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CHEMICAL MANUFACTURERS ASSOCIATION

April 8, 1994

Gordon D. Strickland
Vice President-Technical Services

Dr. Lynn Goldman
Assistant Administrator
Office of Prevention, Pesticides
and Toxic Substances
TS-7101
Environmental Protection Agency
401 M Street, SW, Room 637 East Tower
Washington, DC 20460

Dear Dr. Goldman:

The Chemical Manufacturers Association makes public all final reports developed from research projects that it administers. The following reports, recently completed, are enclosed:

"Subchronic Toxicity Study by the Inhalation Route in Rats and Mice with 4-Vinylcyclohexene," Volumes 1 and 2.

"Rat and Mouse Bone Marrow Micronucleus Assay of 4-Vinylcyclohexene Following Subchronic Inhalation Exposure, Revision No. 1"

"Two-week Range-Finding Study with 4-Vinylcyclohexene in Rats and Mice"

"In Vitro Metabolism of 4-Vinylcyclohexene and Metabolites"

"Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice"

"Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints"

These reports do not include confidential information.

If you have any questions, please call Dr. Elizabeth J. Moran of my staff at 202/887-1182.

RECEIVED
6/24/94

Sincerely,

Gordon D. Strickland

2501 M Street, NW, Washington, DC 20037 202-887-1388 Panafax 202-887-1237 Telex 89617 (CMA WSH)

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Study Title

Subchronic Toxicity Study by the Inhalation Route in Rats and Mice with 4-Vinylcyclohexene

Volume 1 of 2

Laboratory Project ID

Haskell Laboratory Report No. 442-93

Author

Judith C. Stadler, Ph.D., D.A.B.T.

Study Completed on

February 14, 1994

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, Delaware 19714

Chemical Manufacturers Association Study Number

BUT-21.0-COT-DHL-03

Medical Research Project No. 9523-001

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to EPA Good Laboratory Practice Standards (40 CFR 792) except for the deviations documented and explained below.

1. In some instances, standard forms were printed with initials prior to recording data; raw data entered on those forms were not, therefore, initialed and dated at the time of entry.

2. At the time of the study, the Standard Operating Procedure (SOP) for the automated environmental monitoring system used for inhalation chambers in this laboratory did not include the specific instrument used in the study. Although this instrument is similar in design to the others, the requirement for an SOP was not fully satisfied. The SOP has subsequently been revised and the instrument number added to the maintenance logs.

These deviations did not affect the integrity of the study.

Submitter: E. I. du Pont de Nemours and Company

Sponsor: Chemical Manufacturers Association, Washington, D.C.

Date

Study Director: Judith C. Stadler 2/14/94

Judith C. Stadler, Ph.D., D.A.B.T.
Senior Research Toxicologist
Inhalation and Oral Toxicology

Sponsor Representative: 2/2/94

Elizabeth J. Moran, Ph.D., D.A.B.T.
Chemical Manufacturers Association

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

GENERAL INFORMATION

Material Tested: Cyclohexene, 4-ethenyl- | Haskell No. 19,353

Other Codes and/or Synonyms:
4-Ethenylcyclohexene
Vinylcyclohexene (Vinyl cyclohexene)
4-Vinylcyclohexene-1
4-Vinylcyclohexene
VCH
VN MK 6A
ID# IN210G308
4-VCH

C.A.S. Registry Number: 100-40-3

Purity: 99.8% (as supplied)

Contaminants: t-Butylcatechol as a stabilizer

Physical Form: colorless liquid

Material Submitted by: Union Carbide
Institute, West Virginia

Material Tested: 1,3-Butadiene | Haskell No. 19,999

Other Codes and/or Synonyms:
1,3-Butadiene (BD)
Lot# TK1661
CMA Contract BUT-21.0-COT-DHL

C.A.S. Registry Number: 106-99-0

Purity: 99% (as supplied)

Composition: 99% 1,3-Butadiene
100 ppm of TBC (tert-butyl catechol)

Physical Form: colorless gas

Material Submitted by: Exxon
Baton Rouge, Louisiana

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

GENERAL INFORMATION (Continued)

Medical Research Project No.: 9523-001

Sponsor: Chemical Manufacturers Association
2561 M Street, N.W.
Washington, D.C. 20037

Contact Person: E. J. Moran
Chemical Manufacturers Association
Washington, D.C.

Study Initiation/Completion Dates: 12-3-92 / 2-14-94

In-life Study Initiated/Completed: 1-26-93 / 4-30-93

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

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SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

SUMMARY

Groups of ten male and ten female Crl:CD®BR rats and ten male and ten female $B6C3F_1$/CrlBR mice each were exposed for six hours per day, five days per week to 4-vinylcyclohexene (4-VCH). The rats were exposed to concentrations of 4-VCH targeted to 0, 250, 1000 or 1500 ppm and the mice were exposed to targeted concentrations of 0, 50, 250 or 1000 ppm. In addition, another group was exposed to 1,3-butadiene by inhalation to compare the toxicity of the two compounds. Both rats and mice were exposed to 1,3-butadiene targeted to a concentration of 1000 ppm. The exposures were conducted over a period of approximately 13 weeks. The animals were weighed and observed once each week and food consumption was monitored throughout the study. Starting on test day 10, clinical signs of toxicity that were apparent immediately following exposures were also recorded. Animals were examined for ophthalmological abnormalities prior to study start and before the end of the study. Clinical pathology evaluations were conducted 92-95 days after study start. At the end of the study all animals were sacrificed and necropsied, and selected tissues were evaluated microscopically.

The mean concentrations for study exposures in the chambers targeted to 50, 250, 1000 or 1500 ppm 4-VCH were 53, 250, 1000, and 1500 ppm, respectively. The mean concentration measured in the 1,3-butadiene exposure chamber was 980 ppm. Temperature and humidity conditions in the exposure chambers were within acceptable ranges for the study.

Significant body weight effects from exposure to 4-VCH were observed for rats, but not for mice. The male rats in the 1500 ppm 4-VCH exposure group had mean body weights that were significantly lower than mean body weights of controls and the male and female rats in the 1500 ppm group had low weight gains that were statistically significant. None of the other groups of rats and none of the groups of mice exposed to 4-VCH or 1,3-butadiene had significant effects on weight gain. No differences in mean food consumption were noted for any groups of male or female rats or mice; however, mean food

efficiency was affected in rats exposed to 1500 ppm 4-VCH. The decreased efficiency was correlated with the low mean body weight gain of that group.

Compound-related mortality occurred in study mice exposed to 1000 ppm 4-VCH. Ten of ten male mice and five of ten female mice died on test day 11 or 12. Three additional female mice in the 1000 ppm 4-VCH group died prior to study completion. Additional deaths among study mice exposed to 4-VCH or 1,3-butadiene were not directly attributable to compound exposure. There was no compound-related mortality in study rats.

Clinical signs observed in rats and mice immediately following exposures were frequent, but transient. Signs such as discharge from the mouth and nose and stained fur were considered exposure-related effects, and not compound-specific effects. The most notable compound-related effect was lethargy, which was observed in male rats exposed to 1500 ppm 4-VCH.

None of the rats exposed to 4-VCH or 1,3-butadiene had compound-related abnormalities in the hematological or clinical chemical evaluations nor in the urinalysis assays at the end of the study. None of the mice exposed to 50 or 250 ppm 4-VCH had compound-related hematological effects. Insufficient numbers of mice exposed to 1000 ppm 4-VCH were available for meaningful data evaluation. Mice exposed to 1000 ppm 1,3-butadiene had mild hemolytic anemia and mild decreases in platelet counts.

Compound-related increases in organ weights and microscopic abnormalities were observed in study rats; however, they were considered non-adverse effects. Male and female rats exposed to 1000 or 1500 ppm 4-VCH or 1000 ppm 1,3-butadiene had increased absolute and/or relative liver weights, and the male rats in these same exposure groups had increased relative kidney weights. Microscopically, an increased accumulation of hyaline droplets was observed in male rat kidneys. Although related to compound exposure, the droplets were not accompanied by cytotoxicity and were limited to male rats only. For this reason, they were believed to have been associated with α_{2u}-globulin and not to have been an adverse effect in the rats.

The most notable adverse effect observed during pathological evaluations of study mice was ovarian atrophy in the females exposed to 1000 ppm 4-VCH or 1000 ppm 1,3-butadiene. The atrophy was slightly more severe in the group exposed to 4-VCH than in the group exposed to 1,3-butadiene. There were no other pathological effects in male or female mice exposed to 4-VCH. Additionally, male mice exposed to 1,3-butadiene had decreased testicular weights accompanied by slight testicular degeneration and atrophy. The ovarian and testicular effects were considered adverse and attributable to compound exposures.

The no-observable-adverse-effect level for study rats was 1000 ppm 4-VCH and for mice was 250 ppm 4-VCH.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

SIGNATURES

Date

Approved by: Robert W Rickard 2/3/94
Robert W. Rickard, Ph.D., D.A.B.T.
Manager
Inhalation and Oral Toxicology

Authored, Reviewed and
Approved for Issue
by Study Director: Judith C Stadler 2/14/94
Judith C. Stadler, Ph.D, D.A.B.T.
Senior Research Toxicologist
Inhalation and Oral Toxicology

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

QUALITY ASSURANCE DOCUMENTATION

H-19353

Dates of Inspection:

Conduct - 2/3/93; 4/28-29/93

Records, Report(s) - 3/26,29-30/93; 4/1-5/93; 6/29-30/93; 7/1,7-9,22-23/93; 8/3-6,9-11/93; 10/19-20,25-28/93; 11/2-3,22-23,29-31/93; 12/1-3,5-8/93

Findings reported to:

Study Director - 4/5/93; 7/9/93; 8/11/93; 11/4,10/93; 12/8/93

Management - 7/9/93; 8/11/93; 10/7/93; 11/4,10/93; 12/9/93

Reported by: Deborah A. Vick
Deborah A. Vick
Quality Assurance Auditor

2/14/94
Date

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

ACKNOWLEDGMENTS

The following individuals were responsible for the conduct of the study:

Manager:	Robert W. Rickard, Ph.D.
Study Director:	Judith C. Stadler, Ph.D (9/10/93 - present) Anthony S. Panepinto, M.S. Hygiene (12/3/92 - 9/9/93)
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Toxicology Report Preparation:	Maryanne M. Wilford, B.A.

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Managers:	Nancy C. Chromey, Ph.D.
Clinical Pathologist:	Glenn S. Elliott, D.V.M., Ph.D.
Pathologist:	Steven R. Frame, D.V.M., Ph.D.
Peer Review Pathologist:	Theodore W. Slone, D.V.M.
Pathology Report Preparation:	Wanda F. Dinbokowitz

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

ACKNOWLEDGMENTS (Continued)

The following individuals were responsible for quality assurance:

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Auditor: Deborah A. Vick

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15021 Dufief Mill Road
Gaithersburg, Maryland 20878

The following individual was responsible for the health status assessment for the animals on study:

Laboratory Veterinarian: Charles E. Cover, V.M.D.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

INTRODUCTION

The test substance, 4-vinylcyclohexene (4-VCH) is a dimer of 1,3-butadiene. It is formed during 1,3-butadiene production and is also used in polymer manufacturing. In a 13-week oral study, 4-VCH produced mortality and body weight effects, as well as hyaline droplets in the kidneys of male rats and ovarian atrophy in female mice.[1] In a subsequent chronic oral study, 4-VCH caused ovarian tumors in female mice.[1] No previous subchronic or chronic studies have been conducted with 4-VCH by the inhalation route. Extensive toxicity testing has been conducted with 1,3-butadiene, including 13-week and chronic inhalation studies. 1,3-Butadiene was carcinogenic to both rats and mice in the long-term study.[2]

Due to the potential for human exposure and the results of the 1,3-butadiene studies, a subchronic inhalation study with 4-VCH was deemed appropriate. This study was conducted according to requirements of the U. S. Environmental Protection Agency in a consent order on 4-VCH, promulgated under the provisions of Section 4 of the Toxic Substances Control Act.[3]

Prior to initiation of this 13-week study, a two-week range-finding study was conducted with rats and mice exposed to 0, 250, 750, or 1500 ppm 4-VCH.[4] Exposure concentrations for this study were set based on effects observed in rats and mice exposed to 1500 ppm. These effects included significant mortality in mice and significant body weight effects in male rats.

OBJECTIVES

The objective of this study was to evaluate the subchronic toxicity of 4-vinylcyclohexene (4-VCH) when administered by inhalation to male and female Crl:CD®BR rats and to male and female $B6C3F_1$/CrlBR mice for approximately 13 weeks. The inhalation route of administration was selected because it is the potential route of human exposure. A 1,3-butadiene exposure group was added to

the study so that a comparison could be made between effects from exposure to 4-VCH and effects that result from exposure to 1,3-butadiene.

MATERIALS AND METHODS

A. Test Substance

The test substance, 4-vinylcyclohexene (4-VCH), is a colorless liquid at room temperature. It was supplied in a 55-gallon drum by the Union Carbide Corporation, Institute, West Virginia. The analytical purity of 4-VCH was determined by gas chromatography while it was stored at Haskell Laboratory.

The positive control substance, 1,3-butadiene, is a colorless gas at room temperature. It was supplied in DOT 4BW240 cylinders by the Exxon Chemical Company, Baton Rouge, Louisiana. Analytical purity of each cylinder of positive control material was determined by gas chromatography at Haskell Laboratory.

B. Test Species

On 1/12/93, 65 male and 65 female Crl:CD®BR rats, approximately 22 days of age, were acquired from Charles River Laboratories, Inc., Kingston, New York. The rats were weighed on 1/14/93 and the body weights ranged from 47 to 73 grams for the male rats, and 35 to 73 grams for the female rats. The Crl:CD®BR rat was selected based on the request of the study sponsor and the extensive experience with this strain at Haskell Laboratory. The strain has been suitable for hardiness, longevity, sensitivity, and low incidence of spontaneous diseases.

On 1/12/93, 65 male and 65 female B6C3F_1/CrlBR mice, approximately 36 days of age, were acquired from Charles River Laboratories, Inc., Raleigh, North Carolina. On 1/14/93, the body weights ranged from 16 to 25 grams for the male mice, and from 13 to 20 grams for the female mice. The B6C3F_1/CrlBR mouse was

used in previous 4-VCH studies and was requested by the study sponsor. It is suitable in inhalation studies with respect to hardiness, longevity, sensitivity, and low incidence of spontaneous diseases.

C. Animal Husbandry

All animals were housed in stainless steel, wire-mesh cages suspended above cage boards. Males and females were placed on separate cage racks, and the two species were housed in separate rooms during the pretest period and in the same room during the study. Cages were repositioned on the racks and racks were repositioned in the animal room every two weeks. The rats and mice were housed in a Bioclean® room (laminar flow air circulation pattern) in close proximity to the inhalation chambers. The animal room was maintained on a 12-hour light/dark cycle (fluorescent light) and targeted at a temperature of 23° ± 2°C and a relative humidity of 55 ± 15%. Excursions outside the target ranges were considered of insufficient magnitude or duration to have affected the outcome of the study.

D. Pretest Period

Upon arrival at Haskell Laboratory, the animals were removed from shipping cartons and housed with sexes on separate cage racks, and rats and mice in separate quarantine rooms. The rats were housed one per cage and the mice were housed two per cage. Six days before the first exposure (test day -6), mice were moved to one per cage. Each animal was temporarily identified with a one- to two-digit number, and the cages were labeled with the temporary numbers. Animals were weighed three (rats) or four (mice) times during the pretest period and observed daily for any gross signs of disease or injury. On 1/25/93, all animals were examined by a veterinary ophthalmologist (see Section N).

During the pretest period, three female mice were found dead. On the bases of body weight gain and clinical observations, the rats were released from quarantine on 1/21/93, and the mice on 1/20/93 by the laboratory veterinarian.

On test day -1, a female mouse (temporary ID 63) was accidentally killed. It was replaced with another female mouse (temporary ID 60) and assigned study ID 1001 in the 1,3-butadiene exposure group.

E. Study Design

There were five groups of inhalation study animals of each species: a negative (air) control group, three groups exposed to different concentrations of 4-VCH, and one 1,3-butadiene exposure group.

An age-matched group of rats was also maintained during the 90-day period, for use in a micronucleus study which was conducted concurrently with this study. The additional rats were weighed and observed over the 13-week period and injected with cyclophosphamide at the end of the study as a positive control for the micronucleus assay. Results of that study will be reported separately.

Groups of study animals were as follows:

GROUP DESIGNATION	# RATS/SEX	ATMOSPHERIC CONCENTRATION (ppm)
Control	10	0
Low 4-VCH	10	250
Intermediate 4-VCH	10	1000
High 4-VCH	10	1500
1,3-butadiene	10	1000
Cyclophosphamide (positive control)	5	No exposure

GROUP DESIGNATION	# MICE/SEX	ATMOSPHERIC CONCENTRATION (ppm)
Control	10	0
Low 4-VCH	10	50
Intermediate 4-VCH	10	250
High 4-VCH	10	1000
1,3-butadiene	10	1000

F. Assignment to Groups and Study Start

Near the end of the quarantine period, 55 rats and 50 mice of each sex were selected for study use based on adequate body weight gain and freedom from any clinical signs of disease or injury. The selected animals were divided by computerized, stratified randomization into groups. The groups were constructed so that there were no statistically significant differences among group body weight means within a sex. The order of animals within each group was randomized.

After assignment to groups, rats and mice were housed individually. Each animal was permanently identified with a number tattooed on the tail. The study started on 1/26/93, which was designated as Test Day 1. The rats were approximately 36 days of age and the mice were approximately 50 days of age at study start. Rats that were not assigned to a test group were removed from the animal room on 2/23/93 and were sacrificed. Mice that were not assigned to a test group were removed from the animal room on 2/9/93 (males) or 2/10/93 (females) and were sacrificed.

G. Diet and Water

Throughout the pretest and test periods, except during exposure, Purina® Certified Rodent Chow® #5002 (chunk) and water supplied by the Wilmington Suburban Water Corporation were provided ad libitum.

As part of an animal health monitoring program, food and water analyses for contaminants were conducted approximately every month. This program is monitored and administered by the laboratory veterinarian; data are maintained separately from study records. Feed analyses indicated that feed was within recommended limits set by the National Center for Toxicological Research for bacteria, bacterial spores, and filamentous fungi. Pathogenic bacteria were not detected in water samples collected during the course of the study.

H. Test Chambers (Figure 1)

Six chambers were dedicated to the study throughout the duration of the exposure period. One 150-L chamber was used to expose mice to the low 4-VCH concentration of 50 ppm. Five 300-L chambers were used to expose rats only to 1500 ppm 4-VCH, or rats and mice to the other 4-VCH concentrations and to 1,3-butadiene. The chambers and all surfaces that the test material contacted prior to exhaust from the chamber were constructed of stainless steel or glass, and were thoroughly cleaned prior to introduction of the test substance.

The chambers were operated in a one-pass, flow-through mode with an air-flow rate targeted at 30-40 L/min for the 150-L chamber and 60-70 L/min for the 300-L chambers. During the 4-VCH and 1,3-butadiene exposures, the relative humidity of the chamber air, chamber temperature, and chamber air-flow rates were monitored continually and recorded at approximately five-minute intervals. The temperature and humidity of the negative control chamber (0 ppm 4-VCH) were monitored and recorded at approximately 30-minute intervals; and the airflow was recorded once per exposure. Chamber oxygen content was determined at least twice daily.

Chamber exhaust was passed through a carbon bed prior to release through the roof exhaust system. Emissions fell within the bounds of a laboratory permit from the state of Delaware Department of Natural Resources and Environmental Control.

I. Exposures

Groups of ten male and ten female rats, and ten male and ten female mice were exposed to one of three targeted 4-VCH concentrations or one 1,3-butadiene concentration for six hours per day. A negative control group of ten male and ten female rats, and ten male and ten female mice were exposed to air alone. In most cases, the exposures were five days per week, for a total of 65 exposures over a 13-week period.

Groups of study animals were initially exposed to test substance on four separate days so that the total number of exposures would be the same for each group, with sacrifice and necropsy on different days. In general, male mice started on test day 1, female mice on test day 2, male rats on test day 3, and female rats on test day 4. Due to difficulties with the exposure system, however, male mice were initially exposed to 1,3-butadiene on test day 2. These male mice were exposed separately on test day 54 to ensure a study total of 65 exposures. Due to a holiday, all animals were exposed only four days during week 11 and six days during week 12.

Exposures were conducted during approximately the same eight-hour period each day to minimize diurnal physiological variations. During exposure, rats and mice were housed individually in stainless steel cage modules. Throughout the daily exposure periods, animals visible through the chamber window were observed for well-being. Observations made while animals were in the chambers were documented at least once each day. At the time the observations were documented, the response of the animals to a noise (made by rapping on the chamber window) was recorded as a measure of general alertness. Before transfer to housing facilities at the end of each exposure, test groups were left in their respective exposure chambers for a period sufficient to allow clearance of test substances from the chamber atmospheres. Control animals were treated exactly as test animals, except that the negative control chamber atmosphere did not contain a test substance.

J. Atmosphere Generation and Analyses

Atmospheres of 4-VCH were generated by metering the liquid test substance, with either a Harvard Apparatus model 22 Syringe Infusion Pump or a Fluid Metering Incorporated model QG6 Displacement Pump, into a heated, glass Instatherm flask. Vapor was diluted by filtered air to the desired concentrations for each of the four test chambers. Chamber concentrations of 4-VCH were controlled by varying the test substance flow rate into the Instatherm flasks. A baffle located at the inlet of the exposure chamber helped evenly disperse the vapor within the chamber. Filtered air alone was metered in an identical manner into the negative control chamber. The

Instatherm flasks and connecting glass tubing were maintained at a constant temperature, of approximately 28-30°C.

Atmospheres of 1,3-butadiene were generated by metering the gas through a Brooks model 1355 Sho-Rate Flowmeter. Vapor was diluted by filtered air to the desired concentration and controlled by varying the 1,3-butadiene flow rate into the exposure chamber. A baffle located at the inlet of the exposure chamber helped evenly disperse the vapor within the chamber.

Chamber atmospheres were analyzed by gas chromatography at approximately 30-minute intervals during each six-hour exposure period. Samples were drawn by vacuum pump from representative areas of the chambers. A distribution study was conducted prior to study start with samples taken from one of the 300-L chambers. The variability of concentrations at the top, middle, and bottom of the inhalation chamber was tested against variability of four samples taken from one of the sample ports. There were no significant differences; therefore, the test substance (4-VCH) was considered adequately distributed throughout the inhalation chamber. Distribution was assumed to be similar for all of the 300-L chambers. Adequate distribution of 4-VCH in 150-L chambers was confirmed in previous studies.

Gas samples of 4-VCH (approximately 0.5 ml) were analyzed with a Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector. The temperature of the injection port was 100°C, and the temperature of the flame ionization detector was 250°C. Nitrogen, the carrier gas, had a flow rate of 15 ml/min. Samples were chromatographed isothermally at 75°C on a 15m x 0.53mm (inside diameter) SPB-S column. The detector was supplied with air (350 ml/min) and hydrogen (30 ml/min). The atmospheric concentration of 4-VCH was determined by comparing the detector response of the chamber samples to standard curves. The 4-VCH standards were prepared prior to each exposure by injecting known volumes of 4-VCH liquid into gas bags which contained known volumes of air.

Gas samples of 1,3-butadiene (approximately 0.5 ml) were analyzed with a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector. The temperature of the injection port was 50°C, and the temperature

of the flame ionization detector was 250°C. The nitrogen carrier gas had a flow rate of 12 ml/min. Samples were chromatographed isothermally at 35°C on a 10m x 0.53mm (inside diameter) HP-5 or HP-17 column. The detector was supplied with air (350 ml/min) and hydrogen (30 ml/min). The atmospheric concentration of 1,3-butadiene was determined by comparing the detector response of the chamber samples to standard curves. The gas standards were prepared prior to each exposure by injecting known volumes of 1,3-butadiene gas into gas bags which contained known volumes of air.

K. Body Weights

All animals were weighed once per week throughout the study.

L. Food Consumption and Food Efficiency

The amount of food consumed by each test group over each weighing interval was determined throughout the study. Group food consumption was determined by weighing the amount of chow in the food bucket at the beginning of the interval and subtracting the diet remaining and the amount of spillage from feeders at the end of the interval. From these determinations and body weight data, mean individual daily food consumption and food efficiency were calculated.

M. Clinical Observations and Mortality

Cage-site examinations to detect moribund or dead animals and abnormalities in behavior and appearance were conducted twice daily on weekdays and at least once daily on weekends and holidays. Moribund animals were sacrificed. Animals that died or were sacrificed *in extremis* received a pathological examination. At each weighing, each animal was individually handled and examined for abnormal behavior, appearance, and palpable masses. Initially in the study, clinical observations were made and recorded on a group basis when the animals were removed from the exposure chambers. Starting on test day 10, due to the high incidence of clinical signs, observations were made and

recorded for individual animals after each exposure.

N. Ophthalmological Evaluation

Two ophthalmological examinations were conducted by a veterinary ophthalmologist. At least one hour before each examination, one or two drops of 1% tropicamide were placed in each eye of every animal to produce mydriasis. Both eyes were examined by focal illumination and indirect ophthalmoscopy.

The initial examination was performed during the pretest period (test day -1) on all animals received for the study. All surviving animals were examined prior to the 13-week sacrifice (test day 88). A report of the findings from both ocular examinations was submitted by the examining ophthalmologist.

O. Clinical Laboratory Evaluation

The clinical laboratory evaluations of mice were conducted on test days 92 and 93. The evaluations of rats were conducted on test days 94 and 95.

Two days prior to the collection of blood samples for clinical evaluation, the rats were placed in metabolism cages and allowed to acclimate to the new cages. The day prior to blood collection, rats were returned to the metabolism cages after the exposure period. They were allowed access to food and water for at least two hours following the final exposure period. After this two-hour period, the food was removed from the cage and urine collection was initiated. At the end of the overnight urine collection period (approximately 14-16 hours), blood samples were collected. The mice were allowed a two-hour feeding period following the last exposure, were fasted overnight, and had blood samples drawn the next morning. Urine was not collected from mice.

Blood samples for hematological and clinical chemical measurements were collected from the orbital sinus of each animal while the animal was under light carbon dioxide anesthesia.

Bone marrow smears were prepared from all rats and mice at the 13-week sacrifice; however, no further evaluation was necessary to verify any clinical laboratory results. Additional bone marrow smears were collected from each rat and mouse sacrificed by design for evaluation of micronuclei. Evaluation of the bone marrow for micronuclei proceeded under a separate protocol and results have been reported elsewhere.

1. Hematology

The hematological parameters examined for rats and mice were erythrocyte, leukocyte, differential leukocyte, and platelet counts, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration. Blood smears for reticulocyte counts were prepared from each animal and were evaluated for male mice in the 0 and 250 ppm 4-VCH groups, female mice in the 0 and 1000 ppm 4-VCH groups, and male and female mice exposed to 1000 ppm 1,3-butadiene.

2. Clinical Chemistry

Blood serum from rats was evaluated for alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities, and concentrations of blood urea nitrogen, total serum protein, albumin, globulin (calculated), creatinine, cholesterol, glucose, calcium, sodium, potassium, phosphate, chloride, and total bilirubin.

3. Urine Analyses

Urine collected from rats during the fasting period was measured for the following: volume, pH, osmolality, glucose, protein, bilirubin, urobilinogen, ketone, and occult blood. Urine color and transparency were recorded, and sediment from each urine sample was microscopically examined.

P. Pathological Evaluation

All animals that were found dead, accidentally killed, or were sacrificed

in extremis were necropsied. After approximately 13 weeks on test (test days 92-95), all surviving rats and mice were sacrificed and necropsied. The order of sacrifice was random among all groups within a sex.

The spleen, heart, lungs, liver, kidneys, adrenal glands (rats only), testes, ovaries (rats only), and brain of rats and mice sacrificed by design were weighed wet at necropsy. Organ weight/final body weight ratios were calculated.

The following tissues were collected from all rats and mice (tissue integrity permitting): skin, bone marrow (sternal and femoral), mesenteric lymph nodes, mandibular lymph nodes, spleen, thymus, aorta, heart, trachea, lungs, nose, larynx, pharynx, salivary glands, esophagus, stomach, liver, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, kidneys, urinary bladder, pituitary, thyroid, parathyroid, adrenal glands, prostate, testes, epididymides, seminal vesicles, mammary gland, ovaries, uterus, vagina, brain, spinal cord, sciatic nerve, skeletal muscle, sternum, femur/knee joint, eyes (retinas evaluated separately), exorbital lacrimal glands, Harderian glands, Zymbal's glands, gallbladder (mice only), and all gross lesions.

All tissues were fixed in 10% neutral buffered formalin except testes, epididymides, eyes, and skin with mammary gland, which were fixed in Bouins.

All tissues collected from rats and mice in the high-concentration, negative control, and positive control groups, and from all rats and mice that were found dead (tissue integrity permitting), were accidentally killed, or were sacrificed in extremis, were further processed to slides, stained with hematoxylin and eosin, and examined microscopically. Nose, lungs, liver, kidneys, ovaries, and all organs with gross lesions from rats and mice in the low- and intermediate- concentration groups were also processed to slides and examined microscopically.

Tissue samples from the livers and lungs of study animals exposed to 1,3-butadiene were collected for DNA adduct studies and sent to the University of North Carolina. In addition, blood cells from these animals were also sent for adduct studies (not DNA adducts as indicated in Protocol Amendment #2).

Reports of those studies will be documented elsewhere.

Q. Statistical Analyses

Body weights, body weight gains, organ weights, and clinical laboratory measurements were analyzed by a one-way analysis of variance for animals exposed to 4-VCH. When the corresponding F-test for differences among test group means was significant, pairwise comparisons between test and control groups were made with the Dunnett's test. Only pairwise comparisons were made between control and 1,3-butadiene-exposed animals.

Incidence of clinical observations was evaluated by the Fisher's Exact test with a Bonferroni correction. If the Fisher's Exact test was significant, the Cochran-Armitage test for trend was used for groups exposed to 4-VCH.

Bartlett's test for homogeneity of variances was performed on the organ weight and clinical laboratory data and, if significant (alpha = 0.005), was followed by nonparametric procedures.

Except for Bartlett's test, all other significance was judged at alpha = 0.05.

RECORDS AND SAMPLE RETENTION

All original data and the original of this report were retained at Haskell Laboratory or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware. Preserved wet tissues, paraffin blocks, histological slides, and blood and bone marrow smears were retained at Haskell Laboratory. Samples from each drum and/or cylinder of the test substances and records in support of the test material purity analyses were retained by Haskell Laboratory.

RESULTS AND DISCUSSION

A. Sample Stability and Chamber Concentrations of Test Substances
(Purity Analysis Report, Table 1, Figure 2, Appendix B)

The test samples received for use in the study were analyzed prior to study start and following the last exposure. The purity of 4-vinylcyclohexene (4-VCH) prior to study start was 99.59% and following the study was 99.67%. The purity of 1,3-butadiene prior to study start was 99.9% and after the study was 99.9% (see Purity Analysis Report). Both materials were considered stable over the duration of the study.

The mean concentrations ± SEM of 4-VCH in exposure chambers targeted to 50, 250, 1000 or 1500 ppm were 53 ± 1.2, 250 ± 3.3, 1000 ± 9.8, and 1500 ± 9.7 ppm (Table 1). The mean concentration ± SEM of 1,3-butadiene in the chamber targeted to 1000 ppm was 980 ± 16 ppm.

The majority of the daily mean concentrations of 4-VCH and 1,3-butadiene were within ± 20% of targeted concentrations; however, there were a few daily mean concentrations outside this range (Appendix B). The ranges of the daily mean concentrations of these chambers were 32 to 69 ppm, 110 to 300 ppm, 680 to 1100 ppm and 1200 to 1600 ppm 4-VCH for the 50, 250, 1000 and 1500 ppm 4-VCH chambers, respectively. The range of daily mean concentrations was 630 to 1300 ppm for the 1,3-butadiene chamber. The excursions were primarily on the low end of the range (Figure 2), and none of these excursions were considered to have impacted the outcome of the study.

B. Chamber Temperature, Humidity, and Airflow
(Table 1, Appendices C-E)

The mean temperatures in the exposure chambers for all exposure days were between 23 and 27°C (Table 1), and the daily mean temperatures ranged from 21 to 29°C (Appendix C). The mean relative humidity over the course of the study was between 43 and 63% for the six chambers. The daily mean humidity readings

generally ranged from 40 to 70%; however, there were a few excursions outside these readings (Appendix D). The mean chamber airflows ranged from 60 to 66 L/min for the 300-L chambers and the mean airflow for the 150-L chamber (50 ppm 4-VCH) was 33 L/min. The daily mean airflow readings ranged from 43 to 72 L/min for the 300-L chambers and from 29 to 37 L/min for the 150-L chamber (Appendix E). The environmental conditions were considered within ranges to maintain animal comfort during the study. Excursions outside the targeted ranges were considered not to have adversely affected the study outcome.

C. Mean Body Weights and Body Weight Gains
(Tables 2-9, Figures 3-6, Appendices F and G)

Significant body weight effects from exposure to 4-VCH were observed for study rats, but not for mice. Mean body weights and body weight gains for the groups of male and female rats exposed to 1500 ppm 4-VCH were lower than the mean values of their respective control groups. The male rat group had mean body weights that were statistically different from controls starting on test day 31 and continuing to the end of the study (Table 2). By the end of the study, the mean body weight of the male group was 9.3% lower than the male controls and the female group was 10.9% lower than the female controls (Tables 2 and 3). Body weights gains for both of these groups were statistically different from controls (Tables 4 and 5). The mean weight differences were considered compound-related. None of the other groups of rats exposed to 4-VCH or to 1,3-butadiene had mean body weights that were statistically different from their respective controls.

Individual groups of mice exposed to 4-VCH had mean body weights that were, at times, statistically different from controls (Tables 6 and 7), but the differences were sporadic and did not lead to a consistent pattern of weight gain differences over 13 weeks (Tables 8 and 9). The exposure to 4-VCH or 1,3-butadiene had no apparent effect on body weights of mice in the study.

D. Food Consumption and Food Efficiency
(Tables 10-17)

Group food consumption was measured weekly for both rats and mice. Mean food consumption by groups of study rats exposed to 4-VCH or 1,3-butadiene was not appreciably lower than mean food consumption by the groups exposed to air (Tables 10 and 11). The food efficiency values were low for both male and female rats exposed to 1500 ppm. This is consistent with the low mean body weights for these groups and is considered a compound-related effect. There were no apparent food consumption or food efficiency effects observed in any groups of study mice (Tables 14 and 15).

E. Clinical Observations and Mortality
(Tables 18-21, Figures 7 and 8, Appendices H and I)

There were no exposure-related mortalities among study rats. One female rat in the 1000 ppm 4-VCH exposure group was removed from study on test day 66 because it was found outside of its caging, and subsequent evaluations were deemed inappropriate.

There were a number of early mortalities among mice during the study. Of special note were early deaths in the groups of mice exposed to 1000 ppm 4-VCH. Ten of ten male mice and five of ten female mice died on test days 11 or 12. There were no unusual elevations in chamber atmospheres; and abrupt mortality, without prior clinical abnormalities, was also seen in mice exposed to 1500 ppm 4-VCH in a two-week range-finding study. Therefore, these deaths were directly related to compound exposure, although the specific cause of death could not be identified (see Pathology Report). An additional three female mice in this group died prior to the end of the study on test days 70 and 88 (Appendix I). Additional early deaths among study mice included one male control (test day 84) and three females, one exposed to 250 ppm 4-VCH (test day 70), and two exposed to 1000 ppm 1,3-butadiene (test days 38 and 64). In addition, one female mouse (study ID 409, animal #91439) in the 50 ppm exposure group escaped from its caging on test day 4 and was replaced with an extra mouse (temporary ID 52) and assigned study ID 411.

There were minimal clinical signs observed in study animals during the weekly pre-exposure examinations. None of these signs appeared to be directly related to exposure to 4-VCH or to 1,3-butadiene (Tables 18-21).

During exposures, rats in all exposure groups were frequently sleeping and had a slow response to the sound of tapping on the inhalation chamber walls. Mice exposed to the 250 or 1000 ppm 4-VCH concentrations responded in a manner similar to the rats. On test day 10, when a number of the mice were found dead prior to exposure, some of the mice that were still alive in the 1000 ppm exposure group displayed signs of tremors while in the inhalation chambers.

Rats and mice had more clinical signs when they were individually examined following the exposures than during the weekly pre-exposure examinations. Generally, these signs were transient, and they were not seen at subsequent pre-exposure evaluations. Signs such as discharge from the mouth and nose and stained fur, which were seen in male and female rats, were considered exposure related, but not compound related. Statistically significant increased incidences of lethargy occurred in male (250 and 1500 ppm) and female (1500 ppm) rats exposed to 4-VCH. There was also a significant trend across the groups of female rats (Tables 18 and 19). Similarly, there was an increased incidence of lethargy in female mice exposed to 1000 ppm, and a positive trend across all groups of female mice. Given the slow response to sound during exposures, and the frequent occurrences of lethargy following exposures, this effect appeared to be related to exposure to 4-VCH (Tables 20 and 21).

F. Ophthalmological Evaluation

(Ophthalmology Report, Appendices J and K)

When animals were examined for ophthalmological lesions prior to study start, animal selection and grouping had been completed. Two animals had ocular abnormalities, one female rat (temporary ID #53) not designated for placement on study and one female mouse (ID #206, Animal # 21426) designated for the control group (Ophthalmology Report). The mouse had a detached retina, but was replaced on study with pretest temporary ID #62.

On test day 88, when the animals were examined, two rats and one mouse exposed to 4-VCH had ocular abnormalities. One male rat in the 1500 ppm exposure group and one female rat in the 250 ppm exposure group had retinal degeneration; one female mouse in the 50 ppm exposure group had corneal dystrophy. One rat and one mouse exposed to 1000 ppm 1,3-butadiene had ocular abnormalities, which included retinal degeneration in the rat and an enucleated eye in the mouse. A male rat in the positive control group for the micronucleus study was also reported to have retinal degeneration (Ophthalmology Report). None of the abnormalities were related to exposure to either 4-VCH or 1,3-butadiene.

G. Clinical Laboratory Evaluation

(Clinical Pathology Report, Tables 22-29, Appendices L and M)

None of the rats exposed to 4-VCH or to 1,3-butadiene had compound-related abnormalities in hematological, clinical chemical, or urinalysis parameters (Tables 22, 23, and 26-29). There were several statistically significant values in the results, but they were deemed not to be of biological consequence in this study.

Due to the premature deaths in study mice, only two female mice exposed to 1000 ppm 4-VCH were alive at the time blood was taken, and no meaningful conclusions could be drawn from their data. No alterations in hematology parameters among mice exposed to 50 or 250 ppm 4-VCH were reported (Tables 24 and 25). Both male and female mice exposed to 1,3-butadiene had mild hemolytic anemia and mildly decreased platelet counts.

The no-observable-adverse-effect level for clinical pathology parameters among animals exposed to 4-VCH was 1500 ppm for rats and 250 ppm for mice (Clinical Pathology Report).

CONCLUSIONS

A number of adverse effects related to the repeated inhalation exposure of rats and mice to 4-vinylcyclohexene were noted in this study. Rats exposed to 1500 ppm 4-VCH had statistically and/or biologically significant decreases in body weights. Mean food efficiency was also affected in this group of rats. Clinically observed lethargy among rats exposed to 1500 ppm was also a significant effect.

The most notable effect in mice was the early mortality among males and females exposed to 1000 ppm 4-VCH. The microscopically detected ovarian atrophy in female mice exposed to 1000 ppm was also a significant adverse effect.

No adverse effects were seen in rats exposed to 1000 ppm 4-VCH or to 1,3-butadiene; thus, no toxicity comparisons could be made between the two compounds. There were ovarian lesions in female mice exposed to 1000 ppm 1,3-butadiene and 1000 ppm 4-VCH; however, the ovarian lesions in mice exposed to 1000 ppm 4-VCH appeared to be slightly more severe.

The no-observable-adverse-effect level for rats in this 13-week study was 1000 ppm 4-VCH and for mice was 250 ppm 4-VCH.

REFERENCES

1. NTP Technical Report (1986). Toxicology and Carcinogenesis Studies of 4-Vinylcyclohexene in F344/N Rats and B6C3F$_1$ Mice. NIH Publication No. 86-2559.

2. NTP Bioassay Report No. 288 (1983).

3. Environmental Protection Agency, Toxic Substances Control Act; Testing Consent Order for 4-vinylcyclohexene 40 CFR Part 799; published September 9, 1991.

4. DuPont HLR 759-93.

H. Pathological Evaluation

(Pathology Report, Tables 30-41, Appendices N-Q)

The evaluation of mean organ weight data for the 13-week study indicated that there were compound-related effects in several organs of study animals. There were increased mean absolute and/or relative liver weights in male and female rats exposed to 1000 ppm or 1500 ppm 4-VCH and to 1000 ppm 1,3-butadiene. The changes were not accompanied by corresponding microscopic liver changes and were considered normal metabolic responses to a xenobiotic. Increased mean relative kidney weights were also observed in rats. Only male groups exposed to 1000 or 1500 ppm 4-VCH and to 1000 ppm 1,3-butadiene were affected. Microscopically, male rats in all groups had increased hyaline droplet accumulation in renal proximal tubules. The incidence and severity of accumulation was slightly greater for the rats exposed to 1000 ppm 4-VCH than for those exposed to 1000 ppm 1,3-butadiene. The hyaline droplet formation was associated with α_{2u}-globulin since it was apparent only in male rats. The formation of hyaline droplets was not considered an adverse effect since the accumulation was not accompanied by kidney cytotoxicity. No other organ weight or microscopic effects were observed in rats.

There were microscopic, but no organ weight effects, observed in mice exposed to 4-VCH in the study. There were, however, decreased testes weights in mice exposed to 1000 ppm 1,3-butadiene. The weight changes were accompanied by a very slight testicular degeneration and atrophy that could be detected microscopically. The most notable adverse effect in mice exposed to 4-VCH was seen microscopically in the females. Ovarian atrophy was present in mice exposed to 1000 ppm 4-VCH. The atrophy was characterized as a paucity of ovarian follicles and was slightly more severe than a mild atrophy observed in mice exposed to 1000 ppm 1,3-butadiene.

The organ weight changes in study rats were considered not to be adverse; therefore, only the body weight changes in the groups exposed to 1500 ppm 4-VCH were considered adverse, and the no-observable-adverse-effect level for the pathological parameters in rats was 1000 ppm 4-VCH (Pathology Report). Based on the ovarian and testicular effects, the no-observable- adverse-effect level for pathological changes in mice was 250 ppm 4-VCH.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

PURITY ANALYSIS REPORT

SUBCHRONIC INHALATION TOXICITY: 90 DAY STUDY WITH 4-VINYLCYCLOHEXENE IN RATS AND MICE

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

ANALYSIS OF 4-VINYLCYCLOHEXENE AND 1,3-BUTADIENE

Medical Research Project Number:	9523-001
Haskell Sample Numbers:	19353 (VCH) and 19999 (1,3-BD)
Analytical Test Code:	999
Analytical Report Number:	HA-93-070

SUMMARY

Compounds 4-vinylcyclohexene (VCH, H-19353) and 1,3-butadiene (1,3-BD, H-19999) were analyzed by a method using gas chromatography with flame ionization detection.

Analysis of the VCH sample submitted on January 13, 1993 indicated that the major component comprised an average of 99.59% of the total sample with a range of 99.59 to 99.60% for an average of six replicate analyses. Analysis of the compound submitted on May 10, 1993 found the composition to be essentially unchanged at an average of 99.67% with a range of 99.44 to 99.75%.

Analysis of the 1,3-BD sample submitted on January 13, 1993 indicated that the major component comprised greater than 99.9% for an average of six replicate analyses. Analysis of the compound submitted on May 10, 1993 found the composition to be essentially unchanged at 99.9%.

HA-93-070p2

METHODS

Compounds VCH and 1,3-BD were analyzed using the following chromatographic method.

CHROMATOGRAPHIC CONDITIONS

Instrument	Hewlett-Packard Model 5890 Gas Chromatograph (NX2406)	
Column	15m x 0.53 i.d. Nukol (SUPELCO Inc.) 0.5µm film thickness Helium carrier, 1 psi (6.5 mL/min)	
Detector	Flame Ionization, 250°C Air, 300 mL/min Hydrogen, 32 mL/min Helium Make Up, 20.7 mL/min	
Injector	250°C, spilt flow, 30 mL/min. split ratio: 4.6 to 1	
Temperature Program	Initial Temperature:	40°C
	Initial Time:	5.00 min
	Program Rate:	20°C/min
	Final Temperature (VCH):	250°C
	Final Temperature (1,3-BD):	100°C
	Final Time (VCH):	5.00 min
	Final Time (1,3-BD):	0.00 min
Data Analysis	Hewlett-Packard 3365 GC Chemstation Area percent report option enabled	

DETERMINATION OF COMPOSITION

External reference standards were available for this study. Compounds from Aldrich Chemical, VCH (Aldrich V220-8) and 1,3-BD (Aldrich 29,503-5), were used as retention time standards. Identification of the major component of the test materials, VCH (H-19,353) and 1,3-BD (H-19,999), was done by comparing retention times with those of the Aldrich materials.

The area of all eluted peaks was summed and the area percent of each peak was calculated using the area percent option in the Chemstation software.

The polymerization inhibitor, 4-tertbutylcatechol, present in both compounds was not eluted from the column. Therefore, the area percent was not corrected for this component.

HA-93-070p3

SAMPLE PREPARATION

All analyses were done with undiluted (neat) compound. VCH was sampled using a 10 µL syringe with the syringe plunger at zero. A manual injection with the plunger at zero gives an injection volume of 0.1 µL. The injection volume arises from the liquid remaining in the needle.

1,3-BD was sampled by removing 5 µL of gas from a bag containing 1 atmosphere of the compound. This compound was also analyzed using manual injections.

RESULTS

The results of the analyses are shown in Table I of this Analytical Report HA-93-070.

Report and Analyses by: Dennis A. Redder, Chemistry Associate — 8/20/93 Date

Approval by: Charlotte H. Lattin, Research Chemist — 8/20/93 Date

Date issued: August 20, 1993

Analytical Report Number: HA-93-070

Notebook References: E68447: 36, 38, 40, 48 - 56, 86 - 92

Number of Pages in Report: 5

HA-93-070p5

TABLE I

Area Percent of VCH

Submitted on: 13-Jan-93	Submitted on: 10-May-93
99.59	99.44
99.59	99.69
99.59	99.75
99.60	99.73
99.60	99.74
99.59	99.64
99.59 average	99.67 average

Area percent of 1,3-BD

Submitted on: 13-Jan-93	Submitted on: 10-May-93
99.99	99.95
99.99	99.99
99.99	99.95
99.99	99.97
99.99	99.97
99.99	99.97
>99.9 average	>99.9 average

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

OPHTHALMOLOGY REPORT

NANCY M. BROMBERG, V.M.D.,M.S.
15021 DUFIEF MILL ROAD
GAITHERSBURG, MD 20878

STUDY INFORMATION: MR 9523
H# 19353
Species: Rat, Mouse
Study Director: Anthony Panepinto

PRETEST OPHTHALMOLOGICAL EXAMINATION SUMMARY
Examination Date: 1/25/93

METHODS: Indirect ophthalmoscopy following 1% Tropicamide mydriasis

SPECIES	GROUP #	PRETEST ID#	OBSERVATIONS
Rat	PretestF	53/0	Vitreal hemorrhage left eye
Mouse	II	206/91426	Bilateral detached retina

COMMENTS/DISCUSSION/SUMMARY

Two animals were found to have ocular abnormalities and should be excluded from the study.

Nancy M. Bromberg V.M.D. 2-15-93

Nancy M. Bromberg, V.M.D., M.S. Date
Diplomate, A.C.V.O.

NANCY M.BROMBERG, V.M.D.,M.S.
15021 DUFIEF MILL ROAD
GAITHERSBURG, MD 20878

STUDY INFORMATION: MR: 9523
H# 19353
Species: Rat, Mouse
Study Director: Anthony Panepinto

OPHTHALMOLOGICAL EXAMINATION SUMMARY
Examination Date: April 23, 1993
Test Day : 90

METHODS: Indirect Ophthalmoscopy following 1% Tropicamide mydriasis

SPECIES: RAT

SEX	GROUP #	ID#	ANIMAL #	OBSERVATION
Male	VII	701	531453	Focal Retinal degeneration right eye
	IX	906	531468	Focal retinal degeneration right eye
	XI	1104	531476	Focal retinal degeneration left eye
Female	IV	405	531492	Retinal degeneration right eye

SPECIES: MOUSE

Female	IV	408	91438	Corneal dystrophy left eye
	X	1006	91466	Left eye enucleated

COMMENTS/DISCUSSION/SUMMARY

Four rats and one mouse were found to have ocular abnormalities. All four rats had unilateral retinal degenerations. The mouse had unilateral corneal dystrophy. These findings are not considered to be compound-related in etiology.

Nancy M. Bromberg, V.M.D., M.S.
Diplomate, A.C.V.O.

May 10, 1993

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

CLINICAL PATHOLOGY REPORT

CLINICAL PATHOLOGY REPORT NO. 17-93

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

SUMMARY

This report describes the clinical pathology findings from a subchronic inhalation toxicity study with 4-vinylcyclohexene (4-VCH). Groups of ten male and ten female Crl:CD®BR rats and B6C3F_1/CrlBR mice were exposed by inhalation to 4-VCH. Concentrations for rats were 0, 250, 1000, or 1500 ppm, and for mice, they were 0, 50, 250, or 1000 ppm. In addition, for comparison with effects of 4-VCH exposure, groups of 10 male and 10 female rats and mice were exposed to 1000 ppm of 1,3-butadiene as a positive control. All groups were exposed for 6 hours per day, 5 days per week for approximately 13 weeks.

Rats exposed to 4-VCH or 1,3-butadiene did not have toxicologically important clinical pathologic alterations. Several statistically significant findings were evident but they were all biologically inconsequential.

Mice exposed to 1000 ppm of 4-VCH had high mortality and only two females were available for clinical pathology evaluation. Therefore, 250 ppm of 4-VCH was the highest exposure concentration for which there were sufficient numbers of mice to permit valid conclusions to be made. There were no clinical pathologic alterations in mice exposed to 50 or 250 ppm of 4-VCH.

Mice exposed to 1000 ppm of 1,3-butadiene had mild hemolytic anemia and mildly decreased platelet counts. The platelet alterations were mild and would not have caused or indicated biological adversity. These 1,3-butadiene-related effects could not be compared with similar exposure concentration of 4-VCH because of the aforementioned mortality in mice exposed to 1000 ppm of 4-VCH.

Under the conditions of this study and for the clinical pathology parameters measured, the no-observable-adverse-effect level (NOAEL) for male and female rats following exposure to 4-VCH was 1500 ppm. The NOAEL for male and female mice exposed to 4-VCH was 250 ppm, the highest exposure group from which usable clinical pathology data was available.

Report by: Glenn S Elliott

Glenn S. Elliott, D.V.M., Ph.D.
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PROCEDURE

Four groups of ten male and four groups of ten female Crl:CD®BR rats and B6C3F$_1$/CrlBR mice were exposed, by inhalation, for six hours per day, five days a week for approximately 13 weeks, to 4-vinylcyclohexene (4-VCH). Rats were exposed at concentrations of 0 (control), 250, 1000, or 1500 ppm. Mice were exposed at concentrations of 0, 50, 250, or 1000 ppm. In a similar exposure routine, one group each, of ten male and ten female rats and mice were exposed to 1000 ppm of 1,3-butadiene. 1,3-Butadiene-exposed animals were used as positive controls for comparison with effects due to exposure to its dimer, 4-VCH.

Ten male and ten female rats from each group were designated for clinical pathology evaluation on test days 94 and 95 (90-DAY). Only nine female rats from the 1000 ppm 4-VCH group were available for clinical evaluation. Ten male and ten female mice were designated for clinical pathology evaluation on test days 92 and 93 (90-DAY). Due to early deaths, only nine male mice from the 0 ppm group, nine female mice from the 250 ppm group, two female mice from the 1000 ppm 4-VCH group, and eight female mice from the 1000 ppm 1,3-butadiene group were available for clinical pathology evaluation. All male mice from the 1000 ppm 4-VCH group died prior to clinical pathology evaluation.

At the sampling time, blood was taken from the orbital sinus of each fasted rat and fasted mouse while the animal was under light carbon dioxide anethesia. The following hematologic parameters were measured or calculated: number of erythrocytes (RBC), leukocytes (WBC), and platelets (PLAT); hemoglobin concentration (Hb) and hematocrit (Ht); mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC); and relative numbers of neutrophils (Neut), band neutrophils (Band), lymphocytes (Lymph), atypical lymphocytes (Alym), monocytes (Mono), eosinophils (Eosin), and basophils (Baso). Absolute values for the various types of leukocytes were calculated from the leukocytic data. Reticulocyte smears from all animals were prepared and they were evaluated from mice in the 0, 250 (males only), and 1000 ppm (females only) 4-VCH and 1000 ppm 1,3-butadiene groups. Absolute values for reticulocyte counts (Retic) were calculated from the red blood cell data. Blood cell counts, hemoglobin concentration, hematocrit, and mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were determined on a Serono Baker System 9000® hematology analyzer. Differential cell counts and reticulocyte counts were determined on a Hematrak® Automated Differential System cell counter.

