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**ORIGINAL**

July 12, 1996

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PDCN: 88960000052

**8E-0796-13576**

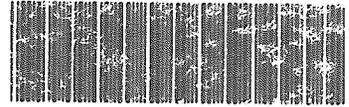
Document Processing Center (TS-790)  
Office of Toxic Substances  
Environmental Protection Agency  
401 M St. S.W.  
Washington, D.C. 20460  
Attention: Section 8(e) Coordinator



8EHQ-96-13576

**Contains No CB**

Subject: TSCA Section 8(e) Submission



89960000185

Dear Sir/Madam:

Elf Atochem North America, Inc. (Elf Atochem) has received the final report of a micronucleus study in mice and is submitting the results of this study to the Environmental Protection Agency (EPA) pursuant to Toxic Substances Control Act (TSCA) Section 8(e). Preliminary results from this study were submitted to the Agency by Elf Atochem on January 25, 1996. This study provides information on methyl mercaptan (CAS No. 74-93-1) and does not involve effects in humans. The title of the study report is Bone Marrow Micronucleus Assay in Male and Female Swiss-Webster Mice Following Acute Nose-Only Inhalation Exposure to Methyl Mercaptan.

Nothing in this letter is considered to be confidential business information of Elf Atochem.

In this study, mice were exposed to methyl mercaptan for six hours by nose-only inhalation at 114, 258 or 512 ppm, and were sacrificed at 24, 48 and 72 hours after exposure for evaluation of micronucleus formation in the bone marrow. Although a statistically significant increase in micronucleated erythrocytes was observed in male mice 24 hours after exposure to 512 ppm methyl mercaptan, the biological significance of this increase is considered equivocal since the increase was slight, the control group had a micronucleus frequency lower than the historical control mean at the laboratory, and the response was observed at a level that was fatal.

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TSCA 8(e) Submission  
Methyl Mercaptan  
July 12, 1996  
Page 2

In conclusion, although the increases in micronucleus frequency observed were weak, methyl mercaptan met the criteria established in the protocol for a positive response in the mouse bone marrow erythrocyte micronucleus assay.

Results from the study report will be incorporated into the Elf Atochem Material Safety Data Sheet for methyl mercaptan.

Further questions regarding this submission may be directed to me at (215) 419-5890.

Sincerely,



Debra Randall, D.A.B.T.  
Product Safety Manager

# SRI International

Final Report - July 9, 1996

## BONE MARROW MICRONUCLEUS ASSAY IN MALE AND FEMALE SWISS- WEBSTER MICE FOLLOWING ACUTE NOSE-ONLY INHALATION EXPOSURE TO METHYL MERCAPTAN

SRI Project No. LSC-6370-070  
SRI Study No. MC20-95

Prepared for:

Elf Atochem North America, Inc.  
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Richard A. Winegar, Ph.D.  
Director, Cytogenetics Program  
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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M020-95

QUALITY ASSURANCE UNIT  
Final Report and  
Conflict of Interest Statement

The Quality Assurance Unit of SRI International assures that the study-- Bone Marrow Micronucleus Assay in Male and Female Swiss-Webster Mice following Acute Nose-Only Inhalation Exposure to Methyl Mercaptan, Study No. M020-95-- has been reviewed for adherence to Good Laboratory Practice regulations as set forth by the U.S. Environmental Agency (40 CFR 792) and the Organization for Economic Cooperation and Development.

The following inspections were conducted during this study:

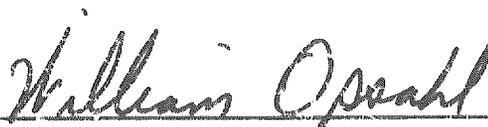
<u>Phase Inspected</u>	<u>Date of Inspection</u>	<u>Date Findings Reported to Management/Study Director</u>
Exposure	10-09-95	10-10-95
Raw Data and Draft Report	01-21-96	04-04-96
	04-02-96	04-04-96
	04-03-96	04-04-96
Final Report Verification	07-09-96	07-09-96

This statement certifies that the personnel listed below participated in the inspections and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Ann Griffin

William P. Opsahl

Pamela A. Pallakoff

  
\_\_\_\_\_  
William P. Opsahl, Ph.D.  
Manager, SRI Quality Assurance Unit

  
\_\_\_\_\_  
Date

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study M020-95

### GOOD LABORATORY PRACTICE STANDARDS COMPLIANCE STATEMENT

The study described in this report was conducted in compliance with the Good Laboratory Practice (GLP) regulations specified in 40 CFR 792 for the Toxic Substances Control Act, with the following exceptions:

1. The analyses of contaminant levels in the animal feed and animal drinking water were conducted outside of SRI and thus were beyond SRI's control. SRI cannot assure that those analyses were conducted according to GLP standards or whether the original data for those analyses have been archived.
2. The Standard Operating Procedures for the use of the gas chromatograph used to determine the concentrations of the test article in the inhalation exposure environments had been written but not yet received final approval at the time the study was conducted.
3. Purity, identity, and stability were the responsibility of the Sponsor (for the test article) and are not included in this report. The supplier (for the control article) provided characterization of the positive control.
4. The computer hardware and software used for determination and recording of environmental conditions were not validated. During the course of the definitive experiment, there was a failure of the computer system that resulted in the irreversible loss of data. There was also a human error that resulted in the unrecoverable loss of environmental conditions data previously collected.
5. The distribution and quantitative use of the test article were not documented. Because of the physical properties of the test article (a liquified gas), the pressure on the gas cylinder would not be an indicator of the amount of test article contained in the cylinder. Thus, the cylinder pressure was neither measured nor recorded.
6. No characterization or analysis of the negative control air was performed at SRI.

  
Richard Winegar  
Study Director

7/9/96  
Date

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**Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study M020-95**

**APPROVAL SIGNATURES**

Written by: Kathleen O'Loughlin 7/9/96  
Kathleen O'Loughlin Date  
Cell Biologist

Written by: Robert C. Baldwin 6/17/96  
Robert C. Baldwin, Ph.D., D.A.B.T. Date  
Inhalation Toxicologist

Approved by: Richard Winegar 7/9/96  
Richard Winegar, Ph.D. Date  
Study Director

Report Final: 7/9/96

**SRI International  
Toxicology Laboratory  
333 Ravenswood Ave.  
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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study M020-95

### SUMMARY

The genotoxic potential of nose-only inhalation exposure of methyl mercaptan to induce micronucleus formation in bone marrow erythrocytes was determined in Swiss-Webster mice.

A dose range finding experiment was performed at doses of 112, 374, or 570 ppm methyl mercaptan for a single 6 hour nose-only inhalation exposure, and sacrificed approximately 72 hours after exposure. Clinical signs observed included shallow breathing at 112 ppm, and shallow breathing and hypoactivity at 374 and 570 ppm. Two male mice were found dead at 570 ppm during the exposure period. No statistically significant polychromatic erythrocyte (PCE) suppression was observed in any dose group in either bone marrow or peripheral blood. Based on the data, the following target exposure concentrations of methyl mercaptan were chosen for the definitive experiment for both sexes: 125, 250, and 500 ppm.

In the definitive experiment, 15 mice per sex per treatment group were exposed to methyl mercaptan by nose-only inhalation at 114, 258, or 512 ppm. Five mice per sex per group were sacrificed 24, 48, and 72 hours after the single 6 hour exposure and bone marrow was evaluated for cytotoxicity and micronucleus formation. An air-exposed control group of male and female mice and a urethane positive control group of male mice were treated similarly and evaluated concurrently with the methyl mercaptan-treated groups. Clinical observations included shallow breathing and hypoactivity at 258 and 512 ppm in all mice. Three female and two male mice were found dead at 512 ppm methyl mercaptan.

A statistically significant increase in micronucleated PCE was observed in male mice at 24 hr after exposure to 512 ppm of methyl mercaptan. Although statistically significant, the biological significance of this increase is equivocal since the control group had a micronucleus frequency lower than the SRI historical mean (0.08% vs. the laboratory mean historical frequency of 0.21%). Using the Cochran-Armitage test for a trend in binomial proportions, statistically significant increases in micronucleated PCE were observed in male mice at 24 and 48 hr and in female mice at 48 hr after exposure to methyl mercaptan.

In conclusion, although the increases in micronucleated frequencies observed are weak, methyl mercaptan meets the criteria established in the protocol for a positive response in the mouse bone marrow erythrocyte micronucleus assay.

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## INTRODUCTION

### STUDY OBJECTIVE

The objective of this study was to evaluate the ability of a single 6 hour nose-only inhalation exposure of methyl mercaptan to induce chromosomal or mitotic spindle abnormalities in bone marrow cells of treated mice, as indicated by an increased incidence of micronuclei in newly formed RNA-containing erythrocytes.

### REGULATORY COMPLIANCE

#### Testing Guidelines:

TSCA Guideline 40 CFR 798.1150, Subpart B for the inhalation exposure activities (where applicable), and TSCA Guideline 40 CFR 798.5395, Subpart F for the micronucleus sampling and evaluations.

OECD Guideline No. 403 for the inhalation exposure activities, and OECD Guideline No. 474 for the micronucleus sampling and evaluations.

#### GLP Regulations:

EPA, TSCA; 40 CFR 792

OECD, Good Laboratory Practice in the Testing of Chemicals (1982)

Animal Husbandry: NIH89-544

### TEST SYSTEM BACKGROUND

The micronucleus assay is an *in vivo* genotoxicity assay used to assess the clastogenic (i.e., chromosome-breaking) or mitotic spindle-damaging potential of chemicals. The target cell population is erythroblasts undergoing final DNA replication and mitosis before the main nucleus is expelled. The newly formed erythrocytes that result from this division can be identified and scored in smears prepared from either bone marrow (Schmid, 1976; Heddle et al., 1983; MacGregor et al., 1987) or peripheral blood (MacGregor et al., 1983).

Newly formed erythrocytes can be distinguished from mature erythrocytes by their higher RNA content, which fluoresces orange when stained with pyronin Y or acridine orange (MacGregor et al., 1983b; Hayashi et al., 1983). When chromosomes break or spindle abnormalities occur during the division of erythroblasts, acentric chromosomal fragments and abnormal and detached chromosomes lag behind during anaphase and are left in the cytoplasm of the daughter cells in the form of "micronuclei." Thus, scoring micronuclei in erythrocytes

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mice following acute nose-only inhalation exposure to methyl mercaptan  
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after dosing the animal with a chemical provides an assessment of the chemical's ability to induce these types of damage (Heddle et al., 1983).

The appropriate doses used in the micronucleus assay should include a maximum tolerated dose (MTD) for the duration of the treatment that is determined by toxicity and the ratio of polychromatic erythrocytes (PCEs) to total erythrocytes (RBCs) (MacGregor et al., 1987; WHO, 1985), unless solubility or dosing volume limits are reached. Moreover, the assessment of micronuclei should take into account any potential time lags caused by metabolic activation of the test article.

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### MATERIALS AND METHODS

#### TEST ARTICLE

Name: Methyl mercaptan (MeSH).  
CAS No.: 74-93-1.  
Lot No.: 449 BM 95B.  
Production date: 5/8/95.  
Physical properties: Extremely disagreeable odor; vapor pressure approx. 11 psig at 70°F.  
Density: 0.89, as per supplier.  
Source: Elf Atochem North America, Inc.  
Date of Receipt: 6/7/95  
Expiration date: 5/8/96, as per supplier.  
Purity and Stability: The identity, purity, stability, and composition of the test article is the responsibility of the Sponsor.  
Storage conditions: Building T, Room 4: 18-22°C; Room 12: 18.6-44.7°C.  
Amount received: Two steel cylinders containing an unspecified amount (approximately 150 ml/container, as per supplier).  
Disposition: Remaining unused portion of the test article will be returned to the Sponsor at the end of study, who will be responsible for maintaining an archive sample.

#### POSITIVE CONTROL ARTICLE

Name: Urethane.  
CAS No.: 51-79-6  
Lot No.: 123H0858.  
Purity: 99.9%, as per supplier.  
Appearance and physical state at room temperature: White powder.  
Stability: Stable, as per supplier.  
Received: November 3, 1994.  
Expiration: November 3, 1999.  
Storage conditions: Stored at room temperature (18-28°C) in a light proof plastic secondary container at the SRI Chemical Repository, Building M, Room 217.  
Supplier: Sigma Chemical Company  
Disposition: Retained as positive control at SRI Chemical Repository, Building M, Room 217.

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### INHALATION EXPOSURE EQUIPMENT

52-port, stainless steel, non-recirculating, nose-only, "flow-past" exposure units (Lab Products, Inc., Maywood, NJ; Model No. 70052-S). Described by W.C. Cannon, *et al.* 1983.

Animal restraining tubes obtained from CH Technologies (Westwood, NJ). Model CHT-247 for mice.

### GENERATION AND CLEAN UP OF THE EXPOSURE ATMOSPHERES

The desired concentrations of the test article in air were achieved by directing metered flow rates of the test article and compressed air into the opposing arms of a stainless steel tee. The gas mixture exiting from the side arm of the tee was directed into the top inlet of an inhalation exposure unit. The compressed air used to expose the negative control animals and used to dilute the test article was provided by an air compressor dedicated to supplying air to the inhalation exposure facility at SRI. Before initiation of animal studies in the laboratory, the compressed air supply was tested to determine the level of carbon monoxide, the most likely contaminant and the contaminant of most concern, in compressed air supplies. No carbon monoxide was detected at a minimum detection level of 5 ppm by use of Drager air sampling tubes.

### MONITORING EXPOSURE CONDITIONS

An IBM compatible Personal Computer with a 486 DX2 66 MHZ microprocessor using Microsoft WINDOWS, V3.1 (Microsoft Corp., Redmond, WA) was used with the software and hardware described below to monitor and record environmental data.

EXCEL for Windows, V5.0 was used for calculation of relative humidity from temperatures monitored by WorkBench PC for WINDOWS, V2.04 and Model DS-12-8-TC DATASHUTTLES (Strawberry Tree, Inc., Sunnyvale, CA)

A Model 605-3C Magnahelix (Dwyer Instruments Co., Michigan City, IN ), reporting pressure differential gauge was used to monitor the difference in pressure between the interior of each nose-only exposure unit and it's surrounding environment.

Temperature and relative humidity were monitored by using pairs of Type J

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### Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan SRI Study M020-95

thermocouples (Omega Model No. 5TC-TT-J-20-72, Omega Engineering, Inc., Stamford, CT). One thermocouple was used as is (i.e., dry); a second thermocouple (i.e., wet) was covered with cotton wicking that was kept wet by deionized water provided from a dedicated reservoir. The difference in temperatures between the wet and dry thermocouples and the dry temperature were used to determine the relative humidity by reference to a table of values derived from CRC (1955).

The temperature of the wet and dry thermocouples and the pressure differential were monitored every 2 sec. The value for each sensor was saved in the computer memory until 300 values had been collected (i.e., 10 min) from all sensors. From each block of 300 values, the mean, minimum, and maximum were determined and those statistics and the starting time of each 10-minute interval were saved to a permanent computer file. The monitoring procedure was begun before the first animal was connected to an exposure unit and continued until the next 10-min calculation period was completed after the last animal was removed from an exposure unit.

Because of the extremely low odor detection threshold of the test article and the absence of a chemical scrubber on the exhaust from the exposure unit enclosures, test article leakage around the animals produced by the small positive pressure inside each exposure unit had to be controlled. The leakage was controlled by enclosing each restraining tube connected to an exposure unit within a polyethylene bag (i.e., Ziploc Brand Storage Bag, Dow Brands L.P., Indianapolis, IN) sealed to the exposure unit with vinyl electrical tape. Because of a concern about the possibility of heat build up inside the bags, an additional thermocouple was installed for each of the exposure units; this thermocouple was held in place inside the plastic bag and above the restraining tube of one animal on each exposure unit. These thermocouples were monitored according to the same schedule used for the thermocouples described above.

The concentration of the test article in the exposure atmosphere was determined by collecting 0.5 mL-samples of the gaseous atmosphere by using 1.0-mL Pressure -Lcc® gas-tight syringes (Precision Sampling Corp., Baton Rouge, LA). The samples collected were injected individually into the injector port of a gas chromatograph. The details of the analysis and calibration procedures are described in Appendix A.

The concentration of the test article was determined in samples collected from a port in the top level of each exposure unit. Before introduction of animals, the homogeneity of the test article concentration was determined by analysis of samples collected from one of the four ports at each of the nine exposure-unit levels used for either monitoring the environment or exposure of animals.

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### INHALATION EXPOSURE PROCEDURES

The nose-only exposure units were configured so that the top nine levels of ports (4 ports per level) were used. The ports on the bottom four levels were sealed with rubber stoppers, and the supply tubes in those ports were replaced with steel rods; by eliminating exposure atmosphere supplied to those unused ports, the exposure atmosphere was provided only to those ports used for exposing animals or for monitoring the exposure atmosphere. When not in use, ports in the top nine levels were also sealed with rubber stoppers.

Shortly before initiation of inhalation exposures, each of the preselected mice was placed into an individual restraining tube. After a group of animals had been restrained, the animals in their tubes were transported to the nearby inhalation exposure room. The flow rate of the exhaust air was increased to produce a net negative relative pressure inside the exposure unit to minimize outward flow of the test article and potential contamination of the environment outside of the laboratory building during the animal loading process. At the appropriate times, a rubber stopper was removed from an exposure port, and a restraining tube containing a mouse was inserted rapidly. During the dose range finding assay, each tube and animal was sealed inside a plastic bag as they were inserted into an exposure port. During the definitive experiment, all animals in an exposure group were inserted into their respective ports before the process of sealing the bags was begun. The difference in procedures was necessitating by the different logistic constraints of the larger group size used in the definitive assay. The time of insertion of each animal or group of three or four animals was recorded. After all of the bags had been sealed for an exposure unit, the exhaust flow rate was reduced to produce a slight net positive pressure inside the exposure unit. Animals were examined at least hourly and a gas sample was collected approximately once per hour during the 6-hr exposure periods. Because of the logistic limitations of placing mice in restraining tubes and sealing the tubes on the exposure units into plastic bags, there were often significant time differences between the exposure initiation of one group and the next; thus each treatment group was exposed according to its own schedule.

At the end of the 6-hr exposure period for each treatment group, the exhaust air flow was again increased to produce a negative pressure inside an exposure unit compared to the immediate external air pressure. The plastic bags were opened, and the animals in their restraining tubes were removed from the exposure unit and examined in their tubes. After all the animals in a treatment group had been removed, they were returned to their original animal holding room and placed into clean cages. Animals were housed no more than five of the same sex per cage until sacrifice.

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### ANIMAL HUSBANDRY

#### TEST SYSTEM SPECIFICATION

##### Dose Range Finding Experiment

Swiss-Webster mice, 16 males and 16 females, born on August 1, 1995, were received by the SRI Laboratory Animal Medicine Department (LAMD) from Simonsen Laboratories on September 19, 1995. On arrival, mice of the same sex were randomly placed in animal cages (10 per cage). The weights of the male mice ranged from 23.9 to 25.0 g, and those of the female mice ranged from 24.5 to 25.8 g at the time of treatment. Twelve male and twelve female mice, 7.5 weeks of age, were used for the dose range finding experiment initiated on September 22, 1995.

##### Definitive Experiment

Swiss-Webster mice, 78 males and 62 females, born on August 14, 1995, were received by the SRI LAMD from Simonsen Laboratories on October 2, 1995. On arrival, mice of the same sex were randomly placed in animal cages (10 per cage). The weights of the male mice ranged from 22.9 to 28.1 g, and those of the female mice ranged from 22.6 to 26.7 g at the time of treatment. Seventy-five male and 60 female mice, 8 weeks of age, were used for the definitive experiment initiated on October 9, 1995.

#### TEST SYSTEM IDENTIFICATION

Animals were randomized by using an IBM PC computer-generated, weight sort randomization form (definitive experiment only), and uniquely identified by ear punch. Before inhalation exposures, the animals were consecutively assigned to treatment groups (i.e., animals numbered one through eight were the first eight animals used in the study). A color-coded card attached to the outside of each cage listed the study number, sex, animal number, test article, concentration level, start date, and sacrifice time. In order to be able to identify animals during the inhalation exposure periods, their identification numbers were also written on their tails in indelible ink.

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### SUPPLIER

Simonsen Laboratories  
1180C Day Road  
Gilroy, CA 95020.

### QUARANTINE

Mice received on September 19, 1995 were quarantined for 6 days and released to the study on September 25, 1995 by Director of the SRI LAMD. Mice received on October 2, 1995 were quarantined for 7 days and released to the study on October 9, 1995 by Director of the SRI LAMD. Composite parasitology examinations performed by CVD, INC. (Consolidated Veterinary Diagnostics, Inc., 2825 KOVR Dr., W. Sacramento, CA, 95605) on September 20, and October 3, 1995 were negative, and no evidence of adverse clinical signs was observed during the quarantine period.

### ANIMAL ROOM ENVIRONMENTAL CONDITIONS

Rooms:	SRI Building T, Rooms T6i (dose range finding experiment) and T61 (definitive experiment).
Temperature range:	68-79°F (during normal housing, not exposure).
Humidity range:	18-65% (during normal housing, not exposure).
Light cycle:	12 hours light/12 hours dark.
Ventilation rate:	10 room volumes per hour.
Degree of recirculated air used:	None.
Cage specification:	Mice were housed no more than 10 to a cage before exposure and no more than 5 to a cage after exposure. 22" x 12.5" x 8" polycarbonate cages with hardwood-chip bedding were used throughout the study.

