July 2007 Date
Study Analyst

Report Reviewed By:

Christopher P. Chengelis, PhD, DABT — 16 JULY 2007 Date
Director, Toxicology

for: Daniel T. Kirkpatrick, PhD, DABT — 16 JUL 2007 Date
Director, Inhalation Toxicology

Misty R. Lee, BA — 16 July 2007 Date
Group Supervisor, Study Analysis and Reports

Charlene A. Weygandt, BS — 16 July 2007 Date
Scheduling Coordinator, Regulatory Affairs and Services

Report Reviewed And Approved By:

Joseph M. Tobin, BS — 09 JULY 07 Date
Dow Corning Corporation
Sponsor Representative

KEY STUDY PERSONNEL AND REPORT SUBMISSION (CONTINUED)

Study Personnel:

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Carol A. Kopp, BS, LAT	Manager, Gross Pathology and Developmental Toxicology Laboratory
Teresa D. Morris, BS	Senior Operations Manager, Toxicology
Theresa M. Rafeld	Group Manager, Formulations Laboratory
Michael A. Safron, AS, HT (ASCP)	Manager, Histology
Robert A. Wally, BS, RAC	Manager, Reporting and Regulatory Technical Services

9. QUALITY ASSURANCE UNIT STATEMENT

9.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
02-Mar-2007	Test Article Exposure	02-Mar-2007	23-Apr-2007	M.Stauffer T.DeVan Booth
23-Apr-2007	Draft Report (Inhalation Report)	24-Apr-2007	24-May-2007	L.Goodrich
23-Apr-2007	Study Records (N-1)	23-Apr-2007	24-May-2007	L.Goodrich
18-Apr-2007 24-Apr-2007	Study Records (Ex-1, Ex-2)	24-Apr-2007	24-May-2007	L.Goodrich
24-Apr-2007	Study Records (Ex-1 Supplemental)	24-Apr-2007	24-May-2007	L.Goodrich
24-Apr-2007	Study Records (I-1)	24-Apr-2007	24-May-2007	L.Goodrich
11-May-2007	Study Records (H-1)	11-May-2007	20-Jun-2007	A.Kinney L.Goodrich
11-May-2007	Study Records (P-1)	11-May-2007	20-Jun-2007	A.Kinney L.Goodrich
12-May-2007 14-May-2007	Draft Report (Pathology Appendix)	14-May-2007	20-Jun-2007	L.Goodrich
13-May-2007 18-May-2007	Draft Report (without Inhalation and Pathology Appendices)	18-May-2007	20-Jun-2007	L.Goodrich

This study was inspected in accordance with the OECD Principles of Good Laboratory Practice, the standard operating procedures of WIL Research Laboratories, LLC and the sponsor's protocol and protocol amendments, with the following exceptions. The data located in Appendix B (Certificate of Analysis) were the responsibility of the sponsor. 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.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments and the standard operating procedures of WIL Research Laboratories, LLC.

The retention sample and the final report will be stored in the Archives at WIL Research Laboratories, LLC or another location specified by the sponsor.

9.2. APPROVAL

This study was inspected according to the criteria discussed in Section 9.1.

Report Audited By:

Patricia A. Brant 16 July 2007
for Lori J. Goodrich, RQAP-GLP Date
Group Supervisor, Quality Assurance

Report Released By:

Heather L Johnson 16 Jul 2007
Heather L. Johnson, BS, RQAP-GLP Date
Manager, Quality Assurance

10. REFERENCES

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Elwell, M. R.; Mahler J.F.; Rao G.N. Have you seen this? Inflammatory lesions in the lungs of rats. *Toxicologic Pathology* **1997**, *25*, 529-31

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Weinberg, Jeffrey. Acute Inhalation Toxicity Study of Trimethylsilanol in Albino Rats (Study No. WIL-401006). WIL Research Laboratories, LLC, Ashland, OH, **2007**.

11. DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 7.5** states that mean chamber temperature and relative humidity and chamber concentrations will be recorded approximately every 60 minutes throughout the exposures. Due to sample timing constraints of the LabVIEW inhalation data system, these parameters were recorded approximately every 37 minutes.
- **Protocol Section 8.2** states that findings noted at the clinical examination will be recorded for individual animals; the condition of animals without signs will be documented as a general comment. On 4 March 2007 (study day 2) during clinical observations, normal findings were documented individually for all animals in the control and 60 ppm groups and male no. 56173 (300 ppm group).
- **Protocol Section 8.3** states that all animals will receive a detailed physical examination weekly, beginning at least 1 week prior to test article administration. On 13 March 2007 (study day 11), detailed physical examinations were collected on all animals. As detailed physical examinations were scheduled prior to necropsy on 14 March 2007 (study day 12), these findings were documented as the prior to exposure findings for study day 11.

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.

FINAL REPORT

Volume 1 of 4
(Text, Figures 1-20, Tables 1-75 and Appendix A - Tables A1-A18)

STUDY TITLE

A WHOLE-BODY INHALATION REPEATED DOSE TOXICITY STUDY WITH THE REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST OF TRIMETHYLSILANOL IN RATS

STUDY NUMBER

WIL-401009

DATA REQUIREMENT

OECD Guideline 422

STUDY DIRECTOR

Tammye L. Fleeman, BS, LAT

STUDY INITIATION DATE

8 February 2008

STUDY COMPLETION DATE

21 November 2008

PERFORMING LABORATORY

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MR 315915

COMPLIANCE STATEMENT

This study, designated WIL-401009, was conducted in compliance with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C(97) 186/Final], 26 November 1997; the standard operating procedures of WIL Research Laboratories, LLC and the protocol as approved by the sponsor.

The protocol was designed to be in general accordance with the OECD Guideline for Testing of Chemicals, Guideline 422, Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test, 22 March 1996.

Tammye L. Fleeman, BS, LAT
Staff Toxicologist, Developmental and
Reproductive Toxicology
Study Director

21 November 2008
Date

TABLE OF CONTENTS

VOLUME 1 Page

Compliance Statement **2**
Table Of Contents **3**
Index Of Figures **8**
Index Of Tables **10**
Index Of Appendices **15**
1. Summary **21**
1.1. Objective 21
1.2. Study Design 21
1.3. Results 22
1.4. Conclusion 23
2. Introduction **24**
2.1. General Study Information 24
2.2. Key Study Dates 24
3. Study Design **25**
4. Experimental Procedures - Materials And Methods **26**
4.1. Test Article Identification 26
4.2. Exposure Methods 26
4.3. Test Atmosphere Generation Methods 28
4.4. Methods For Characterization Of Exposure Atmospheres 28
4.4.1. Nominal Concentrations 28
4.4.2. Analyzed Exposure Concentrations 29
4.4.3. Temporal Stability And Homogeneity Of Vapor Test Atmospheres 29
4.5. Test Article Characterization 30
4.6. Test System 30
4.7. Animal Receipt And Acclimation 30
4.8. Animal Housing (Non-Exposure Periods) 30
4.9. Diet, Drinking Water And Maintenance 31

VOLUME 1 Page

4.10. Environmental Conditions (Non-Exposure Periods) 32
4.11. Assignment Of Animals To Treatment Groups 32
5. Parameters Evaluated 34
5.1. Observations (All Phases) 34
5.1.1. Clinical Observations And Survival 34
5.1.2. Body Weights 34
5.1.3. Food Consumption 35
5.2. F_0 Male And Toxicity Phase Female Parameters 36
5.2.1. Functional Observational Battery (FOB) 36
5.2.1.1. Home Cage Observations 36
5.2.1.2. Handling Observations 36
5.2.1.3. Open Field Observations (Evaluated Over A 2-Minute Observation Period) 36
5.2.1.4. Sensory Observations 37
5.2.1.5. Neuromuscular Observations 37
5.2.1.6. Physiological Observations 37
5.2.2. Locomotor Activity 37
5.2.3. Clinical Pathology 38
5.2.3.1. Hematology And Coagulation Parameters 38
5.2.3.2. Serum Chemistry 39
5.2.4. Macroscopic Examinations 39
5.2.4.1. Unscheduled Deaths 39
5.2.4.2. Scheduled Euthanasia 39
5.2.4.3. Organ Weights 40
5.2.5. Slide Preparation And Microscopic Examination 41
5.3. Reproductive Phase Female Parameters (F_0) 42
5.3.1. Breeding Procedures 42
5.3.2. Parturition 43
5.3.3. Scheduled Euthanasia 43

VOLUME 1 Page

5.3.4. F_1 Litter Parameters 43
5.3.4.1. Litter Viability And Deaths 43
5.3.4.2. Clinical Observations 44
5.3.4.3. Body Weights 44
5.3.4.4. Sex Determination 44
5.3.4.5. Calculation Of Litter Parameters 44
5.3.4.6. Scheduled Euthanasia 45
5.4. Statistical Analyses 45
5.5. Data Retention 47
6. Results 48
6.1. Analyzed Exposure Concentrations 48
6.2. F_0 Males 48
6.2.1. Clinical Observations And Survival 48
6.2.2. Body Weights 49
6.2.3. Food Consumption 49
6.2.4. Functional Observational Battery (FOB) 49
6.2.5. Locomotor Activity 50
6.2.6. Clinical Pathology 51
6.2.6.1. Hematology And Coagulation 51
6.2.6.2. Serum Chemistry 51
6.2.7. Reproductive Performance 52
6.2.8. Anatomic Pathology 52
6.2.8.1. Macroscopic Examinations 52
6.2.8.2. Organ Weights 53
6.2.8.3. Microscopic Examinations 53
6.3. Toxicity Phase Females 53
6.3.1. Clinical Observations And Survival 53
6.3.2. Body Weights 54
6.3.3. Food Consumption 54

VOLUME 1 Page

6.3.4. Functional Observational Battery (FOB) 55
6.3.5. Locomotor Activity 55
6.3.6. Clinical Pathology 56
6.3.6.1. Hematology And Coagulation 56
6.3.6.2. Serum Chemistry 56
6.3.7. Anatomic Pathology 57
6.3.7.1. Macroscopic Examinations 57
6.3.7.2. Organ Weights 57
6.3.7.3. Microscopic Examinations 58
6.4. Reproductive Phase Females (F_0) 59
6.4.1. Clinical Observations And Survival 59
6.4.2. Body Weights 59
6.4.2.1. Pre-Mating 59
6.4.2.2. Gestation 59
6.4.2.3. Lactation 60
6.4.3. Food Consumption 60
6.4.3.1. Pre-Mating 60
6.4.3.2. Gestation 60
6.4.3.3. Lactation 61
6.4.4. Reproductive Performance 61
6.4.5. Gestation Length And Parturition 62
6.4.5.1. Macroscopic Examinations 62
6.5. F_1 Litter Data 62
6.5.1. PND 0 Litter Data And Postnatal Survival 62
6.5.2. General Physical Condition 63
6.5.3. Offspring Body Weights 63
6.5.4. Necropsies Of Pups Found Dead 63
6.5.5. Scheduled Pup Necropsies (PND 4) And Pups Sent To Necropsy Due To Mechanical Trauma 64

VOLUME 1

Page

7. Discussion **65**
8. Conclusions **67**
9. Key Study Personnel And Report Submission **68**
10. Quality Assurance Unit Statement **70**
10.1. Phases Inspected 70
10.2. Approval 72
11. References **73**
12. Deviations From The Protocol **75**

INDEX OF FIGURES

VOLUME 1 (continued) Page

1. Summary Of Body Weights [G] (F_0 Males) 78
2. Summary Of Forelimb Grip Strength [G] (F_0 Males) 79
3. Summary Of Hindlimb Grip Strength [G] (F_0 Males) 80
4. Summary Of Motor Activity Total Counts (F_0 Males - Total Counts In Session) 81
5. Summary Of Ambulatory Motor Activity Counts (F_0 Males - Total Counts In Session) 82
6. Summary Of Motor Activity Total Counts (F_0 Males - Pretest) 83
7. Summary Of Ambulatory Motor Activity Counts (F_0 Males - Pretest) 84
8. Summary Of Motor Activity Total Counts (F_0 Males - Week 4) 85
9. Summary Of Ambulatory Motor Activity Counts (F_0 Males - Week 4) 86
10. Summary Of Body Weights [G] (F_0 Toxicity Phase Females) 87
11. Summary Of Forelimb Grip Strength [G] (F_0 Toxicity Phase Females) 88
12. Summary Of Hindlimb Grip Strength [G] (F_0 Toxicity Phase Females) 89
13. Summary Of Motor Activity Total Counts (F_0 Toxicity Phase Females - Total Counts In Session) 90
14. Summary Of Ambulatory Motor Activity Counts (F_0 Toxicity Phase Females - Total Counts in Session) 91
15. Summary Of Motor Activity Total Counts (F_0 Toxicity Phase Females - Pretest) 92
16. Summary Of Ambulatory Motor Activity Counts (F_0 Toxicity Phase Females - Pretest) 93
17. Summary Of Motor Activity Total Counts (F_0 Toxicity Phase Females - Week 4) 94

VOLUME 1 (continued) Page

18. Summary Of Ambulatory Motor Activity Counts (F_0 Toxicity Phase Females - Week 4) 95

19. Summary Of Body Weights [G] (F_0 Reproductive Phase Females) 96

20. Summary Of Body Weights During Gestation [G] (F_0 Reproductive Phase Females) 97

INDEX OF TABLES

Note: Some table titles have been shortened due to software spacing constraints.

VOLUME 1 (continued) Page

F_0 MALES

1. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (F_0 - Daily/Detailed Physicals) 99

2. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (F_0 - Midpoint Of Exposure) 100

3. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (F_0 - 1 Hour Post-Exposure) 101

4. Summary Of Body Weights [G] (F_0) 102

5. Summary Of Body Weight Changes [G] (F_0) 104

6. Summary Of Food Consumption [Grams/Animal/Day] (F_0) 106

7. Summary Of Functional Observational Battery Data (F_0 Home Cage Observations - Pretest Evaluation) 107

8. Summary Of Functional Observational Battery Data (F_0 Handling Observations - Pretest Evaluation) 108

9. Summary Of Functional Observational Battery Data (F_0 Open Field Observations - Pretest Evaluation) 111

10. Summary Of Functional Observational Battery Data (F_0 Sensory Observations - Pretest Evaluation) 113

11. Summary Of Functional Observational Battery Data (F_0 Neuromuscular Observations - Pretest Evaluation) 115

12. Summary Of Functional Observational Battery Data (F_0 Physiological Observations - Pretest Evaluation) 116

13. Summary Of Functional Observational Battery Data (F_0 Home Cage Observations - Week 4 Evaluation) 117

VOLUME 1 (continued) Page

14. Summary Of Functional Observational Battery Data (F_0 Handling Observations - Week 4 Evaluation) 118

15. Summary Of Functional Observational Battery Data (F_0 Open Field Observations - Week 4 Evaluation) 121

16. Summary Of Functional Observational Battery Data (F_0 Sensory Observations - Week 4 Evaluation) 123

17. Summary Of Functional Observational Battery Data (F_0 Neuromuscular Observations - Week 4 Evaluation) 125

18. Summary Of Functional Observational Battery Data (F_0 Physiological Observations - Week 4 Evaluation) 126

19. Summary Of Motor Activity Counts (F_0) 127

20. Summary Of Hematology Values (F_0 - Day 34 Evaluation) 135

21. Summary Of Serum Chemistry Values (F_0 - Day 34 Evaluation) 141

22. Summary Of Reproductive Performance (F_0) 146

23. Summary Of Macroscopic Findings (F_0 - Scheduled Necropsy) 148

24. Summary Of Organ Weights And Organ Weights Relative To Body Weights (F_0 - Scheduled Necropsy) 149

25. Summary Of Microscopic Findings (F_0 - Scheduled Necropsy) 157

F_0 Toxicity Phase Females

26. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (F_0 - Toxicity Phase - Daily/Detailed Physicals) 165

27. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (F_0 - Toxicity Phase - Midpoint Of Exposure) 166

28. Summary Of Clinical Findings: Total Occurrence/No. Of Animals (F_0 - Toxicity Phase - 1 Hour Post-Exposure) 167

29. Summary Of Body Weights [G] (F_0 - Toxicity Phase) 168

30. Summary Of Body Weight Changes [G] (F_0 - Toxicity Phase) 170

VOLUME 1 (continued) Page

31. Summary Of Food Consumption [Grams/Animal/Day] (F_0 - Toxicity Phase)..... 172

32. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Home Cage Observations - Pretest Evaluation) 173

33. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Handling Observations - Pretest Evaluation) 174

34. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Open Field Observations - Pretest Evaluation) 177

35. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Sensory Observations - Pretest Evaluation)..... 179

36. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Neuromuscular Observations - Pretest Evaluation)..... 181

37. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Physiological Observations - Pretest Evaluation)..... 182

38. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Home Cage Observations - Week 4 Evaluation)..... 183

39. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Handling Observations - Week 4 Evaluation)..... 184

40. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Open Field Observations - Week 4 Evaluation)..... 187

41. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Sensory Observations - Week 4 Evaluation)..... 189

42. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Neuromuscular Observations - Week 4 Evaluation)..... 191

43. Summary Of Functional Observational Battery Data
(F_0 - Toxicity Phase Physiological Observations - Week 4 Evaluation)..... 192

44. Summary Of Motor Activity Counts (F_0 - Toxicity Phase)..... 193

45. Summary Of Hematology Values (F_0 - Toxicity Phase - Day 35 Evaluation).....201

VOLUME 1 (continued) Page

46. Summary Of Serum Chemistry Values
(F_0 - Toxicity Phase - Day 35 Evaluation) 207

47. Summary Of Macroscopic Findings
(F_0 - Toxicity Phase - Unscheduled Death) 212

48. Summary Of Macroscopic Findings
(F_0 - Toxicity Phase - Scheduled Necropsy) 213

49. Summary Of Organ Weights And Organ Weights Relative To Body Weights
(F_0 - Toxicity Phase - Scheduled Necropsy) 214

50. Summary Of Microscopic Findings
(F_0 - Toxicity Phase - Unscheduled Death) 220

51. Summary Of Microscopic Findings
(F_0 - Toxicity Phase - Scheduled Necropsy) 228

F_0 REPRODUCTIVE PHASE FEMALES

52. Summary Of Clinical Findings: Total Occurrence/No. Of Animals
(F_0 - Reproductive Phase - Daily/Detailed Physicals) 237

53. Summary Of Clinical Findings: Total Occurrence/No. Of Animals
(F_0 - Reproductive Phase - Midpoint Of Exposure) 239

54. Summary Of Clinical Findings: Total Occurrence/No. Of Animals
(F_0 - Reproductive Phase - 1 Hour Post-Exposure) 240

55. Summary Of Body Weights [G] (F_0 - Reproductive Phase) 241

56. Summary Of Body Weight Changes [G] (F_0 - Reproductive Phase) 242

57. Summary Of Body Weights During Gestation [G] (F_0 - Reproductive Phase) 243

58. Summary Of Body Weight Changes During Gestation [G]
(F_0 - Reproductive Phase) 245

59. Summary Of Body Weights During Lactation [G] (F_0 - Reproductive Phase) 247

60. Summary Of Body Weight Changes During Lactation [G]
(F_0 - Reproductive Phase) 248

VOLUME 1 (continued) Page

61. Summary Of Food Consumption [Grams/Animal/Day] (F_0 - Reproductive Phase) 249

62. Summary Of Food Consumption During Gestation [G/Animal/Day] (F_0 - Reproductive Phase) 250

63. Summary Of Food Consumption During Lactation [G/Animal/Day] (F_0 - Reproductive Phase) 252

64. Summary Of Reproductive Performance (F_0) 253

65. Summary Of Gestation Lengths [Days] (F_0 - Reproductive Phase) 255

66. Summary Of Macroscopic Findings (F_0 - Reproductive Phase - Failed To Deliver) 256

67. Summary Of Macroscopic Findings (F_0 - Reproductive Phase - Scheduled Necropsy) 257

68. Summary Of Corpora Lutea And Implantation Sites (F_0 - Reproductive Phase) 258

F_1 Litter Data

69. Summary Of PND 0 Litter Data (F_1) 259

70. Summary Of Postnatal Survival [% Per Litter] (F_1) 260

71. Summary Of Pup Clinical Observations (F_1) 261

72. Summary Of Offspring Weights [G] (F_1) 262

73. Summary Of Offspring Weight Changes [G] (F_1) 263

74. Summary Of Pup Necropsy Findings (F_1 - Found Dead Pups) 264

75. Summary Of Pup Necropsy Findings (F_1 - PND 4 Pups And Pups Sent To Necropsy Due To Mechanical Trauma) 265

INDEX OF APPENDICES

VOLUME 1 (continued) Page

A. Individual Animal Data 266

F_0 MALES

A1. Individual Clinical Observations [Positive Findings Only] (F_0 - Daily/Detailed Physicals) 267

A2. Individual Clinical Observations [Positive Findings Only] (F_0 - 1 Hour Post-Exposure) 280

A3. Individual Body Weights [G] (F_0) 281

A4. Individual Body Weight Changes [G] (F_0) 285

A5. Individual Food Consumption [Grams/Animal/Day] (F_0) 289

A6. Individual Functional Observational Battery Data (F_0 Home Cage Observations - Pretest Evaluation) 293

A7. Individual Functional Observational Battery Data (F_0 Handling Observations - Pretest Evaluation) 297

A8. Individual Functional Observational Battery Data (F_0 Open Field Observations - Pretest Evaluation) 309

A9. Individual Functional Observational Battery Data (F_0 Sensory Observations - Pretest Evaluation) 317

A10. Individual Functional Observational Battery Data (F_0 Neuromuscular Observations - Pretest Evaluation) 325

A11. Individual Functional Observational Battery Data (F_0 Physiological Observations - Pretest Evaluation) 333

A12. Individual Functional Observational Battery Data (F_0 Home Cage Observations - Week 4 Evaluation) 337

A13. Individual Functional Observational Battery Data (F_0 Handling Observations - Week 4 Evaluation) 341

VOLUME 1 (continued) Page

A14. Individual Functional Observational Battery Data (F_0 Open Field Observations - Week 4 Evaluation) 353

A15. Individual Functional Observational Battery Data (F_0 Sensory Observations - Week 4 Evaluation) 361

A16. Individual Functional Observational Battery Data (F_0 Neuromuscular Observations - Week 4 Evaluation) 369

A17. Individual Functional Observational Battery Data (F_0 Physiological Observations - Week 4 Evaluation) 377

A18. Individual Motor Activity Counts (F_0) 381

VOLUME 2

A19. Individual Hematology Values (F_0 - Day 34 Evaluation) 391

A20. Individual Serum Chemistry Values (F_0 - Day 34 Evaluation) 403

A21. Individual Macroscopic And Microscopic Findings (F_0 - Scheduled Necropsy) 411

A22. Individual Organ Weights And Final Body Weights [G] (F_0 - Scheduled Necropsy) 458

A23. Individual Organ Weights Relative To Final Body Weights [G/100 G] (F_0 - Scheduled Necropsy) 466

F_0 TOXICITY PHASE FEMALES

A24. Individual Clinical Observations [Positive Findings Only] (F_0 - Toxicity Phase - Daily/Detailed Physicals) 474

A25. Individual Clinical Observations [Positive Findings Only] (F_0 - Toxicity Phase - 1 Hour Post-Exposure) 480

A26. Individual Body Weights [G] (F_0 - Toxicity Phase) 481

A27. Individual Body Weight Changes [G] (F_0 - Toxicity Phase) 485

A28. Individual Food Consumption [Grams/Animal/Day] (F_0 - Toxicity Phase) 489

VOLUME 2 (continued) Page

A29. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Home Cage Observations - Pretest Evaluation) 493

A30. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Handling Observations - Pretest Evaluation).....497

A31. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Open Field Observations - Pretest Evaluation).....509

A32. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Sensory Observations - Pretest Evaluation).....517

A33. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Neuromuscular Observations - Pretest Evaluation).....525

A34. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Physiological Observations - Pretest Evaluation).....533

A35. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Home Cage Observations - Week 4 Evaluation).....537

A36. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Handling Observations - Week 4 Evaluation).....541

A37. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Open Field Observations - Week 4 Evaluation).....553

A38. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Sensory Observations - Week 4 Evaluation).....561

A39. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Neuromuscular Observations - Week 4 Evaluation).....569

A40. Individual Functional Observational Battery Data
(F_0 - Toxicity Phase Physiological Observations - Week 4 Evaluation).....577

A41. Individual Motor Activity Counts (F_0 - Toxicity Phase) 581

A42. Individual Hematology Values
(F_0 - Toxicity Phase - Day 35 Evaluation) 589

VOLUME 2 (continued) Page

A43. Individual Serum Chemistry Values
(F_0 - Toxicity Phase - Day 35 Evaluation) 601

A44. Individual Macroscopic And Microscopic Findings
(F_0 - Toxicity Phase - Unscheduled Death) 609

A45. Individual Macroscopic And Microscopic Findings
(F_0 - Toxicity Phase - Scheduled Necropsy) 611

A46. Individual Organ Weights And Final Body Weights [G]
(F_0 - Toxicity Phase - Scheduled Necropsy) 653

A47. Individual Organ Weights Relative To Final Body Weights [G/100 G]
(F_0 - Toxicity Phase - Scheduled Necropsy) 657

F_0 REPRODUCTIVE PHASE FEMALES

A48. Individual Clinical Observations [Positive Findings Only]
(F_0 - Reproductive Phase - Daily/Detailed Physicals) 661

A49. Individual Clinical Observations [Positive Findings Only]
(F_0 - Reproductive Phase - 1 Hour Post-Exposure) 673

A50. Individual Body Weights [G] (F_0 - Reproductive Phase) 674

A51. Individual Body Weight Changes [G] (F_0 - Reproductive Phase) 678

A52. Individual Body Weights During Gestation [G]
(F_0 - Reproductive Phase) 682

A53. Individual Body Weight Changes During Gestation [G]
(F_0 - Reproductive Phase) 686

A54. Individual Body Weights During Lactation [G]
(F_0 - Reproductive Phase) 690

A55. Individual Body Weight Changes During Lactation [G]
(F_0 - Reproductive Phase) 694

A56. Individual Food Consumption [Grams/Animal/Day]
(F_0 - Reproductive Phase) 698

A57. Individual Food Consumption During Gestation [G/Animal/Day]
(F_0 - Reproductive Phase) 702

VOLUME 2 (continued) Page

A58. Individual Food Consumption During Lactation [G/Animal/Day] (F_0 - Reproductive Phase) 706

A59. Individual Reproductive Performance (F_0 - Reproductive Phase) 710

A60. Individual Gestation Lengths [Days] (F_0 - Reproductive Phase) 714

A61. Individual Macroscopic Findings (F_0 - Reproductive Phase - Failed To Deliver) 715

A62. Individual Macroscopic Findings (F_0 - Reproductive Phase - Scheduled Necropsy) 718

A63. Individual Corpora Lutea And Implantation Sites (F_0 - Reproductive Phase) 755

VOLUME 3

F_1 LITTER DATA

A64. Individual Litter Viability (F_1) 761

A65. Individual Pup Viability (F_1) 766

A66. Individual Pup Clinical Observations (F_1) 772

A67. Individual Offspring Weights [G] (F_1) 795

A68. Individual Offspring Weight Changes [G] (F_1) 824

A69. Individual Pup Necropsy Findings (F_1 - Found Dead Pups) 851

A70. Individual Pup Necropsy Findings (F_1 - PND 4 Pups And Pups Sent To Necropsy Due To Mechanical Trauma) 855

B. Certificate Of Analysis (Sponsor-Supplied Data) 875

C. Exposure Atmosphere Generation Report (WIL Research Laboratories, LLC) 877

D. Animal Room Environmental Conditions 954

E. Scoring Criteria For The Functional Observational Battery 961

VOLUME 4

Page

F. Summary Of Validation Studies (WIL-99263, WIL-99310, WIL-99435 And WIL-99441) 978

G. Clinical Pathology Methods, Procedures And References 1066

H. Pathology Report (WIL Research Laboratories, LLC) 1071

I. Statistical Analysis Report (BioSTAT Consultants, Inc.) 1089

J. WIL Subchronic FOB Historical Control Data Version 1.1 [Crl:CD(SD) Rats] 1111

K. WIL Motor Activity Historical Control Data Version 1.1 [Crl:CD(SD) Rats] 1178

L. WIL Reproductive Historical Control Data Version 2.5 [Crl:CD(SD) Rats] 1199

M. Study Protocol 1208

1. SUMMARY

1.1. OBJECTIVE

This study was designed to investigate the potential toxic effects of trimethylsilanol when administered via whole-body inhalation to rats for a minimum of 28 days and to evaluate the potential of the test article to affect male and female reproductive performance such as gonadal function, mating behavior, conception, parturition and early postnatal development.

1.2. STUDY DESIGN

Three groups of Crl:CD(SD) rats, each group consisting of 10 males, 10 toxicity phase females and 10 reproductive phase females, were exposed via whole-body inhalation to vapor atmospheres of the test article, trimethylsilanol, 6 hours/day, 7 days/week. Target exposure concentrations were 60, 300 and 600 parts per million (ppm). A concurrent control group was exposed to filtered air on a comparable regimen. Mean measured test article exposure concentrations were 0, 61, 303 and 602 ppm (males), 0, 61, 304 and 604 ppm (toxicity phase females) and 0, 61, 304 and 603 ppm (reproductive phase females) for the control, 60, 300 and 600 ppm groups, respectively. Exposures were initiated when the animals were approximately 11-12 weeks of age. F_0 males and reproductive phase females were exposed daily for 14 days prior to mating, throughout the mating period and continuing through the day prior to euthanasia (males) or gestation day 20 (reproductive phase females), for a total of 34 and 35-48 consecutive days, respectively. F_0 reproductive phase females with no evidence of mating or that failed to deliver were exposed through post-mating or post-cohabitation day 24 (the day prior to euthanasia) for a total of 52 consecutive days. F_0 toxicity phase females were exposed for 28 consecutive days.

All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. Functional observational battery (FOB) and locomotor activity data were recorded for all males and toxicity phase females prior to initiation of exposure for each sex (baseline

evaluation) and near the end of exposure (study week 4). F_0 males were euthanized following completion of the mating period and F_0 toxicity phase females were euthanized following 28 days of exposure. Clinical pathology evaluations (hematology and serum chemistry) were performed on all F_0 males and toxicity phase females at necropsy. Complete necropsies were conducted on all F_0 males and toxicity phase females, and selected organs were weighed; selected tissues in the control and high-exposure groups were examined microscopically.

All F_0 reproductive phase females were allowed to deliver and rear their pups until lactation day 4. F_1 clinical observations and body weights were recorded on postnatal days (PND) 1 and 4. Reproductive phase dams and pups were euthanized and examined on lactation day/PND 4.

1.3. RESULTS

One F_0 toxicity phase female in the 300 ppm group was found dead on study day 35, the day of scheduled necropsy. While the cause of death for this female was not determined, there were no mortalities in the 600 ppm group. Therefore, this mortality at 300 ppm was not attributed to test article exposure. All other toxicity phase females and all F_0 males and reproductive phase females survived to the scheduled necropsies. There were no test article-related macroscopic findings or effects on organ weights.

There were no remarkable clinical observations noted at any exposure level. Mean body weights, body weight gains and food consumption for males and females at all exposure levels were similar to the control group for all phases. There were no test article-related effects on FOB parameters, including home cage, handling, open field, sensory, neuromuscular or physiological observations and no effects on locomotor activity patterns for males and toxicity phase females. No remarkable shifts in the pattern of habituation occurred during locomotor activity assessments in any of the test article-exposed groups when the F_0 males and toxicity phase females were evaluated prior to the initiation of exposure or during the study week 4 evaluation.

Decreased mean eosinophil and lymphocyte counts were noted for the 600 ppm group F_0 males. In addition, increased mean alanine aminotransferase values were noted for F_0 males and toxicity phase females in the 600 ppm group when compared with the control group. Although these changes were considered test article-related, they were not considered adverse in the absence of correlating histopathology.

Mean mating, fertility and copulation/conception indices for all exposure concentrations were similar to the control group. Mean gestation lengths, postnatal survival and F_1 body weights were similar to the control group. There were no test article-related macroscopic findings observed for pups.

1.4. CONCLUSION

Test article-related effects were limited to changes in hematology (lower eosinophil and lymphocyte counts for males) and serum chemistry (higher alanine aminotransferase for males and toxicity phase females) at 600 ppm. These changes occurred in the absence of correlating histologic changes and were not considered adverse. Therefore, under the conditions of this screening study, an exposure level of 600 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for reproductive, systemic and neonatal toxicity of trimethylsilanol when administered via whole-body inhalation exposure to Crl:CD(SD) rats.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This report presents the data from "A Whole-Body Inhalation Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test of Trimethylsilanol in Rats." Due to software spacing constraints, the study title is presented as "An Inhalation Repro/Dev Tox Study of Trimethylsilanol in Rats" on the report tables. For clarification of terminology used to differentiate the generations in the report text, the offspring of the F_0 generation were defined as the F_1 litters.