Bone marrow smears were prepared at sacrifice in the event that their evaluation would facilitate interpretation of the toxicologic importance of other findings. Evaluation was not required.

In conjunction with hematological measurements in rats, the following serum chemical parameters were measured: activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST); and serum concentrations of glucose (GLUCO), urea nitrogen (BUN), calcium (CALC), phosphorus (PHOS), bilirubin (BILRN), cholesterol (CHOL), creatinine (CREAT), total protein (TPROT), albumin (ALBMN), sodium (Na), potassium (K), and chloride (Cl). Clinical chemical analytes were measured on a Boerhinger

Mannheim/Hitachi 717 clinical chemistry analyzer using Boerhinger Mannheim reagents. Serum globulin (GLOBN) concentration was calculated from the total protein and albumin concentrations.

One day prior to each bleeding time for rats only, an overnight (approximately 14-hours) urine specimen was collected from each animal to measure volume (VOL), osmolality (OSMOL), urobilinogen (UROBL), and pH; and to determine the presence of hemoglobin or occult blood (BLOOD), glucose, protein, bilirubin, and ketone (acetoacetic acid). Osmolality was determined on a Precision System Multi-Osmette™ 2430 osmometer. Urine biochemical constituents were measured with Ames Multistix® urine chemistry dipsticks on a Clinitek® 200 urine chemistry analyzer. Urine appearance (color and transparency) was recorded and the sediment from each specimen was microscopically examined.

STATISTICAL ANALYSES

Statistical comparisons were made between 0 ppm (control) groups and groups exposed to 4-VCH. Separate statistical comparisons were made between the 0 ppm (control) groups and groups exposed to 1,3-butadiene. A one-way analysis of variance (ANOVA) and Bartlett's test were performed. When the value of the F-test statistic from ANOVA was significant, the Dunnett test was used to compare means from the control groups and each of the exposed groups. When the results of the Bartlett test were significant ($p \leq 0.005$), the Kruskal-Wallis test was employed and the Mann-Whitney U test was used to compare means from the control groups and each of the exposed groups. Significance was judged at the 5% probability level.

RESULTS AND DISCUSSION

Hematology

Rats (Tables 22-23; Appendix L)

Toxicologically important hematologic alterations did not occur in male or female rats exposed to 4-VCH or 1,3-butadiene. The following statistically significant hematologic alterations were not considered to be toxicologically important:

- o A slight, statistically significant increase in mean platelet counts occurred in female rats exposed to 1500 ppm of 4-VCH; however, the platelet counts in this group were within the expected range of normal biological variation and within the historical control range (999,000-1,245,000/µl).
- o Male rats exposed to 1000 ppm of 1,3-butadiene had significantly decreased eosinophil counts. Decreased eosinophil counts do not indicate any biological adversity.
- o Female rats exposed to 1000 ppm of 1,3-butadiene had significantly decreased MCH; however the group mean and individual MCH were within the historical control range (20-22 pg).

Mice (Tables 24-25, Appendix M)

Mice exposed to 1000 ppm of 4-VCH had high mortality and most died before clinical pathology was conducted. All males and eight females in the 1000 ppm 4-VCH groups died. Data from the two surviving female mice was insufficient to permit valid conclusions about the effect of exposure to 1000 ppm of 4-VCH.

Toxicologically important hematologic alterations did not occur in male or female mice exposed to 50 or 250 ppm of 4-VCH.

Male and female mice exposed to 1000 ppm of 1,3-butadiene had a mild, statistically significant reduction in circulating erythrocyte mass (decreased RBC, Hb, Ht). These alterations were accompanied by increased reticulocyte counts (not statistically significant in females exposed to 1000 ppm of 1,3-butadiene). A decreased erythrocyte mass accompanied by increased reticulocyte counts indicates hemolytic anemia with bone marrow regeneration of erythrocytes. Erythrocyte indices (MCV, MCH, MCHC) were also altered in a manner consistent with hemolytic anemia. The erythrocytic alterations observed in this study were similar to the alterations described in another inhalation study with 1,3-butadiene.[1] In the previous study, anemia was observed in male $B6C3F_1$ mice at exposure concentrations of 62.5 ppm and higher, and in females at 625 ppm.

Male and female mice exposed to 1000 ppm of 1,3-butadiene had mildly decreased platelet counts. The group means were outside of the historical control range (1,014,000-1,727,000/µl and 886,000-1,382,000/µl, male and female, respectively) and the alterations may have been compound-related. However, the magnitude of the platelet count decrease was not sufficient to have adverse health effects. Also, similar decreases were not observed in another inhalation study in $B6C3F_1$ mice with high exposure concentration of 625 ppm of 1,3-butadiene.[1]

A statistically significant increase in lymphocyte counts in male mice exposed to 1000 ppm of 1,3-butadiene was not considered to be toxicologically important because individual animal and group mean lymphocyte counts were well within the range of historical controls.

Clinical Chemistry (Tables 26-27, Appendix L)

Toxicologically important clinical chemistry alterations did not occur in male or female rats exposed to 4-VCH or 1,3-butadiene. The following statistically significant clinical chemistry alterations which occurred in male rats were not considered to be toxicologically important:

- Serum calcium concentration were significantly increased in males exposed to 1000 or 1500 ppm of 4-VCH, or 1000 ppm of 1,3-butadiene. However, the magnitude of this serum calcium concentration increase was biologically inconsequential and group means and most individual rat calcium concentrations were within the historical control range (10.4-11.4 mg/dl).
- Males exposed to 1000 or 1500 ppm of 4-VCH had significantly increased serum albumin concentrations. Mean albumin concentrations were within the historical control range (4.3-4.6 g/dl) and the statistically significant findings were not considered to be toxicologically important.
- Males exposed to 1000 ppm of 1,3-butadiene had significantly decreased

serum cholesterol, globulin, and potassium concentrations and an increased glucose concentration. The magnitude of these alterations was miniscule and the findings were not considered to be biologically important.

- o Males exposed to 1000 ppm of 4-VCH had significantly decreased serum sodium concentrations but the change was minimal and a relevant dose-response relationship was not present.

Urinalysis (Tables 28-29, Appendix L)

Toxicologically important urinalysis alterations did not occur in male or female rats exposed to 4-VCH or 1,3-butadiene. The following statistically significant urinalysis alterations which occurred in male rats were not considered to be toxicologically important:

- o Urine volume and osmolality were significantly different from controls in male and female rats exposed to 4-VCH and 1,3-butadiene. Males exposed to 250, 1000 (not statistically significant), or 1500 ppm of 4-VCH or 1000 ppm of 1,3-butadiene excreted increased volume of urine which tended to have decreased osmolality (not statistically significant). Conversely, females exposed to 1000 or 1500 ppm of 4-VCH or 1000 ppm of 1,3-butadiene had significant increases in urine osmolality accompanied by minimally decreased urine volume (not statistically significant). In both instances, male and female alike, the relevant control group values (against which statistical comparisons were made) were at the fringes of the historical control range. (Historical control ranges: Urine volume--5.7-20.2 ml and 2.2-9.3 ml, male and female, respectively; Osmolality--842-1744 mOs and 829-2132 mOs, male and female, respectively) Also, the altered mean values and most individual animal results were within the historical control range. For these reasons the statistically significant alterations were not considered to be toxicologically important.
- o Male rats exposed to 1000 ppm of 4-VCH had significantly decreased urine pH and female rats exposed to 1500 ppm of 4-VCH had significantly decreased urine pH. These observations in males and females were biologically inconsequential and within the historical control range (5.9-8.3 and 5.5-6.7, male and female, respectively).

CONCLUSIONS

In the present study, the comparative toxicities of 1,3-butadiene and its dimer, 4-VCH, were assessed in rats and mice. In rats, neither 4-VCH nor 1,3-butadiene caused toxicologically important alterations of clinical pathology parameters. In male and female mice, 1,3-butadiene exposure caused mild hemolytic anemia and a mild decrease in platelet counts. The platelet alterations were mild and would not have caused or indicated biological adversity. Similar alterations were not evident in surviving female mice exposed to a comparable concentration of 4-VCH; however, only two mice in this group survived to be evaluated. All male mice exposed to 1000 ppm of 4-VCH succumbed before clinical pathology evaluation was conducted.

Under the conditions of this study and for the clinical pathology parameters measured, the no-observable-adverse-effect level (NOAEL) for rats following exposure to 4-VCH was 1500 ppm for males and females. The NOAEL for male and

female mice exposed to 4-VCH was 250 ppm. The NOAEL for male mice was based on absence of clinical pathology alterations in the 250 ppm group, the highest exposure group from which clinical pathology data was available. Two female mice exposed to 1000 ppm of 4-VCH survived to have clinical pathology evaluations and abnormalities were not detected. However, clinical pathology data from two animals does not provide sufficient data on which to draw sound conclusions. Therefore, the NOAEL for female mice was established at 250 ppm, since no clinical pathology alterations occurred at this concentration.

REFERENCE

1. Melnick, RL, Huff, JE, Roycroft, JH, Chou, BJ, Miller, RA. Inhalation Toxicology and Carcinogenicity of 1,3-Butadiene in B6C3F$_1$ Mice Following 65 Weeks of Exposure. Environmental Health Perspectives, 86:27-36, 1990.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

PATHOLOGY REPORT

PATHOLOGY REPORT NO. 74-93

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

SUMMARY

Groups of 10 male and 10 female Crl:CD®BR rats and B6C3F$_1$/CrlBR mice were exposed by inhalation to 4-vinylcyclohexene (4-VCH). Concentrations for rats were 0, 250, 1000, or 1500 ppm. Concentrations for mice were 0, 50, 250, or 1000 ppm. In addition, groups of 10 male and 10 female rats and mice were exposed to 1000 ppm of 1,3-butadiene as a positive control. All groups were exposed to their respective concentrations for 6 hours per day, 5 days per week for 13 weeks.

Final body weights were decreased in male and female rats exposed to 1500 ppm of 4-VCH. Body weight decreases relative to controls were 9% and 11% for male and female rats, respectively; these decreases were statistically significant in male, but not female rats. There were no statistically significant changes in mean final body weights in male rats exposed to 250 or 1000 ppm of 4-VCH or to 1000 ppm of 1,3-butadiene.

Increased mean absolute and/or relative liver weights were present in male and female rats exposed to 1000 or 1500 ppm of 4-VCH or 1000 ppm of 1,3-butadiene. These liver weight changes were not associated with correlative microscopic changes and were considered to represent a normal response of the liver to metabolism of a xenobiotic.

Increased mean relative kidney weights were present in male rats exposed to 1000 or 1500 ppm of 4-VCH or to 1000 ppm of 1,3-butadiene. Microscopically, increased hyaline droplet accumulation in renal proximal tubules was present in male rats at all exposure concentrations of 4-VCH and in rats exposed to 1,3-butadiene. At 1000 ppm, hyaline droplet accumulation occurred with greater incidence and slightly greater severity in the 4-VCH group as compared to the 1,3-butadiene group. Since this finding occurred in male rats but not female rats or mice of either sex, hyaline droplet formation was likely the result of altered degradation of α_{2u}-globulin. At the exposure concentrations tested, hyaline droplet accumulation was not associated with adverse effects in the kidneys such as cytotoxicity.

In mice, exposure to 1000 ppm of 4-VCH was associated with high mortality in both males (10/10) and females (8/10). There was no compound-related mortality in mice exposed to 1000 ppm of 1,3-butadiene. The large number of early deaths precluded statistical evaluation of final body and organ weight data in male and female mice exposed to 1000 ppm of 4-VCH. There were no compound-related changes in final body or organ weights in male or female mice exposed to 50 or 250 ppm of 4-VCH. However, decreased testes weights were present in male mice exposed to 1000 ppm of 1,3-butadiene. These testicular weight changes were associated microscopically with very slight testicular degeneration and atrophy.

4-VCH-related microscopic changes in mice were limited to the ovaries of animals exposed to 1000 ppm. These changes were characterized by a paucity of ovarian follicles. Similar, but slightly less severe ovarian changes were present in mice exposed to 1000 ppm of 1,3-butadiene.

Based upon final body weight effects and upon the consideration of liver weight changes and microscopic renal effects as non-adverse, the no-observable-adverse-effect level (NOAEL) for male and female rats following subchronic exposure to 4-VCH was 1000 ppm (the intermediate concentration). Based upon mortality effects and microscopic changes in the ovaries, the NOAEL for both male and female mice was 250 ppm (the intermediate exposure concentration). At the comparable exposure concentration of 1000 ppm, most compound-related effects following exposure to the test compounds were more severe in 4-VCH-exposed animals as compared to those exposed to 1,3-butadiene.

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INTRODUCTION AND METHODS

This report summarizes final body and organ weight data and gross and microscopic findings in male and female Crl:CD®BR rats and B6C3F$_1$/CrlBR mice exposed by inhalation to 4-vinylcyclohexene (4-VCH). Group designations for rats were as follows:

Group Designation Male	Female	Number/Group	Concentration Of 4-VCH (ppm)
I	II	10	0 (Control)
III	IV	10	250 (Low)
V	VI	10	1000 (Intermediate)
VII	VIII	10	1500 (High)
IX	X	10	1000 1,3-butadiene (Positive control)

Group designations for mice were as follows:

Group Designation Male	Female	Number/Group	Concentration Of 4-VCH (ppm)
I	II	10	0 (Control)
III	IV	10	50 (Low)
V	VI	10	250 (Intermediate)
VII	VIII	10	1000 (High)
IX	X	10	1000 1,3-butadiene (Positive control)

Animals were exposed to their respective concentrations for 6 hours per day, 5 days per week for approximately 13 weeks.

Animals sacrificed in extremis or sacrificed by design at the termination of the study were euthanatized by CO_2 anesthesia and exsanguination. All animals, including those found dead or accidentally killed, were examined for gross alterations. For animals sacrificed by design, organ weights were determined for lungs, liver, kidneys, brain, heart, spleen, adrenal glands (rats only), ovaries (rats only), and testes. For all animals, representative samples of the following organs and tissues were collected in fixative: liver, gallbladder (mouse only), kidneys, lungs, heart, skeletal muscle, spleen, aorta, brain, spinal cord, stomach, duodenum, jejunum, ileum,

pancreas, cecum, colon, rectum, mesenteric lymph node, salivary glands, mandibular lymph nodes, Harderian glands, exorbital lacrimal glands, thymus, adrenal glands, pituitary gland, sciatic nerve, thyroid gland, parathyroid glands, trachea, esophagus, larynx/pharynx, Zymbal's glands, prostate, urinary bladder, seminal vesicles, femur/knee joint, sternum, bone marrow (sternal and femoral), nose, skin, mammary gland, eyes,* testes, epididymides, ovaries, uterus, vagina, and gross lesions. Eyes, skin, mammary gland, testes, and epididymides were fixed in Bouin's fixative. All other tissues were fixed in 10% neutral buffered formalin. Following decalcification, the nose was sectioned to produce 4 levels according to the method described by Young (1981). Fixed tissues to be processed to slides were embedded in paraffin, sectioned at 5 micrometers and stained with hematoxylin and eosin.

All tissues collected from animals in the control, high-dose, and positive control groups, and from all animals found dead, accidentally killed, or sacrificed in extremis, were processed to slides for microscopic evaluation. In addition, since all male mice in the 1000 ppm 4-VCH group died by test day 12, all collected tissues from 250 ppm males were processed to slides for microscopic evaluation. Liver, kidney, lung, nose, ovaries and gross lesions from animals in the remaining low and intermediate dose groups were also processed to slides and examined microscopically. The remaining tissues from animals in the low and intermediate dose groups were archived in fixative.

Mean body weight and mean absolute and relative (% of body weight) organ weights were analyzed using a one-way analysis of variance (ANOVA). When the F-test from the ANOVA was significant, Dunnett's test was used to compare means from the control group with the test groups. Significance was judged at the 0.05 probability level.

RESULTS

Mortality

Rats (Appendix P)

One female rat in the 1000 ppm 4-VCH group was removed from the study on test day 66. All other rats survived to the termination of the study and were sacrificed by design.

Mice (Appendix Q)

All male mice and 8/10 female mice exposed to 1000 ppm 4-VCH were found dead prior to the termination of the study. All male mice were found dead within 12 days on test. A cause of death for 1000 ppm mice was not apparent from the

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* Retina was evaluated as a separate organ due to its greater susceptibility to autolytic change.

pathological evaluation. There was no compound-related mortality in male or female mice exposed to 50 or 250 ppm of 4-VCH or to 1000 ppm of 1,3-butadiene. All other early deaths (2/10 and 1/10 males exposed to 0 and 50 ppm of 4-VCH, respectively, and 1/10 and 2/10 females exposed to 250 ppm of 4-VCH and 1000 ppm of 1,3-butadiene, respectively) were accidental or were associated with spontaneous lesions.

Body and Organ Weights

Rats (Tables 30-31; Appendix N)

A compound-related and statistically significant decrease (9%) in mean final body weight was present in male rats exposed to 1500 ppm of 4-VCH. There were no statistically significant changes in mean final body weights in male rats exposed to 250 or 1000 ppm of 4-VCH or to 1000 ppm of 1,3-butadiene.

Also in male rats, compound-related and statistically significant increases were present in mean absolute (15%) and relative (27%) liver weights at 1500 ppm and in mean relative liver weight (17%) at 1000 ppm of 4-VCH. Though not statistically significant, an 11% increase in mean absolute liver weight in 1000 ppm males was likely compound related. Exposure to 1,3-butadiene produced a statistically significant increase in mean relative (8%), but not mean absolute, liver weight in male rats.

Statistically significant increases in mean relative kidney weights were present in male rats at 1000 (16%) and 1500 (20%) ppm of VCH and at 1000 (12%) ppm of 1,3-butadiene. Based on compound-related microscopic changes (see Microscopic Findings) in the kidney, these kidney weight effects were likely compound-related. However, absolute kidney weights in these groups were not statistically increased. Thus, the magnitude of the relative kidney weight increases was influenced, to some extent, by decreases in body weights.

A statistically significant increase in mean relative testes weight was present in male rats exposed to 1500 ppm of 4-VCH. However, mean absolute testes weight was not statistically increased nor were compound-related microscopic changes present in the testes. Thus, this testes weight change was considered secondary to decreased body weight.

In female rats, an 11% reduction in mean final body weight was present in the 1500 ppm 4-VCH group. Though not statistically significant, the body weight change was considered compound related based upon the quantitatively similar and statistically significant body weight finding in male rats. There were no statistically significant changes in mean final body weights in female rats exposed to 250 or 1000 ppm of 4-VCH or to 1000 ppm of 1,3-butadiene.

Compound-related increases in mean relative liver weights were present in female rats exposed to 1000 (11%) or 1500 (22%) ppm of 4-VCH or 1000 (10%) ppm of 1,3-butadiene. Mean absolute liver weights were not statistically increased.

Statistically significant increases in mean relative (but not mean absolute) spleen and kidney weights in 1500 ppm female rats were considered secondary to decreased body weights inasmuch as absolute weights for these organs were not statistically increased and compound-related microscopic changes were not present. A statistically significant decrease in mean absolute lung weight in 1,3-butadiene-exposed female rats was not associated with changes in the mean relative weight or with microscopic changes in the lungs. Thus, this increase was considered spurious and not due to exposure to 1,3-butadiene.

Mice (Tables 32-33; Appendix O)

Because of early deaths, final body and organ weight data were not available for male mice exposed to 1000 ppm of 4-VCH. Similarly, high mortality in female mice (8/10 found dead prior to the study termination) precluded meaningful statistical evaluation of body and organ weight data. There were no compound-related changes in final body or organ weights in male or female mice exposed to 50 or 250 ppm of 4-VCH. A statistically decreased mean absolute brain weight in female mice at 50 ppm of 4-VCH was not dose-related and was not associated with relative weight changes or microscopic findings in the brain. Thus, this decrease was considered spurious and unrelated to exposure to 4-VCH.

In male mice, exposure to 1000 ppm of 1,3-butadiene produced statistically significant decreases in mean absolute and relative testes weight (34% and 36%, respectively). A statistically significant decrease in mean relative brain weight was also present in male mice exposed to 1,3-butadiene. However, in the absence of changes in absolute brain weights or corroborative microscopic findings, this increase in relative brain weight was considered spurious and unrelated to exposure to 1,3-butadiene.

In female mice, exposure to 1000 ppm of 1,3-butadiene produced statistically significant increases in mean absolute and relative liver weights (8% for both absolute and relative weights). In addition, the mean relative spleen weight in 1,3-butadiene-exposed female mice had a statistically significant decrease (12%) compared to controls. The biological significance of this latter finding is uncertain (see Microscopic Findings).

Gross Findings

Rats (Tables 34-35; Appendix P)

There were no gross findings related to exposure to the test compounds. All gross observations were considered incidental occurrences of changes common in rats of this strain and age.

Mice (Tables 36-37; Appendix Q)

There were no gross findings considered related to exposure to 4-VCH or

1,3-butadiene. All gross lesions were considered to represent incidental occurrences of changes common in mice of this strain and age. Lung discoloration, noted with a slightly increased incidence in 1000 ppm 4-VCH mice, was a sequela to post mortem congestion.

Microscopic Findings

Rats (Tables 38-39; Appendix P)

In male rats, a compound-related increase in hyaline droplet accumulation was present in the proximal tubules of the kidney at all 4-VCH-exposure levels. Increased hyaline droplet accumulation was present in all rats in the 1500 and 1000 ppm groups and was graded as mild. The lesion was present in 7/10 male rats at 250 ppm and was graded as minimal. Minimal increased hyaline droplet accumulation was present in 2/10 rats exposed to 1000 ppm of 1,3-butadiene. For both 4-VCH and 1,3-butadiene, increased hyaline droplet formation was not associated with cytotoxicity of tubular epithelium at any of the concentrations tested.

There were no other microscopic findings in male or female rats which were considered to be related to exposure to the test compounds. Ovarian atrophy was diagnosed in 2/10 female rats in the 1500 ppm group. Unlike ovarian atrophy observed in the mice (see below), the ovarian change in these rats was characterized by a decreased number of corpora lutea and was considered to most likely represent an incidental finding (see Discussion).

Mice (Tables 40-41; Appendix Q)

In male mice, thymic lymphoid atrophy was present in 6/10 animals exposed to 1000 ppm of 4-VCH. This finding likely represents a secondary, stress-related effect to exposure to lethal concentrations of 4-VCH. Atrophy of lymphoid organs, especially the thymus, is common in severely stressed animals. There were no other 4-VCH-related microscopic findings in male mice at any of the exposure concentrations tested.

Male mice exposed to 1000 ppm of 1,3-butadiene had an increased incidence of testicular degeneration/atrophy (either unilateral or bilateral). Given the magnitude of the testes weight changes in 1,3-butadiene-exposed mice, these microscopic testicular changes were remarkably minimal in severity. Further, the lesions were often unilateral and were generally not associated with concomitant changes in epididymal sperm quantity or quality. However, because of the magnitude of testicular weight changes, the testicular degeneration/atrophy noted in 1,3 butadiene-exposed mice was considered compound-related.

In females, compound-related minimal to mild ovarian atrophy was present in 5/10 mice (5/5 mice on study beyond test day 12) exposed to 1000 ppm of 4-VCH and in 6/10 mice exposed to 1,3-butadiene. Ovarian atrophy was primarily characterized by a paucity of all developmental stages of ovarian follicles and was slightly more severe in the 4-VCH group as compared to 1,3-butadiene-exposed animals. There were no compound-related microscopic ovarian changes in the 50 or 250 ppm 4-VCH groups.

A slight increased incidence of splenic lymphoid atrophy was present in female mice exposed to 1000 ppm of 1,3-butadiene. This finding was correlative to the slight decrease in mean relative spleen weights noted in 1,3-butadiene-exposed female mice. The significance of this finding is uncertain but lymphoid atrophy is a common nonspecific response to stress.

All other microscopic findings in mice exposed to 4-VCH or to 1,3-butadiene were considered incidental and unrelated to exposure to the test compounds. Included among such lesions were increased mitotic figures in the livers of some male and female mice exposed to 1000 ppm of 4-VCH. This finding was considered age-related inasmuch as cell proliferation rates are normally higher in young mice, and in the present study increased mitotic figures occurred only in mice on study for 12 days or less.

DISCUSSION AND CONCLUSIONS

In rats, 4-VCH did not produce mortality at any of the concentrations tested. However, in both male and female rats, exposure to 4-VCH was associated with decreased mean final body weights at 1500 ppm.

Increased mean absolute and/or relative liver weights were present in male and female rats exposed to 1000 or 1500 ppm of 4-VCH or 1000 ppm of 1,3-butadiene. At the 1000 ppm level, liver weight increases were slightly greater in 4-VCH exposed rats as compared to those exposed to 1,3-butadiene. These liver weight changes were not associated with correlative microscopic changes. Since 4-VCH is oxidized to epoxides by liver microsomes (Smith et al., 1990), increased liver weights likely represent a normal physiological response to metabolism of a xenobiotic.

Increased formation of hyaline droplets within proximal renal tubules was noted in all 4-VCH-exposed groups of male rats. A similar finding, but with lower incidence and severity at the comparable concentration (1000 ppm), was present in 1,3-butadiene-exposed rats. Increased hyaline droplet accumulation was also observed in male rats in a previous 13-week gavage study with 4-VCH (Collins, 1987). Hyaline droplets, in low density, are a normal feature of the mature male rat proximal tubule. These droplets represent secondary lysosomes which, in most instances, contain the low molecular weight protein α_{2u}-globulin. A number of chemicals have been demonstrated to produce "α_{2u}-globulin nephropathy" via interference with lysosomal hydrolysis of the

protein (Hard and Snowden, 1991). In the present inhalation study with 4-VCH, hyaline droplet accumulation was not associated with the other constitutive features of α_{2u}-globulin nephropathy such as single cell necrosis, cell sloughing, granular cast formation or mineralization. Thus, this change in male rats exposed to 4-VCH (or to 1,3-butadiene) did not appear to represent an adverse effect at the exposure concentrations tested. Further, though compounds inducing increased hyaline droplet formation cause renal tubular tumors in male rats in long-term studies, this male-rat-specific response is not considered predictive for female rats or other common mammalian species including humans (Alden and Frith, 1991).

Ovarian atrophy was noted in two rats exposed to 1500 ppm of 4-VCH. Atrophy in these two rats was morphologically distinct from that seen in the mice in that the primary change was a decrease in the numbers of corpora lutea. Further, though the diepoxide of 4-VCH is a known ovarian toxicant in rats (Salyers et al.), 4-VCH at gavage concentrations of up to 800 mg/kg for 13 weeks did not produce ovarian changes in rats (Collins, 1987). Thus, the ovarian change seen in 2/10 1500 ppm rats in the present study likely represents a spontaneous change unrelated to 4-VCH exposure.

In contrast to rats, exposure of mice to 1000 ppm of 4-VCH produced high mortality in both males and females. Compound-related mortality did not occur in mice exposed to 50 or 250 ppm of 4-VCH or 1000 ppm of 1,3-butadiene.

Exposure of female mice to 1000 ppm of 4-VCH or 1,3-butadiene produced ovarian atrophy which was characterized by decreased numbers of all developmental stages of ovarian follicles. 1,3-Butadiene is a known ovarian toxicant in mice (Melnick et al., 1990), and 4-VCH, a dimer of 1,3-butadiene, produced ovarian atrophy in mice in a 13-week gavage study (Collins, 1987). In the present inhalation study, the incidences of ovarian atrophy, especially in the 4-VCH group, were likely mitigated by early deaths. The relative paucity of ovarian follicles appeared somewhat greater in the 1000 ppm 4-VCH group as compared to 1,3-butadiene-exposed animals. No ovarian changes were present in the 50 or 250 ppm 4-VCH groups.

Decreased testes weights and slight testicular degeneration occurred in 1,3-butadiene-exposed male mice but were not present as a compound-related effect in 4-VCH-exposed mice. These findings are consistent with previous reports for both 1,3-butadiene and 4-VCH ((Melnick et al., 1990; Collins, 1987). However, it should again be noted that in the present study, no male mice exposed to 1000 ppm of 4-VCH were on study beyond day 12.

In the present study, the comparative toxicities of 1,3-butadiene and its dimer, 4-VCH, were assessed in rats and mice. At the comparable exposure concentration of 1000 ppm, most compound-related effects--liver and kidney effects in rats and mortality (4-VCH only) and ovarian effects in mice--were more severe in 4-VCH-exposed animals as compared to those exposed to 1,3-butadiene. However, compound-related testicular effects in mice were present following exposure to 1,3-butadiene but not to 4-VCH.

Based on final body weight effects, the no-observable-adverse-effect level (NOAEL) for rats following subchronic exposure to 4-VCH was 1000 ppm (the intermediate concentration) for both males and females. This conclusion is also based upon the consideration of liver weight changes and microscopic renal effects as non-adverse under the conditions of this study. Based upon mortality and microscopic changes in the ovaries, the NOAEL for both male and female mice was 250 ppm (the intermediate exposure concentration).

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SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLES

SUBCHRONIC INHALATION TOXICITY: 90-DAY STUDY WITH 4-VINYLCYCLOHEXENE

TABLES

EXPLANATORY NOTES

NOTES:

Table 1
All values are reported to two significant figures.

Tables 10-17
All per animal values calculated based on food consumed by group and total animals per group.

Tables 28-29

	Normal	Abnormal
Appearance		
Color	Light to dark yellow or amber	Color other than yellow or amber
Microscopic		
Red Blood Cells	Average of 0 to 4 red blood cells per high power field	Average of more than 4 red blood cells per high power field
Casts	None observed	Average of more than 0 per low power field
Epithelial Cells	Average of 0 to 9 per high power field	Average of more than 9 per high power field
White Blood Cells	Average of 0 to 9 per high power field	Average of more than 9 per high power field

ABBREVIATIONS:

Tables 2-9
AOV = Analysis of Variance

Tables 22-25

RBC - erythrocytes
Hb - hemoglobin concentration
Ht - hematocrit
MCV - mean corpuscular volume
MCH - mean corpuscular hemoglobin
MCHC - mean corpuscular hemoglobin concentration
PLAT - platelets
WBC - leukocytes
Neut - neutrophils
Band - band neutrophils
Lymph - lymphocytes
Alym - atypical lymphocytes
Mono - monocytes
Eosin - eosinophils
Baso - basophils

SUBCHRONIC INHALATION TOXICITY: 90-DAY STUDY WITH 4-VINYLCYCLOHEXENE

TABLES

EXPLANATORY NOTES (Continued)

ABBREVIATIONS: (Continued)

Tables 26-27

ALP - alkaline phosphatase
ALT - alanine aminotransferase
AST - aspartate aminotransferase
CPK - creatine phosphokinase
GGT - gammaglutamyl transferase
GLUCO - glucose
BUN - blood urea nitrogen
CALC - calcium
PHOS - phosphorous
BILRN - bilirubin
CREAT - creatinine
CK - creatinine kinase
TPROT - total protein
TRIG - triglycerides
ALBUM - albumin
GLOBN - globulin
Na - sodium
K - potassium
Cl - chloride
CHOL - cholesterol

Tables 28-29

VOL - volume
OSMOL - osmolality
UROBL - urobilinogen

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 1

SUMMARY OF CHAMBER CONCENTRATION, TEMPERATURE, RELATIVE HUMIDITY, AND AIRFLOW

DESIGN CONCENTRATION (ppm):	0	50	250	1000	1500	1000 1,3-BUTADIENE
PARAMETER MEASURED[a]						
Chamber Concentration (ppm):	0 (0.0)[b] 0[c]	53 (1.2) 32 - 69	250 (3.3) 110 - 300	1000 (9.8) 680 - 1100	1500 (9.7) 1200 - 1600	980 (16) 630 - 1300
Chamber Temperature (°C):	27 (0.11) 23 - 28	23 (0.11) 21 - 25	26 (0.16) 22 - 28	26 (0.15) 22 - 28	26 (0.14) 23 - 29	25 (0.09) 23 - 26
Chamber Relative Humidity (%):	43 (0.42) 37 - 49	57 (0.49) 48 - 68	57 (0.86) 40 - 98	44 (0.58) 36 - 61	55 (1.4) 40 - 88	63 (0.86) 45 - 87
Chamber Airflow (L/min):	60 (0.0) 60	33 (0.16) 29 - 37	64 (0.42) 47 - 68	66 (0.25) 58 - 68	65 (0.84) 43 - 72	65 (0.51) 54 - 71

[a] Each value is the mean of the daily means, calculated over the 90-day test period.
[b] Standard error of the mean is reported in parentheses.
[c] Mean daily ranges.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 2

MEAN BODY WEIGHTS OF MALE RATS

	MEAN BODY WEIGHTS (g)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
3	171.2(16.8)[b]	175.2(14.6)	176.8(13.0)	173.0(12.0)	177.8(13.4)
10	224.1(19.1)	228.0(16.9)	224.2(12.5)	214.2(12.7)	216.7(14.2)
17	281.9(22.1)	285.0(19.3)	277.1(15.2)	262.5(14.5)	266.1(15.2)
24	334.5(27.1)	336.1(19.8)	325.6(8.4)	313.9(14.9)	323.0(25.6)
31	375.4(27.2)	379.9(22.6)	364.8(11.9)	348.6(19.5)*	363.9(30.7)
38	409.4(30.5)	414.1(26.1)	398.5(17.2)	380.8(18.2)*	400.0(32.5)
45	441.4(35.0)	443.9(26.2)	427.1(15.8)	407.6(16.3)*	434.2(39.3)
52	464.9(46.5)	471.2(29.8)	446.8(23.6)	427.9(21.7)*	458.8(41.2)
59	486.8(45.4)	496.7(35.6)	469.9(20.1)	447.8(22.6)*	485.1(47.6)
66	502.0(42.5)	510.1(34.7)	486.7(25.2)	460.3(25.3)*	498.4(54.1)
73	521.4(46.8)	526.1(35.5)	505.4(22.0)	480.2(26.0)*	512.0(55.3)
80	535.2(48.0)	544.8(37.1)	515.7(29.7)	485.5(24.7)*	527.9(51.8)
87	552.1(48.2)	556.2(37.9)	526.7(30.4)	500.0(31.5)*	539.5(56.1)
94	523.4(45.0)	526.4(37.2)	500.1(29.5)	474.7(28.1)*	514.0(56.7)

[a] The first day-on-test and first day-of-exposure for male rats were test day 3.
[b] Standard deviation is reported in parentheses.
Statistical methods: One-way AOV and Dunnett's tests were performed on weekly interval data. (See Statistical Analyses)
* Statistically significant difference from control at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 3

MEAN BODY WEIGHTS OF FEMALE RATS

	MEAN BODY WEIGHTS (g)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
4	160.2(15.8)[b]	155.2(10.6)	159.3(12.5)	154.7(15.9)	157.0(12.2)
11	186.1(17.4)	183.3(12.4)	181.6(15.5)	179.2(21.0)	180.5(13.9)
18	214.0(20.4)	209.2(14.7)	206.2(17.9)	203.4(23.6)	205.4(14.8)
25	241.7(23.6)	233.8(20.0)	230.6(19.7)	223.3(25.7)	230.8(13.9)
32	259.9(21.8)	252.9(22.3)	247.5(23.2)	235.7(23.1)	251.6(15.9)
39	279.2(25.5)	269.4(25.0)	266.8(24.6)	251.0(27.5)	265.8(16.3)
46	288.4(22.8)	279.4(27.2)	277.3(22.9)	261.7(30.4)	278.3(16.0)
53	301.2(22.4)	286.0(28.3)	282.5(24.2)	269.4(34.8)	287.4(16.9)
60	307.6(25.3)	296.1(28.4)	291.0(26.9)	274.7(28.6)	300.3(16.6)
67	313.7(25.9)	302.7(30.0)	293.3(30.5)	281.3(27.8)	301.3(15.7)
74	324.1(27.7)	309.2(31.6)	302.7(30.7)	290.9(30.9)	306.1(16.0)
81	332.3(26.6)	319.1(34.6)	313.1(32.6)	294.7(29.8)	322.2(20.7)
88	336.8(31.9)	327.3(34.9)	312.2(30.9)	303.7(35.1)	324.0(23.4)
95	323.0(34.7)	306.6(31.3)	299.5(32.5)	287.9(35.1)	309.5(21.3)

[a] The first day-on-test and first day-of-exposure for female rats were test day 4.

[b] Standard deviation is reported in parentheses.
Statistical methods: One-way AOV and Dunnett's tests were performed on weekly interval data. (See Statistical Analyses)
There were no statistically significant differences at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 4

MEAN BODY WEIGHT GAINS OF MALE RATS

	MEAN BODY WEIGHT GAINS (g)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
3-10	52.9(5.6)[b]	52.7(6.8)	47.4(5.3)	41.2(7.7)*	38.9(7.4)*
10-17	57.8(5.1)	57.0(8.6)	53.0(10.4)	48.3(7.5)	49.4(8.0)*
17-24	52.6(7.1)	51.2(6.9)	48.4(9.6)	51.3(13.5)	56.9(16.8)
24-31	41.0(5.0)	43.8(9.8)	39.3(8.9)	34.7(16.4)	40.9(21.0)
31-38	34.0(9.9)	34.2(14.8)	33.6(7.8)	32.3(6.1)	36.1(15.9)
38-45	32.0(10.0)	29.7(5.7)	28.7(7.9)	26.8(6.4)	34.2(7.8)
45-52	23.5(12.4)	27.4(6.4)	19.6(20.9)	20.2(9.3)	24.5(4.0)
52-59	21.9(4.9)	25.5(10.4)	23.1(14.6)	19.9(3.6)	26.3(8.6)
59-66	15.2(6.5)	13.3(5.4)	16.9(6.2)	12.6(5.0)	13.3(9.6)
66-73	19.4(10.0)	16.1(5.3)	18.7(4.5)	19.8(7.5)	13.6(3.9)
73-80	13.8(10.8)	18.7(5.0)	10.3(11.5)	5.3(15.1)	16.0(10.3)
80-87	16.9(9.1)	11.3(5.5)	11.0(8.2)	14.6(17.7)	11.6(10.9)
87-94	-28.7(6.4)	-29.8(4.3)	-26.6(6.0)	-25.3(7.1)	-25.5(8.2)
3-94	352.1(31.1)	351.1(32.6)	323.3(37.3)	301.6(23.2)*	336.3(50.3)

[a] The first day-on-test and first day-of-exposure for male rats were test day 3.
[b] Standard deviation is reported in parentheses
Statistical methods: One-way AOV and Dunnett's tests were performed on both weekly and summary interval data (see Statistical Analyses).
* Statistically significant difference from control at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 5

MEAN BODY WEIGHT GAINS OF FEMALE RATS

	MEAN BODY WEIGHT GAINS (g)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
4-11	25.9(3.6)[b]	28.1(6.7)	22.3(5.4)	24.6(7.5)	23.5(7.2)
11-18	27.8(5.7)	26.0(13.6)	24.6(3.9)	24.2(4.9)	24.9(6.8)
18-25	27.7(8.9)	24.6(16.1)	24.4(7.1)	19.9(6.1)	25.4(5.1)
25-32	18.2(4.9)	19.1(10.8)	16.8(6.7)	12.4(10.8)	20.8(4.6)
32-39	19.4(8.2)	16.5(6.3)	19.3(8.2)	15.3(5.8)	14.3(6.8)
39-46	9.1(9.9)	9.9(5.4)	10.6(5.8)	10.7(6.8)	12.4(7.0)
46-53	12.8(12.8)	6.7(6.3)	5.1(6.4)	7.7(7.0)	9.2(5.2)
53-60	6.5(10.2)	10.0(8.9)	8.6(8.1)	5.3(8.1)	12.9(6.7)
60-67	6.0(8.8)	6.7(8.8)	3.1(6.5)	6.6(6.9)	1.0(9.8)
67-74	10.5(10.0)	6.5(4.1)	9.4(5.2)	9.5(5.6)	4.8(5.2)
74-81	8.2(9.7)	9.9(9.8)	10.4(4.6)	3.8(6.7)	16.1(11.4)
81-88	4.5(8.6)	8.2(8.8)	-0.9(7.0)	9.0(7.3)	1.8(5.2)
88-95	-13.8(9.4)	-20.7(5.2)	-12.7(8.4)	-15.7(9.4)	-14.4(5.9)
4-95	162.7(24.2)	151.4(25.8)	141.2(27.5)	133.3(25.3)*	152.5(17.3)

[a] The first day-on-test and first day-of-exposure for female rats were test day 4.
[b] Standard deviation is reported in parentheses
Statistical methods: One-way AOV and Dunnett's tests were performed on both weekly and summary interval data (see Statistical Analyses).
* Statistically significant difference from control at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 6

MEAN BODY WEIGHTS OF MALE MICE

	MEAN BODY WEIGHTS (g)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
1	25.6(1.3)[b]	25.4(1.5)	24.9(1.0)	25.2(1.1)	24.9(1.1)
8	26.1(1.7)	26.2(2.5)	25.1(2.1)	25.8(1.7)	26.0(1.3)
15	27.2(1.9)	27.1(2.2)	27.0(1.3)	[c]	26.9(1.4)
22	28.0(2.1)	23.1(2.4)*	24.4(3.0)*		27.9(1.3)
29	29.4(2.1)	29.5(2.1)	29.1(1.4)		29.4(1.7)
36	30.3(1.7)	30.2(2.5)	29.8(2.5)		29.7(1.8)
43	31.2(2.3)	31.4(2.2)	31.8(1.6)		30.6(2.0)
50	31.8(2.4)	31.6(2.0)	31.9(1.1)		30.4(1.6)
57	32.4(2.4)	32.8(2.1)	33.1(2.0)		31.6(1.8)
64	32.8(2.0)	33.0(2.1)	33.0(1.5)		32.2(1.5)
71	33.3(2.6)	33.4(2.1)	33.6(1.8)		32.8(1.8)
78	33.5(1.7)	34.0(2.2)	34.3(1.5)		33.1(1.5)
85	33.9(2.0)	34.7(2.2)	34.5(1.8)		34.3(1.7)
92	27.7(1.4)	28.1(2.5)	27.5(1.8)		28.7(1.7)

[a] The first day-on-test and first day-of-exposure for male mice exposed to 4-vinylcyclohexene were test day 1. The first day-on-test and first day-of-exposure for male mice exposed to 1,3-butadiene were test day 2.
[b] Standard deviation is reported in parentheses.
[c] Ten of ten male mice died on test day 11 or 12.
Statistical methods: One-way AOV and Dunnett's tests were performed on weekly interval data. (See Statistical Analyses)
* Statistically significant difference from control at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 7

MEAN BODY WEIGHTS OF FEMALE MICE

	MEAN BODY WEIGHTS (g)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
2	20.6(0.7)[b]	20.5(0.8)	20.5(0.8)	20.5(0.4)	19.9(1.0)
9	20.8(1.0)	20.7(0.8)	20.9(1.0)	21.4(0.8)	20.7(1.0)
16	22.4(1.4)	20.4(2.8)	20.2(2.3)	22.4(1.1)[c]	20.6(2.6)
23	23.3(1.3)	23.0(1.3)	23.3(1.2)	23.1(1.1)	23.8(1.1)
30	23.5(1.4)	23.0(1.2)	22.8(1.4)	23.4(1.1)	24.1(1.7)
37	25.7(0.9)	24.5(1.4)	24.0(1.1)*	24.6(1.9)	24.4(2.8)
44	25.6(1.0)	25.1(1.1)	25.0(1.4)	25.0(1.9)	25.2(1.2)
51	25.8(1.2)	25.7(1.1)	24.6(1.5)	25.0(1.8)	26.2(1.8)
58	25.5(1.5)	25.8(1.2)	25.1(1.7)	25.7(1.7)	26.4(1.6)
65	27.3(1.0)	26.4(1.3)	25.5(1.3)*	27.0(1.7)	26.4(2.0)
72	26.9(1.0)	26.9(1.3)	25.6(1.8)	27.0(2.2)	26.6(1.8)
79	27.1(1.7)	26.7(1.2)	26.2(2.0)	27.0(1.7)	27.2(1.2)
86	27.7(1.0)	26.8(1.4)	26.5(2.2)	27.1(1.8)	27.5(1.8)
93	22.6(1.4)	21.7(0.9)	21.1(1.8)	21.1(1.6)	22.6(1.1)

[a] The first day-on-test and first day-of-exposure for female mice were test day 2.
[b] Standard deviation is reported in parentheses.
[c] Five of ten female mice died on test day 11 or 12.
Statistical methods: One-way AOV and Dunnett's tests were performed on weekly interval data. (See Statistical Analyses)
* Statistically significant difference from control at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 8

MEAN BODY WEIGHT GAINS OF MALE MICE

	MEAN BODY WEIGHT GAINS (g)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
1- 8	0.5(1.1)[b]	0.8(1.9)	0.3(2.0)	0.6(1.2)	1.2(0.7)
8-15	1.1(1.6)	1.0(2.2)	1.9(1.5)	[c]	0.9(0.8)
15-22	0.7(1.5)	-4.1(2.1)*	-2.7(2.4)*		0.9(0.7)
22-29	1.4(1.6)	6.4(2.4)*	4.7(2.7)*		1.5(1.1)
29-36	0.9(1.1)	0.7(1.5)	0.7(2.6)		0.3(1.7)
36-43	1.0(1.8)	1.2(0.6)	1.9(2.6)		0.9(1.7)
43-50	0.6(0.9)	0.3(1.2)	0.1(0.7)		-0.2(1.6)
50-57	0.6(1.7)	1.2(0.7)	1.3(1.2)		1.2(1.7)
57-64	0.4(2.1)	0.2(1.3)	-0.1(0.8)		0.7(1.2)
64-71	0.5(1.6)	0.4(1.1)	0.6(1.3)		0.5(0.5)
71-78	0.2(1.4)	0.6(0.8)	0.7(0.7)		0.4(1.2)
78-85	0.4(0.7)	0.8(1.9)	0.3(1.2)		1.2(2.3)
85-92	-6.1(0.9)	-6.5(1.1)	-7.1(1.6)		-5.6(1.1)
1-92	2.3(1.3)	2.7(1.0)	2.6(1.9)		3.9(1.8)

[a] The first day-on-test and first day-of-exposure for male mice exposed to 4-vinylcyclohexene were test day 1. The first day-on-test and first day-of-exposure for male mice exposed to 1,3-butadiene were test day 2.

[b] Standard deviation is reported in parentheses

[c] Ten of ten male mice died on test day 11 or 12.

Statistical methods: One-way AOV and Dunnett's tests were performed on both weekly and summary interval data (see Statistical Analyses).

* Statistically significant difference from control at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 9

MEAN BODY WEIGHT GAINS OF FEMALE MICE

	MEAN BODY WEIGHT GAINS (g)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
2- 9	0.2(0.8)[b]	0.4(0.5)	0.4(0.8)	1.0(1.0)	0.9(0.9)
9-16	1.6(1.8)	-0.3(2.7)	-0.7(2.3)	1.0(1.5)[c]	-0.2(2.6)
16-23	0.9(1.5)	2.5(3.6)	3.1(2.2)	0.8(1.6)	3.2(2.7)*
23-30	0.2(1.6)	0.0(2.0)	-0.5(1.2)	0.3(0.8)	0.3(1.2)
30-37	2.2(1.2)	1.5(1.1)	1.2(1.3)	1.2(1.8)	0.4(2.3)*
37-44	-0.1(1.0)	0.6(2.1)	1.0(1.0)	0.3(1.3)	-0.1(0.7)
44-51	0.2(1.6)	0.6(1.4)	-0.4(1.6)	0.0(1.0)	1.0(2.0)
51-58	-0.3(1.3)	0.1(1.2)	0.4(1.4)	0.7(0.6)	0.2(1.2)
58-65	1.8(1.1)	0.6(1.2)	0.4(1.1)	1.2(1.7)	0.4(1.2)*
65-72	-0.4(0.9)	0.5(2.1)	-0.1(1.4)	-0.1(0.5)	0.2(1.6)
72-79	0.2(1.4)	-0.2(1.9)	0.6(1.7)	0.0(1.0)	0.6(0.9)
79-86	0.7(1.1)	0.1(1.1)	0.2(1.5)	0.0(0.9)	0.3(1.8)
86-93	-5.2(1.1)	-5.1(1.0)	-5.4(1.7)	-5.3(3.9)	-4.9(2.1)
2-93	2.0(1.1)	1.5(0.9)	0.6(1.4)	0.8(1.6)	2.6(1.5)

[a] The first day-on-test and first day-of-exposure for female mice were test day 2.
[b] Standard deviation is reported in parentheses
[c] Five of ten female mice died on test day 11 or 12.
Statistical methods: One-way AOV and Dunnett's tests were performed on both weekly and summary interval data (see Statistical Analyses).
* Statistically significant differences at alpha = 0.05.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 10

MEAN DAILY FOOD CONSUMPTION BY MALE RATS

	MEAN DAILY FOOD CONSUMED PER RAT (g)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
3-10	23.0	23.6	23.3	22.0	22.5
10-17	25.9	26.2	25.6	25.1	25.3
17-24	28.7	28.9	29.3	28.8	26.0
24-31	28.7	29.6	29.2	28.6	29.9
31-38	29.1	29.8	29.0	29.2	31.4
38-45	29.0	30.8	29.9	29.6	32.1
45-52	28.8	31.1	29.4	29.4	31.4
52-59	30.7	30.3	30.0	28.5	31.8
59-66	28.8	30.0	29.2	29.0	30.2
66-73	28.1	29.2	28.5	28.7	29.0
73-80	29.9	32.3	30.1	30.0	30.0
80-87	29.2	30.6	28.6	29.2	31.6
87-94	29.3	28.6	29.5	28.8	30.4
3-94	28.4	29.3	28.6	28.2	29.3

[a] The first day-on-test and first day-of-exposure for male rats were test day 3.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 11

MEAN DAILY FOOD CONSUMPTION BY FEMALE RATS

	MEAN DAILY FOOD CONSUMED PER RAT (g)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
4-11	18.6	19.2	19.0	18.2	18.5
11-18	19.9	19.9	20.0	19.1	19.8
18-25	22.7	21.1	20.8	21.9	20.8
25-32	22.9	22.4	21.8	21.3	21.9
32-39	22.6	23.0	21.7	21.4	22.9
39-46	23.4	22.7	22.2	21.7	23.5
46-53	25.2	24.8	23.5	23.0	25.0
53-60	22.2	22.1	22.2	20.6	22.5
60-67	22.6	22.5	21.5	21.4	22.4
67-74	22.7	21.7	21.8	21.7	21.8
74-81	23.6	24.4	23.1	22.5	25.0
81-88	22.6	23.4	31.3	21.8	22.7
88-95	22.3	22.2	[b]	18.2	23.4
4-95	22.4	22.3	22.3[c]	21.0	22.3

[a] The first day-on-test and first day-of-exposure for female rats were test day 4.
[b] Due to technical error, inadequate food consumption data were recorded for this period to calculate food consumed.
[c] Interval represents test days 4-88.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 12

MEAN FOOD EFFICIENCY OF MALE RATS

	MEAN FOOD EFFICIENCY (g WT GAIN/g FOOD CONSUMED)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
3-10	0.328	0.319	0.290	0.267	0.248
10-17	0.319	0.311	0.295	0.275	0.279
17-24	0.261	0.253	0.236	0.255	0.313
24-31	0.204	0.211	0.192	0.173	0.196
31-38	0.167	0.164	0.166	0.158	0.165
38-45	0.157	0.138	0.137	0.130	0.152
45-52	0.117	0.126	0.095	0.098	0.112
52-59	0.102	0.120	0.110	0.100	0.118
59-66	0.075	0.064	0.083	0.062	0.063
66-73	0.098	0.079	0.094	0.099	0.067
73-80	0.066	0.083	0.049	0.025	0.076
80-87	0.082	0.053	0.055	0.071	0.052
87-94	-0.140	-0.149	-0.129	-0.126	-0.120
3-94	0.136	0.132	0.124	0.117	0.126

[a] The first day-on-test and first day-of-exposure for male rats were test day 3.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 13

MEAN FOOD EFFICIENCY OF FEMALE RATS

	MEAN FOOD EFFICIENCY (g WT GAIN/g FOOD CONSUMED)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
TEST DAYS[a]					
4-11	0.199	0.210	0.167	0.193	0.181
11-18	0.200	0.186	0.176	0.181	0.180
18-25	0.174	0.166	0.168	0.130	0.174
25-32	0.114	0.122	0.110	0.083	0.135
32-39	0.122	0.103	0.127	0.102	0.089
39-46	0.056	0.062	0.068	0.070	0.075
46-53	0.073	0.038	0.031	0.048	0.052
53-60	0.042	0.065	0.055	0.037	0.082
60-67	0.038	0.042	0.021	0.044	0.006
67-74	0.066	0.043	0.062	0.063	0.032
74-81	0.049	0.058	0.064	0.024	0.092
81-88	0.028	0.050	-0.004	0.059	0.011
88-95	-0.089	-0.133	[b]	-0.124	-0.088
4-95	0.080	0.075	0.082[c]	0.070	0.075

[a] The first day-on-test and first day-of-exposure for female rats were test day 4.
[b] Due to technical error, inadequate food consumption data were recorded for this period.
[c] Interval represents test days 4-88.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 14

MEAN DAILY FOOD CONSUMPTION BY MALE MICE

	MEAN DAILY FOOD CONSUMED PER MOUSE (g)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
1- 8	4.7	4.8	4.8	4.9	4.6
8-15	5.3	5.4	5.2	5.0	5.4
15-22	6.3	4.9	4.8	[b]	5.1
22-29	3.7	5.8	5.5		5.2
29-36	5.4	5.6	5.1		5.0
36-43	5.1	5.5	5.2		[c]
43-50	5.4	5.5	5.4		[c]
50-57	5.6	5.8	5.5		5.4
57-64	5.5	5.4	5.4		5.1
64-71	5.4	5.3	5.2		5.0
71-78	5.4	5.5	5.3		5.2
78-85	5.5	5.9	5.4		5.3
85-92	5.8	5.8	5.6		5.6
1-92	5.3	5.5	5.2		5.5

[a] The first day-on-test and first day-of-exposure for male mice exposed to 4-vinylcyclohexene were test day 1. The first day-on-test and first day-of-exposure for male mice exposed to 1,3-butadiene were test day 2.