There were no alterations in environmental conditions that could be expected to adversely affect the results of this study.

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**FEED AND WATER SUPPLY**

- Feed: Purina Certified Rodent Chow #5002 *ad libitum*, except during inhalation exposures.  
Purina Mills, Inc., St. Louis, MO.  
Lot Nos. MAR20952A, JUNE28953A, and MAY24951B.
- Water: UV purified/deionized water *ad libitum*, except during inhalation exposures. Water purity is analyzed quarterly, and results are filed by SRI LAMD.

Feed and water were withheld during the time the animals were in restraining tubes. Based on the results of the feed and water analyses, there were no known contaminants in the water or feed that could be expected to alter the results of this study.

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### STUDY DESIGN

#### STUDY DATES

Study initiation: June 9, 1995.  
Study completion: July 9, 1996.

Experimental start date: September 25, 1995.  
Experimental end date: October 12, 1995.

#### TREATMENT AND SACRIFICE

##### Dose Range Finding Experiment

This preliminary dose range finding experiment was performed to determine the appropriate concentration levels for the definitive experiment and to define the effects of methyl mercaptan on the development of erythrocytes in bone marrow. The route of exposure models a potential route of human exposure to the test article.

Mice were treated with a single 6 hour nose-only inhalation exposure to methyl mercaptan. Three mice per sex per treatment group were individually weighed and exposed to test-article concentrations of 112, 374, and 570 ppm (target concentrations were 100, 350, or 600 ppm, respectively). An air-exposed control group, consisting of three male and three female mice, was treated similarly and concurrently with the test article-exposed groups. Approximately 72 hours after the inhalation exposure, final body weights were taken, and peripheral blood was sampled and smears prepared. Animals were then anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight), and euthanized by cervical dislocation. Bone marrow was collected and smears were prepared from each surviving mouse.

Based on the results of the dose range finding experiment, the high concentration for the definitive experiment was selected based on the following criteria:

1. it was to be the minimum concentration which caused a 50 to 80% suppression of the PCE/RBC ratio relative to the negative control,

or if no suppression of the PCE/RBC ratio was observed,

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2. it was to be the minimum concentration which caused animal death or compound-related signs of toxicity,

or, if no death or observable signs of toxicity occurred,

3. it was to be the maximum practical concentration that could be administered, as determined by physical bulk and solubility.

### Definitive Experiment

The treatment groups for the definitive experiment are shown in Table 1. Mice were exposed for a single 6-hour nose-only inhalation period to the test article. Five mice per sex per treatment level were weighed individually and exposed to test article concentrations of 114, 258, or 512 ppm (target concentrations were 125, 250, and 500 ppm, respectively). A vehicle control group was exposed similarly and concurrently to compressed air from the same source supplying the dilution air used for the three test article-exposed groups. A group of 15 male mice was treated by gavage with 300 mg/kg of urethane in water on the same day as the inhalation exposure was conducted. Urethane was diluted in sterile water before dose administration. Animals were anesthetized by carbon dioxide inhalation and euthanized by cervical dislocation. Bone marrow smears then were prepared from each mouse.

Table 1  
EXPERIMENTAL DESIGN OF THE DEFINITIVE EXPERIMENT

Treatment	Total No. Mice Treated/(Sacrificed) <sup>a</sup>	
	Male	Female
Air Control	15(15)	15(15)
Methyl mercaptan		
114 ppm	15(15)	15(15)
258 ppm	15(15)	15(15)
512 ppm	15(13) <sup>b</sup>	15(12) <sup>c</sup>
Urethane		
300 mg/kg	15(15)	0(15)

<sup>a</sup>Five mice of each sex were sacrificed 24, 48, or 72 hours after exposure.

<sup>b</sup>Three of five mice were sacrificed 72 hours after exposure because of two mortalities.

<sup>c</sup>Four of five mice were sacrificed 24 hours after exposure because of one mortality; three of five mice were sacrificed 72 hours after exposure because of two mortalities.

**Negative Control:** Air.

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**Positive Control:** Urethane (300 mg/kg in sterile water) was administered to male mice only. The purpose of the positive control was to ensure that the assay procedures were properly performed.

### PERIPHERAL BLOOD ANALYSIS

Peripheral blood smears were analyzed from the dose range finding experiment. Blood samples were obtained by snipping approximately 1/16 in. off the tip of the animal's tail. The blood from each mouse was blotted on three clean, prelabeled microscope slides, spread, air-dried, fixed in absolute methanol for 5 minutes, and stored until coded and stained. Slides from each test animal were examined visually, and the two slides with the most uniform preparation were coded and one coded slide was then stained with acridine orange (Hayashi et al., 1983). Unstained slides were filed but not used.

### BONE MARROW ANALYSIS

Bone marrow smears were analyzed in both the dose range finding and the definitive experiments. Femurs from each mouse was removed and flushed gently with 0.2 mL of fetal bovine serum (FBS) into 0.5 mL of FBS in a 1.5-mL conical tube. Cells were concentrated by centrifugation, then resuspended in a volume of supernatant, approximately equal to the pellet volumes. The sample from each mouse was transferred to three clean, prelabeled microscope slides, spread, air-dried, fixed in absolute methanol for 5 minutes, and stored until coded and stained. Slides from each test animal were visually examined, coded, and the one slide with the most uniform preparation was stained with acridine orange (Hayashi et al., 1983). Unstained slides were filed but not used.

### DATA COLLECTION

Slides for micronucleus evaluation were coded by an individual not involved in the microscopic evaluation using random-letter codes generated by an SRI-developed software package on an IBM PC program (VIVOBOK). Slide labels were printed directly from the computer.

### CYTOLOGICAL ANALYSIS

Peripheral blood smears and bone marrow smears were evaluated by using epifluorescence microscopy at a magnification of 630X. In the dose range finding experiment, peripheral blood smears and bone marrow smears were analyzed for the number of RNA-positive (polychromatic) erythrocytes (PCEs) in at least 1000 and 200 erythrocytes,

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respectively, per animal. In the definitive assay, two parameters were determined in the bone marrow smears: (1) the number of micronucleated PCEs in at least 1000 PCEs per animal, which provides an index of chromosomal damage, and (2) the number of PCEs in at least 1000 erythrocytes per animal, which provides an index of cytotoxicity.

The criteria used for identifying micronuclei are those described by Schmid (1976), with the additional requirement that the micronuclei exhibit the bright yellow fluorescence characteristic of acridine orange stain. The data from a given slide were registered directly to an IBM PC data file during scoring (using the SRI-developed software package MNSCORE). After analysis, the slides were decoded and the data summarized by using a decoding program in an IBM PC (using the SRI-developed software package MNSUM). Individual animal data summaries for the definitive experiment are included in Appendix B.

## DATA ANALYSIS AND INTERPRETATION

### Criteria for a Valid Assay

The data from this assay were considered acceptable if (1) the frequency of micronucleated cells in the air control group was within the normal historical range, (2) the administration of the urethane positive control article resulted in a statistically significant elevation of micronucleated cells, and (3) at least three surviving animals of each sex in two or more treatment groups showed a ratio of PCEs to total erythrocytes in bone marrow greater than or equal to 0.1.

### Statistical Tests

The results of the analyses of the exposure concentrations of the test articles were analyzed using means and standard deviations calculated by use of EXCEL 5.0c for Windows (Microsoft Corporation, Redmond, WA). The results of the analyses of the gas standards were evaluated using linear regression analysis (EXCEL 5.0c) with the concentration as the independent variable and the peak area as the dependent variable.

For analysis of micronucleus frequency, the data were analyzed separately for each sex. The ratio of micronucleated PCEs to total PCEs and the ratio of PCEs to total erythrocytes in percentages were calculated for each animal.

The statistical significance of differences in the percentage of PCEs to total erythrocytes among methyl mercaptan-treated and air control groups was evaluated by using

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the Kruskal-Wallis analysis of variance on ranks (calculated by using the SRI-developed software program KRUSKAL in an IBM PC).

The frequency of micronucleated PCEs statistically analyzed using the Cochran-Armitage test (using the SRI developed software COCHRAN) for trend in binomial proportions, to determine if a significant dose-response relationship was present, and the normal test for equality of binomial proportions (Kastenbaum and Bowman, 1970) to determine if individual treatment groups were statistically different from those from controls (using the SRI-developed software package MNSUM). These tests and their rationale are discussed in the ASTM Standard Guide for Conduct of Micronucleus Assays in mammalian Bone Marrow Erythrocytes and other publications (ASTM Committee, 1988; Margolin et al., 1983).

### Interpretation of Response

**Positive.** The test article was considered positive if the incidence of micronucleated PCEs was significantly higher than that in the air control group ( $p < 0.05$ ) in either (1) two different treatment groups from one experiment, (2) at a single treatment level if confirmed by a separate experiment (e.g., an increased frequency of micronucleated cells in the dose range finding experiment), or (3) a positive, dose-related increase in the incidence of micronucleated cells.

**Negative.** The test article was considered negative if the criteria for a positive or inconclusive response were not met.

**Inconclusive.** The results were considered inconclusive if there was reason to believe that the concentrations of the test article selected for evaluation were inappropriate (e.g., excessive toxicity) or if a statistically significant elevation in the frequency of micronucleated PCEs was observed in only one treatment group and the dose-response trend was not significant.

### AMENDMENTS TO THE PROTOCOL

Two modifications were made to the original protocol. Protocol Amendment Number 1 modified the following items: storage conditions of the test article, method for assuring correct dosing, proposed study schedule for the dose range finding experiment, and record retention at SRI. Protocol Amendment Number 2 addressed the study schedule for the definitive experiment, and specified the target concentrations for exposure in the definitive experiment. The study protocol and amendments are included as Appendix C.

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### DEVIATIONS FROM THE PROTOCOL

During the dose range finding experiment, the coefficient of variation (CV) for the low concentration exceeded 10%, and the ranges for the low and mid concentrations in the definitive experiment exceeded 10%.

The data system collected and analyzed environmental conditions every 10 minutes rather than every 15 minutes.

Because of logistic limitations, the exposure atmospheres in the dose range finding and definitive experiments could not be sampled and analyzed at least hourly.

In the animal rooms, the humidity ranged from 18-65%. Humidity excursions occurred beyond those recommended by the NIH Guideline (40-70%) for approximately 117 hours.

In the definitive experiment, mice were euthanized by carbon dioxide inhalation rather than sodium pentobarbital overdose and cervical dislocation.

No quarantine necropsies were performed on either shipment of mice for preliminary health evaluation.

During the exposure, plastic bags were used to control leakage of the test article in the animal restraining tubes (see Monitoring Exposure Conditions above).

These deviations should have no affect on the outcome of the study or the integrity of the data generated.

### KEY PERSONNEL

Richard Winegar	Program Director, Study Director
Robert Baldwin	Program Director, Inhalation Toxicology
Kathleen O'Loughlin	Cell Biologist
Kathleen Stewart	Microbiologist
Carolyn Reed	Director LAMD

### OUTSIDE SCIENTISTS

No outside scientists were used in the conduct of this study.

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### ARCHIVES AND REPORTS TO BE MAINTAINED

The original copy of the Final Report and the bound study records, including all raw data, computer-generated data sheets, the protocol, data disk, relevant communications and supporting documents will be stored at the SRI Records Center, located in Building B, for the period of time required by the applicable regulatory guidelines. Microscope slides will be retained in the SRI International Tissue Archive until submission of the final report. Following that time, the slides will be transferred to CVD, and will be stored there for at least ten years. Parasitology records will be stored in the archive facility of the performing laboratory (CVD, INC. 3911 West Capital Ave., Sacramento, CA 95606) for at least 10 years. The Sponsor will be asked about further storage or disposition of the retained materials after the ten-year storage period.

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### RESULTS AND DISCUSSION

#### DOSE RANGE FINDING EXPERIMENT

For this experiment, three mice per sex per treatment group received a single 6-hour nose-only inhalation exposure to methyl mercaptan at 112, 374, and 570 ppm. A control group, consisting of three male and three female mice, received air only. Mice were observed daily from the start of treatment until death or sacrifice. The concentration ranges for the low- and mid-concentrations exceeded the protocol criterion of 10%. These deviations are judged not to have had a significant adverse effect on the study (See Table A-2).

The mean body weights recorded immediately before exposure and immediately before euthanasia are summarized in Table 2. Comparing the differences between terminal and pre-exposure body weights of each of the treatment groups at each of the sacrifice times reveals no significant differences.

Clinical signs observed included shallow breathing at the fourth hour of exposure at 112 ppm, shallow breathing at the third hour of exposure at 374 and 570 ppm with hypoactivity at the mid and high dose levels in all mice when observed after completion of exposure. Two male mice were found dead near the end of the second hour and during the sixth hour of exposure at 570 ppm. Any mouse showing clinical signs appeared normal on Day 2. Surviving mice were sacrificed approximately 72 hours after the inhalation exposure, and cytotoxicity was determined based on the ratio of RNA-positive erythrocytes (PCEs) to total red blood cells (RBCs).

The number of PCEs among total RBCs was counted in both peripheral blood and bone marrow smears to estimate the frequency of PCEs among erythrocytes. These data are summarized in Tables 3 and 4, respectively. No significant PCE suppression was observed in any of the methyl mercaptan treatment groups when compared to the air control group in either peripheral blood or bone marrow.

Based on the criteria specified in the study protocol, the target concentrations selected, in consultation with the Sponsor, for the definitive experiment were 125, 250, and 500 ppm of methyl mercaptan for both sexes.

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### DEFINITIVE EXPERIMENT

#### Conditions of Exposure

For this experiment, fifteen mice per sex per treatment group were treated with a single 6-hour nose-only inhalation exposure to methyl mercaptan at 114, 258, or 512 ppm. An air-exposed control group and a urethane-treated positive control group (male mice only) were treated similarly and evaluated concurrently with the methyl mercaptan-exposed groups. Mice were observed daily from the start of treatment until death or sacrifice.

Across the 6-hr exposure period for each treatment group, there was no noticeable trend in the test article concentrations (Table A-6). The coefficients of variation across time were 7.1, 4.3, and 3.0% for the low, mid, and high concentrations, respectively. This degree of variability is within the  $\pm 10\%$  specified in the protocol. Logistics limited the frequency of gas sample collection and analysis during the first part of the exposure session. As a result, the sampling frequency deviated from the hourly requirement of the protocol. This deviation did not substantially affect the validity of the study.

#### In-Life Responses of the Animals

Clinical observations in this experiment included shallow breathing and hypoactivity at the fourth and fifth hours, respectively, of exposure at 258 ppm in all mice. All mice at 258 ppm appeared normal on Day 2 and on all subsequent experiment days. Shallow breathing at the third and fourth hours of exposure, and hypoactivity at the fifth hour of exposure were observed at 512 ppm in all mice. One female mouse was found dead after 2 hours of exposure at 512 ppm, and two female and two male mice were found dead at 512 ppm on Day 2. All surviving mice at 512 ppm appeared normal on Day 2 and on all subsequent experiment days. Mice treated with 114 ppm methyl mercaptan, air control, or urethane appeared normal throughout the experiment.

#### Post-Mortem Evaluations

Mice were evaluated for toxicity and micronucleus formation in bone marrow erythrocytes. Erythrocytes in bone marrow from treated mice were examined, and the percent of PCEs among total RBCs and of micronucleated PCEs among PCEs were determined. The results from the definitive experiment are presented in Tables 5 and 6.

The percentages of PCEs among RBCs in groups treated with methyl mercaptan did not differ significantly from those of the air control groups in any of the dose groups for

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either sex by the non-parametric Kruskal-Wallis analysis of variance on ranks. Analysis of ranks compares all methyl mercaptan treatment groups and controls together; therefore individual treatment groups were not compared to their specific controls.

In bone marrow, male mice treated with air control averaged background micronucleus incidences at 0.08, 0.20, and 0.12% at the 24-, 48-, and 72-hr harvests, respectively. Female mice treated with air control averaged background micronucleus incidences at 0.16, 0.20, and 0.16% at the 24-, 48-, and 72-hour harvests, respectively. The percentage of micronucleated PCEs of male mice treated with urethane were approximately 9, 3, and 2 times greater, respectively, than that of the air control groups at the 24-, 48-, and 72-hour harvests.

The binomial proportions test (Kastenbaum and Bowman) found a statistically significant difference between male mice treated with 512 ppm methyl mercaptan when compared to the respective air control group at the 24-hr harvest. Although statistically significant, the biological significance of this increase is equivocal since the control group had a micronucleus frequency lower than the SRI historical mean (0.08% vs. the laboratory mean historical frequency of 0.21%). Additionally, it is important to note that at 512 ppm methyl mercaptan five out of thirty mice died.

Using the Cochran-Armitage test for a trend in binomial proportions ( $p \leq 0.05$ ), significant increases in micronucleated PCE were observed in male mice at 24 and 48 hr and in female mice at 48 hr after exposure to methyl mercaptan.

The binomial proportions test found statistically significant differences between urethane-treated groups when compared to their respective air control groups at the 24- and 48-hour harvests. At 72 hours, urethane showed a slightly elevated frequency of micronucleated PCEs when compare to the air control group.

The data from this assay was considered acceptable because the frequency of micronucleated PCEs in the air control groups was within the SRI normal historical range (see Appendix D), the administration of urethane positive control resulted in a statistically significant elevation of micronucleated cells, and there were at least three surviving animals of each sex in two or more treatment groups with a PCE/RBC ratio greater than or equal to 0.1 in two or more treatment groups.

In conclusion, although the increases in micronucleated frequencies observed are weak, methyl mercaptan meets the criteria established in the protocol for a positive response in the mouse bone marrow erythrocyte micronucleus assay.

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Table 2

DOSE RANGE FINDING EXPERIMENT IN MALE AND FEMALE SWISS-WEBSTER MICE TREATED WITH A SINGLE EXPOSURE OF METHYL MERCAPTAN: MEAN BODY WEIGHTS

MALE MICE

Treatment	Conc. (ppm)	Time (hr) <sup>a</sup>	(n) <sup>b</sup>	Initial Body Wt (g)		Final Body Wt (g)		Weight Gain <sup>d</sup> (%)	
				Mean ± S.D.	(n) <sup>c</sup>	Mean ± S.D.	(n) <sup>c</sup>	Mean ± S.D.	(n) <sup>c</sup>
Control, air	0.0	72	3	29.6 ± 1.6	3	30.7 ± 1.3	3	3.66 ± 2.05	3
MeSH	112.0	72	3	30.2 ± 1.7	3	29.8 ± 2.7	3	-1.21 ± 3.57	3
MeSH	374.0	72	3	31.7 ± 0.6	3	30.7 ± 1.9	3	-2.95 ± 5.24	3
MeSH	570.0	72	3	30.7 ± 1.1	1	31.1	1	-1.88	1

FEMALE MICE

Treatment	Conc. (ppm)	Time (hr) <sup>a</sup>	(n) <sup>b</sup>	Initial Body Wt (g)		Final Body Wt (g)		Weight Gain <sup>d</sup> (%)	
				Mean ± S.D.	(n) <sup>c</sup>	Mean ± S.D.	(n) <sup>c</sup>	Mean ± S.D.	(n) <sup>c</sup>
Control, air	0.0	72	3	26.6 ± 1.0	3	28.2 ± 1.6	3	5.96 ± 2.04	3
MeSH	112.0	72	3	27.9 ± 0.2	3	27.8 ± 1.3	3	-0.35 ± 4.72	3
MeSH	374.0	72	3	28.3 ± 0.5	3	27.6 ± 0.9	3	-2.59 ± 1.79	3
MeSH	570.0	72	3	28.5 ± 0.3	3	29.2 ± 0.8	3	2.46 ± 2.80	3

<sup>a</sup>All surviving mice were sacrificed 72 hours after exposure.

<sup>b</sup>Number of treated animals.

<sup>c</sup>Number of surviving animals.

<sup>d</sup>Weight Gain (%) = 100 x (Final weight - Initial weight) / Initial weight.

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Table 3

DOSE RANGE FINDING EXPERIMENT IN MALE AND FEMALE SWISS-WEBSTER MICE TREATED WITH A SINGLE EXPOSURE OF METHYL MERCAPTAN: PERIPHERAL BLOOD DATA

Treatment	Concentration (ppm)	Sex	Time (hr) <sup>a</sup>	(n) <sup>b</sup>	PCE/RBC <sup>c</sup> (%) Mean ± S.E.
Control, air	0.0	Male	72	3	2.13 ± 0.43
MeSH	112.0	Male	72	3	1.84 ± 0.14
MeSH	374.0	Male	72	3	3.05 ± 0.13
MeSH	570.0	Male	72	1	2.21
Control, air	0.0	Female	72	3	1.33 ± 0.41
MeSH	112.0	Female	72	3	1.77 ± 0.54
MeSH	374.0	Female	72	3	2.12 ± 0.34
MeSH	570.0	Female	72	3	3.02 ± 0.28

<sup>a</sup>Surviving mice sacrificed approximately 72 hours after exposure.

<sup>b</sup>Number of surviving animals.

<sup>c</sup>PCE/RBC = polychromatic erythrocyte/red blood cell ratio.