The following computer protocols were used for data collection during the study:

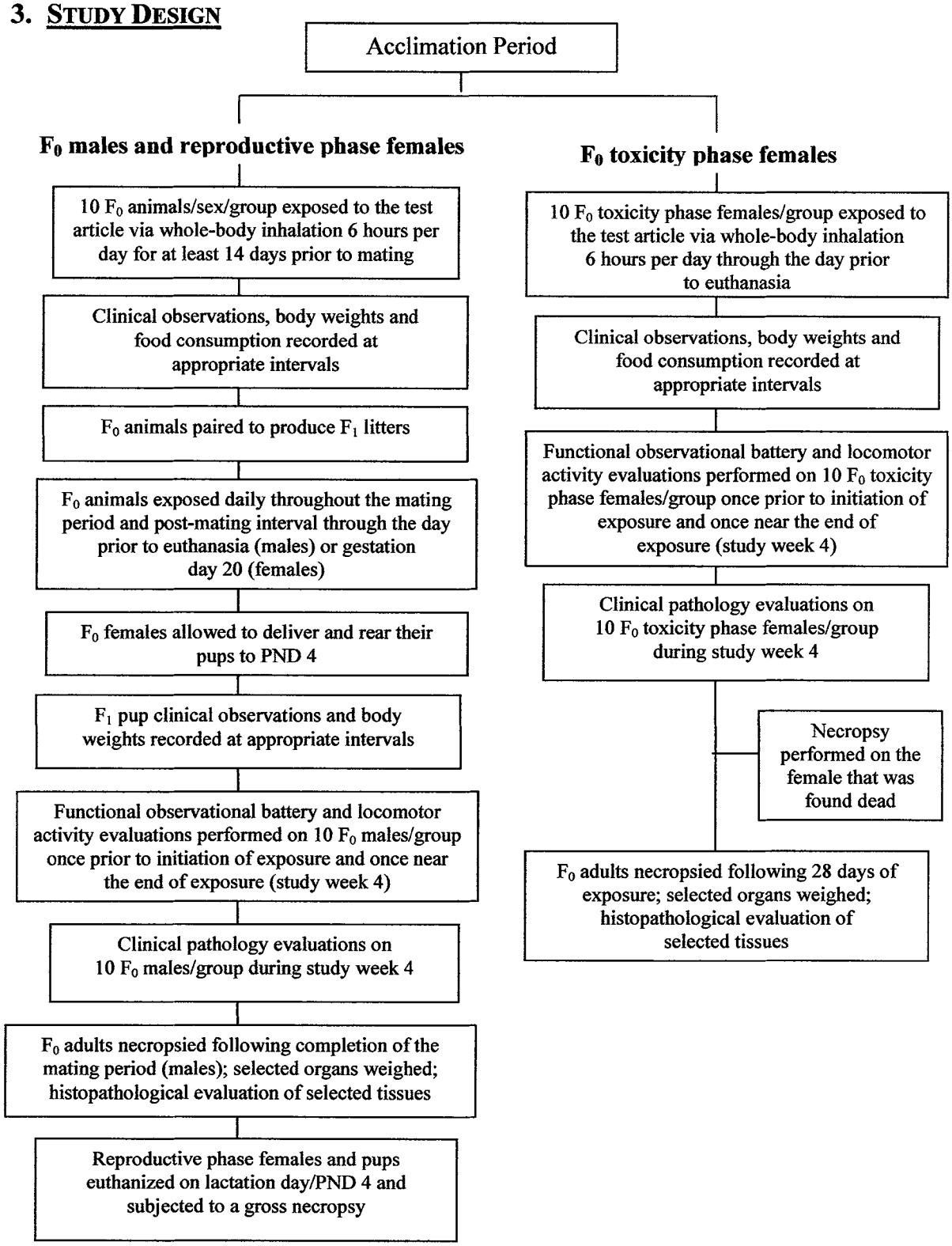
Computer Protocol	Type of Data Collected
WIL-401009M	Male main study data
WIL-401009F	Female toxicity phase data
WIL-401009R	Female reproductive phase data
WIL-401009I	Inhalation data

2.2. KEY STUDY DATES[1]

Date(s)	Event(s)
18 March 2008	Experimental starting date (animal receipt)
3 April 2008	Experimental start date (initiation of test article exposure)
3 April 2008 - 6 May 2008	Test article exposure (males)
3 April 2008 - 24 May 2008	Test article exposure (reproductive phase females)
10 April 2008 - 7 May 2008	Test article exposure (toxicity phase females)
16-30 April 2008	Mating period
7 May 2008	Male necropsy
8 May 2008	Toxicity phase necropsy
13-26 May 2008	Lactation day 4 necropsies
31 May 2008	Experimental termination/completion date (last microscopic examination)

[1] Date ranges represent the first and last dates of evaluation.

3. STUDY DESIGN



4. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

4.1. TEST ARTICLE IDENTIFICATION

The test article, trimethylsilanol, was received from Shin-Etsu Silicones of America, Inc., Akron, OH, on 20 February 2008, as follows:

Identification	Quantity Received	Physical Description
31-OH-RM Lot no. 801004 Exp. date: 24 July 2008 [WIL log no. 7910A]	14 buckets Total gross weight: 228.0 g	Clear, colorless liquid

Documentation regarding the purity and stability of the test article is on file with the sponsor and WIL Research Laboratories, LLC. A Certificate of Analysis for the test article was provided by the sponsor and is presented in Appendix B. The purity of the test article was 99.75%. The test article was stored at room temperature and was considered stable under this condition. A reserve sample of the test article (3.5 g) was collected on 19 March 2008, and stored in the Archives of WIL Research Laboratories, LLC.

4.2. EXPOSURE METHODS

Exposures were conducted in four 1.0 m^3 (1000-L) stainless steel and glass whole-body exposure chambers. One chamber was dedicated for each group for the duration of the study. The chambers were operated under dynamic conditions at a slight negative pressure with at least 12 air changes per hour. The exposure period was approximately 6 hours per day. Males and reproductive phase females were exposed daily for at least 14 days prior to mating and continuing throughout mating for a minimum of 34 days (males) or through gestation day 20 (reproductive phase females). Toxicity phase females were exposed daily for a minimum of 28 days.

All animals were housed in a normal animal colony room during non-exposure hours. For each day of exposure, the animals were transferred from their home cages to the

exposure chamber caging, transported to the exposure chamber room, exposed for the requisite duration and then returned to their home cages. In order to minimize any potential variation occurring due to positioning within the chamber, the cages were sequentially 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, LLC. Food and water were withheld during the exposure periods. Animal exposures were conducted at approximately the same time each day.

The following table presents the study group assignment:

Group Number	Test Article	Target Exposure Concentration (ppm)	Number of Animals: Males	Toxicity Phase Females	Reproductive Phase Females
1	Filtered air	0	10	10	10
2	Trimethylsilanol	60	10	10	10
3	Trimethylsilanol	300	10	10	10
4	Trimethylsilanol	600	10	10	10

Exposure levels were selected based on results of a previous 2-week whole-body inhalation toxicity study of trimethylsilanol in rats (Weinberg, 2007; WIL-401008); target exposures were 60, 300 and 600 ppm. In that study, exposure-related effects were limited to the 600 ppm group and consisted of CNS/behavior-related clinical signs of hypoactivity (males) and impaired equilibrium (females), and macroscopic findings of diffusely pale lungs. Based on these data, exposure levels of 60, 300 and 600 ppm were selected for the current study.

The selected route of administration for this study was whole-body inhalation exposure because this is a reliable and accurate method of evaluating toxicity and is a possible route of human exposure. The number of animals selected for this study was the minimum required to yield statistically and scientifically meaningful data and was consistent with regulatory agency expectations.

4.3. TEST ATMOSPHERE GENERATION METHODS

Trimethylsilanol was generated for exposure as a vapor using a bubbler-type vaporization system located within a heated containment box. Nitrogen flowed into the inlet stem of a gas washing bottle containing an appropriate quantity of test article and bubbled through a fritted disc at the bottom of the bottle in order to create vapors of the test article. The concentrated vapors were piped to the heated chamber inlet where the concentration was reduced by mixing with chamber ventilation air. The control group was exposed to clean, filtered air. A detailed description of the generation methods is presented in Appendix C.

The exposure period was adjusted for calculated t_{99} equilibrium time (approximately 20 minutes) for this study. The animals were placed into the exposure chambers and the generation of the test article vapor was started. The initiation for exposure was the time following one T_{99} interval. The exposure period was terminated after 6 hours when the test article vapor generation was stopped.

Chamber temperature, relative humidity, chamber ventilation rate, and negative pressure within the chambers were monitored continuously and were recorded approximately every 35 minutes. The daily mean chamber temperature ranged from 19°C to 26°C. The daily mean chamber humidity ranged from 38% to 68%. Oxygen content within the chambers was measured during prestudy method development and daily during the exposure phase; mean oxygen content was 20.9% throughout the study. Chamber environmental conditions are summarized in Appendix C.

4.4. METHODS FOR CHARACTERIZATION OF EXPOSURE ATMOSPHERES

4.4.1. NOMINAL CONCENTRATIONS

Nominal exposure concentrations were determined by weighing the gas washing bottle for each test article chamber prior to and after each day's exposure. The total volume of air that passed through each chamber was calculated from the daily average chamber ventilation flowrate in liters per minute and the exposure duration. The nominal concentration of each phase was calculated as follows.

$$\text{Concentration (ppm)} = \frac{WT \times MV \times 10^6}{GMW \times CF \times DUR}$$

Where:

WT	=	Weight of test article used, grams
MV	=	Molar volume at 730 mmHg and 21°C, 25.11 L/mole
10^6	=	ppm conversion factor
GMW	=	Test article molecular weight, 90.20 g/mole
CF	=	Daily average chamber flowrate for a given day, LPM
DUR	=	Duration of the daily exposure, minutes

4.4.2. ANALYZED EXPOSURE CONCENTRATIONS

Actual exposure concentrations within each chamber were measured at approximately 35-minute intervals during each daily exposure period by a validated gas chromatographic method. Exposure atmosphere samples were delivered to the gas chromatograph from the approximate middle of each chamber by a pump and multi-port sampling valve. At least 1 standard was analyzed each day prior to exposure to confirm gas chromatographic calibration. Overall mean measured test article exposure concentrations were 0, 61, 303 and 602 ppm (males), 0, 61, 304 and 604 ppm (toxicity phase females) and 0, 61, 304 and 603 ppm (reproductive phase females) for the filtered air, 60, 300 and 600 ppm groups, respectively.

4.4.3. TEMPORAL STABILITY AND HOMOGENEITY OF VAPOR TEST ATMOSPHERES

Homogeneity and temporal stability of the exposure concentrations were evaluated during the method development phase of the study. Four test locations and a reference location were used for these determinations. The test locations were top rear, top front, bottom rear and bottom front of each exposure chamber. Samples were collected and analyzed on the GC as rapidly as possible by alternating from the reference location to a test location. The measured concentration was calculated as a percent difference for each

position from the reference location. Homogeneity was performed in triplicate for each test article exposure chamber. Details of the homogeneity and temporal stability analyses are presented in Appendix C, and the results are summarized in Section 6.1.

4.5. TEST ARTICLE CHARACTERIZATION

Test article characterization, including purity and stability analyses, was conducted by the sponsor and therefore, no purity or stability analyses were conducted during the testing portion of this study.

4.6. TEST SYSTEM

Sexually mature male and virgin female Crl:CD(SD) rats from Charles River Laboratories, Inc., Raleigh, NC, were used as the test system on this study.

The animal model, the Crl:CD(SD) rat, is recognized as appropriate for general toxicity and reproductive toxicity studies. WIL Research Laboratories, LLC has reproductive historical control data in the Crl:CD(SD) rat. This animal model has been proven to be susceptible to the effects of reproductive toxicants.

4.7. ANIMAL RECEIPT AND ACCLIMATION

Fifty male and 100 female Crl:CD(SD) rats were received in good health from Charles River Laboratories, Inc., Raleigh, NC on 18 March 2008. The animals were approximately 61 days old upon receipt. Each animal was examined by a qualified technician on the day of receipt and weighed the following day. Each rat was uniquely identified by a Monel® metal ear tag displaying the animal number. The males and reproductive phase females were housed for an acclimation period of 16 days prior to the first day of treatment. The toxicity phase females were housed for an acclimation period of 23 days prior to the first day of treatment. During the acclimation period, the animals were observed twice daily for mortality and general changes in appearance and behavior.

4.8. ANIMAL HOUSING (NON-EXPOSURE PERIODS)

Following receipt and until pairing, all F_0 animals were housed individually in clean, stainless steel wire-mesh cages suspended above cage-board. The cage-board was

changed at least 3 times per week. The males and reproductive phase females were paired for mating in the home cage of the male. Following positive evidence of mating, the males were housed in suspended wire-mesh cages until the scheduled necropsy, and the females were transferred to plastic maternity cages with nesting material, ground corncob bedding (Bed-O'Cobs®; The Andersons, Cob Products Division, Maumee, OH). The nesting material is periodically analyzed by the manufacturer for contaminants. No contaminants were present in the bedding at concentrations sufficient to interfere with the outcome of the study. The results of these analyses are maintained at WIL Research Laboratories, LLC. The dams and their litters were housed in these cages until euthanasia on lactation day 4. Females with no evidence of mating or that failed to deliver were housed in plastic maternity cages until post-cohabitation or post-mating day 25. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The animal facilities at WIL Research Laboratories, LLC are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

4.9. DIET, DRINKING WATER AND MAINTENANCE

The basal diet used in this study, PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research Laboratories, LLC. Feed lots used during the study are documented in the study records. Feeders were changed and sanitized once per week. Municipal water supplying the facility is sampled for contaminants according to standard operating procedures. The results of the diet and water analyses are maintained at WIL Research Laboratories, LLC. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study. Reverse osmosis-purified (on-site) drinking water, delivered by an automatic watering system, and the basal diet were provided ad libitum throughout the acclimation period and during the study, except during exposure periods and FOB and locomotor activity assessments. In addition, food was not available during the overnight fasting period prior to blood collection for clinical pathology assessments (toxicology phase).

4.10. ENVIRONMENTAL CONDITIONS (NON-EXPOSURE PERIODS)

All rats were housed throughout the acclimation period and during the study in an environmentally controlled room. The room environmental controls were set to maintain daily averages of 71°F ± 5°F (22°C ± 3°C) temperature and 50% ± 20% relative humidity. Room temperature and relative humidity were controlled and monitored using the Metasys® DDC Electronic Environmental control system. These data were recorded approximately hourly and are summarized in Appendix D. Actual mean daily temperature ranged from 68.9°F to 71.1°F (20.5°C to 21.7°C) and mean daily relative humidity ranged from 37.2% to 45.9% during the study. Light timers were calibrated to provide a 12-hour light (0600 hours to 1800 hours)/12-hour dark photoperiod. Air handling units were set to provide a minimum of 10 fresh air changes per hour.

4.11. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

At the conclusion of the acclimation period, all available males and females were weighed and examined in detail for physical abnormalities. At the discretion of the study director, each animal judged to be in good health and meeting acceptable body weight requirements (300 g to 500 g for males and 200 g to 300 g for females) was selected for use in the computerized randomization procedure. At that time, the individual body weights and corresponding animal identification numbers were entered into the WIL Toxicology Data Management System (WTDMS™). A printout containing the animal numbers, corresponding body weights and individual group assignments was generated using a computer randomization procedure. The animals then were arranged into groups according to the printout. Individual body weights at randomization were within ± 20% of the mean for each sex. These animals were then randomized into 2 study replicates to allow for the reasonable conduct of the functional observational battery and locomotor activity assessments. Each dose group and sex was approximately equally represented within each study replicate. Once randomized into groups, the control animals were housed in cages on a separate cage rack from the test article-exposed animals.

The experimental design for the WIL-401009 consisted of 3 test article-exposed groups and 1 control group, each composed of 10 males, 10 toxicity phase females and 10 reproductive phase females. Test article exposure was initiated on study day 0 for the males and the reproductive phase females. These animals were approximately 11 weeks old; body weights ranged from 341 g to 418 g (males) and from 218 g to 280 g (reproductive phase females). Test article exposure was initiated on study day 7 for the toxicity phase females; body weights ranged from 231 g to 282 g. The males and reproductive phase females were approximately 13 weeks old when paired on study day 14; reproductive phase female body weights ranged from 239 g to 314 g on gestation day 0.

5. PARAMETERS EVALUATED

5.1. OBSERVATIONS (ALL PHASES)

5.1.1. CLINICAL OBSERVATIONS AND SURVIVAL

All rats were observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality. Individual clinical observations were recorded daily. Once prior to the initiation of exposure, on the first day of exposure and on a weekly basis throughout the study, all rats were observed outside the home cage in a standard arena and evaluated for changes in gait, posture and response to handling, as well as the presence of clonic or tonic movements, stereotypies or bizarre behavior. Observations were conducted prior to test article exposure during the treatment period and were not performed on the day of Functional Observational Battery (FOB) assessments. Animals visible in the exposure chamber were also observed for signs of toxicity at the mid-point of exposure (3 hours following beginning of exposure) and any positive findings were recorded. All animals were observed for signs of toxicity approximately 1 hour following the exposure period. The absence or presence of findings was recorded for individual animals; however, for reporting purposes, only positive findings are presented on the individual tables.