[b] Ten of ten male mice died on test day 11 or 12.

[c] Due to technical error, inadequate food consumption data were recorded for this period to calculate food consumed.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 15

MEAN DAILY FOOD CONSUMPTION BY FEMALE MICE

	MEAN DAILY FOOD CONSUMED PER MOUSE (g)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
2- 9	4.3	4.2	4.4	4.4	4.0
9-16	4.6	4.5	4.5	5.2[b]	4.4
16-23	5.0	5.1	5.0	5.1	5.0
23-30	5.0	4.8	4.6	5.2	4.6
30-37	4.4	4.5	4.9	5.0	4.7
37-44	4.8	5.0	9.8	5.2	4.6
44-51	5.3	5.3	5.0	5.4	5.2
51-58	5.8	5.3	4.8	5.5	5.2
58-65	5.5	5.3	5.3	5.3	4.7
65-72	5.5	5.5	4.8	5.7	5.4
72-79	5.3	5.1	5.0	4.7	5.2
79-86	5.4	5.4	5.1	6.2	5.4
86-93	5.6	5.6	5.2	5.2	5.5
2-93	5.1	5.0	5.3	5.2	4.9

[a] The first day-on-test and first day-of-exposure for female mice were test day 2.
[b] Five of ten female mice died on test day 11 or 12.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 16

MEAN FOOD EFFICIENCY OF MALE MICE

	MEAN FOOD EFFICIENCY (g WT GAIN/g FOOD CONSUMED)				
GROUP:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
1- 8	0.016	0.023	0.008	0.018	0.036
8-15	0.031	0.025	0.052	[b]	0.025
15-22	0.016	-0.119	-0.079		0.026
22-29	0.056	0.157	0.122		0.041
29-36	0.023	0.018	0.020		0.009
36-43	0.027	0.030	0.054		0.009
43-50	0.015	0.006	0.002		[c]
50-57	0.015	0.029	0.032		0.032
57-64	0.010	0.005	-0.003		0.018
64-71	0.013	0.009	0.016		0.015
71-78	0.006	0.016	0.018		0.011
78-85	0.010	0.019	0.007		0.033
85-92	-0.150	-0.161	-0.181		-0.143
1-92	0.005	0.005	0.005		0.008

[a] The first day-on-test and first day-of-exposure for male mice exposed to 4-vinylcyclohexene were test day 1. The first day-on-test and first day-of-exposure for male mice exposed to 1,3-butadiene were test day 2.

[b] Ten of ten male mice died on test day 11 or 12.

[c] Due to technical error, inadequate food consumption data were recorded for this period to calculate food consumed.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 17

MEAN FOOD EFFICIENCY OF FEMALE MICE

	MEAN FOOD EFFICIENCY (g WT GAIN/g FOOD CONSUMED)				
GROUP:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
TEST DAYS[a]					
2- 9	0.005	0.013	0.013	0.031	0.032
9-16	0.050	-0.009	-0.021	0.027[b]	-0.006
16-23	0.027	0.071	0.087	0.021	0.093
23-30	0.007	0.001	-0.015	0.008	0.008
30-37	0.071	0.047	0.036	0.033	0.012
37-44	-0.004	0.018	0.015	0.009	-0.002
44-51	0.005	0.016	-0.011	0.001	0.027
51-58	-0.007	0.004	0.013	0.019	0.005
58-65	0.047	0.016	0.012	0.033	0.013
65-72	-0.012	0.013	-0.002	-0.003	0.006
72-79	0.005	-0.006	0.018	0.000	0.016
79-86	0.018	0.002	0.006	0.001	0.007
86-93	-0.132	-0.130	-0.148	-0.147	-0.127
2-93	0.004	0.003	0.001	0.002	0.006

[a] The first day-on-test and first day-of-exposure for female mice were test day 2.
[b] Five of ten female mice died on test day 11 or 12.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 18

SUMMARY OF CLINICAL OBSERVATIONS IN MALE RATS

	NUMBER OF RATS WITH GIVEN SIGN				
GROUP[a]:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
OBSERVATION (WEEKLY PREEXPOSURE)					
AGGRESSIVE BEHAVIOR	0	1 (59)[b]	0	0	1 (52)
DISCHARGE EYE(S)	0	0	0	1 (94)	0
DISCHARGE MOUTH	0	0	0	1 (52)	0
DIARRHEA	0	1 (45)	0	2 (21)	0
STAINED FUR	0	0	0	4 (24)	0

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 18 (Continued)

SUMMARY OF CLINICAL OBSERVATIONS IN MALE RATS

	NUMBER OF RATS WITH GIVEN SIGN				
GROUP[a]:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
OBSERVATION (DAILY POSTEXPOSURE)					
AGGRESSIVE BEHAVIOR	0	2 (69)[b]	0	2 (64)	1 (59)
ALOPECIA	0	1 (67)	0	0	0
DISCHARGE CHIN	0	0	0	1 (79)	0
DISCHARGE EYE(S)	0	1 (42)	0	1 (56)	0
DISCHARGE MOUTH	0	10 (28)*	10 (33)*	10 (10)*	10 (31)*
DISCHARGE NOSE	0	4 (54)	5 (42)*	0	0
DIARRHEA	0	0	1 (42)	0	0
HUNCHED POSTURE	0	3 (45)	1 (60)	0	0
LETHARGY	0	7 (24)*	2 (23)	9 (23)*	1 (53)
RUFFLED FUR	0	0	0	2 (67)	0
SKIN SORE	0	0	2 (71)	2 (88)	3 (65)
STAINED FUR	1 (31)	7 (67)*	9 (15)*	9 (24)*	10 (23)*
VOCALIZATION	0	2 (65)	1 (77)	2 (60)	1 (67)

a Total rats per group at study start = 10.
b Values in parentheses are the medians for test days when the given signs were first observed.
Statistical methods: Fisher's Exact Test with Bonferroni Correction and Cochran Armitage Test for trend. (See Statistical Analyses)
* Statistically significant difference from control at alpha = 0.05.
No significant trends.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 19

SUMMARY OF CLINICAL OBSERVATIONS IN FEMALE RATS

	NUMBER OF RATS WITH GIVEN SIGN				
GROUP[a]:	II	IV	VI	VIII	X
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
OBSERVATION (WEEKLY PREEXPOSURE)					
ALOPECIA	0	0	0	0	1 (74)[b]
STAINED FUR	0	3 (74)	0	0	1 (60)
OBSERVATION (DAILY POSTEXPOSURE)					
AGGRESSIVE BEHAVIOR	0	1 (53)	0	0	0
ALOPECIA	0	0	0	1 (39)	0
DISCHARGE MOUTH	0	9 (35)*	10 (29)*	10 (21)*	10 (31)*
DISCHARGE NOSE	0	3 (42)	2 (42)	3 (49)	0
LETHARGY	0+	0+	1 (22)+	6 (21)*+	2 (39)
RUFFLED FUR	0	0	0	1 (67)	0
SKIN SORE	0	2 (75)	2 (73)	1 (87)	0
STAINED FUR	0	8 (35)*	10 (35)*	10 (26)*	10 (26)*
WET UNDERBODY	0	0	0	1 (58)	0

a Total rats per group at study start = 10.
b Values in parentheses are the medians for test days when the given signs were first observed.
Statistical methods: Fisher's Exact Test with Bonferroni Correction and Cochran Armitage Test for trend.
(See Statistical Analyses)
* Statistically significant difference from control at alpha = 0.05.
+ Positive trend for groups exposed to 4-vinylcyclohexene.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 20

SUMMARY OF CLINICAL OBSERVATIONS IN MALE MICE

	NUMBER OF MICE WITH GIVEN SIGN				
GROUP[a]:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
OBSERVATION (WEEKLY PREEXPOSURE)					
DISCHARGE NOSE	1 (84)	0	0	0	0
DEHYDRATED	1 (84)[b]	0	0	0	0
GASPING	1 (84)	0	0	0	0
HUNCHED POSTURE	1 (84)	1 (22)	0	0	0
LETHARGY	0	1 (22)	0	0	0
MASS ON NOSE	1 (84)	0	0	0	0
NO FECES	1 (84)	0	0	0	0
RUFFLED FUR	3 (71)	0	0	0	0
SKIN SORE	1 (84)	0	0	0	0
TREMORS	0	0	0	1 (11)	0
WEAKNESS	1 (84)	1 (22)	0	0	0

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 20 (Continued)

SUMMARY OF CLINICAL OBSERVATIONS IN MALE MICE

	NUMBER OF MICE WITH GIVEN SIGN				
GROUP[a]:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
OBSERVATION (DAILY POSTEXPOSURE)					
LETHARGY	0	1 (22)	0	0	0
SKIN SORE	0	1 (51)	0	0	0
TREMORS	0	3 (22)	0	3 (11)	0

Total mice per group at study start = 10.
Values in parentheses are the medians for test days when the given signs were first observed.
Statistical methods: Fisher's Exact Test with Bonferroni Correction and Cochran Armitage Test for trend. (See Statistical Analyses)
There were no statistically significant differences at alpha = 0.05.
No significant trends.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 21

SUMMARY OF CLINICAL OBSERVATIONS IN FEMALE MICE

	NUMBER OF MICE WITH GIVEN SIGN				
GROUP[a]:	II	VI	VI	VIII	X
CONCENTRATION (ppm):	0	50	250	1000	1000 1,3-BUTADIENE
OBSERVATION (WEEKLY PREEXPOSURE)					
DIARRHEA	0	0	0	0	1 (63)
HUNCHED POSTURE	0	0	1 (70)	0	1 (63)
LETHARGY	0	0	1 (70)	0	1 (63)
WEAKNESS	0	0	1 (70)	0	1 (63)
OBSERVATION (DAILY POSTEXPOSURE)					
ALOPECIA	0	1 (77)[b]	0	0	0
CONVULSIONS	0	0	0	1 (11)	0
GASPING	0	0	0	0	1 (60)
HUNCHED POSTURE	0	1 (22)	1 (68)	0	0
IRREGULAR RESPIRATION	0	0	0	0	1 (60)
LETHARGY	0+	2 (22)+	1 (68)+	5 (16)**	0
LUNG NOISE	0	0	0	0	1 (60)
SKIN SORE	3 (18)	0	0	0	0
TREMORS	0	3 (22)	0	2 (11)	0

a Total mice per group at study start = 10.
b Values in parentheses are the medians for test days when the given signs were first observed.
Statistical methods: Fisher's Exact Test with Bonferroni Correction and Cochran Armitage Test for trend.
(See Statistical Analyses)
* Statistically significant difference from control at alpha = 0.05.
+ Positive trend for groups exposed to 4-vinylcyclohexene.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 22

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
RBC x10^6/ul	0	8.14(0.55)[a]	
	250	8.06(0.52)	
	1,000	8.11(0.40)	
	1,500	7.98(0.32)	
	1,000	8.15(0.50)	[1,3-butadiene]
Hb g/dl	0	15.6(0.9)	
	250	15.4(0.6)	
	1,000	15.4(0.6)	
	1,500	15.6(0.3)	
	1,000	15.5(0.6)	[1,3-butadiene]
Ht %	0	42.(4.)	
	250	42.(3.)	
	1,000	42.(2.)	
	1,500	42.(1.)	
	1,000	42.(1.)	[1,3-butadiene]
MCV fl	0	52.(2.)	
	250	52.(2.)	
	1,000	52.(1.)	
	1,500	52.(1.)	
	1,000	52.(2.)	[1,3-butadiene]
MCH pg	0	19.(1.)	
	250	19.(1.)	
	1,000	19.(1.)	
	1,500	20.(1.)	
	1,000	19.(1.)	[1,3-butadiene]
MCHC g/dl	0	37.(1.)	
	250	37.(1.)	
	1,000	37.(1.)	
	1,500	37.(1.)	
	1,000	37.(1.)	[1,3-butadiene]

[a] Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 22 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
PLAT $x10^3/ul$	0	1035.(173.)[a]	
	250	1078.(90.)	
	1,000	1127.(51.)	
	1,500	1154.(92.)	
	1,000	1070.(172.)	[1,3-butadiene]

a Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 22 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
WBC x10^3/ul	0	17.1(5.3)[a]	
	250	20.0(5.6)	
	1,000	17.8(4.6)	
	1,500	16.6(4.2)	
	1,000	18.5(4.2)	[1,3-butadiene]
Neut WBCx%	0	2789.(2348.)	
	250	2366.(1419.)	
	1,000	1812.(627.)	
	1,500	1971.(945.)	
	1,000	1806.(651.)	[1,3-butadiene]
Band WBCx%	0	0.(0.)	
	250	0.(0.)	
	1,000	0.(0.)	
	1,500	0.(0.)	
	1,000	0.(0.)	[1,3-butadiene]
Lymph WBCx%	0	12915.(2918.)	
	250	15814.(4320.)	
	1,000	14387.(3847.)	
	1,500	12909.(3206.)	
	1,000	14899.(3528.)	[1,3-butadiene]
Alym WBCx%	0	0.(0.)	
	250	0.(0.)	
	1,000	0.(0.)	
	1,500	0.(0.)	
	1,000	0.(0.)	[1,3-butadiene]

a Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 22 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
	0	1210.(576.)[a]	
Mono	250	1691.(684.)	
WBCx%	1,000	1409.(539.)	
	1,500	1646.(667.)	
	1,000	1794.(920.)	[1,3-butadiene]
	0	196.(261.)	
Eosin	250	159.(151.)	
WBCx%	1,000	182.(232.)	
	1,500	115.(113.)	
	1,000	41.(86.)+	[1,3-butadiene]
	0	0.(0.)	
Baso	250	0.(0.)	
WBCx%	1,000	0.(0.)	
	1,500	0.(0.)	
	1,000	0.(0.)	[1,3-butadiene]

[a] Group means and standard deviations (SD)

\+ Significantly different from control at 5% level by Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 23

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
RBC x10^6/ul	0	7.49(0.36)[a]	
	250	7.58(0.28)	
	1,000	7.58(0.23)	
	1,500	7.44(0.49)	
	1,000	7.44(0.23)	[1,3-butadiene]
Hb g/dl	0	15.7(0.5)	
	250	15.7(0.4)	
	1,000	15.7(0.5)	
	1,500	15.5(0.8)	
	1,000	15.3(0.4)	[1,3-butadiene]
Ht %	0	43.(1.)	
	250	43.(1.)	
	1,000	43.(1.)	
	1,500	43.(3.)	
	1,000	42.(1.)	[1,3-butadiene]
MCV fl	0	57.(1.)	
	250	56.(1.)	
	1,000	56.(1.)	
	1,500	57.(1.)	
	1,000	56.(1.)	[1,3-butadiene]
MCH pg	0	21.(1.)	
	250	21.(1.)	
	1,000	21.(1.)	
	1,500	21.(1.)	
	1,000	21.(1.)*	[1,3-butadiene]
MCHC g/dl	0	37.(1.)	
	250	37.(1.)	
	1,000	37.(1.)	
	1,500	37.(1.)	
	1,000	37.(1.)	[1,3-butadiene]

a Group means and standard deviations(SD)
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 23 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
	0	1049.(107.)[a]	
PLAT	250	1014.(65.)	
$x10^3/ul$	1,000	1057.(139.)	
	1,500	1182.(145.)*	
	1,000	1089.(63.)	[1,3-butadiene]

[a] Group means and standard deviations(SD)

* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 23 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
WBC x10^3/ul	0	12.8(2.5)[a]	
	250	12.2(1.8)	
	1,000	11.2(2.8)	
	1,500	12.4(3.1)	
	1,000	11.8(3.1)	[1,3-butadiene]
Neut WBCx%	0	821.(388.)	
	250	881.(490.)	
	1,000	1246.(859.)	
	1,500	1847.(1397.)	
	1,000	1274.(993.)	[1,3-butadiene]
Band WBCx%	0	0.(0.)	
	250	0.(0.)	
	1,000	0.(0.)	
	1,500	0.(0.)	
	1,000	0.(0.)	[1,3-butadiene]
Lymph WBCx%	0	10957.(2418.)	
	250	10481.(1435.)	
	1,000	9005.(1857.)	
	1,500	9629.(2356.)	
	1,000	9647.(2772.)	[1,3-butadiene]
Alym WBCx%	0	37.(82.)	
	250	14.(45.)	
	1,000	57.(139.)	
	1,500	27.(85.)	
	1,000	52.(117.)	[1,3-butadiene]

[a] Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 23 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
Mono WBCx%	0	889.(342.)[a]	
	250	710.(251.)	
	1,000	742.(398.)	
	1,500	814.(329.)	
	1,000	715.(624.)	[1,3-butadiene]
Eosin WBCx%	0	117.(114.)	
	250	134.(114.)	
	1,000	128.(136.)	
	1,500	93.(100.)	
	1,000	113.(95.)	[1,3-butadiene]
Baso WBCx%	0	0.(0.)	
	250	0.(0.)	
	1,000	0.(0.)	
	1,500	0.(0.)	
	1,000	0.(0.)	[1,3-butadiene]

a Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 24

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY
RBC $x10^6$/ul	0	10.53(0.41)[a]
	50	10.26(1.29)
	250	10.59(0.22)
	1,000	[b]
	1,000	8.26(0.28)* [1,3-butadiene]
Hb g/dl	0	17.3(0.8)
	50	17.0(1.8)
	250	17.7(0.5)
	1,000	[b]
	1,000	15.8(0.4)* [1,3-butadiene]
Ht %	0	51.(2.)
	50	50.(6.)
	250	51.(1.)
	1,000	[b]
	1,000	45.(1.)* [1,3-butadiene]
Retic $x10^3$/ul	0	261.(95.)
	50	++
	250	276.(139.)
	1,000	[b]
	1,000	388.(115.)* [1,3-butadiene]

[a] Group means and standard deviations(SD)
[b] All animals in group died prior to sample collection.
* Significantly different form control at 5% level by Dunnett criteria
++ Retics not performed for this group

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 24 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
	0	49.(1.)[a]	
MCV	50	49.(2.)	
fl	250	49.(1.)	
	1,000	b	
	1,000	54.(0.)*	[1,3-butadiene]
	0	16.(1.)	
MCH	50	17.(1.)	
pg	250	17.(0.)	
	1,000	b	
	1,000	19.(0.)*	[1,3-butadiene]
	0	34.(1.)	
MCHC	50	35.(1.)	
g/dl	250	35.(1.)	
	1,000	b	
	1,000	36.(1.)*	[1,3-butadiene]
	0	1014.(203.)	
PLAT	50	998.(216.)	
$x10^3$/ul	250	843.(86.)	
	1,000	b	
	1,000	775.(100.)*	[1,3-butadiene]

[a] Group means and standard deviations(SD)
[b] All animals in group died prior to sample collection.
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 24 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
WBC x10^3/ul	0	2.4(0.7)[a]	
	50	2.6(1.0)	
	250	1.6(0.5)	
	1,000	b	
	1,000	2.9(0.4)	[1,3-butadiene]
Neut WBCx%	0	567.(293.)	
	50	378.(196.)	
	250	310.(254.)	
	1,000	b	
	1,000	381.(137.)	[1,3-butadiene]
Band WBCx%	0	0.(0.)	
	50	2.(5.)	
	250	2.(6.)	
	1,000	b	
	1,000	0.(0.)	[1,3-butadiene]
Lymph WBCx%	0	1762.(482.)	
	50	2191.(965.)	
	250	1320.(381.)	
	1,000	b	
	1,000	2403.(309.)*	[1,3-butadiene]

a Group means and standard deviations(SD)
b All animals in group died prior to sample collection.
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 24 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR MALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
	0	2.(5.)[a]	
Alym	50	0.(0.)	
WBCx%	250	0.(0.)	
	1,000	[b]	
	1,000	0.(0.)	[1,3-butadiene]
	0	27.(25.)	
Mono	50	59.(62.)	
WBCx%	250	16.(23.)	
	1,000	[b]	
	1,000	52.(55.)	[1,3-butadiene]
	0	9.(14.)	
Eosin	50	11.(15.)	
WBCx%	250	2.(8.)	
	1,000	[b]	
	1,000	14.(16.)	[1,3-butadiene]
	0	0.(0.)	
Baso	50	0.(0.)	
WBCx%	250	0.(0.)	
	1,000	[b]	
	1,000	0.(0.)	[1,3-butadiene]

[a] Group means and standard deviations(SD)
[b] All animals in group died prior to sample collection.

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 25

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
RBC x10^6/ul	0	10.58(0.19)[a]	
	50	10.81(0.19)	
	250	10.67(0.24)[b]	
	1,000	10.56(0.32)[c]	
	1,000	9.25(0.32)*[d]	[1,3-butadiene]
Hb g/dl	0	17.2(0.5)	
	50	17.6(0.5)	
	250	17.1(0.4)[b]	
	1,000	17.0(0.1)[c]	
	1,000	16.4(0.3)*[d]	[1,3-butadiene]
Ht %	0	50.(1.)	
	50	51.(1.)	
	250	51.(1.)[b]	
	1,000	53.(2.)[c]	
	1,000	48.(2.)*[d]	[1,3-butadiene]
Retic x10^3/ul	0	209.(137.)	
	50	++	
	250	++	
	1,000	343.(210.)[c]	
	1,000	336.(167.)[d]	[1,3-butadiene]

a Group means and standard deviations(SD)
b Nine animals in group
c Two animals in group
d Eight animals in group
* Significantly different from control at 5% level by Dunnett criteria
++ Retics not performed for this group

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 25 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
	0	48.(1.)[a]	
MCV	50	47.(1.)	
fl	250	47.(1.)[b]	
	1,000	50.(0.)*[c]	
	1,000	52.(1.)*[d]	[1,3-butadiene]
	0	16.(0.)	
MCH	50	16.(0.)	
pg	250	16.(0.)[b]	
	1,000	17.(1.)[c]	
	1,000	18.(1.)*[d]	[1,3-butadiene]
	0	34.(1.)	
MCHC	50	35.(1.)	
g/dl	250	34.(1.)[b]	
	1,000	32.(1.)*[c]	
	1,000	34.(1.)[d]	[1,3-butadiene]
	0	886.(167.)	
PLAT	50	779.(150.)	
$x10^3/ul$	250	964.(76.)[b]	
	1,000	1136.(37.)[c]	
	1,000	717.(97.)*[d]	[1,3-butadiene]

[a] Group means and standard deviations(SD)
[b] Nine animals in group
[c] Two animals in group
[d] Eight animals in group
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 25 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
WBC x10^3/ul	0	2.8(1.2)[a]	
	50	2.1(0.7)	
	250	2.5(0.9)[b]	
	1,000	3.3(1.8)[c]	
	1,000	3.1(1.0)[d]	[1,3-butadiene]
Neut WBCx%	0	551.(432.)	
	50	502.(485.)	
	250	624.(483.)[b]	
	1,000	423.(230.)[c]	
	1,000	320.(155.)[d]	[1,3-butadiene]
Band WBCx%	0	0.(0.)	
	50	0.(0.)	
	250	0.(0.)[b]	
	1,000	0.(0.)[c]	
	1,000	0.(0.)[d]	[1,3-butadiene]
Lymph WBCx%	0	2233.(1078.)	
	50	1626.(406.)	
	250	1852.(804.)[b]	
	1,000	2828.(1538.)[c]	
	1,000	2752.(888.)[d]	[1,3-butadiene]

a Group means and standard deviations(SD)
b Nine animals in group
c Two animals in group
d Eight animals in group

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 25 (continued)

SUMMARY OF HEMATOLOGIC FINDINGS FOR FEMALE MICE

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
Alym WBCx%	0	0.(0.)[a]	
	50	0.(0.)	
	250	0.(0.)[b]	
	1,000	0.(0.)[c]	
	1,000	0.(0.)[d]	[1,3-butadiene]
Mono WBCx%	0	48.(101.)	
	50	12.(16.)	
	250	21.(19.)[b]	
	1,000	0.(0.)[c]	
	1,000	22.(33.)[d]	[1,3-butadiene]
Eosin WBCx%	0	18.(39.)	
	50	0.(0.)	
	250	3.(10.)[b]	
	1,000	0.(0.)[c]	
	1,000	6.(17.)[d]	[1,3-butadiene]
Baso WBCx%	0	0.(0.)	
	50	0.(0.)	
	250	0.(0.)[b]	
	1,000	0.(0.)[c]	
	1,000	0.(0.)[d]	[1,3-butadiene]

a Group means and standard deviations(SD)
b Nine animals in group
c Two animals in group
d Eight animals in group

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 26

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
ALP U/L	0	97.(33.)[a]	
	250	98.(28.)	
	1,000	91.(12.)	
	1,500	99.(27.)	
	1,000	89.(15.)	[1,3-butadiene]
ALT U/L	0	33.(5.)	
	250	32.(4.)	
	1,000	33.(6.)	
	1,500	34.(5.)	
	1,000	36.(8.)	[1,3-butadiene]
AST U/L	0	84.(20.)	
	250	71.(15.)	
	1,000	73.(11.)	
	1,500	66.(10.)	
	1,000	75.(11.)	[1,3-butadiene]
GLUCO mg/dl	0	102.(8.)	
	250	105.(8.)	
	1,000	100.(7.)	
	1,500	103.(17.)	
	1,000	111.(8.)*	[1,3-butadiene]
BUN mg/dl	0	16.(2.)	
	250	16.(2.)	
	1,000	16.(2.)	
	1,500	17.(3.)	
	1,000	15.(2.)	[1,3-butadiene]
CREAT mg/dl	0	0.63(0.07)	
	250	0.59(0.02)	
	1,000	0.63(0.06)	
	1,500	0.63(0.07)	
	1,000	0.58(0.03)	[1,3-butadiene]

a Group means and standard deviations(SD)
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 26 (continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
	0	7.92(0.57)[a]	
PHOS	250	7.86(0.40)	
mg/dl	1,000	7.59(0.54)	
	1,500	7.75(0.91)	
	1,000	7.54(0.77)	[1,3-butadiene]
	0	0.14(0.02)	
BILRN	250	0.15(0.06)	
mg/dl	1,000	0.15(0.02)	
	1,500	0.14(0.03)	
	1,000	0.14(0.03)	[1,3-butadiene]
	0	10.5(0.2)	
CALC	250	10.7(0.3)	
mg/dl	1,000	10.9(0.2)*	
	1,500	10.9(0.4)*	
	1,000	10.8(0.3)*	[1,3-butadiene]
	0	65.(9.)	
CHOL	250	62.(14.)	
mg/dl	1,000	74.(15.)	
	1,500	66.(14.)	
	1,000	53.(9.)*	[1,3-butadiene]

[a] Group means and standard deviations(SD)
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 26 (continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
	0	6.7(0.2)[a]	
TPROT	250	6.6(0.3)	
g/dl	1,000	6.9(0.3)	
	1,500	6.9(0.4)	
	1,000	6.6(0.4)	[1,3-butadiene]
	0	4.3(0.2)	
ALBMN	250	4.3(0.2)	
g/dl	1,000	4.6(0.2)*	
	1,500	4.6(0.2)*	
	1,000	4.4(0.2)	[1,3-butadiene]
	0	2.4(0.1)	
GLOBN	250	2.3(0.3)	
g/dl	1,000	2.3(0.1)	
	1,500	2.3(0.4)	
	1,000	2.2(0.2)*	[1,3-butadiene]
	0	146.(2.)	
Na	250	145.(1.)	
mmol/L	1,000	144.(1.)*	
	1,500	146.(1.)	
	1,000	146.(1.)	[1,3-butadiene]
	0	6.1(0.2)	
K	250	6.1(0.3)	
mmol/L	1,000	6.2(0.3)	
	1,500	6.1(0.4)	
	1,000	5.8(0.3)*	[1,3-butadiene]
	0	98.(1.)	
Cl	250	98.(2.)	
mmol/L	1,000	98.(1.)	
	1,500	98.(1.)	
	1,000	99.(1.)	[1,3-butadiene]

[a] Group means and standard deviations(SD)
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 27

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
ALP U/L	0	58.(16.)[a]	
	250	55.(13.)	
	1,000	68.(20.)	
	1,500	65.(25.)	
	1,000	67.(26.)	[1,3-butadiene]
ALT U/L	0	31.(5.)	
	250	32.(5.)	
	1,000	34.(7.)	
	1,500	36.(9.)	
	1,000	31.(6.)	[1,3-butadiene]
AST U/L	0	66.(11.)	
	250	70.(11.)	
	1,000	73.(15.)	
	1,500	67.(10.)	
	1,000	64.(12.)	[1,3-butadiene]
GLUCO mg/dl	0	103.(11.)	
	250	107.(8.)	
	1,000	105.(7.)	
	1,500	102.(5.)	
	1,000	107.(8.)	[1,3-butadiene]
BUN mg/dl	0	15.(2.)	
	250	16.(2.)	
	1,000	18.(3.)	
	1,500	18.(4.)	
	1,000	15.(3.)	[1,3-butadiene]
CREAT mg/dl	0	0.72(0.06)	
	250	0.73(0.02)	
	1,000	0.74(0.09)	
	1,500	0.69(0.05)	
	1,000	0.69(0.04)	[1,3-butadiene]

a Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 27 (continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
PHOS mg/dl	0	5.99(0.52)[a]	
	250	5.39(0.70)	
	1,000	5.54(0.87)	
	1,500	5.88(0.63)	
	1,000	5.96(0.90)	[1,3-butadiene]
BILRN mg/dl	0	0.18(0.03)	
	250	0.20(0.06)	
	1,000	0.16(0.03)	
	1,500	0.16(0.02)	
	1,000	0.17(0.04)	[1,3-butadiene]
CALC mg/dl	0	10.9(0.3)	
	250	10.8(0.4)	
	1,000	10.7(0.3)	
	1,500	10.7(0.3)	
	1,000	11.0(0.3)	[1,3-butadiene]
CHOL mg/dl	0	71.(21.)	
	250	74.(23.)	
	1,000	74.(18.)	
	1,500	93.(26.)	
	1,000	77.(20.)	[1,3-butadiene]

a Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 27 (continued)

SUMMARY OF CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
TPROT g/dl	0	7.3(0.4)[a]	
	250	7.3(0.4)	
	1,000	7.4(0.3)	
	1,500	7.3(0.3)	
	1,000	7.2(0.4)	[1,3-butadiene]
ALBMN g/dl	0	5.0(0.3)	
	250	5.1(0.4)	
	1,000	5.1(0.3)	
	1,500	5.0(0.3)	
	1,000	5.1(0.5)	[1,3-butadiene]
GLOBN g/dl	0	2.3(0.3)	
	250	2.2(0.2)	
	1,000	2.3(0.2)	
	1,500	2.3(0.2)	
	1,000	2.1(0.2)	[1,3-butadiene]
Na mmol/L	0	144.(2.)	
	250	144.(2.)	
	1,000	143.(2.)	
	1,500	143.(2.)	
	1,000	144.(2.)	[1,3-butadiene]
K mmol/L	0	5.6(0.2)	
	250	5.4(0.3)	
	1,000	5.4(0.3)	
	1,500	5.6(0.3)	
	1,000	5.5(0.3)	[1,3-butadiene]
Cl mmol/L	0	98.(2.)	
	250	99.(1.)	
	1,000	98.(2.)	
	1,500	99.(3.)	
	1,000	98.(2.)	[1,3-butadiene]

[a] Group means and standard deviations(SD)

No statistically significant differences found at 5% level by Dunnett or Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 28

SUMMARY OF CLINICAL URINALYSIS FINDINGS FOR MALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
VOL ml	0	6.5(1.6)[a]	
	250	12.3(5.1)+	
	1,000	10.6(8.2)	
	1,500	11.0(4.5)+	
	1,000	8.9(2.5)*	[1,3-butadiene]
OSMOL mOs	0	1363.(186.)	
	250	965.(266.)	
	1,000	1229.(420.)	
	1,500	1159.(335.)	
	1,000	1236.(430.)	[1,3-butadiene]
pH	0	6.6(0.6)	
	250	6.8(0.3)	
	1,000	6.1(0.4)*	
	1,500	6.3(0.4)	
	1,000	6.7(0.4)	[1,3-butadiene]
UROBL mg/dl	0	0.2(0.0)	
	250	0.2(0.0)	
	1,000	0.2(0.0)	
	1,500	0.2(0.0)	
	1,000	0.2(0.0)	[1,3-butadiene]

a Group means and standard deviations(SD)
* Significantly different from control at 5% level by Dunnett criteria
+ Significantly different from control at 5% level by Mann-Whitney U criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 28 (continued)

SUMMARY OF CLINICAL URINALYSIS FINDINGS FOR MALE RATS

Measurement	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY
Blood	0	1/10++
Number Positive	250	1/10
	1,000	0/10
	1,500	0/10
	1,000	0/10 1,3-butadiene
Glucose	0	0/10
Number Positive	250	0/10
	1,000	0/10
	1,500	0/10
	1,000	0/10 1,3-butadiene
Protein	0	0/10
Number Abnormal	250	0/10
$\geq$ +3	1,000	0/10
	1,500	0/10
	1,000	1/10 1,3-butadiene
Bilirubin	0	1/10
Number Positive	250	0/10
	1,000	0/10
	1,500	0/10
	1,000	3/10 1,3-butadiene
Ketone	0	6/10
Number Abnormal	250	7/10
$\geq$ +1	1,000	6/10
	1,500	8/10
	1,000	7/10 1,3-butadiene
Appearance (Color	0	0/10
and Transparency)	250	0/10
Number Abnormal	1,000	0/10
	1,500	0/10
	1,000	0/10 1,3-butadiene
Microscopic	0	0/10
Number Abnormal	250	3/10
	1,000	2/10
	1,500	1/10
	1,000	1/10 1,3-butadiene

++ Number of abnormal or positive findings/number of individual specimens examined

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 29

SUMMARY OF CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS

TESTS	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY	
VOL ml	0	9.1(4.3)[a]	
	250	8.3(5.0)	
	1,000	6.3(3.8)	
	1,500	7.6(5.7)	
	1,000	6.1(3.6)	[1,3-butadiene]
OSMOL mOs	0	829.(277.)	
	250	946.(306.)	
	1,000	1414.(663.)*	
	1,500	1461.(689.)*	
	1,000	1392.(611.)*	[1,3-butadiene]
pH	0	6.6(0.4)	
	250	6.2(0.3)	
	1,000	6.1(0.5)	
	1,500	5.8(0.6)*	
	1,000	6.2(0.6)	[1,3-butadiene]
UROBL mg/dl	0	0.2(0.0)	
	250	0.2(0.0)	
	1,000	0.3(0.3)	
	1,500	0.3(0.3)	
	1,000	0.4(0.3)	[1,3-butadiene]

a Group means and standard deviations(SD)
* Significantly different from control at 5% level by Dunnett criteria

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 29 (continued)

SUMMARY OF CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS

Measurement	CONCENTRATION (ppm)	SAMPLING TIME 90-DAY
Blood Number Positive	0	1/10++
	250	0/10
	1,000	0/9
	1,500	1/10
	1,000	3/10 1,3-butadiene
Glucose Number Positive	0	0/10
	250	0/10
	1,000	0/9
	1,500	0/10
	1,000	1/10 1,3-butadiene
Protein Number Abnormal ≥ +3	0	0/10
	250	0/10
	1,000	0/9
	1,500	1/10
	1,000	0/10 1,3-butadiene
Bilirubin Number Positive	0	0/10
	250	0/10
	1,000	0/9
	1,500	0/10
	1,000	3/10 1,3-butadiene
Ketone Number Abnormal ≥ +1	0	0/10
	250	0/10
	1,000	0/9
	1,500	0/10
	1,000	0/10 1,3-butadiene
Appearance (Color and Transparency) Number Abnormal	0	0/10
	250	0/10
	1,000	0/9
	1,500	0/10
	1,000	0/10 1,3-butadiene
Microscopic Number Abnormal	0	0/10
	250	1/10
	1,000	0/9
	1,500	4/10
	1,000	3/10 1,3-butadiene

++ Number of abnormal or positive findings/number of individual specimens examined

TABLE 30
MEAN FINAL BODY AND ORGAN WEIGHTS FROM MALE RATS@

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	FINAL BODY
I	0	523.3 (45.0)
III	250	526.4 (37.2)
V	1000	500.1 (29.5)
VII	1500	474.7 (28.1)#
IX	1000 1,3-BUTADIENE	514.0 (56.7)

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS
I	0	14.751 (1.941)	3.843 (0.477)	2.004 (0.194)	1.732 (0.207)	0.814 (0.201)	0.069 (0.007)
III	250	15.280 (1.980)	4.161 (0.396)	2.117 (0.249)	1.794 (0.175)	0.840 (0.108)	0.071 (0.010)
V	1000	16.387 (0.698)	4.233 (0.430)	1.987 (0.118)	1.645 (0.096)	0.792 (0.129)	0.062 (0.005)
VII	1500	16.930 (1.784)#	4.189 (0.467)	2.017 (0.169)	1.604 (0.162)	0.757 (0.113)	0.068 (0.007)
IX	1000 1,3-BUTADIENE	15.633 (1.928)	4.243 (0.587)	1.928 (0.145)	1.705 (0.154)	0.806 (0.130)	0.068 (0.009)

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS
I	0	2.8114 (.1957)	0.7326 (.0431)	0.3836 (.0279)	0.3307 (.0260)	0.1546 (.0303)	0.0132 (.0013)
III	250	2.8979 (.2635)	0.7899 (.0419)	0.4027 (.0469)	0.3411 (.0263)	0.1593 (.0156)	0.0135 (.0020)
V	1000	3.2841 (.1829)#	0.8480 (.0869)#	0.3982 (.0290)	0.3299 (.0246)	0.1580 (.0218)	0.0124 (.0012)
VII	1500	3.5627 (.2443)#	0.8816 (.0721)#	0.4254 (.0327)	0.3380 (.0291)	0.1597 (.0229)	0.0143 (.0014)
IX	1000 1,3-BUTADIENE	3.0403 (.1492)#	0.8236 (.0432)#	0.3770 (.0259)	0.3329 (.0215)	0.1565 (.0167)	0.0133 (.0018)

TABLE 30 (Continued)
MEAN FINAL BODY AND ORGAN WEIGHTS FROM MALE RATS@

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	FINAL BODY
I	0	523.3 (45.0)
III	250	526.4 (37.2)
V	1000	500.1 (29.5)
VII	1500	474.7 (28.1)#
IX	1000	514.0 (56.7)
	1,3-BUTADIENE	

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	TESTES	BRAIN
I	0	3.613 (0.227)	2.211 (0.171)
III	250	3.781 (0.276)	2.149 (0.096)
V	1000	3.553 (0.317)	2.129 (0.081)
VII	1500	3.690 (0.209)	2.103 (0.059)
IX	1000	3.649 (0.436)	2.240 (0.168)
	1,3-BUTADIENE		

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONCENTRATION (ppm)	TESTES	BRAIN
I	0	0.6939 (.0618)	0.4244 (.0371)
III	250	0.7210 (.0663)	0.4101 (.0345)
V	1000	0.7122 (.0692)	0.4274 (.0335)
VII	1500	0.7806 (.0750)#	0.4441 (.0243)
IX	1000	0.7109 (.0543)	0.4386 (.0366)
	1,3-BUTADIENE		

STANDARD DEVIATION IN PARENTHESES
- SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY DUNNETT'S TEST
@ - STATISTICAL COMPARISONS TO CONTROL GROUP WERE CARRIED OUT SEPARATELY FOR 4-VCH- AND 1,3-BUTADIENE-EXPOSED GROUPS

TABLE 31
MEAN FINAL BODY AND ORGAN WEIGHTS FROM FEMALE RATS@

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	FINAL BODY
II	0	323.0 (34.7)
IV	250	306.6 (31.3)
VI	1000	299.5 (32.5)
VIII	1500	287.9 (35.1)
X	1000 1,3-BUTADIENE	309.5 (21.3)

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS
II	0	8.809 (0.737)	2.303 (0.219)	1.707 (0.237)	1.118 (0.073)	0.590 (0.102)	0.083 (0.011)
IV	250	8.881 (0.802)	2.255 (0.236)	1.671 (0.351)	1.098 (0.063)	0.565 (0.068)	0.088 (0.013)
VI	1000	9.080 (1.251)	2.246 (0.277)	1.601 (0.145)	1.061 (0.102)	0.557 (0.041)	0.086 (0.008)
VIII	1500	9.600 (1.497)	2.447 (0.429)	1.636 (0.230)	1.116 (0.171)	0.620 (0.106)	0.080 (0.011)
X	1000 1,3-BUTADIENE	9.270 (0.587)	2.399 (0.198)	1.530 (0.093)#	1.132 (0.091)	0.547 (0.059)	0.086 (0.007)

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS
II	0	2.7385 (.1708)	0.7180 (.0811)	0.5293 (.0534)	0.3496 (.0438)	0.1819 (.0190)	0.0262 (.0055)
IV	250	2.9009 (.0929)	0.7372 (.0599)	0.5505 (.1363)	0.3602 (.0256)	0.1850 (.0213)	0.0290 (.0053)
VI	1000	3.0276 (.2020)#	0.7526 (.0833)	0.5372 (.0506)	0.3563 (.0399)	0.1876 (.0220)	0.0290 (.0027)
VIII	1500	3.3284 (.2385)#	0.8460 (.0581)#	0.5693 (.0492)	0.3886 (.0432)	0.2143 (.0167)#	0.0279 (.0018)
X	1000 1,3-BUTADIENE	2.9991 (.1566)#	0.7753 (.0450)	0.4948 (.0188)	0.3665 (.0294)	0.1771 (.0193)	0.0278 (.0025)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 31 (Continued)
MEAN FINAL BODY AND ORGAN WEIGHTS FROM FEMALE RATS@

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	FINAL BODY
II	0	323.0 (34.7)
IV	250	306.6 (31.3)
VI	1000	299.5 (32.5)
VIII	1500	287.9 (35.1)
X	1000 1,3-BUTADIENE	309.5 (21.3)

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	OVARIES	BRAIN
II	0	0.146 (0.021)	2.047 (0.194)
IV	250	0.150 (0.030)	1.944 (0.056)
VI	1000	0.131 (0.018)	1.949 (0.064)
VIII	1500	0.134 (0.042)	1.970 (0.225)
X	1000 1,3-BUTADIENE	0.144 (0.025)	2.042 (0.180)

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONCENTRATION (ppm)	OVARIES	BRAIN
II	0	0.0461 (.0108)	0.6368 (.0532)
IV	250	0.0497 (.0128)	0.6386 (.0494)
VI	1000	0.0446 (.0089)	0.6567 (.0659)
VIII	1500	0.0466 (.0136)	0.6885 (.0699)
X	1000 1,3-BUTADIENE	0.0464 (.0079)	0.6598 (.0411)

STANDARD DEVIATION IN PARENTHESES
- SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY DUNNETT'S TEST
@ - STATISTICAL COMPARISONS TO CONTROL GROUP WERE CARRIED OUT SEPARATELY FOR 4-VCH- AND 1,3-BUTADIENE-EXPOSED GROUPS

TABLE 32
MEAN FINAL BODY AND ORGAN WEIGHTS FROM MALE MICE@

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	FINAL BODY
I	0	27.7 (1.4)
III	50	28.1 (2.5)
V	250	27.5 (1.8)
IX	1000	28.7 (1.7)

1,3-BUTADIENE

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES
I	0	1.283 (0.099)	0.611 (0.064)	0.249 (0.052)	0.188 (0.026)	0.060 (0.014)	0.232 (0.017)
III	50	1.323 (0.197)	0.641 (0.084)	0.237 (0.032)	0.181 (0.015)	0.064 (0.031)	0.238 (0.013)
V	250	1.251 (0.146)	0.588 (0.064)	0.260 (0.044)	0.173 (0.014)	0.054 (0.011)	0.237 (0.015)
IX	1000	1.365 (0.086)	0.624 (0.074)	0.263 (0.025)	0.181 (0.029)	0.063 (0.006)	0.153 (0.011)#

1,3-BUTADIENE

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES
I	0	4.6393 (.3039)	2.2044 (.1386)	0.8990 (.1735)	0.6799 (.0868)	0.2165 (.0433)	0.8397 (.0744)
III	50	4.6894 (.3508)	2.2766 (.1747)	0.8429 (.0622)	0.6450 (.0564)	0.2244 (.1007)	0.8494 (.0445)
V	250	4.5532 (.4561)	2.1407 (.1963)	0.9464 (.1412)	0.6306 (.0514)	0.1952 (.0410)	0.8623 (.0411)
IX	1000	4.7539 (.1822)	2.1720 (.1977)	0.9150 (.0678)	0.6296 (.0804)	0.2203 (.0220)	0.5339 (.0141)#

1,3-BUTADIENE

TABLE 32 (Continued)
MEAN FINAL BODY AND ORGAN WEIGHTS FROM MALE MICE@

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	FINAL BODY
I	0	27.7 (1.4)
III	50	28.1 (2.5)
V	250	27.5 (1.8)
IX	1000	28.7 (1.7)
	1,3-BUTADIENE	

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONC. (ppm)	BRAIN
I	0	0.481 (0.020)
III	50	0.479 (0.016)
V	250	0.475 (0.013)
IX	1000	0.471 (0.020)
	1,3-BUTADIENE	

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONC. (ppm)	BRAIN
I	0	1.7408 (.0807)
III	50	1.7137 (.1266)
V	250	1.7350 (.1071)
IX	1000	1.6434 (.0812)#
	1,3-BUTADIENE	

STANDARD DEVIATION IN PARENTHESES
- SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY DUNNETT'S TEST
@ - STATISTICAL COMPARISONS TO CONTROL GROUP WERE CARRIED OUT SEPARATELY FOR 4-VCH- AND 1,3-BUTADIENE-EXPOSED GROUPS

TABLE 33
MEAN FINAL BODY AND ORGAN WEIGHTS FROM FEMALE MICE@

MEAN FINAL BODY WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	FINAL BODY
II	0	22.6 (1.4)
IV	50	21.7 (0.9)
VI	250	21.1 (1.8)
VIII^	1000	21.1 (1.6)
X	1000 1,3-BUTADIENE	22.6 (1.1)

MEAN ABSOLUTE ORGAN WEIGHTS (grams)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
II	0	1.032 (0.090)	0.391 (0.034)	0.229 (0.064)	0.149 (0.029)	0.069 (0.011)	0.485 (0.013)
IV	50	1.024 (0.117)	0.374 (0.022)	0.231 (0.031)	0.136 (0.014)	0.065 (0.013)	0.457 (0.019)#
VI	250	0.991 (0.182)	0.368 (0.029)	0.212 (0.034)	0.131 (0.015)	0.062 (0.015)	0.468 (0.020)
VIII	1000	1.145 (0.144)	0.400 (0.037)	0.192 (0.020)	0.126 (0.013)	0.049 (0.004)	0.434 (0.012)
X	1000 1,3-BUTADIENE	1.118 (0.078)#	0.398 (0.022)	0.241 (0.025)	0.147 (0.010)	0.060 (0.007)	0.478 (0.021)

MEAN RELATIVE ORGAN WEIGHTS (% of body weight)

GROUP	CONCENTRATION (ppm)	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
II	0	4.5678 (.3035)	1.7333 (.1165)	1.0110 (.2422)	0.6563 (.1050)	0.3037 (.0366)	2.1537 (.1447)
IV	50	4.7238 (.5851)	1.7252 (.1561)	1.0634 (.1501)	0.6282 (.0761)	0.2995 (.0656)	2.1084 (.1162)
VI	250	4.6837 (.5894)	1.7504 (.0885)	1.0060 (.1165)	0.6197 (.0426)	0.2940 (.0553)	2.2346 (.1654)
VIII	1000	5.4161 (.2843)	1.8920 (.0381)	0.9090 (.0268)	0.5941 (.0199)	0.2346 (.0005)	2.0580 (.0948)
X	1000 1,3-BUTADIENE	4.9513 (.2289)#	1.7608 (.0431)	1.0666 (.1104)	0.6538 (.0659)	0.2674 (.0259)#	2.1200 (.1283)

STANDARD DEVIATION IN PARENTHESES
- SIGNIFICANTLY DIFFERENT (P<0.05) FROM CONTROL GROUP BY DUNNETT'S TEST
@ - STATISTICAL COMPARISONS TO CONTROL GROUP WERE CARRIED OUT SEPARATELY FOR 4-VCH- AND 1,3 BUTADIENE-EXPOSED GROUPS
^ - GROUP VIII NOT INCLUDED IN STATISTICAL ANALYSIS (ONLY 2/10 ANIMALS SURVIVED TO FINAL SACRIFICE)

TABLE 34
INCIDENCES OF GROSS OBSERVATIONS IN MALE RATS

GROUP DESIGNATION:	I	III	V	VII	IX
CONCENTRATION (ppm):	0	250	1000	1500	1000 1,3-BUTADIENE
NUMBER IN GROUP:	10	10	10	10	10
SITE/LESION:					
STOMACH	0	0	1	0	0
ULCER/EROSION	0	0	0	0	1
DISCOLORATION					
LUNGS	2	0	0	0	0
DISCOLORATION					
KIDNEYS	0	0	1	0	0
DILATATION	0	1	2	1	0
DISCOLORATION					
MANDIBULAR LYMPH NODE	2	1	0	1	0
LARGE	0	1	0	0	0
DISCOLORATION					
THYMUS	1	0	1	0	0
DISCOLORATION					
PITUITARY GLAND	1	0	0	0	0
DEFORMITY					
EPIDIDYMIDES	0	0	0	0	1
MASS					
TESTES	1	0	0	0	0
SMALL	0	0	0	0	1
HEMORRHAGE					
SKIN	0	0	0	1	0
CHROMODACRYORRHEA					

NOTE:
o INCIDENCES REFLECT THE NUMBER OF ANIMALS WITH A GIVEN LESION.

TABLE 35
INCIDENCES OF GROSS OBSERVATIONS IN FEMALE RATS

SITE/LESION:	GROUP DESIGNATION: / CONCENTRATION (ppm): / NUMBER IN GROUP:	II 0 10	IV 250 10	VI 1000 9	VIII 1500 10	X 1000 1,3-BUTADIENE 10
LIVER						
DISCOLORATION		1	0	0	1	0
KIDNEYS						
DISCOLORATION		1	0	1	0	0
MISSING		0	0	1	0	0
MANDIBULAR LYMPH NODE						
LARGE		0	0	0	1	0
SPLEEN						
CYST		1	0	0	1	0
THYMUS						
DISCOLORATION		1	0	1	2	0
PITUITARY GLAND						
LARGE		0	0	1	0	0

NOTE:
- o INCIDENCES REFLECT THE NUMBER OF ANIMALS WITH A GIVEN LESION.

TABLE 36
INCIDENCES OF GROSS OBSERVATIONS IN MALE MICE

SITE/LESION:	GROUP DESIGNATION: I CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	III 50 10	V 250 10	VII 1000 10	IX 1000 1,3-BUTADIENE 10
LIVER					
DISCOLORATION	0	1	1	0	0
SMALL INTESTINE					
LESION, OTHER	0	0	0	1	0
LARGE INTESTINE					
LESION, OTHER	0	0	0	1	0
LUNGS					
DISCOLORATION	1	1	0	4	0
SPLEEN					
SMALL	3	4	3	0	0
SKULL					
DEFORMITY	1	0	0	0	0
TAIL					
AMPUTATION	2	0	0	0	0
CHRONIC DERMATITIS	0	0	1	0	0
SKIN					
STAIN	1	0	0	0	0
EYES					
DISCOLORATION	0	0	1	0	0

NOTE:
- o INCIDENCES REFLECT THE NUMBER OF ANIMALS WITH A GIVEN LESION.