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Table 4

DOSE RANGE FINDING EXPERIMENT IN MALE AND FEMALE SWISS-WEBSTER MICE TREATED WITH A SINGLE EXPOSURE OF METHYL MERCAPTAN: BONE MARROW DATA

Treatment	Concentration (ppm)	Sex	Time (hr) <sup>a</sup>	(n) <sup>b</sup>	PCE/RBC <sup>c</sup> (%) Mean ± S.E.
Control, air	0.0	Male	72	3	49.44 ± 1.35
MeSH	112.0	Male	72	3	51.50 ± 5.04
MeSH	374.0	Male	72	3	54.88 ± 5.26
MeSH	570.0	Male	72	1	56.85
Control, air	0.0	Female	72	3	56.52 ± 2.57
MeSH	112.0	Female	72	3	55.97 ± 4.78
MeSH	374.0	Female	72	3	54.07 ± 3.69
MeSH	570.0	Female	72	3	54.78 ± 4.35

<sup>a</sup>Surviving mice sacrificed approximately 72 hours after exposure.

<sup>b</sup>Number of surviving animals.

<sup>c</sup>PCE/RBC = polychromatic erythrocyte/red blood cell ratio.

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Table 5

DEFINITIVE EXPERIMENT IN MALE SWISS-WEBSTER MICE TREATED WITH A SINGLE EXPOSURE OF METHYL MERCAPTAN: MICRONUCLEUS FREQUENCY

Treatment	Conc. (ppm)	Time (hr) <sup>a</sup>	(n) <sup>b</sup>	PCE/RBC <sup>c</sup> (%) Mean ± S.E.	No. of PCE	PCE with MN	
						No.	(%) Mean ± S.E.
Control, air	0.0	24	5	57.10 ± 4.18	5012	4	0.08 ± 0.02
MeSH	114.0	24	5	57.61 ± 5.09	5014	10	0.20 ± 0.06
MeSH	258.0	24	5	52.27 ± 7.70	5011	10	0.20 ± 0.06
MeSH	512.0	24	5	45.46 ± 5.45	5012	13	0.26 ± 0.07 <sup>d</sup>
Urethane <sup>f</sup>	300.0	24	5	52.84 ± 5.69	5011	38	0.76 ± 0.14 <sup>e</sup>
Control, air	0.0	48	5	51.31 ± 4.83	5007	10	0.20 ± 0.05
MeSH	114.0	48	5	52.93 ± 6.80	5009	4	0.08 ± 0.02
MeSH	258.0	48	5	47.05 ± 3.88	5010	9	0.18 ± 0.09
MeSH	512.0	48	5	49.25 ± 2.22	5010	19	0.38 ± 0.11
Urethane <sup>f</sup>	300.0	48	5	40.09 ± 3.66	5008	27	0.54 ± 0.10 <sup>e</sup>
Control, air	0.0	72	5	52.76 ± 4.12	5012	6	0.12 ± 0.05
MeSH	114.0	72	5	44.16 ± 5.90	5011	6	0.12 ± 0.05
MeSH	258.0	72	5	42.56 ± 6.87	5013	6	0.12 ± 0.02
MeSH	512.0	72	3	52.35 ± 2.47	3007	4	0.13 ± 0.03
Urethane <sup>f</sup>	300.0	72	5	42.54 ± 3.08	5008	8	0.16 ± 0.05

<sup>a</sup>All surviving mice were sacrificed 24, 48, or 72 hours after exposure.

<sup>b</sup>Number of surviving animals.

<sup>c</sup>PCE/RBC = polychromatic erythrocyte/red blood cell ratio.

<sup>d</sup>Statistically different from control (p < 0.05) by test for binomial proportions.

<sup>e</sup>Statistically different from control (p < 0.01) by test for binomial proportions.

<sup>f</sup>Urethane was administered to mice at 300 mg/kg body weight.

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Table 6

DEFINITIVE EXPERIMENT IN FEMALE SWISS-WEBSTER MICE TREATED WITH A SINGLE EXPOSURE OF METHYL MERCAPTAN: MICRONUCLEUS FREQUENCY

Treatment	Conc. (ppm)	Time (hr) <sup>a</sup>	(n) <sup>b</sup>	PCE/RBC <sup>c</sup> (%) Mean ± S.E.	No. of PCE	PCE with MN	
						No.	(%) Mean ± S.E.
Control, air	0.0	24	5	47.39 ± 4.67	5005	8	0.16 ± 0.05
MeSH	114.0	24	5	52.83 ± 4.60	5010	6	0.12 ± 0.02
MeSH	258.0	24	5	53.68 ± 3.04	5005	9	0.18 ± 0.07
MeSH	512.0	24	4	49.98 ± 2.07	4006	11	0.27 ± 0.09
Control, air	0.0	48	5	49.06 ± 2.24	5010	10	0.20 ± 0.09
MeSH	114.0	48	5	56.58 ± 2.60	5009	6	0.12 ± 0.06
MeSH	258.0	48	5	58.73 ± 3.76	5006	8	0.16 ± 0.04
MeSH	512.0	48	5	50.07 ± 3.96	5007	15	0.30 ± 0.08
Control, air	0.0	72	5	59.41 ± 7.54	5008	8	0.16 ± 0.05
MeSH	114.0	72	5	55.66 ± 4.10	5007	8	0.16 ± 0.06
MeSH	258.0	72	5	52.46 ± 3.85	5009	9	0.18 ± 0.06
MeSH	512.0	72	3	51.96 ± 4.98	3005	5	0.17 ± 0.03

<sup>a</sup>All mice were sacrificed 24, 48, or 72 hours after exposure.

<sup>b</sup>Number of surviving animals.

<sup>c</sup>PCE/RBC = polychromatic erythrocyte/red blood cell ratio.

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**Appendix A**

**CLEAN UP, ANALYSIS, AND MONITORING  
OF EXPOSURE CONDITIONS**

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### I. Introduction

In order to confirm the concentrations of the test article in the inhalation exposure atmospheres to which the animals were exposed, samples of the atmospheres were collected and the concentrations of the test article determined. In order to verify the accuracy of those analyses, standard samples of known concentrations of the test article in air were prepared.

The temperature and relative humidity of the exposure atmospheres were determined over 10-minute increments beginning before the first animal was introduced to the exposure atmosphere and continued until after the last animal was removed. Because the protocol specified the number of determinations over 15-minute increments, the use of 10-minute increments constitutes a technical deviation from the protocol.

In order to assure that external "clean" air was not drawn into an exposure unit from around an animal and thereby possibly diluting the desired exposure atmosphere, the rates of supply and exhaust for each exposure unit was controlled to produce a net slight positive pressure inside each exposure unit compared to the surrounding environment. The pressure differential of each exposure unit was determined over 10-minute increments beginning before the first animal was introduced to the exposure atmosphere and continued until the last animal was removed.

### II. Materials and Methods

#### A. Gas Chromatographic Conditions

Instrument:	Varian Model 3400 gas chromatograph (S/N 5627) equipped with a thermal conductivity detector and a Hewlett-Packard Model 3390A integrator
Detection:	Electron capture
Temperatures:	Column: 30°C Injector: 150°C Detector: 150°C
Carrier gas:	Nitrogen
Flow rate:	32 mL/min at a column head pressure of 32 psig. Flow rate was measured with a digital flow meter
Column:	1/8" X 10' aluminum packed with Haysep DB, 100/120 mesh (Alltech Associates, Inc., Deerfield, IL). Support was received in bulk, and the column was packed at SRI

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**Injections:** 0.5 mL using a 1.0 mL gas-tight syringe (Precision Corp., Baton Rouge, LA)

### B. Preparation of Methyl Mercaptan Standards

Samples of known concentrations of the test article in air were prepared fresh daily by injecting measured volumes of undiluted test article into sealed glass vessels of known volume. The glass vessels had been previously calibrated so that their volume was known to at least  $\pm 0.1\%$ . The vessels were sealed with Teflon® valves and were equipped with injection ports sealed with Teflon-lined silastic septa.

Before a sample of the test article was injected, each vessel was flushed with a stream of air to remove all detectable traces of any previous injections of the test article. Periodically, samples of the air in the standards vessels were analyzed for detectable levels of test article. If any level of test article were detected, the flushing procedure was repeated.

Samples of the test article were collected with either a 1.0-mL or 100- $\mu$ L gas tight syringe (Precision Sampling Corp., Baton Rouge, LA) from a metered stream of the test article. Sample volumes and standards vessel were selected to obtain test article concentrations ranging from 46 to 879m (v/v) for the definitive assay. The resulting concentrations are summarized below.

<u>Volume (mL)</u>		<u>Calculated Conc. (ppm)</u>
<u>MeSH</u>	<u>Dil. Vessel</u>	
0.25	5382.0	46
0.10	1009.0	99
0.20	345.1	580
0.10	361.2	277
0.30	341.3	879

Samples (0.5 mL) were collected from the vessels containing the gas standards and injected into the GC. The resulting peak areas (the dependent variable) and the calculated concentrations (the independent variable) were analyzed by linear regression analysis (Microsoft EXCEL 5.0, Microsoft Corp., Redmond, WA) with the intercept assumed to be zero. A series of gas standard samples were prepared and analyzed with each group of samples collected and analyzed from the exposure units. Each gas standard was sampled and analyzed in duplicate and sometimes in triplicate.

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### C. Determination of Test Article Concentrations in Exposure Atmospheres

Samples (0.5 mL) of the exposure atmospheres were collected from a sampling port on Level 1 (top most) of each exposure unit. These samples were injected into the GC and analyzed as described above. From the slope (units of peak area units per ppm) of the linear regression analysis of the gas standards and the peak areas of samples from the exposure atmospheres, the test article concentrations were calculated.

$$\text{Conc. of MeSH} = (\text{peak area})/(\text{regression slope})$$

The results of the individual analyses of the exposure atmospheres are summarized in Table A-1.

### D. Determination of the Homogeneity of Test Article Concentrations in the Exposure Units

During the animal exposure sessions, the concentration of the test article was determined in samples collected from a port in the top level of each exposure unit. However, before introduction of animals, the homogeneity of the test article concentration was determined by analysis of a single sample collected from one of the four ports at each of the nine exposure-unit levels to be used for either monitoring or exposure of animals. The results of those determinations are summarized in Tables A-2 and A-6.

### E. Determination of Temperature and Relative Humidity in Exposure Atmospheres

Temperature and relative humidity were monitored by using pairs of Type J thermocouples (Omega Model No. 5TC-TT-J-20-72, Omega Engineering, Inc., Stamford, CT). One thermocouple was used as is (i.e., dry); a second thermocouple (i.e., wet) was covered with cotton wicking that was kept wet by deionized water provided from a dedicated reservoir. The difference in temperatures between the wet and dry thermocouples and the dry temperature were used to determine the relative humidity by reference to a table of values derived from CRC (1955).

The output signals from the thermocouples were monitored by using a data monitoring and collection software (WorkBench PC for Windows, V2.04, Strawberry Tree, Inc., Sunnyvale, CA) and analog to digital conversions units (Model DS-12-8-TC DATASHUTTLES, Strawberry Tree, Inc.) operating on an IBM-compatible Personal Computer with a 486 DX2 66 MHZ microprocessor. Relative humidity was calculated by using direct data links to Microsoft EXCEL V5.0 (Microsoft Corp., Redmond, WA). Both EXCEL and WorkBench operated under Microsoft WINDOWS, V3.1 (Microsoft Corp.).

The temperature of the wet and dry thermocouples were monitored every 2 sec. The values for each sensor was saved in the computer memory until 300 values had been collected (i.e., 10 min) from

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all sensors. From each block of 300 values, the mean, minimum, and maximum were determined and those statistics saved to a permanent computer file. The monitoring procedure was begun before the first animal was introduced to an exposure unit and continued until the next 10-min calculation period was completed after the last animal was removed from an exposure unit. The mean temperatures and relative humidities experienced by the animals are summarized in Tables A-3, A-4, A-7 and A-8.

At a time not recorded, it was noticed that the humidity data display was not being updated. Upon investigation, it was discovered that for an unknown reason, the data link between WorkBench and Excel had been lost, and the Excel file that calculated relative humidity had been corrupted. An uncorrupted copy of the Excel file was loaded, but the data link could not be re-established without halting the data collection. Because the temperature of the wet and dry thermocouples was being monitored and recorded independently from the humidity data, data collection was continued with the knowledge that the relative humidity could be calculated later. However, at some time after 3 hr 40 min into this first data collection session, data collection was halted as a result of some as yet undetermined problem with the software or hardware. The computer was restarted, the first set of data storage files was saved, and a second monitoring session was initiated. The system halted again approximately 1 hr 20 min later. After an approximate 17-min delay during which time the cause of the problem was investigated unsuccessfully, the system was restarted, and the second set of data files was saved as just described. The third data collection session lasted approximately 1 hr. Because of the very late hour (20:30 hr) and the level of fatigue of the staff, it was decided that the third set of data files would be left in their original state until the next morning in order to reduce the possibility of loss due to human error. However, upon attempting to exit the WorkBench data collection software, the wrong icon was activated. Instead of activating the upper left most icon to exit the program, the adjacent icon to start data collection was activated. The error was noted almost immediately, and data collection was halted within 1 sec. Nevertheless, restarting data collection irreversibly deleted the last hour of data collected during the exposures.

The lost humidity data was replaced by using the mean dry and wet thermocouple data and an intact copy of the Excel file to calculate relative humidity. Because only mean wet temperatures were recorded, only mean relative humidity values could be calculated. During this procedure, it was discovered that temperatures were not recorded for a 2 hr 46 min period beginning at approximately 3 hr 40 min into the first data collection session; the reason for this data loss is unknown. These missing values and the environmental data lost at the end of the study could not be recalculated or retrieved. These deviations from the protocol are judged not to have had a significant adverse effect on the scientific integrity of the study, because during the time that the environmental data were being recorded reliably, there was relatively little variability in the values. During the times that data was lost, there were no known alterations in the operating parameters of the supply or exhaust systems for the exposure units. It is reasonable, therefore, to extrapolate the conditions to the lost final hour and to interpolate across the missing 2 hr 46 min. On this basis, the exposure environment for the air-exposed negative

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control group remained at, but did not exceed 70°F for most of the exposure period; none of the other groups exceeded the temperature or humidity limits specified in the protocol.

### F. Determination of Pressure Differentials in Exposure Units

The difference in pressure between the interior of a nose-only exposure unit and the surrounding environment was measured by using a Model 605-3 C Magnahelix® (Dwyer Instruments Co., Michigan City, IN) Differential Pressure Indicating Transmitter. The electrical signal from each Magnahelix was monitored by using the hardware and software system described above for temperature monitoring. The magnitude of the pressure differential is not important as long as the differential is greater than zero, i.e., the pressure inside each exposure unit containing a concentration of the test article is greater than the pressure of the external environment. As long as the pressure differential is greater than zero, the direction of air flow around each animal will be from the interior of the exposure unit out towards the external environment. If the direction of "leakage" air flow were the reverse, then external air would dilute the exposure atmosphere and the exposure concentration experienced by the animals would not be known. The pressure differentials measured during the animal studies are summarized in Tables A-5 and A-9.

### G. Clean Up of the Exposure Atmospheres

Because of the very low odor detection threshold of the test article (i.e., 11 ppb according to information provided by the Sponsor), the exhaust from the exposure units could not be vented to the environment without treatment. Based on recommendation by the Sponsor, an exhaust clean up system was developed and used on the exhaust from each of the three exposure units used for dilutions of the test article. Each clean up system consisted of a first stage recirculating scrubber in which 5 M sodium hydroxide in water was sprayed over a column of approximately 4 in. by 12 in. of 3/4-in. hollow polypropylene spheres. The exhaust air flowed counter to the direction of the spray and the gravity flow of the caustic. The exhaust from the scrubber entered the bottom of an absorber unit containing approximately 800 to 1000 mL of 3/16-in. alumina beads coated with potassium permanganate (Carasorb® Air Filtration Media, Carus Chemical Co., LaSalle, IL). The top exhaust from the absorber unit entered the facility's air exhaust system where it was subsequently diluted approximately 1000-fold before exiting the laboratory building. The caustic solution in the scrubbers and the Carasorb were replaced before the range-finding assay and again before the definitive assay. Because the analytical method used to determine exposure concentrations had a limit of quantification of approximately 20 to 40 ppm and a limit of visual detection of a gas chromatographic peak of approximately half of that level, this analytical method would not be applicable to determining the very low levels of test article that might escape into the exhaust line. Therefore a more sensitive but qualitative detection method was used. When the prototype clean up system was operating with approximately 15 L/min of air containing 600 to 700 ppm

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MeSH, a 1-mL sample was collected from the air exhausting the adsorber unit. The needle was removed from the end of the gas-tight syringe and the gaseous contents of the syringe was expelled. No mercaptan odor could be detected, indicating that the MeSH concentration must be in the low parts-per-billion range. With the additional subsequent dilution of this air stream, it was decided that the cleanup system was acceptably effective.

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**Table A-1**  
**Concentrations of MeSH at the Exposure**  
**Ports Used to Expose Animals and Monitor Conditions**

<b>Port ID<sup>b</sup></b>	<b>MeSH Concentrations (ppm)</b>		
	<b>EU 1<sup>a</sup></b>	<b>EU 2</b>	<b>EU 3</b>
	<b>Group 2</b>	<b>Group 3</b>	<b>Group 4</b>
1A	128	355	597
2A	116	340	620
3A	122	338	629
4A	123	344	613
5A	119	360	600
6A	123	340	610
7A	123	347	617
8A	129	340	619
9A	124	355	616
<b>Mean</b>	<b>123.0</b>	<b>346.6</b>	<b>613.4</b>
<b>SD</b>	<b>4.0</b>	<b>8.2</b>	<b>10.0</b>
<b>CV<sup>c</sup></b>	<b>3.3%</b>	<b>2.4%</b>	<b>1.6%</b>
<b>Min.</b>	<b>116</b>	<b>338</b>	<b>597</b>
<b>Max.</b>	<b>129</b>	<b>360</b>	<b>629</b>
<b>Range<sup>d</sup></b>	<b>10.6%</b>	<b>6.3%</b>	<b>5.2%</b>

a. Exposure unit number, assigned during construction of the facility.

b. Non-radial designates the vertical level of the exposure port. Level 1 is the top level nearest the gas inlet. Level 9 is the lowest level used and nearest the gas exhaust. The letter is an arbitrary designation of the radial position of each port.

c. Coefficient of variation = 100% x SD / mean.

d. Range = 100% x (max - min) / mean.

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**Table A-2**  
**Exposure Concentrations of MeSH During**  
**the Range-Finding Study**

Group 1		Group 2		Group 3		Group 4	
ET <sup>a</sup>	Conc. <sup>b</sup>	ET	Conc.	ET	Conc.	ET	Conc.
0:00		0:00		0:00		0:00	
1:40	0	1:09	79	0:17	353	1:34	559
4:38	0	1:43	108	0:54	379	1:17	598
6:06		2:50	83	2:00	391	2:19	575
		3:20	136	3:01	383	3:24	574
		3:49	134	3:50	361	4:26	527
		4:41	108	4:39	389	4:38	578
		5:49	133	5:19	362	5:42	577
		6:06		6:05		6:06	
<b>Mean</b>	<b>0</b>		<b>112</b>		<b>374</b>		<b>570</b>
<b>SD</b>	<b>0</b>		<b>24</b>		<b>15</b>		<b>22</b>
<b>CV<sup>c</sup></b>			<b>21.6%</b>		<b>4.0%</b>		<b>3.9%</b>
<b>N</b>	<b>2</b>		<b>7</b>		<b>7</b>		<b>7</b>

a. Elapsed time (hr:min) from start of the exposure period for each group of animals.

b. Concentration of MeSH in ppm (v/v).

c. Coefficient of Variation = 100% x SD/mean.