5.1.2. BODY WEIGHTS

Individual male and toxicity phase female body weights were recorded weekly, beginning 1 week prior to test article exposure, on the first day of exposure and weekly thereafter until the scheduled euthanasia. Body weights were also recorded for the toxicity phase animals assigned to FOB and locomotor activity assessments on the days of these evaluations; however, these data are maintained in the study records and are not presented in this report. Individual reproductive phase female body weights were recorded weekly, beginning 1 week prior to test article exposure, on the first day of exposure and on a weekly basis thereafter until evidence of copulation was observed. Mean weekly body weights and body weight changes are presented for each interval. In addition, cumulative mean body weight changes are presented for the pre-mating period

(males and reproductive phase females) and for the entire generation (males and toxicity phase females). Once evidence of mating was observed, reproductive phase female body weights were recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Mean gestation body weights and corresponding mean body weight changes are presented for these intervals and for the overall gestation interval (days 0-20); mean lactation weight changes are presented for lactation days 1-4. Weekly body weights for females for which there was no evidence of mating are presented in the individual report tables until necropsy. When body weights could not be determined for an animal during a given interval (due to an unscheduled death, as females enter gestation/lactation, weighing error, etc.), group mean values were calculated for that interval using the available data. The time periods when body weight values were unavailable for a given animal were designated as "NA" (Not Applicable) on the individual report tables.

5.1.3. FOOD CONSUMPTION

Individual food consumption was recorded on the corresponding weekly body weight days until pairing. Food intake was not recorded during the mating period. Once evidence of mating was observed, reproductive phase female food consumption was recorded on gestation days 0, 4, 7, 11, 14, 17 and 20 and on lactation days 1 and 4. Food consumption for reproductive phase females with no evidence of mating and for all males was measured on a weekly basis until the scheduled euthanasia. Food consumption was reported as g/animal/day and g/kg/day for the corresponding body weight change intervals. Calculation of the comprehensive intervals excludes all erroneous values such as total food spillage.

When food consumption could not be determined for an animal during a given interval (due to a weighing error, food spillage, obvious erroneous value, etc.), group mean values were calculated for that interval using the available data. The time periods when food consumption values were unavailable for a given animal were designated as "NA" (Not Applicable) on the individual report tables.

5.2. F_0 MALE AND TOXICITY PHASE FEMALE PARAMETERS

5.2.1. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Functional observational battery (FOB) observations were recorded for all F_0 males and toxicity phase females during the week prior to initiation of test article exposure (baseline evaluation) and prior to exposure during study week 4 (the last week of the study). The FOB used at WIL Research Laboratories, LLC, is based on previously developed protocols (Moser, 1991; Moser et al., 1991; Irwin, 1968; Gad, 1982; Moser et al., 1988; Haggerty, 1989; O'Donoghue, 1989). FOB Testing was performed by the same technicians, whenever possible, without knowledge of the animal's group assignment. The FOB was performed in a sound-attenuated room equipped with a white noise generator set to operate at 70 ± 10 dB. All animals were observed for the following parameters as described below (refer to Appendix E for a detailed description of the scoring criteria used for each observation and to Appendix F for positive control/validation data):

5.2.1.1. HOME CAGE OBSERVATIONS

Posture
Convulsions/Tremors
Feces consistency
Biting
Palpebral (eyelid) closure

5.2.1.2. HANDLING OBSERVATIONS

Ease of removal from cage
Lacrimation/Chromodacryorrhea
Piloerection
Palpebral closure
Eye prominence
Red/Crusty deposits
Ease of handling animal in hand
Salivation
Fur appearance
Respiratory rate/character
Mucous membranes/Eye/Skin color
Muscle tone

5.2.1.3. OPEN FIELD OBSERVATIONS (EVALUATED OVER A 2-MINUTE OBSERVATION PERIOD)

Mobility
Rearing
Convulsions/Tremors
Grooming
Bizarre/Stereotypic behavior
Time to first step (seconds)
Gait
Arousal
Urination/Defecation
Gait score
Backing

5.2.1.4. SENSORY OBSERVATIONS

Approach response
Startle response
Pupil response
Forelimb extension
Air righting reflex
Touch response
Tail pinch response
Eyeblink response
Hindlimb extension
Olfactory orientation

5.2.1.5. NEUROMUSCULAR OBSERVATIONS

Hindlimb extensor strength
Hindlimb foot splay
Grip strength-hind and forelimb
Rotarod performance

5.2.1.6. PHYSIOLOGICAL OBSERVATIONS

Catalepsy
Body temperature
Body weight

5.2.2. LOCOMOTOR ACTIVITY

Locomotor activity was assessed for all F_0 males and toxicity phase females during the week prior to initiation of exposure (baseline evaluation) and prior to exposure during study week 4. Locomotor activity, recorded after the completion of the FOB, was measured automatically using the Kinder Scientific Motor Monitor System (Kinder Scientific Company, LLC, Poway, California). This personal computer-controlled system utilizes a series of infrared photobeams surrounding a clear plastic, rectangular cage to quantify each animal's motor activity. Four-sided black plastic enclosures were used to surround the clear plastic boxes to decrease the potential for distraction from extraneous environmental stimuli or stimuli from technicians or adjacent animals. The black enclosures rested on top of the photobeam frame and did not interfere with the path of the beams. The testing of treatment groups was done according to replicate sequence. Each animal was tested separately. Data were collected in 5-minute epochs and the test session duration was 60 minutes. These data were compiled as six, 10-minute subintervals for tabulation.

Data for ambulatory and total motor activity were tabulated. Total motor activity was defined as a combination of fine motor skills (i.e., grooming, interruption of

1 photobeam) and ambulatory motor activity (interruption of 2 or more consecutive photobeams). Refer to Appendix F for motor activity validation and positive control data.

5.2.3. CLINICAL PATHOLOGY

Blood samples for clinical pathology evaluations (hematology, serum chemistry and coagulation parameters) were collected from all F_0 males and toxicity phase females at the scheduled necropsies (study day 34 for males and study day 35 for females). The animals were fasted overnight prior to blood collection. Blood for serum chemistry and hematology was collected from the retro-orbital sinus following isoflurane anesthesia. Blood for coagulation parameters was collected from the vena cava at the time of necropsy. Blood was collected into tubes containing potassium (K_3) EDTA (hematology), sodium citrate (clotting determinations) or no anticoagulant (serum chemistry). Clinical pathology methods, procedures and references are presented in Appendix G. The interpretation of the clinical pathology data was the responsibility of the principal investigator for clinical pathology, Ellen L. Ziemer, DVM, PhD, DACVIM, DACVP, WIL Research Laboratories, LLC (Appendix H). The following parameters were evaluated:

5.2.3.1. HEMATOLOGY AND COAGULATION PARAMETERS

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Prothrombin time (ProTime)
Activated partial thromboplastin time (APTT)

Reticulocyte count
  Percent (Reticulocyte)
  Absolute (Retic Absolute)
Differential leukocyte count - Percent and absolute
  -Neutrophil
  -Lymphocyte
  -Monocyte
  -Eosinophil
  -Basophil
  -Large unstained cell

( ) - Designates tabular abbreviation

5.2.3.2. SERUM CHEMISTRY

Albumin
Total protein
Globulin [by calculation]
Albumin/globulin ratio (A/G Ratio) [by calculation]
Total bilirubin (Total Bili)
Urea nitrogen
Creatinine
Alkaline phosphatase (AlkalinePhos'tse)
Alanine aminotransferase (Alanine Transfer)
Aspartate aminotransferase (AspartatTransfer)
Gamma glutamyltransferase (GlutamylTransfer)
Glucose
Total cholesterol (Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium

( ) - Designates tabular abbreviation

5.2.4. MACROSCOPIC EXAMINATIONS

5.2.4.1. UNSCHEDULED DEATHS

A gross necropsy was performed on the toxicity phase female that was found dead during the study. In addition, tissues were collected and preserved as noted in Section 5.2.4.2.

5.2.4.2. SCHEDULED EUTHANASIA

All surviving F_0 males and toxicity phase females were euthanized by isoflurane inhalation followed by exsanguination. Males were euthanized following completion of the mating period and 34 days of exposure; toxicity phase females were euthanized following 28 days of exposure. Necropsy included examination of the external surface, all orifices, the cranial cavity, the external surface of the brain, and the thoracic, abdominal and pelvic cavities, including viscera. At the time of necropsy, the following tissues and organs were placed in 10% neutral-buffered formalin (except as noted):

Adrenal glands (2)
Aorta
Bone with marrow (femur and sternebrae)
Bone marrow smear[a]
Brain (forebrain, midbrain, hindbrain)[b]
Cervix
Coagulating glands
Eyes with optic nerve (2)[c]
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Heart
Kidneys (2)
Liver (sections of 2 lobes)
Lungs (including bronchi, fixed by inflation with fixative)
Lymph node (mesenteric and mandibular)
Mammary gland (females only)
Ovaries and oviducts (2)[d]
Pancreas
Peripheral nerve (sciatic)
Pituitary gland
Prostate gland
Salivary gland [mandibular (2)]
Seminal vesicles (2)
Skeletal muscle (rectus femoris)
Skin
Spinal cord (cervical, thoracic and lumbar)[e]
Spleen
Testes with epididymides[f] (2)
Thymus gland
Thyroids [with parathyroids, if present (2)]
Trachea
Urinary bladder
Uterus with vagina
All gross lesions[g]

[a] = Not collected from female that was found dead. Bone marrow smears were not placed in formalin; slides were examined only if scientifically warranted.
[b] = All levels of the brain are designated as "Brain" in the raw data.
[c] = Placed in Davidson's solution.
[d] = Oviducts and parathyroids were examined microscopically if in the plane of the section, and in all cases where gross lesions were present.
[e] = Cervical, thoracic and lumbar spinal cord are designated as "Spinal Cord" in the raw data.
[f] = Fixed in Bouin's solution.
[g] = Representative sections of corresponding organs from a sufficient number of control animals were retained for comparison, if possible.

5.2.4.3. ORGAN WEIGHTS

The following organs from all surviving F_0 males and toxicity phase females were weighed at the scheduled necropsies:

Adrenal glands	Ovaries with oviducts
Brain	Prostate gland
Epididymides[a]	Seminal vesicles
Heart	Spleen
Kidneys	Testes[a]
Liver	Thymus gland
Lungs	Thyroids with parathyroids[b]

[a] = These paired organs were weighed separately.
[b] = Fixed in 10% neutral-buffered formalin prior to weighing.

Except as noted, paired organs were weighed together. Absolute weights and organ to final body weight ratios were reported.

5.2.5. SLIDE PREPARATION AND MICROSCOPIC EXAMINATION

After fixation, protocol-specified tissues were trimmed according to standard operating procedures and the protocol. Trimmed tissues were processed into paraffin blocks, sectioned at 4 to 8 microns, mounted on glass microscope slides and stained with hematoxylin and eosin. Sections of the testes and epididymides were also stained with PAS.

Microscopic examination was performed on all tissues listed in Section 5.2.4.2. from all F_0 males and toxicity phase females in the control and 600 ppm groups at the scheduled necropsies, and from the toxicity phase female that was found dead. Special emphasis was placed on stages of spermatogenesis in the male gonads and histopathology of interstitial cell structure. Missing tissues were identified as not found at necropsy, lost at necropsy, lost during processing, not in plane of section or other reasons as appropriate. Tissues may appear on the report tables as not examined due to the tissue not being in the plane of section, not present at trimming, etc. Microscopic examination was performed by Catherine Picut, VMD, DACVP, DABT (Appendix H).

5.3. REPRODUCTIVE PHASE FEMALE PARAMETERS (F_0)

5.3.1. BREEDING PROCEDURES

Following a minimum of 14 days of exposure, 1 female assigned to the reproductive toxicity phase was cohabited with 1 male rat of the same treatment group. A breeding record containing the male and female identification numbers and the start date of cohabitation was prepared. Each female was housed in the home cage of the male. Positive evidence of mating was confirmed by the presence of a vaginal copulatory plug or the presence of sperm following a vaginal lavage. Each mating pair was examined daily. The day when evidence of mating was identified was termed gestation day 0. If evidence of copulation was not detected after 14 days of pairing, any females that had not shown evidence of mating were placed in plastic maternity cages.

For the purpose of calculating pre-coital intervals, rats paired over a 12-hour dark cycle were considered to have been paired for 1 day.

Mating, fertility and copulation/conception indices were calculated as follows:

$$\text{Male (Female) Mating Index (\%)} = \frac{\text{No. of Males (Females) with Evidence of Mating (or Confirmed Pregnancy)}}{\text{Total No. of Males (Females) Used for Mating}} \times 100$$

$$\text{Male Fertility Index (\%)} = \frac{\text{No. of Males Siring a Litter}}{\text{Total No. of Males Used for Mating}} \times 100$$

$$\text{Male Copulation Index (\%)} = \frac{\text{No. of Males Siring a Litter}}{\text{No. of Males with Evidence of Mating (or Females Confirmed Pregnant)}} \times 100$$

$$\text{Female Fertility Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{Total No. of Females Used for Mating}} \times 100$$

$$\text{Female Conception Index (\%)} = \frac{\text{No. of Females with Confirmed Pregnancy}}{\text{No. of Females with Evidence of Mating (or Confirmed Pregnancy)}} \times 100$$

5.3.2. PARTURITION

All females were allowed to deliver naturally and rear their young to PND 4. During the period of expected parturition, the females were observed twice daily for initiation and completion of parturition and for signs of dystocia. On the day parturition was initiated (PND 0), pups were sexed and examined for gross malformations, and the numbers of stillborn and live pups were recorded. Individual gestation length was calculated using the date delivery started.

5.3.3. SCHEDULED EUTHANASIA

Females that delivered were euthanized (by carbon dioxide inhalation) on lactation day 4; the number of former implantation sites and corpora lutea were recorded. Females that failed to deliver were euthanized on post-mating day 25 (females with evidence of mating) or post-cohabitation day 25 (females with no evidence of mating). Uteri with no macroscopic evidence of implantation were opened and subsequently placed in 10% ammonium sulfide solution for detection of early implantation loss (Salewski, 1964).

5.3.4. F_1 LITTER PARAMETERS

5.3.4.1. LITTER VIABILITY AND DEATHS

Each litter was examined daily for survival, and all deaths were recorded. All pups were individually identified by application of tattoo markings on the digits following completion of parturition. A daily record of litter size was maintained. Standardization of litter size was not performed. Intact offspring that died were necropsied using a fresh dissection technique including the heart and major vessels and examination of the brain by a mid-coronal slice (Stuckhardt and Poppe, 1984). If a skeletal anomaly was suspected, the pups were eviscerated, cleared, stained with Alizarin Red S as described by Dawson (Dawson, 1926) and examined. Cannibalized pups were discarded without necropsy. Tissues were preserved in 10% neutral-buffered formalin for possible future histopathologic examination only as deemed necessary by the gross findings. The carcass of each pup was then discarded. Histopathologic examination was deemed

unnecessary during consultation between the study director and the sponsor; therefore, tissues that were retained from F_1 pups will be discarded upon issuance of the final report.

5.3.4.2. CLINICAL OBSERVATIONS

Litters were examined daily for survival and any adverse changes in appearance or behavior. Each pup received a detailed physical examination on PND 1 and 4. Any abnormalities in nursing behavior were recorded.

5.3.4.3. BODY WEIGHTS

Pups were individually weighed on PND 1 and 4. Mean pup weights were presented by sex for each litter and by dose group. When body weights could not be determined for a pup during a given interval (due to an unscheduled death, weighing error, etc.), group mean values were calculated for that interval using the available data. The time periods a given pup was not weighed were designated as "NA" (Not Applicable) on the individual report tables.

5.3.4.4. SEX DETERMINATION

Pups were individually sexed on PND 0 and 4.

5.3.4.5. CALCULATION OF LITTER PARAMETERS

Litter parameters were defined as follows:

$$\text{Mean Live Litter Size} = \frac{\text{Total No. of Viable Pups on PND 0}}{\text{No. of Litters with Viable Pups PND 0}}$$

$$\text{Postnatal Survival Between Birth and PND 0 or PND 4 (\% Per Litter)} = \frac{\Sigma\,(\text{Viable Pups Per Litter on PND 0 or PND 4/No. of Pups Born Per Litter})}{\text{No. of Litters Per Group}} \times 100$$

$$\text{Postnatal Survival for All Other Intervals (\% Per Litter)} = \frac{\Sigma\,(\text{Viable Pups Per Litter at End of Interval N/Viable Pups Per Litter at Start of Interval N})}{\text{No. of Litters Per Group}} \times 100$$

Where N = PND 0-1 and 1-4

5.3.4.6. SCHEDULED EUTHANASIA

On PND 4, surviving F_1 pups were euthanized via an intraperitoneal injection of sodium pentobarbital and necropsied with emphasis on developmental morphology. Organs and tissues with gross lesions were preserved in 10% neutral-buffered formalin for possible future histopathologic examination. All carcasses were then discarded. Histopathologic examination was deemed unnecessary during consultation between the study director and the sponsor; therefore, tissues retained from F_1 pups will be discarded upon issuance of the final report.