TABLE 37
INCIDENCES OF GROSS OBSERVATIONS IN FEMALE MICE

SITE/LESION:	GROUP DESIGNATION: II CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	IV 50 10	VI 250 10	VIII 1000 10	X 1000 1,3-BUTADIENE 10
LUNGS					
DISCOLORATION	0	1	1	3	0
NOSE					
FRACTURE	0	0	0	0	2
KIDNEYS					
LARGE	0	0	1	0	0
DILATATION	0	0	1	0	0
DEFORMITY	0	0	1	0	0
SPLEEN					
LARGE	0	0	1	0	0
SMALL	0	1	1	0	0
SKIN					
ALOPECIA	0	1	0	0	0
EYES					
MISSING	0	0	0	0	1

NOTE:
o INCIDENCES REFLECT THE NUMBER OF ANIMALS WITH A GIVEN LESION.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 38
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I	III	V	VII
		CONCENTRATION (ppm): 0	250	1000	1500
		NUMBER IN GROUP: 10	10	10	10
DIGESTIVE SYSTEM					
LIVER		10	10	10	10
FATTY CHANGE, MEDIAN CLEFT		1 (-,1,-,-,-)	5 (-,5,-,-,-)	1 (-,1,-,-,-)	-
FIBROSIS, CAPSULE		1 (-,1,-,-,-)	-	-	-
INFLAMMATION, SUBACUTE		10 (-,1,-,-,-) (-,0,-,-,-)	10 (-,1,-,-,-) (-,0,-,-,-)	10 (-,1,-,-,-) (-,0,-,-,-)	9 (-,1,-,-,-) (-,8,1,-,-) 1 (-,1,-,-,-)
NECROSIS, FOCAL		-	-	-	
ESOPHAGUS		10	0	0	10
STOMACH		10	0	1 (-,1,-,-,-)	10
EROSION/ULCERATION, GLANDULAR MUCOSA		-	-	-	-
HEMORRHAGE, NONGLANDULAR MUCOSA		-	-	-	
DUODENUM		10	0	0	10
JEJUNUM		10	0	0	10
ILEUM		10	0	0	10
CECUM		10	0	0	10
COLON		10	0	0	10
RECTUM		10	0	0	10
PANCREAS		10	0	0	10
ATROPHY, ACINAR		1 (-,1,-,-,-)	-	-	-
INFLAMMATION, SUBACUTE		2 (-,2,-,-,-)	-	-	-
SALIVARY GLANDS		10	0	0	10
RESPIRATORY SYSTEM					
NOSE		10	10	10	10
DEGENERATION, OLFACTORY EPITHELIUM		-	-	-	1 (-,1,-,-,-)
HYPERPLASIA/SQUAMOUS METAPLASIA, RESP EPITH		1 (-,1,-,-,-)	-	-	-
INFLAMMATION, SUPPURATIVE		1 (-,1,-,-,-)	-	-	-

TABLE 38 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I	III	V	VII
		CONCENTRATION (ppm): 0	250	1000	1500
		NUMBER IN GROUP: 10	10	10	10
RESPIRATORY SYSTEM (Cont'd)					
TRACHEA		10	0	0	10
LARYNX/PHARYNX		10	0	0	10
LUNGS		10	10	10	10
HISTIOCYTOSIS, ALVEOLAR INFLAMMATION, SUBACUTE/CHRONIC		1 (-,1,-,-,-)	-	1 (-,1,-,-,-)	2 (-,2,-,-,-)
CARDIOVASCULAR SYSTEM					
HEART		10	0	0	10
INFLAMMATION/DEGENERATION		4 (-,4,-,-,-)	-	-	2 (-,2,-,-,-)
AORTA		10	0	0	10
URINARY SYSTEM					
KIDNEYS		10	10	10	10
HYALINE DROPLETS, INCREASED		-	7 (-,7,-,-,-)	10 (-,-,1,-,-) (-,-,0,-,-)	- (-,-,1,-,-) (-,-,0,-,-)
HYDRONEPHROSIS, UNILATERAL NEPHROPATHY		5 (-,5,-,-,-)	10 (-,1,-,-,-) (-,0,-,-,-)	1 (-,-,1,-,-) 8 (-,8,-,-,-)	9 (-,8,1,-,-)
URINARY BLADDER		10	0	0	10
MUSCULOSKELETAL SYSTEM					
SKELETAL MUSCLE		10	0	0	10
FEMUR/KNEE JOINT		10	0	0	10
STERNUM		10	0	0	10
HEMOLYMPHATIC SYSTEM					
SPLEEN		10	0	0	10

TABLE 38 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	III 250 10	V 1000 10	VII 1500 10
HEMOLYMPHATIC SYSTEM (Cont'd)					
THYMUS		10	0	1	10
MESENTERIC LYMPH NODE		10	0	0	10
MANDIBULAR LYMPH NODES		10	2	0	10
CONGESTION/HEMORRHAGE		-	1 (-,1,-,-,-)	-	-
BONE MARROW		10	0	0	10
NERVOUS SYSTEM					
BRAIN		10	0	0	10
SPINAL CORD		10	0	0	10
SCIATIC NERVE		10	0	0	10
ENDOCRINE SYSTEM					
PITUITARY		10	0	0	10
CYST(S)		1 (-,1,-,-,-)	-	-	1 (-,-,1,-,-)
ADRENAL GLANDS		10	0	0	10
THYROID GLAND		10	0	0	10
PARATHYROID GLANDS		10	0	0	10
REPRODUCTIVE SYSTEM					
TESTES		10	0	0	10
DEGENERATION, UNILATERAL		2 (-,1,-,-,1)	-	-	1 (-,1,-,-,-)
GRANULOMA		1 (-,-,1,-,-)	-	-	-
HEMORRHAGE		-	-	-	-
EPIDIDYMIDES		10	0	0	10
OLIGOSPERMIA/GERM CELL DEBRIS, UNILATERAL		1 (-,-,-,1,-)	-	-	-
SPERM GRANULOMA		-	-	-	-

TABLE 38 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I	III	V	VII
		CONCENTRATION (ppm): 0	250	1000	1500
		NUMBER IN GROUP: 10	10	10	10
REPRODUCTIVE SYSTEM (Cont'd)					
PROSTATE		10	0	0	10
ATROPHY/FIBROSIS		1 (-,1,-,-,-)	-	-	-
INFLAMMATION, SUBACUTE		4 (-,4,-,-,-)	-	-	5 (-,4,1,-,-)
SEMINAL VESICLES		10	0	0	10
MISCELLANEOUS					
EYES		10	0	0	10
RETINA		10	0	0	10
DEGENERATION/ATROPHY, UNILATERAL		-	-	-	1 (-,-,1,-,-)
HARDERIAN GLANDS		10	0	0	10
EXORBITAL LACRIMAL GLANDS		10	0	0	10
SKIN		10	0	0	10
ZYMBAL'S GLANDS		10	0	0	10
OTHER		1	0	0	0
MESENTERY: INFLAMMATION, PERIVASCULAR		1 (-,1,-,-,-)	-	-	-

TABLE 38 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: IX CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
DIGESTIVE SYSTEM		
LIVER		10
FATTY CHANGE, MEDIAN CLEFT		3 (-,2,-,1,-)
FIBROSIS, CAPSULE		-
INFLAMMATION, SUBACUTE		10 (,1,,,) (-,0,-,-,-)
NECROSIS, FOCAL		-
ESOPHAGUS		10
STOMACH		10
EROSION/ULCERATION, GLANDULAR MUCOSA		-
HEMORRHAGE, NONGLANDULAR MUCOSA		1 (-,1,-,-,-)
DUODENUM		10
JEJUNUM		10
ILEUM		10
CECUM		10
COLON		10
RECTUM		10
PANCREAS		10
ATROPHY, ACINAR		1 (-,1,-,-,-)
INFLAMMATION, SUBACUTE		2 (-,2,-,-,-)
SALIVARY GLANDS		10
RESPIRATORY SYSTEM		
NOSE		10
DEGENERATION, OLFACTORY EPITHELIUM		-
HYPERPLASIA/SQUAMOUS METAPLASIA, RESP EPITH		-
INFLAMMATION, SUPPURATIVE		-

TABLE 38 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: IX CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
RESPIRATORY SYSTEM (Cont'd)		
TRACHEA		10
LARYNX/PHARYNX		10
LUNGS		10
HISTIOCYTOSIS, ALVEOLAR		2 (-,2,-,-,-)
INFLAMMATION, SUBACUTE/CHRONIC		1 (-,1,-,-,-)
CARDIOVASCULAR SYSTEM		
HEART		10
INFLAMMATION/DEGENERATION		5 (-,4,1,-,-)
AORTA		10
URINARY SYSTEM		
KIDNEYS		10
HYALINE DROPLETS, INCREASED		2 (-,2,-,-,-)
HYDRONEPHROSIS, UNILATERAL		-
NEPHROPATHY		9 (-,9,-,-,-)
URINARY BLADDER		10
MUSCULOSKELETAL SYSTEM		
SKELETAL MUSCLE		10
FEMUR/KNEE JOINT		10
STERNUM		10
HEMOLYMPHATIC SYSTEM		
SPLEEN		10
THYMUS		10

TABLE 38 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: IX CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
HEMOLYMPHATIC SYSTEM (Cont'd)		
MESENTERIC LYMPH NODE		10
MANDIBULAR LYMPH NODES		10
CONGESTION/HEMORRHAGE		-
BONE MARROW		10
NERVOUS SYSTEM		
BRAIN		10
SPINAL CORD		10
SCIATIC NERVE		10
ENDOCRINE SYSTEM		
PITUITARY		10
CYST(S)		-
ADRENAL GLANDS		10
THYROID GLAND		10
PARATHYROID GLANDS		10
REPRODUCTIVE SYSTEM		
TESTES		10
DEGENERATION, UNILATERAL		-
GRANULOMA		-
HEMORRHAGE		1 (-,1,-,-,-)
EPIDIDYMIDES		10
OLIGOSPERMIA/GERM CELL DEBRIS, UNILATERAL		1 (1,-,-,-,-)
SPERM GRANULOMA		
PROSTATE		10
ATROPHY/FIBROSIS		-
INFLAMMATION, SUBACUTE		4 (-,3,1,-,-)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 38 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: 1X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
REPRODUCTIVE SYSTEM (Cont'd)		
SEMINAL VESICLES		10
MISCELLANEOUS		
EYES		10
RETINA		10
DEGENERATION/ATROPHY, UNILATERAL		1 (-,-,1,-,-)
HARDERIAN GLANDS		10
EXORBITAL LACRIMAL GLANDS		10
SKIN		10
ZYMBAL'S GLANDS		10
OTHER		0
MESENTERY: INFLAMMATION, PERIVASCULAR		-

NOTES:
- o THE NUMBER OF ORGANS EXAMINED FOR EACH GROUP IS UNDERLINED.
- o LESION GRADES: P = PRESENT; 1 = MINIMAL; 2 = MILD; 3 = MODERATE; 4 = SEVERE.
- o LESION GRADES CORRESPOND BY POSITION WITH THE NUMBERS IN PARENTHESES, WHICH INDICATE HOW OFTEN EACH GRADE WAS OBSERVED. DOUBLE DIGIT NUMBERS ARE EXPRESSED VERTICALLY, FOR EXAMPLE: (,1, ,1,1) MEANS NO LESIONS (-,0,6,1,5)

WERE GRADED "PRESENT", 10 LESIONS WERE "MINIMAL", 6 LESIONS WERE "MILD", 11 LESIONS WERE "MODERATE", AND 15 LESIONS WERE "SEVERE".

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 39
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: II	IV	VI	VIII
		CONCENTRATION (ppm): 0	250	1000	1500
		NUMBER IN GROUP: 10	10	9	10
DIGESTIVE SYSTEM					
LIVER		10	10	9	10
FATTY CHANGE, FOCAL		3 (-,1,2,-,-)	3 (-,3,-,-,-)	1 (-,1,-,-,-)	1 (-,1,-,-,-)
FATTY CHANGE, MEDIAN CLEFT		1 (-,1,-,-,-)			2 (-,2,-,-,-)
FOCUS/FOCI, EOSINOPHILIC					
INFLAMMATION, SUBACUTE		8 (-,8,-,-,-)	10 (-,1,-,-,-)	9 (-,9,-,-,-)	10 (-,1,-,-,-)
ESOPHAGUS		10	0	0	10
STOMACH		9	0	0	10
DUODENUM		9	0	0	10
JEJUNUM		9	0	0	10
ILEUM		9	0	0	10
CECUM		8	0	0	10
COLON		10	0	0	10
RECTUM		10	0	0	10
PANCREAS		1 (-,1,-,-,-)			
ATROPHY, ACINAR					
FOCUS, BASOPHILIC					
INFLAMMATION, SUBACUTE					
SALIVARY GLANDS		10	0	0	10
INFLAMMATION, SUBACUTE					1 (-,1,-,-,-)
RESPIRATORY SYSTEM					
NOSE		10	10	9	10
INFLAMMATION, SUPPURATIVE					1 (-,1,-,-,-)
TRACHEA		10	0	0	10

TABLE 39 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: II CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	IV 250 10	VI 1000 9	VIII 1500 10
RESPIRATORY SYSTEM (Cont'd)					
LARYNX/PHARYNX		10	0	0	10
LUNGS		10	10	9	10
HISTIOCYTOSIS, ALVEOLAR		1 (-,1,-,-,-)	1 (-,1,-,-,-)	2 (-,2,-,-,-)	1 (-,1,-,-,-)
CARDIOVASCULAR SYSTEM					
HEART		10	0	0	10
INFLAMMATION/DEGENERATION		1 (-,1,-,-,-)	-	-	-
AORTA		10	0	0	10
URINARY SYSTEM					
KIDNEYS		10	10	9	10
INFLAMMATION, SUBACUTE		-	3 (-,3,-,-,-)	-	-
MINERALIZATION		-	1 (-,1,-,-,-)	-	-
NEPHROPATHY		1 (-,1,-,-,-)	1 (-,1,-,-,-)	3 (-,3,-,-,-)	2 (-,2,-,-,-)
ONE OF PAIR PRESENT		-	-	1 (1,-,-,-,-)	-
URINARY BLADDER		10	0	0	10
MUSCULOSKELETAL SYSTEM					
SKELETAL MUSCLE		10	0	0	10
FEMUR/KNEE JOINT		10	0	0	10
STERNUM		10	0	0	10
HEMOLYMPHATIC SYSTEM					
SPLEEN		10	0	0	10
CYST(S), CAPSULE		-	-	-	1 (-,1,-,-,-)
THYMUS		10	0	1	10
HYPERPLASIA, EPITHELIAL		1 (-,1,-,-,-)	-	-	-

TABLE 39 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: II CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	IV 250 10	VI 1000 9	VIII 1500 10
HEMOLYMPHATIC SYSTEM (Cont'd)					
MESENTERIC LYMPH NODE		9	0	0	10
MANDIBULAR LYMPH NODES		10	0	0	10
BONE MARROW		10	0	0	10
NERVOUS SYSTEM					
BRAIN		10	0	0	10
SPINAL CORD		10	0	0	10
SCIATIC NERVE		10	0	0	10
ENDOCRINE SYSTEM					
PITUITARY		10	0	1	10
CYST(S)		-	-	1 (-,-,-,1,-)	-
ADRENAL GLANDS		10	0	0	10
MEDULLA NOT PRESENT		1 (1,-,-,-,-)	-	-	-
THYROID GLAND		10	0	0	10
PARATHYROID GLANDS		10	0	0	10
REPRODUCTIVE SYSTEM					
MAMMARY GLAND		10	0	0	10
OVARIES		10	10	9	10
ATROPHY		-	-	-	2 (-,2,-,-,-)
CYST(S)		1 (-,1,-,-,-)	2 (-,2,-,-,-)	1 (-,1,-,-,-)	4 (-,4,-,-,-)
UTERUS		10	0	0	10
VAGINA		10	0	0	10
INFLAMMATION, SUBACUTE		-	-	-	1 (-,1,-,-,-)

TABLE 39 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: II CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	IV 250 10	VI 1000 9	VIII 1500 10
MISCELLANEOUS					
EYES		10	0	0	10
RETINA		10	0	0	10
DEGENERATION/ATROPHY, UNILATERAL		1 (-,1,-,-,-)	-	-	-
ROSETTES		1 (-,1,-,-,-)	-	-	-
HARDERIAN GLANDS		10	0	0	10
EXORBITAL LACRIMAL GLANDS		10	0	0	10
ATROPHY		-	-	-	1 (-,1,-,-,-)
SKIN		10	0	0	10
ZYMBAL'S GLANDS		10	0	0	10
OTHER		0	0	0	0

TABLE 39 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
DIGESTIVE SYSTEM		
LIVER		10
FATTY CHANGE, FOCAL		1 (-,1,-,-,-)
FATTY CHANGE, MEDIAN CLEFT		7 (-,7,-,-,-)
FOCUS/FOCI, EOSINOPHILIC		
INFLAMMATION, SUBACUTE		
ESOPHAGUS		10
STOMACH		10
DUODENUM		10
JEJUNUM		10
ILEUM		10
CECUM		10
COLON		10
RECTUM		10
PANCREAS		10
ATROPHY, ACINAR		1 (-,1,-,-,-)
FOCUS, BASOPHILIC		1 (-,1,-,-,-)
INFLAMMATION, SUBACUTE		
SALIVARY GLANDS		10
INFLAMMATION, SUBACUTE		1 (-,1,-,-,-)
RESPIRATORY SYSTEM		
NOSE		10
INFLAMMATION, SUPPURATIVE		
TRACHEA		10
LARYNX/PHARYNX		10

TABLE 39 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
RESPIRATORY SYSTEM (Cont'd)		
LUNGS		10
HISTIOCYTOSIS, ALVEOLAR		-
CARDIOVASCULAR SYSTEM		
HEART		10
INFLAMMATION/DEGENERATION		2 (-,2,-,-,-)
AORTA		10
URINARY SYSTEM		
KIDNEYS		10
INFLAMMATION, SUBACUTE		1 (-,1,-,-,-)
MINERALIZATION		-
NEPHROPATHY		1 (-,1,-,-,-)
ONE OF PAIR PRESENT		-
URINARY BLADDER		10
MUSCULOSKELETAL SYSTEM		
SKELETAL MUSCLE		10
FEMUR/KNEE JOINT		10
STERNUM		10
HEMOLYMPHATIC SYSTEM		
SPLEEN		10
CYST(S), CAPSULE		-
THYMUS		10
HYPERPLASIA, EPITHELIAL		-
MESENTERIC LYMPH NODE		10

TABLE 39 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
HEMOLYMPHATIC SYSTEM (Cont'd)		
MANDIBULAR LYMPH NODES		10
BONE MARROW		10
NERVOUS SYSTEM		
BRAIN		10
SPINAL CORD		10
SCIATIC NERVE		10
ENDOCRINE SYSTEM		
PITUITARY		10
CYST(S)		-
ADRENAL GLANDS		10
MEDULLA NOT PRESENT		-
THYROID GLAND		10
PARATHYROID GLANDS		9
REPRODUCTIVE SYSTEM		
MAMMARY GLAND		10
OVARIES		10
ATROPHY		-
CYST(S)		4 (-,4,-,-,-)
UTERUS		10
VAGINA		10
INFLAMMATION, SUBACUTE		-

TABLE 39 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE RATS

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
MISCELLANEOUS		
EYES		10
RETINA		10
DEGENERATION/ATROPHY, UNILATERAL		1 (-,1,-,-,-)
ROSETTES		-
HARDERIAN GLANDS		10
EXORBITAL LACRIMAL GLANDS		10
ATROPHY		-
SKIN		10
ZYMBAL'S GLANDS		10
OTHER		0

NOTES:
- o THE NUMBER OF ORGANS EXAMINED FOR EACH GROUP IS UNDERLINED.
- o LESION GRADES: P = PRESENT; 1 = MINIMAL; 2 = MILD; 3 = MODERATE; 4 = SEVERE.
- o LESION GRADES CORRESPOND BY POSITION WITH THE NUMBERS IN PARENTHESES, WHICH INDICATE HOW OFTEN EACH GRADE WAS OBSERVED. DOUBLE DIGIT NUMBERS ARE EXPRESSED VERTICALLY, FOR EXAMPLE: (,1, ,1,1) MEANS NO LESIONS (-,0,6,1,5)

WERE GRADED "PRESENT", 10 LESIONS WERE "MINIMAL", 6 LESIONS WERE "MILD", 11 LESIONS WERE "MODERATE", AND 15 LESIONS WERE "SEVERE".

TABLE 40
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I	III	V	VII
		CONCENTRATION (ppm): 0	50	250	1000
		NUMBER IN GROUP: 10	10	10	10
DIGESTIVE SYSTEM					
LIVER		10	10	10	10
FATTY CHANGE, MEDIAN CLEFT		3 (-,3,-,-,-)	4 (-,4,-,-,-)	2 (-,2,-,-,-)	-
INFLAMMATION, SUBACUTE		1 (-,1,-,-,-)	2 (-,2,-,-,-)	1 (-,1,-,-,-)	-
MITOTIC FIGURES, INCREASED		-	-	-	2 (-,2,-,-,-)
GALLBLADDER		10	1	10	9
ESOPHAGUS		10	1	10	10
STOMACH		10	1	10	10
EROSION/ULCERATION, NONGLANDULAR MUCOSA		-	-	-	-
DUODENUM		10	1	10	8
JEJUNUM		10	1	10	9
ILEUM		10	1	10	9
CECUM		10	1	10	9
COLON		10	1	10	10
RECTUM		10	1	10	10
PANCREAS		10	1	10	10
SALIVARY GLANDS		10	1	10	10
INFLAMMATION, SUBACUTE		-	-	-	-
RESPIRATORY SYSTEM					
NOSE		10	10	10	10
INFLAMMATION/SEPSIS		1 (-,-,1,-,-)	-	-	-
TRACHEA		10	1	10	10
LARYNX/PHARYNX		10	1	10	10

TABLE 40 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	III 50 10	V 250 10	VII 1000 10
RESPIRATORY SYSTEM (Cont'd)					
LUNGS		10	10	10	10
INFLAMMATION/TYPE II CELL HYPERPLASIA		-	-	-	-
CARDIOVASCULAR SYSTEM					
HEART		10	1	10	10
AORTA		10	1	10	10
URINARY SYSTEM					
KIDNEYS		10	10	10	10
INFARCT		-	1 (-,1,-,-,-)	-	-
NEPHROPATHY		-	-	-	-
URINARY BLADDER		10	1	10	10
MUSCULOSKELETAL SYSTEM					
SKELETAL MUSCLE		10	1	10	10
FEMUR/KNEE JOINT		10	1	10	10
STERNUM		10	1	10	10
HEMOLYMPHATIC SYSTEM					
SPLEEN		10	5	10	9
ATROPHY, LYMPHOID		1 (-,1,-,-,-)	1 (-,1,-,-,-)	-	1 (-,1,-,-,-)
EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		1 (-,-,1,-,-)	-	1 (-,1,-,-,-)	-
THYMUS		9	1	10	10
ATROPHY, LYMPHOID		-	-	1 (-,1,-,-,-)	6 (-,3,3,-,-)
MESENTERIC LYMPH NODE		10	1	10	10
MANDIBULAR LYMPH NODES		9	1	10	9
FOAMY MACROPHAGE AGGREGATES		1 (-,-,1,-,-)	-	-	-

TABLE 40 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	III 50 10	V 250 10	VII 1000 10
HEMOLYMPHATIC SYSTEM (Cont'd)					
BONE MARROW		10	1	10	10
- MYELOID HYPERPLASIA		2 (-,2,-,-,-)	-	-	-
NERVOUS SYSTEM					
BRAIN		10	1	10	10
SPINAL CORD		10	1	10	10
SCIATIC NERVE		10	1	10	10
ENDOCRINE SYSTEM					
PITUITARY		9	1	8	6
ADRENAL GLANDS		10	1	10	10
HYPERPLASIA, SUBCAPSULAR CELLS		5 (-,5,-,-,-)	1 (-,1,-,-,-)	5 (-,5,-,-,-)	1 (-,1,-,-,-)
THYROID GLAND		10	1	10	9
PARATHYROID GLANDS		9	1	10	3
REPRODUCTIVE SYSTEM					
TESTES		10	1	10	10
DEGENERATION/ATROPHY, BILATERAL		1 (-,1,-,-,-)	-	-	-
DEGENERATION/ATROPHY, UNILATERAL		3 (-,3,-,-,-)	-	1 (-,1,-,-,-)	1 (-,1,-,-,-)
EPIDIDYMIDES		10	1	10	10
GERM CELL DEBRIS, INCREASED		-	-	-	-
PROSTATE		10	1	10	9
SEMINAL VESICLES		10	1	10	10
MISCELLANEOUS					
EYES		10	1	10	9
LENS NOT PRESENT		-	-	-	-
ONE OF PAIR PRESENT		-	-	-	2 (2,-,-,-,-)

TABLE 40 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: I	III	V	VII
		CONCENTRATION (ppm): 0	50	250	1000
		NUMBER IN GROUP: 10	10	10	10
MISCELLANEOUS (Cont'd)					
RETINA		10	1	10	8
HARDERIAN GLANDS		10	1	10	10
EXORBITAL LACRIMAL GLANDS		10	1	10	10
LYMPHOID AGGREGATES, INCREASED		-	-	-	-
SKIN		10	1	10	10
ZYMBAL'S GLANDS		10	1	10	10
OTHER		0	0	1	0
TEETH: ODONTODYSPLASIA		-	-	1 (1,-,-,-,-)	-

TABLE 40 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: IX CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
DIGESTIVE SYSTEM		
LIVER		10
FATTY CHANGE, MEDIAN CLEFT		1 (-,1,-,-,-)
INFLAMMATION, SUBACUTE		6 (-,6,-,-,-)
MITOTIC FIGURES, INCREASED		-
GALLBLADDER		10
ESOPHAGUS		10
STOMACH		10
EROSION/ULCERATION, NONGLANDULAR MUCOSA		1 (-,1,-,-,-)
DUODENUM		10
JEJUNUM		10
ILEUM		10
CECUM		10
COLON		10
RECTUM		10
PANCREAS		10
SALIVARY GLANDS		10
INFLAMMATION, SUBACUTE		2 (-,2,-,-,-)
RESPIRATORY SYSTEM		
NOSE		10
INFLAMMATION/SEPSIS		-
TRACHEA		10
LARYNX/PHARYNX		10

TABLE 40 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: IX CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
RESPIRATORY SYSTEM (Cont'd)		
LUNGS		10
INFLAMMATION/TYPE II CELL HYPERPLASIA		1 (-,1,-,-,-)
CARDIOVASCULAR SYSTEM		
HEART		10
AORTA		10
URINARY SYSTEM		
KIDNEYS		10
INFARCT		-
NEPHROPATHY		2 (-,2,-,-,-)
URINARY BLADDER		10
MUSCULOSKELETAL SYSTEM		
SKELETAL MUSCLE		10
FEMUR/KNEE JOINT		10
STERNUM		10
HEMOLYMPHATIC SYSTEM		
SPLEEN		10
ATROPHY, LYMPHOID		-
EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		1 (-,1,-,-,-)
THYMUS		10
ATROPHY, LYMPHOID		-
MESENTERIC LYMPH NODE		10
MANDIBULAR LYMPH NODES		9
FOAMY MACROPHAGE AGGREGATES		-

TABLE 40 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: IX CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
HEMOLYMPHATIC SYSTEM (Cont'd)		
BONE MARROW		10
MYELOID HYPERPLASIA		-
NERVOUS SYSTEM		
BRAIN		10
SPINAL CORD		10
SCIATIC NERVE		10
ENDOCRINE SYSTEM		
PITUITARY		6
ADRENAL GLANDS		10
HYPERPLASIA, SUBCAPSULAR CELLS		4 (-,4,-,-,-)
THYROID GLAND		9
PARATHYROID GLANDS		7
REPRODUCTIVE SYSTEM		
TESTES		10
DEGENERATION/ATROPHY, BILATERAL		5 (-,5,-,-,-)
DEGENERATION/ATROPHY, UNILATERAL		5 (-,5,-,-,-)
EPIDIDYMIDES		10
GERM CELL DEBRIS, INCREASED		1 (-,1,-,-,-)
PROSTATE		10
SEMINAL VESICLES		10
MISCELLANEOUS		
EYES		10
LENS NOT PRESENT		1 (1,-,-,-,-)
ONE OF PAIR PRESENT		-

TABLE 40 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN MALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: IX CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
MISCELLANEOUS (Cont'd)		
RETINA		10
HARDERIAN GLANDS		10
EXORBITAL LACRIMAL GLANDS		10
LYMPHOID AGGREGATES, INCREASED		1 (-,1,-,-,-)
SKIN		10
ZYMBAL'S GLANDS		10
OTHER		0
TEETH: ODONTODYSPLASIA		-

NOTES:
- o THE NUMBER OF ORGANS EXAMINED FOR EACH GROUP IS UNDERLINED.
- o LESION GRADES: P = PRESENT; 1 = MINIMAL; 2 = MILD; 3 = MODERATE; 4 = SEVERE.
- o LESION GRADES CORRESPOND BY POSITION WITH THE NUMBERS IN PARENTHESES, WHICH INDICATE HOW OFTEN EACH GRADE WAS OBSERVED. FOR EXAMPLE: (-,1,2,-,-) MEANS NO LESIONS WERE GRADED "PRESENT" (NON-GRADED LESIONS), 1 LESION WAS GRADED "MINIMAL", 2 LESIONS WERE GRADED "MILD", NO LESIONS WERE GRADED "MODERATE", AND NO LESIONS WERE GRADED "SEVERE".

TABLE 41
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: II	IV	VI	VIII
		CONCENTRATION (ppm): 0	50	250	1000
		NUMBER IN GROUP: 10	10	10	10
DIGESTIVE SYSTEM					
LIVER		10	10	10	10
FATTY CHANGE, MEDIAN CLEFT		1 (-,1,-,-,-)	2 (-,2,-,-,-)	-	3 (-,3,-,-,-)
INFLAMMATION, SUBACUTE		4 (-,4,-,-,-)	5 (-,5,-,-,-)	4 (-,4,-,-,-)	4 (-,4,-,-,-)
MITOTIC FIGURES, INCREASED		-	-	-	-
NECROSIS, FOCAL		-	2 (-,2,-,-,-)	-	-
GALLBLADDER		10	0	1	9
ESOPHAGUS		10	0	1	10
STOMACH		10	0	1	10
DUODENUM		10	0	1	10
JEJUNUM		10	0	1	10
ILEUM		10	0	1	9
CECUM		10	0	1	10
COLON		10	0	1	10
RECTUM		10	0	1	9
PANCREAS		10	0	1	10
SALIVARY GLANDS		10	0	1	10
INFLAMMATION, SUBACUTE		-	-	-	-
RESPIRATORY SYSTEM					
NOSE		10	10	10	10
DEGENERATION, TRIGEMINAL NERVE		1 (-,1,-,-,-)	-	-	-
FRACTURE/INFLAMMATION		-	-	-	-
HEMORRHAGE, NASOTURBINATE		1 (-,-,1,-,-)	-	-	-
INFLAMMATION, SUPPURATIVE		-	-	-	-
TRACHEA		10	0	1	10

TABLE 41 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: II CONCENTRATION (ppm): 0 NUMBER IN GROUP: 10	IV 50 10	VI 250 10	VIII 1000 10
RESPIRATORY SYSTEM (Cont'd)					
LARYNX/PHARYNX		10	0	1	10
LUNGS		10	10	10	10
INFLAMMATION, SUBACUTE/CHRONIC		-	-	1 (-,1,-,-,-)	-
CIRCULATORY SYSTEM					
HEART		10	0	1	10
INFLAMMATION/DEGENERATION		-	-	1 (-,1,-,-,-)	-
AORTA		10	0	1	10
URINARY SYSTEM					
KIDNEYS		10	10	10	10
INFLAMMATION, CAPSULE		-	-	-	-
PYELONEPHRITIS		-	-	1 (-,-,-,-,1)	-
URINARY BLADDER		10	0	1	9
HYPERPLASIA, MUCOSA		-	-	1 (-,-,1,-,-)	-
MUSCULOSKELETAL SYSTEM					
SKELETAL MUSCLE		10	0	1	10
FEMUR/KNEE JOINT		10	0	1	10
STERNUM		10	0	1	10
HEMOLYMPHATIC SYSTEM					
SPLEEN		10	1	2	10
ATROPHY, LYMPHOID		-	1 (-,1,-,-,-)	1 (-,-,-,1,-)	-
EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		-	-	1 (-,-,-,-,1)	-
PIGMENT, INCREASED		-	-	-	2 (-,2,-,-,-)
THYMUS		10	0	1	10
ATROPHY, LYMPHOID		1 (-,1,-,-,-)	-	1 (-,-,1,-,-)	-
NECROSIS/APOPTOSIS, INCREASED		-	-	-	-

TABLE 41 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: II	IV	VI	VIII
		CONCENTRATION (ppm): 0	50	250	1000
		NUMBER IN GROUP: 10	10	10	10
HEMOLYMPHATIC SYSTEM (Cont'd)					
MESENTERIC LYMPH NODE		10	0	1	10
MANDIBULAR LYMPH NODES		10	0	1	9
BONE MARROW		10	0	1	10
MYELOID HYPERPLASIA		-	-	-	-
NERVOUS SYSTEM					
BRAIN		10	0	1	10
SPINAL CORD		10	0	1	10
SCIATIC NERVE		10	0	1	10
ENDOCRINE SYSTEM					
PITUITARY		9	0	1	6
ADRENAL GLANDS		10	0	1	10
HYPERPLASIA, SUBCAPSULAR CELLS		10 (-,1,-,-,-) (-,0,-,-,-)	-	1 (-,1,-,-,-)	9 (-,9,-,-,-)
MEDULLA NOT PRESENT		-	-	1 (1,-,-,-,-)	-
ONE OF PAIR PRESENT		-	-	1 (1,-,-,-,-)	-
THYROID GLAND		10	0	1	10
PARATHYROID GLANDS		8	0	1	9
REPRODUCTIVE SYSTEM					
MAMMARY GLAND		10	0	1	9
OVARIES		10	10	10	10
*B TERATOMA		-	1 (1,-,-,-,-)	-	-
ATROPHY		-	-	-	5 (-,-,5,-,-)
CYST(S)		1 (-,1,-,-,-)	1 (-,1,-,-,-)	-	-
ONE OF PAIR PRESENT		-	-	-	1 (1,-,-,-,-)

TABLE 41 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION:	II	IV	VI	VIII
		CONCENTRATION (ppm):	0	50	250	1000
		NUMBER IN GROUP:	10	10	10	10
REPRODUCTIVE SYSTEM (Cont'd)						
UTERUS			10	0	1	10
CYST/CYSTIC HYPERPLASIA, ENDOMETRIAL			4 (-,4,-,-,-)	-	-	-
VAGINA			10	0	1	10
ATROPHY, MUCOSA			-	-	-	1 (-,-,1,-,-)
MISCELLANEOUS						
EYES			10	0	1	10
LENS NOT PRESENT			1 (1,-,-,-,-)	-	-	-
ONE OF PAIR PRESENT			-	-	-	-
RETINA			10	0	0	8
HARDERIAN GLANDS			10	0	1	10
EXORBITAL LACRIMAL GLANDS			10	0	1	10
ATROPHY			1 (-,1,-,-,-)	-	-	-
SKIN			10	1	1	10
ALOPECIA			-	1 (-,-,1,-,-)	-	-
ZYMBAL'S GLANDS			10	0	1	10
OTHER			0	0	0	0
ORAL MUCOSA: INFLAMMATION/ULCERATION			-	-	-	-

TABLE 41 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
DIGESTIVE SYSTEM		
LIVER		10
FATTY CHANGE, MEDIAN CLEFT		2 (-,2,-,-,-)
INFLAMMATION, SUBACUTE		4 (-,4,-,-,-)
MITOTIC FIGURES, INCREASED		-
NECROSIS, FOCAL		-
GALLBLADDER		8
ESOPHAGUS		10
STOMACH		10
DUODENUM		10
JEJUNUM		10
ILEUM		10
CECUM		10
COLON		10
RECTUM		10
PANCREAS		10
SALIVARY GLANDS		10
INFLAMMATION, SUBACUTE		1 (-,1,-,-,-)
RESPIRATORY SYSTEM		
NOSE		10
DEGENERATION, TRIGEMINAL NERVE		-
FRACTURE/INFLAMMATION		1 (-,-,1,-,-)
HEMORRHAGE, NASOTURBINATE		-
INFLAMMATION, SUPPURATIVE		1 (-,-,-,1,-)
TRACHEA		10

TABLE 41 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
RESPIRATORY SYSTEM (Cont'd)		
LARYNX/PHARYNX		10
LUNGS		10
INFLAMMATION, SUBACUTE/CHRONIC		-
CIRCULATORY SYSTEM		
HEART		10
INFLAMMATION/DEGENERATION		-
AORTA		10
URINARY SYSTEM		
KIDNEYS		10
INFLAMMATION, CAPSULE		1 (-,1,-,-,-)
PYELONEPHRITIS		-
URINARY BLADDER		10
HYPERPLASIA, MUCOSA		-
MUSCULOSKELETAL SYSTEM		
SKELETAL MUSCLE		10
FEMUR/KNEE JOINT		10
STERNUM		10
HEMOLYMPHATIC SYSTEM		
SPLEEN		10
ATROPHY, LYMPHOID		3 (-,1,1,1,-)
EXTRAMEDULLARY HEMATOPOIESIS, INCREASED		-
PIGMENT, INCREASED		-
THYMUS		10
ATROPHY, LYMPHOID		2 (-,1,1,-,-)
NECROSIS/APOPTOSIS, INCREASED		1 (-,1,-,-,-)

TABLE 41 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
HEMOLYMPHATIC SYSTEM (Cont'd)		
MESENTERIC LYMPH NODE		10
MANDIBULAR LYMPH NODES		9
BONE MARROW		10
MYELOID HYPERPLASIA		1 (-,1,-,-,-)
NERVOUS SYSTEM		
BRAIN		10
SPINAL CORD		10
SCIATIC NERVE		9
ENDOCRINE SYSTEM		
PITUITARY		8
ADRENAL GLANDS		10
HYPERPLASIA, SUBCAPSULAR CELLS		9 (-,9,-,-,-)
MEDULLA NOT PRESENT		-
ONE OF PAIR PRESENT		-
THYROID GLAND		10
PARATHYROID GLANDS		10
REPRODUCTIVE SYSTEM		
MAMMARY GLAND		10
OVARIES		10
*B TERATOMA		-
ATROPHY		6 (-,4,2,-,-)
CYST(S)		1 (-,1,-,-,-)
ONE OF PAIR PRESENT		-

TABLE 41 (Continued)
INCIDENCES OF MICROSCOPIC OBSERVATIONS IN FEMALE MICE

TISSUE/LESION	LESION GRADES (P,1,2,3,4)	GROUP DESIGNATION: X CONCENTRATION (ppm): 1000 1,3-BUTADIENE NUMBER IN GROUP: 10
REPRODUCTIVE SYSTEM (Cont'd)		
UTERUS		10
CYST/CYSTIC HYPERPLASIA, ENDOMETRIAL		1 (-,1,-,-,-)
VAGINA		10
ATROPHY, MUCOSA		-
MISCELLANEOUS		
EYES		10
LENS NOT PRESENT		-
ONE OF PAIR PRESENT		1 (1,-,-,-,-)
RETINA		10
HARDERIAN GLANDS		10
EXORBITAL LACRIMAL GLANDS		10
ATROPHY		-
SKIN		9
ALOPECIA		-
ZYMBAL'S GLANDS		10
OTHER		2
ORAL MUCOSA: INFLAMMATION/ULCERATION		2 (-,1,-,1,-)

NOTES:
- o THE NUMBER OF ORGANS EXAMINED FOR EACH GROUP IS UNDERLINED.
- o LESION GRADES: P = PRESENT; 1 = MINIMAL; 2 = MILD; 3 = MODERATE; 4 = SEVERE.
- o LESION GRADES CORRESPOND BY POSITION WITH THE NUMBERS IN PARENTHESES, WHICH INDICATE HOW OFTEN EACH GRADE WAS OBSERVED. DOUBLE DIGIT NUMBERS ARE EXPRESSED VERTICALLY, FOR EXAMPLE: (,1, ,1,1) MEANS NO LESIONS (-,0,6,1,5) WERE GRADED "PRESENT", 10 LESIONS WERE "MINIMAL", 6 LESIONS WERE "MILD", 11 LESIONS WERE "MODERATE", AND 15 LESIONS WERE "SEVERE".
- o TUMOR CODES: *B = BENIGN

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

FIGURES

SUBCHRONIC INHALATION TOXICITY STUDY WITH 4-VINYLCYCLOHEXENE

FIGURES

EXPLANATORY NOTES

NOTES:

Figures 3-6 do not include body weights taken immediately prior to necropsy (test days 92-95). At that time, the animals were weighed following clinical pathology exams and/or fasting, and the weight losses do not adequately represent the group weight gains.

ABBREVIATIONS:

Figure 1
FMI = Fluid Metering Incorporated

Figures 3-8
BUT = 1,3-butadiene

FIGURE 1

DIAGRAM OF EXPOSURE APPARATUS

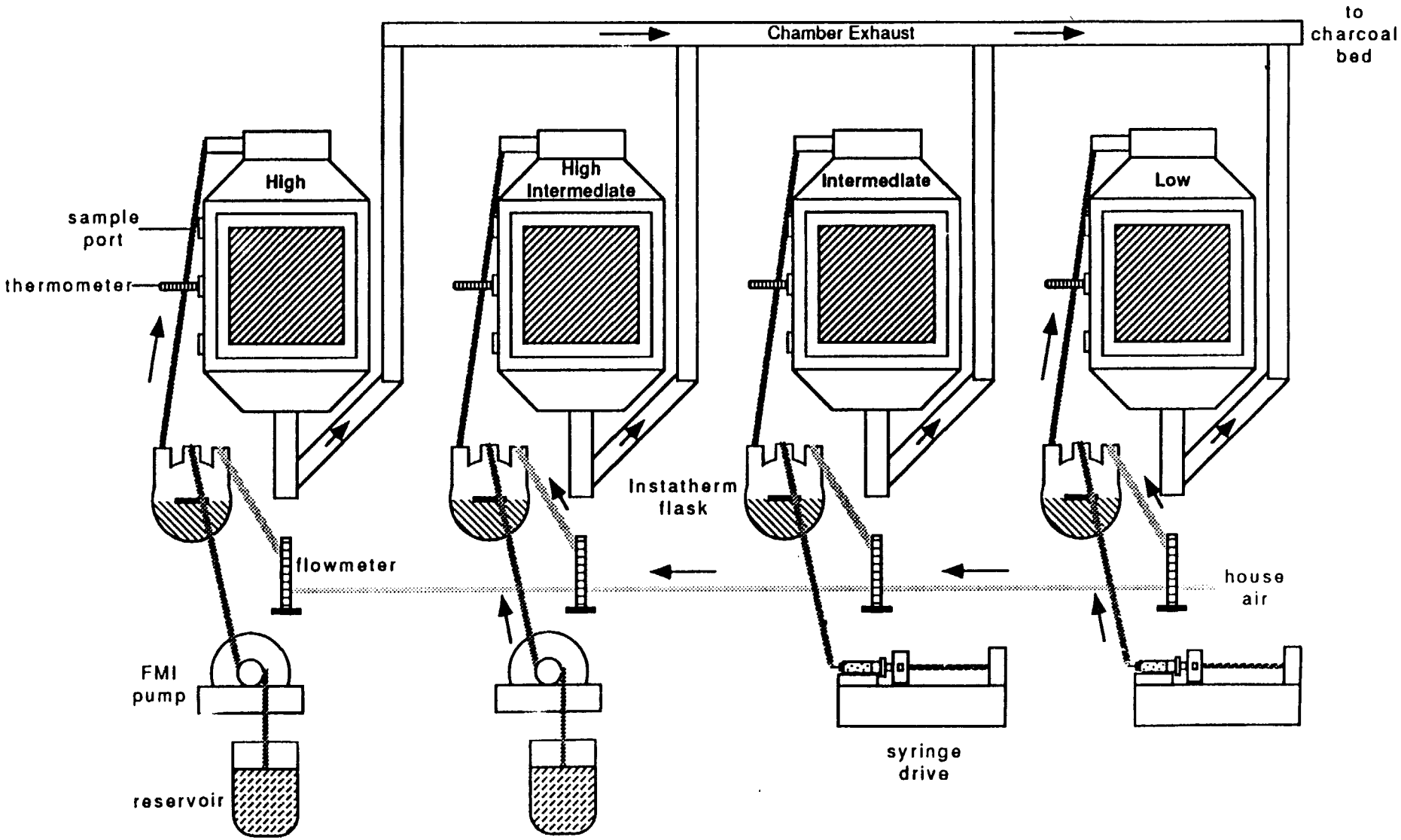
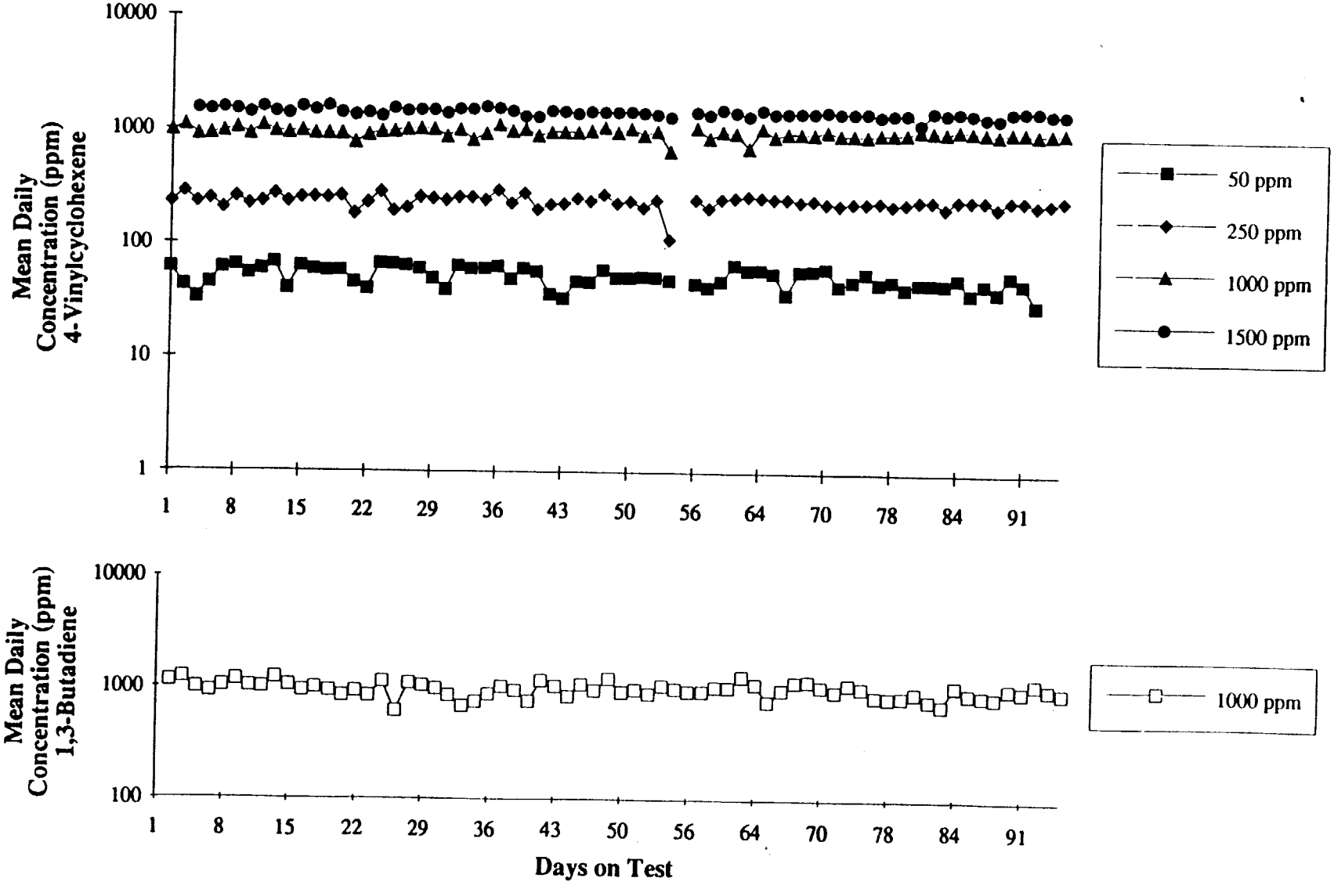