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Table A-3  
Temperature (°C) of Exposure Atmospheres During the Range-Finding Study

Group 1				Group 2				Group 3				Group 4			
ET <sup>a</sup>	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.
-0:07	19.6	19.4	20.0	-0:05	20.6	20.3	20.9	-0:05	20.1	19.9	20.5	-0:04	20.5	20.3	20.9
0:03	9.6	-94.6 <sup>b</sup>	21.3	0:05	20.6	20.4	20.8	0:05	20.2	19.8	20.6	0:06	20.5	20.3	20.8
0:13	19.8	19.6	20.1	0:15	20.7	20.4	20.9	0:15	20.3	20.1	20.5	0:16	20.5	20.3	20.8
0:23	19.8	19.6	20.1	0:25	20.7	20.4	20.9	0:25	20.3	20.0	20.6	0:26	20.5	20.2	20.8
0:33	19.7	19.5	20.0	0:35	20.6	20.3	20.9	0:35	20.3	19.9	20.5	0:36	20.7	20.4	20.9
0:43	19.8	19.5	20.0	0:45	20.5	20.2	20.9	0:45	20.4	20.1	20.6	0:46	20.7	20.4	20.9
0:53	19.7	19.5	20.1	0:55	20.6	20.3	21.0	0:55	20.4	20.1	20.6	0:56	20.7	20.4	20.9
1:03	19.8	19.4	20.2	1:05	20.6	20.4	20.8	1:05	20.4	20.1	20.7	1:06	20.8	20.5	21.1
1:13	19.8	19.5	20.0	1:15	20.6	20.3	20.9	1:15	20.5	20.3	20.8	1:16	20.8	20.5	21.0
1:23	19.9	19.6	20.1	1:25	20.6	20.2	20.9	1:25	20.5	20.2	20.7	1:26	20.8	20.5	21.1
1:33	19.9	19.5	20.1	1:35	20.7	20.4	21.0	1:35	20.5	20.2	20.7	1:36	20.7	20.5	20.9
1:43	20.0	19.7	20.3	1:45	20.7	20.5	21.0	1:45	20.5	20.2	20.6	1:46	20.7	20.5	20.9
1:53	20.0	19.7	20.2	1:55	20.7	20.4	20.9	1:55	20.5	20.3	20.8	1:56	20.7	20.4	20.9
2:03	20.0	19.7	20.3	2:05	20.8	20.5	21.0	2:05	20.5	20.1	20.7	2:06	20.7	20.4	21.1
2:13	20.1	19.8	20.4	2:15	20.8	20.5	21.0	2:15	20.5	20.2	20.9	2:16	20.7	20.5	21.0
2:23	20.1	19.9	20.3	2:25	20.8	20.6	21.2	2:25	20.5	20.2	20.7	2:26	20.7	20.4	21.0
2:33	20.1	19.9	20.4	2:35	20.8	20.6	21.1	2:35	20.5	20.2	20.7	2:36	20.7	20.4	21.0
2:43	20.1	19.8	20.3	2:45	20.9	20.6	21.2	2:45	20.5	20.2	20.8	2:46	20.7	20.5	21.0
2:53	20.2	19.9	20.3	2:55	20.8	20.6	21.1	2:55	20.5	20.2	20.8	2:56	20.8	20.5	21.1
3:03	20.1	19.8	20.4	3:05	20.9	20.6	21.2	3:05	20.6	20.3	20.9	3:06	20.7	20.4	20.9
3:13	20.2	19.9	20.5	3:15	20.8	20.5	21.0	3:15	20.4	20.3	20.6	3:16	20.4	-9.4 <sup>b</sup>	20.8
3:23	20.1	19.8	20.4	3:25	20.8	20.5	21.1	3:25	20.5	20.1	20.7	3:26	20.7	20.4	21.0
3:33	20.2	19.9	20.5	3:35	20.8	20.4	21.1	3:35	20.5	20.3	20.8	3:36	20.7	20.5	21.1
3:43	20.2	19.9	20.4	3:45	20.9	20.7	21.2	3:45	20.5	20.2	20.8	3:46	20.8	20.6	21.0
3:53	20.2	20.0	20.5	3:55	21.0	20.6	21.3	3:55	20.5	20.3	20.7	3:56	20.8	20.5	21.0
4:03	20.3	19.9	20.6	4:05	20.9	20.6	21.1	4:05	20.5	20.2	20.8	4:06	20.7	20.4	20.9
4:13	20.1	19.9	20.3	4:15	20.8	20.5	21.0	4:15	20.4	20.2	20.5	4:16	20.7	20.3	20.9
4:23	20.1	19.8	20.4	4:25	20.8	20.5	21.1	4:25	20.5	20.1	20.8	4:26	20.6	20.4	21.3
4:33	20.1	19.9	20.4	4:35	20.8	20.5	21.1	4:35	20.3	20.0	20.5	4:36	20.6	20.3	20.8
4:43	20.1	19.8	20.3	4:45	20.8	20.6	21.0	4:45	20.2	20.0	20.5	4:46	20.5	20.1	20.9
4:53	20.1	19.9	20.4	4:55	20.9	20.6	21.2	4:55	20.3	19.9	20.6	4:56	20.5	20.2	20.6
5:03	20.1	19.8	20.4	5:05	20.8	20.5	21.0	5:05	20.2	20.0	20.4	5:06	20.5	20.2	20.8
5:13	20.0	19.8	20.3	5:15	20.8	20.5	21.2	5:15	20.2	19.9	20.5	5:16	20.7	20.4	21.0
5:23	20.1	19.8	20.5	5:25	20.6	20.3	20.9	5:25	20.4	20.1	20.7	5:26	20.5	20.3	20.7
5:33	20.0	19.7	20.2	5:35	20.6	20.3	20.8	5:35	20.3	20.1	20.5	5:36	20.6	20.3	20.8
5:43	19.9	19.7	20.2	5:45	20.5	20.1	20.9	5:45	20.4	20.2	20.8	5:46	20.6	20.3	21.0
5:53	19.9	19.5	20.3	5:55	20.5	20.3	20.8	5:55	20.5	20.3	20.8	5:56	20.7	20.5	21.0
6:03	20.0	19.7	20.2	6:05	20.6	20.3	20.9	6:05	20.6	20.2	21.1				
Mean	19.7				20.7				20.4				20.7		
Min.		19.4				20.1				19.8				20.1	
Max.			21.3				21.3				21.1				21.3
N	38				38				38				37		

a. Elapsed Time. Indicates the 10-min interval beginning at the indicated time.

b. Value excluded from statistics because it was judged to have resulted from an electronic artifact.

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Table A-4  
Relative Humidity (%) of Exposure Atmospheres During the Range-Finding Study

Group 1				Group 2				Group 3				Group 4			
ET <sup>a</sup>	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.
-0:07	62.9	59.0	66.0	-0:05	62.9	60.0	64.0	-0:05	62.5	59.0	63.0	-0:04	66.3	63.0	67.0
0:03	65.2	59.0	75.0	0:05	60.8	59.0	64.0	0:05	62.8	59.0	64.0	0:06	66.1	63.0	67.0
0:13	63.1	59.0	66.0	0:15	60.4	56.0	64.0	0:15	62.4	59.0	63.0	0:16	66.4	63.0	67.0
0:23	63.0	63.0	66.0	0:25	60.6	59.0	64.0	0:25	62.0	59.0	64.0	0:26	66.5	63.0	67.0
0:33	63.1	62.0	66.0	0:35	60.6	59.0	64.0	0:35	62.2	59.0	63.0	0:36	66.8	64.0	67.0
0:43	63.0	59.0	66.0	0:45	60.8	59.0	64.0	0:45	62.8	59.0	64.0	0:46	66.7	64.0	67.0
0:53	63.0	59.0	66.0	0:55	61.0	59.0	64.0	0:55	62.5	59.0	64.0	0:56	66.6	64.0	67.0
1:03	62.9	59.0	66.0	1:05	60.8	59.0	64.0	1:05	62.7	59.0	64.0	1:06	66.3	64.0	67.0
1:13	62.9	59.0	63.0	1:15	61.1	56.0	64.0	1:15	63.0	60.0	64.0	1:16	66.9	64.0	67.0
1:23	62.7	59.0	66.0	1:25	60.8	59.0	64.0	1:25	62.0	59.0	64.0	1:26	66.7	64.0	67.0
1:33	62.8	59.0	66.0	1:35	61.2	60.0	64.0	1:35	63.3	59.0	64.0	1:36	66.6	64.0	67.0
1:43	62.9	59.0	66.0	1:45	61.2	60.0	64.0	1:45	62.7	59.0	64.0	1:46	66.7	64.0	67.0
1:53	62.8	59.0	66.0	1:55	61.6	59.0	64.0	1:55	63.2	59.0	64.0	1:56	66.8	64.0	67.0
2:03	62.9	59.0	63.0	2:05	61.1	59.0	64.0	2:05	62.4	59.0	64.0	2:06	66.7	64.0	67.0
2:13	62.8	59.0	63.0	2:15	61.3	60.0	64.0	2:15	62.9	59.0	64.0	2:16	66.8	64.0	67.0
2:23	62.4	59.0	63.0	2:25	61.2	60.0	64.0	2:25	62.2	60.0	64.0	2:26	66.8	64.0	67.0
2:33	62.9	59.0	63.0	2:35	61.3	60.0	64.0	2:35	62.7	59.0	64.0	2:36	66.8	64.0	67.0
2:43	62.8	59.0	63.0	2:45	61.3	56.0	64.0	2:45	62.5	59.0	64.0	2:46	66.8	64.0	67.0
2:53	62.8	59.0	63.0	2:55	61.0	60.0	64.0	2:55	63.0	59.0	64.0	2:56	66.8	64.0	67.0
3:03	62.3	59.0	63.0	3:05	61.3	56.0	64.0	3:05	63.2	59.0	64.0	3:06	66.5	64.0	67.0
3:13	62.7	59.0	63.0	3:15	60.9	60.0	64.0	3:15	62.7	59.0	64.0	3:16	67.1	64.0	71.0
3:23	61.9	59.0	63.0	3:25	61.5	60.0	64.0	3:25	61.9	59.0	64.0	3:26	66.8	64.0	67.0
3:33	62.0	59.0	63.0	3:35	61.6	60.0	64.0	3:35	62.1	59.0	64.0	3:36	66.8	64.0	67.0
3:43	62.2	59.0	66.0	3:45	61.4	60.0	64.0	3:45	62.2	59.0	64.0	3:46	66.8	64.0	67.0
3:53	62.4	59.0	63.0	3:55	60.7	60.0	64.0	3:55	62.1	59.0	64.0	3:56	66.9	64.0	67.0
4:03	62.7	59.0	64.0	4:05	60.5	60.0	64.0	4:05	62.5	60.0	64.0	4:06	66.6	64.0	67.0
4:13	62.6	59.0	63.0	4:15	61.0	60.0	64.0	4:15	62.8	59.0	64.0	4:16	66.7	64.0	67.0
4:23	61.8	59.0	63.0	4:25	61.2	60.0	64.0	4:25	61.6	59.0	64.0	4:26	66.9	64.0	71.0
4:33	61.7	59.0	63.0	4:35	61.3	60.0	64.0	4:35	61.7	59.0	63.0	4:36	66.5	64.0	67.0
4:43	60.1	59.0	63.0	4:45	61.2	60.0	64.0	4:45	62.6	59.0	63.0	4:46	66.4	63.0	67.0
4:53	59.4	59.0	63.0	4:55	60.7	56.0	64.0	4:55	62.3	59.0	64.0	4:56	66.2	63.0	67.0
5:03	59.2	55.0	63.0	5:05	60.3	60.0	64.0	5:05	62.7	59.0	63.0	5:06	66.3	63.0	70.0
5:13	59.2	59.0	63.0	5:15	61.2	60.0	64.0	5:15	62.1	59.0	63.0	5:16	66.9	66.0	70.0
5:23	59.0	55.0	63.0	5:25	61.0	59.0	64.0	5:25	62.7	59.0	64.0	5:26	66.0	66.0	71.0
5:33	58.9	55.0	63.0	5:35	61.5	59.0	64.0	5:35	63.1	59.0	66.0	5:36	67.8	66.0	71.0
5:43	59.0	55.0	61.0	5:45	62.3	59.0	64.0	5:45	63.8	63.0	67.0	5:46	67.3	66.0	71.0
5:53	58.9	55.0	63.0	5:55	61.4	56.0	64.0	5:55	63.8	63.0	67.0	5:56	68.2	62.0	71.0
6:03	58.8	55.0	63.0	6:05	62.0	52.0	64.0	6:05	64.2	60.0	67.0				
Mean	61.9				61.2				62.6				66.8		
Min.		55.0				56.0				59.0				63.0	
Max.			75.0				64.0				67.0				71.0
N	38				38				38				37		

a. Elapsed Time. Indicates the 10-min interval beginning on the indicated date.

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
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Table A-5  
Pressure Differential (in. H<sub>2</sub>O) of Exposure Environment During the Range-Finding Study

Group 1				Group 2				Group 3				Group 4			
ET <sup>a</sup>	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.
-0:07	-0.15	-0.15	-0.05	-0:05	-0.13	-0.14	-0.05	-0:05	-0.07	-0.08	-0.06	-0:04	0.06	-0.10	0.11
0:03	-0.01	-0.15	0.07	0:05	0.00	-0.14	0.05	0:05	-0.07	-0.08	-0.05	0:06	0.10	0.09	0.11
0:13	0.06	0.05	0.07	0:15	0.03	0.02	0.05	0:15	0.04	-0.07	0.06	0:16	0.09	0.02	0.11
0:23	0.06	0.05	0.07	0:25	0.03	0.02	0.04	0:25	0.05	0.03	0.06	0:26	0.03	0.02	0.04
0:33	0.05	0.05	0.07	0:35	0.03	0.02	0.05	0:35	0.05	0.03	0.06	0:36	0.03	0.02	0.04
0:43	0.05	0.04	0.07	0:45	0.03	0.02	0.04	0:45	0.05	0.04	0.06	0:46	0.03	0.02	0.04
0:53	0.05	0.04	0.07	0:55	0.03	0.02	0.04	0:55	0.05	0.04	0.06	0:56	0.03	0.02	0.04
1:03	0.05	0.04	0.06	1:05	0.03	0.02	0.05	1:05	0.05	0.04	0.06	1:06	0.03	0.02	0.04
1:13	0.05	0.05	0.06	1:15	0.03	0.02	0.05	1:15	0.05	0.04	0.06	1:16	0.03	0.02	0.04
1:23	0.05	0.04	0.07	1:25	0.04	0.03	0.06	1:25	0.05	0.05	0.06	1:26	0.03	0.02	0.05
1:33	0.05	0.04	0.07	1:35	0.04	0.03	0.05	1:35	0.05	0.04	0.07	1:36	0.05	0.05	0.06
1:43	0.05	0.03	0.07	1:45	0.04	0.03	0.05	1:45	0.05	0.03	0.06	1:46	0.05	0.04	0.06
1:53	0.04	0.02	0.06	1:55	0.05	0.04	0.06	1:55	0.05	0.03	0.06	1:56	0.05	0.04	0.05
2:03	0.04	0.03	0.06	2:05	0.04	0.03	0.06	2:05	0.05	0.03	0.08	2:06	0.03	0.02	0.05
2:13	0.04	0.03	0.05	2:15	0.05	0.03	0.06	2:15	0.06	0.05	0.08	2:16	0.03	0.02	0.05
2:23	0.04	0.04	0.05	2:25	0.04	0.03	0.06	2:25	0.06	0.04	0.07	2:26	0.04	0.03	0.04
2:33	0.04	0.03	0.06	2:35	0.05	0.03	0.06	2:35	0.05	0.03	0.07	2:36	0.04	0.02	0.10
2:43	0.04	0.03	0.06	2:45	0.04	0.03	0.06	2:45	0.04	0.03	0.06	2:46	0.04	-0.02	0.07
2:53	0.04	0.03	0.06	2:55	0.04	0.03	0.05	2:55	0.04	0.03	0.06	2:56	0.04	0.03	0.04
3:03	0.05	0.03	0.06	3:05	0.05	0.03	0.06	3:05	0.04	0.03	0.07	3:06	0.03	0.02	0.04
3:13	0.05	0.03	0.07	3:15	0.04	0.02	0.06	3:15	0.05	0.03	0.06	3:16	0.03	-0.03	0.07
3:23	0.05	0.04	0.06	3:25	0.04	0.03	0.06	3:25	0.04	0.03	0.06	3:26	0.03	0.02	0.04
3:33	0.05	0.04	0.06	3:35	0.04	0.03	0.06	3:35	0.04	0.03	0.06	3:36	0.03	0.03	0.04
3:43	0.04	0.04	0.07	3:45	0.06	0.04	0.08	3:45	0.04	0.03	0.06	3:46	0.05	0.02	0.07
3:53	0.04	0.03	0.06	3:55	0.05	0.04	0.07	3:55	0.04	0.02	0.06	3:56	0.04	0.01	0.07
4:03	0.04	0.04	0.06	4:05	0.04	0.03	0.07	4:05	0.04	0.02	0.05	4:06	0.04	-0.03	0.05
4:13	0.05	0.03	0.06	4:15	0.04	0.04	0.05	4:15	0.05	0.04	0.06	4:16	0.03	0.03	0.04
4:23	0.04	0.03	0.06	4:25	0.04	0.04	0.05	4:25	0.05	0.04	0.06	4:26	0.03	-0.04	0.11
4:33	0.05	0.04	0.06	4:35	0.04	0.03	0.05	4:35	0.06	0.05	0.07	4:36	0.04	0.03	0.04
4:43	0.05	0.04	0.05	4:45	0.04	0.03	0.09	4:45	0.05	0.04	0.07	4:46	0.04	0.03	0.04
4:53	0.05	0.04	0.07	4:55	0.05	0.03	0.07	4:55	0.05	0.04	0.06	4:56	0.04	0.02	0.11
5:03	0.05	0.04	0.07	5:05	0.05	0.04	0.08	5:05	0.05	0.04	0.06	5:06	0.06	0.05	0.07
5:13	0.06	0.05	0.07	5:15	0.05	0.04	0.07	5:15	0.05	0.03	0.06	5:16	0.05	0.02	0.13
5:23	0.05	0.04	0.07	5:25	0.05	0.04	0.07	5:25	0.04	0.02	0.05	5:26	0.04	0.02	0.05
5:33	0.06	0.04	0.07	5:35	0.05	0.04	0.06	5:35	0.04	0.03	0.05	5:36	0.04	0.04	0.05
5:43	0.05	0.03	0.08	5:45	0.04	0.02	0.05	5:45	0.04	0.03	0.06	5:46	-0.02	-0.10	0.10
5:53	-0.01	-0.15	0.06	5:55	-0.06	-0.14	0.05	5:55	0.05	0.04	0.06	5:56	-0.02	-0.10	-0.02
6:03	-0.14	-0.15	-0.02	6:05	-0.13	-0.14	-0.13	6:05	-0.01	-0.07	0.06				
Mean	0.04				0.03				0.04				0.04		
Min.		-0.15				-0.14				-0.08				-0.10	
Max.			0.08				0.09				0.08				0.13
N	36				36				36				37		

a. Elapsed Time indicates the 10-min interval beginning at the indicated time.

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
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**Table A-6**  
**Exposure Concentrations of MeSH During**  
**the Definitive Study**

Group 1		Group 2		Group 3		Group 4	
ET <sup>a</sup>	Conc. <sup>b</sup>	ET	Conc.	ET	Conc.	ET	Conc.
0:00		0:00		0:00		0:00	
4:06	0	2:03	111	1:21	262	0:44	491
6:06		2:55	125	2:14	247	1:11	525
		4:06	112	3:34	271	2:36	519
		5:16	106	4:33	256	3:39	495
		6:09		5:25	244	4:25	517
				5:57	269	5:12	525
				6:09		6:08	
Mean	0		113.8		258.2		511.9
SD			8.1		11.1		15.5
CV <sup>c</sup>			7.1%		4.3%		3.0%
Min			106.2		244.1		490.9
Max			125.3		271.5		525.0
Range <sup>d</sup>			16.8%		10.6%		6.7%

a. Elapsed time (hr: min) from start of the exposure period for each group of animals.

b. Concentration of MeSH in ppm (v/v).

c. Coefficient of Variation = 100% x SD/mean

d. Range = 100% x (max - min)/mean

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
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Table A-7  
Temperature (°C) of Exposure Atmospheres During the Definitive Study

Group 1				Group 2				Group 3				Group 4			
ET <sup>a</sup>	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.	ET	Mean	Min.	Max.
0:01	20.2	19.8	20.7	-0:01	20.7	20.5	21.1	-0:02	20.8	20.5	21.2	-0:02	20.9	20.7	21.3
0:11	20.5	20.1	20.8	0:09	21.2	20.8	21.5	0:08	21.0	20.6	21.4	0:08	21.1	20.8	21.4
0:21	20.6	20.4	20.9	0:19	21.4	21.1	21.6	0:18	21.6	21.3	21.8	0:18	21.3	21.0	21.6
0:31	20.8	20.5	21.1	0:29	21.4	21.2	21.7	0:28	21.8	21.5	22.0	0:28	21.5	21.3	21.8
0:41	21.1	20.7	21.3	0:39	21.5	21.2	21.9	0:38	21.9	21.6	22.1	0:38	21.7	21.4	21.9
0:51	21.1	20.7	21.3	0:49	21.8	21.6	22.1	0:48	21.9	21.6	22.2	0:48	21.6	21.5	21.8
1:01	21.0	20.8	21.2	0:59	21.6	21.4	22.0	0:58	21.9	21.7	22.2	0:58	21.6	21.4	21.8
1:11	21.0	20.7	21.1	1:09	21.7	21.4	22.0	1:08	22.0	21.8	22.2	b			
1:21	21.0	20.7	21.2	1:19	21.6	21.4	21.8	1:18	21.9	21.5	22.2	3:44	21.2	20.9	21.5
1:31	21.0	20.8	21.3	1:29	21.6	21.4	21.8	1:28	21.6	21.4	22.0	3:54	21.1	20.9	21.3
1:41	20.9	20.7	21.2	1:39	21.4	21.1	21.7	1:38	21.5	21.2	21.7	4:04	21.1	20.9	21.3
1:51	21.1	20.8	21.3	1:49	21.4	21.2	21.7	1:48	21.4	21.2	21.7	4:14	21.0	20.8	21.3
2:01	21.1	20.8	21.3	1:59	21.5	21.3	21.8	1:58	21.4	21.3	21.6	4:24	21.0	20.8	21.3
2:11	21.1	20.8	21.3	2:09	21.5	21.3	21.7	2:08	21.5	21.3	21.7	4:34	20.9	20.7	21.1
2:21	20.9	20.6	21.2	2:19	21.4	21.1	21.7	b				4:44	20.9	20.6	21.1
2:31	21.0	20.7	21.2	2:29	21.4	21.2	21.7	4:54	21.4	21.1	21.7	4:54	21.0	20.7	21.3
2:41	21.1	20.9	21.3	2:39	21.5	21.3	21.7	5:04	21.4	21.2	21.7	5:04	20.9	20.6	21.1
2:51	21.0	20.7	21.3	2:49	21.5	21.3	21.8	5:14	21.5	21.3	21.7	c			
3:01	21.0	20.7	21.2	2:59	21.5	21.2	21.7	5:24	21.4	21.1	21.8				
3:11	21.1	20.8	21.2	b				5:34	21.5	21.3	21.7				
3:21	21.1	20.9	21.4	5:45	21.3	20.9	21.6	5:44	21.5	21.3	21.7				
3:31	21.2	20.9	21.5	5:55	21.3	21.1	22.0	5:54	21.4	21.1	21.6				
3:41	21.3	21.0	21.5	6:05	21.4	21.1	21.7	6:04	21.5	21.0	21.8				
b				6:15	21.2	20.9	21.5	6:14	21.4	21.1	21.7				
6:27	21.0	20.5	21.5	6:25	21.1	20.8	21.3								
Mean	21.0				21.4				21.5				21.2		
Min.		19.8				20.5				20.5				20.6	
Max.			21.5				22.1				22.2				21.9
N	24				24				23				16		

a. Elapsed Time (hr:min). Indicates the 10-min interval beginning at the indicated time.

b. The data collection system failed because of unknown cause, resulting in an interruption in data collection between approx. 15:28 and 18:14 hours PDT.

c. An operator error inadvertently deleted data collected between approx. 15:59 and 20:09 hours PDT.