5.4. STATISTICAL ANALYSES

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 1% and 5%, comparing each test article-treated group to the control group by sex. Each mean was presented with the standard deviation (S.D.), standard error (S.E.) and the number of animals (N) used to calculate the mean. Due to the use of significant figures and the different rounding conventions inherent in the types of software used, the means and standard deviations on the summary and individual tables may differ slightly. Data obtained from nongravid females were excluded from statistical analyses following the mating period. Where applicable, the litter was used as the experimental unit.

Parental mating, fertility, conception and copulation indices were analyzed using the Chi-square test with Yates' correction factor (Hollander and Wolfe, 1999). Mean body weights (weekly, gestation and lactation), body weight changes and food consumption, precoital intervals, offspring body weights and body weight changes, gestation length, numbers of corpora lutea and implantation sites, number of pups born, live litter size on PND 0, unaccounted-for sites, absolute and relative organ weights, clinical pathology values (except gamma glutamyltransferase) and FOB data values were subjected to a parametric one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1980) to determine intergroup differences. If the ANOVA revealed statistically significant

($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test article-exposed groups to the control group. FOB parameters that yielded scalar or descriptive data and qualitative histopathological findings in the test article-exposed groups were compared to the control group using Fisher's Exact test (Steel and Torrie, 1980). Mean litter proportions (percent per litter) of males at birth and postnatal survival, as well as gamma glutamyltransferase values, were subjected to the Kruskal-Wallis nonparametric ANOVA (Kruskal and Wallis, 1952) to determine intergroup differences. If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunn's test (Dunn, 1964) was used to compare the test article-exposed groups to the control group.

All repeated measures analysis of variance (RANOVA) statistical analyses for total and ambulatory locomotor activity counts collected during pretest and after dosing were conducted by BioSTAT Consultants, Inc., Portage, Michigan, using SAS version 9.1 software (SAS Institute, Inc., 2002-2003). The results of these analyses are presented in Appendix I.

Each analysis endpoint was analyzed, by sex and session, with a RANOVA. Factors in the model included treatment group (TRT), time interval (TIME), and the interaction of time interval and treatment group (TRT*TIME). The SAS procedure PROC MIXED was used for analysis with the random effect of animal included as the repeated measurement. The covariance structure across time was selected by comparing Akaike's Information Criterion (AIC).

The monotonic dose-response relationship was evaluated using sequential linear trend tests based on ordinal spacing of dosage levels. The linear dose by time interaction (LinTrt*Time) was evaluated, and if significant at the 0.05 level, trend tests on treatment means were performed at the 0.05 level for each time interval. If the linear dose by time interaction was not significant, the trend test was conducted across the pooled time intervals by session only.

Nonmonotonic dose responses were evaluated whenever no significant linear trends were detected by TRT and/or TRT*TIME interaction was significant at the 0.01 level. Within the framework of the RANOVA, pair-wise comparisons were made for each individual treated group with the control group through linear contrasts. If TRT*TIME was significant, the comparisons were conducted for each time interval. If only the TRT effect was significant, the comparisons were conducted across the pooled time intervals of the entire session. These nonmonotonic dose response comparisons were conducted at the 0.01 significance level.

All statistical analyses were conducted using SAS version 9.1 (SAS Institute, Inc., 2002-2003) software. Total and ambulatory locomotor activity data were analyzed by BioSTAT Consultants, Inc., Portage, Michigan. The statistical summary report is presented in Appendix I.

5.5. DATA RETENTION

The sponsor has title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol. Appropriate supporting documentation for statistical analyses conducted and reported by BioSTAT Consultants, Inc., is maintained in the Archives at WIL Research Laboratories, LLC.

Reserve samples of the test article, pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC in compliance with regulatory requirements.

6. RESULTS

6.1. ANALYZED EXPOSURE CONCENTRATIONS

Exposure Atmosphere Generation Report: Appendix C

Overall mean measured test article exposure concentrations are summarized below.

Males

Group:	Control	60 ppm	300 ppm	600 ppm
Mean Concentration (ppm):	0	61	303	602
Standard Deviation:	0.0	1.9	10.1	11.8
N:	34	34	34	34

Toxicity Phase Females

Group:	Control	60 ppm	300 ppm	600 ppm
Mean Concentration (ppm):	0	61	304	604
Standard Deviation:	0.0	2.0	10.1	10.1
N:	28	28	28	28

Reproductive Phase Females

Group:	Control	60 ppm	300 ppm	600 ppm
Mean Concentration (ppm):	0	61	304	603
Standard Deviation:	0.0	2.2	11.8	15.5
N:	52	38	48	52

6.2. F_0 MALES

6.2.1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 1, 2, 3

Individual Data: Appendix A - Tables A1, A2

All F_0 males survived to the scheduled necropsy. No test article-related clinical findings were noted during the daily examinations, at the midpoint of exposure or approximately 1 hour following the end of exposure. Clinical findings were observed in the exposed groups during the daily examinations and approximately 1 hour following the end of exposure, and included hair loss, scabbing and/or red material on various body surfaces. However, these findings were observed in single animals, similarly in the control group and/or in a manner that was not exposure-related. Therefore, no relationship to the test article was apparent.

6.2.2. BODY WEIGHTS

Summary Data: Tables 4, 5; Figure 1

Individual Data: Appendix A - Tables A3, A4

Mean body weights and body weight gains for the 60, 300 and 600 ppm group males were similar to the control group throughout the premating (study days 0-13) and entire exposure (study days 0-34) periods. No statistically significant differences were observed. During study days 27-34, mean body weight losses were noted for all groups, including the control group. This was likely due to the fasting period (study day 33-34) prior to blood collection for clinical pathology evaluations.

6.2.3. FOOD CONSUMPTION

Summary Data: Table 6

Individual Data: Appendix A - Table A5

Mean food consumption in the 60, 300 and 600 ppm group males was similar to that in the control group throughout the study. No statistically significant differences were noted.

6.2.4. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Summary Data: Tables 7 through 18; Figures 2, 3

Individual Data: Appendix A - Tables A6 through A17

Positive Control Data: Appendix F

Historical Control Data: Appendix J

While no test article-related findings were observed for F_0 males at any exposure level during the functional observational battery (home cage, handling, open field, sensory, neuromuscular and physiological observations), 1 male in the 600 ppm group (no. 11420) was observed with Straub tail (increased tail elevation). However, this finding was only noted in 1 of the 10 males observed. Therefore, no relationship to the test article was apparent. No remarkable or statistically significant differences were apparent between

the control and test article groups prior to the initiation of exposure (baseline evaluations) or prior to exposure during the last week of the study (study week 4).

6.2.5. LOCOMOTOR ACTIVITY

Summary Data: Table 19; Figures 4 through 9

Individual Data: Appendix A - Table A18

Statistical Summary Report: Appendix I

Historical Control Data: Appendix K

Locomotor activity patterns (total activity as well as ambulatory activity counts) in F_0 males were unaffected by test article exposure during the study week 4 evaluation. Values obtained from the 6 subintervals evaluated (0-10 minutes, 11-20 minutes, 21-30 minutes, 31-40 minutes, 41-50 minutes and 51-60 minutes) and the overall 60-minute test session were comparable to the concurrent control values. While no statistically significant differences in locomotor activity were observed during the exposure period, mean ambulatory counts for the 60 ppm group males were significantly ($p=0.003$) higher than the control group during the first 10-minute subinterval of the pretest evaluation. Because this change occurred prior to the initiation of the exposure period, there was no relationship to the test article. In addition, mean total motor activity counts for the overall 60-minute session were 24.9% higher for the 600 ppm males when compared to the control group during study week 4. However, this apparent increase was primarily due to 1 male that also had Straub tail. Therefore, no relationship to the test article was apparent. All other differences from the control group were slight, not statistically significant when analyzed by a repeated measures analysis and/or did not occur in an exposure-related manner. No remarkable shifts in the pattern of habituation occurred in any of the test article-exposed groups when the F_0 males were evaluated prior to the initiation of exposure or during the study week 4 evaluation.

6.2.6. CLINICAL PATHOLOGY

6.2.6.1. HEMATOLOGY AND COAGULATION

Summary Data: Table 20

Individual Data: Appendix A - Table A19

Pathology Report: Appendix H

Mean absolute eosinophil and lymphocyte counts for the 600 ppm group males were 42.9% and 26.4% lower, respectively, than the control group. The difference for mean absolute eosinophils was significant ($p<0.05$). Because these changes were within historical limits for approximately age-matched animals (0.05-0.25 and 5.20-13.78 thousand/μL for eosinophils and lymphocytes, respectively) and did not correlate with any other clinical or pathological consequences, the decreased absolute eosinophil and lymphocyte counts were considered test article-related but nonadverse.

There were no test article-related alterations in coagulation parameters in males at any exposure level.

6.2.6.2. SERUM CHEMISTRY

Summary Data: Table 21

Individual Data: Appendix A - Table A20

Pathology Report: Appendix H

The mean alanine aminotransferase value for the 600 ppm group males was 20.5% higher than the control group. The difference was significant ($p<0.05$). While this change was considered test article-related, it was not considered adverse because the magnitude of change from the control group was small, the value was within historical limits and it did not correlate with any other clinical or pathological abnormalities.

6.2.7. REPRODUCTIVE PERFORMANCE

Summary Data: Table 22

Individual Data: Appendix A - Table A59

Historical Control Data: Appendix L

F_0 male reproductive parameters are presented in the following table.

Text Table 1. Results of Reproductive Performance

	Exposure Level (ppm)				WIL HC[a]
Parameter	0	60	300	600	Mean (Range)
Male Mating Index (%)	90.0	100.0	100.0	90.0	97.0 (86.7-100.0)
Male Fertility Index (%)	90.0	100.0	100.0	80.0	90.6 (63.2-100.0)
Male Copulation Index (%)	100.0	100.0	100.0	88.9	93.5 (66.7-100.0)

Note: Mating, fertility and copulation indices evaluated using Chi-Square test
[a] = WIL historical control data

No exposure-related effects on reproductive performance were observed at any exposure level. No statistically significant differences were noted between the control and test article-exposed groups. Males that did not sire a litter numbered 1, 0, 0 and 2 in the control, 60, 300 and 600 ppm groups, respectively.

6.2.8. ANATOMIC PATHOLOGY

6.2.8.1. MACROSCOPIC EXAMINATIONS

Summary Data: Table 23

Individual Data: Appendix A - Table A21

Pathology Report: Appendix H

No test article-related internal findings were observed at any exposure level in males at the scheduled necropsy. Macroscopic findings observed in the test article-exposed groups occurred infrequently and/or in a manner that was not exposure-related.

6.2.8.2. ORGAN WEIGHTS

Summary Data: Table 24

Individual Data: Appendix A - Tables A22, A23

Pathology Report: Appendix H

There were no test article-related changes in organ weights for the 60, 300 and 600 ppm group F_0 males. Differences from the control group were slight, did not occur in an exposure-related manner and/or were not statistically significant.

6.2.8.3. MICROSCOPIC EXAMINATIONS

Summary Data: Table 25

Individual Data: Appendix A - Table A21

Pathology Report: Appendix H

There were no test article-related histopathologic findings in F_0 males at any exposure level. All histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than exposure to the test article. There was no test article-related alteration in the prevalence, severity or histologic character of those incidental tissue alterations.

6.3. TOXICITY PHASE FEMALES

6.3.1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 26, 27, 28

Individual Data: Appendix A - Tables A24, A25

One toxicity phase female (no. 11518) in the 300 ppm group was found dead on study day 35, the day of scheduled necropsy. The cause of death for this female could not be determined and there were no clinical findings observed for this female during the study. However, because there were no mortalities at 600 ppm, this mortality at 300 ppm was not considered test article-related. All other animals survived to the scheduled necropsy.

No test article-related clinical findings were noted during the daily examinations, at the midpoint of exposure or approximately 1 hour following the end of exposure. Clinical findings were observed in the test article-exposed groups during the daily examinations and approximately 1 hour following the end of exposure, and included hair loss and red material on various body surfaces, scabbing and swollen or reddened ears. However, these findings were observed in single animals, similarly in the control group and/or in a manner that was not exposure-related. Therefore, no relationship to the test article was apparent.

6.3.2. Body Weights

Summary Data: Tables 29, 30; Figure 10

Individual Data: Appendix A - Tables A26, A27

Mean body weights and body weight gains for the 60, 300 and 600 ppm group toxicity phase females was similar to the control group when evaluated during the entire exposure period (study days 7-35). Differences from the control group were slight and not statistically significant. During study days 28-35, mean body weight losses were noted for all groups, including the control group. This was likely primarily due to the fasting period (study day 34-35) prior to blood collection for clinical pathology evaluations.

6.3.3. Food Consumption

Summary Data: Table 31

Individual Data: Appendix A - Table A28

Mean food consumption in the 60, 300 and 600 ppm group toxicity phase females was similar to that in the control group throughout the study. No statistically significant differences were noted.

6.3.4. FUNCTIONAL OBSERVATIONAL BATTERY (FOB)

Summary Data: Tables 32 through 43; Figures 11, 12

Individual Data: Appendix A - Tables A29 through A40

Positive Control Data: Appendix F

Historical Control Data: Appendix J

No test article-related findings were observed for the toxicity phase females at any exposure level during the functional observational battery (home cage, handling, open field, sensory, neuromuscular and physiological observations). No remarkable or statistically significant differences were apparent between the control and test article groups prior to the initiation of exposure (baseline evaluations) or prior to exposure during the last week of the study (study week 4).

6.3.5. LOCOMOTOR ACTIVITY

Summary Data: Table 44; Figures 13 through 18

Individual Data: Appendix A - Table A41

Statistical Summary Report: Appendix I

Historical Control Data: Appendix K

Locomotor activity patterns (total activity as well as ambulatory activity counts) for the toxicity phase females were unaffected by test article exposure when evaluated during study week 4. Values obtained from the 6 subintervals evaluated (0-10 minutes, 11-20 minutes, 21-30 minutes, 31-40 minutes, 41-50 minutes and 51-60 minutes) and the overall 60-minute test session were comparable to the concurrent control values. Differences from the control group were slight, not statistically significant when analyzed by a repeated measures analysis and/or were similar to pretest values. No remarkable shifts in the pattern of habituation occurred in any of the test article-exposed groups when the toxicity phase females were evaluated prior to the initiation of exposure or during the study week 4 evaluation.

6.3.6. CLINICAL PATHOLOGY

6.3.6.1. HEMATOLOGY AND COAGULATION

Summary Data: Table 45

Individual Data: Appendix A - Table A42

Pathology Report: Appendix H

A significantly ($p<0.05$) higher mean corpuscular volume was noted for the 600 ppm group toxicity phase females (55.8 fL) when compared with the control group (53.9 fL). This finding was not considered to be test article-related because there were no correlating clinical pathological findings. Furthermore, the change was considered toxicologically insignificant because the small magnitude of change and because the increase in MCV fell within the WIL historical control data range. There were no test article-related alterations in coagulation parameters in toxicity phase females at any exposure level.

6.3.6.2. SERUM CHEMISTRY

Summary Data: Table 46

Individual Data: Appendix A - Table A43

Pathology Report: Appendix H

The mean alanine aminotransferase value for the 600 ppm group females was 11.1% higher than the control group, but did not achieve statistical significance. While this change was considered test article-related, it was not considered adverse because the magnitude of change from the control group was small, the value was within WIL historical limits and it did not correlate with any other clinical or pathological abnormalities. In addition, a significantly ($p<0.05$) lower creatinine level was noted for the 300 ppm toxicity phase females when compared with the control group. However, because this decrease was not observed in an exposure-related manner, no relationship to the test article was apparent.

6.3.7. ANATOMIC PATHOLOGY

6.3.7.1. MACROSCOPIC EXAMINATIONS

Summary Data: Tables 47, 48

Individual Data: Appendix A - Tables A44, A45

Pathology Report: Appendix H

One toxicity phase female (no. 11518) in the 300 ppm group was found dead on study day 35 (day of scheduled necropsy). This female had no macroscopic findings that indicated a cause of death. Congestion, which would be an expected post-mortem change, was noted histologically for multiple tissues (see Section 6.3.7.3.).

No test article-related internal findings were observed at any exposure level in toxicity phase females at the scheduled necropsy. Macroscopic findings observed in the test article-exposed groups occurred infrequently and/or in a manner that was not exposure-related.