FIGURE 2

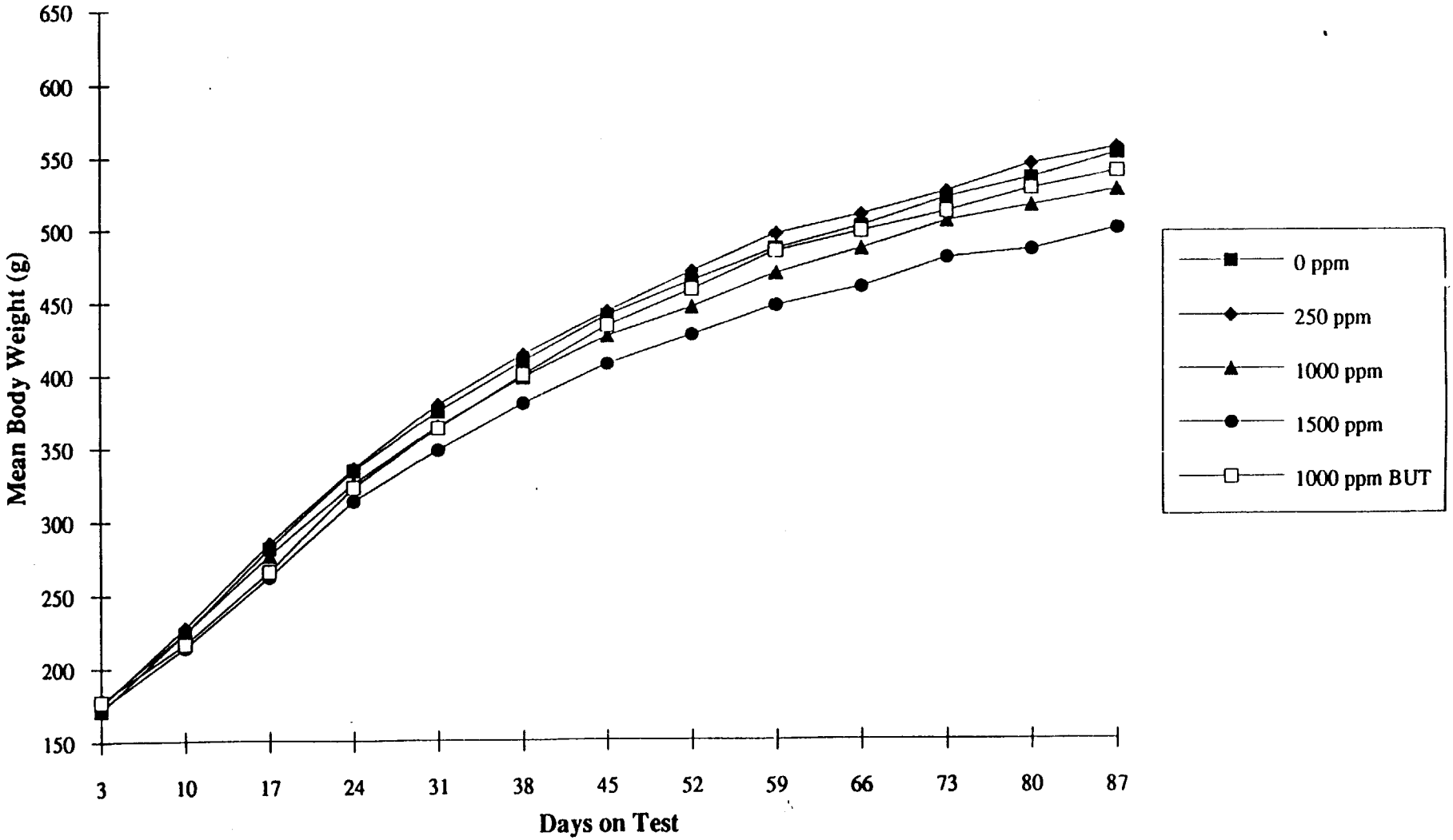
MEAN DAILY EXPOSURE CONCENTRATIONS



SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 3

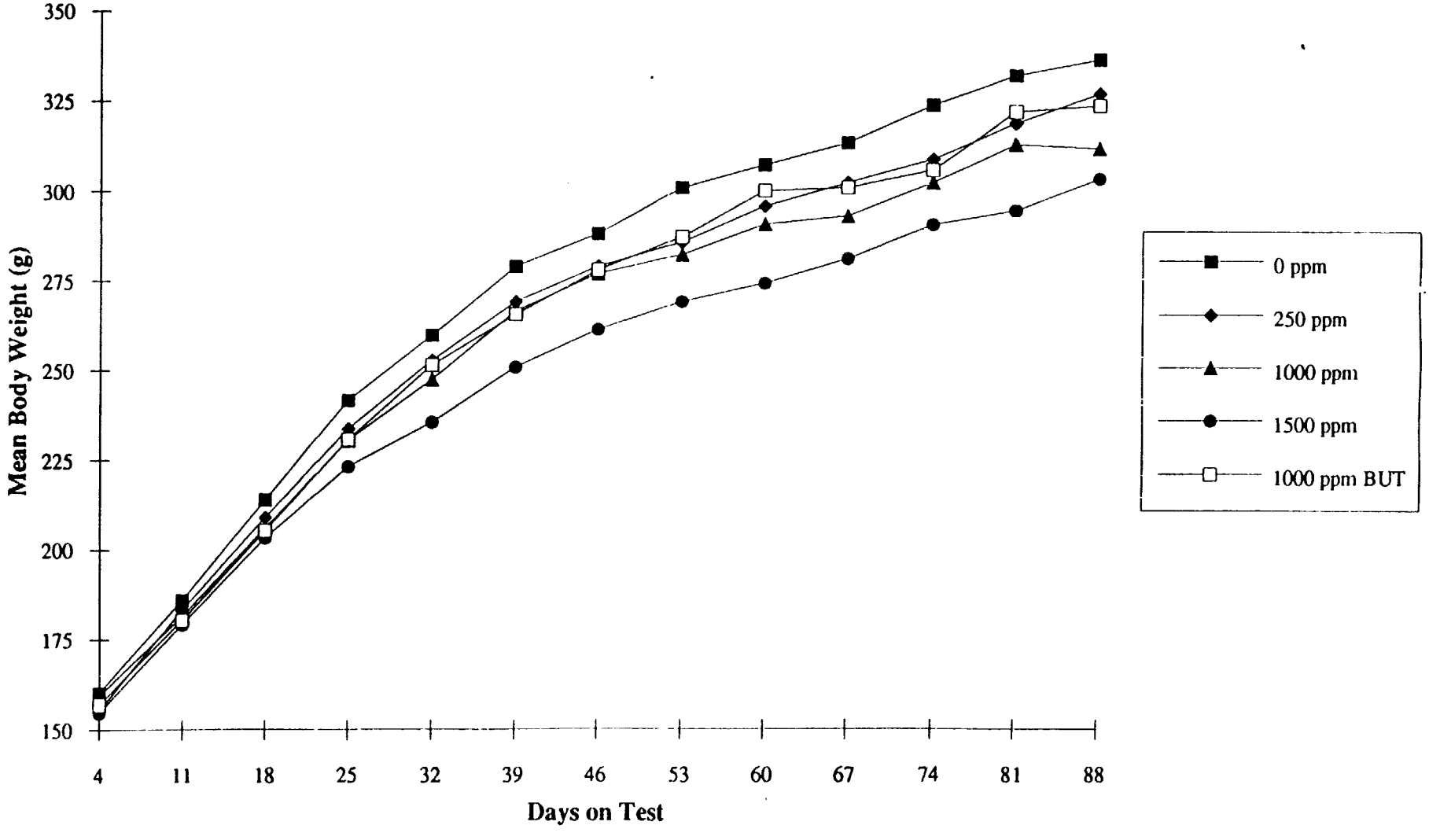
MEAN BODY WEIGHTS OF MALE RATS



SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 4

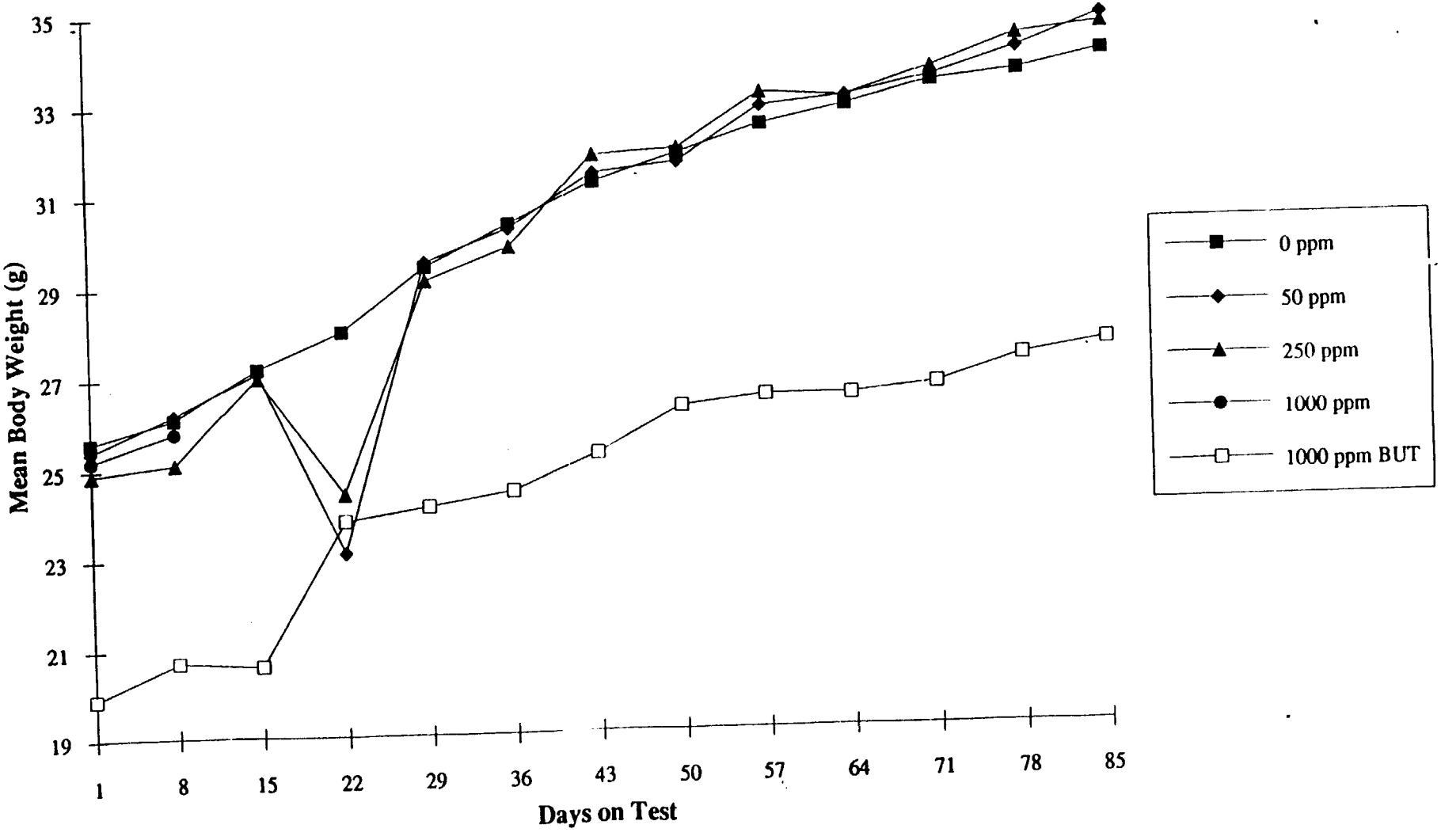
MEAN BODY WEIGHTS OF FEMALE RATS



SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 5

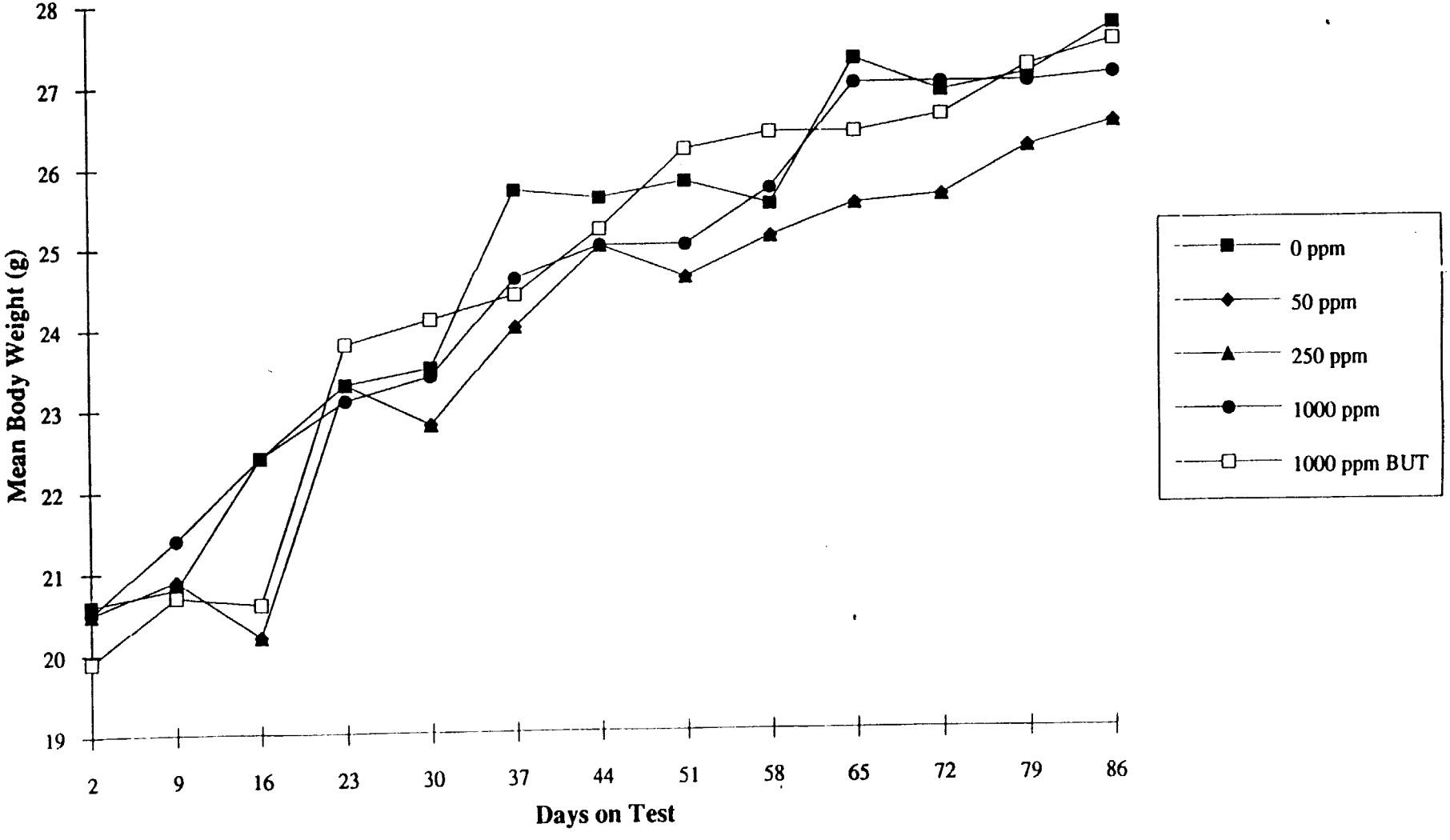
MEAN BODY WEIGHTS OF MALE MICE



SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 6

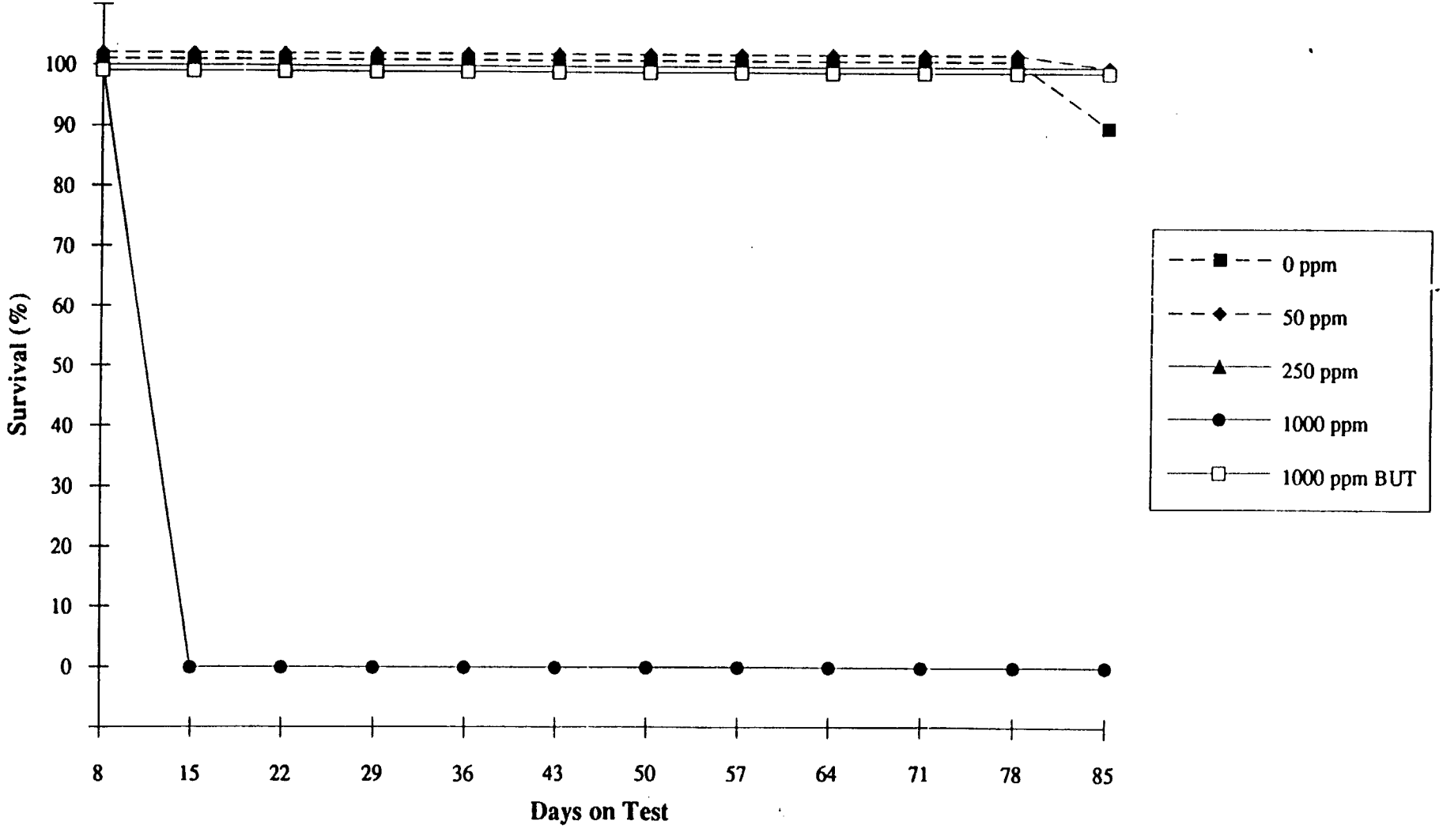
MEAN BODY WEIGHTS OF FEMALE MICE



SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 7

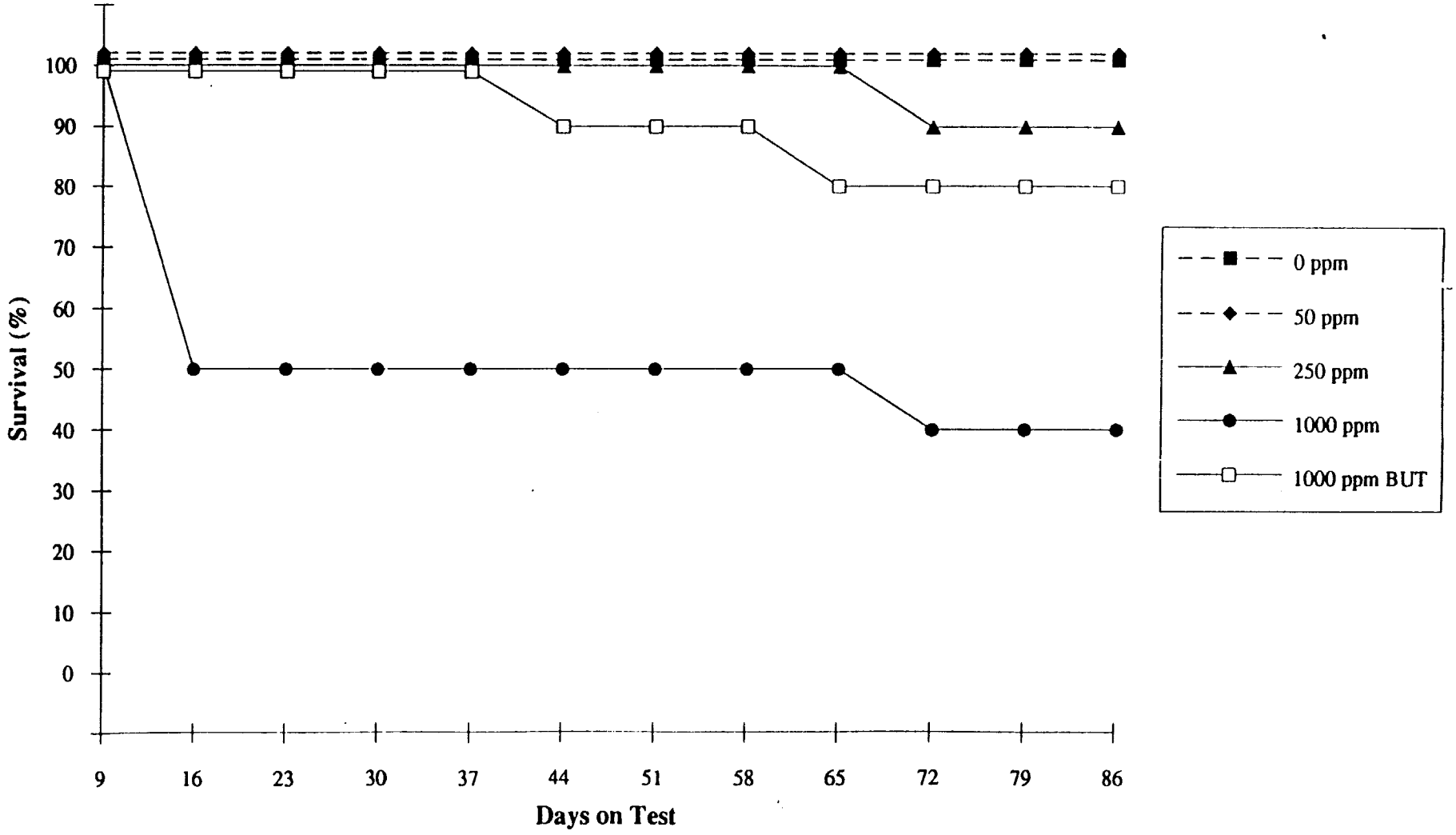
SURVIVAL OF MALE MICE



SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 8

SURVIVAL OF FEMALE MICE



SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDICES

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX A

Protocol and Protocol Amendments

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

CHEMICAL MANUFACTURERS ASSOCIATION STUDY NO. BUT-21.0-COT-DHL-03

PROTOCOL

Stine Haskell Animal Welfare Committee No. CBT28-GP and CBT36-GP

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
RATS AND MICE WITH 4-VINYLCYCLOHEXENE

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SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

INTRODUCTION

4-Vinylcyclohexene is a colorless liquid formed during the production of 1,3 butadiene. When pure 4-vinylcyclohexene was used in acute toxicity tests, an LC_{50} in rats of 10,554 ppm was observed. When tested in mice an LC_{50} of 6,102 ppm was observed.

This study was required by the U. S. Environmental Protection Agency in the consent order on 4-vinylcyclohexene promulgated under the provisions of Section 4 of the Toxic Substances Control Act.(1)

OBJECTIVE

The objective of this study is to evaluate the subchronic toxicity of 4-vinylcyclohexene when administered by inhalation to male and female Crl:CD®BR rats and to male and female B6C3F1/CrlBR mice for approximately 13 weeks. The inhalation route of administration was selected because it is the potential route of human exposure.

SPONSOR AND TEST FACILITY

This study is sponsored by the Chemical Manufacturing Association, Washington, District of Columbia. The study will be conducted at Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware, in accordance with all applicable Good Laboratory Practice regulations.(2)

STUDY DESIGN

The experimental design for rats is as follows:

Male	Female	# Rats/Sex/Group	Atmospheric Concentration (ppm)
I	II	10	0 (control)
III	IV	10	250 (Low)
V	VI	10	1,000 (Intermediate)
VII	VIII	10	1,500 (High)
IX	X	5	(Positive Control: cyclophosphamide)*

* A single intraperitoneal injection of cylclophosphamide will be administered as a positive control in rats for the micronucleus evaluation.

The experimental design for mice is as follows:

Male	Female	# Mice/Sex/Group	Atmospheric Concentration (ppm)
XI	XII	10	0 (control)
XIII	XIV	10	50 (Low)
XV	XVI	10	250 (Intermediate)
XVII	XVIII	10	1,000 (High)
XIX	XX	10	1,000 (Positive Control: 1,3-butadiene)

The schedule for study functions is as follows:

Function	Approximate Study Week			
	0	1	7	13
Clinical Sign Observation	<---------- Daily ---------->			
Body Weights	<---------- Weekly ---------->			
Group Food Consumption	<---------- Weekly ---------->			
Ophthalmological Exam	a	-	-	b
Clinical Pathology	-	-	-	b
Pathology	-	-	-	b

a Conducted on all animals prior to the start of the study

b Conducted on all surviving rats and mice

SELECTION OF EXPOSURE CONCENTRATIONS

In a two-week rangefinding study, four groups of 5 male and 5 female rats and 5 male and 5 female mice were exposed for two weeks by inhalation to nominal 4-vinylcyclohexene concentrations of 0, 250, 750, or 1500 ppm. Actual mean concentrations were 0, 240, 720, and 1500 ppm. Exposures were for six hours/day, five days/week with only one day of rest separating the two weeks.

One female rat in the 720 ppm exposure group was found dead on study day 2 and 5/5 male mice and 4/5 female mice were found dead prior to exposure on test day 4 in the 1500 ppm exposure group. The fifth female mouse at 1500 ppm was moribund and was sacrificed in extremis without further inhalation exposure. There were no other mortalities, and the clinical signs were unremarkable in the animals that survived the study. Significant body weight effects were observed in male rats exposed to 1500 ppm. The mean body weight gain of this group during the exposure period was lower (approximately 11%) than the mean weight gain of control males. All male and female mice that died had low mean body weights one to two days prior to death. These body weight effects were considered to be related to the exposure to 4-vinylcyclohexene.

Based on these results, three dose levels of 4-vinylcyclohexene have been selected. The high level selected for rats will be 1500 ppm with intermediate and low concentrations of 1000 and 250 ppm, respectively. For mice, the high level will be 1000 ppm with intermediate and low concentrations of 250 and 50 ppm, respectively.

TEST MATERIAL

The test substance, 4-vinylcyclohexene (H-19353), is a colorless liquid at ambient temperature. The test substance, and information on the synonyms, CAS registry number, basic physical properties, hazards, and hazardous substance classification(s) was provided by the Union Carbide Corporation, Institute, West Virginia. The sample was shipped December 10, 1991 and was identified as batch number 1. This information will be provided on the Haskell Laboratory Sample Evaluation form. The original form will be retained at Haskell Laboratory. A copy of the form will be included in the study records.

The purity and stability of the test substance will be confirmed at Haskell Laboratory near and prior to the initiation of the exposure phase of this study and within a week following the completion of the exposure phase of the study.

The positive control substance, 1,3-butadiene, is a colorless gas at ambient temperature. The positive control substance, and information on the synonyms, CAS registry number, basic physical properties, hazards, and hazardous substance classification(s) will be provided by the Exxon Chemical Company. This information will be provided on the Haskell Laboratory Sample Evaluation form. The original form will be retained at Haskell Laboratory. A copy of the form will be included in the study records.

The purity and stability of the positive control substance will be confirmed at Haskell Laboratory near and prior to the initiation of the exposure phase of this study and within a week following the completion of the exposure phase of the study.

MATERIALS AND METHODS

A. Test Species

Approximately 60 male and 60 female Crl:CD®BR rats, approximately 21 days of age, with body weights for males ranging from 45 to 65 grams and body weight for females ranging from 35 to 55 grams, will be acquired from Charles River Breeding Laboratories, Inc., Kingston, New York. Approximately 60 male and 60 female B6C3F_1/CrlBR mice, approximately 28 days of age, with body weights for males ranging from 19 to 25 grams and body weight for females ranging from 16 to 22 grams, will be acquired from Charles River Breeding Laboratories, Inc., St. Constant, Quebec, Canada. These strains were selected because NTP oral studies with 4-vinylcyclohexene used B6 mice, and inhalation studies with 1,3-butadiene used the CD rat and B6 mouse.

B. Pretest Procedures

Following arrival at Haskell Laboratory, all rats and mice will be removed from shipping cartons. Rats will be housed 1 per cage and mice housed 3 per cage in stainless steel, wire-mesh cages suspended above cage boards. Rats and mice will be housed in separate rooms; males and females will be placed on separate cage racks. Rats and mice will be:

- o quarantined for approximately two weeks.
- o fed Purina® Certified Rodent Chow® #5002 (IPCRC) and provided tap water ad libitum.
- o identified temporarily with colored tail marks and cage identification.
- o weighed twice within a week of arrival and weekly thereafter.
- o observed with respect to weight gain and any gross signs of disease or injury.
- o given an ophthalmological evaluation.
- o grouped and tattooed as described below.

Rats or mice that die or that are sacrificed in extremis during the quarantine period will be necropsied to check for the presence of disease. Based on these findings, further diagnostic procedures may be employed at the

discretion of the study director, the pathologist assigned to the study, or the laboratory veterinarian.

Rats and mice will be released from quarantine by the laboratory veterinarian on the basis of body weights and clinical signs of all rats.

C. Assignment to Study Groups

During the quarantine period, 50 animals of each sex and species, will be selected on the bases of body weight gain and freedom from any clinical signs of disease or injury. The rats and mice will be divided by computerized-stratified ramdomization into groups of 10 male and 10 female rats and groups of 10 male and 10 female mice so that there are no statistically significant differences among body weight means within a sex. The order of rats and mice within each group shall be randomized. Each group of rats and mice within a sex will be designated as either a control, low-, intermediate-, high-, or positive control concentration group. After assignment to treatment groups, each rat or mouse will be identified by a number tattooed on the tail.

Rats or mice that have not been assigned to a test group may, at the discretion of the study director, be released for use in other studies or will be sacrificed by carbon dioxide asphyxiation and discarded without pathological evaluation.

D. Animal Husbandry

After assignment to treatment groups, each rat and mouse within a sex will be identified by a number tattooed on the tail (see appendix I). All rats and mice will be individually housed in stainless steel, wire-mesh cages suspended above Upjohn DACB® or R-2 Reemay®-backed cage boards. Male and female rats and mice will be housed on separate cage racks. The position of cage racks within the animal room will be rotated weekly. The position of cages on the racks

will be rotated every two weeks.

The animal room will be maintained on a timer-controlled 12 hour light/dark cycle with relative humidity and temperature targeted at 55 ± 15% and 23 ± 2°C, respectively.

E. Diet and Water

Throughout pretest and test periods, except during exposure, Certified Purina® Laboratory Chow® #5002 (chunk) and water will be available ad libitum.

F. Test Chambers and Inhalation Exposure

Six chambers (approximately 150-300L) will be dedicated to this study throughout the duration of the exposure period. The chambers and all surfaces that the test material will contact prior to exhaust from the chamber are constructed of stainless steel or glass. All chambers will be free of any contamination from previous tests.

The chambers will be operated in a one-pass, flow-through mode with air flow rates adequate to provide adequate distribution of the test material, sufficient oxygen for test animals and to prevent contamination from volatiles derived from animal excreta. Atmospheres of 4-vinylcyclohexene will be generated by evaporating the liquid test substance and mixing it with conditioned, filtered air to the sweep the test substance into the chamber at the desired chamber concentrations. The chamber shall be operated to ensure that the chamber air is changed at least 12 times per hour. The atmospheric concentration of 4-vinylcyclohexene will be determined at least once per hour by a gas chromatographic method. The methodology used for preparing standards and sampling chamber atmospheres will be documented in the study records. Adequate chamber distribution of the test article shall be verified prior to the study start.

Atmospheric concentrations of the positive control substance (1,3-butadiene), will be generated by metering the gas into a mixing flask and mixing it with conditioned, filtered air to the sweep the positive control substance into the chamber at the desired chamber concentration. The chamber shall be operated to ensure that the chamber air is changed at least 12 times per hour. The atmospheric concentration of 1,3-butadiene will be determined at least once per hour by a gas chromatographic method. The methodology used for preparing standards and sampling chamber atmospheres will be documented in the study records. Adequate chamber distribution of the positive control substance shall be verified prior to the study start.

Chamber exhaust will be passed through a carbon bed prior to being exhausted through the roof exhaust system, using a lab wide permit exemption from the State of Delaware Department of Natural Resources and Environmental Control.

Ideally, the chamber environmental conditions will be similar to housing conditions (i.e., temperature of 22 $\pm$ 3oC, relative humidity of 40%-60%). However, temperature and humidity variations outside of these ranges are commonly encountered due to the generation procedures used and the characteristics of the test substance itself. During exposure, the relative humidity of the chamber air, the chamber temperature, and chamber airflow rate will be measured at least every 30 minutes. Chamber oxygen content is targeted at 19% and will be measured and recorded at least twice daily.

G. Exposures

Groups of 10 male and 10 female rats and 10 male and 10 female mice will be exposed to one of three targeted concentrations for six hours per day, approximately five days per week, for a minimum of 65 exposures over an approximately 13 week period (weekends and holidays excluded). Control groups of 10 male and 10 female rats and 10 male and 10 female mice will be exposed to air alone., A positive control group consisting of 10 male and 10 female mice

will be exposed to 1,3-butadiene.

All exposures will be conducted during the same eight-hour period of the day to minimize diurnal physiological variations. During exposure, rats and mice will be housed individually in exposure modules. Prior to transfer to housing facilities at the end of each exposure, test groups will be left in their respective exposure chambers for a period sufficient to allow clearance of test material from the chamber atmosphere as determined by atmospheric analysis. Cage positions within the exposure chambers will be rotated daily. Control rats and mice will be treated exactly as test animals, except that the control chamber atmospheres will not contain the test material.

During the exposure period, at approximately hourly intervals, animals visible through the chamber window will be checked for well being.

H. Body Weights, Clincial Observations and Food Consumption

Cage-site examinations to detect moribund or dead rats and mice and abnormal behavior or appearance among rats and mice will be conducted at least once daily throughout the study. On exposure days, a second observation will be conducted when animals are removed from the chamber. Moribund rats and mice will be sent to pathology for sacrifice and pathological examination. Dead rats and mice will be given a gross pathological examination.

Individual body weights and clinical signs will be recorded weekly throughout the test, unless clinical observations warrant a more frequent schedule. At each weighing, each rat and mouse will be individually handled and examined for abnormal behavior, appearance, and palpable masses. Records of the amount of food consumed by each test group over each weighing interval will be determined over the course of the study. These data will be used to calculate mean individual food consumption. Should a 10% decrease in body weight gain occur in any group(s), a weekly determination of individual food

consumption will be instituted for the group(s) with the decrease and the control group.

I. Ophthalmological Evaluation

Two ocular examinations will be conducted by a veterinary ophthalmologist. The initial examination will be performed during the quarantine period on all rats and mice received for the study, prior to selection and grouping. Both eyes of each animal will be examined by focal illumination and indirect opthalmoscopy. The eyes will be examined in subdued light after mydriasis has been produced by 1% tropicsamide solution. Any rats or mice with pre-existing ocular damage may be eliminated from consideration for use in the study. All rats and mice in the groups that will be sacrificed at 90 days will be given ocular examinations prior to sacrifice.

A report of the findings from all ophthalmological examinations will be submitted by the examining veterinary ophthalmologist.

J. Clinical Laboratory Evaluation

A clinical laboratory evaluation will be conducted at approximately 90 days after initiation of the study on all surviving rats and mice.

Two days prior to collection of blood samples for clinical evaluation, the rats will be placed in metabolism cages and allowed to acclimate. The day prior to blood collection, the rats will be returned to metabolism cages following the exposure period. The rats will be allowed to access food and water for at least 2 hours. Following this period food and water shall be removed, and urine collection initiated. At the conclusion of this period (approximately 14 hours), blood samples for hematological and clinical chemical measurements will be collected from the orbital sinus of each rat while the rat is under light carbon dioxide anesthesia. Blood samples will also be drawn from mice in a similar manner; however, these mice will not be

acclimated in metabolism cages, nor will urine be collected.

1. Hematology

The hematological parameters examined at each sampling time for rats and mice will include erythrocyte, leukocyte, differential leukocyte, and platelet counts, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration. Blood smears for reticulocyte counts will be prepared from each rat and each mouse at each sampling time and will be evaluated, if required, to substantiate or clarify the results of hematologic evaluation.

2. Clinical Chemistry

Blood serum from rats will be evaluated for alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase activities, and concentrations of blood urea nitrogen, total serum protein, albumin, globulin (calculated), creatinine, cholesterol, glucose, calcium, sodium, potassium, phosphate, chloride, and total bilirubin.

3. Urine Analyses (rats only)

Urine collected during each fasting period for rats will be measured for the following: volume, pH, and osmolality and measures of glucose, protein, bilirubin, urobilinogen, ketone, and occult blood. Urine color and transparency will be recorded, and sediment from each urine sample will be microscopically examined.

K. Pathological Evaluation

All rats or mice that die or are sacrificed *in extremis* before the end will be necropsied and undergo pathological examination. After approximately 13

weeks, all surviving rats will be sacrificed by pentobarbital anesthesia and exsanguination, then necropsied. The order of sacrifice for the pathological evaluation will be random among all treatment groups within a sex.

Bone marrow smears will be prepared from all rats and mice at the 13 week sacrifice and will be evaluated if warranted by experimental findings. Additional bone marrow will be collected from each rat and mouse sacrificed by design for evaluation of micronuclei. Evaluation of the bone marrow for micronuclei will proceed under a separate protocol.

The brain, heart, liver, lungs, spleen, kidneys, adrenals (rats only), testes, and ovaries (rats only) will be weighed wet at necropsy and organ weight/final body weight ratios will be calculated. Organs from rats or mice found dead or sacrificed in extremis may be weighed at the discretion of the pathologist or the study director, but will not be included in the statistical analyses.

The following tissues will be collected from all rats and mice (tissue integrity permitting).

Integumentary System
- Skin

Hematopoietic System
- Bone marrow (sternum and femur)
- Lymph nodes (mandibular and mesenteric)
- Spleen*
- Thymus

Circulatory System
- Aorta (thoracic)
- Heart*

Respiratory System
- Trachea
- Lungs*
- Nose (4 cross sections)
- Larynx
- Pharynx

Digestive System
- Salivary glands
- Esophagus
- Stomach
- Liver*
- Pancreas
- Small intestine (duodenum, jejunum, and ileum)
- Large intestine (cecum, colon, and rectum)
- Gall bladder+

Urinary System
- Kidneys*
- Bladder

Endocrine System
- Pituitary
- Thyroid - Parathyroid
- Adrenals*

Reproductive System

Males	Females
Prostate	Mammary gland
Testes*	Ovaries*
Epididymides	Uterus
Seminal vesicles	Vagina

Nervous System
- Brain* (includes sections of medulla/pons, cerebellar cortex, cerebral cortex)
- Spinal cord (3 levels)
- Peripheral nerve (sciatic)

Musculoskeletal System
- Muscle (thigh)
- Bone (sternum and femur)

Other
- Eyes
- Exorbital lacrimal glands
- Harderian glands
- Zymbal gland
- All gross lesions

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* Organs will be weighed. Ovary and adrenal weights will be measured in rats only.

\+ Mice only

All tissues will be fixed in 10% neutral buffered formalin except testes, eyes, skin, and mammary gland which will be fixed in Bouins.

All tissues from rats and mice in the high-concentration, control, and positive control groups, and from all rats and mice that are found dead (tissue integrity permitting) or are sacrificed *in extremis* will be further processed to slides, stained with hematoxylin and eosin, and examined microscopically. Lungs, liver, kidneys, nose, ovaries, all gross lesions, and target organs from rats and mice in the low- and intermediate-concentration groups will also be examined microscopically. The remaining tissues collected from the low- and intermediate-concentration groups will be evaluated microscopically if needed to establish a no-observable-effect level for potential compound-related abnormalities.

STATISTICAL ANALYSES

Body weights, body weight gains, organ weights, and clinical laboratory measurements will be analyzed by a one-way analysis of variance. When the test for differences among test group means (F test) is significant, pairwise comparisons between test and control groups will be made with the Dunnett's tests. Significance will be judged at alpha = 0.05

Incidence of clinical observations will be evaluated by the Fisher's Exact test with a Bonferroni correction and, if significant, will be followed by the Cochran-Armitage test for trend.

Bartlett's test for homogeneity of variances will be performed on the clinical laboratory data and, if significant (alpha = 0.005), will be followed by nonparametric procedures.

Except for Bartlett's test, all other significance will be judged at alpha = 0.05. Other methods will be used, if appropriate, at the time of analyses.

SAFETY AND HOUSEKEEPING

4-vinylcyclohexene is of low acute toxicity by the inhalation route. Good industrial hygiene practices will be followed during conduct of the study. Prior to initiation of the study, the test methods and equipment will be reviewed by the Process Hazards Review Committee at Haskell Laboratory. Handling of test material, chamber waste, and animal carcasses will follow procedures outlined in standard operating procedures and the Stine-Haskell Safety and Occupational Health Manual.

RECORDS RETENTION

All original records will be retained at Haskell Laboratory or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware. Preserved wet tissues, paraffin blocks, histological slides, and blood and bone marrow smears will be retained at Haskell Laboratory.

Any protocol change, addition, or deletion will be approved by the sponsor and documented.

REPORT

Following the conclusion of the study, a comprehensive report will be prepared which accurately and completely describes the study addressed in this protocol. The final report will include as a minimum, the following:

- o Title and date of the report
- o Information on the test material
- o Identification of key people responsible for various portions of the study
- o Information on animals and animal husbandry
- o Methods used in the study (including statistics)
- o Results of the study in tabular form including:

Clinical signs
Food Consumption (mean values) and Body weight data (individual and mean values)
Chamber environmental measurements (individual and mean values)
Test material concentration (individual and mean values)
Survival Data
Hematology, clinical chemistry, and urinanalysis data (individual and mean values)
Gross pathological examinations
Microscopic pathology observations
Organ weights and organ/body weight ratios (individual and mean values)

- o Quality Assurance Statement
- o Good Laboratory Practice Statement

MISCELLANEOUS ITEMS

Haskell Laboratory has an animal health monitoring program which consists of periodic food and water analyses for contaminants, and sampling freshly washed cages and cage racks for bacteria. This program is monitored and administered by the laboratory veterinarian; data are maintained separately from study records.

REFERENCES

1. Environmental Protection Agency, Toxic Substance Control Act; Testing Consent Order for 4-vinylcyclohexene 40 CFR Part 799; published September 9, 1991.>

2. Part III. Environmental Protection Agency, Toxic Substances Control Act; Good Laboratory Practice Standards 40 CFR Part 792; published August 17, 1989.>

APPENDIX I

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

Animal Identification Marking System (AIMS)

Rats will be identified by a tail tattoo. The numbering system will be according to the following scheme:

Groups		Tattoo Number		Exposure Concentration (ppm)
Male	Female	Male	Female	4-vinylcyclohexene
I	II	101-110	201-210	0 (CONTROL)
III	IV	301-310	401-410	250 (LOW)
V	VI	501-510	601-610	1,000 (INTERMEDIATE)
VII	VIII	701-710	801-810	1,500 (HIGH)
IX	X	901-910	1001-1010	** (POSITIVE CONTROL)

** A single intraperitoneal injection of cylclophosphamide will be administered as a positive control in rats for the micronucleus evaluation.

Mice will be identified by a tail tattoo. The numbering system will be according to the following scheme:

Groups		Tattoo Number		Exposure Concentration (ppm)
Male	Female	Male	Female	4-vinylcyclohexene
XI	XII	101-110	201-210	0 (CONTROL)
XIII	XIV	301-310	401-410	50 (LOW)
XV	XVI	501-510	601-610	250 (INTERMEDIATE)
XVII	XVIII	701-710	801-810	1,000 (HIGH)
XIX	XX	901-910	1001-1010	1,000 (POSITIVE CONTROL)

APPENDIX II

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

Study Personnel and Study Dates

Study Function	Study Personnel
Study Direction	A. S. Panepinto Toxicologist
Veterinary Ophthalmology	J. M. Clinton Veterinarian
Clinical Pathology	G. S. Elliott Clinical Pathologist
Pathology	S. R. Frame Pathologist
Consulting Toxicologist	D. A. Keller Research Toxicologist

Study Dates

Proposed Experiment Start	January, 1993
Ophthalmological Evaluations	
Pretest	January,1993
13 Week	April, 1993
Clinical Laboratory Evaluations	
13 Week	April, 1993
Scheduled Sacrifice	April, 1993
Proposed Draft Report Date: Draft to Sponsor:	November, 1993

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

PROTOCOL

Date

Approved by: Anthony S. Panepinto 12-3-92
Anthony S. Panepinto
Study Director

Nancy C Chroney for SRF 12-3-92
Steven R. Frame
Pathologist

Nancy C Chroney for GSE 12-3-92
Glenn S. Elliot
Clinical Pathologist

Robert W. Rickard 12-3-92
Robert W. Rickard
Manager
Chronic, Biochemical, & Inhalation Toxicology

cc: J.C. Hamill
W.F. Dinbokowitz
J. R. Pennington, Jr.
T. W. Slone, Jr.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

CHEMICAL MANUFACTURERS ASSOCIATION STUDY NO. BUT-21.0-COT-DHL-03

PROTOCOL AMENDMENT #1

The STUDY DESIGN on page 4 presently reads.

The experimental design for rats is as follows:

Male	Female	# Rats/Sex/Group	Atmospheric Concentration (ppm)
I	II	10	0 (control)
III	IV	10	250 (Low)
V	VI	10	1,000 (Intermediate)
VII	VIII	10	1,500 (High)
IX	X	5	(Positive Control: cyclophosphamide)*

* A single intraperitoneal injection of cylclophosphamide will be administered as a positive control in rats for the micronucleus evaluation.

The experimental design for mice is as follows:

Male	Female	# Mice/Sex/Group	Atmospheric Concentration (ppm)
XI	XII	10	0 (control)
XIII	XIV	10	50 (Low)
XV	XVI	10	250 (Intermediate)
XVII	XVIII	10	1,000 (High)
XIX	XX	10	1,000 (Positive Control: 1,3-butadiene)

The STUDY DESIGN on page 4 is amended as follows.

The experimental design for rats is as follows:

Male	Female	# Rats/Sex/Group	Atmospheric Concentration (ppm)
I	II	10	0 (control)
III	IV	10	250 (Low)
V	VI	10	1,000 (Intermediate)
VII	VIII	10	1,500 (High)
IX	X	10	1,000 1,3-butadiene
XI	XII	5	(Positive Control: cyclophosphamide)*

* A single intraperitoneal injection of cylclophosphamide will be administered as a positive control in rats for the micronucleus evaluation.

The experimental design for mice is as follows:

Male	Female	# Mice/Sex/Group	Atmospheric Concentration (ppm)
I	II	10	0 (control)
III	IV	10	50 (Low)
V	VI	10	250 (Intermediate)
VII	VIII	10	1,000 (High)
IX	X	10	1,000 (Positive Control: 1,3-butadiene)

REASON FOR CHANGE: Protocol was amended to reflect changes in study design. An additional group of rats was added for inhalation exposure to 1,3-Butadiene following technical clarification of study goals by sponsor.

The MATERIALS AND METHODS on page 7 presently read.

A. Test Species

Approximately 60 male and 60 female B6C3F$_1$/CrlBR mice, approximately 28 days of age, with body weights for males ranging from 19 to 25 grams and body weight for females ranging from 16 to 22 grams, will be acquired from Charles River Breeding Laboratories, Inc., St. Constant, Quebec, Canada.

B. Pretest Procedures

Rats will be housed 1 per cage and mice housed 3 per cage in stainless steel, wire-mesh cages suspended above cage boards.

The MATERIALS AND METHODS on page 7 are amended to read.

A. Test Species

Approximately 60 male and 60 female B6C3F$_1$/CrlBR mice, approximately 35 days of age, with body weights for males ranging from 19 to 25 grams and body weight for females ranging from 16 to 22 grams, will be acquired from Charles River Breeding Laboratories, Inc., St. Constant, Quebec, Canada.

B. Pretest Procedures

Rats will be housed 3 per cage and mice housed 3 per cage in stainless steel, wire-mesh cages suspended above cage boards.

REASON FOR CHANGE:

A. Test Species

The supplier was unable to supply mice for this study at 28 days of age.

B. Pretest Procedures

Changed to provide appropriate housing environment for young rats.

The MATERIALS AND METHODS on page 10 and 11 presently read.

G. Exposures

A positive control group consisting of 10 male and 10 female mice will be exposed to 1,3-butadiene.

H. Body Weights, Clincial Observations and Food Consumption

Cage-site examinations to detect moribund or dead rats and mice and abnormal behavior or appearance among rats and mice will be conducted at least once daily throughout the study. On exposure days, a second observation will be conducted when animals are removed from the chamber.

The MATERIALS AND METHODS on page 10 and 11 are amended to read.

G. Exposures

A positive control group consisting of 10 male and 10 female rats and 10 male and 10 female mice will be exposed to 1,3-butadiene.

H. Body Weights, Clincial Observations and Food Consumption

Cage-site examinations to detect moribund or dead rats and mice and abnormal behavior or appearance among rats and mice will be conducted at least once daily throughout the study. On exposure days, a second observation will be conducted when animals are removed from the chamber. Any observed signs shall be recorded in the computer system. Otherwise the rat or mouse will be assumed to have been NAD.

REASON FOR CHANGE:

G. Exposures

Additional group of rats was added to be exposed by inhalation to 1,3-Butadiene to enhance study design.

H. Body Weights, Clincial Observations and Food Consumption

A second computer database was created to allow collection of post-exposure clinical observations.

The MATERIALS AND METHODS on page 13, 14, and 15 presently read.

K. Pathological Evaluation

All rats or mice that die or are sacrificed in extremis before the end will be necropsied and undergo pathological examination. After approximately 13 weeks, all surviving rats will be sacrificed by pentobarbital anesthesia and exsanguination, then necropsied.

All tissues will be fixed in 10% neutral buffered formalin except testes, eyes, skin, and mammary gland which will be fixed in Bouins.

The MATERIALS AND METHODS on page 13, 14, and 15 are amended to read.

K. Pathological Evaluation

All rats or mice that die or are sacrificed in extremis before the end will be necropsied and undergo pathological examination. After approximately 13 weeks, all surviving rats will be sacrificed by carbon dioxide anesthesia and exsanguination, then necropsied.

All tissues will be fixed in 10% neutral buffered formalin except testes, eyes, skin, epididymides, and mammary gland which will be fixed in Bouins.

REASON FOR CHANGE:

K. Pathological Evaluation

Changes made in method of sacrifice and fixing of epididymides in Bouins were procedural changes made at the suggestion of the pathologist.

The animal numbering system was revised as follows:

APPENDIX I

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

Animal Identification Marking System (AIMS)

Rats will be identified by a tail tattoo. The numbering system will be according to the following scheme:

Groups		Tattoo Number		Exposure Concentration (ppm)
Male	Female	Male	Female	4-vinylcyclohexene
I	II	101-110	201-210	0 (CONTROL)
III	IV	301-310	401-410	250 (LOW)
V	VI	501-510	601-610	1,000 (INTERMEDIATE)
VII	VIII	701-710	801-810	1,500 (HIGH)
IX	X	901-910	1001-1010	1,000 (1,3-butadiene)
XI	XII	1101-1105	1201-1205	** (POSITIVE CONTROL)

** A single intraperitoneal injection of cylclophosphamide will be administered as a positive control in rats for the micronucleus evaluation.

Mice will be identified by a tail tattoo. The numbering system will be according to the following scheme:

Groups		Tattoo Number		Exposure Concentration (ppm)
Male	Female	Male	Female	4-vinylcyclohexene
I	II	101-110	201-210	0 (CONTROL)
III	IV	301-310	401-410	50 (LOW)
V	VI	501-510	601-610	250 (INTERMEDIATE)
VII	VIII	701-710	801-810	1,000 (HIGH)
IX	X	901-910	1001-1010	1,000 (1,3-butadiene)

REASON FOR CHANGE:

Changes in numbering system reflect changes in study design.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

PROTOCOL AMENDMENT #1

Date

Approved by: _____ 3/8/93
Anthony S. Panepinto
Study Director

_____ 3/4/93
Elizabeth J. Moran
Manager, Butadiene Panel
Chemical Manufacturers Association

cc: J.C. Hamill
W.F. Dinbokowitz
J. R. Pennington, Jr.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

CHEMICAL MANUFACTURERS ASSOCIATION STUDY NO. BUT-21.0-COT-DHL-03

PROTOCOL AMENDMENT #2

GENERAL STATEMENT

This protocol refers to all mice and rats when addressing clinical pathology and histopathological evaluation. Rats from Group XI and XII are not included in these evaluations and were intended for induction of micronuclei by injection with cyclophosphamide. They were assigned to this study as a positive control for the micronucleus portion of this test.

The MATERIALS AND METHODS on page 12 presently read.

Two days prior to collection of blood samples for clinical evaluation, the rats will be placed in metabolism cages and allowed to acclimate. The day prior to blood collection, the rats will be returned to metabolism cages following the exposure period. Rats will be allowed to access food and water for at least 2 hours. Following this period food and water shall be removed, and urine collection initiated. At the conclusion of this period (approximately 14 hours), blood samples for hematological and clinical chemical measurements will be collected from the orbital sinus of each rat while the rat is under light carbon dioxide anesthesia. Blood samples will also be drawn from mice in a similar manner; however, these mice will not be acclimated in metabolism cages, nor will urine be collected.

The MATERIALS AND METHODS on page 12 are amended to read.

Two days prior to collection of blood samples for clinical evaluation, the rats will be placed in metabolism cages and allowed to acclimate. The day prior to blood collection, the rats will be returned to metabolism cages following the exposure period. Both rats and mice will be allowed to access food and water for at least 2 hours following the final exposure period. Following this period food shall be removed, and in rats urine collection initiated. At the conclusion of this period (approximately 14 hours), blood samples for hematological and clinical chemical measurements will be collected from the orbital sinus of each rat while the rat is under light carbon dioxide anesthesia. Blood samples will also be drawn from mice in a similar manner; however, these mice will not be acclimated in metabolism cages, nor will urine be collected.

REASON FOR CHANGE:

The period following the final exposure was meant to include a feeding period and a fasting period. However, water is not to be removed. These periods will also include mice.

The MATERIALS AND METHODS on page 13, 14, and 15 read.

K. Pathological Evaluation

All rats or mice that die or are sacrificed in extremis before the end will be necropsied and undergo pathological examination. After approximately 13 weeks, all surviving rats will be sacrificed by carbon dioxide anesthesia and exsanguination, then necropsied.

The MATERIALS AND METHODS on page 13, 14, and 15 are amended to read.

K. Pathological Evaluation

All rats or mice that die or are sacrificed in extremis before the end will be necropsied and undergo pathological examination. After approximately 13 weeks, all surviving rats and mice will be sacrificed by carbon dioxide anesthesia and exsanguination, then necropsied.

Rats in Group(s) IX and X and mice in Group(s) XIX and XX will be deeply anesthetized by carbon dioxide at sacrifice in order to faciltitate further sampling of blood. This will be done to avoid pain or suffering of the animals. These blood samples will be collected using EDTA or Heparin as an anticoagulant. Packed red blood cells will be isolated and washed. These cells will be stored at -70°C and shipped in dry ice to Dr. James A. Swenberg at the University of North Carolina at Chapel Hill for DNA adduct studies.

Samples of liver and lung tissue from rats and mice in Groups IX, X, XIX, and XX will be frozen in liquid and sent to Dr. Swenberg for similar studies.

REASON FOR CHANGE:

K. Pathological Evaluation

Changes made reflect error of not including mice in the statement about surviving animals to be necropsied. In addition the sponsor requested that additional blood and liver and lung tissue from the Butadiene exposure groups be supplied to the above-mentioned for DNA adduct analysis.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

PROTOCOL AMENDMENT #2

Date

Approved by: _____ 4/28/93
Anthony S. Panepinto
Study Director

_____ 4/28/93
Elizabeth J. Moran
Manager, Butadiene Panel
Chemical Manufacturers Association

cc: J.C. Hamill
W.F. Dinbokowitz
J. R. Pennington, Jr.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

CHEMICAL MANUFACTURERS ASSOCIATION STUDY NO. BUT-21.0-COT-DHL-03

PROTOCOL AMENDMENT #3

Stine Haskell Animal Welfare Committee No. CBT28-GP and CBT36-GP

The **TEST MATERIAL** section on page 6 presently reads.

The test substance, 4-vinylcyclohexene (H-19353), is a colorless liquid at ambient temperature. The test substance, and information on the synonyms, CAS registry number, basic physical properties, hazards, and hazardous substance classification(s) was provided by the Union Carbide Corporation, Institute, West Virginia. The sample was shipped December 10, 1991 and was identified as batch number 1. This information will be provided on the Haskell Laboratory Sample Evaluation form. The original form will be retained at Haskell Laboratory. A copy of the form will be included in the study records.

The **TEST MATERIAL** section on page 6 is amended as follows.