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**Table A-8**  
**Relative Humidity (%) of Exposure Atmospheres**  
**During the Definitive Study**

Group 1		Group 2		Group 3		Group 4	
ET <sup>a</sup>	Mean <sup>b</sup>	ET	Mean	ET	Mean	ET	Mean
0:01	70	-0:01	63	-0:02	63	-0:02	66
0:11	70	0:09	59	0:08	63	0:09	66
0:21	66	0:19	59	0:18	60	0:18	63
0:31	66	0:29	55	0:28	60	0:28	60
0:41	70	0:39	60	0:38	60	0:38	60
0:51	70	0:49	64	0:48	60	0:48	60
1:01	70	0:59	64	0:58	60	0:58	60
1:11	70	1:09	64	1:08	60	c	
1:21	70	1:19	64	1:18	60	3:44	64
1:31	70	1:29	64	1:28	60	3:54	64
1:41	70	1:39	63	1:38	60	4:04	64
1:51	70	1:49	63	1:48	63	4:14	64
2:01	70	1:59	64	1:58	63	4:24	63
2:11	70	2:09	64	2:08	60	4:34	64
2:21	70	2:19	63	c		4:44	64
2:31	70	2:29	63	4:54	62	4:54	63
2:41	70	2:39	64	5:04	61	5:04	63
2:51	70	2:49	64	5:14	61	d	
3:01	70	2:59	64	5:24	69		
3:11	70	c		5:34	60		
3:21	70	5:45	64	5:44	60		
3:31	70	5:55	64	5:54	59		
3:41	70	6:05	60	6:04	52		
c							
6:27	72						
Mean	69.8		62.5		60.6		63.0
SD	1.3		2.4		1.2		2.0
N	24		22		22		16
Min.	66		55		59		60
Max.	72		64		63		66
Range <sup>e</sup>	9.2%		14.4%		6.3%		9.5%

- a. Elapsed Time (hr:min). Indicates the 10-min interval beginning at the indicated time.
- b. Only mean values can be reported because a failure in the data collection system eliminated direct calculation and recording of relative humidity. The values shown were calculated from the mean wet and dry temperatures recorded separately.
- c. The data collection system failed because of unknown cause, resulting in an interruption in data collection between approx. 15:28 and 16:14 hours EDT.
- d. An operator error inadvertently deleted data collected between approx. 19:59 and 20:40 hours EDT.
- e. 100% x (Maximum - minimum)/mean

CLIENT PRIVATE

Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study M020-95

Table A-9  
Pressure Differential (in. H<sub>2</sub>O) of Exposure Environment During the Definitive Study

Group 1				Group 2				Group 3				Group 4			
ET	Mean	Min.	Max.												
0:01	-0.11	-0.15	-0.04	0:01	-0.11	-0.14	-0.04	0:02	-0.06	-0.09	-0.05	0:02	-0.05	-0.08	-0.02
0:11	-0.12	-0.15	-0.04	0:09	-0.06	-0.09	-0.04	0:08	-0.05	-0.06	-0.05	0:08	-0.03	-0.03	-0.02
0:21	-0.12	-0.15	-0.04	0:19	-0.07	-0.08	-0.05	0:18	-0.06	-0.06	-0.05	0:18	-0.03	-0.04	-0.03
0:31	-0.12	-0.15	-0.05	0:29	-0.08	-0.10	-0.06	0:28	-0.06	-0.06	-0.05	0:28	-0.04	-0.05	-0.03
0:41	0.03	-0.15	0.09	0:39	-0.06	-0.11	0.07	0:38	-0.03	-0.07	0.19	0:38	-0.01	-0.06	0.19
0:51	0.06	0.04	0.08	0:49	0.06	0.05	0.07	0:48	0.14	0.08	0.21	0:48	0.04	0.03	0.05
1:01	0.06	0.04	0.09	0:59	0.07	0.06	0.07	0:58	0.17	0.08	0.21	0:58	0.04	0.03	0.06
1:11	0.07	0.05	0.09	1:09	0.06	0.05	0.08	1:08	0.17	0.11	0.22	1:08	0.05	0.04	0.06
1:21	0.08	0.06	0.10	1:19	0.06	0.04	0.07	1:18	0.19	0.13	0.22	1:18	0.05	0.04	0.06
1:31	0.10	0.07	0.13	1:29	0.06	0.04	0.07	1:28	0.19	0.14	0.22	1:28	0.05	0.04	0.06
1:41	0.10	0.09	0.12	1:39	0.05	0.04	0.06	1:38	0.22	0.17	0.25	1:38	0.05	-0.03	0.08
1:51	0.10	0.08	0.12	1:49	0.05	0.05	0.07	1:48	0.19	0.07	0.23	1:48	0.03	-0.03	0.06
2:01	0.10	0.07	0.11	1:59	0.05	0.05	0.07	1:58	0.20	0.17	0.22	1:58	0.04	0.03	0.05
2:11	0.09	0.07	0.12	2:09	0.06	0.04	0.07	2:08	0.14	-0.05	0.22	2:08	0.04	0.03	0.05
2:21	0.10	0.08	0.13	2:19	0.06	0.05	0.07	2:18	0.07	0.05	0.08	2:18	0.03	0.03	0.05
2:31	0.12	0.10	0.13	2:29	0.06	0.05	0.08	2:28	0.08	0.06	0.09	2:28	0.03	0.02	0.08
2:41	0.11	0.08	0.13	2:39	0.07	0.05	0.08	2:38	0.08	0.06	0.10	2:38	0.03	0.03	0.05
2:51	0.09	0.05	0.14	2:49	0.08	0.07	0.08	2:48	0.08	0.06	0.10	2:48	0.03	0.03	0.04
3:01	0.05	0.03	0.08	2:59	0.07	0.06	0.09	2:58	0.08	0.07	0.09	2:58	0.03	0.03	0.04
3:11	0.06	0.03	0.08	3:09	0.08	0.07	0.09	3:08	0.07	0.05	0.09	3:08	0.03	0.03	0.05
3:21	0.07	0.05	0.09	3:19	0.07	0.05	0.10	3:18	0.09	0.07	0.09	3:18	0.04	0.03	0.05
3:31	0.08	0.06	0.10	3:29	0.07	0.06	0.09	3:28	0.08	0.05	0.09	b			
3:41	0.08	0.05	0.10	3:39	0.06	-0.04	0.11	3:38	0.08	0.07	0.10	3:44	0.04	0.03	0.05
3:51	0.06	0.03	0.09	3:49	0.08	0.06	0.10	3:48	0.07	0.03	0.09	3:54	0.04	0.04	0.05
4:01	0.06	0.03	0.09	3:59	0.07	0.05	0.09	3:58	0.07	0.05	0.08	4:04	0.05	0.04	0.05
4:11	0.07	0.04	0.09	4:09	0.08	0.05	0.10	4:08	0.06	-0.05	0.09	4:14	0.04	0.04	0.05
4:21	0.07	0.05	0.09	4:19	0.06	0.05	0.08	4:18	0.04	0.02	0.06	4:24	0.04	0.03	0.04
4:31	0.09	0.07	0.11	4:29	0.06	0.05	0.07	4:28	0.05	0.03	0.06	4:34	0.04	0.03	0.05
4:41	0.10	0.08	0.12	4:39	0.07	0.06	0.08	b				4:44	0.04	0.03	0.05
4:51	0.10	0.08	0.12	4:49	0.06	0.05	0.07	4:54	0.03	0.02	0.05	4:54	0.04	0.04	0.05
5:01	0.09	0.06	0.11	4:59	0.05	0.04	0.08	5:04	0.02	0.01	0.04	5:04	0.04	0.04	0.05
5:11	0.07	0.04	0.09	5:09	0.05	0.04	0.06	5:14	0.03	0.02	0.04	c			
5:21	0.07	0.06	0.09	5:19	0.04	0.03	0.06	5:24	0.01	0.00	0.03				
5:31	0.07	0.05	0.09	b				5:34	0.00	-0.01	0.02				
5:41	0.07	0.04	0.09	5:45	0.05	0.04	0.06	5:44	0.05	-0.01	0.11				
5:51	0.08	0.06	0.09	5:55	-0.01	-0.10	0.06	5:54	0.09	-0.06	0.13				
6:01	-0.07	-0.15	0.09	6:05	-0.04	-0.04	-0.04	6:04	-0.06	-0.07	-0.04				
b															
6:27	-0.03	-0.04	-0.03												
Mean	0.05			0.04				0.07				0.03			
Min.		-0.15				-0.14				-0.09				-0.08	
Max.			0.14				0.11				0.25				0.19
N	38			36				36				30			

a. Elapsed Time (hr:min). Indicates the 10-min interval beginning at the indicated time.  
 b. The data collection system failed because of unknown cause, resulting in an interruption in data collection between approx. 15:28 and 18:14 hours PDT.  
 c. An operator error inadvertently deleted data collected between approx. 19:59 and 20:40 hours PDT.

**CLIENT PRIVATE**

**Bone marrow erythrocyte micronucleus assay in Swiss-Webster  
mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study M020-95**

**Appendix B**

**INDIVIDUAL ANIMAL DATA SUMMARIES FOR  
THE DEFINITIVE MICRONUCLEUS EXPERIMENT**

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020M-95, Data File "M020M-95.msu"  
 Study No.: M020-95

Treatment: Control, air  
 Sacrifice: 24 hrs

Dose: 0.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN	
ZC	M	1004	643	64.04	1003	1	0.10	
TOTAL for Animal #:M020M-95-1				64.04			0.10	
ZL	M	1004	558	55.58	1002	0	0.00	
TOTAL for Animal #:M020M-95-2				55.58			0.00	
WD	M	1003	420	41.87	1003	1	0.10	
TOTAL for Animal #:M020M-95-3				41.87			0.10	
DP	M	1001	652	65.13	1002	1	0.10	
TOTAL for Animal #:M020M-95-4				65.13			0.10	
AY	M	1002	590	58.88	1002	-	0.10	
TOTAL for Animal #:M020M-95-5				58.88			0.10	
Dose group total:				Mean:	57.10	5012	4	0.08
				SE:	4.18			0.02

Treatment: MeSH  
 Sacrifice: 24 hrs

Dose: 114.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN	
ZH	M	1010	570	56.44	1004	0	0.00	
TOTAL for Animal #:M020M-95-6				56.44			0.00	
ES	M	1006	705	70.08	1002	2	0.20	
TOTAL for Animal #:M020M-95-7				70.08			0.20	
EK	M	1005	583	58.01	1002	2	0.20	
TOTAL for Animal #:M020M-95-8				58.01			0.20	
DJ	M	1015	648	63.84	1004	2	0.20	
TOTAL for Animal #:M020M-95-9				63.84			0.20	
KY	M	1003	398	39.68	1002	4	0.40	
TOTAL for Animal #:M020M-95-10				39.68			0.40	
Dose group total:				Mean:	57.61	5014	10	0.20
				SE:	5.09			0.06

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020M-95, Data File "M020M-95.msu"  
 Study No.: M020-95

Treatment: MeSH  
 Sacrifice: 24 hrs

Dose: 258.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN	
TJ	M	1004	481	47.91	1002	3	0.30	
TOTAL for Animal #:M020M-95-11				47.91			0.30	
EY	M	1006	303	30.12	1001	3	0.30	
TOTAL for Animal #:M020M-95-12				30.12			0.30	
ED	M	1004	462	46.02	1003	1	0.10	
TOTAL for Animal #:M020M-95-13				46.02			0.10	
DE	M	1002	759	75.75	1003	0	0.00	
TOTAL for Animal #:M020M-95-14				75.75			0.00	
DS	M	1004	618	61.55	1002	3	0.30	
TOTAL for Animal #:M020M-95-15				61.55			0.30	
Dose group total:				Mean:	52.27	5011	10	0.20
				SE:	7.70			0.06

Treatment: MeSH  
 Sacrifice: 24 hrs

Dose: 512.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN	
BR	M	1011	396	39.17	1003	4	0.40	
TOTAL for Animal #:M020M-95-16				39.17			0.40	
AS	M	1002	495	49.40	1003	3	0.30	
TOTAL for Animal #:M020M-95-17				49.40			0.30	
WW	M	1002	291	29.04	1002	1	0.10	
TOTAL for Animal #:M020M-95-18				29.04			0.10	
AA	M	1054	650	61.67	1002	1	0.10	
TOTAL for Animal #:M020M-95-19				61.67			0.10	
ZS	M	1004	482	48.01	1002	4	0.40	
TOTAL for Animal #:M020M-95-20				48.01			0.40	
Dose group total:				Mean:	45.46	5012	13	0.25
				SE:	5.45			0.07

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020M-95, Data File "M020M-95.msu"  
 Study No.: M020-95

Treatment: Urethane  
 Sacrifice: 24 hrs

Dose: 300.0 mg/kg  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
EM	M	1002	455	45.41	1002	9	0.90
TOTAL for Animal #:M020M-95-21				45.41			0.90
ZA	M	1002	722	72.06	1002	11	1.10
TOTAL for Animal #:M020M-95-22				72.06			1.10
ZR	M	1008	442	43.85	1002	6	0.60
TOTAL for Animal #:M020M-95-23				43.85			0.60
CC	M	1002	432	43.11	1003	3	0.30
TOTAL for Animal #:M020M-95-24				43.11			0.30
DT	M	1002	599	59.78	1002	9	0.90
TOTAL for Animal #:M020M-95-25				59.78			0.90
Dose group total:				Mean:	5011	38	0.76
				SE:	5.69		0.14

Treatment: Control, air  
 Sacrifice: 48 hrs

Dose: 0.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
TP	M	1002	480	47.90	1002	2	0.20
TOTAL for Animal #:M020M-95-26				47.90			0.20
WR	M	1013	705	69.60	1001	2	0.20
TOTAL for Animal #:M020M-95-27				69.60			0.20
DL	M	1002	481	48.00	1000	4	0.40
TOTAL for Animal #:M020M-95-28				48.00			0.40
EA	M	1001	409	40.86	1002	1	0.10
TOTAL for Animal #:M020M-95-29				40.86			0.10
ZZ	M	1002	503	50.20	1002	1	0.10
TOTAL for Animal #:M020M-95-30				50.20			0.10
Dose group total:				Mean:	5007	10	0.20
				SE:	4.83		0.05



**INDIVIDUAL ANIMAL DATA SUMMARIES**

Expt. #: M020M-95, Data File "M020M-95.msu"  
 Study No.: M020-95

Treatment: MeSE  
 Sacrifice: 48 hrs

Dose: 512.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
CZ	M	1004	527	52.49	1002	2	0.20
TOTAL for Animal #:M020M-95-41				52.49			0.20
CW	M	1002	439	43.81	1002	4	0.40
TOTAL for Animal #:M020M-95-42				43.81			0.40
TW	M	1004	558	55.58	1002	8	0.80
TOTAL for Animal #:M020M-95-43				55.58			0.80
TC	M	1006	452	44.93	1001	2	0.20
TOTAL for Animal #:M020M-95-44				44.93			0.20
BX	M	1003	496	49.45	1003	3	0.30
TOTAL for Animal #:M020M-95-45				49.45			0.30
Dose group total:		Mean:		49.25	5010	19	0.38
		SE:		2.22			0.11

Treatment: Urethane  
 Sacrifice: 48 hrs

Dose: 300.0 mg/kg  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
EW	M	1002	406	40.52	1001	9	0.90
TOTAL for Animal #:M020M-95-46				40.52			0.90
ZK	M	1004	324	32.27	1000	6	0.60
TOTAL for Animal #:M020M-95-47				32.27			0.60
TL	M	1003	448	44.67	1002	3	0.30
TOTAL for Animal #:M020M-95-48				44.67			0.30
HX	M	1004	321	31.97	1003	4	0.40
TOTAL for Animal #:M020M-95-49				31.97			0.40
AB	M	1003	512	51.05	1002	5	0.50
TOTAL for Animal #:M020M-95-50				51.05			0.50
Dose group total:		Mean:		40.09	5008	27	0.54
		SE:		3.66			0.10

**INDIVIDUAL ANIMAL DATA SUMMARIES**

Expt. #: M020M-95, Data File "M020M-95.msu"  
 Study No.: M020-95

Treatment: Control, air  
 Sacrifice: 72 hrs

Dose: 0.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
AC	M	1002	568	56.69	1003	2	0.20
TOTAL for Animal #:M020M-95-51				56.69			0.20
CD	M	1002	595	59.38	1002	2	0.20
TOTAL for Animal #:M020M-95-52				59.38			0.20
ZD	M	1002	423	42.22	1002	0	0.00
TOTAL for Animal #:M020M-95-53				42.22			0.00
CP	M	1004	622	61.95	1002	2	0.20
TOTAL for Animal #:M020M-95-54				61.95			0.20
CH	M	1003	437	43.57	1003	0	0.00
TOTAL for Animal #:M020M-95-55				43.57			0.00
Dose group total:		Mean:		52.76	5012	6	0.12
		SE:		4.12			0.05

Treatment: MeSE  
 Sacrifice: 72 hrs

Dose: 114.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
ZB	M	1013	430	42.45	1003	1	0.10
TOTAL for Animal #:M020M-95-56				42.45			0.10
JD	M	1003	620	61.81	1002	1	0.10
TOTAL for Animal #:M020M-95-57				61.81			0.10
CJ	M	1004	288	28.69	1002	1	0.10
TOTAL for Animal #:M020M-95-58				28.69			0.10
AT	M	1003	356	35.49	1002	3	0.30
TOTAL for Animal #:M020M-95-59				35.49			0.30
TY	M	1003	525	52.34	1002	0	0.00
TOTAL for Animal #:M020M-95-60				52.34			0.00
Dose group total:		Mean:		44.16	5011	6	0.12
		SE:		5.90			0.05

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020M-95, Data File "M020M-95.msu"  
 Study No.: M020-95

Treatment: MeSH  
 Sacrifice: 72 hrs

Dose: 258.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
EZ	M	1014	678	66.86	1002	1	0.10
TOTAL for Animal #:M020M-95-61				66.86			0.10
WX	M	1003	425	42.37	1002	1	0.10
TOTAL for Animal #:M020M-95-62				42.37			0.10
BZ	M	1008	308	30.56	1002	2	0.20
TOTAL for Animal #:M020M-95-63				30.56			0.20
TT	M	1001	448	44.76	1003	1	0.10
TOTAL for Animal #:M020M-95-64				44.76			0.10
ZT	M	1001	283	28.27	1004	1	0.10
TOTAL for Animal #:M020M-95-65				28.27			0.10
Dose group total:		Mean:		42.56	5013	6	0.12
		SE:		6.87			0.02

Treatment: MeSH  
 Sacrifice: 72 hrs

Dose: 512.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
ZM	M	1002	490	48.90	1003	2	0.20
TOTAL for Animal #:M020M-95-66				48.90			0.20
HZ	M	1001	572	57.14	1002	1	0.10
TOTAL for Animal #:M020M-95-67				57.14			0.10
DR	M	1002	511	51.00	1002	1	0.10
TOTAL for Animal #:M020M-95-68				51.00			0.10
TOTAL for Animal #:M020M-95-69				No data for this animal			
TOTAL for Animal #:M020M-95-70				No data for this animal			
Dose group total:		Mean:		52.35	3007	4	0.13
		SE:		2.47			0.03

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020M-95, Data File "M020M-95.msu"  
 Study No.: M020-95