6.3.7.2. ORGAN WEIGHTS

Summary Data: Table 49

Individual Data: Appendix A - Tables A46, A47

Pathology Report: Appendix H

Higher mean adrenal gland, kidney and liver weights relative to final body weight were noted for the 600 ppm group toxicity phase females when compared with the control group. Although the differences in mean adrenal gland weight achieved statistical significance ($p<0.01$), the value for the 600 ppm group (0.031 g) was within the range of the WIL historical control data (0.02 g-0.05 g) and was not considered toxicologically important. The increases in mean liver and kidney weights relative to final body weight were not toxicologically significant and fell within the WIL historical control data range. In addition, the elevation in mean liver and kidney weights in the high dose females may in part be due to one animal (animal no. 11487) with histopathologic findings consistent with Caroli's disease, which is a combination of polycystic kidney disease and bile duct

dilatation. These findings in the liver and kidney of this particular animal were associated with relatively high liver and kidney weights, which would have contributed to the statistically significant higher mean kidney and liver weights (relative to final body weight) in this group.

There were no test article-related changes in organ weights for the 60 and 300 ppm group toxicity phase females.

6.3.7.3. MICROSCOPIC EXAMINATIONS

Summary Data: Tables 50, 51

Individual Data: Appendix A - Tables A44, A45

Pathology Report: Appendix H

One toxicity phase female (no. 11518) in the 300 ppm group was found dead on study day 35 (day of scheduled necropsy). Congestion, which would be an expected post-mortem change, was noted histologically for multiple tissues (lungs, heart, thymus, kidney and adrenal cortex). Furthermore, this female had a hemorrhagic lymph node and a dilatation in the lumen of the uterus.

There were no test article-related histopathologic findings in toxicity phase females at any exposure level. One 600 ppm group female (no. 11487) was noted with lesions of the kidney and liver compatible with congenital polycystic kidney disease and cystic dilatation of intrahepatic bile ducts. This combination of changes is a congenital autosomal recessive disease recognized in Crl:CD rats (Sanzen, 2001) and therefore, these occurrences in 1 animal were considered unrelated to test article exposure.

All other histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than exposure to the test article. There was no test article-related alteration in the prevalence, severity or histologic character of those incidental tissue alterations.

6.4. REPRODUCTIVE PHASE FEMALES (F_0)

6.4.1. CLINICAL OBSERVATIONS AND SURVIVAL

Summary Data: Tables 52, 53, 54

Individual Data: Appendix A - Tables A48, A49

All reproductive phase females survived to the scheduled necropsy on lactation day 4, post-mating day 25 or post-cohabitation day 25. No test article-related clinical findings were noted during the daily examinations, at the midpoint of exposure or approximately 1 hour following the end of exposure. Clinical findings were observed in the exposed groups during the daily examinations and approximately 1 hour following the end of exposure, and included hair loss and red or yellow material on various body surfaces. However, these findings were observed in single animals, similarly in the control group and/or in a manner that was not exposure-related. Therefore, no relationship to the test article was apparent.

6.4.2. BODY WEIGHTS

6.4.2.1. PRE-MATING

Summary Data: Tables 55, 56; Figure 19

Individual Data: Appendix A - Tables A50, A51

Mean body weights and body weight gains for the 60, 300 and 600 ppm group reproductive phase females were similar when compared with the control group during the entire premating period (study days 0-13). Differences from the control group were slight and not statistically significant.

6.4.2.2. GESTATION

Summary Data: Tables 57, 58; Figure 20

Individual Data: Appendix A - Tables A52, A53

Mean body weights and body weight gains for the 60, 300 and 600 ppm group reproductive phase females were similar to the control group during the entire gestation

treatment period. Differences from the control group were slight and not statistically significant.

6.4.2.3. LACTATION

Summary Data: Tables 59, 60

Individual Data: Appendix A - Tables A54, A55

Following the end of exposure, mean body weights and body weight gains in the 60, 300 and 600 ppm groups were similar to those in the control group. None of the differences from the control group were statistically significant.

6.4.3. FOOD CONSUMPTION

6.4.3.1. PRE-MATING

Summary Data: Table 61

Individual Data: Appendix A - Table A56

Mean food consumption in the 60, 300 and 600 ppm group reproductive phase females was similar to that in the control group throughout the pre-mating period. No statistically significant differences were noted.

6.4.3.2. GESTATION

Summary Data: Tables 62

Individual Data: Appendix A - Tables A57

Mean food consumption in the 60, 300 and 600 ppm group reproductive phase females was similar to that in the control group throughout gestation. No statistically significant differences were noted.

6.4.3.3. LACTATION

Summary Data: Tables 63

Individual Data: Appendix A - Tables A58

Mean food consumption in the 60, 300 and 600 ppm group reproductive phase females was similar to that in the control group during lactation. None of the differences from the control group were statistically significant.

6.4.4. REPRODUCTIVE PERFORMANCE

Summary Data: Table 64

Individual Data: Appendix A - Table A59

Historical Control Data: Appendix L

Reproductive phase female reproductive parameters are presented in the following table.

Text Table 2. Results of Reproductive Performance

	Exposure Level (ppm)				WIL HC[a]
Parameter	0	60	300	600	Mean (Range)
Female Mating Index (%)	90.0	100.0	100.0	90.0	98.2 (86.7-100.0)
Female Fertility Index (%)	90.0	100.0	100.0	80.0	92.6 (63.2-100.0)
Female Conception Index (%)	100.0	100.0	100.0	88.9	93.9 (66.7-100.0)
Pre-Coital Interval (days)	2.6	2.7	3.6	3.9	2.9 (1.8-4.7)

Note: Mating, fertility, copulation and conception indices evaluated using Chi-Square test
Mean pre-coital intervals evaluated using Dunnett's test
[a] = WIL historical control data

No exposure-related effects on reproductive performance were observed at any exposure level. No statistically significant differences were noted between the control and test article-exposed groups. Females that had evidence of mating but did not deliver numbered 0, 0, 0 and 1 in the control, 60, 300 and 600 ppm groups, respectively.

The mean numbers of days between pairing and coitus in the test article-exposed groups were similar to the control group value. None of these differences were statistically significant.

6.4.5. GESTATION LENGTH AND PARTURITION

Summary Data: Table 65

Individual Data: Appendix A - Table A60

Historical Control Data: Appendix L

Mean gestation lengths in the 60, 300 and 600 ppm groups were similar to those in the control group. No statistically significant differences were noted. No signs of dystocia were noted in these groups.

6.4.5.1. MACROSCOPIC EXAMINATIONS

Summary Data: Tables 66, 67, 68

Individual Data: Appendix A - Tables A61, A62

Pathology Report: Appendix H

No test article-related internal findings were observed at any exposure level in females that failed to deliver or females examined at the scheduled necropsy.

The mean numbers of unaccounted-for sites and implantation sites in the 60, 300 and 600 ppm groups were similar to the control group values.

6.5. F_1 LITTER DATA

6.5.1. PND 0 LITTER DATA AND POSTNATAL SURVIVAL

Summary Data: Tables 69, 70

Individual Data: Appendix A - Tables A63, A64, A65

Historical Control Data: Appendix L

The mean number of pups born, live litter size and the percentage of males at birth in the 60, 300 and 600 ppm groups were similar to the control group values. Postnatal survival in the 60, 300 and 600 ppm groups was unaffected by reproductive phase female (maternal) test article exposure through gestation day 20.

6.5.2. General Physical Condition

Summary Data: Table 71

Individual Data: Appendix A - Tables A66

The general physical condition of all F_1 pups in this study was unaffected by F_0 maternal test article exposure. Pups (litters) that were found dead numbered 17(4), 14(7), 2(2) and 1(1) in the control, 60, 300 and 600 ppm groups, respectively. Two, 2 and 1 pups in the control, 60 and 300 ppm groups, respectively, were missing and 1 pup in the 60 ppm group was found dead due to a mechanical injury and therefore sent to necropsy.

6.5.3. Offspring Body Weights

Summary Data: Tables 72, 73

Individual Data: Appendix A - Tables A67, A68

Historical Control Data: Appendix L

Mean F_1 male and female pup body weights and body weight changes in the 60, 300 and 600 ppm groups were similar to the control group during PND 1-4. No statistically significant differences were noted.

6.5.4. Necropsies Of Pups Found Dead

Summary Data: Table 74

Individual Data: Appendix A - Tables A69

The numbers of pups (litters) found dead during PND 0-4 numbered 17(4), 14(7), 2(2) and 1(1) in the control, 60, 300 and 600 ppm groups, respectively. One fetus (no. 11534-01) in the 60 ppm group had mandibular micrognathia (skeletally, the mandible was smaller than normal and fused) and anophthalmia (skeletally, the orbit was smaller than normal). However, because these findings were limited to a single fetus in the low-exposure group, these findings were not attributed to parental exposure to the test article.

6.5.5. SCHEDULED PUP NECROPSIES (PND 4) AND PUPS SENT TO NECROPSY DUE TO MECHANICAL TRAUMA

Summary Data: Table 75

Individual Data: Appendix A - Tables A70

No internal findings that could be attributed to parental test article exposure were noted at the necropsy of pups euthanized on PND 4. Internal findings in the test article-exposed groups were limited to 4 pups in the 300 ppm group and included dark red areas on the eyes and brain and renal papilla not fully developed (Woo and Hoar Grade 1); one of the pups with renal papillae not fully developed was noted with a developmental variation (ipsilateral distended ureter). The pup with dark red areas on the brain was also noted with a developmental variation (pale liver). There were no internal findings for the 60 and 600 ppm group pups.

7. DISCUSSION

This study was designed to investigate the potential toxic effects of trimethylsilanol when administered via whole-body inhalation to rats for a minimum of 28 days and to evaluate the potential of the test article to affect male and female reproductive performance such as gonadal function, mating behavior, conception, parturition and early postnatal development.

One toxicity phase female in the 300 ppm group was found dead on study day 35, the day of scheduled necropsy. The cause of death for this female was not determined; however, because there were no mortalities in the 600 ppm group, this death was not attributed to test article exposure. All other animals survived to the scheduled necropsy.

Test article-related changes in hematology and serum chemistry parameters were noted for the 600 ppm group F_0 males and toxicity phase females. In the 600 ppm group males, mean absolute eosinophil and lymphocyte counts were 42.9% and 26.4% lower (statistically significant), respectively, than the control group, and in toxicity phase females, mean corpuscular volume was 3.5% higher (statistically significant) than the control group. Furthermore, mean alanine aminotransferase values were 20.5% and 11.1% higher for the 600 ppm group males and toxicity phase females, respectively, when compared with the control group. Although the differences for the 600 ppm group were considered to be test article-related, they were not considered to be adverse in the absence of correlating histologic changes.

In a previous 2-week whole body inhalation toxicity study (WIL-401008; Weinberg, 2007) with the test article, CNS/behavior-related findings were observed in the 600 ppm group. In the aforementioned study, hypoactivity and impaired equilibrium were noted for 2 of the 5 males and 2 of the 5 females, respectively, in the 600 ppm group approximately 1-hour following exposure during the first week of exposure. In contrast, there were no test article-related clinical findings observed during the daily examination, at the midpoint of exposure or approximately 1 hour following exposure at any test article concentration in the current study. Furthermore, mean body weights, body weight gains

and food consumption for males, toxicity phase females and reproductive phase females were unaffected by test article exposure. In addition, there were no test article-related effects on reproductive performance, male and female mating, fertility and copulation/conception, FOB and locomotor activity, organ weights and macroscopic and microscopic data. There were no test article-related effects on F_1 litter data, postnatal survival, general physical condition or offspring body weights, and no exposure-related pup macroscopic findings.

8. CONCLUSIONS

Test article-related effects were limited to changes in hematology (lower eosinophil and lymphocyte counts for males) and serum chemistry (higher alanine aminotransferase for males and toxicity phase females) at 600 ppm. These changes occurred in the absence of correlating histologic changes and were not considered adverse. Therefore, under the conditions of this screening study, an exposure level of 600 ppm was considered to be the no-observed-adverse-effect level (NOAEL) for reproductive, systemic and neonatal toxicity of trimethylsilanol when administered via whole-body inhalation exposure to Crl:CD(SD) rats.

9. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Tammye L. Fleeman, BS, LAT
Staff Toxicologist, Developmental and Reproductive Toxicology
Study Director

21 November 2008
Date

Report Prepared By:

Kristin M. Roll, MS
Study Analyst

21 Nov 2008
Date

Report Reviewed By:

Melissa J. Beck, PhD
Assistant Director, Neurosciences

21 Nov 2008
Date

Daniel T. Kirkpatrick, PhD, DABT
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21 Nov 2008
Date

Mark D. Nemec, BS, DABT
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21 Nov. 2008
Date

Michelle L. Pershing, MS
Group Supervisor, Study Analysis and Reports

20 Nov 2008
Date

KEY STUDY PERSONNEL AND REPORT SUBMISSION (CONTINUED)

Donald G. Stump, PhD, DABT
Associate Director, Developmental and Reproductive Toxicology

20 Nov 2008
Date

The following personnel representing Silicones Environmental Health and Safety Council have reviewed this report and agree with the interpretation of the data presented herein:

Joseph M. Tobin, BS
Sponsor Representative

Nov. 11, 2008
Date

Study Personnel:

Susan C. Haley, BS	Manager, Clinical Pathology
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Bennett J. Varsho, MPH, DABT	Operations Manager, Developmental and Reproductive Toxicology and the Formulations Laboratory
Robert A. Wally, BS, RAC	Manager, Reporting and Regulatory Technical Services

10. QUALITY ASSURANCE UNIT STATEMENT

10.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
03-Apr-2008	Test Article Exposure	03-Apr-2008	20-May-2008	S.Power
06-May-2008	Functional Observation Battery	06-May-2008	23-Jun-2008	P.Rusnak
07-May-2008	Blood Collection and Analysis	07-May-2008	23-Jun-2008	A.Deppe
07-May-2008	Necropsy	07-May-2008	23-Jun-2008	A.Deppe
19-May-2008	Trimming of Tissues	19-May-2008	23-Jun-2008	P.Rusnak
16-Jul-2008	Study Records (I-1)	16-Jul-2008	22-Aug-2008	M.Karna
16-Jul-2008, 17-Jul-2008	Study Records (I-2)	17-Jul-2008	22-Aug-2008	M.Karna
17-Jul-2008, 18-Jul-2008	Study Records (I-3)	18-Jul-2008	22-Aug-2008	M.Karna
21-Jul-2008, 30-Jul-2008	Study Records (I-6)	30-Jul-2008	22-Aug-2008	M.Karna
21-Jul-2008, 22-Jul-2008, 08-Aug-2008	Draft Report (Inhalation Appendix)	08-Aug-2008	22-Sep-2008	S.Power
24-Jul-2008	Study Records (I-5)	24-Jul-2008	22-Aug-2008	M.Karna
24-Jul-2008	Study Records (I-7)	24-Jul-2008	22-Aug-2008	M.Karna
25-Jul-2008	Study Records (N-1)	25-Jul-2008	22-Aug-2008	M.Karna
28-Jul-2008	Study Records (N-2)	28-Jul-2008	22-Aug-2008	M.Karna
28-Jul-2008	Study Records (C-1)	28-Jul-2008	22-Aug-2008	M.Karna
28-Jul-2008	Study Records (C-2)	28-Jul-2008	22-Aug-2008	M.Karna
28-Jul-2008, 29-Jul-2008	Study Records (C-3)	29-Jul-2008	22-Aug-2008	M.Karna
29-Jul-2008	Study Records (H-1)	29-Jul-2008	22-Aug-2008	M.Karna

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
29-Jul-2008, 06-Aug-2008	Study Records (P-1)	06-Aug-2008	22-Sep-2008	M.Karna
05-Aug-2008, 06-Aug-2008	Study Records (Ex-1)	08-Aug-2008	22-Sep-2008	S.Power
07-Aug-2008	Study Records (Ex-2)	08-Aug-2008	22-Sep-2008	S.Power
07-Aug-2008	Study Records (Ex-3, Ex-6)	08-Aug-2008	22-Sep-2008	S.Power
07-Aug-2008	Study Records (Ex-4, Ex-5)	12-Aug-2008	22-Sep-2008	S.Power
15-Aug-2008, 16-Aug-2008	Study Records (I-4)	16-Aug-2008	22-Sep-2008	M.Karna
25-Aug-2008	Draft Report (without Inhalation, Pathology and Statistical Analysis Summary Appendices)	25-Aug-2008	22-Sep-2008	M.Karna
25-Aug-2008	Draft Report (Statistical Analysis Summary Appendix)	25-Aug-2008	22-Sep-2008	M.Karna
26-Aug-2008	Draft Report (Pathology Appendix)	26-Aug-2008	22-Sep-2008	M.Karna

This study was inspected in accordance with the OECD Principles of Good Laboratory Practice, the standard operating procedures of WIL Research Laboratories, LLC and the sponsor's protocol and protocol amendments with the following exception. The data located in Appendix B (Certificate of Analysis) were the responsibility of the sponsor. 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.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments and the standard operating procedures of WIL Research Laboratories, LLC.