The test substance, 4-vinylcyclohexene (H-19353), is a colorless liquid at ambient temperature. The test substance, and information on the synonyms, CAS registry number, basic physical properties, hazards, and hazardous substance classification(s) was provided by the Union Carbide Corporation, Institute, West Virginia. **The sample was shipped December 10, 1991 and was identified as batch number 1 as reported by the sponsor. The test substance was received on January 24, 1992 with the lot number of #IN210G308.** This information will be provided on the Haskell Laboratory Sample Evaluation form. The original form will be retained at Haskell Laboratory. A copy of the form will be included in the study records.

REASON FOR AMENDMENT: To provide more information on the shipment, receipt, and identification of the Test Substance 4-VCH.

The **MATERIALS AND METHODS** section on page 7 presently reads.

A. Test Species

Mice will be acquired from Charles River Breeding Laboratories, Inc., St. Constant, Quebec, Canada.

The **MATERIALS AND METHODS** section on page 7 is amended to read.

A. Test Species

Mice will be acquired from Charles River Breeding Laboratories, Inc., Raleigh, North Carolina.

REASON FOR AMENDMENT: Due to problems with the availability of mice the location of where they were bred was changed.

The **MATERIALS AND METHODS** section on page 8 presently reads.

C. Assignment to Study Groups

The rats and mice will be divided by computerized-stratified ramdomization into groups of 10 male and 10 female rats and groups of 10 male and 10 female mice so that there are no statistically significant differences among body weight means within a sex. The order of rats and mice within each group shall be randomized. Each group of rats and mice within a sex will be designated as either a control, low-, intermediate-, high-, or positive control concentration group. After assignment to treatment groups, each rat or mouse will be identified by a number tattooed on the tail.

Rats or mice that have not been assigned to a test group may, at the discretion of the study director, be released for use in other studies or will be sacrificed by carbon dioxide asphyxiation and discarded without pathological evaluation.

The **MATERIALS AND METHODS** section on page 8 is amended to read.

C. Assignment to Study Groups

The rats and mice will be divided by computerized-stratified ramdomization into groups of 10 male and 10 female rats and groups of 10 male and 10 female mice so that there are no statistically significant differences among body weight means within a sex. **A group of 5 male and 5 female rats will be grouped in the same way. The rats will be housed with the other rats and mice on study but will not be exposed during the inhalation portion of the study. This group is intended for micronuclei induction by injection of the positive control substance, cyclophosphamide.** The order of rats and mice within each group shall be randomized. Each group of rats and mice within a sex will be designated as either a control, low-, intermediate-, high-, or positive control concentration group. **After assignment to treatment groups, each rat or mouse will be identified by a 5 or 6 digit number tattooed on the tail.**

Rats or mice that have not been assigned to a test group may, at the discretion of the study director, be released for use in other studies or will be sacrificed by carbon dioxide asphyxiation and discarded without pathological evaluation.

REASON FOR AMENDMENT: Clarification of the protocol.

The **MATERIALS AND METHODS** section on page 14 presently reads.

K. Pathological Evaluation

Bone marrow smears will be prepared from all rats and mice at the 13 week sacrifice and will be evaluated if warranted by experimental findings. Additional bone marrow will be collected from each rat and mouse sacrificed by design for evaluation of micronuclei. Evaluation of the bone marrow for micronuclei will proceed under a separate protocol.

The **MATERIALS AND METHODS** section on page 14 is amended to read.

K. Pathological Evaluation

Bone marrow smears will be prepared from all rats and mice at the 13 week sacrifice and will be evaluated if warranted by experimental findings. **Additional bone marrow will be collected from 5 rats/sex/group and 5 mice/sex group for evaluation of micronuclei.** Evaluation of the bone marrow for micronuclei will proceed under a separate protocol.

REASON FOR AMENDMENT: Clarification of study protocol.

STUDY DIRECTION

The Study Director is currently Anthony S. Panepinto, M.S. Hygiene.

The Study Directorship for this study is transferred to Judith Stadler, Ph.D.

REASON FOR CHANGE: Present study director will resign from company as of 9/15/93.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

PROTOCOL AMENDMENT #3

Date

Approved by: _____ 9-9-93
Anthony S. Panepinto

_____ 9/9/93
Judith C. Stadler
Sr. Research Toxicologist

_____ 9/16/93
Elizabeth J. Moran
Manager, Butadiene Panel
Chemical Manufacturers Association

cc: D. Vick
W.F. Dinbokowitz
J. R. Pennington, Jr.

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE
IN RATS AND MICE WITH 4-VINYLCYCLOHEXENE

MEDICAL RESEARCH PROJECT NO. 9523

CHEMICAL MANUFACTURERS ASSOCIATION STUDY NO. BUT-21.0-COT-DHL-03

PROTOCOL AMENDMENT #4

The Haskell number for the butadiene used in the subchronic inhalation toxicity study was inadvertantly not included in the original protocol. The protocol is amended as follows:

Page 4, Test Material, Paragraph 3, Line 1

Presently reads: "The positive control substance, 1,3-butadiene, is a colorless gas at..."

Amend to read: "The positive control substance, 1,3-butadiene (H-19999), is a colorless gas at..."

Judith C. Stadler 11/29/93
Judith C. Stadler Date
Study Director

12/6/93
Elizabeth J. Moran Date
Manager, Butadiene Panel
Chemical Manufacturers Association

cc: J. C. Hamill

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX B

Mean Daily Chamber Concentration

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER CONCENTRATION

EXPLANATORY NOTES

FOOTNOTES:

[a] Daily mean and standard deviation of 23 to 28 measurements, except as noted when there were fewer samples due to equipment failure:

Exposure 16: All 4-Vinylcyclohexene chambers 20 to 22 measurements.
Exposure 35: 250, 1000, and 1500 ppm chambers 21 to 22 measurements.
Exposure 41: All 4-Vinylcyclohexene chambers 20 to 22 measurements.
Exposure 60: All 4-Vinylcyclohexene chambers 15 to 16 measurements.

[b] Daily mean and standard deviation of 13 to 18 measurements, except as noted when there were more or fewer samples due to equipment failure:

Exposure 15: 8 measurements.
Exposure 24: 23 measurements.
Exposure 50: 7 measurements.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Make-up exposure for male mice exposed to 1,3-Butadiene. The number of days of exposure was subsequently the same for all male mice.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
21	15	46.0	(16.2)	186.1	(59.2)	795.8	(66.3)	1387.5	(191.1)	853.5	(210.6)
22	16	40.3	(10.4)	234.6	(76.8)	922.8	(126.5)	1444.7	(138.6)	932.7	(182.7)
23	17	67.3	(9.6)	293.4	(7.5)	983.1	(46.7)	1337.8	(412.3)	849.6	(54)
24	18	66.9	(27)	198.5	(67.7)	998.5	(67.3)	1563.8	(205.8)	1142.7	(1044.1)
25	19	65.8	(25.4)	210.7	(60.5)	1040.7	(72.2)	1486.2	(80.9)	633.2	(146.5)
WEEK 4 MEAN		57.3	(13)	224.7	(42.4)	948.2	(95.1)	1444.0	(87.5)	882.3	(183.2)
28	20	61.7	(10.3)	263.1	(7.5)	1060.2	(23.8)	1541.0	(46.9)	1111.1	(178)
29	21	50.4	(11.1)	253.6	(32.4)	1033.4	(37.1)	1526.8	(41.8)	1052.9	(82)
30	22	39.8	(6.9)	247.9	(8)	905.4	(37.4)	1421.3	(304.5)	982.8	(445.8)
31	23	65.8	(24.4)	264.9	(25.9)	1026.4	(88)	1541.9	(83.6)	868.6	(338.5)
32	24	60.8	(10.9)	264.8	(38)	843.3	(316)	1555.1	(294.7)	695.0	(537.6)
WEEK 5 MEAN		55.7	(10.5)	258.9	(7.7)	973.7	(94.2)	1517.2	(54.5)	942.1	(165)
35	25	62.0	(7.3)	252.0	(22.1)	959.0	(82.1)	1630.6	(146.4)	748.3	(842)
36	26	64.7	(4.5)	303.6	(7.8)	1139.6	(13.9)	1582.5	(66.5)	878.6	(149.6)
37	27	50.2	(13.8)	234.4	(73.4)	1016.7	(20.5)	1523.3	(107.1)	1026.6	(294.4)
38	28	62.5	(8.2)	286.6	(17.9)	1052.5	(89.9)	1363.5	(555.7)	951.4	(145)
39	29	58.0	(19.7)	206.5	(52.9)	919.7	(53.7)	1346.1	(381.7)	752.3	(480.3)
WEEK 6 MEAN		59.5	(5.7)	256.6	(39.2)	1017.5	(85.3)	1489.2	(128.6)	871.4	(122.3)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
42	30	37.0	(2.7)	229.4	(15.9)	1004.7	(62.5)	1528.3	(85.6)	1159.5	(94.7)
43	31	33.7	(6)	233.7	(8.1)	1003.3	(18.4)	1519.8	(48.6)	998.3	(196)
44	32	48.1	(7.3)	263.0	(18.6)	998.8	(127)	1452.1	(140.2)	839.3	(327.6)
45	33	47.3	(4.5)	248.6	(4.1)	1029.7	(29.3)	1524.8	(47.3)	1083.3	(278.7)
46	34	60.5	(8.2)	282.6	(10.7)	1110.2	(36)	1486.7	(98.6)	966.2	(114.6)
WEEK 7 MEAN		45.3	(10.6)	251.5	(21.9)	1029.3	(46.8)	1502.3	(32.6)	1009.3	(121.4)
49	35	51.8	(11.1)	237.9	(17.8)	1000.8	(72.3)	1494.1	(89.5)	1144.6	(1126.0)
50	36	51.7	(21.5)	247.9	(55.3)	1083.7	(61.3)	1518.8	(43.3)	918.1	(265.7)
51	37	53.3	(4.2)	216.7	(9.7)	951.1	(44.9)	1476.0	(89.2)	966.8	(185.6)
52	38	52.1	(7.2)	253.1	(12.6)	1029.7	(82.6)	1448.0	(241)	891.6	(331.9)
53	39	49.1	(25.7)	114.4	(106.3)	683.6	(106.4)	1368.8	(242.4)	1080.3	(307.2)
54	[f]									988.3	(107)
WEEK 8 MEAN		51.6	(1.5)	214.0	(57.4)	949.8	(156.3)	1461.1	(57.7)	998.3	(97.0)
56	40	46.3	(10.6)	255.3	(8.8)	1101.3	(58.3)	1516.0	(86.9)	948.3	(316.9)
57	41	43.3	(5.7)	218.2	(7.6)	892.5	(104.2)	1425.8	(223.6)	943.3	(207.3)
58	42	48.6	(8.7)	260.9	(5.1)	1023.1	(30.5)	1588.4	(69.4)	1037.5	(57.3)
59	43	67.5	(10)	268.3	(3.6)	992.5	(43)	1521.2	(80)	1025.7	(192.8)
60	44	61.4	(6)	278.5	(7.5)	747.2	(35.8)	1400.5	(66.3)	1292.8	(110.3)
WEEK 9 MEAN		53.4	(10.5)	256.2	(23)	951.3	(136.4)	1490.4	(76.6)	1049.5	(142.7)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS-	EXPOSURE #										
64	45	62.5	(19.3)	271.4	(17.4)	1098.8	(32.5)	1576.9	(343.1)	1117.0	(66.5)
65	46	57.3	(9.7)	264.4	(68.6)	939.3	(273)	1465.6	(174.2)	781.0	(478.6)
66	47	37.6	(9.4)	263.3	(5.6)	1005.0	(30.5)	1480.1	(82.4)	987.0	(353.3)
67	48	59.9	(6.6)	250.3	(6.3)	998.1	(34.7)	1498.7	(82.1)	1148.9	(296.8)
68	49	61.6	(9.4)	256.3	(7.2)	987.0	(34.1)	1518.7	(59.1)	1182.5	(171)
WEEK 10 MEAN		55.8	(10.4)	261.1	(8.1)	1005.6	(58.1)	1508.0	(43.4)	1043.3	(164.3)
70	50	63.6	(21.7)	241.1	(76.6)	1061.9	(51.8)	1554.3	(32.1)	1048.1	(37.6)
71	51	44.7	(5.1)	237.2	(8)	972.3	(46.7)	1512.9	(70.8)	962.7	(145.4)
72	52	49.0	(10)	243.9	(4)	975.9	(84.7)	1514.0	(54.9)	1112.6	(245.5)
73	53	58.5	(4.9)	244.4	(7.5)	963.1	(63.4)	1541.4	(55.1)	1027.3	(189.5)
WEEK 11 MEAN		54.0	(8.6)	241.7	(3.3)	993.3	(46)	1530.7	(20.6)	1037.7	(61.8)
77	54	47.0	(10)	249.9	(11.5)	995.6	(25)	1432.6	(47.3)	869.0	(81.5)
78	55	49.7	(9.2)	234.8	(6.5)	991.1	(63.3)	1478.2	(140.3)	836.8	(92.8)
79	56	43.3	(6.6)	243.6	(5.5)	1009.8	(30.4)	1480.6	(50.9)	859.4	(247.2)
80	57	47.3	(6)	256.3	(4.6)	1078.3	(48.9)	1224.2	(594.9)	934.2	(208.5)
81	58	46.9	(6.3)	255.7	(12.3)	1064.4	(20.1)	1566.0	(18.8)	819.1	(93.3)
82	59	46.7	(11)	224.6	(59.5)	1037.9	(27.3)	1507.8	(47.1)	731.5	(348.5)
WEEK 12 MEAN		46.8	(2)	244.2	(12.5)	1029.5	(36.6)	1448.2	(118.2)	841.7	(66.8)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
84	60	52.4	(6.4)	262.6	(21.2)	1092.9	(63.3)	1552.1	(45.8)	1081.9	(129.2)
85	61	39.0	(6.9)	260.6	(3.4)	1070.8	(54.2)	1514.5	(66.1)	926.1	(101.2)
86	62	46.4	(14.8)	258.7	(19.5)	1041.4	(34.1)	1403.1	(404)	886.9	(252.5)
87	63	39.8	(6.1)	226.6	(6.6)	999.2	(70.9)	1382.7	(338.2)	868.3	(293.8)
88	64	55.9	(10.2)	260.0	(4.8)	1055.9	(15.8)	1571.8	(35.9)	1022.1	(119.3)
WEEK 13 MEAN		46.7	(7.5)	253.7	(15.2)	1052.0	(35.1)	1484.8	(86.7)	957.1	(91.6)
91	65	48.2	(9.1)	262.1	(11.1)	1048.4	(46.5)	1620.8	(28.4)	969.5	(77.8)
92	66	31.5	(10.2)	239.2	(6.5)	1010.1	(40.4)	1620.9	(27.7)	1135.6	(210)
93	67	[d]		247.9	(48)	1026.3	(40.9)	1532.8	(40.3)	1022.0	(108.7)
94	68	[d]		263.5	(110.1)	1050.0	(28.2)	1500.6	(47.2)	960.5	(190.3)
WEEK 14 MEAN		39.9	(11.8)	253.2	(11.7)	1033.7	(19.1)	1568.8	(61.6)	1021.9	(80.5)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
1	1	61.0	(17.1)[c]	231.4	(51.5)	982.9	(81)	[d]		[d]	
2	2	42.6	(8.4)	283.4	(56)	1090.0	(54)	[d]		1142.6	(127.4)
3	3	33.4	(18.7)	232.3	(45.8)	918.4	(210.1)	1528.5	(317.7)	1235.0	(377.3)
4	4	45.1	(26.6)	248.3	(14.5)	923.5	(110.9)	1485.1	(258.7)	994.8	(486.7)
WEEK 1 MEAN[e]		45.5	(11.5)	248.9	(24.3)	978.7	(79.8)	1506.8	(30.7)	1124.1	(121.1)
7	5	61.3	(6.9)	205.8	(98.5)	976.0	(27.5)	1548.0	(43.9)	924.3	(116.7)
8	6	64.6	(11.1)	258.2	(24.9)	1042.4	(79.5)	1523.5	(126.3)	1033.6	(97.3)
9	7	54.7	(7.6)	227.3	(9.6)	920.5	(75.2)	1411.5	(206.3)	1193.1	(189)
10	8	59.4	(7.8)	238.7	(63.5)	1104.7	(40.7)	1592.3	(71)	1026.2	(37.2)
11	9	68.8	(9.3)	279.7	(16.8)	984.0	(46.5)	1459.4	(25.8)	1000.6	(552.4)
WEEK 2 MEAN		61.8	(5.3)	241.9	(28.4)	1005.5	(70.3)	1506.9	(71.8)	1035.6	(98.1)
14	10	40.6	(35.4)	237.9	(50.9)	960.2	(36.4)	1391.5	(443.4)	1233.9	(126.7)
15	11	63.5	(6.2)	257.0	(10)	993.7	(79.7)	1613.8	(110.5)	1064.7	(116.8)
16	12	59.8	(7.5)	261.0	(14.7)	953.3	(61.1)	1506.0	(231.9)	954.3	(124.9)
17	13	58.2	(6.2)	255.6	(9.8)	941.3	(88.9)	1635.2	(73.9)	1017.0	(59.4)
18	14	59.1	(5.8)	267.4	(22.2)	957.1	(48.4)	1423.1	(109.1)	942.6	(125.2)
WEEK 3 MEAN		56.2	(9)	255.8	(11)	961.1	(19.6)	1513.9	(109.5)	1042.5	(117.8)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX C

Mean Daily Chamber Temperature

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER TEMPERATURE

EXPLANATORY NOTES

FOOTNOTES:

[a] Daily mean and standard deviation of approximately 12 measurements.

[b] Daily mean and standard deviation of 60 to 77 measurements, except where noted.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Make-up exposure for male mice exposed to 1,3-Butadiene. Daily mean and standard deviation of 12 measurements. The number of days of exposure was subsequently the same for all male mice.

[g] One hour of data not collected due to technical error. Mean and standard deviation of 54 to 60 measurements.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
1	1	23.7	(0.6)[c]	22.0	(1)	22.0	(1)	22.0	(0)	[d]		[d]	
2	2	23.3	(0.3)	23.0	(1)	22.0	(1)	23.0	(1)	[d]		23.0	(1)
3	3	23.9	(0.9)	23.0	(1)	24.0	(1)	24.0	(1)	24.0	(1)	24.0	(1)
4	4	25.1	(1)	23.0	(1)	26.0	(1)	25.0	(1)	25.0	(1)	25.0	(1)
WEEK 1 MEAN[e]		24.0	(0.8)	22.8	(0.5)	23.5	(1.9)	23.5	(1.3)	24.5	(1)	24.0	(1)
7	5	26.6	(0.6)	23.0	(1)	26.0	(1)	26.0	(1)	25.0	(1)	25.0	(1)
8	6	26.4	(0.6)	22.0	(1)	24.0	(1)	24.0	(1)	23.0	(1)	25.0	(1)
9	7	26.7	(1.1)	22.0	(1)	25.0	(1)	25.0	(1)	24.0	(1)	25.0	(1)
10	8	26.7	(0.9)	23.0	(1)	26.0	(1)	26.0	(1)	25.0	(1)	25.0	(1)
11	9	26.4	(0.9)	23.0	(1)	25.0	(2)	25.0	(1)	25.0	(1)	25.0	(1)
WEEK 2 MEAN		26.6	(0.2)	22.6	(0.5)	25.2	(0.8)	25.2	(0.8)	24.4	(0.9)	25.0	(0)
14	10	26.3	(0.5)	23.0	(1)	26.0	(1)	25.0	(0)	25.0	(0)	24.0	(1)
15	11	26.4	(1.2)	23.0	(1)	25.0	(1)	25.0	(0)	25.0	(1)	25.0	(1)
16	12	26.7	(0.2)	22.0	(1)	26.0	(1)	25.0	(1)	25.0	(1)	25.0	(1)
17	13	26.9	(0.2)	23.0	(1)	26.0	(1)	26.0	(1)	25.0	(1)	25.0	(1)
18	14	27.0	(0.4)	23.0	(1)	26.0	(1)	25.0	(1)	25.0	(1)	25.0	(1)
WEEK 3 MEAN		26.7	(0.3)	22.8	(0.4)	25.8	(0.4)	25.2	(0.4)	25.0	(0)	24.8	(0.4)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
21	15	26.4 (0.5)	22.0 (1)[g]	25.0 (1)[g]	24.0 (1)[g]	24.0 (1)[g]	24.0 (1)[g]
22	16	26.2 (0.7)	23.0 (1)	26.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)
23	17	26.3 (0.8)	23.0 (1)	26.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)
24	18	26.9 (1)	22.0 (1)	26.0 (1)	25.0 (1)	25.0 (0)	25.0 (1)
25	19	26.0 (0.8)	21.0 (1)	24.0 (1)	24.0 (1)	24.0 (1)	25.0 (1)
WEEK 4 MEAN		26.4 (0.3)	22.2 (0.8)	25.4 (0.9)	25.0 (1)	25.0 (1)	24.8 (0.4)
28	20	27.2 (0.4)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
29	21	26.5 (1)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
30	22	26.9 (0.9)	22.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
31	23	27.5 (0.8)	22.0 (1)	25.0 (1)	25.0 (0)	25.0 (1)	26.0 (1)
32	24	26.5 (0.3)	22.0 (1)	26.0 (1)	25.0 (0)	25.0 (1)	25.0 (1)
WEEK 5 MEAN		26.9 (0.4)	22.4 (0.5)	25.8 (0.4)	25.0 (0)	25.0 (0)	25.2 (0.4)
35	25	27.0 (0.9)	22.0 (1)	26.0 (1)	25.0 (0)	25.0 (0)	25.0 (1)
36	26	26.7 (0.6)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	26.0 (1)
37	27	27.1 (0.6)	24.0 (1)	27.0 (1)	26.0 (1)	27.0 (0)	26.0 (1)
38	28	27.1 (0.3)	23.0 (1)	27.0 (1)	26.0 (0)	26.0 (0)	25.0 (1)
39	29	27.0 (0.5)	23.0 (1)	26.0 (1)	26.0 (0)	26.0 (0)	26.0 (1)
WEEK 6 MEAN		27.0 (0.2)	23.0 (0.7)	26.4 (0.5)	25.6 (0.5)	25.8 (0.8)	25.6 (0.5)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
42	30	27.6	(0.9)	24.0	(1)	27.0	(1)	26.0	(0)	26.0	(0)	26.0	(1)
43	31	27.2	(0.7)	24.0	(1)	27.0	(1)	26.0	(1)	26.0	(0)	26.0	(1)
44	32	26.8	(0.4)	23.0	(1)	27.0	(1)	26.0	(0)	26.0	(0)	25.0	(1)
45	33	26.8	(1.2)	23.0	(1)	27.0	(1)	26.0	(0)	26.0	(0)	26.0	(1)
46	34	27.1	(0.8)	23.0	(1)	26.0	(1)	26.0	(1)	25.0	(1)	25.0	(1)
EEK 7 MEAN		27.1	(0.3)	23.4	(0.5)	26.8	(0.4)	26.0	(0)	25.8	(0.4)	25.6	(0.5)
49	35	26.4	(0.7)	22.0	(1)	25.0	(1)	24.0	(1)	24.0	(1)	24.0	(1)
50	36	26.5	(0.9)	22.0	(1)	26.0	(1)	25.0	(1)	25.0	(0)	25.0	(1)
51	37	26.6	(0.9)	23.0	(1)	27.0	(1)	26.0	(1)	26.0	(0)	25.0	(1)
52	38	26.1	(0.5)	22.0	(1)	25.0	(1)	25.0	(0)	24.0	(1)	24.0	(1)
53	39	26.3	(0.4)	24.0	(1)	27.0	(1)	26.0	(1)	26.0	(1)	25.0	(1)
54	[f]											23.2	(1)
EEK 8 MEAN		26.4	(0.2)	22.6	(0.9)	26.0	(1)	25.2	(0.8)	25.0	(1)	24.4	(0.8)
56	40	27.0	(1.2)	24.0	(1)	28.0	(1)	27.0	(0)	27.0	(0)	26.0	(1)
57	41	27.4	(0.4)	24.0	(1)	27.0	(1)	27.0	(1)	27.0	(0)	26.0	(1)
58	42	27.3	(0.6)	24.0	(1)	27.0	(1)	27.0	(0)	27.0	(0)	26.0	(1)
59	43	27.1	(0.7)	24.0	(1)	27.0	(1)	27.0	(0)	26.0	(0)	26.0	(1)
60	44	27.1	(0.9)	24.0	(1)	27.0	(1)	27.0	(0)	27.0	(0)	26.0	(1)
WEEK 9 MEAN		27.2	(0.2)	24.0	(0)	27.2	(0.4)	27.0	(0)	26.8	(0.4)	26.0	(0)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
64	45	28.0	(0.7)	24.0	(1)	28.0	(1)	27.0	(1)	27.0	(1)	26.0	(1)
65	46	27.2	(1)	24.0	(1)	27.0	(1)	27.0	(0)	27.0	(0)	26.0	(1)
66	47	27.1	(1.3)	23.0	(1)	27.0	(1)	27.0	(0)	26.0	(0)	26.0	(1)
67	48	26.8	(0.9)	24.0	(1)	27.0	(1)	26.0	(0)	26.0	(0)	25.0	(1)
68	49	26.5	(1)	23.0	(1)	27.0	(1)	25.0	(1)	25.0	(0)	26.0	(1)
EEK 10 MEAN		27.1	(0.6)	23.6	(0.5)	27.2	(0.4)	26.4	(0.9)	26.2	(0.8)	25.8	(0.4)
70	50	27.4	(0.7)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
71	51	27.7	(0.4)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
72	52	27.1	(0.7)	25.0	(1)[g]	28.0	(1)[g]	28.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
73	53	27.3	(0.7)	25.0	(1)[g]	28.0	(1)[g]	28.0	(0)[g]	29.0	(0)[g]	26.0	(1)[g]
EEK 11 MEAN		27.4	(0.3)	24.5	(0.6)	28.0	(0)	27.5	(0.6)	27.5	(1)	26.0	(0)
77	54	27.5	(1.1)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
78	55	27.5	(0.5)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
79	56	27.6	(0.7)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
80	57	27.7	(0.7)	24.0	(1)	27.0	(1)	27.0	(1)	27.0	(1)	26.0	(1)
81	58	27.7	(1.2)	25.0	(1)	28.0	(1)	28.0	(1)	27.0	(0)	26.0	(1)
82	59	27.0	(0.7)	24.0	(1)	27.0	(1)	27.0	(1)	27.0	(1)	26.0	(1)
WEEK 12 MEAN		27.5	(0.3)	24.2	(0.4)	27.7	(0.5)	27.2	(0.4)	27.0	(0)	26.0	(0)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
84	60	27.6 (0.9)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (1)	26.0 (1)
85	61	28.1 (1.3)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
86	62	27.8 (1.4)	24.0 (1)	28.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
87	63	27.8 (1.2)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
88	64	27.0 (1.4)	24.0 (1)	27.0 (1)	27.0 (1)	27.0 (1)	26.0 (1)
EEK 13 MEAN		27.7 (0.4)	24.0 (0)	27.2 (0.4)	27.0 (0)	27.0 (0)	26.0 (0)
91	65	27.5 (0.5)	24.0 (1)	28.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
92	66	27.0 (0.3)	23.0 (1)	27.0 (1)	26.0 (0)	27.0 (0)	25.0 (1)
93	67	26.4 (0.3)	[d]	26.0 (1)	26.0 (0)	26.0 (0)	25.0 (1)
94	68	24.5 (0.3)	[d]	24.0 (1)	24.0 (1)	24.0 (1)	24.0 (1)
EEK 14 MEAN		26.4 (1.3)	23.5 (1)	26.3 (1.7)	25.8 (1.3)	26.0 (1.4)	25.0 (0.8)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX D

Mean Daily Chamber Relative Humidity

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER RELATIVE HUMIDITY

EXPLANATORY NOTES

FOOTNOTES:

[a] Daily mean and standard deviation of approximately 12 measurements.

[b] Daily mean and standard deviation of 60 to 77 measurements, except where noted.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Readings in error due to dry wet-bulb thermometer.

[g] Make-up exposure for male mice exposed to 1,3-Butadiene. Daily mean and standard deviation of 12 measurements. The number of days of exposure was subsequently the same for all male mice.

[h] One hour of data not collected due to technical error. Mean and standard deviation of 54 to 60 measurements.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
1	1	43.0	(1.8)[c]	50.0	(9)	40.0	(10)	40.0	(9)	[d]		[d]	
2	2	43.0	(3)	53.0	(11)	45.0	(10)	39.0	(10)	[d]		50.0	(11)
3	3	41.8	(3.4)	54.0	(12)	52.0	(13)	50.0	(13)	47.0	(12)	56.0	(9)
4	4	39.9	(4)	50.0	(8)	56.0	(9)	49.0	(9)	59.0[c]	(8)	55.0	(9)
WEEK 1 MEAN[e]		41.9	(1.5)	51.8	(2.1)	48.3	(7.1)	44.5	(5.8)	53.0	(8.5)	53.7	(3.2)
7	5	41.9	(3.3)	54.0	(9)	61.0	(9)	52.0	(12)	66.0	(10)	51.0	(12)
8	6	46.5	(1.5)	56.0	(10)	69.0	(11)	56.0	(12)	75.0	(11)	55.0	(10)
9	7	47.8	(2.8)	55.0	(11)	63.0	(12)	48.0	(11)	65.0	(16)	64.0	(11)
10	8	41.1	(2.4)	53.0	(11)	59.0	(11)	52.0	(14)	64.0	(12)	57.0	(12)
11	9	40.3	(0.9)	52.0	(11)	61.0	(11)	48.0	(12)	62.0	(13)	63.0	(11)
WEEK 2 MEAN		43.5	(3.4)	54.0	(1.6)	62.6	(3.8)	51.2	(3.3)	66.4	(5)	58.0	(5.5)
14	10	40.7	(1.5)	48.0	(7)	53.0	(9)	52.0	(10)	72.0	(10)	65.0	(12)
15	11	40.9	(0.7)	60.0	(13)	57.0	(8)	48.0	(6)	64.0	(18)	54.0	(11)
16	12	40.7	(0.8)	52.0	(8)	55.0	(6)	46.0	(5)	61.0	(12)	55.0	(9)
17	13	41.5	(1.3)	52.0	(10)	55.0	(10)	45.0	(9)	61.0	(14)	61.0	(12)
18	14	44.0	(2.4)	55.0	(10)	55.0	(10)	49.0	(10)	56.0	(16)	62.0	(11)
WEEK 3 MEAN		41.6	(1.4)	53.4	(4.4)	55.0	(1.4)	48.0	(2.7)	62.8	(5.9)	59.4	(4.7)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
21	15	42.2	(0.8)	60.0	(11)[h]	63.0	(17)[h]	61.0	(12)[h]	88.0	(13)[h]	70.0	(9)[h]
22	16	48.9	(0.4)	56.0	(8)	54.0	(8)	45.0	(8)	54.0	(10)	57.0	(8)
23	17	49.3	(0.4)	58.0	(6)	54.0	(6)	42.0	(7)	79.0	(7)	58.0	(9)
24	18	49.1	(0.4)	57.0	(10)	58.0	(7)	48.0	(11)	62.0	(14)	66.0	(16)
25	19	49.1	(0.3)	56.0	(8)	61.0	(6)	49.0	(10)	64.0	(13)	55.0	(10)
EEK 4 MEAN		47.7	(3.1)	57.4	(1.7)	58.0	(4.1)	49.0	(7.2)	69.4	(13.8)	61.2	(6.5)
28	20	48.7	(0.5)	52.0	(8)	58.0	(8)	48.0	(12)	74.0	(17)	60.0	(8)
29	21	48.5	(0.4)	57.0	(13)	63.0	(11)	47.0	(14)	61.0	(10)	[f]	
10	22	48.6	(0.4)	59.0	(9)	62.0	(7)	45.0	(7)	59.0	(11)	62.0	(14)
31	23	49.0	(0.5)	57.0	(6)	64.0	(5)	45.0	(6)	50.0	(11)	58.0	(8)
32	24	49.1	(0.4)	62.0	(8)	61.0	(6)	43.0	(7)	51.0	(13)	70.0	(10)
EEK 5 MEAN		48.8	(0.3)	57.4	(3.6)	61.6	(2.3)	45.6	(1.9)	59.0	(9.7)	62.5	(5.3)
35	25	45.9	(5.1)	56.0	(6)	60.0	(6)	45.0	(6)	59.0	(10)	64.0	(10)
36	26	41.3	(1.3)	56.0	(9)	59.0	(7)	42.0	(7)	50.0	(10)	61.0	(10)
37	27	46.3	(2.8)	58.0	(6)	61.0	(7)	44.0	(8)	48.0	(9)	59.0	(9)
38	28	49.1	(0.4)	59.0	(8)	57.0	(6)	42.0	(7)	51.0	(10)	61.0	(10)
39	29	42.8	(2.1)	61.0	(7)	59.0	(7)	43.0	(7)	49.0	(10)	57.0	(9)
WEEK 6 MEAN		45.1	(3.1)	58.0	(2.1)	59.2	(1.5)	43.2	(1.3)	51.4	(4.4)	60.4	(2.6)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
42	30	46.3	(3.5)	55.0	(8)	44.0	(5)	45.0	(7)	64.0	(14)	65.0	(11)
43	31	45.4	(1.1)	59.0	(8)	[f]		41.0	(7)	50.0	(13)	61.0	(8)
44	32	46.9	(1.2)	61.0	(10)	47.0	(15)	41.0	(5)	47.0	(8)	58.0	(9)
45	33	47.6	(0.4)	57.0	(7)	54.0	(6)	40.0	(7)	49.0	(14)	61.0	(8)
46	34	46.9	(1.6)	52.0	(7)	58.0	(5)	41.0	(7)	49.0	(11)	63.0	(9)
EEK 7 MEAN		46.6	(0.8)	56.8	(3.5)	50.8	(6.4)	41.6	(1.9)	51.8	(6.9)	61.6	(2.6)
49	35	47.3	(1.3)	63.0	(9)	61.0	(9)	52.0	(14)	69.0	(18)	79.0	(13)
50	36	45.2	(1)	62.0	(7)	55.0	(4)	43.0	(5)	49.0	(8)	66.0	(8)
51	37	44.8	(1)	59.0	(10)	98.0	(6)	41.0	(7)	49.0	(11)	65.0	(9)
52	38	45.8	(1.3)	61.0	(6)	59.0	(4)	42.0	(7)	69.0	(9)	61.0	(14)
53	39	44.6	(0.8)	56.0	(8)	53.0	(7)	40.0	(7)	68.0	(11)	65.0	(12)
54	[g]											44.9	(13.7)
EEK 8 MEAN		45.5	(1.1)	60.2	(2.8)	65.2	(18.6)	43.6	(4.8)	60.8	(10.8)	63.5	(11)
56	40	45.1	(0.6)	52.0	(8)	53.0	(3)	41.0	(7)	81.0	(12)	62.0	(7)
57	41	45.7	(1.2)	58.0	(9)	59.0	(5)	41.0	(8)	68.0	(15)	63.0	(12)
58	42	45.0	(2.2)	60.0	(9)	58.0	(6)	41.0	(8)	69.0	(13)	60.0	(10)
59	43	44.6	(1.3)	58.0	(8)	56.0	(5)	41.0	(7)	67.0	(12)	59.0	(11)
60	44	45.5	(2.1)	57.0	(8)	58.0	(7)	41.0	(8)	59.0	(18)	64.0	(10)
WEEK 9 MEAN		45.2	(0.4)	57.0	(3)	56.8	(2.4)	41.0	(0)	68.8	(7.9)	61.6	(2.1)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
64	45	44.2 (1.2)	61.0 (12)	60.0 (7)	50.0 (12)	53.0 (12)	75.0 (13)
65	46	44.1 (1.2)	56.0 (8)	59.0 (6)	43.0 (8)	42.0 (7)	68.0 (8)
66	47	43.5 (1.6)	66.0 (7)	53.0 (5)	39.0 (6)	47.0 (7)	71.0 (7)
67	48	41.7 (1.1)	56.0 (6)	59.0 (3)	38.0 (3)	44.0 (6)	69.0 (8)
68	49	37.9 (3.1)	59.0 (9)	61.0 (6)	40.0 (6)	46.0 (7)	66.0 (10)
EEK 10 MEAN		42.3 (2.6)	59.6 (4.2)	58.4 (3.1)	42.0 (4.8)	46.4 (4.2)	69.8 (3.4)
70	50	41.7 (0.5)	56.0 (5)[h]	53.0 (2)[h]	39.0 (4)[h]	45.0 (4)[h]	69.0 (7)[h]
71	51	40.7 (1.4)	56.0 (6)[h]	50.0 (2)[h]	38.0 (2)[h]	40.0 (4)[h]	87.0 (17)[h]
72	52	40.5 (1.4)	51.0 (5)[h]	51.0 (2)[h]	36.0 (1)[h]	40.0 (3)[h]	67.0 (6)[h]
73	53	40.1 (1.5)	54.0 (4)[h]	53.0 (2)[h]	38.0 (2)[h]	41.0 (4)[h]	58.0 (6)[h]
EEK 11 MEAN		40.8 (0.7)	54.3 (2.4)	51.8 (1.5)	37.8 (1.3)	41.5 (2.4)	70.3 (12.1)
77	54	40.6 (1.7)	58.0 (5)[h]	51.0 (3)[h]	42.0 (8)[h]	47.0 (8)[h]	73.0 (9)[h]
78	55	41.2 (2)	59.0 (6)[h]	55.0 (2)[h]	37.0 (2)[h]	45.0 (7)[h]	55.0 (6)[h]
79	56	36.5 (1)	58.0 (6)[h]	59.0 (3)[h]	37.0 (2)[h]	41.0 (3)[h]	58.0 (6)[h]
80	57	39.5 (1.8)	61.0 (7)	56.0 (7)	42.0 (9)	45.0 (8)	65.0 (9)
81	58	40.6 (1.7)	61.0 (8)	56.0 (9)	42.0 (11)	44.0 (8)	65.0 (10)
82	59	38.8 (0.9)	62.0 (9)	56.0 (9)	43.0 (11)	44.0 (8)	64.0 (12)
WEEK 12 MEAN		39.5 (1.7)	59.8 (1.7)	55.5 (2.6)	40.5 (2.7)	44.3 (2)	63.3 (6.3)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
84	60	40.5 (1.5)	55.0 (8)	54.0 (6)	41.0 (8)	48.0 (10)	74.0 (10)
85	61	38.8 (2.1)	60.0 (8)	54.0 (6)	41.0 (8)	41.0 (7)	65.0 (10)
86	62	39.2 (1.2)	64.0 (11)	56.0 (6)	40.0 (8)	44.0 (9)	72.0 (10)
87	63	39.1 (2.4)	63.0 (8)	55.0 (6)	40.0 (7)	41.0 (6)	68.0 (10)
88	64	37.4 (1.7)	63.0 (8)	55.0 (6)	41.0 (7)	44.0 (9)	64.0 (13)
EEK 13 MEAN		39.0 (1.1)	61.0 (3.7)	54.8 (0.8)	40.6 (0.5)	43.6 (2.9)	68.6 (4.3)
91	65	42.5 (2.1)	68.0 (9)	59.0 (10)	45.0 (9)	51.0 (10)	73.0 (12)
92	66	39.9 (1.3)	58.0 (6)	52.0 (4)	42.0 (5)	46.0 (7)	67.0 (9)
93	67	41.4 (0.7)	[d]	51.0 (4)	40.0 (5)	46.0 (8)	63.0 (9)
94	68	39.9 (2)	[d]	51.0 (7)	39.0 (9)	40.0 (8)	51.0 (9)
EEK 14 MEAN		40.9 (1.3)	63.0 (7.1)	53.3 (3.9)	41.5 (2.6)	45.8 (4.5)	63.5 (9.3)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX E

Mean Daily Chamber Airflow

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER AIRFLOW

EXPLANATORY NOTES

FOOTNOTES:

[a] Represents one reading taken at start of exposure. Airflow was set and remained constant throughout exposure period.

[b] Daily mean and standard deviation of 60 to 77 measurements, except where noted.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Make-up exposure for male mice exposed to 1,3-Butadiene. One airflow reading was taken at exposure start. The number of days of exposure was subsequently the same for all male mice.

[g] One hour of data not collected due to technical error. Mean and standard deviation of 54 to 60 measurements.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
1	1	60	(0)[c]	35.0	(3)	58.0	(9)	61.0	(9)	[d]		[d]	
2	2	60	(0)	33.0	(3)	60.0	(10)	60.0	(9)	[d]		58.0	(9)
3	3	60	(0)	36.0	(4)	47.0	(10)	58.0	(13)	56.0	(11)	55.0	(10)
4	4	60	(0)	34.0	(2)	50.0	(5)	62.0	(4)	58.0	(3)	59.0	(6)
WEEK 1 MEAN[e]		60	(0)	34.5	(1.3)	53.8	(6.2)	60.3	(1.7)	57.0	(1.4)	57.3	(2.1)
7	5	60	(0)	34.0	(3)	58.0	(13)	64.0	(11)	62.0	(10)	60.0	(9)
8	6	60	(0)	34.0	(2)	65.0	(10)	65.0	(10)	65.0	(9)	62.0	(7)
9	7	60	(0)	34.0	(4)	67.0	(10)	65.0	(10)	66.0	(9)	61.0	(6)
10	8	60	(0)	37.0	(3)	66.0	(13)	63.0	(12)	65.0	(11)	60.0	(10)
11	9	60	(0)	37.0	(3)	67.0	(11)	64.0	(11)	66.0	(11)	58.0	(10)
WEEK 2 MEAN		60	(0)	35.2	(1.6)	64.6	(3.8)	64.2	(0.8)	64.8	(1.6)	60.2	(1.5)
14	10	60	(0)	37.0	(3)	67.0	(6)	66.0	(4)	68.0	(4)	60.0	(1)
15	11	60	(0)	35.0	(2)	67.0	(6)	66.0	(4)	68.0	(4)	60.0	(2)
16	12	60	(0)	36.0	(1)	68.0	(6)	65.0	(4)	68.0	(4)	61.0	(3)
17	13	60	(0)	33.0	(2)	67.0	(5)	66.0	(4)	68.0	(4)	59.0	(1)
18	14	60	(0)	33.0	(2)	65.0	(10)	64.0	(10)	66.0	(9)	59.0	(1)
WEEK 3 MEAN		60	(0)	34.8	(1.8)	66.8	(1.1)	65.4	(0.9)	67.6	(0.9)	59.8	(0.8)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
21	15	60	(0)	34.0	(2)[g]	64.0	(10)[g]	64.0	(11)[g]	67.0	(10)[g]	55.0	(2)[g]
22	16	60	(0)	34.0	(1)	64.0	(6)	66.0	(5)	68.0	(3)	54.0	(1)
23	17	60	(0)	34.0	(1)	63.0	(5)	66.0	(4)	64.0	(16)	58.0	(2)
24	18	60	(0)	34.0	(2)	62.0	(8)	64.0	(8)	66.0	(8)	63.0	(7)
25	19	60	(0)	33.0	(1)	62.0	(6)	65.0	(4)	68.0	(4)	71.0	(28)
WEEK 4 MEAN		60	(0)	33.8	(0.4)	63.0	(1)	65.0	(1)	66.6	(1.7)	60.2	(7)
28	20	60	(0)	33.0	(1)	64.0	(8)	67.0	(8)	65.0	(7)	65.0	(3)
29	21	60	(0)	32.0	(3)	58.0	(16)	59.0	(19)	59.0	(17)	63.0	(12)
30	22	60	(0)	33.0	(1)	64.0	(6)	67.0	(4)	65.0	(3)	65.0	(1)
31	23	60	(0)	33.0	(1)	64.0	(6)	67.0	(5)	67.0	(4)	65.0	(2)
32	24	60	(0)	33.0	(1)	64.0	(6)	67.0	(4)	67.0	(3)	63.0	(2)
WEEK 5 MEAN		60	(0)	32.8	(0.4)	62.8	(2.7)	65.4	(3.6)	64.6	(3.3)	64.2	(1.1)
35	25	60	(0)	32.0	(1)	65.0	(6)	66.0	(4)	66.0	(3)	63.0	(4)
36	26	60	(0)	33.0	(1)	65.0	(6)	66.0	(4)	65.0	(4)	61.0	(2)
37	27	60	(0)	33.0	(1)	66.0	(5)	66.0	(4)	66.0	(3)	63.0	(3)
38	28	60	(0)	33.0	(1)	66.0	(6)	66.0	(4)	65.0	(4)	65.0	(2)
39	29	60	(0)	33.0	(1)	66.0	(6)	66.0	(4)	66.0	(3)	64.0	(1)
WEEK 6 MEAN		60	(0)	32.8	(0.4)	65.6	(0.5)	66.0	(0)	65.6	(0.5)	63.2	(1.5)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
42	30	60	(0)	33.0	(1)	67.0	(6)	67.0	(4)	67.0	(4)	66.0	(3)
43	31	60	(0)	33.0	(1)	67.0	(5)	67.0	(4)	67.0	(3)	64.0	(2)
44	32	60	(0)	33.0	(1)	66.0	(6)	67.0	(4)	67.0	(3)	68.0	(2)
45	33	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	67.0	(4)	69.0	(2)
46	34	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	67.0	(4)	70.0	(2)
WEEK 7 MEAN		60	(0)	33.0	(0)	66.0	(1)	67.0	(0)	67.0	(0)	67.4	(2.4)
49	35	60	(0)	32.0	(2)	61.0	(13)	63.0	(15)	62.0	(13)	68.0	(11)
50	36	60	(0)	33.0	(1)	64.0	(10)	66.0	(10)	66.0	(9)	71.0	(2)
51	37	60	(0)	33.0	(1)	66.0	(5)	67.0	(4)	67.0	(6)	69.0	(2)
52	38	60	(0)	33.0	(1)	65.0	(6)	67.0	(5)	43.0	(2)	70.0	(2)
53	39	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	43.0	(2)	69.0	(1)
54	[f]											60.0	(0)
WEEK 8 MEAN		60	(0)	32.8	(0.4)	64.2	(1.9)	66.0	(1.7)	56.2	(12.2)	67.8	(4)
56	40	60	(0)	33.0	(1)	66.0	(5)	68.0	(4)	43.0	(2)	68.0	(2)
57	41	60	(0)	33.0	(1)	66.0	(5)	68.0	(4)	50.0	(3)	68.0	(1)
58	42	60	(0)	32.0	(1)	66.0	(5)	67.0	(4)	50.0	(3)	67.0	(1)
59	43	60	(0)	33.0	(1)	66.0	(5)	67.0	(4)	50.0	(3)	68.0	(1)
60	44	60	(0)	33.0	(1)	66.0	(5)	68.0	(4)	60.0	(11)	67.0	(1)
WEEK 9 MEAN		60	(0)	32.8	(0.4)	66.0	(0)	67.6	(0.5)	50.6	(6.1)	67.6	(0.5)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
64	45	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	68.0	(4)	66.0	(1)
65	46	60	(0)	33.0	(2)	65.0	(8)	66.0	(7)	67.0	(7)	67.0	(1)
66	47	60	(0)	32.0	(1)	66.0	(6)	68.0	(4)	68.0	(4)	66.0	(1)
67	48	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	69.0	(3)	66.0	(1)
68	49	60	(0)	33.0	(1)	64.0	(6)	67.0	(4)	70.0	(4)	67.0	(2)
WEEK 10 MEAN		60	(0)	32.8	(0.4)	65.0	(0.7)	67.0	(0.7)	68.4	(1.1)	66.4	(0.5)
70	50	60	(0)	33.0	(1)[g]	66.0	(5)[g]	67.0	(4)[g]	70.0	(4)[g]	67.0	(1)[g]
71	51	60	(0)	33.0	(1)[g]	68.0	(5)[g]	68.0	(3)[g]	70.0	(4)[g]	67.0	(1)[g]
72	52	60	(0)	33.0	(1)[g]	65.0	(5)[g]	67.0	(4)[g]	72.0	(4)[g]	67.0	(2)[g]
73	53	60	(0)	33.0	(1)[g]	64.0	(5)[g]	67.0	(4)[g]	71.0	(4)[g]	67.0	(1)[g]
WEEK 11 MEAN		60	(0)	33.0	(0)	65.8	(1.7)	67.3	(0.5)	70.8	(1)	67.0	(0)
77	54	60	(0)	33.0	(1)[g]	63.0	(8)[g]	66.0	(8)[g]	69.0	(7)[g]	66.0	(2)[g]
78	55	60	(0)	33.0	(1)[g]	64.0	(6)[g]	67.0	(4)[g]	70.0	(3)[g]	66.0	(1)[g]
79	56	60	(0)	29.0	(1)[g]	64.0	(6)[g]	67.0	(4)[g]	69.0	(4)[g]	67.0	(2)[g]
80	57	60	(0)	33.0	(1)	64.0	(5)	67.0	(4)	70.0	(4)	69.0	(1)
81	58	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	70.0	(4)	66.0	(3)
82	59	60	(0)	30.0	(2)	64.0	(8)	67.0	(8)	70.0	(8)	66.0	(7)
WEEK 12 MEAN		60	(0)	31.8	(1.8)	64.0	(0.6)	66.8	(0.4)	69.7	(0.5)	66.7	(1.2)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
84	60	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	71.0	(4)	67.0	(2)
85	61	60	(0)	32.0	(1)	65.0	(6)	67.0	(4)	71.0	(4)	67.0	(2)
86	62	60	(0)	32.0	(1)	65.0	(6)	67.0	(4)	70.0	(4)	69.0	(2)
87	63	60	(0)	32.0	(1)	64.0	(5)	67.0	(5)	70.0	(4)	69.0	(1)
88	64	60	(0)	32.0	(1)	64.0	(6)	67.0	(4)	70.0	(4)	69.0	(1)
WEEK 13 MEAN		60	(0)	32.2	(0.4)	64.6	(0.5)	67.0	(0)	70.4	(0.5)	68.2	(1.1)
91	65	60	(0)	32.0	(1)	64.0	(6)	67.0	(4)	70.0	(4)	69.0	(1)
92	66	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	70.0	(3)	69.0	(1)
93	67	60	(0)	[d]		65.0	(6)	68.0	(4)	71.0	(4)	71.0	(2)
94	68	60	(0)	[d]		63.0	(9)	66.0	(10)	69.0	(10)	68.0	(10)
WEEK 14 MEAN		60	(0)	32.5	(1)	64.3	(1)	67.0	(0.8)	70.0	(0.8)	69.3	(1.3)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX F

Individual Body Weights of Rats

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS OF RATS

EXPLANATORY NOTES

NOTES:

All weights are reported in grams

ABBREVIATIONS:

RS - Removed from Study

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP: I CONCENTRATION: 0 ppm

TEST DAYS:	3	10	17	24	31	38	45	52
ANIMAL #								
531423	185.9	241.1	301.9	353.9	395.3	414.2	467.7	499.4
531424	191.2	242.8	307.9	367.1	402.2	425.0	464.1	499.5
531425	165.0	221.5	271.2	314.9	349.4	373.0	398.4	406.4
531426	166.9	205.5	259.9	312.1	355.2	388.2	421.4	450.5
531427	163.6	218.1	279.2	327.0	376.7	422.1	445.5	467.6
531428	180.9	237.8	293.0	354.6	393.3	438.4	467.6	495.2
531429	179.1	237.4	292.8	341.8	383.7	420.6	454.7	484.9
531430	188.6	242.8	308.1	370.0	415.5	461.3	492.0	527.7
531431	141.4	191.5	246.9	288.8	332.9	364.0	380.2	378.0
531432	149.6	202.4	257.9	314.3	349.8	387.6	422.4	439.8

TEST DAYS:	59	66	73	80	87	94
ANIMAL #						
531423	526.2	542.0	561.1	573.1	582.0	553.4
531424	514.7	518.4	558.1	550.9	580.8	542.7
531425	428.7	454.8	460.2	470.0	491.3	469.3
531426	472.4	483.1	500.6	509.9	534.2	509.1
531427	488.9	501.0	513.0	519.4	548.9	519.9
531428	511.7	532.2	557.5	587.3	606.2	564.5
531429	506.6	515.6	531.8	560.6	570.8	546.6
531430	554.2	569.5	593.4	608.5	618.9	593.2
531431	408.4	428.1	454.1	472.0	476.5	452.4
531432	456.4	475.4	483.9	500.2	511.0	482.4

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP: III CONCENTRATION: 250 ppm

TEST DAYS:	3	10	17	24	31	38	45	52
ANIMAL #								
531433	176.8	229.7	281.1	337.9	378.0	412.9	448.4	471.3
531434	169.6	231.6	295.0	351.4	384.6	449.7	481.4	507.4
531435	188.5	242.4	301.7	347.3	373.4	387.0	419.2	450.5
531436	175.6	236.8	297.6	349.4	407.1	422.7	445.2	464.1
531437	202.4	250.3	301.5	350.7	405.0	443.6	465.5	502.0
531438	145.0	189.3	239.1	286.4	326.3	358.4	386.9	402.9
531439	176.7	236.2	278.0	343.7	386.1	414.4	449.3	477.8
531440	171.3	226.5	298.1	342.0	392.1	421.3	458.3	489.7
531441	169.5	214.2	270.8	322.0	374.0	420.6	451.2	482.9
531442	176.9	222.7	286.6	330.6	372.5	410.9	433.3	463.8