Treatment: Urethane  
 Sacrifice: 72 hrs

Dose: 300.0 mg/kg  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
ZY	M	1003	492	49.05	1001	2	0.20
TOTAL for Animal #:M020M-95-71				49.05			0.20
DX	M	1005	500	49.75	1002	1	0.10
TOTAL for Animal #:M020M-95-72				49.75			0.10
AR	M	1004	340	33.86	1002	0	0.00
TOTAL for Animal #:M020M-95-73				33.86			0.00
WY	M	1009	423	41.92	1002	3	0.30
TOTAL for Animal #:M020M-95-74				41.92			0.30
YL	M	1002	382	38.12	1001	2	0.20
TOTAL for Animal #:M020M-95-75				38.12			0.20
Dose group total:		Mean:		42.54	5008	8	0.16
		SE:		3.08			0.05

**INDIVIDUAL ANIMAL DATA SUMMARIES**

Expt. #: M020F-95, Data File "M020F-95.msu"  
 Study No.: M020-95

Treatment: Control, air  
 Sacrifice: 24 hrs

Dose: 0.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
ZW	F	1000	532	53.20	1000	0	0.00
TOTAL for Animal #:M020F-95-76				53.20			0.00
TR	F	1003	384	38.29	1003	1	0.10
TOTAL for Animal #:M020F-95-77				38.29			0.10
BX	F	1003	407	40.58	1000	2	0.20
TOTAL for Animal #:M020F-95-78				40.58			0.20
TX	F	1009	423	41.92	1001	3	0.30
TOTAL for Animal #:M020F-95-79				41.92			0.30
ED	F	1002	631	62.97	1001	2	0.20
TOTAL for Animal #:M020F-95-80				62.97			0.20
Dose group total:		Mean:		47.39	5005	8	0.16
		SE:		4.67			0.05

Treatment: MeSH  
 Sacrifice: 24 hrs

Dose: 114.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
WD	F	1016	461	45.37	1003	1	0.10
TOTAL for Animal #:M020F-95-81				45.37			0.10
ZS	F	1002	495	49.40	1002	1	0.10
TOTAL for Animal #:M020F-95-82				49.40			0.10
EJ	F	1007	427	42.40	1002	1	0.10
TOTAL for Animal #:M020F-95-83				42.40			0.10
EY	F	1008	672	66.67	1001	1	0.10
TOTAL for Animal #:M020F-95-84				66.67			0.10
KP	F	1003	605	60.32	1002	2	0.20
TOTAL for Animal #:M020F-95-85				60.32			0.20
Dose group total:		Mean:		52.83	5010	6	0.12
		SE:		4.60			0.02

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020F-95, Data File "M020F-95.msu"  
 Study No.: M020-95

Treatment: MeSH  
 Sacrifice: 24 hrs

Dose: 258.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
EH	F	1010	587	58.12	1001	1	0.10
TOTAL for Animal #:M020F-95-86				58.12			0.10
WS	F	1008	460	45.63	1001	0	0.00
TOTAL for Animal #:M020F-95-87				45.63			0.00
CR	F	1009	623	61.74	1001	4	0.40
TOTAL for Animal #:M020F-95-88				61.74			0.40
ZT	F	1001	479	47.85	1001	1	0.10
TOTAL for Animal #:M020F-95-89				47.85			0.10
AB	F	1017	560	55.06	1001	3	0.30
TOTAL for Animal #:M020F-95-90				55.06			0.30
Dose group total: Mean:				53.68	5005	9	0.18
SE:				3.04			0.07

Treatment: MeSH  
 Sacrifice: 24 hrs

Dose: 512.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
TC	F	1000	496	48.60	1002	3	0.30
TOTAL for Animal #:M020F-95-91				48.60			0.30
TZ	F	1002	472	47.11	1001	5	0.50
TOTAL for Animal #:M020F-95-92				47.11			0.50
EA	F	1005	475	47.26	1001	2	0.20
TOTAL for Animal #:M020F-95-93				47.26			0.20
AJ	F	1003	561	55.93	1002	1	0.10
TOTAL for Animal #:M020F-95-94				55.93			0.10
TOTAL for Animal #:M020F-95-95				No data for this animal			
Dose group total: Mean:				49.98	4006	11	0.27
SE:				2.07			0.09

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020F-95, Data File "M020F-95.msu"  
 Study No.: M020-95

Treatment: Control, air  
 Sacrifice: 48 hrs

Dose: 0.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
WH	F	1019	571	56.04	1002	5	0.50
TOTAL for Animal #:M020F-95-96				56.04			0.50
DB	F	1015	433	42.66	1003	3	0.30
TOTAL for Animal #:M020F-95-97				42.66			0.30
WC	F	1017	511	50.25	1002	1	0.10
TOTAL for Animal #:M020F-95-98				50.25			0.10
DT	F	1000	462	46.20	1001	1	0.10
TOTAL for Animal #:M020F-95-99				46.20			0.10
ZR	F	1009	506	50.15	1002	0	0.00
TOTAL for Animal #:M020F-95-100				50.15			0.00
Dose group total:		Mean:		49.06	50.0	10	0.20
		SE:		2.24			0.09

Treatment: MeSH  
 Sacrifice: 48 hrs

Dose: 114.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
EW	F	1002	538	53.69	1002	0	0.00
TOTAL for Animal #:M020F-95-101				53.69			0.00
TW	F	1001	632	63.14	1001	0	0.00
TOTAL for Animal #:M020F-95-102				63.14			0.00
WT	F	1000	517	51.70	1002	1	0.10
TOTAL for Animal #:M020F-95-103				51.70			0.10
TT	F	1009	522	51.73	1002	3	0.30
TOTAL for Animal #:M020F-95-104				51.73			0.30
AT	F	1004	629	62.65	1002	2	0.20
TOTAL for Animal #:M020F-95-105				62.65			0.20
Dose group total:		Mean:		56.58	5009	6	0.12
		SE:		2.60			0.06

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020F-95, Data File "M020F-95.msu"  
 Study No.: M020-95

Treatment: MeSH  
 Sacrifice: 48 hrs

Dose: 258.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
JR	F	1002	515	51.40	1001	2	0.20
TOTAL for Animal #:M020F-95-106				51.40			0.20
TL	F	1003	527	52.54	1002	0	0.00
TOTAL for Animal #:M020F-95-107				52.54			0.00
DX	F	1018	598	58.74	1000	2	0.20
TOTAL for Animal #:M020F-95-108				58.74			0.20
TP	F	1015	736	72.51	1001	2	0.20
TOTAL for Animal #:M020F-95-109				72.51			0.20
CA	F	1006	588	58.45	1002	2	0.20
TOTAL for Animal #:M020F-95-110				58.45			0.20
Dose group total:		Mean:		58.73	5006	8	0.16
		SE:		3.75			0.04

Treatment: MeSH  
 Sacrifice: 48 hrs

Dose: 512.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN
ZZ	F	1005	554	55.12	1001	0	0.00
TOTAL for Animal #:M020F-95-111				55.12			0.00
AW	F	1007	541	53.72	1001	3	0.30
TOTAL for Animal #:M020F-95-112				53.72			0.30
ZA	F	1004	603	60.06	1002	5	0.50
TOTAL for Animal #:M020F-95-113				60.06			0.50
MR	F	1002	402	40.12	1003	4	0.40
TOTAL for Animal #:M020F-95-114				40.12			0.40
ZM	F	1002	414	41.32	1000	3	0.30
TOTAL for Animal #:M020F-95-115				41.32			0.30
Dose group total:		Mean:		58.07	5007	15	0.30
		SE:		3.96			0.08

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020F-95, Data File "M020F-95.msu"  
 Study No.: M020-95

Treatment: Control, air  
 Sacrifice: 72 hrs

Dose: 0.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN	
NX	F	1003	411	40.98	1002	2	0.20	
TOTAL for Animal #:M020F-95-116				40.98			0.20	
AR	F	1005	854	84.98	1002	1	0.10	
TOTAL for Animal #:M020F-95-117				84.98			0.10	
BC	F	1008	589	58.43	1000	0	0.00	
TOTAL for Animal #:M020F-95-118				58.43			0.00	
BY	F	1005	487	48.46	1002	3	0.30	
TOTAL for Animal #:M020F-95-119				48.46			0.30	
AA	F	1006	646	64.21	1002	2	0.20	
TOTAL for Animal #:M020F-95-120				64.21			0.20	
Dose group total:				Mean:	59.41	5008	8	0.16
				SE:	7.54			0.05

Treatment: MeSH  
 Sacrifice: 72 hrs

Dose: 114.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC (%)	No. of PCE	PCE with MN	% PCE with MN	
DZ	F	1015	620	61.08	1001	3	0.30	
TOTAL for Animal #:M020F-95-121				61.08			0.30	
WZ	F	1004	460	45.82	1001	0	0.00	
TOTAL for Animal #:M020F-95-122				45.82			0.00	
CD	F	1000	675	67.50	1002	1	0.10	
TOTAL for Animal #:M020F-95-123				67.50			0.10	
AC	F	1000	474	47.40	1002	3	0.30	
TOTAL for Animal #:M020F-95-124				47.40			0.30	
AY	F	1002	566	56.49	1001	1	0.10	
TOTAL for Animal #:M020F-95-125				56.49			0.10	
Dose group total:				Mean:	55.66	5007	8	0.16
				SE:	4.10			0.06

INDIVIDUAL ANIMAL DATA SUMMARIES

Expt. #: M020F-95, Data File "M020F-95.msu"  
 Study No.: M020-95

Treatment: MeSH  
 Sacrifice: 72 hrs

Dose: 258.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
EC	F	1004	651	64.84	1001	1	0.10
TOTAL for Animal #:M020F-95-126				64.84			0.10
AK	F	1003	417	41.58	1002	2	0.20
TOTAL for Animal #:M020F-95-127				41.58			0.20
CJ	F	1006	540	53.68	1002	1	0.10
TOTAL for Animal #:M020F-95-128				53.68			0.10
WR	F	1003	481	47.96	1002	1	0.10
TOTAL for Animal #:M020F-95-129				47.96			0.10
WL	F	1003	544	54.24	1002	4	0.40
TOTAL for Animal #:M020F-95-130				54.24			0.40
Dose group total:				Mean:	5009	9	0.18
				SE:	3.85		0.06

Treatment: MeSH  
 Sacrifice: 72 hrs

Dose: 512.0 ppm  
 Vehicle: air

Slide Code	Sex	No. of RBC	No. of PCE	PCE/RBC(%)	No. of PCE	PCE with MN	% PCE with MN
TK	F	1002	547	54.59	1001	1	0.10
TOTAL for Animal #:M020F-95-131				54.59			0.10
TOTAL for Animal #:M020F-95-132				No data for this animal			
BB	F	1009	595	58.97	1002	2	0.20
TOTAL for Animal #:M020F-95-133				58.97			0.20
TOTAL for Animal #:M020F-95-134				No data for this animal			
EM	F	1016	430	42.32	1002	2	0.20
TOTAL for Animal #:M020F-95-135				42.32			0.20
Dose group total:				Mean:	3005	5	0.17
				SE:	4.98		0.03

**CLIENT PRIVATE**

**Bone marrow erythrocyte micronucleus assay in Swiss-Webster  
mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study M020-95**

**Appendix C**

**PROTOCOL AND PROTOCOL AMENDMENTS NO. 1 AND 2**

**CLIENT PRIVATE**

**PROTOCOL TITLE:** Bone marrow micronucleus assay in male and female Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan

**STUDY IDENTIFICATION:** SRI Study No. M020-95

**SPONSOR:** Elf Atochem North America, Inc.  
2000 Market Street  
Philadelphia, PA 19103-3222

**Sponsor Representative:** Roy M. Bannister, Ph.D., D.A.B.T.  
Phone: 215-419-5875  
FAX: 215-419-5800

**TESTING LABORATORY:** SRI International  
Toxicology Laboratory  
333 Ravenswood Avenue  
Menlo Park, CA 94025

**Study Director:** R. A. Winegar, Ph.D.  
Phone: 415-859-6457  
FAX: 415-859-2889

**Inhalation Toxicologist:** R. C. Baldwin, Ph.D., D.A.B.T.  
Phone: 415-859-3174

**APPROVAL OF PROTOCOL**

  
\_\_\_\_\_  
Sponsor Representative

5/25/95  
Date

  
\_\_\_\_\_  
SRI Study Director

6/9/95  
Date

  
\_\_\_\_\_  
SRI Quality Assurance Unit

6/9/95  
Date

## CLIENT PRIVATE

Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M020-95

### I. STUDY OBJECTIVE

To evaluate the ability of a single 6-hr nose-only inhalation exposure to induce chromosomal or mitotic spindle abnormalities in bone marrow cells of treated mice, as indicated by an increased incidence of micronuclei in newly formed, RNA-containing erythrocytes.

### II. PROPOSED STUDY SCHEDULE (to be specified by amendment)

Receive animals:

Release from quarantine:

Experimental start date:

Experimental termination date:

### III. REGULATORY COMPLIANCE

Testing Guidelines:

TSCA Guideline 40 CFR 798.1150, Subpart B for the inhalation exposure activities (where applicable), and

TSCA Guideline 40 CFR 798.5395, Subpart F for the micronucleus sampling and evaluations.

OECD Guideline No. 403 for the inhalation exposure activities, and

OECD Guideline No. 474 for the micronucleus sampling and evaluations.

GLP Regulations:

EPA, TSCA; 40 CFR 792.

OECD, Good Laboratory Practice in the Testing of Chemicals (1982).

Applicable SOPs: SRI International.

Animal Care and Welfare: NIH Guidelines, Public Law 89-544.

### IV. ROUTE OF ADMINISTRATION: nose-only inhalation.

### V. TEST ARTICLE

A. Identification: methyl mercaptan (MeSH, methanethiol, CAS No. 74-93-1).

B. Physical and Chemical Properties

1. Gas: bp at 1 atm. is 5.96°C; shipped in steel cylinders.
2. Vapor pressure: approx 11 psig at 76°F.
3. Color: colorless.
4. Odor: extremely disagreeable; odor of rotten cabbage.
5. Flash point: below 0°F; flammable.

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M820-95

6. **Solubility:** 2.3 to 2.4 g/L of water at 15° to 20°C.
- C. **Purity and Stability**  
The determinations of identity, purity, stability, and composition of the test article are the responsibility of the Sponsor.
- D. **Safe Handling Procedures**
1. **Storage and use conditions for test article**  
Controlled room temperature.  
Out of direct sunlight.  
Well ventilated area.
  2. **Personal safety equipment required when handling**  
Test article: OSHA-approved respirator, safety glasses, lab coat or smock.  
Treated animals: same as above.  
Soiled cages and bedding: no additional precautions beyond what is usually required.  
Blood, urine, or tissues (fresh or fixed): no additional precautions beyond what is usually required.
- E. **Disposition of Remaining Test Article**  
Returned to Sponsor upon submission of Final Report.
- F. **Maintenance of Archival Sample**  
Sponsor is responsible.

## VI. INHALATION EXPOSURE CONDITIONS

- A. **Exposure Atmosphere**  
Gas.
- B. **Exposure Equipment**  
52-port, stainless steel, non-recirculating, nose-only, "flow-past" exposure units (Lab Products, Inc., Maywood, NJ; Model No. 70052-S). Described by W.C. Cannon, *et al.* 1983.  
Animal restraining tubes obtained from CH Technologies (Westwood, NJ).  
Model CHT-247 for mice.
- C. **Generation of Exposure Atmosphere**  
Atmosphere will be generated by external mixing of a metered flow of the test article and a metered flow of compressed air.

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M020-95

- D. Duration of Inhalation Exposure**  
6 hours.
- E. Determination of Exposure Concentrations**
- 1. Agreement with Target Concentrations**  
Criterion: (target - actual)  $\leq$  10% of target.  
Frequency of sampling: at least hourly.
  - 2. Stability Across Time**  
Criterion: (maximum - minimum)  $\leq$  10% of mean across time.  
Frequency of sampling: at least hourly.
  - 3. Homogeneity**  
Criterion: (maximum - minimum)  $\leq$  10% of mean across locations.  
Sampling locations: each location where animals will be exposed or the environment monitored during an animal exposure.  
When determined: before exposing animals.
  - 4. Sample Collection Methods**  
Gas samples drawn into gas-tight syringes.
  - 5. Analytical Method**  
Direct injection into a gas chromatograph (operating conditions will be specified in the final report).
- F. Determination of Particle Size Distributions**  
Not required for a gaseous exposure atmospheres.
- G. Monitoring of Other Exposure Conditions**
- 1. Temperature**  
Criterion: for mice, the recommended air temperature is 18° to 26°C for ambient air.  
Sampling location: one location previously shown to be representative of the animal-exposure conditions.  
Frequency: monitored at 1-min intervals; recorded at 15-min intervals.  
Homogeneity: verified during study set-up phase.
  - 2. Relative Humidity**  
Criterion: for mice, the recommended air temperature is 40 to 70% for ambient air. May be dependent on test article composition, however.  
Sampling location: one location previously shown to be representative of the animal-exposure conditions.  
Frequency: monitored at 1-min intervals; recorded at 15-min intervals.

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**Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M920-95**

**Homogeneity: verified during study set-up phase.**

**How measured: from temperatures of a dry thermocouple and a water-wetted thermocouple.**

**3. Oxygen Concentration**

**Criterion:  $\geq 19\%$  (v/v).**

**Sampling method: will not be measured because exposure system will not permit oxygen depletion below normal atmospheric concentration.**

**4. Air Flow Rate**

**Criterion: at each exposure port,  $\geq 5$  times estimated minute volume for each animal.**

**How monitored: control of air flow rate into the exposure unit and the pressure difference between the air supply and the air exhaust manifolds in the exposure unit.**

**Frequency: pressure differential monitored at 1-min intervals, recorded at 15-min intervals. Actual flow rates measured during development phase of the study.**

**H. Method for Assuring Correct Dosing**

**Representative samples of the exposure atmosphere will be collected and analyzed for the concentration of the test article.**

**Weights measured with calibrated balances.**

**Volumes measured with the appropriate calibrated equipment.**

**Gas flow rates monitored by equipment calibrated against bubble flow meters or other appropriate reference standards.**

**Calibrations, volume measurements, weighings, and control equipment settings will be properly documented and the records will be maintained.**

## **VII. TEST SYSTEM**

**A. Species: mouse.**

**B. Strain: Swiss-Webster.**

**C. Sex: male and female.**

**D. Source of Animals**

**Charles River Laboratories**

**Park Center Drive, Building A**

**Hollister, CA 95023.**

**E. Number of Animals Assigned to the Study: 75 males and 60 females.**

## CLIENT PRIVATE

Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl xanthines  
SRI Study No. M020-95

- F. **Body Weight at Initiation of Testing:** approximately 17-35 g.
- G. **Maximum Variability of Body Weight:**  $\pm 20\%$  within each sex.
- H. **Age at Initiation of Testing:** approximately 7 weeks of age.
- I. **Allocation to Treatment Groups:** computer-generated random-number tables.
- J. **Criteria for Excluding Animals from Selection**  
Health or inappropriate body weight.
- K. **Justification for Selection of the Test System**  
The mouse is a model for the assessment of acute inhalation toxicity and for conducting micronucleus assays.  
The mouse is recommended by toxicity testing guidelines of all regulatory agencies.  
A large comparative data base exists for acute inhalation toxicity and for micronucleus assay results.

### VIII. ANIMAL HUSBANDRY

- A. **Quarantine and Acclimatization**
  - 1. **Duration:**  $\geq 5$  days.
  - 2. **Health Evaluations**  
Two or four mice of each sex (depending on the size of the animal shipment) will be necropsied and examined for body and major organ weights, fecal parasites, and the pinworm *Syphacia*.
  - 3. **Release criteria**  
Results of the above evaluations and general appearance of the animals will be evaluated by the attending veterinarian.
- B. **Housing**
  - 1. **Caging:** 22- x 12½- x 8-in. suspended polycarbonate cages.
  - 2. **Bedding:** hardwood chips (Sani-Chips, P.J. Murphy Forest Products, Montville, NJ).
  - 3. **Number per cage**  
Before exposure:  $\leq 10$  per cage.  
After exposure:  $\leq 5$  per cage.
- C. **Feed**
  - 1. **Type:** Purina Certified Rodent Chow No. 5002.
  - 2. **Availability:** *ad libitum*, except during inhalation exposures.
  - 3. **Contaminants:** none expected based on previous reports from Purina.
  - 4. **Frequency of analysis:** batch analysis provided by Purina.

## CLIENT PRIVATE

Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M028-95

### D. Drinking Water

1. **Type:** water from the San Francisco Municipal Water District will be filtered, deionized, and UV-exposed at SRI.
2. **Availability:** *ad libitum*, except during inhalation exposures.
3. **Contaminants:** none expected based on previous reports.
4. **Frequency of analysis:** bimonthly.

### E. Environmental Control (during normal housing, not exposure)

1. **Temperature range:** 18° to 26°C (64° to 79°F).
2. **Relative humidity:** will be monitored and recorded continuously.
3. **Ventilation rate:** 10 room volumes per hour.
4. **Degree of recirculated air used:** none.
5. **Light/dark cycle:** 12 hr/12 hr.

### F. Unique Identification of the Test System

1. **Cages:** color-coded cards with the study number, cage number, animal identification numbers, sex, test article, dose level, and treatment and sacrifice dates.
2. **Animals:** ear punches.
3. **Identification numbers:** consecutive, beginning at 1; unique to the study.