The raw data, the retention sample and the final report will be stored in the Archives at WIL Research Laboratories, LLC or another location specified by the sponsor.

10.2. APPROVAL

This study was inspected according to the criteria discussed in Section 10.1.

Report Audited By:

Milan Karna, BA
Compliance Specialist

21 Nov 2008
Date

Steven P. Power
Associate Compliance Specialist

21 Nov. 2008
Date

Report Released By:

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

21 Nov 2008
Date

11. REFERENCES

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12. DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 7.6** states that oxygen content of the exposure atmosphere would be measured daily during the exposure phase of the study. On 3, 4, 5 and 23 April 2008 (study days 0, 1, 2 and 20), the oxygen content of the exposure atmosphere was not measured.
- **Protocol Section 7.6** states that mean chamber temperature would be 22 ± 2°C. On 3 and 6 April 2008, the average temperature of Chamber 3 was 25°C. Furthermore, on 18-20 April 2008 and 10, 13 and 17 May 2008, the mean temperature of Chambers 1, 2, 3 and/or 4 were 19°C or 25-26°C.
- **Protocol Section 7.8.1** states that nominal concentration would be within ±10% of the measured chamber concentration. On 8 different dates during study day 1 to 41, the nominal concentration of Chambers 2, 3 or 4 was -27% to 13% of the measured chamber concentration.
- **Protocol Section 7.8.2** states that actual test article concentrations in the exposure atmospheres would be determined approximately every 35 minutes for each chamber, resulting in 10 samples collected per day. On 19 April 2008 (study day 16), 1 sample collected from Chamber 1 was not used. Therefore, only 9 samples were used to calculate the daily mean for that day.
- **Protocol Section 8.1** states that each animal would be observed twice daily, once in the morning and once in the afternoon, for moribundity and mortality for the duration of the study. On 6 April 2008 (study day 3), completion of the afternoon observation was not documented. On 15 April 2008 (study day 12), completion of the morning observation was not documented.
- **Protocol Section 8.1** states that animals would be observed approximately 1 hour following exposure. On several days throughout the exposure period, animals were observed 1 hour 16 minutes to 1 hour 47 minutes following exposure. See the following table for the numbers of animals in each group.

Date	Study Day	Number of Animals	Sex	Group (Phase)
3 April 2008	0	10	F	300 ppm (Reproductive)
		10	F	300 ppm (Toxicity)
4 April 2008	1	2	F	300 ppm (Reproductive)
		10	F	600 ppm (Reproductive)
		10	M	600 ppm
9 April 2008	6	2	F	300 ppm (Reproductive)
		10	F	600 ppm (Reproductive)

Date	Study Day	Number of Animals	Sex	Group (Phase)
11 April 2008	8	2	F	60 ppm (Toxicity)
		10	F	300 ppm (Toxicity)
		10	F	600 ppm (Toxicity)
18 April 2008	15	6	F	600 ppm (Toxicity)
26 April 2008	23	1	F	600 ppm (Toxicity)
		9	F	600 ppm (Reproductive)
27 April 2008	24	10	M	Control
		10	M	60 ppm
		10	M	300 ppm
		10	M	600 ppm
		1	F	60 ppm (Toxicity)
		10	F	300 ppm (Toxicity)
		10	F	600 ppm (Toxicity)

- **Protocol Section 8.2** states that all animals would receive a weekly detailed physical examination, beginning 1 week prior to test article exposure, including the first day of exposure and just prior to the scheduled necropsy. Two, 2, 2 and 1 reproductive phase females in the control, 60, 300 and 600 ppm groups, respectively, did not receive a detailed physical examination just prior to the scheduled necropsy.

These deviations did not negatively impact the quality or integrity of the data or the outcome of the study.

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FINAL REPORT

STUDY TITLE

ACUTE INHALATION TOXICITY STUDY
OF TRIMETHYLSILANOL IN ALBINO RATS

STUDY NUMBER

WIL-401006

DATA REQUIREMENT

OECD Guideline 403

STUDY DIRECTOR

Jeffrey T. Weinberg, BS

STUDY INITIATION DATE

4 January 2007

STUDY COMPLETION DATE

10 May 2007

PERFORMING LABORATORY

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MR 315915

COMPLIANCE STATEMENT

This study, designated WIL-401006, was conducted in compliance with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C (97) 186/Final], 26 November 1997; the standard operating procedures of WIL Research Laboratories, LLC and the protocol as approved by the sponsor.

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10 May 2007
Date

TABLE OF CONTENTS

Page

Compliance Statement **2**
Table Of Contents **3**
Index Of Figures **4**
Index Of Tables **5**
Index Of Appendices **5**
1. Summary **6**
1.1. Objective 6
1.2. Test Guidelines 6
1.3. Study Design 6
1.4. Results 6
1.5. Conclusions 7
2. Introduction **8**
2.1. General Study Information 8
2.2. Key Study Dates 8
2.3. Experimental Design 9
3. Experimental Procedures - Materials And Methods **10**
3.1. Test Article Identification 10
3.2. Exposure Methods 10
3.3. Test Atmosphere Generation Methods 11
3.4. Methods Of Characterization Of Exposure Atmospheres 12
3.4.1. Nominal Concentrations 12
3.4.2. Analyzed Concentrations 13
3.5. Route And Rationale Of Test Article Administration 16
3.6. Animal Receipt And Acclimation 16
3.7. Animal Housing 16
3.8. Diet, Drinking Water And Maintenance 17
3.9. Environmental Conditions 17

Page

3.10. Assignment Of Animals To Treatment Groups 17
3.11. Data Retention 18
4. Parameters Evaluated 18
4.1. Mortality 18
4.2. Clinical Observations 18
4.3. Body Weights 18
4.4. Necropsy 18
5. Results And Discussion 19
5.1. Characterization Of Exposure Atmospheres 19
5.1.1. Environmental Conditions 19
5.1.2. Nominal Concentrations 19
5.1.3. Actual Concentrations 20
5.2. Mortality 20
5.3. Clinical Observations 22
5.4. Body Weights 25
5.5. Necropsy 25
6. Conclusions 26
7. Key Study Personnel And Report Submission 27
8. Quality Assurance Unit Statement 28
8.1. Phases Inspected 28
8.2. Approval 29
9. Deviations From The Protocol 30

INDEX OF FIGURES

1. Atmosphere Generation And Exposure System 32

INDEX OF TABLES

Page

1. Individual Test Atmosphere Concentrations 34
2. Summary Of Clinical Findings: Total Occurrence/No. Of Animals 37
3. Summary Of Body Weights [g] 45
4. Summary Of Body Weight Changes [g] 51
5. Summary Of Macroscopic Findings (Unscheduled Deaths) 57
6. Summary Of Macroscopic Findings (Scheduled Necropsy) 60
7. Individual Clinical Observations 62
8. Individual Body Weights [g] 73
9. Individual Body Weight Changes [g] 85
10. Individual Macroscopic Findings (Unscheduled Deaths) 97
11. Individual Macroscopic Findings (Scheduled Necropsy) 115

INDEX OF APPENDICES

A. Certificate Of Analysis (Sponsor-Provided Data) 127
B. Animal Room Environmental Conditions 129
C. Study Protocol 134

1. SUMMARY

1.1. OBJECTIVE

The objective of this study was to determine the acute inhalation toxicity of Trimethylsilanol when administered as a vapor for a single, 4-hour, whole-body inhalation exposure to rats.

1.2. TEST GUIDELINES

The protocol was designed to be in compliance with the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals, Section 403.

1.3. STUDY DESIGN

The acute inhalation toxicity of Trimethylsilanol was evaluated in this 4-hour, single exposure study in rats. The test article was administered to 3 groups of 5 male and 5 female Crl:CD(SD) albino rats via whole-body inhalation exposure as a vapor at concentrations of 1824, 3517 and 7443 ppm.

Mortality, clinical observations, body weights and body weight changes were evaluated over a 14-day post-exposure observation period. Gross necropsies were conducted on all animals.

1.4. RESULTS

Mortality was 0/10, 8/10 and 10/10 animals for the 1824, 3517 and 7443 ppm groups, respectively. All animals in the 7443 ppm group died during the exposure, and no clinical observations were collected. Clinical observations immediately following exposure included decreased respiration, shallow respiration, rales, labored respiration, lacrimation of the eyes, partial closure of the eyes and yellow material on the urogenital area for the 1824 and 3517 ppm groups; prostration for the 3517 ppm group; and hypoactivity, ataxia, extremities cool to touch and clear material around the mouth, facial area and urogenital area for the 1824 ppm group.

Significant clinical observations for the surviving animals during the 14-day post-exposure observation period consisted of yellow material on the urogenital area for the 1824 and 3517 ppm groups; decreased respiration, shallow respiration, rales, hypoactivity, prostration, ataxia, lacrimation of the eyes, partial closure of the eyes, decreased defecation, decreased urination, opacity of the eyes, brown material on the urogenital area and red material around the right eye, facial area and urogenital area for the 3517 ppm group; and yellow material on the dorsal rump and red material around the nose and on the dorsal trunk for the 1824 ppm group. All surviving animals in the 1824 and 3517 ppm groups were considered normal by study days 2 and 5, respectively.

There were no remarkable changes in body weight. From study day 0 to 7, one female in the 1824 ppm group lost 1 gram. All animals surpassed their initial (study day 0) body weight by study day 14 and were considered normal.

Internal macroscopic findings noted for animals that died prior to the scheduled necropsy were dark red areas on the lungs, dark red discoloration of the lungs and clear fluid contents in the uterus for the 1824 and 3517 ppm groups; red discoloration of the cortico-medullary junction in the kidneys, dark red discoloration of the liver, accessory spleen and cysts on the ovaries for the 3517 ppm group; and dark red discoloration of the adrenal glands, distended urinary bladder, opacity of the eyes and dark red areas on the urinary bladder for the 1824 ppm group.

At the scheduled necropsy, white areas on the spleen, clear fluid contents in the uterus and pale lungs were noted in the 1824 ppm group. There were no other macroscopic findings for animals at the scheduled necropsy.

1.5. CONCLUSIONS

Based on the results of this study, the 4 hour LC_{50} of Trimethylsilanol was 3151 ppm when male and female albino rats were exposed to a single, 4-hour, whole-body vapor concentration of the test article.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This study presents the data from an "Acute Inhalation Toxicity Study of Trimethylsilanol in Albino Rats". Due to software spacing constraints, the study title is presented as "Acute Inhalation Toxicity Study of Trimethylsilanol in Rats" on the report tables.

The following computer protocol was used for data collection during the study:

Computer Protocol(s)	Type of Data Collected
WIL-401006	Main study data

2.2. KEY STUDY DATES

Date(s)	Event(s)
22 January 2007	Experimental starting date (First exposure day)
13 February 2007	Experimental completion date (gross necropsy)

2.3. EXPERIMENTAL DESIGN

The following table presents the animal exposures conducted for the study:

Exposure Number	Target Exposure Concentration (ppm)	Analyzed Exposure Concentration (ppm)	Number of Animals Males	Number of Animals Females
1	7250	7443	5	5
2	1800	1824	5	5
3	3500	3517	5	5

The target exposure concentration for the limit test was based on the recommended safe working level of one-half of the lower explosion limit (LEL). Given the vapor pressure and LEL of the test article (1.45 volume % in air), the target limit test exposure concentration was 7250 ppm. The target exposure concentration for exposures 2 and 3 were provided by the sponsor/study director based on the results of the previous exposure(s).

3. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

3.1. TEST ARTICLE IDENTIFICATION

The test article, 31-OH-RM (Trimethylsilanol), was received from Shin-Etsu Silicones, Akron, Ohio, on 8 December 2006, as follows:

Identification	No. of Containers Received	Description
Trimethylsilanol Lot no. 609230 [WIL Log No. 7236A] CAS No. 1066-40-6	3 cans Total gross weight: 3614.9 g	Clear, colorless liquid

Purity and stability data were the responsibility of the sponsor. A Certificate of Analysis is presented in Appendix A. The purity of the test article was 95.04%. The test article was stored at room temperature. A reserve sample of the test article (approximately 8 g) was collected on 10 January 2007, and stored in the Archives of WIL Research Laboratories, LLC.

3.2. EXPOSURE METHODS

Exposures were conducted using a 130-L stainless steel and glass whole-body inhalation exposure chamber. Food and water were withheld during the exposures. Exposure atmosphere environmental conditions were recorded at approximately 30-minute intervals during each exposure. An Omega model no. HX94C temperature and humidity transmitter probe (Omega Engineering, Inc., Stamford, CT) provided monitoring of temperature and percent relative humidity. A rotameter-type flowmeter (model 127MM, Gilmont Instruments, A Division on Barnant Company, Barrington, IL) was used to control the supply airflow. Chambers were operated under at least 12 to 15 air changes per hour. Negative pressure within the chamber was monitored using a Dwyer Magnehelic Indicating Transmitter pressure gauge (Dwyer Instruments, Inc., Michigan City, IN). Oxygen content was measured on 17 January 2007 during the pre-exposure

method development phase using a Drager PAC III calibrated oxygen sensor (Draeger Safety, Inc., Pittsburgh, PA). The oxygen content was 19.5%.

The time required to attain 99% of the equilibrium concentration (or clearance time for the concentration to decrease from the equilibrium concentration) was calculated by the following formula:

$$t_{99} = \frac{V \times K}{F}$$

Where:

V = volume of the chamber, L

K = constant for 99%, 4.60

F = flow rate of air through the chamber, L/minute

The calculated t_{99} equilibrium time for this study was approximately 20 minutes. The exposure period was adjusted for the t_{99} time. The exposure was initiated after the animals were placed in the exposure chamber and generation of the test article vapor had been ongoing for one t_{99} interval. The exposure period was terminated after 4 hours when the test article vapor generation was stopped. The exposure chambers were opened after the t_{99} clearance time.

3.3. TEST ATMOSPHERE GENERATION METHODS

Vapor atmospheres of the test article were generated using a system similar to that shown in Figure 1. The system operated as follows: The test article was metered at a known flow rate by a liquid metering pump (Model QG20, ⅛-inch piston, Fluid Metering Inc., Oyster Bay, NY) to the top of a glass bead vaporization column (2.4 cm I.D. × 80 cm) filled with 3-, 6-, 12- and 16-mm glass beads. The bead column and delivery line were wrapped with an electric heating tape (Omega Engineering Co., Stamford, CT) controlled using a J-type thermocouple and digital temperature controller set at 100°C and 118°C, respectively. Nitrogen was metered to the bottom of the vaporization column using a

regulator (model 8802K, Coilhose Pneumatics, Middlesex, NJ) and a Gilmont rotameter-type flowmeter (model no. GF-6541-1225). Vaporization occurred as the liquid test article coated the surface of the heated beads and nitrogen flowed up through the column. The test article vapors were piped to the chamber inlet where the concentration was reduced to the desired concentration by mixing with the chamber supply air. The exhaust atmosphere passed through the in-house exhaust system before being released from the facility. The parameters used for this study are summarized in the following table:

Group (ppm)	Nitrogen Flow Rate (L/minute)	Supply Airflow Rate (L/minute)	Total Airflow Rate (L/minute)	Test Article Delivery Rate (mL/minute)
1824	1.5	27.7-28.6	29.2-30.1	0.24-0.30
3517	1.6	28.6-29.6	30.2-31.2	0.49-0.51
7443	2.3	28.6	30.9	1.06-1.11

3.4. METHODS OF CHARACTERIZATION OF EXPOSURE ATMOSPHERES

3.4.1. NOMINAL CONCENTRATIONS

The nominal exposure concentration was calculated as the total amount of test article used during the 260-minute generation period divided by the total volume of air that passed through the chamber during the generation period, using the following equation:

$$\text{Nominal Concentration (ppm)} = \frac{\text{Test Article Used (g)} \times \text{MV} \times 10^6}{\text{TAV} \times \text{GMW}}$$

MV = Molar volume at 730 mmHg and 21°C, 25.11 L/mole

GMW = Gram molecular weight of the test article, 90.20 g/mole

TAV = Total air volume of generation period, L

10^6 = ppm conversion

3.4.2. ANALYZED CONCENTRATIONS

Analyzed exposure concentrations were determined at approximately 30-minute intervals using a gas chromatograph (GC). Samples of the exposure atmosphere were collected from the chamber using an internal gas-sampling valve and sample loop. The chromatograph was displayed, and the area under the sample peak was calculated and stored. The concentration in parts per million (ppm) was calculated using an ln-quadratic formula based on the GC calibration curve. The following table summarizes the gas chromatograph conditions:

Gas Chromatograph:	Hewlett Packard 5890 Series II
Detector:	Flame ionization
Column:	J&W Scientific DB-5 30 m × 0.530 mm, 0.5 micron film thickness
Gases	
Carrier-Helium:	23 mL/minute
Fuel-Hydrogen:	44 mL/minute
Air:	262 mL/minute
Temperatures (°C)	
Column:	150
Detector:	300
Injector:	115
Auxiliary:	125
Retention Time:	Approximately 0.536 minutes

Integrator: Hewlett Packard 3396 Series II

Run Parameters

Zero Offset:	0
Attenuation:	8-10
Chart Speed:	1.0 cm/minute
Peak Area Rejection:	0
Peak Threshold:	5
Peak Width:	0.04

The GC was calibrated using gas-phase standards prepared to contain known vapor concentrations of the test article prepared in 10-L Tedlar® gas bags (SKC, Inc., Eighty Four, PA). Standard bags were prepared by metering a known amount of air into the bag using a dry test meter (Model DTM-200A, American Meter Co., Horsham, PA) and injecting a known volume of test article. Using a hand-held heated blower, the bag was warmed until total volatilization of the test article was visually observed. A sample was collected from the bag and run through the GC. The concentrations of the gas-phase standards were calculated as follows:

$$\text{Concentration (ppm)} = \frac{VOL \times R \times T \times D \times 10^{-3} \times 10^{6}}{L \times GMW \times P}$$

Where:

VOL	=	Volume of test article vaporized into bag, µL
R	=	Universal constant, 62.36 L mmHg/mole K
T	=	Nominal laboratory temperature in K (273 + 21°C = 294 K)
D	=	Density of the test article, 0.8174 g/mL
L	=	Volume of air used to prepare bag, 8 L
GMW	=	Gram molecular weight, 90.20 g/mole
P	=	Nominal laboratory barometric pressure, 730 mmHg
10^{-3}	=	µL to mL conversion factor
10^{6}	=	Conversion factor to ppm

Prior to initiating animal exposure, the GC was calibrated with standards prepared and analyzed in triplicate. Two calibration curves were defined using standards prepared at vapor concentrations spanning an appropriate range relative to the target exposure concentrations for the study. The 569, 1422, 3556 and 5690 ppm standards were used for the 1824 and 3517 ppm groups and the 5690, 7112 and 8535 ppm standards were used for the 7443 ppm group. The integrated peak area (y) and the theoretical concentrations of the calibration standards (x) were fit with least-squares regression analysis to the ln-quadratic formula:

$$\ln(y) = a \times [\ln(x)]^2 + b \times \ln(x) + c$$

On the day of exposure, one of the standards was prepared and analyzed, and the prime calibration curve was used to perform a calibration check of the GC. The following table summarizes the standards used for this study:

Standard Concentration (ppm)	Volume of Test Article (µL)	Volume of Air (L)
569	20	8
1422	50	8
3556	125	8
5690	200	8
7112	250	8
8535	300	8

3.5. ROUTE AND RATIONALE OF TEST ARTICLE ADMINISTRATION

The selected route of administration for this study was whole-body inhalation exposure since this is a reliable and accurate method of evaluating toxicity and is a possible route of human exposure. The animal model, the albino rat, is generally recognized as appropriate for acute inhalation toxicity studies.

3.6. ANIMAL RECEIPT AND ACCLIMATION

The Crl:CD(SD) rats used for this study were received in good health from Charles River Laboratories, Raleigh, North Carolina, on 9 and 16 January 2007. The rats were uniquely identified by an ear tag displaying the animal number and were acclimated to the laboratory for a minimum of 7 days. During the acclimation period, the rats were observed twice daily for mortality and moribundity.

3.7. ANIMAL HOUSING

Upon arrival, all animals were housed in individual suspended wire-mesh cages. The animals were maintained by the animal husbandry staff of WIL Research Laboratories, LLC in accordance with standard operating procedures (SOP). On the day of exposure, the animals were placed in wire mesh batteries containing separate cages, transported to the exposure room, exposed for the requisite duration then returned to their home cages.

The individual cage dimensions (height × width × length) for the exposure battery were 6 × 4 × 8 inches.

3.8. DIET, DRINKING WATER AND MAINTENANCE

The basal diet used in this study, PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, is a certified feed with appropriate analyses performed by the manufacturer and provided to WIL Research Laboratories, LLC. Municipal water supplying the facility is sampled for contaminants according to SOP. The results of the diet and water analyses are maintained at WIL Research Laboratories, LLC. No contaminants were present in animal feed or water at concentrations sufficient to interfere with the objectives of this study. The basal diet and municipal water, delivered by an automatic watering system, were provided ad libitum, except during the exposure periods.

3.9. ENVIRONMENTAL CONDITIONS

The animal room was maintained with controlled temperature, humidity and light (12 hours light/12 hours dark). The room temperature and humidity controls were set to maintain daily averages of 71 ± 5°F (22 ± 3°C) and 50 ± 20% relative humidity. Room temperature and relative humidity were monitored using the Metasys DDC Electronic Environmental control system and were scheduled for data collection on an hourly basis. These data are summarized in Appendix B. Actual mean daily temperature ranged from 70.3°F to 70.6°F (21.3°C to 21.4°C) and mean daily relative humidity ranged from 34.7% to 39.7% during the study.

3.10. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

Animals used in the study were selected from available stock and assigned to groups using an arbitrary assignment. The animals were selected based on body weight requirements and on the appearance of general good health. The selected animals were approximately 9 weeks old at initiation of exposure; body weight values ranged from

310 g to 346 g for males and from 207 g to 242 g for females. Individual body weights at assignment were within ± 20% of the mean for each sex.

3.11. DATA RETENTION

The sponsor has title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol.

Pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC, in compliance with regulatory requirements.

4. PARAMETERS EVALUATED

4.1. MORTALITY

Each animal was observed for mortality at the approximate midpoint of exposure, immediately following exposure on study day 0 and twice daily thereafter for 14 days.

4.2. CLINICAL OBSERVATIONS

Each animal was observed immediately following exposure on study day 0 and once daily thereafter for 14 days.

4.3. BODY WEIGHTS

Body weights were obtained immediately prior to exposure on study day 0 and once daily thereafter for 14 days. A final body weight was collected for all animals that died on study.

4.4. NECROPSY

Animals in extremis or at the scheduled necropsy were euthanized by isoflurane anesthesia followed by exsanguination. The major organ systems of the cranial, thoracic and abdominal cavities were examined for all animals.

5. RESULTS AND DISCUSSION

5.1. CHARACTERIZATION OF EXPOSURE ATMOSPHERES

5.1.1. ENVIRONMENTAL CONDITIONS

Mean temperature and relative humidity of the animal exposure atmospheres are summarized in the following table:

Group	Temperature (°C)			Relative Humidity (%)		
(ppm)	Mean	S.D.	N	Mean	S.D.	N
1824	25	0.6	8	31	5.6	8
3517	26	0.5	8	29	3.6	8
7443	26	0.5	8	29	5.2	8

5.1.2. NOMINAL CONCENTRATIONS

The following table summarizes the nominal exposure concentration data for the exposures.

Group (ppm)	Test Article Used (g)	Total Volume of Chamber Air (L)	Nominal Exposure Concentration (ppm)	Actual to Nominal Ratio (%)
1824	54.8	7722	1976	92.3
3517	103.8	7878	3668	95.9
7443	219.4	8034	7602	97.9

5.1.3. ACTUAL CONCENTRATIONS

Individual Data: Table 1

The following table summarizes the actual exposure concentration data for the animal exposures:

Group (ppm)	Mean Concentration (ppm)	Standard Deviation	Number of Samples
1824	1824	86.2	8
3517	3517	142.1	8
7443	7443	218.3	8

5.2. MORTALITY

Summary Data: Table 2

Individual Data: Table 7

All animals in the 7443 ppm group died during the exposure. Four animals in the 3517 ppm group were found dead by study day 2. In addition, 4 animals in the 3517 ppm group were euthanized in extremis by study day 2. The justification for euthanasia of 4 animals in the 3517 ppm group was based on the severity and persistence of clinical signs. Total mortality was 0/10, 8/10 and 10/10 animals for the 1824, 3517 and 7443 ppm groups, respectively.

Group	During Exposure		Study Days 0-1		2-6		7-14		Total Mortality		
(ppm)	M	F	M	F	M	F	M	F	M	F	C
1824	0	0	0	0	0	0	0	0	0	0	0
3517	0	0	2	1	2	3	0	0	4	4	8
7443	5	5	-	-	-	-	-	-	5	5	10

M = Male F = Female C = Combined Sexes
- = No animals remaining; total mortality occurred prior to that interval.

Based on the data obtained, the following LC_{50} values (in ppm) and slopes (with 95% confidence limits) were calculated by the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949):

	LC_{50} (95% confidence limits)	Slope (95% confidence limits)
Males:	3151 (2681-3702)	1.14 (1.09-1.19)
Females:	3151 (2681-3702)	1.14 (1.09-1.19)
Combined:	3151 (2811-3531)	1.14 (1.11-1.17)

5.3. CLINICAL OBSERVATIONS

Summary Data: Table 2

Individual Data: Table 7

Animals in the 7443 ppm group died during exposure; therefore, no clinical observations were recorded for that group. The following table summarizes the clinical observations (number of animals with finding) noted immediately following exposure:

	Group (ppm)			
	1824		3517	
Clinical Observation	M	F	M	F
Decreased Respiration	4	3	5	5
Shallow Respiration	0	2	1	5
Rales	1	0	2	0
Labored Respiration	0	0	1	0
Prostration	0	0	5	5
Hypoactivity	5	5	0	0
Ataxia	5	5	0	0
Extremities Cool To Touch	0	1	0	0
Lacrimation Right Eye	0	3	2	5
Lacrimation Left Eye	0	2	2	3
Partial Closure Right Eye	0	2	1	1
Partial Closure Left Eye	0	2	1	1
Dried Yellow Material, Urogenital Area	1	1	2	3
Wet Yellow Material, Urogenital Area	0	0	1	1
Dried Clear Material Around Mouth	1	0	0	0
Dried Clear Material, Facial Area	0	1	0	0
Wet Clear Material, Urogenital Area	2	2	0	0

M = Male F = Female

There were no other toxicologically significant clinical signs immediately following exposure.

Animals in the 7443 ppm group died during exposure; therefore, no clinical observations were recorded for that group. The following table summarizes the clinical observations (number of occurrences/number of animals) noted during the 14-day post-exposure observation period:

	Group (ppm)			
	1824		3517	
Clinical Observation	M	F	M	F
Decreased Respiration	0/0	0/0	8/4	7/5
Shallow Respiration	0/0	0/0	8/4	9/5
Rales	0/0	0/0	4/3	0/0
Hypoactivity	0/0	0/0	8/4	8/5
Prostration	0/0	0/0	8/4	7/5
Ataxia	0/0	0/0	0/0	1/1
Body Cool To Touch	0/0	0/0	7/4	8/5
Decreased Defecation	0/0	0/0	7/4	9/5
Decreased Urination	0/0	0/0	7/4	9/5
Lacrimation Right Eye	0/0	0/0	6/4	5/5
Lacrimation Left Eye	0/0	0/0	6/4	5/5
Partial Closure Right Eye	0/0	0/0	1/1	2/2
Partial Closure Left Eye	0/0	0/0	1/1	3/2
Opacity Right Eye	0/0	0/0	1/1	0/0
Opacity Left Eye	0/0	0/0	1/1	0/0
Wet Yellow Material, Urogenital Area	0/0	0/0	3/2	6/4
Dried Yellow Material, Urogenital Area	1/1	1/1	2/1	0/0
Dried Brown Material, Urogenital Area	0/0	0/0	0/0	3/1
Dried Red Material Around Right Eye	0/0	0/0	0/0	1/1
Dried Red Material, Facial Area	0/0	0/0	0/0	1/1
Wet Red Material, Urogenital Area	0/0	0/0	0/0	1/1
Dried Yellow Material, Dorsal Rump	0/0	1/1	0/0	0/0
Dried Red Material Around Nose	1/1	0/0	0/0	0/0
Dried Red Material, Dorsal Trunk	1/1	0/0	0/0	0/0

M = Male F = Female

There were no other toxicologically significant clinical signs during the 14-day post-exposure observation period. All surviving animals in the 1824 and 3517 ppm groups were considered normal by study days 2 and 5, respectively.

5.4. BODY WEIGHTS

Summary Data: Tables 3, 4

Individual Data: Tables 8, 9

There were no remarkable changes in body weight. From study day 0 to 7, one female in the 1824 ppm group lost 1 gram. All animals surpassed their initial (study day 0) body weight by study day 14 and were considered normal.

5.5. NECROPSY

Summary Data: Tables 5, 6

Individual Data: Tables 10, 11

The following table summarizes the gross macroscopic findings (number of animals with finding) noted for animals in the 3517 and 7443 ppm groups that died prior to the scheduled necropsy:

	Group (ppm)			
	3517		7443	
Macroscopic Findings	M	F	M	F
Dark Red Areas on the Lungs	0	1	2	3
Dark Red Discoloration of the Lungs	1	0	2	2
Clear Fluid Contents in the Uterus	0	1	0	1
Red Discoloration of the Cortico-Medullary Junction in the Kidneys	0	0	1	2
Dark Red Discoloration of the Liver	0	0	1	0
Accessory Spleen	0	0	0	1
Cysts on the Ovaries	0	0	0	1
Dark Red Discoloration of the Adrenal Glands	1	1	0	0
Distended Urinary Bladder	1	1	0	0
Opacity of the Eyes	1	1	0	0
Dark Red Areas on the Urinary Bladder	0	1	0	0

At the scheduled necropsy, macroscopic findings noted for animals in the 1824 ppm group were white areas on the spleen for 2 females, clear fluid contents in the uterus for 2 females and pale lungs for 1 male. There were no other macroscopic findings for animals at the scheduled necropsy.

6. CONCLUSIONS

Based on the results of this study, the LC_{50} of Trimethylsilanol was 3151 ppm when male and female albino rats were exposed to a vapor of the test article as a single, 4-hour, whole-body inhalation exposure.

7. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

Jeffrey T. Weinberg, BS — 10 May 2007 Date
Project Specialist, Inhalation Toxicology
Study Director

Report Prepared By:

Christine A. Heston, BA — 10 May 2007 Date
Quality Control Coordinator, Inhalation Toxicology

Report Reviewed By:

Daniel T. Kirkpatrick, PhD, DABT — 10 May 2007 Date
Director, Inhalation Toxicology

Robert A. Wally, BS, RAC — 10 May 2007 Date
Manager, Reporting and Regulatory Technical Services

Study Personnel:

Carol A. Kopp, BS, LAT	Manager, Gross Pathology and Developmental Toxicology Laboratory
Randy M. Barrett, BS	Publishing Specialist, Regulatory Affairs and Services

8. QUALITY ASSURANCE UNIT STATEMENT

8.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to WIL Management	Auditor(s)
22-Jan-2007	Test Article Exposure	22-Jan-2007	27-Feb-2007	T. DeVan Booth, M. Stauffer
27-Mar-2007	Study Records (N-1)	27-Mar-2007	23-Apr-2007	L. Goodrich
27-Mar-2007, 28-Mar-2007, 02-Apr-2007	Study Records (Ex-1)	02-Apr-2007	10-May-2007	L. Goodrich
02-Apr-2007	Draft Report	02-Apr-2007	10-May-2007	L. Goodrich

This study was inspected in accordance with the OECD Principles of Good Laboratory Practice, the standard operating procedures of WIL Research Laboratories, LLC, and the sponsor's protocol and protocol amendments with the following exception. The data located in Appendix A (Certificate Of Analysis) were the responsibility of the sponsor. 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.

This report accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments and the standard operating procedures of WIL Research Laboratories, LLC.

The raw data, the retention sample and the final report will be stored in the Archives at WIL Research Laboratories, LLC, or another location specified by the sponsor.

8.2. APPROVAL

This study was inspected according to the criteria discussed in Section 8.1.

Report Audited By:

Lori J. Goodrich, RQAP-GLP
Group Supervisor, Quality Assurance

10 May 2007
Date

Report Released By:

Heather L. Osborn, BS, RQAP-GLP
Manager, Quality Assurance

10 May 2007
Date

9. DEVIATIONS FROM THE PROTOCOL

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 7.5** states that mean chamber temperature will be 22 ± 2°C. During animal exposure, the mean temperature was 25°C, 26°C and 26°C for the 1824, 3517 and 7443 ppm groups, respectively.
- **Protocol Section 7.5** states that mean chamber relative humidity will be 30% to 70%. During animal exposure, the mean relative humidity was 29% for the 3517 and 7443 ppm groups.

These deviations did not negatively impact the quality or integrity of the data or the outcome of the study.