TEST DAYS:	59	66	73	80	87	94
ANIMAL #						
531433	506.4	518.1	539.3	553.3	565.0	529.7
531434	551.1	560.7	580.5	594.9	608.1	583.1
531435	465.3	487.3	504.1	515.7	526.6	493.4
531436	488.1	494.6	514.7	530.2	541.9	508.9
531437	532.8	546.2	555.3	580.3	590.9	559.8
531438	423.6	436.1	447.5	462.9	470.3	447.9
531439	484.1	502.6	527.6	549.2	569.8	536.2
531440	513.4	520.1	532.1	552.5	558.0	531.5
531441	511.0	531.7	545.9	570.5	573.2	546.6
531442	491.6	503.2	514.4	538.7	557.9	526.5

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP: V CONCENTRATION: 1000 ppm

TEST DAYS:	3	10	17	24	31	38	45	52
ANIMAL #								
531443	165.1	207.6	275.8	319.0	368.7	408.4	434.2	459.6
531444	182.0	224.9	276.0	320.9	353.2	375.7	406.2	433.1
531445	197.6	243.1	294.3	330.9	374.5	400.5	424.4	454.1
531446	157.1	203.7	257.7	309.8	348.8	378.2	410.0	433.6
531447	165.6	217.5	271.8	324.8	380.7	427.8	439.8	469.9
531448	172.7	217.7	248.6	318.7	350.6	387.8	421.9	453.3
531449	181.1	229.2	284.1	330.3	356.3	386.7	408.7	438.4
531450	182.1	233.4	297.4	334.6	370.1	410.0	442.3	465.4
531451	194.2	235.4	278.5	329.7	365.8	392.2	430.5	392.2
531452	170.5	229.1	287.0	336.9	379.7	417.3	453.4	468.0

TEST DAYS:	59	66	73	80	87	94
ANIMAL #						
531443	478.8	491.3	513.1	517.3	539.6	512.7
531444	451.9	460.9	483.1	497.8	503.4	470.3
531445	459.8	470.0	488.5	502.7	507.9	492.6
531446	455.7	475.3	493.9	504.6	516.5	491.7
531447	500.7	526.2	540.1	570.5	574.7	539.3
531448	464.9	482.5	502.2	493.0	518.5	494.0
531449	451.9	466.8	483.3	481.1	491.3	467.1
531450	484.1	507.2	523.6	546.3	555.1	534.2
531451	449.4	460.9	488.4	493.2	493.2	461.7
531452	501.4	526.1	537.9	550.2	566.5	537.0

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP: VII CONCENTRATION: 1500 ppm

TEST DAYS:	3	10	17	24	31	38	45	52
ANIMAL #								
531453	167.1	214.0	273.2	323.7	357.9	386.1	409.5	425.1
531454	170.1	221.9	259.0	311.5	380.5	409.2	432.8	452.4
531455	177.4	205.2	250.5	335.8	339.7	371.9	404.5	420.1
531456	181.2	220.4	273.7	309.8	339.3	374.2	394.5	428.8
531457	196.0	239.4	281.0	333.0	365.8	386.9	401.6	429.4
531458	153.9	189.3	230.3	287.6	310.4	347.3	379.3	386.0
531459	169.8	215.5	265.4	308.1	349.9	380.1	413.8	437.2
531460	158.9	209.0	260.5	307.6	350.1	389.3	419.9	437.9
531461	175.7	213.7	272.7	321.8	359.7	401.4	426.5	.459.2
531462	180.2	213.7	259.0	299.6	332.5	361.9	393.9	402.6

TEST DAYS:	59	66	73	80	87	94
ANIMAL #						
531453	439.4	444.8	472.4	485.0	491.7	466.7
531454	475.0	488.2	511.2	523.1	535.9	506.2
531455	436.9	451.1	470.7	481.6	501.3	478.9
531456	448.5	458.3	473.5	491.4	497.7	482.7
531457	452.2	469.5	500.7	511.9	536.3	506.9
531458	407.0	413.7	434.8	437.2	447.1	422.2
531459	460.2	481.9	492.0	503.7	503.8	472.7
531460	463.1	471.9	479.6	476.9	477.0	463.2
531461	476.0	492.0	517.3	482.9	542.7	505.7
531462	419.4	432.0	449.5	461.0	466.7	441.5

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE RATS

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TEST DAYS:	3	10	17	24	31	38	45	52
ANIMAL #								
531463	186.9	227.6	287.4	346.6	367.2	390.9	416.7	439.7
531464	157.0	193.1	241.9	286.2	318.5	359.1	387.7	408.5
531465	158.9	202.5	259.1	302.8	342.1	375.3	406.8	431.6
531466	170.2	199.4	249.4	286.5	311.7	349.2	369.8	392.5
531467	187.6	231.8	287.8	329.5	368.6	396.4	432.2	449.9
531468	187.3	229.0	268.1	343.5	389.9	437.0	481.1	505.8
531469	198.6	222.5	265.2	352.8	374.2	440.6	484.7	509.3
531470	179.4	218.8	256.1	309.5	403.1	409.2	443.9	469.9
531471	170.2	211.5	269.2	322.6	371.1	405.2	446.3	477.5
531472	181.4	230.4	276.8	350.0	392.6	437.4	473.2	503.2

TEST DAYS:	59	66	73	80	87	94
ANIMAL #						
531463	459.6	468.3	485.5	489.1	498.4	472.3
531464	431.7	441.6	458.8	474.7	489.5	465.5
531465	460.2	457.1	471.1	497.9	502.7	468.6
531466	408.5	418.3	423.8	442.9	451.8	429.2
531467	468.8	483.1	493.6	514.8	527.2	491.8
531468	550.5	585.2	601.2	595.4	633.8	606.2
531469	539.8	550.3	566.6	589.3	592.9	568.1
531470	499.1	513.5	527.8	549.1	554.5	529.7
531471	497.3	511.5	527.2	551.7	551.9	546.3
531472	535.4	555.3	564.3	574.5	592.7	562.8

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE RATS

GROUP: II CONCENTRATION: 0 ppm

TEST DAYS:	4	11	18	25	32	39	46	53
ANIMAL #								
531478	162.4	185.7	206.0	249.2	262.3	283.8	299.2	310.5
531479	183.7	209.9	247.2	282.0	296.7	331.2	314.6	345.8
531480	138.3	165.3	188.1	205.2	223.9	243.1	246.8	269.1
531481	155.0	176.6	198.0	229.8	247.4	262.4	271.4	289.2
531482	166.2	194.1	228.5	255.3	274.2	296.1	314.2	300.8
531483	158.0	189.2	220.0	241.4	262.4	267.8	283.1	306.1
531484	174.1	201.8	225.0	258.6	271.0	279.8	293.3	312.9
531485	177.3	207.1	236.7	258.4	279.1	302.6	314.2	316.3
531486	136.5	160.9	190.7	222.5	238.0	257.6	270.6	278.0
531487	150.8	170.7	199.4	214.4	243.6	267.8	276.2	282.9

TEST DAYS:	60	67	74	81	88	95
ANIMAL #						
531478	302.1	317.3	319.7	335.1	337.4	299.5
531479	354.6	346.3	375.4	381.0	394.5	380.3
531480	260.9	261.7	272.0	276.2	270.4	252.3
531481	299.0	299.5	311.2	319.1	321.9	312.5
531482	319.5	342.9	352.7	336.7	355.4	350.8
531483	306.5	306.9	319.9	333.5	333.4	317.5
531484	318.5	322.9	338.0	349.2	359.5	349.2
531485	326.9	336.0	325.7	344.9	337.0	330.8
531486	282.7	290.2	305.4	318.4	318.5	306.7
531487	305.8	313.2	321.5	329.1	340.1	330.1

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE RATS

GROUP: IV CONCENTRATION: 250 ppm

TEST DAYS:	4	11	18	25	32	39	46	53
ANIMAL #								
531488	136.1	167.4	192.6	214.0	220.2	243.6	248.5	252.9
531489	152.9	182.9	192.2	222.7	255.8	263.7	273.9	277.6
531490	147.5	184.4	208.5	232.6	240.9	257.0	260.0	280.3
531491	158.6	181.0	203.3	226.9	243.4	263.2	268.9	270.7
531492	166.2	199.4	208.3	273.3	302.6	326.9	340.5	352.8
531493	171.6	206.5	234.4	257.1	263.2	284.3	292.0	296.9
531494	153.1	184.7	232.6	252.0	267.0	287.0	300.5	303.8
531495	144.2	166.1	215.6	217.1	234.0	243.5	250.7	259.2
531496	161.2	179.7	198.9	220.6	254.4	269.8	282.3	292.0
531497	160.3	180.6	205.8	221.5	247.3	255.0	276.2	274.0

TEST DAYS:	60	67	74	81	88	95
ANIMAL #						
531488	264.0	271.6	280.0	294.8	297.0	280.4
531489	293.7	294.4	299.0	315.6	320.5	294.0
531490	275.3	285.8	292.3	292.7	303.7	284.2
531491	284.3	282.6	287.7	305.5	313.0	296.1
531492	358.3	369.3	383.6	406.6	411.0	379.1
531493	297.1	316.4	321.3	328.2	341.5	319.8
531494	326.1	327.5	328.0	321.4	348.8	332.0
531495	266.9	275.8	278.2	280.7	291.2	276.1
531496	298.5	316.2	322.6	325.9	333.4	314.0
531497	296.4	287.6	299.4	319.3	312.7	290.0

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE RATS

GROUP: VI CONCENTRATION: 1000 ppm

TEST DAYS:	4	11	18	25	32	39	46	53
ANIMAL #								
531498	158.3	189.7	216.3	236.9	239.3	272.8	287.7	298.1
531499	168.6	192.2	216.8	235.8	253.6	268.6	286.3	282.8
531500	145.0	164.3	184.5	208.8	226.4	246.0	255.9	263.6
531501	150.8	172.1	189.4	221.7	240.5	258.2	267.5	275.8
531502	165.5	184.5	212.2	231.1	252.0	274.4	273.7	286.7
531503	158.8	179.2	208.0	232.1	252.4	254.6	268.5	271.3
531504	163.5	178.6	199.6	211.1	221.1	238.2	249.2	246.8
531505	185.6	217.1	245.2	279.2	305.9	328.5	332.7	338.3
531506	143.2	165.9	189.8	219.6	239.7	266.5	274.6	287.2
531507	154.2	172.8	200.6	229.9	243.8	259.9	277.3	274.0

TEST DAYS:	60	67	74	81	88	95
ANIMAL #						
531498	293.8	307.9	320.0	330.6	337.9	321.7
531499	298.5	RS (DAY	66)			
531500	272.1	275.1	273.1	285.0	295.1	277.0
531501	289.0	290.8	298.2	312.9	317.0	301.5
531502	298.7	296.3	304.6	310.3	302.5	279.4
531503	283.3	276.6	286.9	290.4	288.6	287.4
531504	257.7	255.8	266.9	280.3	280.6	260.4
531505	358.4	363.9	371.5	388.9	381.9	372.1
531506	284.0	294.8	308.4	314.0	303.9	305.6
531507	274.9	278.5	294.8	305.2	302.3	290.6

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE RATS

GROUP: VIII CONCENTRATION: 1500 ppm

TEST DAYS:	4	11	18	25	32	39	46	53
ANIMAL #								
531508	131.1	154.6	168.8	185.2	194.3	204.2	208.2	215.0
531509	148.5	170.2	192.6	211.1	225.9	240.9	252.4	249.6
531510	147.4	164.7	189.8	209.7	223.9	237.5	245.9	249.1
531511	153.4	177.1	195.3	213.7	219.3	229.9	235.8	238.2
531512	169.7	189.1	220.2	249.3	266.4	287.6	288.3	308.1
531513	189.3	231.6	257.7	278.9	270.9	294.1	316.0	328.8
531514	144.9	164.3	189.5	211.6	229.5	237.5	249.3	258.3
531515	145.6	178.5	206.5	229.3	251.9	273.5	283.3	293.0
531516	158.3	181.8	208.9	214.7	244.4	253.7	274.8	290.0
531517	158.3	180.2	205.1	229.5	230.9	251.0	262.9	263.9

TEST DAYS:	60	67	74	81	88	95
ANIMAL #						
531508	229.7	237.6	241.2	253.1	255.4	249.5
531509	257.9	267.6	275.3	277.3	281.8	262.7
531510	261.6	265.6	267.7	280.8	284.6	275.6
531511	250.6	252.5	263.6	263.7	263.0	255.4
531512	315.2	308.7	329.6	324.4	347.2	328.1
531513	316.0	327.0	334.8	339.7	355.8	341.2
531514	264.7	269.9	276.5	271.7	283.0	247.6
531515	297.5	306.7	314.9	327.0	333.7	321.7
531516	290.1	293.8	309.4	312.3	320.0	311.8
531517	263.9	283.8	295.7	296.5	312.0	285.7

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE RATS

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TEST DAYS:	4	11	18	25	32	39	46	53
ANIMAL #								
531518	151.9	170.8	186.6	219.6	232.6	254.1	259.1	276.5
531519	161.9	177.1	203.7	225.2	242.9	258.8	269.8	277.4
531520	163.4	185.9	213.5	231.0	251.2	257.4	281.9	283.2
531521	168.7	189.5	211.9	243.9	264.7	268.9	288.8	293.9
531522	130.1	151.1	174.4	201.7	220.2	235.5	252.3	261.5
531523	157.6	186.4	211.7	233.5	259.3	276.7	285.9	298.3
531524	147.6	167.1	200.9	222.8	250.8	256.2	267.2	270.1
531525	152.7	190.1	219.9	243.2	267.4	283.7	291.7	299.5
531526	163.9	196.8	209.1	239.3	255.8	279.4	281.4	295.0
531527	172.4	190.0	221.8	247.3	270.6	287.7	304.5	318.9

TEST DAYS:	60	67	74	81	88	95
ANIMAL #						
531518	284.6	292.6	291.4	311.0	315.4	300.6
531519	292.4	287.7	290.9	305.8	302.9	293.2
531520	288.2	305.8	316.5	310.5	306.2	294.4
531521	308.3	314.0	310.1	330.2	330.4	307.6
531522	281.9	274.3	285.1	297.3	296.8	285.9
531523	316.2	311.9	323.7	333.8	339.1	325.9
531524	287.3	290.9	294.8	299.4	305.9	292.9
531525	298.8	309.1	311.3	342.6	348.3	321.5
531526	313.1	297.7	302.4	330.5	324.5	317.0
531527	332.4	328.7	334.7	360.8	370.4	356.5

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX G

Individual Body Weights of Mice

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS OF MICE

EXPLANATORY NOTES

NOTES:

All weights are reported in grams

ABBREVIATIONS:

SE - Sacrificed *in extremis*
NW - Not Weighed
FD - Found Dead
RS - Replaced on Study

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICE

GROUP: I CONCENTRATION: 0 ppm

TEST DAYS:	1	8	15	22	29	36	43	50
ANIMAL #								
91371	24.6	24.8	25.2	26.0	28.8	30.8	33.2	33.6
91372	24.9	25.4	25.0	26.1	28.0	28.2	30.0	29.5
91373	25.8	25.9	28.0	29.2	29.8	29.5	29.9	31.7
91374	27.1	26.7	28.6	29.5	32.0	32.1	33.3	34.6
91375	24.4	26.0	28.2	28.0	29.3	29.0	30.3	30.9
91376	25.2	24.0	25.1	26.3	26.5	29.8	29.3	30.3
91377	25.1	24.9	25.9	25.6	26.8	28.1	28.2	29.7
91378	26.0	26.3	27.6	27.2	29.1	30.5	30.0	29.5
91379	28.3	30.2	28.1	32.3	30.5	30.8	35.7	36.4
91380	24.4	26.8	30.7	29.3	33.1	33.7	32.4	31.7

TEST DAYS:	57	64	71	78	85	92
ANIMAL #						
91371	31.7	30.8	31.8	32.3	32.3	26.2
91372	29.5	30.6	29.7	30.5	31.5	26.7
91373	31.0	33.6	33.7	34.5	34.8	29.2
91374	33.9	33.5	35.9	36.1	37.3	29.9
91375	31.4	32.5	31.4	32.9 SE (DAY 84)		
91376	31.3	31.9	34.3	34.4	33.8	27.6
91377	30.3	34.2	31.6	33.1	34.0	26.9
91378	34.1	30.3	31.2	31.8	31.1	26.1
91379	37.6	36.8	38.1	34.9	35.5	NW
91380	33.2	33.5	35.0	34.3	35.1	28.7

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICE

GROUP: III CONCENTRATION: 50 ppm

TEST DAYS:	1	8	15	22	29	36	43	50
ANIMAL #								
91381	25.2	26.2	28.7	24.0	30.2	31.2	32.0	32.2
91382	25.7	26.0	23.5	23.7	26.4	28.6	30.2	31.1
91383	25.8	26.6	26.6	24.0	29.9	30.3	30.6	31.1
91384	28.6	30.3	31.2	26.0	34.3	35.4	36.6	36.4
91385	23.9	24.5	24.9	16.8	28.8	26.4	28.6	31.1
91386	25.8	27.5	28.2	23.5	30.0	30.8	30.9	30.1
91387	26.4	28.9	28.8	24.4	30.2	30.7	31.7	33.5
91388	25.4	21.3	27.5	23.6	28.5	31.7	32.8	31.6
91389	24.0	26.2	26.5	23.2	29.0	29.5	31.2	29.9
91390	23.4	24.5	25.6	21.8	28.0	27.7	29.3	29.4

TEST DAYS:	57	64	71	78	85	92
ANIMAL #						
91381	33.1	32.8	32.4	32.9	35.3	28.4
91382	31.3	32.6	33.5	34.6	33.0	27.9
91383	31.9	32.0	34.4	35.3	36.4	29.5
91384	38.1	37.6	38.3	38.9	39.6	33.2
91385	32.4	34.6	33.7	34.7	32.6	24.9
91386	30.5	30.9	31.3	30.7	35.5	NW
91387	34.2	32.2	33.5	33.9	34.5	28.9
91388	33.1	34.3	33.0	32.4	33.2	28.2
91389	31.7	32.6	33.4	34.1	34.8	26.6
91390	31.8	30.5	30.1	32.2	32.5	25.4

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICE

GROUP: V CONCENTRATION: 250 ppm

TEST DAYS:	1	8	15	22	29	36	43	50
ANIMAL #								
91391	25.5	22.0	26.6	22.4	28.4	31.2	32.7	32.2
91392	25.9	26.4	28.1	22.9	30.7	24.5	33.2	33.1
91393	25.9	26.5	27.6	24.2	30.3	32.5	31.5	32.1
91394	26.1	25.4	26.9	22.5	28.7	30.2	31.9	32.3
91395	23.4	25.1	27.3	23.4	28.0	28.7	30.5	30.9
91396	24.2	23.3	24.9	20.8	27.1	28.9	29.8	30.5
91397	24.3	22.8	27.4	28.0	28.5	29.8	31.4	31.5
91398	24.9	26.2	26.4	22.7	29.9	31.3	31.3	31.7
91399	25.5	29.0	29.6	30.0	31.3	33.3	35.3	33.7
91400	23.3	24.8	25.7	27.1	28.1	27.7	30.0	30.5

TEST DAYS:	57	64	71	78	85	92
ANIMAL #						
91391	34.2	33.3	34.2	34.8	35.9	27.2
91392	34.3	34.7	34.0	35.2	35.8	27.3
91393	35.6	34.2	32.9	33.5	36.1	26.4
91394	32.7	32.5	35.3	34.5	35.0	27.5
91395	31.3	30.8	32.2	32.6	33.7	28.0
91396	30.7	31.5	32.6	33.9	32.8	25.1
91397	32.9	32.7	32.0	33.7	33.2	26.7
91398	32.0	32.4	32.8	33.6	33.5	27.1
91399	36.5	35.8	37.8	37.8	37.5	32.1
91400	30.9	32.0	32.0	33.1	31.8	27.3

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICE

GROUP: VII CONCENTRATION: 1000 ppm

TEST DAYS:	1	8
ANIMAL #		
91401	24.4	25.3 FD (DAY 12)
91402	24.8	25.7 FD (DAY 11)
91403	26.1	27.6 FD (DAY 11)
91404	27.0	27.4 FD (DAY 11)
91405	24.0	25.9 FD (DAY 12)
91406	25.1	25.0 FD (DAY 11)
91407	25.6	26.5 FD (DAY 11)
91408	24.4	21.9 FD (DAY 12)
91409	26.5	27.4 FD (DAY 11)
91410	23.8	25.2 FD (DAY 11)

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF MALE MICE

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TEST DAYS:	1	8	15	22	29	36	43	50
ANIMAL #								
91411	24.2	26.3	25.8	28.0	29.0	30.1	30.1	32.3
91412	25.0	26.1	27.2	28.5	30.0	28.6	33.4	30.1
91413	26.1	27.7	28.7	29.2	31.5	31.8	33.0	31.9
91414	26.5	26.8	29.2	29.8	31.4	32.3	30.6	29.8
91415	23.1	24.3	24.9	26.6	26.9	30.0	30.1	29.8
91416	24.5	25.4	26.4	26.7	27.1	27.6	27.8	28.1
91417	24.3	24.5	25.4	26.3	29.2	26.7	27.3	27.7
91418	24.8	26.9	27.4	27.6	27.6	28.9	30.7	30.5
91419	26.2	27.6	28.2	29.5	30.9	29.4	30.3	32.2
91420	23.9	24.6	26.3	26.4	29.9	31.3	32.3	31.1

TEST DAYS:	57	64	71	78	85	92
ANIMAL #						
91411	33.9	33.0	33.9	32.6	36.2	30.1
91412	32.7	33.9	35.1	34.2	36.3	29.6
91413	31.2	31.9	32.0	35.0	33.9	27.9
91414	28.9	29.9	30.7	30.6	36.3	30.0
91415	31.7	34.0	34.5	33.8	33.4	27.2
91416	33.0	31.3	32.3	32.9	31.3	26.7
91417	28.3	29.9	29.4	30.5	32.7	26.4
91418	31.2	31.7	32.2	33.2	34.5	28.3
91419	32.9	33.0	33.4	34.1	33.9	29.6
91420	31.8	33.6	34.0	34.5	35.0	31.4

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE MICE

GROUP: II CONCENTRATION: 0 ppm

TEST DAYS:	2	9	16	23	30	37	44	51
ANIMAL #								
91421	21.3	21.4	23.3	25.3	24.4	27.6	26.8	26.5
91422	20.3	22.0	21.4	22.3	25.1	27.2	25.5	25.5
91423	19.7	19.6	23.8	23.7	23.1	25.3	25.3	25.3
91424	19.6	19.6	21.7	21.1	21.5	24.9	24.2	24.4
91425	21.8	21.3	23.5	21.8	24.4	25.3	26.1	26.9
91426	20.6	19.7	22.2	23.6	21.9	25.4	26.6	25.7
91427	21.4	21.9	23.5	24.0	25.7	25.3	27.0	24.8
91428	20.7	20.4	23.2	24.5	23.0	25.3	24.7	28.4
91429	20.4	21.5	19.1	22.9	22.7	25.4	24.8	26.0
91430	20.2	20.2	21.9	23.9	23.6	25.7	25.1	24.4

TEST DAYS:	58	65	72	79	86	93
ANIMAL #						
91421	26.8	28.7	27.4	27.7	28.8	23.9
91422	26.5	27.2	26.2	29.7	29.0	23.8
91423	24.6	27.5	27.7	28.4	29.0	22.0
91424	23.8	26.8	26.2	24.9	26.9	20.6
91425	25.2	27.8	28.2	27.7	26.8	22.9
91426	25.8	27.4	26.2	26.1	27.5	22.9
91427	27.1	28.7	27.3	28.0	28.7	23.8
91428	27.5	26.8	28.0	27.8	27.4	24.0
91429	23.9	25.8	25.0	25.5	26.5	20.3
91430	23.7	26.2	26.3	24.7	26.8	21.7

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE MICE

GROUP: IV CONCENTRATION: 50 ppm

TEST DAYS:	2	9	16	23	30	37	44	51
ANIMAL #								
91431	21.3	21.4	21.8	24.4	23.5	24.9	24.0	24.5
91432	19.5	18.7	20.0	21.7	22.4	21.9	27.1	27.2
91433	20.0	20.3	22.2	24.2	21.2	23.0	23.5	25.7
91434	19.9	20.7	22.6	22.6	23.9	24.2	26.5	25.6
91435	20.9	21.6	24.8	20.9	25.5	26.3	25.6	25.1
91436	20.2	20.4	16.0	22.9	22.7	24.2	24.4	25.9
91437	20.3	21.1	16.5	25.1	23.8	25.7	24.8	24.8
91438	20.2	21.2	21.7	22.7	22.8	25.4	24.6	27.9
91439	22.3 RS (DAY 4)							
91440	20.3	20.7	18.1	23.0	22.7	25.7	24.7	25.6
91471	NW	20.9	20.4	22.0	21.5	23.6	26.0	24.8

TEST DAYS:	58	65	72	79	86	93
ANIMAL #						
91431	26.6	26.9	25.5	27.6	28.2	21.9
91432	26.2	26.6	28.9	24.8	24.4	20.5
91433	25.8	26.3	26.7	27.0	27.5	22.7
91434	23.6	24.1	26.8	26.0	25.5	21.3
91435	25.7	25.7	28.9	27.0	27.7	21.9
91436	25.3	24.8	27.6	29.3	27.6	23.6
91437	26.7	26.6	26.1	25.7	25.9	21.2
91438	27.9	27.3	24.8	26.6	25.4	21.2
91439						
91440	25.7	28.8	27.5	26.7	28.7	22.2
91471	24.9	27.1	26.5	26.6	27.0	20.7

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE MICE

GROUP: VI CONCENTRATION: 250 ppm

TEST DAYS:	2	9	16	23	30	37	44	51
ANIMAL #								
91441	21.6	22.3	23.1	25.1	24.5	25.0	26.1	27.0
91442	20.8	22.5	19.7	24.5	23.7	23.5	26.0	24.8
91443	19.6	21.0	17.4	22.7	20.0	23.7	25.9	25.0
91444	20.4	21.0	18.6	23.0	23.0	25.5	24.9	24.8
91445	19.9	20.2	21.6	23.1	22.6	21.8	21.9	25.2
91446	19.9	20.2	16.2	22.7	22.1	23.7	23.8	21.6
91447	20.7	20.6	20.9	24.1	22.7	24.0	25.3	24.0
91448	20.3	19.7	21.5	21.0	22.5	23.4	24.7	24.9
91449	19.6	19.9	20.2	22.5	21.6	23.8	24.5	23.2
91450	22.0	21.3	22.8	23.9	25.0	25.6	27.1	25.9

TEST DAYS:	58	65	72	79	86	93
ANIMAL #						
91441	28.1	27.2	27.6	28.2	27.3	22.7
91442	25.8	26.3	28.3	29.0	27.8	23.0
91443	26.5	25.7	25.3	25.5	26.8	20.8
91444	23.5	25.8	24.2	27.5	25.6	22.6
91445	24.9	25.0	23.1	25.3	26.1	18.8
91446	24.0	23.8	25.7	24.1	24.1	20.4
91447	24.8	24.3 SE (DAY 70)				
91448	22.7	23.7	23.2	23.5	23.7	18.3
91449	23.7	25.9	26.7	24.9	25.7	20.1
91450	26.8	27.5	26.3	28.2	31.1	22.8

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE MICE

GROUP: VIII CONCENTRATION: 1000 ppm

TEST DAYS:	2	9	16	23	30	37	44	51
ANIMAL #								
91451	21.2	20.7	22.8	24.6	24.0	23.0	24.5	24.1
91452	20.8	22.1	23.9	21.9	23.0	26.7	26.1	27.4
91453	20.7	22.7	22.0	22.5	22.9	24.8	23.4	24.1
91454	20.1	22.3 FD (DAY 12)						
91455	20.7	20.8 FD (DAY 11)						
91456	20.4	20.1	22.4	24.0	25.1	26.3	27.6	26.3
91457	20.4	21.2 FD (DAY 12)						
91458	20.3	21.4	20.8	22.7	22.2	22.3	23.2	23.1
91459	20.2	21.1 FD (DAY 12)						
91460	20.0	22.0 FD (DAY 11)						

TEST DAYS:	58	65	72	79	86	93
ANIMAL #						
91451	24.4	28.0	28.3	27.6	28.9 FD (DAY 88)	
91452	27.4	26.4 FD (DAY 70)				
91453	25.2	25.8	25.0	26.5	26.4 FD (DAY 88)	
91454						
91455						
91456	27.7	29.4	29.4	28.9	28.1	20.0
91457						
91458	23.9	25.2	25.3	25.0	24.8	22.2
91459						
91460						

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL BODY WEIGHTS (g) OF FEMALE MICE

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TEST DAYS:	2	9	16	23	30	37	44	51
ANIMAL #								
91503								
91461	20.4	20.5	22.4	24.3	24.4	25.4	25.2	26.7
91462	20.0	21.6	22.4	24.8	25.5	24.8	25.0	26.8
91463	18.9	20.0	20.8	25.0	23.8	26.9	27.8	28.2
91464	21.8	22.9	20.2	24.7	26.6	26.9	25.8	26.4
91465	19.1	20.8	22.4	23.1	23.9	24.2	24.7	27.3
91466	19.4	21.4	17.3	24.9	25.2	24.5	24.0	24.1
91467	21.0	20.7	22.4	23.5	22.6	26.9	25.8	22.9
91468	19.5	19.6	21.6	23.1	25.2	23.5	23.7	28.1
91469	19.5	19.4	14.8	22.5	21.2	17.4 SE (DAY 38)		
91470	18.9	20.5	21.2	22.0	22.2	24.0	24.6	25.1

TEST DAYS:	58	65	72	79	86	93
ANIMAL #						
91503						
91461	26.3	26.7	27.2	27.6	30.2	23.5
91462	26.8	26.5	28.9	28.1	25.4	21.5
91463	29.5 FD (DAY 64)					
91464	27.4	29.3	27.5	28.2	27.5	22.2
91465	26.6	28.7	27.2	27.6	27.3	22.8
91466	25.5	25.2	26.9	26.7	27.8	22.5
91467	24.8	23.2	24.0	25.8	24.8	23.6
91468	26.4	26.8	27.7	28.4	29.0	20.7
91469						
91470	24.1	25.0	23.7	25.3	27.8	23.8

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX H

Individual Clinical Observations and Mortality Records of Rats

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF RATS

EXPLANATORY NOTES

NOTES:

Post Exposure Observation

At the start of the study, individual clinical signs were recorded weekly and post-exposure observations were noted only on a group basis. Individual post-exposure animal observations were recorded starting on test day 22. No post-exposure clinical signs of toxicity were observed in rats for group II. For all other groups, only those rats with noted signs were reported here.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: I CONCENTRATION: 0 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531423	SACRIFICED BY DESIGN TEST DAY 94		
531424	SACRIFICED BY DESIGN TEST DAY 94		
531425	SACRIFICED BY DESIGN TEST DAY 94		
531426	SACRIFICED BY DESIGN TEST DAY 94		
531427	SACRIFICED BY DESIGN TEST DAY 94		
531428	SACRIFICED BY DESIGN TEST DAY 94		
531429	SACRIFICED BY DESIGN TEST DAY 94		
531430	SACRIFICED BY DESIGN TEST DAY 94		
531431	SACRIFICED BY DESIGN TEST DAY 94		
531432	SACRIFICED BY DESIGN TEST DAY 94		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: III CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531433	SACRIFICED BY DESIGN TEST DAY 94		
531434	SACRIFICED BY DESIGN TEST DAY 94		
531435	SACRIFICED BY DESIGN TEST DAY 94		
531436	SACRIFICED BY DESIGN TEST DAY 94		
531437	SACRIFICED BY DESIGN TEST DAY 94		
531438	AGGRESSIVE	59	94
	SACRIFICED BY DESIGN TEST DAY 94		
531439	SACRIFICED BY DESIGN TEST DAY 94		
531440	SACRIFICED BY DESIGN TEST DAY 94		
531441	DIARRHEA	45	73
	SACRIFICED BY DESIGN TEST DAY 94		
531442	SACRIFICED BY DESIGN TEST DAY 94		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: V CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531443	SACRIFICED BY DESIGN TEST DAY 94		
531444	SACRIFICED BY DESIGN TEST DAY 94		
531445	SACRIFICED BY DESIGN TEST DAY 94		
531446	SACRIFICED BY DESIGN TEST DAY 94		
531447	SACRIFICED BY DESIGN TEST DAY 94		
531448	SACRIFICED BY DESIGN TEST DAY 94		
531449	SACRIFICED BY DESIGN TEST DAY 94		
531450	SACRIFICED BY DESIGN TEST DAY 94		
531451	SACRIFICED BY DESIGN TEST DAY 94		
531452	SACRIFICED BY DESIGN TEST DAY 94		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531453	SACRIFICED BY DESIGN TEST DAY 94		
531454	STAINED CHIN RED	52	66
	SACRIFICED BY DESIGN TEST DAY 94		
531455	STAINED CHIN BROWN SLIGHT	24	87
	DISCHARGE LEFT EYE(S) RED	94	94
	SACRIFICED BY DESIGN TEST DAY 94		
531456	STAINED FACE RED SLIGHT	24	59
	SACRIFICED BY DESIGN TEST DAY 94		
531457	DIARRHEA	17	31
	DISCHARGE MOUTH CLEAR	52	59
	SACRIFICED BY DESIGN TEST DAY 94		
531458	SACRIFICED BY DESIGN TEST DAY 94		
531459	SACRIFICED BY DESIGN TEST DAY 94		
531460	STAINED CHIN RED SLIGHT	24	24
	DIARRHEA	24	24
	SACRIFICED BY DESIGN TEST DAY 94		
531461	SACRIFICED BY DESIGN TEST DAY 94		
531462	SACRIFICED BY DESIGN TEST DAY 94		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531463	SACRIFICED BY DESIGN TEST DAY 94		
531464	SACRIFICED BY DESIGN TEST DAY 94		
531465	AGGRESSIVE SACRIFICED BY DESIGN TEST DAY 94	52	94
531466	SACRIFICED BY DESIGN TEST DAY 94		
531467	SACRIFICED BY DESIGN TEST DAY 94		
531468	SACRIFICED BY DESIGN TEST DAY 94		
531469	SACRIFICED BY DESIGN TEST DAY 94		
531470	SACRIFICED BY DESIGN TEST DAY 94		
531471	SACRIFICED BY DESIGN TEST DAY 94		
531472	SACRIFICED BY DESIGN TEST DAY 94		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: II CONCENTRATION: 0 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531478	SACRIFICED BY DESIGN TEST DAY 95		
531479	SACRIFICED BY DESIGN TEST DAY 95		
531480	SACRIFICED BY DESIGN TEST DAY 95		
531481	SACRIFICED BY DESIGN TEST DAY 95		
531482	SACRIFICED BY DESIGN TEST DAY 95		
531483	SACRIFICED BY DESIGN TEST DAY 95		
531484	SACRIFICED BY DESIGN TEST DAY 95		
531485	SACRIFICED BY DESIGN TEST DAY 95		
531486	SACRIFICED BY DESIGN TEST DAY 95		
531487	SACRIFICED BY DESIGN TEST DAY 95		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: IV CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531488	STAINED NOSE RED BROWN	74	81
	STAINED FACE RED BROWN	74	74
	SACRIFICED BY DESIGN TEST DAY 95		
531489	STAINED BOTH FRONT SHOULDER(S) YELLOW BROWN	74	81
	SACRIFICED BY DESIGN TEST DAY 95		
531490	SACRIFICED BY DESIGN TEST DAY 95		
531491	STAINED FACE YELLOW BROWN	74	81
	SACRIFICED BY DESIGN TEST DAY 95		
531492	SACRIFICED BY DESIGN TEST DAY 95		
531493	SACRIFICED BY DESIGN TEST DAY 95		
531494	SACRIFICED BY DESIGN TEST DAY 95		
531495	SACRIFICED BY DESIGN TEST DAY 95		
531496	SACRIFICED BY DESIGN TEST DAY 95		
531497	SACRIFICED BY DESIGN TEST DAY 95		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531498	SACRIFICED BY DESIGN TEST DAY 95		
531499	REMOVED FROM STUDY TEST DAY 66		
531500	SACRIFICED BY DESIGN TEST DAY 95		
531501	SACRIFICED BY DESIGN TEST DAY 95		
531502	SACRIFICED BY DESIGN TEST DAY 95		
531503	SACRIFICED BY DESIGN TEST DAY 95		
531504	SACRIFICED BY DESIGN TEST DAY 95		
531505	SACRIFICED BY DESIGN TEST DAY 95		
531506	SACRIFICED BY DESIGN TEST DAY 95		
531507	SACRIFICED BY DESIGN TEST DAY 95		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531508	SACRIFICED BY DESIGN TEST DAY 95		
531509	SACRIFICED BY DESIGN TEST DAY 95		
531510	SACRIFICED BY DESIGN TEST DAY 95		
531511	SACRIFICED BY DESIGN TEST DAY 95		
531512	SACRIFICED BY DESIGN TEST DAY 95		
531513	SACRIFICED BY DESIGN TEST DAY 95		
531514	SACRIFICED BY DESIGN TEST DAY 95		
531515	SACRIFICED BY DESIGN TEST DAY 95		
531516	SACRIFICED BY DESIGN TEST DAY 95		
531517	SACRIFICED BY DESIGN TEST DAY 95		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531518	SACRIFICED BY DESIGN TEST DAY 95		
531519	STAINED NOSE RED SLIGHT	60	88
	SACRIFICED BY DESIGN TEST DAY 95		
531520	SACRIFICED BY DESIGN TEST DAY 95		
531521	SACRIFICED BY DESIGN TEST DAY 95		
531522	SACRIFICED BY DESIGN TEST DAY 95		
531523	SACRIFICED BY DESIGN TEST DAY 95		
531524	SACRIFICED BY DESIGN TEST DAY 95		
531525	SACRIFICED BY DESIGN TEST DAY 95		
531526	ALOPECIA RIGHT FRONT PAW(S)	74	95
	SACRIFICED BY DESIGN TEST DAY 95		
531527	SACRIFICED BY DESIGN TEST DAY 95		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: I CONCENTRATION: 0 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531423	STAINED FACE RED SLIGHT	31	35

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: III CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531433	LETHARGY SLIGHT	23	28
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	DISCHARGE NOSE RED SLIGHT	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	46	46
	DISCHARGE MOUTH CLEAR SLIGHT	51	52
	DISCHARGE MOUTH CLEAR SLIGHT	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	82	82
531434	LETHARGY SLIGHT	24	28
	DISCHARGE MOUTH CLEAR SLIGHT	44	44
	DISCHARGE MOUTH CLEAR SLIGHT	46	46
	DISCHARGE MOUTH CLEAR SLIGHT	50	50
	DISCHARGE MOUTH CLEAR RED SLIGHT	51	53
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	DISCHARGE MOUTH CLEAR SLIGHT	67	71
531435	LETHARGY SLIGHT	24	28
	DISCHARGE MOUTH SLIGHT	43	44
	HUNCHED OVER	45	45
	LETHARGY	45	45
	DISCHARGE MOUTH CLEAR SLIGHT	45	46
	DISCHARGE MOUTH CLEAR SLIGHT	50	51
	HUNCHED OVER	51	51
	LETHARGY	52	53
	HUNCHED OVER	53	57
	STAINED FACE YELLOW	59	59
	STAINED HEAD RED SLIGHT	65	65
	STAINED FACE RED	73	73
531436	DISCHARGE MOUTH CLEAR SLIGHT	37	38
	DISCHARGE MOUTH CLEAR SLIGHT	44	44
	HUNCHED OVER	51	51
	LETHARGY	51	51
	DISCHARGE MOUTH CLEAR SLIGHT	60	60
	DISCHARGE MOUTH CLEAR RED SLIGHT	72	72
	STAINED CHIN RED	73	73
	DISCHARGE MOUTH CLEAR SLIGHT	81	81
	STAINED CHIN BROWN	82	82

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: III CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531437	LETHARGY SLIGHT	23	28
	DISCHARGE MOUTH CLEAR SLIGHT	23	28
	DISCHARGE MOUTH CLEAR	37	42
	DISCHARGE MOUTH CLEAR SLIGHT	43	44
	DISCHARGE MOUTH CLEAR SLIGHT	46	46
	DISCHARGE MOUTH CLEAR SLIGHT	50	57
	STAINED NOSE RED BROWN YELLOW	65	65
	STAINED CHIN YELLOW BROWN	65	65
	DISCHARGE MOUTH CLEAR SLIGHT	68	71
	STAINED CHIN RED	84	84
	DISCHARGE MOUTH CLEAR SLIGHT	85	86
531438	DISCHARGE MOUTH CLEAR SLIGHT	24	29
	DISCHARGE MOUTH CLEAR SLIGHT	39	39
	DISCHARGE MOUTH CLEAR SLIGHT	43	44
	DISCHARGE MOUTH CLEAR	45	45
	DISCHARGE MOUTH CLEAR SLIGHT	51	57
	VOCALIZATION	59	59
	STAINED FACE RED BROWN	65	65
	AGGRESSIVE	67	67
	AGGRESSIVE	70	70
	DISCHARGE MOUTH CLEAR SLIGHT	77	77
	DISCHARGE MOUTH CLEAR	84	84
	DISCHARGE MOUTH CLEAR SLIGHT	85	93
531439	DISCHARGE MOUTH CLEAR	39	39
	DISCHARGE RIGHT EYE(S) RED SLIGHT	42	42
	DISCHARGE MOUTH CLEAR	44	44
	DISCHARGE MOUTH CLEAR SLIGHT	45	46
	DISCHARGE MOUTH CLEAR SLIGHT	50	50
	DISCHARGE MOUTH CLEAR MODERATE	51	51
	DISCHARGE MOUTH CLEAR	52	56
	DISCHARGE BOTH EYE(S) RED	53	57
	DISCHARGE BOTH EYE(S) RED SLIGHT	58	60
	DISCHARGE MOUTH CLEAR MODERATE	60	60
	DISCHARGE MOUTH CLEAR SLIGHT	65	66
	DISCHARGE MOUTH	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	68	71
	DISCHARGE NOSE RED	71	71
	STAINED CHIN RED SLIGHT	72	73
	DISCHARGE MOUTH CLEAR SLIGHT	77	77

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: III CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531439	DISCHARGE MOUTH CLEAR SLIGHT	81	92
	STAINED CHIN RED	84	84
531440	DISCHARGE MOUTH CLEAR SLIGHT	24	28
	DISCHARGE MOUTH CLEAR SLIGHT	39	39
	DISCHARGE MOUTH	43	44
	DISCHARGE MOUTH CLEAR SLIGHT	50	58
	DISCHARGE MOUTH CLEAR MODERATE	60	60
	DISCHARGE NOSE RED BROWN	65	65
	DISCHARGE MOUTH CLEAR SLIGHT	66	66
	STAINED CHIN RED	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	68	68
	AGGRESSIVE	70	70
	DISCHARGE MOUTH CLEAR MODERATE	71	71
	DISCHARGE MOUTH CLEAR SLIGHT	77	77
	STAINED CHIN RED	84	84
	DISCHARGE MOUTH CLEAR SLIGHT	85	85
	DISCHARGE MOUTH CLEAR MODERATE	86	88
531441	DISCHARGE MOUTH CLEAR SLIGHT	21	28
	DISCHARGE MOUTH CLEAR	37	37
	DISCHARGE MOUTH CLEAR SLIGHT	39	39
	LETHARGY	42	42
	HUNCHED OVER	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	43	45
	DISCHARGE MOUTH CLEAR SLIGHT	57	58
	DISCHARGE MOUTH CLEAR SLIGHT	67	67
	STAINED CHIN BROWN YELLOW	70	70
	VOCALIZATION	71	71
	STAINED CHIN RED	73	73
	DISCHARGE MOUTH CLEAR SLIGHT	86	86
531442	DISCHARGE MOUTH CLEAR SLIGHT	24	28
	LETHARGY SLIGHT	39	39
	DISCHARGE MOUTH CLEAR SLIGHT	42	42
	DISCHARGE NOSE CLEAR SLIGHT	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	44	44
	LETHARGY	52	53
	DISCHARGE MOUTH CLEAR SLIGHT	66	66
	ALOPECIA FRONT PAW(S)	67	93

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: III CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531442	DISCHARGE NOSE RED	68	68
	LETHARGY	85	85

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: V CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531443	STAINED HEAD BROWN	15	15
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SLIGHT	44	44
	DISCHARGE MOUTH CLEAR SLIGHT	46	46
	HUNCHED OVER	60	60
	STAINED NOSE RED	66	66
	DISCHARGE MOUTH CLEAR	82	82
531444	STAINED CHIN NOSE BROWN SLIGHT	14	14
	DISCHARGE MOUTH NOSE CLEAR MODERATE	21	21
	STAINED CHIN RED SLIGHT	22	35
	LETHARGY	22	28
	DISCHARGE MOUTH CLEAR BROWN MODERATE	28	28
	DISCHARGE MOUTH CLEAR RED SEVERE	35	36
	DISCHARGE MOUTH CLEAR	37	39
	DISCHARGE MOUTH CLEAR SEVERE	42	44
	DISCHARGE NOSE CLEAR SEVERE	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	45	46
	DISCHARGE NOSE CLEAR MODERATE	49	49
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	LETHARGY SLIGHT	50	50
	DISCHARGE MOUTH SLIGHT	51	53
	DISCHARGE MOUTH CLEAR SEVERE	56	58
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	STAINED FACE YELLOW	59	59
	STAINED HEAD YELLOW BROWN	65	65
	STAINED FACE RED BROWN	65	65
	STAINED CHIN YELLOW BROWN	65	65
	STAINED NOSE BROWN RED	67	68
	DISCHARGE MOUTH CLEAR SLIGHT	73	73
	COLORED DISCHARGE MOUTH CLEAR SLIGHT	79	84
	STAINED CHIN BROWN RED	85	85
	STAINED FACE BROWN RED	85	85
531445	STAINED CHIN BROWN	22	23
	LETHARGY SLIGHT	24	29
	DISCHARGE MOUTH CLEAR SLIGHT	31	32
	DISCHARGE MOUTH CLEAR MODERATE	35	37
	DISCHARGE MOUTH CLEAR SLIGHT	38	42
	DISCHARGE NOSE CLEAR SEVERE	42	42
	LETHARGY	42	43

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: V CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531445	DISCHARGE MOUTH CLEAR SLIGHT	45	50
	LETHARGY SLIGHT	50	50
	DISCHARGE MOUTH CLEAR SLIGHT	52	56
	STAINED CHIN YELLOW	57	57
	DISCHARGE MOUTH CLEAR SLIGHT	58	60
	STAINED CHIN YELLOW	66	66
	DISCHARGE MOUTH CLEAR MODERATE	70	70
	COLORED DISCHARGE MOUTH CLEAR SLIGHT	79	81
	STAINED CHIN RED	82	85
531446	DISCHARGE MOUTH CLEAR SLIGHT	36	37
	DISCHARGE MOUTH CLEAR SLIGHT	52	52
	STAINED FACE BROWN YELLOW	65	65
	DISCHARGE MOUTH CLEAR SLIGHT	72	73
	DISCHARGE MOUTH CLEAR SLIGHT	81	84
	DISCHARGE MOUTH CLEAR SLIGHT	91	91
531447	DISCHARGE MOUTH RED SLIGHT	35	39
	DISCHARGE MOUTH CLEAR SLIGHT	45	49
	DISCHARGE MOUTH CLEAR SLIGHT	52	53
	DISCHARGE NOSE RED	59	59
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	DISCHARGE MOUTH RED	67	68
	DISCHARGE MOUTH CLEAR	70	70
531448	STAINED MOUTH BROWN SLIGHT	14	14
	DISCHARGE MOUTH CLEAR SLIGHT	16	25
	DISCHARGE MOUTH CLEAR	28	36
	DISCHARGE MOUTH CLEAR SLIGHT	37	39
	STAINED FACE RED	39	39
	DISCHARGE MOUTH BROWN SEVERE	42	42
	DISCHARGE MOUTH BROWN SLIGHT	43	44
	DISCHARGE MOUTH CLEAR MODERATE	45	46
	DISCHARGE MOUTH CLEAR BROWN SEVERE	49	49
	DISCHARGE NOSE BROWN CLEAR SEVERE	49	49
	STAINED FACE RED YELLOW	50	50
	DISCHARGE MOUTH CLEAR SLIGHT	52	53
	SORE TOE(S)	56	56
	DISCHARGE MOUTH CLEAR MODERATE	56	56
	STAINED FACE RED BROWN	57	57
	DISCHARGE MOUTH CLEAR SLIGHT	59	59

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: V CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531448	DISCHARGE MOUTH RED SLIGHT	60	65
	STAINED CHIN BROWN	66	66
	STAINED CHIN BROWN YELLOW	67	70
	DISCHARGE MOUTH CLEAR RED	70	70
	STAINED CHIN BROWN RED	71	77
531449	STAINED MOUTH RED	14	14
	DISCHARGE MOUTH BROWN SLIGHT	21	22
	DISCHARGE MOUTH CLEAR RED SLIGHT	23	24
	DISCHARGE MOUTH RED SLIGHT	25	38
	DISCHARGE MOUTH CLEAR SEVERE	42	42
	DISCHARGE MOUTH BROWN MODERATE	49	49
	STAINED CHIN YELLOW	53	53
	DISCHARGE MOUTH CLEAR MODERATE	56	56
	STAINED CHIN BROWN YELLOW	57	58
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED CHIN BROWN SLIGHT	65	65
	STAINED CHIN BROWN	66	66
	STAINED CHIN BROWN	68	70
	DISCHARGE MOUTH CLEAR	71	71
	STAINED CHIN BROWN	72	73
	DISCHARGE MOUTH CLEAR SEVERE	77	77
	DISCHARGE MOUTH CLEAR	78	78
	STAINED CHIN RED	82	82
531450	STAINED CHIN BROWN RED	49	49
	STAINED CHIN RED	51	51
	DISCHARGE MOUTH CLEAR SLIGHT	52	56
	STAINED CHIN BROWN	60	60
	STAINED CHIN BROWN	68	72
	STAINED CHIN BROWN RED	77	77
	STAINED CHIN BROWN	79	80
	DISCHARGE MOUTH BROWN CLEAR SEVERE	84	84
531451	STAINED MOUTH BROWN SLIGHT	14	16
	DISCHARGE MOUTH NOSE CLEAR BROWN SEVERE	21	21
	STAINED CHIN BROWN	22	23
	COLORED DISCHARGE NOSE RED	24	30
	COLORED DISCHARGE NOSE CLEAR SLIGHT	31	31
	DISCHARGE NOSE CLEAR SLIGHT	35	35

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: V CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531451	DISCHARGE MOUTH CLEAR SEVERE	35	36
	DISCHARGE MOUTH CLEAR	37	38
	DISCHARGE MOUTH CLEAR MODERATE	42	42
	DISCHARGE NOSE CLEAR MODERATE	42	42
	STAINED CHIN YELLOW BROWN	43	43
	DISCHARGE MOUTH CLEAR SLIGHT	46	46
	STAINED CHIN YELLOW	56	57
	STAINED NOSE RED	66	66
	DISCHARGE MOUTH CLEAR SLIGHT	66	66
	DISCHARGE MOUTH CLEAR SEVERE	77	77
	SORE RIGHT FRONT TOE(S)	86	93
531452	DIARRHEA	42	42
	STAINED CHIN BROWN	44	44
	STAINED FACE RED	49	49
	DISCHARGE MOUTH CLEAR SLIGHT	53	56
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED CHIN BROWN SLIGHT	65	65
	STAINED CHIN BROWN	68	70
	DISCHARGE MOUTH CLEAR SLIGHT	71	71
	VOCALIZATION	77	77

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531453	DISCHARGE MOUTH CLEAR	10	10
	LETHARGY SLIGHT	23	23
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED CHIN BROWN SEVERE	35	35
	DISCHARGE MOUTH CLEAR SLIGHT	37	38
	STAINED FACE RED BROWN	53	53
	LETHARGY	60	60
	STAINED NOSE RED	67	67
	STAINED CHIN RED	82	82
531454	DISCHARGE MOUTH CLEAR	10	14
	STAINED FACE BROWN	18	18
	STAINED MOUTH BROWN	18	18
	DISCHARGE MOUTH CLEAR	21	30
	STAINED CHIN BROWN	22	39
	DISCHARGE MOUTH CLEAR	37	37
	STAINED FACE BROWN SLIGHT	39	39
	LETHARGY SLIGHT	42	42
	DISCHARGE MOUTH CLEAR MODERATE	42	42
	STAINED CHIN BROWN YELLOW	43	44
	STAINED CHIN BROWN YELLOW	46	46
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	DISCHARGE MOUTH CLEAR SEVERE	56	56
	STAINED FACE BROWN YELLOW	57	65
	AGGRESSIVE	60	65
	VOCALIZATION	60	60
	DISCHARGE MOUTH CLEAR MODERATE	65	65
	STAINED FACE BROWN RED	66	66
	STAINED NOSE RED	67	70
	STAINED CHIN BROWN	68	70
	DISCHARGE MOUTH CLEAR SEVERE	77	77
	STAINED CHIN RED	82	82
	STAINED CHIN RED BROWN	86	92
531455	DISCHARGE MOUTH CLEAR	10	15
	DISCHARGE MOUTH CLEAR SLIGHT	16	23
	STAINED NOSE BROWN	18	18
	STAINED BACK YELLOW	18	18
	LETHARGY SLIGHT	21	23
	STAINED FACE BROWN SLIGHT	24	43
	DISCHARGE MOUTH MODERATE	42	43