### G. Cleaning and Sanitation

1. **Cages:** changed twice weekly.
2. **Feeders:** changed biweekly.
3. **Racks:** changed biweekly.
4. **Rooms:** sanitized before receipt of the animals; monthly thereafter.
5. **Cleaning agents:** detergents and disinfectants free of essential oils; approved for use in animal facilities.

### H. Pest/Vermin Controls

1. **Provider:** a commercial pest control contractor.
2. **Records:** SRI's Health and Safety Department will maintain records of all requests for services and any application of chemicals by date, location, kind, and quantity.
3. **Limitations:** at no time during an ongoing study will chemicals be introduced into an animal room without specific approval of the Sponsor.

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M820-95

### I. Welfare of the Animals

1. **Objective:** to minimize, if not eliminate, pain and suffering in all animals in this study.
2. **Moribund animals:** euthanized at the discretion of the Study Director.
3. **Pain and suffering:** animals experiencing undue pain and suffering will be euthanized at the discretion of the Study Director in consultation with the attending veterinarian.
4. **Integrity of the study:** every effort will be made to protect the scientific validity of the study.

### J. Euthanasia

1. **Agent:** sodium pentobarbital anesthesia followed by cervical dislocation.
2. **Route of administration:** intraperitoneal injection.
3. **Amount:** 60 mg/kg body weight or to effect.

## IX. EXPERIMENTAL DESIGN

### A. Selection of Exposure Concentrations

1. **Basis:** range-finding (see below).
2. **Responsibility for selection:** sponsor.
3. **Maximum concentration:** limit concentration of 2 mg/L (per "Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity Studies" EPA memo from J. Whalen and J. Redden, dated Dec. 12, 1992)
4. **How specified:** by an amendment to the protocol.

### B. Group Designations

1. **Form:** consecutive numbers.
2. **Order of assignment:** from lowest to highest concentration or chronologically, as appropriate.

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Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M920-95

### 3. Numbers of animals assigned

Treatment	Number of Animals						Totals	
	24 Hr Sample		48 Hr Sample		72 Hr Sample		Male	Female
	Male	Female	Male	Female	Male	Female		
Air Control	5	5	5	5	5	5	15	15
Positive Control <sup>a</sup>	5	0	5	0	5	0	15	0
Low [NF <sub>3</sub> ]	5	5	5	5	5	5	15	15
Mid [NF <sub>3</sub> ]	5	5	5	5	5	5	15	15
High [NF <sub>3</sub> ]	5	5	5	5	5	5	15	15
Totals	25	20	25	20	25	20	75	60

<sup>a</sup> Animals will be treated once with urethane (CAS No. 51-79-6), 300 mg/kg by gavage, in water.

- C. Frequency and Duration of Exposure: a single 6-hr exposure.
- D. Route of Exposure: spontaneous inhalation.
- E. Method of Exposure: nose-only inhalation exposure.
- F. Physical Form of the Test-Article Exposure Atmosphere: gas.
- G. Justification for Inhalation Exposure  
Models a potential route of human exposure to the test article.

### X. RANGE-FINDING STUDY

- A. Exposure Conditions  
One 6-hr inhalation exposure.
- B. Group Size  
3 males and 3 females per concentration level.
- C. Selection of the Three Exposure Concentrations  
Based on lethality data provided by the Sponsor.
- D. Animal Responses Examined  
Clinical signs of toxicity observed frequently during and immediately after inhalation exposure; daily thereafter.  
Blood smears prepared at approximately 72 hr after the exposure. Blood taken from the ventral tail vessel.

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### **E. Postmortem Evaluations**

Bone marrow smears prepared from the femurs of each animal according to the general method of Schmid (1976) at approximately 72 hr after the exposure.

Three slides prepared from each animal.

Two slides coded with computer-generated random letter codes. One coded slide stained with acridine orange (Hayashi et al., 1983; MacGregor et al., 1983).

### **F. Cytological Analysis of Slides**

One thousand erythrocytes from bone marrow and from peripheral blood, will be classified as RNA-positive or RNA-negative. The PCE/RBC ratio at 72 hr in blood provides an estimate of the expected bone marrow cytotoxicity at 48 hr.

### **G. Method of Examination: epifluorescence microscopy at 630 or 1000X.**

### **H. Selection Criteria for Exposure Concentrations for the Definitive Study**

The high concentration for the definitive study will be 1) the minimum concentration that caused a 60 to 80% suppression of the PCE/RBC (polychromatic erythrocyte/red blood cell, i.e. erythrocyte) ratio relative to the negative control, or if no suppression is observed 2) the minimum concentration that caused animal death or compound-related signs of toxicity, or if no signs of toxicity occur 3) the maximum practical concentration that can be administered. The mid and low concentrations will be  $\frac{1}{2}$  and  $\frac{1}{4}$  of the high concentration, respectively. A negative control group will be exposed to uncontaminated air.

## **XI. DEFINITIVE STUDY**

### **A. Exposure Conditions**

6-hr inhalation exposure.

### **B. Inhalation Exposure Group Size**

15 males and 15 females per inhalation-exposed group.

15 males for the positive control group.

### **C. Sacrifice Times**

5 males and 5 females sacrificed from each exposure group at 24, 48, and 72 hours after inhalation exposure. Five males from the positive control group will be sacrificed at 24, 48, and 72 hr post-treatment.

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- D. **Selection of Concentrations**  
Based on range-finding study with the approval of the Sponsor; added to protocol by amendment.
- E. **Animal Responses Examined In Life**  
Clinical signs of toxicity observed frequently during and immediately after inhalation exposure; daily thereafter.
- F. **Postmortem Evaluations**  
Bone marrow smears prepared from the femurs of each animal according to the general method of Schmid (1976).  
Three slides prepared from each animal.  
Two slides coded with computer-generated random letter codes. One coded slide stained with acridine orange.
- G. **Cytological Analysis of Bone Marrow Slides**  
Number of micronucleated RNA positive erythrocytes (PCE) among a total of 1,000 PCE per animal.  
Number of PCE among 1000 total erythrocytes (RBC) per animal (5000 per group of 5 animals of each sex for each inhalation-exposed group).
- H. **Method of Examination:** epifluorescence microscopy at 630 or 1000X.

### XII. CRITERIA FOR VALID ASSAY

- The frequency of micronucleated cells in the negative control group is within the normal historical range, and
- The positive control group had a statistically significant elevation in the incidence of micronucleated cells, and
- There are a minimum of three surviving animals of each sex with a PCE/RBC ratio in the bone marrow greater than or equal to 0.1 in two or more dose groups.

### XIII. PROPOSED STATISTICAL METHODS

- Data analyzed separately by sex.
- The frequency of micronucleated PCE among PCE, and the percentage of PCE among RBC, will be calculated for each animal.
- The statistical significance of differences in the percentage of PCE among groups will be evaluated using the Kruskal-Wallis analysis of variance on ranks.
- The micronucleus frequency data will be analyzed by using the Cochran-Armitage test for trend in binomial proportions, to determine if a significant dose-response relationship is present.

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The normal test for equality of binomial proportions (Kastenbaum and Bowman, 1970) will be used to determine if individual dose groups are statistically elevated above the negative control.

These statistical tests are based on recommended methods (ASTM Committee, 1988; Margolin et al., 1983).

### XIV. CRITERIA FOR INTERPRETATION

#### A. Positive

The incidence of micronucleated PCE is significantly higher than that in the negative control group ( $p < 0.05$ ) in either 1) two different dose groups from one experiment or 2) at a single dose if confirmed by a separate experiment (e.g., an increased frequency of micronucleated cells in the range-finding study). A positive dose-related increase in the incidence of micronucleated cells.

#### B. Negative

The criteria for a positive or inconclusive response are not met.

#### C. Inconclusive

The exposure concentrations were inappropriate (e.g., excessive cytotoxicity), or, a statistically significant elevation in micronucleated PCE observed in only one treatment group and the dose-response trend is not significant.

### XV. CONTROL OF BIAS

Animals will be randomly assigned to treatment groups by using computer-generated random numbers.

Evaluation of bone marrow slides in the definitive study will be conducted "blind".

### XVI. REPORTING

#### A. Format

Suitable for submission to the US EPA.

#### B. Degree of Detail

Sufficient to permit independent evaluation or replication by another laboratory.

Data for individual animals and, where appropriate, group means and standard errors.

Statistical methods, other than means and standard errors, will be described in detail.

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- Text of protocol and any amendments included.
- Any protocol deviations and their impact on the study.
- Summary of the results of the range-finding study.
- C. **Data Tabulations - Exposure Conditions**
  - Exposure concentrations and times measured.
  - Mean, standard error, and range of exposure temperatures and, where appropriate, relative humidity.
  - Complete description of the exposure atmosphere generator and its operating parameters.
- D. **Data Tabulations - Animal Responses by Sex**
  - Mortality and time of occurrence or discovery.
  - Clinical signs and day of observation.
  - Frequency of micronucleated PCE among all PCE.
  - Frequency of PCE among RBC.
- E. **Sponsor Review**
  - Sponsor will have 28 days to review an audited draft report.
  - Audited final report issued after 28 days or receipt of Sponsor's comments on draft report, whichever is sooner.

## XVII. RECORD RETENTION

- A. **Records Retained**

One copy of the final report and protocol with original signatures, correspondence concerning the conduct or interpretation of the study, all original raw data records, properly-signed and -dated print out of electronically captured raw data, any other records generated during the conduct of the study. Slides of bone marrow preparations will be retained as well.
- B. **Ownership**

All raw data and samples generated by SRI, supporting documents, and records are the property of the Sponsor.
- C. **Archive Location**

Paper: Records Retention Center, SRI International, 333 Ravenswood Ave., Menlo Park, CA 94025  
Slides: CVD Archives, Inc., 2825 KOVR Drive, West Sacramento, CA 95605, a dedicated commercial archiving facility.
- D. **Period of Retention**

10 years by SRI; Sponsor responsibility beyond that period.

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- E. Further Disposition  
Sponsor's option after specified retention period.
- F. QA Records  
Master schedule, data audit, and inspection records retained by the SRI International Quality Assurance Unit.

### XVIII. REFERENCES

- ASTM Committee E-47 on Biological Effects and Environmental Fate (1988). "Standard Guide for Conduct of Micronucleus Assays in Mammalian Bone Marrow Erythrocytes". ASTM Standard No. E 1263-88.
- Cannon, W. C., Blanton, E. F., and McDonald, K. E. (1983). The flowpast chamber: an improved nose-only exposure system for rodents. *Am. Ind. Hyg. Assoc. J.* 44 (12), 923-928.
- Hayashi M., Sofuni, T., and Ishidate, M., Jr. (1983). An application of acridine orange fluorescent staining to the micronucleus test. *Mutat. Res.* 120, 241-247.
- Kastenbaum, M. A. and Bowman, K. O. (1970). Tables for determining the statistical significance of mutation frequencies. *Mutat. Res.* 9, 527-549.
- MacGregor, J. T., Wehr, C. M., and Langlois, R.G. (1983). A simple fluorescent staining procedure for micronuclei and RNA-containing erythrocytes using Hoechst 33258 and pyronin Y. *Mutat. Res.* 120, 269-275.
- Margolin, B. H., Collings, B. J., and Mason, J. M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* 5, 705-716.
- Schmid, W. (1976). The micronucleus test for cytogenetic analysis. In *Chemical Mutagens*, Vol. 4. (A. Hollander, Ed.), pp. 31-53. Plenum Press, New York.
- U.S. Department of Health and Human Services (1985). "Guide for the Care and Use of Laboratory Animals". PHS/NIH Publication No. 85-23.

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SRI Study No. M020-95

### PROTOCOL AMENDMENT NO. 1

#### I. PROTOCOL SECTIONS CHANGED

Section X.D. Storage Conditions for the Test Article

Section XI.H. Method for Assuring Correct Dosing

Section XV. Pilot Study

Section XXII. Record Retention

#### II. DESCRIPTION OF THE CHANGES

Section X.D. The test article will be stored at controlled room temperature.

Section XI.H. Add to this section the following text: Samples of the exposure atmosphere will be collected from an area representative of the breathing zone of the animals and analyzed for the concentration of the test article.

Section XV. The pilot study will be conducted at exposure concentrations of approximately 100, 350, and 600 ppm (v/v) of the test article. The schedule for conduct of the pilot study will be:

Date of receipt of the animals: 9/19/95

Date of release from quarantine: 9/25/95

Date of initiation of treatment: 9/25/95

Date of collection of bone marrow  
and blood: 9/28/95

Section XXII. All raw data, samples generated by SRI, supporting documents, and correspondence relating to the conduct and/or interpretation of the study will be retained by SRI and archived as described.

#### III. JUSTIFICATION FOR THE CHANGES

Section X.D. The storage temperature was omitted from the protocol inadvertently.

Section XI.H. The text described above was left out of this section of the protocol inadvertently.

Section XV. The exposure concentrations must be specified in the protocol to be in compliance with GLPS.

Section XXII. In an effort to be concise, a statement that SRI would actually retain the specified items was not included in the protocol inadvertently. Addition of this text brings the protocol into compliance with GLPS.

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**Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
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**IV. IMPACT OF THE CHANGES ON THE STUDY**

Section X.D. The change has no impact on the study or test article because the test article has been stored at controlled room temperature from the time of its receipt at SRI.

Section XI.H. The added text has no impact on the procedures to be used in the study because the procedures have been described elsewhere in the protocol.

Section XV. The changes complete information required to be in the protocol. There are no adverse effects on the study.

Section XXII. The added text describes what would have been done in any event; thus, the change has no effect on the conduct of the study but will bring the protocol wording into compliance with GLPS.

**V. DATES OF IMPLEMENTATION**

Section X.D. 6/7/95 (date of receipt of the test article)

Section XI.H. Date of this amendment

Section XV. Date of this amendment

Section XXII. Date of this amendment

**VI. APPROVAL OF THE AMENDMENT**

  
Richard Winegar, Ph.D.  
Study Director

Sept 21, 1995  
Date

  
Roy Bannister, Ph.D., D.A.B.T.  
Sponsor's Representative

9/22/95  
Date

## CLIENT PRIVATE

Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M628-95

### PROTOCOL AMENDMENT NO. 2

#### I. PROTOCOL SECTIONS CHANGED

Section II. Proposed Study Schedule

Section XI.D. Selection of Concentrations (Definitive Study)

#### II. DESCRIPTION OF THE CHANGES

Section II. The schedule for the definitive study will be as follows:

Receive animals: 10/02/95

Release from quarantine: 10/09/95

Experimental start date: 10/09/95

Experimental termination date: 10/12/95

Section XI.D. The target concentrations for exposing the animals in the definitive study will be 0, 125, 250, and 500 ppm (v/v) of methyl mercaptan.

#### III. JUSTIFICATION FOR THE CHANGES

Section II. The protocol did not specify the schedule for the definitive study.

Section XI.D. The target concentrations were not specified in the protocol because they were to be decided upon based on the results from the pilot study. The bone marrow and peripheral blood evaluations from the pilot study indicated no suppression of the PCE/RBC ratio caused by the test substance. Because of the mortality and extreme acute toxicity seen in the animals exposed to the high concentration of 570 ppm during the pilot study, it was decided that the highest concentration in the definitive study should be reduced to 500 ppm in order to minimize the potential of invalidating the high concentration group because of excess mortality.

#### IV. IMPACT OF THE CHANGES ON THE STUDY

Section II. The change adds required information to the protocol, and insures continued compliance with Good Laboratory Practice Standards

Section XI.D. The change adds required information to the protocol, and insures continued compliance with Good Laboratory Practice Standards.

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**Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study No. M020-95**

**PROTOCOL AMENDMENT NO. 2 (Concluded)**

**V. DATES OF IMPLEMENTATION**

Section II. The schedule was approved as of the date of approval of this amendment.

Section XI.D. The change was implemented with verbal approval of this amendment by the Sponsor on 10/06/95.

**VI. APPROVAL OF THE AMENDMENT**

  
\_\_\_\_\_  
Richard Winegar, Ph.D.  
Study Director

10/6/95  
Date

  
\_\_\_\_\_  
Roy Banister, Ph.D., D.A.B.T.  
Sponsor's Representative

10/13/95  
Date

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**Bone marrow erythrocyte micronucleus assay in Swiss-Webster mice following acute nose-only inhalation exposure to methyl mercaptan  
SRI Study M020-95**

**Appendix D**

**HISTORICAL SRI BONE MARROW  
MICRONUCLEUS FREQUENCY VALUES: WATER**

SRI HISTORICAL MN FREQUENCIES OF NICE  
TREATED WITH WATER: BONE MARROW SMEAR EVALUATIONS

Animal No.	C S S E E P L			Dose mg/kg	Time	# RBC	PCE/ RBC	# PCE	XPCE w/MN	
	X E L	Chemical								
MN90-15F-1	F	SM	B	Control/water	0.0	24h	200	51.90	1000	0.10
MN90-15F-2	F	SM	B	Control/water	0.0	24h	200	47.19	1000	0.20
MN90-15F-3	F	SM	B	Control/water	0.0	24h	200	52.05	1000	0.10
MN90-15F-4	F	SM	B	Control/water	0.0	24h	200	47.57	1000	0.29
MN90-15F-5	F	SM	B	Control/water	0.0	24h	200	54.03	1000	0.00
MN90-15F-21	F	SM	B	Control/water	0.0	48h	200	48.15	1000	0.10
MN90-15F-22	F	SM	B	Control/water	0.0	48h	200	45.91	1000	0.10
MN90-15F-23	F	SM	B	Control/water	0.0	48h	200	52.07	1000	0.20
MN90-15F-24	F	SM	B	Control/water	0.0	48h	200	51.27	1000	0.10
MN90-15F-25	F	SM	B	Control/water	0.0	48h	200	45.77	1000	0.10
MN90-15M-1	M	SM	B	Control/water	0.0	24h	200	50.71	1000	0.10
MN90-15M-2	M	SM	B	Control/water	0.0	24h	200	41.36	1000	0.10
MN90-15M-3	M	SM	B	Control/water	0.0	24h	200	40.00	1000	0.30
MN90-15M-4	M	SM	B	Control/water	0.0	24h	200	41.59	1000	0.20
MN90-15M-5	M	SM	B	Control/water	0.0	24h	200	56.59	1000	0.00
MN90-15M-26	M	SM	B	Control/water	0.0	48h	200	49.28	1000	0.10
MN90-15M-27	M	SM	B	Control/water	0.0	48h	200	49.75	1000	0.19
MN90-15M-28	M	SM	B	Control/water	0.0	48h	200	50.86	1000	0.10
MN90-15M-29	M	SM	B	Control/water	0.0	48h	200	53.21	1000	0.10
MN90-15M-30	M	SM	B	Control/water	0.0	48h	200	50.89	1000	0.30
MN90-21F-1	F	SM	B	Control/water	0.0	24h	200	57.61	1000	0.00
MN90-21F-2	F	SM	B	Control/water	0.0	24h	200	51.85	1000	0.10
MN90-21F-3	F	SM	B	Control/water	0.0	24h	200	47.06	1000	0.30
MN90-21F-4	F	SM	B	Control/water	0.0	24h	200	55.87	1000	0.19
MN90-21F-5	F	SM	B	Control/water	0.0	24h	200	53.37	1000	0.30
MN90-21F-21	F	SM	B	Control/water	0.0	48h	200	54.59	1000	0.20
MN90-21F-22	F	SM	B	Control/water	0.0	48h	200	58.21	1000	0.20
MN90-21F-23	F	SM	B	Control/water	0.0	48h	200	57.55	1000	0.20
MN90-21F-24	F	SM	B	Control/water	0.0	48h	200	49.33	1000	0.10
MN90-21F-25	F	SM	B	Control/water	0.0	48h	200	54.05	1000	0.20
MN90-21F-41	F	SM	B	Control/water	0.0	72h	200	51.40	1000	0.40
MN90-21F-42	F	SM	B	Control/water	0.0	72h	200	56.40	1000	0.10
MN90-21F-43	F	SM	B	Control/water	0.0	72h	200	56.46	1000	0.20
MN90-21F-44	F	SM	B	Control/water	0.0	72h	200	47.57	1000	0.00
MN90-21F-45	F	SM	B	Control/water	0.0	72h	200	60.78	1000	0.20
MN90-21M-1	M	SM	B	Control/water	0.0	24h	200	53.08	1000	0.20
MN90-21M-2	M	SM	B	Control/water	0.0	24h	200	53.74	1000	0.10
MN90-21M-3	M	SM	B	Control/water	0.0	24h	200	51.20	1000	0.20
MN90-21M-4	M	SM	B	Control/water	0.0	24h	200	55.83	1000	0.20
MN90-21M-5	M	SM	B	Control/water	0.0	24h	200	57.62	1000	0.29

SRI HISTORICAL MN FREQUENCIES OF NICE  
TREATED WITH WATER: BONE MARROW SHEAR EVALUATIONS

Animal No.	S S E			Chemical	Dose mg/kg	Time	# RBC	PCE/ RBC	# PCE	XPCE u/MN
	E	P	L							
	X	E	L							
MN90-21M-26	M	SM	B	Control/water	0.0	48h	200	50.93	1000	0.00
MN90-21M-27	M	SM	B	Control/water	0.0	48h	200	52.40	1000	0.20
MN90-21M-28	M	SM	B	Control/water	0.0	48h	200	48.17	1000	0.29
MN90-21M-29	M	SM	B	Control/water	0.0	48h	200	53.55	1000	0.00
MN90-21M-30	M	SM	B	Control/water	0.0	48h	200	57.07	1000	0.10
MN90-21M-51	M	SM	B	Control/water	0.0	72h	200	57.35	1000	0.30
MN90-21M-52	M	SM	B	Control/water	0.0	72h	200	52.13	1000	0.20
MN90-21M-53	M	SM	B	Control/water	0.0	72h	200	58.94	1000	0.29
MN90-21M-54	M	SM	B	Control/water	0.0	72h	200	56.46	1000	0.00
MN90-21M-55	M	SM	B	Control/water	0.0	72h	200	46.08	1000	0.19
MN90-24M-1	M	SM	R	Control/Water	0.0	24h	200	51.54	1000	0.39
MN90-24M-2	M	SM	B	Control/Water	0.0	24h	200	56.87	1000	0.29
MN90-24M-3	M	SM	B	Control/Water	0.0	24h	200	56.93	1000	0.30
MN90-24M-4	M	SM	B	Control/Water	0.0	24h	200	53.69	1000	0.20
MN90-24M-5	M	SM	B	Control/Water	0.0	24h	200	55.00	1000	0.29
MN90-24M-26	M	SM	B	Control/Water	0.0	48h	200	53.18	1000	0.10
MN90-24M-27	M	SM	B	Control/Water	0.0	48h	200	51.95	1000	0.20
MN90-24M-28	M	SM	B	Control/Water	0.0	48h	200	50.69	1000	0.20
MN90-24M-29	M	SM	B	Control/Water	0.0	48h	200	50.96	1000	0.30
MN90-24M-30	M	SM	B	Control/Water	0.0	48h	200	54.76	1000	0.20
MN90-24M-51	M	SM	B	Control/Water	0.0	72h	200	53.36	1000	0.30
MN90-24M-52	M	SM	B	Control/Water	0.0	72h	200	46.73	1000	0.20
MN90-24M-53	M	SM	B	Control/Water	0.0	72h	200	57.08	1000	0.10
MN90-24M-54	M	SM	B	Control/Water	0.0	72h	200	55.72	1000	0.10
MN90-24M-55	M	SM	B	Control/Water	0.0	72h	200	54.81	1000	0.30
MN90-24F-1	F	SM	B	Control/Water	0.0	24h	200	51.87	1000	0.30
MN90-24F-2	F	SM	B	Control/Water	0.0	24h	200	54.93	1000	0.29
MN90-24F-3	F	SM	B	Control/Water	0.0	24h	200	52.74	1000	0.20
MN90-24F-4	F	SM	B	Control/Water	0.0	24h	200	58.96	1000	0.10
MN90-24F-5	F	SM	B	Control/Water	0.0	24h	200	57.92	1000	0.10
MN90-24F-21	F	SM	B	Control/water	0.0	48h	200	48.78	1000	0.30
MN90-24F-22	F	SM	B	Control/water	0.0	48h	200	53.64	1000	0.40
MN90-24F-23	F	SM	B	Control/water	0.0	48h	200	56.74	1000	0.30
MN90-24F-24	F	SM	B	Control/water	0.0	48h	200	46.72	1000	0.20
MN90-24F-25	F	SM	B	Control/water	0.0	48h	200	48.61	1000	0.20
MN90-24F-41	F	SM	B	Control/Water	0.0	72h	200	53.67	1000	0.20
MN90-24F-42	F	SM	B	Control/Water	0.0	72h	200	54.21	1000	0.20
MN90-24F-43	F	SM	B	Control/Water	0.0	72h	200	55.81	1000	0.29
MN90-24F-44	F	SM	B	Control/Water	0.0	72h	200	47.62	1000	0.20
MN90-24F-45	F	SM	B	Control/Water	0.0	72h	200	45.89	1000	0.30

SRI HISTORICAL NM FREQUENCIES OF NICE  
TREATED WITH WATER: BONE MARROW SHEAR EVALUATIONS

Animal No.	S S E E P L X E L			Chemical	Dose mg/kg	Time	# RBC	PCE/ RBC	# PCE	%PCE w/w
	C									
MN90-25M-1	M	SM	B	Control/Water	0.0	24h	200	51.40	1000	0.30
MN90-25M-2	M	SM	B	Control/Water	0.0	24h	200	59.20	1000	0.30
MN90-25M-3	M	SM	B	Control/Water	0.0	24h	200	50.67	1000	0.10
MN90-25M-4	M	SM	B	Control/Water	0.0	24h	200	57.53	1000	0.10
MN90-25M-5	M	SM	B	Control/Water	0.0	24h	200	58.53	1000	0.29
MN90-25M-26	M	SM	B	Control/Water	0.0	48h	200	47.62	1000	0.10
MN90-25M-27	M	SM	B	Control/Water	0.0	48h	200	44.04	1000	0.20
MN90-25M-28	M	SM	B	Control/Water	0.0	48h	200	55.72	1000	0.10
MN90-25M-29	M	SM	B	Control/Water	0.0	48h	200	59.62	1000	0.39
MN90-25M-30	M	SM	B	Control/Water	0.0	48h	200	56.65	1000	0.10
MN90-25M-51	M	SM	B	Control/Water	0.0	72h	200	57.62	1000	0.20
MN90-25M-52	M	SM	B	Control/Water	0.0	72h	200	50.46	1000	0.19
MN90-25M-53	M	SM	B	Control/Water	0.0	72h	200	62.25	1000	0.10
MN90-25M-54	M	SM	B	Control/Water	0.0	72h	200	47.83	1000	0.38
MN90-25M-55	M	SM	B	Control/Water	0.0	72h	200	53.18	1000	0.49
MN90-25F-1	F	SM	B	Control/Water	0.0	24h	200	52.45	1000	0.40
MN90-25F-2	F	SM	B	Control/Water	0.0	24h	200	54.73	1000	0.20
MN90-25F-3	F	SM	B	Control/Water	0.0	24h	200	51.18	1000	0.20
MN90-25F-4	F	SM	B	Control/Water	0.0	24h	200	46.08	1000	0.20
MN90-25F-5	F	SM	B	Control/Water	0.0	24h	200	52.65	1000	0.20
MN90-25F-21	F	SM	B	Control/Water	0.0	48h	200	48.86	1000	0.19
MN90-25F-22	F	SM	B	Control/Water	0.0	48h	200	51.79	1000	0.30
MN90-25F-23	F	SM	B	Control/Water	0.0	48h	200	55.00	1000	0.30
MN90-25F-24	F	SM	B	Control/Water	0.0	48h	200	51.08	1000	0.10
MN90-25F-25	F	SM	B	Control/Water	0.0	48h	200	50.72	1000	0.40
MN90-25F-41	F	SM	B	Control/Water	0.0	72h	200	54.63	1000	0.40
MN90-25F-42	F	SM	B	Control/Water	0.0	72h	200	63.55	1000	0.29
MN90-25F-43	F	SM	B	Control/Water	0.0	72h	200	55.92	1000	0.29
MN90-25F-44	F	SM	B	Control/Water	0.0	72h	200	49.27	1000	0.20
MN90-25F-45	F	SM	B	Control/Water	0.0	72h	200	59.31	1000	0.40
MN91-5M-1	M	SM	B	Control/water	0.0	24h	200	53.33	1000	0.10
MN91-5M-2	M	SM	B	Control/water	0.0	24h	200	50.94	1000	0.59
MN91-5M-3	M	SM	B	Control/water	0.0	24h	200	50.49	1000	0.30
MN91-5M-4	M	SM	B	Control/water	0.0	24h	200	44.81	1000	0.10
MN91-5M-5	M	SM	B	Control/water	0.0	24h	200	48.54	1000	0.20
MN91-5M-26	M	SM	B	Control/water	0.0	48h	200	52.80	1000	0.00
MN91-5M-27	M	SM	B	Control/water	0.0	48h	200	54.63	1000	0.20
MN91-5M-28	M	SM	B	Control/water	0.0	48h	200	41.87	1000	0.49
MN91-5M-29	M	SM	B	Control/water	0.0	48h	200	57.56	1000	0.00
MN91-5M-30	M	SM	B	Control/water	0.0	48h	200	57.14	1000	0.30

SRI HISTORICAL MN FREQUENCIES OF MICE  
TREATED WITH WATER: BONE MARROW SMEAR EVALUATIONS

Animal No.	S S E E P L X E L			Chemical	Dose mg/kg	Time	# RBC	PCE/ RBC	# PCE	2PCE u/MN
	C									
MN91-5F-1	F	SM	B	Control/water	0.0	24h	200	61.61	1000	0.20
MN91-5F-2	F	SM	B	Control/water	0.0	24h	200	47.03	1000	0.00
MN91-5F-3	F	SM	B	Control/water	0.0	24h	200	50.66	1000	0.10
MN91-5F-4	F	SM	B	Control/water	0.0	24h	200	62.75	1000	0.30
MN91-5F-5	F	SM	B	Control/water	0.0	24h	200	53.85	1000	0.20
MN91-5F-21	F	SM	B	Control/water	0.0	48h	200	50.71	1000	0.29
MN91-5F-22	F	SM	B	Control/water	0.0	48h	200	55.76	1000	0.20
MN91-5F-23	F	SM	B	Control/water	0.0	48h	200	52.94	1000	0.10
MN91-5F-24	F	SM	B	Control/water	0.0	48h	200	59.83	1000	0.30
MN91-5F-25	F	SM	B	Control/water	0.0	48h	200	48.71	1000	0.20
MN91-9M-1	M	SM	B	Control/water	0.0	24h	200	56.86	2000	0.15
MN91-9M-2	M	SM	B	Control/water	0.0	24h	200	64.79	2000	0.30
MN91-9M-3	M	SM	B	Control/water	0.0	24h	200	50.50	2000	0.25
MN91-9M-4	M	SM	B	Control/water	0.0	24h	200	68.08	2000	0.25
MN91-9M-5	M	SM	B	Control/water	0.0	24h	200	77.23	2000	0.30
MN91-9M-31	M	SM	B	Control/water	0.0	48h	200	54.88	2000	0.05
MN91-9M-32	M	SM	B	Control/water	0.0	48h	200	52.00	2000	0.05
MN91-9M-33	M	SM	B	Control/water	0.0	48h	200	73.95	2000	0.45
MN91-9M-34	M	SM	B	Control/water	0.0	48h	200	70.65	2000	0.15
MN91-9M-35	M	SM	B	Control/water	0.0	48h	200	64.55	2000	0.15
MN91-9F-1	F	SM	B	Control/water	0.0	24h	200	62.04	2000	0.30
MN91-9F-2	F	SM	B	Control/water	0.0	24h	200	57.01	2000	0.35
MN91-9F-3	F	SM	B	Control/water	0.0	24h	200	61.23	2000	0.45
MN91-9F-4	F	SM	B	Control/water	0.0	24h	200	61.82	2000	0.10
MN91-9F-5	F	SM	B	Control/water	0.0	24h	200	64.29	2000	0.25
MN91-9F-31	F	SM	B	Control/water	0.0	48h	200	55.74	2000	0.15
MN91-9F-32	F	SM	B	Control/water	0.0	48h	200	55.00	2000	0.45
MN91-9F-33	F	SM	B	Control/water	0.0	48h	200	67.79	2000	0.20
MN91-9F-34	F	SM	B	Control/water	0.0	48h	200	55.66	2000	0.25
MN91-9F-35	F	SM	B	Control/water	0.0	48h	200	51.89	2000	0.30
MN91-12M-1	M	SM	B	Control/water	0.0	24h	200	59.72	1000	0.10
MN91-12M-2	M	SM	B	Control/water	0.0	24h	200	44.95	1000	0.39
MN91-12M-3	M	SM	B	Control/water	0.0	24h	200	51.74	1000	0.20
MN91-12M-4	M	SM	B	Control/water	0.0	24h	200	55.98	1000	0.20
MN91-12M-5	M	SM	B	Control/water	0.0	24h	200	56.95	1000	0.50
MN91-12M-26	M	SM	B	Control/water	0.0	48h	200	50.99	1000	0.19
MN91-12M-27	M	SM	B	Control/water	0.0	48h	200	57.94	1000	0.40
MN91-12M-28	M	SM	B	Control/water	0.0	48h	200	58.85	1000	0.49
MN91-12M-29	M	SM	B	Control/water	0.0	48h	200	45.97	1000	0.10
MN91-12M-30	M	SM	B	Control/water	0.0	48h	200	50.00	1000	0.39

SRI HISTORICAL MN FREQUENCIES OF NICE  
TREATED WITH WATER: BONE MARROW SMEAR EVALUATIONS

Animal No.	S S E E P L X E L			Chemical	Dose mg/kg	Time	# RBC	PCE/ RBC	# PCE	2PCE %/MN
	C									
MN91-12F-1	F	SM	B	Control/water	0.0	24h	200	54.81	1000	0.30
MN91-12F-2	F	SM	B	Control/water	0.0	24h	200	63.00	1000	0.39
MN91-12F-3	F	SM	B	Control/water	0.0	24h	200	46.57	1000	0.20
MN91-12F-4	F	SM	B	Control/water	0.0	24h	200	51.85	1000	0.40
MN91-12F-5	F	SM	B	Control/water	0.0	24h	200	57.60	1000	0.39
MN91-12F-21	F	SM	B	Control/water	0.0	48h	200	56.54	1000	0.20
MN91-12F-22	F	SM	B	Control/water	0.0	48h	200	63.60	1000	0.39
MN91-12F-23	F	SM	B	Control/water	0.0	48h	200	53.88	1000	0.20
MN91-12F-24	F	SM	B	Control/water	0.0	48h	200	66.83	1000	0.30
MN91-12F-25	F	SM	B	Control/water	0.0	48h	200	48.57	1000	0.20
MN91-14M-1	M	SM	B	Control/water	0.0	24h	200	64.11	1000	0.30
MN91-14M-2	M	SM	B	Control/water	0.0	24h	200	45.00	1000	0.00
MN91-14M-3	M	SM	B	Control/water	0.0	24h	200	61.19	1000	0.70
MN91-14M-4	M	SM	B	Control/water	0.0	24h	200	55.13	1000	0.20
MN91-14M-5	M	SM	B	Control/water	0.0	24h	200	67.33	1000	0.20
MN91-14M-26	M	SM	B	Control/water	0.0	48h	200	52.00	1000	0.00
MN91-14M-27	M	SM	B	Control/water	0.0	48h	200	74.38	1000	0.10
MN91-14M-28	M	SM	B	Control/water	0.0	48h	200	58.05	1000	0.50
MN91-14M-29	M	SM	B	Control/water	0.0	48h	200	66.35	1000	0.00
MN91-14M-30	M	SM	B	Control/water	0.0	48h	200	63.86	1000	0.30
MN91-14F-1	F	SM	B	Control/water	0.0	24h	200	66.50	1000	0.10
MN91-14F-2	F	SM	B	Control/water	0.0	24h	200	64.00	1000	0.20
MN91-14F-3	F	SM	B	Control/water	0.0	24h	200	51.69	1000	0.50
MN91-14F-4	F	SM	B	Control/water	0.0	24h	200	50.00	1000	0.40
MN91-14F-5	F	SM	B	Control/water	0.0	24h	200	53.85	1000	0.10
MN91-14F-21	F	SM	B	Control/water	0.0	48h	200	54.23	1000	0.10
MN91-14F-22	F	SM	B	Control/water	0.0	48h	200	51.58	1000	0.20
MN91-14F-23	F	SM	B	Control/water	0.0	48h	200	49.08	1000	0.30
MN91-14F-24	F	SM	B	Control/water	0.0	48h	200	58.22	1000	0.20
MN91-14F-25	F	SM	B	Control/water	0.0	48h	200	61.50	1000	0.40
MN93-8M-1	M	SM	B	Control/soft water	0.0	24h	206	58.74	1005	0.20
MN93-8M-2	M	SM	B	Control/soft water	0.0	24h	201	48.26	1004	0.20
MN93-8M-3	M	SM	B	Control/soft water	0.0	24h	214	60.28	1007	0.20
MN93-8M-4	M	SM	B	Control/soft water	0.0	24h	217	49.77	1005	0.20
MN93-8M-5	M	SM	B	Control/soft water	0.0	24h	220	38.18	1001	0.10
MN93-8M-26	M	SM	B	Control/soft water	0.0	48h	210	57.62	1005	0.40
MN93-8M-27	M	SM	B	Control/soft water	0.0	48h	256	37.50	1010	0.20
MN93-8M-28	M	SM	B	Control/soft water	0.0	48h	224	57.59	1009	0.10
MN93-8M-29	M	SM	B	Control/soft water	0.0	48h	224	65.52	1012	0.30
MN93-8M-30	M	SM	B	Control/soft water	0.0	48h	201	69.15	1006	0.10

SRI HISTORICAL MN FREQUENCIES OF NICE  
TREATED WITH WATER: BONE MARROW SMEAR EVALUATIONS

Animal No.	S S E			Chemical	Dose mg/kg	Time	# RBC	PCE/RBC	# PCE	%PCE w/MN
	X	E	L							
MN93-8F-1	F	SM	B	Control/soft water	0.0	24h	231	45.89	1014	0.30
MN93-8F-2	F	SM	B	Control/soft water	0.0	24h	213	68.54	1003	0.10
MN93-8F-3	F	SM	B	Control/soft water	0.0	24h	204	64.22	1008	0.00
MN93-8F-4	F	SM	B	Control/soft water	0.0	24h	203	54.19	1015	0.20
MN93-8F-5	F	SM	B	Control/soft water	0.0	24h	225	41.33	1015	0.10
MN93-8F-21	F	SM	B	Control/soft water	0.0	48h	203	58.62	1001	0.20
MN93-8F-22	F	SM	B	Control/soft water	0.0	48h	237	64.98	1015	0.39
MN93-8F-23	F	SM	B	Control/soft water	0.0	48h	227	65.20	1015	0.20
MN93-8F-24	F	SM	B	Control/soft water	0.0	48h	236	58.05	1016	0.00
MN93-8F-25	F	SM	B	Control/soft water	0.0	48h	223	81.61	1010	0.10
MN94D48M-1	M	SM	B	Control/water	0.0	24h	201	56.72	2009	0.25
MN94D48M-2	M	SM	B	Control/water	0.0	24h	202	46.04	2009	0.10
MN94D48M-3	M	SM	B	Control/water	0.0	24h	235	57.45	2003	0.10
MN94D48M-4	M	SM	B	Control/water	0.0	24h	217	59.36	2001	0.20
MN94D48M-5	M	SM	B	Control/water	0.0	24h	222	59.91	2004	0.10
MN94D48M-26	M	SM	B	Control/water	0.0	48h	216	56.94	2021	0.20
MN94D48M-27	M	SM	B	Control/water	0.0	48h	201	47.76	2004	0.10
MN94D48M-28	M	SM	B	Control/water	0.0	48h	201	58.71	2008	0.20
MN94D48M-29	M	SM	B	Control/water	0.0	48h	238	50.84	2066	0.24
MN94D48M-30	M	SM	B	Control/water	0.0	48h	213	69.95	2050	0.10
MN94D48M-51	M	SM	B	Control/water	0.0	72h	204	61.65	2010	0.20
MN94D48M-52	M	SM	B	Control/water	0.0	72h	238	55.04	2003	0.15
MN94D48M-53	M	SM	B	Control/water	0.0	72h	204	51.96	2014	0.05
MN94D48M-54	M	SM	B	Control/water	0.0	72h	214	50.00	2015	0.25
MN94D48M-55	M	SM	B	Control/water	0.0	72h	206	32.52	2013	0.05
MN94D58M-1	F	SM	B	Control/water	0.0	24h	232	52.16	2001	0.05
MN94D58M-2	F	SM	B	Control/water	0.0	24h	219	53.88	2012	0.15
MN94D58M-3	F	SM	B	Control/water	0.0	24h	214	68.22	2032	0.05
MN94D58M-4	F	SM	B	Control/water	0.0	24h	219	45.66	2052	0.05
MN94D58M-5	F	SM	B	Control/water	0.0	24h	205	54.63	2023	0.15
MN94D58M-21	F	SM	B	Control/water	0.0	48h	210	57.14	2023	0.05
MN94D58M-22	F	SM	B	Control/water	0.0	48h	210	56.19	2017	0.05
MN94D58M-23	F	SM	B	Control/water	0.0	48h	207	49.28	2001	0.05
MN94D58M-24	F	SM	B	Control/water	0.0	48h	200	54.50	2016	0.25
MN94D58M-25	F	SM	B	Control/water	0.0	48h	206	61.17	2029	0.10
MN94D58M-41	F	SM	B	Control/water	0.0	72h	207	52.66	2029	0.05
MN94D58M-42	F	SM	B	Control/water	0.0	72h	211	54.98	2001	0.15
MN94D58M-43	F	SM	B	Control/water	0.0	72h	212	46.04	2006	0.20
MN94D58M-44	F	SM	B	Control/water	0.0	72h	227	43.61	2016	0.00
MN94D58M-45	F	SM	B	Control/water	0.0	72h	213	79.34	2011	0.20

Minimum MN value: 0.00%  
 Maximum MN value: 0.70%  
 Mean average value: 0.21%

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