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531455	STAINED CHIN BROWN	44	44
	STAINED FACE RED	44	44
	DISCHARGE MOUTH CLEAR	45	45
	DISCHARGE MOUTH CLEAR SLIGHT	46	49
	STAINED FACE RED BROWN YELLOW	50	52
	STAINED FACE RED BROWN	53	56
	STAINED CHIN BROWN RED	56	60
	STAINED NOSE BROWN RED	58	60
	STAINED FACE BROWN RED	58	60
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED CHIN YELLOW BROWN SLIGHT	65	66
	STAINED HEAD YELLOW	65	65
	STAINED FACE BROWN RED	66	66
	STAINED NOSE BROWN	67	67
	STAINED FACE BROWN	67	67
	STAINED CHIN BROWN RED	68	72
	DISCHARGE MOUTH CLEAR SEVERE	77	77
	DISCHARGE MOUTH CLEAR SLIGHT	82	82
	STAINED FACE BROWN RED	84	86
	STAINED CHIN BROWN RED	84	84
	STAINED CHIN RED BROWN	86	86
531456	COLORED DISCHARGE MOUTH	10	10
	DISCHARGE MOUTH CLEAR	14	31
	STAINED CHIN RED	23	24
	STAINED FACE BROWN SLIGHT	30	37
	DISCHARGE MOUTH CLEAR SLIGHT	39	39
	DISCHARGE MOUTH CLEAR BROWN RED SEVERE	42	42
	STAINED FACE BROWN	43	43
	LETHARGY	43	43
	DISCHARGE MOUTH CLEAR SLIGHT	44	53
	STAINED FACE RED BROWN	56	56
	STAINED CHIN RED BROWN	56	57
	DISCHARGE MOUTH CLEAR SLIGHT	58	60
	STAINED HEAD YELLOW	65	65
	STAINED CHIN YELLOW BROWN	65	65
	STAINED CHIN BROWN RED	66	66
	STAINED FACE BROWN RED	66	66
	STAINED CHIN RED BROWN	67	67
	STAINED NOSE BROWN RED	68	73
	STAINED CHIN BROWN YELLOW	70	73

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531456	DISCHARGE MOUTH CLEAR SEVERE	77	77
	SORE LEFT REAR TOE(S)	84	93
	STAINED FACE RED BROWN	85	86
	STAINED CHIN RED BROWN	85	86
531457	DISCHARGE MOUTH CLEAR	14	15
	DISCHARGE MOUTH CLEAR	21	21
	LETHARGY SLIGHT	22	28
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED CHIN RED SLIGHT	35	43
	DISCHARGE MOUTH CLEAR MODERATE	37	38
	DISCHARGE MOUTH CLEAR SLIGHT	39	39
	DISCHARGE MOUTH CLEAR SEVERE	42	42
	DISCHARGE MOUTH CLEAR MODERATE	44	44
	LETHARGY	46	46
	DISCHARGE MOUTH CLEAR SEVERE	49	49
	LETHARGY SLIGHT	50	52
	DISCHARGE MOUTH CLEAR MODERATE	50	52
	DISCHARGE MOUTH CLEAR MODERATE	56	56
	STAINED FACE BROWN YELLOW	57	59
	DISCHARGE MOUTH CLEAR SLIGHT	59	60
	STAINED UNDERBODY YELLOW	65	65
	RUFFLED FUR	66	66
	STAINED FACE BROWN RED	66	66
	STAINED CHIN BROWN RED	66	66
	STAINED CHIN BROWN	67	73
	DISCHARGE MOUTH CLEAR	70	71
	DISCHARGE MOUTH CLEAR SEVERE	77	77
	COLORED DISCHARGE MOUTH CLEAR	79	81
	DISCHARGE MOUTH CLEAR SLIGHT	84	91
531458	DISCHARGE MOUTH CLEAR	10	10
	DISCHARGE MOUTH CLEAR SLIGHT	16	18
	DISCHARGE MOUTH CLEAR	21	21
	LETHARGY SLIGHT	22	23
	STAINED CHIN BROWN	24	29
	STAINED CHIN BROWN SLIGHT	30	35
	LETHARGY	31	31
	DISCHARGE MOUTH CLEAR	36	38
	LETHARGY SLIGHT	38	38
	STAINED CHIN BROWN SLIGHT	39	43

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531458	DISCHARGE MOUTH CLEAR SEVERE	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	45	49
	DISCHARGE MOUTH CLEAR MODERATE	56	56
	DISCHARGE MOUTH CLEAR SLIGHT	60	60
	STAINED FACE RED BROWN	65	65
	STAINED CHIN BROWN	67	67
	STAINED CHIN BROWN YELLOW	71	71
	DISCHARGE MOUTH CLEAR	77	77
	STAINED FACE RED	85	88
531459	DISCHARGE MOUTH CLEAR	28	31
	STAINED FACE BROWN SLIGHT	35	35
	STAINED CHIN BROWN SEVERE	35	35
	DISCHARGE MOUTH CLEAR	36	38
	DISCHARGE MOUTH SEVERE	42	42
	STAINED CHIN RED BROWN	53	56
	STAINED FACE RED BROWN YELLOW	56	57
	STAINED HEAD RED BROWN	65	65
	STAINED FACE RED BROWN	65	66
	STAINED CHIN RED	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	81	81
531460	DISCHARGE MOUTH CLEAR	10	10
	DISCHARGE MOUTH CLEAR	16	18
	DISCHARGE MOUTH CLEAR MODERATE	21	31
	LETHARGY SLIGHT	29	29
	STAINED FACE RED SLIGHT	35	35
	DISCHARGE MOUTH CLEAR	36	38
	STAINED CHIN BROWN SLIGHT	38	38
	DISCHARGE MOUTH CLEAR SEVERE	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	43	44
	DISCHARGE MOUTH CLEAR MODERATE	46	46
	STAINED FACE BROWN	49	49
	STAINED FACE RED YELLOW	52	53
	DISCHARGE BOTH EYE(S)	56	56
	STAINED FACE BROWN YELLOW	57	57
	STAINED FACE BROWN YELLOW RED	58	60
	DISCHARGE MOUTH CLEAR SLIGHT	59	60
	VOCALIZATION	60	60
	STAINED FACE RED BROWN	65	65
	STAINED CHIN RED BROWN	65	65

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531460	AGGRESSIVE	67	67
	DISCHARGE MOUTH CLEAR MODERATE	71	71
	STAINED CHIN BROWN	72	72
	VOCALIZATION	78	78
	STAINED CHIN RED	84	84
	DISCHARGE MOUTH CLEAR SLIGHT	85	86
531461	DISCHARGE MOUTH CLEAR	14	14
	LETHARGY SLIGHT	21	23
	STAINED FACE RED SLIGHT	24	35
	DISCHARGE MOUTH CLEAR	28	30
	DISCHARGE MOUTH CLEAR SLIGHT	35	38
	DISCHARGE MOUTH CLEAR SEVERE	42	42
	STAINED CHIN YELLOW	44	44
	STAINED NOSE RED	45	46
	DISCHARGE MOUTH CLEAR	45	45
	DISCHARGE MOUTH CLEAR	49	49
	STAINED CHIN YELLOW	52	52
	DISCHARGE MOUTH CLEAR SLIGHT	59	60
	STAINED NOSE RED BROWN	65	65
	STAINED CHIN YELLOW	66	66
	STAINED CHIN BROWN	67	67
	RUFFLED FUR	67	67
	STAINED CHIN BROWN YELLOW	70	72
	DISCHARGE MOUTH CLEAR SEVERE	77	77
	COLORED DISCHARGE CHIN RED	79	80
531462	DISCHARGE MOUTH CLEAR	21	23
	DISCHARGE MOUTH CLEAR SLIGHT	28	31
	DISCHARGE MOUTH CLEAR SLIGHT	38	38
	DISCHARGE MOUTH CLEAR SEVERE	42	42
	DISCHARGE MOUTH CLEAR MODERATE	45	45
	LETHARGY	65	65
	DISCHARGE MOUTH CLEAR	77	77
	SORE TOE(S)	92	93

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531463	STAINED FACE BROWN	14	14
	DISCHARGE MOUTH CLEAR	37	50
	STAINED FACE RED SLIGHT	38	38
	STAINED NOSE RED BROWN SLIGHT	42	43
	STAINED FACE RED	50	53
	DISCHARGE MOUTH CLEAR SLIGHT	51	65
	STAINED FACE RED BROWN	56	65
	STAINED CHIN RED BROWN	56	56
	STAINED CHIN RED BROWN	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	71	71
	STAINED CHIN BROWN	71	73
	DISCHARGE MOUTH CLEAR SLIGHT	77	78
531464	STAINED NOSE BROWN	14	14
	DISCHARGE MOUTH CLEAR	37	43
	STAINED NOSE BROWN SLIGHT	42	43
	STAINED FACE RED SLIGHT	44	44
	DISCHARGE MOUTH CLEAR	46	67
	STAINED CHIN RED BROWN	50	51
	STAINED FACE RED	51	56
	STAINED CHIN BROWN RED	56	66
	STAINED UNDERBODY YELLOW	58	60
	SORE TOE(S)	65	93
	STAINED NOSE RED BROWN	65	67
	STAINED CHIN BROWN RED	71	72
	STAINED NOSE RED BROWN	71	71
	STAINED CHIN BROWN	77	80
	STAINED FACE BROWN RED	84	86
	STAINED NOSE RED BROWN	86	86
531465	STAINED FACE RED SLIGHT	30	31
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SEVERE	42	43
	STAINED NOSE RED SLIGHT	42	43
	STAINED CHIN RED SLIGHT	42	51
	DISCHARGE MOUTH CLEAR SLIGHT	44	51
	STAINED FACE RED	53	58
	DISCHARGE MOUTH CLEAR SLIGHT	53	57
	AGGRESSIVE	59	71
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	STAINED CHIN RED BROWN	65	66

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531465	VOCALIZATION	67	71
	DISCHARGE MOUTH CLEAR SLIGHT	67	67
	STA ED CHIN BROWN YELLOW	71	72
	AGG SSIVE	77	78
	DISCHARGE MOUTH CLEAR SLIGHT	85	86
	VOCALIZATION	86	87
531466	STAINED CHIN BROWN SLIGHT	14	43
	STAINED FACE RED SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SLIGHT	35	42
	STAINED FACE RED BROWN SLIGHT	38	65
	STAINED CHIN RED	49	56
	DISCHARGE MOUTH CLEAR	50	57
	SORE TOE(S)	59	71
	STAINED CHIN BROWN	66	66
	DISCHARGE MOUTH CLEAR SLIGHT	67	67
	STAINED CHIN YELLOW BROWN	71	72
	STAINED FACE YELLOW BROWN	71	71
	DISCHARGE MOUTH CLEAR SLIGHT	77	78
	COLORED DISCHARGE MOUTH CLEAR	81	82
	DISCHARGE MOUTH CLEAR	84	87
	SORE TOE(S)	84	86
	SORE LEFT REAR TOE(S)	93	93
531467	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SLIGHT	42	51
	STAINED CHIN RED BROWN MODERATE	42	51
	STAINED CHIN RED BROWN	50	51
	DISCHARGE MOUTH SLIGHT	53	56
	STAINED FACE BROWN RED	56	56
	STAINED FACE RED BROWN	58	58
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	STAINED CHIN BROWN	70	71
	DISCHARGE MOUTH CLEAR SLIGHT	70	70
	STAINED FACE RED	73	73
531468	STAINED CHIN BROWN YELLOW	21	25
	STAINED CHIN BROWN SLIGHT	29	35
	STAINED FACE RED BROWN SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	DISCHARGE MOUTH CLEAR	37	52

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531468	STAINED NOSE RED SLIGHT	42	42
	STAINED CHIN RED BROWN SLIGHT	42	52
	STAINED FACE RED BROWN	44	50
	DISCHARGE MOUTH CLEAR SLIGHT	56	58
	STAINED CHIN YELLOW	71	73
	STAINED CHIN YELLOW SLIGHT	77	81
	DISCHARGE MOUTH CLEAR MODERATE	86	86
	STAINED FACE RED	87	88
531469	DISCHARGE MOUTH CLEAR SLIGHT	30	31
	STAINED CHIN BROWN	31	51
	DISCHARGE MOUTH CLEAR SEVERE	38	39
	DISCHARGE MOUTH CLEAR MODERATE	43	44
	STAINED NOSE RED BROWN SLIGHT	43	43
	DISCHARGE MOUTH CLEAR MODERATE	49	50
	DISCHARGE MOUTH CLEAR SLIGHT	51	51
	LETHARGY SLIGHT	53	56
	DISCHARGE MOUTH CLEAR SLIGHT	53	57
	STAINED CHIN RED	53	56
	STAINED CHIN BROWN RED	58	66
	STAINED FACE RED BROWN	60	65
	STAINED UNDERBODY YELLOW BROWN	65	65
	DISCHARGE MOUTH CLEAR SLIGHT	70	70
	STAINED FACE RED BROWN	85	88
	STAINED CHIN BROWN RED	85	85
531470	STAINED CHIN BROWN SLIGHT	23	25
	DISCHARGE MOUTH CLEAR SLIGHT	31	32
	DISCHARGE MOUTH CLEAR	37	43
	STAINED CHIN BROWN SLIGHT	43	51
	STAINED FACE RED BROWN	49	51
	DISCHARGE MOUTH CLEAR SLIGHT	50	51
	DISCHARGE MOUTH CLEAR SLIGHT	53	53
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED CHIN RED BROWN YELLOW	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	70	70
	STAINED FACE RED	73	73
531471	DISCHARGE MOUTH CLEAR SLIGHT	30	31
	STAINED CHIN BROWN SLIGHT	31	31

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE RATS

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531471	STAINED CHIN BROWN SLIGHT	39	53
	DISCHARGE MOUTH CLEAR SLIGHT	42	42
	DISCHARGE MOUTH CLEAR MODERATE	43	43
	DISCHARGE MOUTH RED	49	57
	STAINED FACE RED BROWN SLIGHT	51	53
	STAINED CHIN BROWN SLIGHT	65	65
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED CHIN RED	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	67	67
	STAINED CHIN YELLOW BROWN	71	80
	STAINED FACE YELLOW BROWN	71	71
	STAINED CHIN RED	82	82
	STAINED CHIN YELLOW	84	87
	SORE LEFT REAR TOE(S)	85	86
	DISCHARGE MOUTH CLEAR SLIGHT	86	86
531472	STAINED CHIN BROWN SLIGHT	23	32
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED CHIN BROWN MODERATE	35	38
	STAINED FACE BROWN SLIGHT	38	38
	STAINED NOSE BROWN SLIGHT	42	51
	STAINED CHIN BROWN SLIGHT	42	50
	DISCHARGE MOUTH CLEAR MODERATE	43	43
	STAINED FACE RED BROWN	50	50
	DISCHARGE MOUTH CLEAR SLIGHT	53	53
	DISCHARGE MOUTH CLEAR SLIGHT	59	65
	STAINED CHIN BROWN SLIGHT	65	65
	DISCHARGE MOUTH CLEAR SLIGHT	70	70
	STAINED CHIN RED BROWN	71	71

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: IV CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531488	STAINED FACE RED	22	30
	STAINED CHIN RED SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SLIGHT	39	39
	STAINED NOSE RED BROWN	65	65
	DISCHARGE MOUTH RED CLEAR	70	70
	STAINED FACE RED	71	73
	STAINED FACE RED BROWN	86	86
531489	DISCHARGE MOUTH CLEAR SLIGHT	52	52
	AGGRESSIVE	53	53
	STAINED LEFT SHOULDER(S) BROWN	58	60
	STAINED NOSE BROWN RED	65	65
	STAINED FACE RED	70	71
531490	STAINED CHIN RED SLIGHT	22	22
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SLIGHT	35	39
	STAINED FACE RED	50	51
	DISCHARGE MOUTH CLEAR SLIGHT	53	56
	STAINED FACE BROWN	56	56
	STAINED NOSE BROWN	59	59
	STAINED NOSE BROWN RED	65	65
	STAINED FACE BROWN RED	66	68
	DISCHARGE MOUTH CLEAR RED	70	70
	STAINED NOSE RED	71	71
	DISCHARGE MOUTH CLEAR SLIGHT	72	72
	STAINED FACE BROWN	73	73
	STAINED CHIN BROWN RED	73	73
531491	STAINED FACE RED SLIGHT	29	29
	STAINED FACE RED MODERATE	30	35
	DISCHARGE MOUTH CLEAR SLIGHT	37	38
	STAINED FACE BROWN	39	39
	DISCHARGE NOSE RED SLIGHT	42	42
	STAINED FACE RED	42	43
	DISCHARGE MOUTH CLEAR SLIGHT	44	44
	STAINED NOSE RED	45	46
	STAINED FACE RED	50	51
	DISCHARGE MOUTH CLEAR SLIGHT	53	56
	STAINED FACE BROWN	65	65
	STAINED NOSE BROWN RED	65	77

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: IV CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531491	STAINED CHIN BROWN RED	65	65
	STAINED FACE BROWN RED	67	67
	STAINED FACE RED BROWN	70	71
	SORE RIGHT REAR TOE(S	78	94
	STAINED NOSE RED BROWN	86	86
531492	DISCHARGE MOUTH CLEAR SLIGHT	24	38
	STAINED FACE BROWN	39	39
	DISCHARGE MOUTH CLEAR SLIGHT	43	45
	DISCHARGE MOUTH CLEAR	49	50
	DISCHARGE MOUTH CLEAR SLIGHT	51	51
	DISCHARGE MOUTH CLEAR SLIGHT	53	71
	STAINED FACE RED BROWN	65	65
	STAINED CHIN BROWN	72	77
	DISCHARGE MOUTH CLEAR SLIGHT	82	82
531493	STAINED FACE RED SLIGHT	31	31
	STAINED FACE RED SLIGHT	35	35
	STAINED FACE BROWN	39	39
	STAINED FACE RED	43	44
	STAINED NOSE RED	45	46
	STAINED FACE RED	49	51
	STAINED FACE RED	57	57
	STAINED NOSE RED	66	68
	STAINED NOSE RED BROWN	86	86
531494	DISCHARGE MOUTH CLEAR SLIGHT	31	32
	DISCHARGE NOSE RED	45	45
	DISCHARGE MOUTH CLEAR	91	91
531495	DISCHARGE MOUTH CLEAR SLIGHT	53	56
	STAINED NOSE RED BROWN	86	86
531496	DISCHARGE NOSE RED SLIGHT	35	35
	DISCHARGE MOUTH CLEAR SLIGHT	35	35
	DISCHARGE MOUTH CLEAR SLIGHT	39	39
	DISCHARGE MOUTH RED SLIGHT	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	44	44
	DISCHARGE MOUTH CLEAR SLIGHT	52	56
	DISCHARGE MOUTH CLEAR SLIGHT	60	66
	DISCHARGE MOUTH CLEAR SLIGHT	70	70

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: IV CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531496	STAINED CHIN RED	72	72
	DISCHARGE MOUTH CLEAR SLIGHT	84	85
531497	DISCHARGE MOUTH CLEAR SLIGHT	35	38
	DISCHARGE MOUTH CLEAR SLIGHT	49	49
	SORE LEFT REAR PAW(S)	71	80
	DISCHARGE MOUTH CLEAR SLIGHT	81	81

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531498	STAINED FACE BROWN RED SLIGHT	39	39
	DISCHARGE MOUTH CLEAR	50	50
	DISCHARGE MOUTH CLEAR SLIGHT	52	52
	STAINED FACE RED	53	53
	DISCHARGE MOUTH CLEAR SLIGHT	56	56
	STAINED NOSE RED	58	65
	STAINED NOSE BROWN YELLOW	65	65
	STAINED FACE RED	66	66
	STAINED NOSE RED	67	67
	DISCHARGE MOUTH CLEAR SLIGHT	72	73
	STAINED FACE BROWN RED	86	86
	DISCHARGE MOUTH CLEAR SLIGHT	92	93
531499	DISCHARGE MOUTH CLEAR	14	14
	STAINED CHIN BROWN SLIGHT	31	31
	DISCHARGE MOUTH CLEAR SLIGHT	37	45
	DISCHARGE MOUTH CLEAR	50	56
	STAINED NOSE RED BROWN	59	65
	STAINED FACE RED BROWN	65	65
531500	DISCHARGE MOUTH CLEAR	14	14
	DISCHARGE MOUTH RED	24	29
	DISCHARGE MOUTH CLEAR SLIGHT	30	38
	STAINED FACE BROWN SLIGHT	39	39
	DISCHARGE NOSE CLEAR	42	42
	DISCHARGE MOUTH CLEAR	45	45
	DISCHARGE MOUTH CLEAR SEVERE	49	50
	DISCHARGE NOSE RED SEVERE	49	50
	DISCHARGE MOUTH CLEAR SLIGHT	52	56
	STAINED FACE BROWN YELLOW RED	53	53
	STAINED NOSE RED	59	59
	STAINED CHIN BROWN	60	65
	STAINED FACE RED BROWN	65	66
	STAINED CHIN BROWN	67	73
	DISCHARGE MOUTH	77	81
	STAINED FACE BROWN RED	86	86
	DISCHARGE MOUTH CLEAR SLIGHT	92	93
	STAINED CHIN BROWN	92	92
531501	LETHARGY	22	25
	DISCHARGE MOUTH CLEAR SLIGHT	36	39

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531501	STAINED FACE BROWN RED SLIGHT	39	42
	STAINED FACE RED	44	44
	DISCHARGE MOUTH CLEAR SLIGHT	46	46
	DISCHARGE MOUTH BROWN SLIGHT	49	49
	STAINED FACE RED BROWN	50	50
	STAINED FACE RED	53	53
	DISCHARGE MOUTH CLEAR SEVERE	56	56
	DISCHARGE MOUTH CLEAR RED SLIGHT	57	57
	STAINED FACE RED BROWN	65	65
	STAINED CHIN BROWN RED	67	67
	STAINED CHIN BROWN	70	70
	DISCHARGE MOUTH RED BROWN MODERATE	77	77
531502	STAINED FACE RED	22	22
	DISCHARGE MOUTH CLEAR	36	37
	DISCHARGE MOUTH CLEAR MODERATE	45	45
	STAINED NOSE RED	67	67
	DISCHARGE MOUTH RED BROWN MODERATE	77	77
	DISCHARGE MOUTH CLEAR SLIGHT	81	81
	DISCHARGE MOUTH CLEAR	91	92
531503	STAINED CHIN BROWN RED	22	25
	DISCHARGE MOUTH CLEAR SEVERE	28	38
	DISCHARGE MOUTH BROWN SLIGHT	42	42
	STAINED CHIN RED	43	43
	DISCHARGE MOUTH CLEAR SLIGHT	44	45
	STAINED CHIN YELLOW	46	46
	DISCHARGE MOUTH CLEAR SEVERE	49	50
	DISCHARGE MOUTH CLEAR SEVERE	56	56
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED NOSE RED	67	67
	STAINED CHIN BROWN YELLOW	70	70
	DISCHARGE MOUTH CLEAR SEVERE	77	77
	SORE TOE(S)	78	94
531504	STAINED FACE RED	22	29
	DISCHARGE MOUTH CLEAR SLIGHT	30	38
	DISCHARGE MOUTH CLEAR MODERATE	42	43
	DISCHARGE MOUTH RED SLIGHT	44	44
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	DISCHARGE MOUTH CLEAR SLIGHT	50	52

SUBCHRQNIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531504	DISCHARGE MOUTH	56	56
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED FACE RED BROWN	65	65
	STAINED NOSE RED	67	67
	STAINED CHIN RED	67	67
	SORE RIGHT FRONT PAW(S)	67	67
	STAINED FACE RED	73	73
531505	DISCHARGE MOUTH CLEAR SLIGHT	23	24
	DISCHARGE MOUTH SLIGHT	25	25
	DISCHARGE MOUTH CLEAR MODERATE	28	30
	DISCHARGE MOUTH CLEAR SLIGHT	31	38
	STAINED FACE RED SLIGHT	39	39
	DISCHARGE NOSE CLEAR SEVERE	42	42
	DISCHARGE NOSE CLEAR SEVERE	42	42
	STAINED CHIN RED	43	44
	DISCHARGE MOUTH CLEAR SLIGHT	46	46
	DISCHARGE MOUTH CLEAR SLIGHT	53	53
531506	DISCHARGE MOUTH CLEAR SLIGHT	23	42
	STAINED FACE RED SLIGHT	35	35
	DISCHARGE MOUTH CLEAR SLIGHT	45	45
	DISCHARGE MOUTH RED SLIGHT	50	52
	STAINED FACE BROWN	56	56
531507	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED CHIN RED SLIGHT	35	35
	DISCHARGE MOUTH CLEAR SLIGHT	37	37
	DISCHARGE MOUTH CLEAR SLIGHT	67	67

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531508	STAINED CHIN BROWN SLIGHT	14	21
	DISCHARGE MOUTH CLEAR	15	31
	LETHARGY SLIGHT	21	22
	STAINED CHIN	31	38
	STAINED FACE BROWN SLIGHT	35	38
	DISCHARGE MOUTH CLEAR MODERATE	37	38
	STAINED CHIN YELLOW	43	43
	STAINED FACE YELLOW	49	49
	STAINED CHIN YELLOW	51	51
	DISCHARGE MOUTH SLIGHT	53	57
	STAINED CHIN YELLOW	58	59
	DISCHARGE MOUTH CLEAR MODERATE	77	77
	SORE LEFT FRONT TOE(S)	87	94
531509	STAINED CHIN BROWN SLIGHT	14	15
	DISCHARGE MOUTH CLEAR	22	22
	DISCHARGE MOUTH CLEAR	28	29
	DISCHARGE MOUTH CLEAR SLIGHT	30	31
	STAINED FACE BROWN SLIGHT	35	37
	STAINED CHIN BROWN SLIGHT	35	35
	DISCHARGE MOUTH CLEAR	37	37
	DISCHARGE MOUTH SLIGHT	39	39
	DISCHARGE MOUTH CLEAR MODERATE	42	42
	DISCHARGE NOSE CLEAR MODERATE	49	49
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	DISCHARGE MOUTH CLEAR SLIGHT	51	51
	DISCHARGE MOUTH SLIGHT	53	56
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	DISCHARGE MOUTH CLEAR SLIGHT	65	65
	STAINED CHIN YELLOW	67	67
	STAINED CHIN RED	73	73
	DISCHARGE MOUTH CLEAR MODERATE	77	77
531510	STAINED UNDERBODY YELLOW	14	14
	LETHARGY	21	23
	DISCHARGE MOUTH BROWN CLEAR SEVERE	21	22
	STAINED FACE YELLOW	22	22
	STAINED FACE BROWN	24	38
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	DISCHARGE NOSE CLEAR MODERATE	49	49
	DISCHARGE MOUTH CLEAR MODERATE	49	49

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531510	DISCHARGE MOUTH CLEAR SLIGHT	51	56
	DISCHARGE MOUTH CLEAR SLIGHT	59	59
	RUFFLED FUR	67	67
	STAINED CHIN YELLOW	67	71
	DISCHARGE MOUTH CLEAR MODERATE	77	77
	DISCHARGE MOUTH CLEAR	82	82
	STAINED CHIN YELLOW BROWN	84	87
531511	LETHARGY	21	21
	ALOPECIA LEFT PAW(S)	39	42
	DISCHARGE MOUTH CLEAR MODERATE	42	42
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	STAINED CHIN YELLOW	67	67
	DISCHARGE MOUTH CLEAR RED	82	82
531512	DISCHARGE MOUTH CLEAR	21	22
	DISCHARGE MOUTH CLEAR SLIGHT	37	43
	DISCHARGE NOSE CLEAR MODERATE	49	49
	DISCHARGE MOUTH CLEAR SLIGHT	51	51
	DISCHARGE MOUTH CLEAR MODERATE	56	56
	STAINED FACE RED BROWN	56	56
	DISCHARGE MOUTH CLEAR SLIGHT	58	59
	STAINED CHIN BROWN	60	60
	STAINED NOSE BROWN SLIGHT	65	66
	STAINED CHIN BROWN	67	67
	STAINED CHIN BROWN YELLOW	71	73
	DISCHARGE MOUTH CLEAR SLIGHT	86	86
531513	LETHARGY	21	21
	STAINED FACE YELLOW	22	22
	STAINED FACE RED SLIGHT	23	23
	DISCHARGE MOUTH CLEAR SLIGHT	29	31
	DISCHARGE MOUTH CLEAR MODERATE	42	43
	STAINED PERINEUM YELLOW	44	44
	STAINED FACE RED	49	49
	STAINED CHIN YELLOW	50	51
	DISCHARGE MOUTH SLIGHT	53	56
	STAINED CHIN BROWN YELLOW	57	57
	DISCHARGE MOUTH CLEAR SLIGHT	59	60
	STAINED PERINEUM BROWN YELLOW	65	65
	STAINED CHIN BROWN YELLOW	71	73

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531513	DISCHARGE MOUTH CLEAR MODERATE	77	77
	STAINED CHIN BROWN YELLOW	84	84
531514	DISCHARGE MOUTH CLEAR SLIGHT	36	39
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	STAINED NOSE YELLOW	65	65
	STAINED FACE RED	73	73
531515	DISCHARGE MOUTH CLEAR	21	21
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	STAINED NOSE YELLOW	65	65
531516	LETHARGY	21	23
	DISCHARGE MOUTH CLEAR	21	21
	DISCHARGE MOUTH CLEAR	28	28
	STAINED FACE BROWN	28	29
	DISCHARGE MOUTH CLEAR SLIGHT	29	31
	DISCHARGE MOUTH CLEAR SLIGHT	42	42
	DISCHARGE MOUTH CLEAR SLIGHT	52	56
	STAINED UNDERBODY YELLOW	57	57
	WET UNDERBODY	58	59
	STAINED NOSE YELLOW BROWN	65	65
	STAINED FACE BROWN YELLOW	65	65
	STAINED CHIN BROWN	72	72
531517	DISCHARGE MOUTH CLEAR	21	21
	LETHARGY	22	22
	STAINED FACE RED SLIGHT	23	31
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED FACE RED	37	39
	DISCHARGE MOUTH CLEAR MODERATE	49	49
	STAINED FACE BROWN	58	59
	DISCHARGE MOUTH CLEAR	60	65
	STAINED CHIN YELLOW	60	65
	DISCHARGE MOUTH CLEAR SLIGHT	67	67
	DISCHARGE MOUTH CLEAR MODERATE	77	77

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531518	STAINED MOUTH BROWN RED	14	14
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED FACE BROWN SLIGHT	35	35
	DISCHARGE MOUTH CLEAR	37	72
	STAINED NOSE BROWN SLIGHT	42	50
	STAINED CHIN RED BROWN SLIGHT	42	50
	STAINED FACE RED BROWN SLIGHT	49	50
	STAINED FACE RED	52	58
	STAINED UNDERBODY RED BROWN	53	56
	STAINED UNDERBODY RED BROWN	60	67
	STAINED CHIN RED BROWN	60	77
	STAINED NOSE BROWN RED	65	71
	STAINED PERINEUM RED YELLOW	67	67
	STAINED FACE RED BROWN	84	85
	COLORED DISCHARGE MOUTH CLEAR	88	91
531519	STAINED CHIN YELLOW BROWN	14	42
	LETHARGY	24	25
	STAINED FACE RED SLIGHT	30	31
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED FACE RED SLIGHT	38	50
	STAINED CHIN RED BROWN	43	57
	DISCHARGE MOUTH CLEAR	50	50
	STAINED FACE RED SLIGHT	52	56
	STAINED UNDERBODY RED BROWN	53	56
	DISCHARGE MOUTH CLEAR MODERATE	56	58
	STAINED NOSE RED	59	65
	STAINED CHIN RED BROWN	60	65
	STAINED FACE BROWN RED	67	94
	DISCHARGE MOUTH CLEAR SLIGHT	86	86
531520	DISCHARGE MOUTH CLEAR SLIGHT	30	30
	DISCHARGE MOUTH CLEAR SLIGHT	38	43
	STAINED CHIN BROWN SLIGHT	43	52
	DISCHARGE MOUTH RED BROWN SEVERE	49	50
	DISCHARGE MOUTH CLEAR SLIGHT	52	58
	LETHARGY SLIGHT	53	57
	STAINED FACE RED	53	58
	STAINED UNDERBODY RED BROWN	53	56
	STAINED CHIN RED	53	57
	DISCHARGE MOUTH CLEAR SLIGHT	60	65

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531521	STAINED CHIN YELLOW	22	31
	STAINED FACE BROWN SLIGHT	23	28
	STAINED CHIN BROWN SLIGHT	35	36
	DISCHARGE MOUTH CLEAR	37	46
	STAINED CHIN BROWN MODERATE	39	43
	STAINED CHIN BROWN SLIGHT	44	50
	DISCHARGE MOUTH CLEAR SEVERE	49	49
	STAINED FACE RED	49	50
	DISCHARGE MOUTH CLEAR MODERATE	50	51
	DISCHARGE MOUTH CLEAR SEVERE	52	56
	STAINED FACE RED SLIGHT	52	66
	DISCHARGE MOUTH CLEAR SLIGHT	57	66
	STAINED UNDERBODY BROWN RED YELLOW	60	60
	STAINED CHIN BROWN RED	68	70
	STAINED FACE RED	73	77
	STAINED CHIN RED	81	81
	STAINED FACE BROWN RED	85	87
	STAINED CHIN RED BROWN YELLOW	86	86
	STAINED NOSE RED BROWN	86	86
531522	STAINED FACE BROWN	28	35
	DISCHARGE MOUTH CLEAR SLIGHT	39	53
	STAINED FACE RED BROWN	44	66
	STAINED CHIN RED BROWN	53	60
	DISCHARGE MOUTH CLEAR SEVERE	56	66
	STAINED FACE RED BROWN	84	84
	STAINED CHIN RED BROWN	84	84
	STAINED FACE RED BROWN	86	87
531523	STAINED CHIN BROWN SLIGHT	29	31
	STAINED FACE BROWN SLIGHT	35	43
	DISCHARGE MOUTH CLEAR MODERATE	38	38
	STAINED NOSE RED BROWN SLIGHT	42	42
	STAINED CHIN BROWN	44	49
	STAINED FACE RED BROWN SEVERE	49	58
	DISCHARGE MOUTH CLEAR MODERATE	49	65
	DISCHARGE MOUTH CLEAR SLIGHT	66	66
	STAINED CHIN BROWN	67	70
	DISCHARGE MOUTH CLEAR SLIGHT	71	71
	STAINED CHIN RED	72	73
	STAINED CHIN RED BROWN	77	81

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531524	STAINED CHIN BROWN	21	21
	STAINED FACE BROWN SLIGHT	23	28
	STAINED FACE RED SLIGHT	30	30
	DISCHARGE MOUTH CLEAR SLIGHT	31	31
	STAINED FACE YELLOW SLIGHT	35	49
	DISCHARGE MOUTH CLEAR	37	37
	DISCHARGE MOUTH CLEAR SLIGHT	42	43
	STAINED CHIN RED BROWN SLIGHT	44	56
	STAINED FACE YELLOW RED SLIGHT	50	60
	DISCHARGE MOUTH CLEAR SLIGHT	53	57
	STAINED FACE RED BROWN	65	66
	STAINED NOSE RED SLIGHT	65	65
	DISCHARGE MOUTH CLEAR SLIGHT	77	77
	STAINED FACE BROWN	84	93
531525	STAINED CHIN RED SLIGHT	42	66
	STAINED FACE RED	49	66
	DISCHARGE MOUTH CLEAR MODERATE	56	57
	DISCHARGE MOUTH CLEAR MODERATE	60	66
	STAINED PERINEUM YELLOW BROWN	65	66
531526	DISCHARGE MOUTH RED BROWN	29	31
	DISCHARGE MOUTH CLEAR SLIGHT	38	43
	STAINED FACE RED	49	57
	STAINED CHIN RED BROWN	49	50
	DISCHARGE MOUTH CLEAR MODERATE	49	58
	STAINED NOSE RED BROWN	59	66
	STAINED CHIN RED BROWN	60	65
	STAINED UNDERBODY RED BROWN	60	60
	STAINED UNDERBODY RED BROWN SLIGHT	65	65
	STAINED FACE BROWN RED	84	86
	STAINED FACE BROWN	91	94
531527	DISCHARGE MOUTH CLEAR SLIGHT	31	42
	STAINED FACE RED SLIGHT	31	31
	STAINED FACE RED	49	56
	STAINED CHIN RED	49	56
	DISCHARGE MOUTH CLEAR	50	57
	DISCHARGE MOUTH CLEAR SLIGHT	58	58
	STAINED NOSE RED BROWN	59	60
	STAINED FACE RED BROWN	60	60

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE RATS

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
531527	STAINED PERINEUM YELLOW	60	60
	STAINED CHIN BROWN	70	70
	STAINED FACE BROWN	70	70
	DISCHARGE MOUTH CLEAR SLIGHT	70	70

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX I

Individual Clinical Observations and Mortality Records of Mice

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MICE

EXPLANATORY NOTES

NOTES:

Post Exposure Observation

At the start of the study, individual clinical signs were recorded weekly and post-exposure observations were noted only on a group basis. Individual post-exposure animal observations were recorded starting on test day 10. No post-exposure clinical signs of toxicity were observed in mice for groups I, V, and IX. For all other groups, only those mice with noted signs were reported here.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE MICE

GROUP: I CONCENTRATION: 0 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91371	SACRIFICED BY DESIGN TEST DAY 92		
91372	SACRIFICED BY DESIGN TEST DAY 92		
91373	SACRIFICED BY DESIGN TEST DAY 92		
91374	SACRIFICED BY DESIGN TEST DAY 92		
91375	RUFFLED FUR	71	84
	GASPING	84	84
	DISCHARGE NOSE RED	84	84
	SORE NOSE	84	84
	MASS ON NOSE	84	84
	HUNCHED OVER	84	84
	DEHYDRATED	84	84
	NO FECES ON CAGEBOARD	84	84
	WEAK	84	84
	SACRIFICED IN EXTREMIS TEST DAY 84		
91376	SACRIFICED BY DESIGN TEST DAY 92		
91377	RUFFLED FUR	85	92
	SACRIFICED BY DESIGN TEST DAY 92		
91378	RUFFLED FUR	64	92
	SACRIFICED BY DESIGN TEST DAY 92		
91379	ACCIDENTLY KILLED TEST DAY 92		
91380	SACRIFICED BY DESIGN TEST DAY 92		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE MICE

GROUP: III CONCENTRATION: 50 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91381	SACRIFICED BY DESIGN TEST DAY 92		
91382	SACRIFICED BY DESIGN TEST DAY 92		
91383	SACRIFICED BY DESIGN TEST DAY 92		
91384	SACRIFICED BY DESIGN TEST DAY 92		
91385	HUNCHED OVER	22	22
	LETHARGY	22	22
	WEAK	22	22
	SACRIFICED BY DESIGN TEST DAY 92		
91386	ACCIDENTLY KILLED TEST DAY 92		
91387	SACRIFICED BY DESIGN TEST DAY 92		
91388	SACRIFICED BY DESIGN TEST DAY 92		
91389	SACRIFICED BY DESIGN TEST DAY 92		
91390	SACRIFICED BY DESIGN TEST DAY 92		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE MICE

GROUP: V CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91391	SACRIFICED BY DESIGN TEST DAY 92		
91392	SACRIFICED BY DESIGN TEST DAY 92		
91393	SACRIFICED BY DESIGN TEST DAY 92		
91394	SACRIFICED BY DESIGN TEST DAY 92		
91395	SACRIFICED BY DESIGN TEST DAY 92		
91396	SACRIFICED BY DESIGN TEST DAY 92		
91397	SACRIFICED BY DESIGN TEST DAY 92		
91398	SACRIFICED BY DESIGN TEST DAY 92		
91399	SACRIFICED BY DESIGN TEST DAY 92		
91400	SACRIFICED BY DESIGN TEST DAY 92		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE MICE

GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91401	FOUND DEAD TEST DAY 12		
91402	FOUND DEAD TEST DAY 11		
91403	FOUND DEAD TEST DAY 11		
91404	FOUND DEAD TEST DAY 11		
91405	FOUND DEAD TEST DAY 12		
91406	FOUND DEAD TEST DAY 11		
91407	FOUND DEAD TEST DAY 11		
91408	TREMORS FOUND DEAD TEST DAY 12	11	11
91409	FOUND DEAD TEST DAY 11		
91410	FOUND DEAD TEST DAY 11		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE MICE

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91411	SACRIFICED BY DESIGN TEST DAY 92		
91412	SACRIFICED BY DESIGN TEST DAY 92		
91413	SACRIFICED BY DESIGN TEST DAY 92		
91414	SACRIFICED BY DESIGN TEST DAY 92		
91415	SACRIFICED BY DESIGN TEST DAY 92		
91416	SACRIFICED BY DESIGN TEST DAY 92		
91417	SACRIFICED BY DESIGN TEST DAY 92		
91418	SACRIFICED BY DESIGN TEST DAY 92		
91419	SACRIFICED BY DESIGN TEST DAY 92		
91420	SACRIFICED BY DESIGN TEST DAY 92		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: II CONCENTRATION: 0 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91421	SACRIFICED BY DESIGN TEST DAY 93		
91422	SACRIFICED BY DESIGN TEST DAY 93		
91423	SACRIFICED BY DESIGN TEST DAY 93		
91424	SACRIFICED BY DESIGN TEST DAY 93		
91425	SACRIFICED BY DESIGN TEST DAY 93		
91426	SACRIFICED BY DESIGN TEST DAY 93		
91427	SACRIFICED BY DESIGN TEST DAY 93		
91428	SACRIFICED BY DESIGN TEST DAY 93		
91429	SACRIFICED BY DESIGN TEST DAY 93		
91430	SACRIFICED BY DESIGN TEST DAY 93		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: IV CONCENTRATION: 50 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91431	SACRIFICED BY DESIGN TEST DAY 93		
91432	SACRIFICED BY DESIGN TEST DAY 93		
91433	SACRIFICED BY DESIGN TEST DAY 93		
91434	SACRIFICED BY DESIGN TEST DAY 93		
91435	SACRIFICED BY DESIGN TEST DAY 93		
91436	SACRIFICED BY DESIGN TEST DAY 93		
91437	SACRIFICED BY DESIGN TEST DAY 93		
91438	SACRIFICED BY DESIGN TEST DAY 93		
91440	SACRIFICED BY DESIGN TEST DAY 93		
91471	SACRIFICED BY DESIGN TEST DAY 93		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: VI CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91441	SACRIFICED BY DESIGN TEST DAY 93		
91442	SACRIFICED BY DESIGN TEST DAY 93		
91443	SACRIFICED BY DESIGN TEST DAY 93		
91444	SACRIFICED BY DESIGN TEST DAY 93		
91445	SACRIFICED BY DESIGN TEST DAY 93		
91446	SACRIFICED BY DESIGN TEST DAY 93		
91447	WEAK	70	70
	HUNCHED OVER	70	70
	LETHARGY	70	70
	SACRIFICED IN EXTREMIS TEST DAY 70		
91448	SACRIFICED BY DESIGN TEST DAY 93		
91449	SACRIFICED BY DESIGN TEST DAY 93		
91450	SACRIFICED BY DESIGN TEST DAY 93		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: VIII CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91451	FOUND DEAD TEST DAY 88		
91452	FOUND DEAD TEST DAY 70		
91453	FOUND DEAD TEST DAY 88		
91454	FOUND DEAD TEST DAY 12		
91455	CONVULSIONS OBSERVED PRIOR TO DEATH FOUND DEAD TEST DAY 11	11	11
91456	SACRIFICED BY DESIGN TEST DAY 93		
91457	FOUND DEAD TEST DAY 12		
91458	SACRIFICED BY DESIGN TEST DAY 93		
91459	FOUND DEAD TEST DAY 12		
91460	FOUND DEAD TEST DAY 11		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

WEEKLY (PREEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91461	SACRIFICED BY DESIGN TEST DAY 93		
91462	SACRIFICED BY DESIGN TEST DAY 93		
91463	LETHARGY	63	63
	HUNCHED OVER	63	63
	DIARRHEA	63	63
	WEAK	63	63
	FOUND DEAD TEST DAY 64		
91464	SACRIFICED BY DESIGN TEST DAY 93		
91465	SACRIFICED BY DESIGN TEST DAY 93		
91466	SACRIFICED BY DESIGN TEST DAY 93		
91467	SACRIFICED BY DESIGN TEST DAY 93		
91468	SACRIFICED BY DESIGN TEST DAY 93		
91469	SACRIFICED IN EXTREMIS TEST DAY 38		
91470	SACRIFICED BY DESIGN TEST DAY 93		

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE MICE

GROUP: III CONCENTRATION: 50 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91381	SORE TAIL	51	67
91385	LETHARGY	22	29
	TREMORS	22	22
91386	TREMORS SLIGHT	22	24
91389	TREMORS SLIGHT	22	24

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF MALE MICE

GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91401	TREMORS	11	11
91403	TREMORS	10	10
91408	TREMORS SEVERE	11	11

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: II CONCENTRATION: 0 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91421	SORE TAIL	18	31
91424	SORE TAIL	18	18
91425	SORE TAIL	18	18

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: IV CONCENTRATION: 50 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91434	LETHARGY	22	24
	TREMORS SLIGHT	22	22
91435	TREMORS	22	24
	HUNCHED OVER	22	22
	LETHARGY	22	22
91436	ALOPECIA LEFT SHOULDER(S)	77	92
91471	TREMORS SLIGHT	22	24

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: VI CONCENTRATION: 250 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91447	HUNCHED OVER	68	68
	LETHARGY	68	68

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: VIII CONCENTRATION: 1000 ppm

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91451	LETHARGY	16	17
	LETHARGY SLIGHT	28	28
	LETHARGY	31	31
91452	TREMORS	11	11
	LETHARGY	16	17
91453	LETHARGY	16	17
91456	LETHARGY	16	17
	LETHARGY	31	31
91458	LETHARGY	16	17
91459	TREMORS	11	11

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

DAILY (POSTEXPOSURE)
INDIVIDUAL CLINICAL OBSERVATIONS AND MORTALITY RECORDS OF FEMALE MICE

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	OBSERVATION	FIRST DAY OBSERVED	LAST DAY OBSERVED
91463	GASPING	60	60
	IRREGULAR RESPIRATION	60	60
	LUNG NOISE	60	60

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX J

Individual Ophthalmological Observations of Rats

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS OF RATS

EXPLANATORY NOTES

ABBREVIATIONS:

NAD - No Abnormalities Detected

FOOTNOTES:

[a] The animal was removed from study prior to the time of the examination.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN MALE RATS

GROUP: I CONCENTRATION: 0 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
101	531423	NAD	NAD
102	531424	NAD	NAD
103	531425	NAD	NAD
104	531426	NAD	NAD
105	531427	NAD	NAD
106	531428	NAD	NAD
107	531429	NAD	NAD
108	531430	NAD	NAD
109	531431	NAD	NAD
110	531432	NAD	NAD

GROUP: III CONCENTRATION: 250 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
301	531433	NAD	NAD
302	531434	NAD	NAD
303	531435	NAD	NAD
304	531436	NAD	NAD
305	531437	NAD	NAD
306	531438	NAD	NAD
307	531439	NAD	NAD
308	531440	NAD	NAD
309	531441	NAD	NAD
310	531442	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN MALE RATS

GROUP: V CONCENTRATION: 1000 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
501	531443	NAD	NAD
502	531444	NAD	NAD
503	531445	NAD	NAD
504	531446	NAD	NAD
505	531447	NAD	NAD
506	531448	NAD	NAD
507	531449	NAD	NAD
508	531450	NAD	NAD
509	531451	NAD	NAD
510	531452	NAD	NAD

GROUP: VII CONCENTRATION: 1500 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
701	531453	NAD	Right Eye: focal retinal degeneration
702	531454	NAD	NAD
703	531455	NAD	NAD
704	531456	NAD	NAD
705	531457	NAD	NAD
706	531458	NAD	NAD
707	531459	NAD	NAD
708	531460	NAD	NAD
709	531461	NAD	NAD
710	531462	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN MALE RATS

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
901	531463	NAD	NAD
902	531464	NAD	NAD
903	531465	NAD	NAD
904	531466	NAD	NAD
905	531467	NAD	NAD
906	531468	NAD	Right Eye: focal retinal degeneration
907	531469	NAD	NAD
908	531470	NAD	NAD
909	531471	NAD	NAD
910	531472	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN FEMALE RATS

GROUP: II CONCENTRATION: 0 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
201	531478	NAD	NAD
202	531479	NAD	NAD
203	531480	NAD	NAD
204	531481	NAD	NAD
205	531482	NAD	NAD
206	531483	NAD	NAD
207	531484	NAD	NAD
208	531485	NAD	NAD
209	531486	NAD	NAD
210	531487	NAD	NAD

GROUP: IV CONCENTRATION: 250 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
401	531488	NAD	NAD
402	531489	NAD	NAD
403	531490	NAD	NAD
404	531491	NAD	NAD
405	531492	NAD	Right Eye: retinal degeneration (generalized)
406	531493	NAD	NAD
407	531494	NAD	NAD
408	531495	NAD	NAD
409	531496	NAD	NAD
410	531497	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN FEMALE RATS

GROUP: VI CONCENTRATION: 1000 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
601	531498	NAD	NAD
602	531499	NAD	•
603	531500	NAD	NAD
604	531501	NAD	NAD
605	531502	NAD	NAD
606	531503	NAD	NAD
607	531504	NAD	NAD
608	531505	NAD	NAD
609	531506	NAD	NAD
610	531507	NAD	NAD

GROUP: VIII CONCENTRATION: 15000 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
801	531508	NAD	NAD
802	531509	NAD	NAD
803	531510	NAD	NAD
804	531511	NAD	NAD
805	531512	NAD	NAD
806	531513	NAD	NAD
807	531514	NAD	NAD
808	531515	NAD	NAD
809	531516	NAD	NAD
810	531517	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN FEMALE RATS

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
1001	531518	NAD	NAD
1002	531519	NAD	NAD
1003	531520	NAD	NAD
1004	531521	NAD	NAD
1005	531522	NAD	NAD
1006	531523	NAD	NAD
1007	531524	NAD	NAD
1008	531525	NAD	NAD
1009	531526	NAD	NAD
1010	531527	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX K

Individual Ophthalmological Observations of Mice

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS OF MICE

EXPLANATORY NOTES

ABBREVIATIONS:

NAD - No Abnormalities Detected

FOOTNOTES:

[a] The animal was not alive at the time of the examination.

[b] The animal was removed from study prior to the time of the examination.

[c] The animal was not on study at the time of the examination.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN MALE MICE

GROUP: I CONCENTRATION: 0 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
101	91371	NAD	NAD
102	91372	NAD	NAD
103	91373	NAD	NAD
104	91374	NAD	NAD
105	91375	NAD	*
106	91376	NAD	NAD
107	91377	NAD	NAD
108	91378	NAD	NAD
109	91379	NAD	NAD
110	91380	NAD	NAD

GROUP: III CONCENTRATION: 50 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
301	91381	NAD	NAD
302	91382	NAD	NAD
303	91383	NAD	NAD
304	91384	NAD	NAD
305	91385	NAD	NAD
306	91386	NAD	NAD
307	91387	NAD	NAD
308	91388	NAD	NAD
309	91389	NAD	NAD
310	91390	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN MALE MICE

GROUP: V CONCENTRATION: 250 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
501	91391	NAD	NAD
502	91391	NAD	NAD
503	91393	NAD	NAD
504	91394	NAD	NAD
505	91395	NAD	NAD
506	91396	NAD	NAD
507	91397	NAD	NAD
508	91398	NAD	NAD
509	91399	NAD	NAD
510	91400	NAD	NAD

GROUP: VII CONCENTRATION: 1000 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
701	91401	NAD	a
702	91402	NAD	a
703	91403	NAD	a
704	91404	NAD	a
705	91405	NAD	a
706	91406	NAD	a
707	91407	NAD	a
708	91408	NAD	a
709	91409	NAD	a
710	91410	NAD	a

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN MALE MICE

GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
901	91411	NAD	NAD
902	91412	NAD	NAD
903	91413	NAD	NAD
904	91414	NAD	NAD
905	91415	NAD	NAD
906	91416	NAD	NAD
907	91417	NAD	NAD
908	91418	NAD	NAD
909	91419	NAD	NAD
910	91420	NAD	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN FEMALE MICE

GROUP: II CONCENTRATION: 0 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
201	91421	NAD	NAD
202	91422	NAD	NAD
203	91423	NAD	NAD
204	91424	NAD	NAD
205	91425	NAD	NAD
206	91426	NAD	NAD
207	91427	NAD	NAD
208	91428	NAD	NAD
209	91429	NAD	NAD
210	91430	NAD	NAD

GROUP: IV CONCENTRATION: 50 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
401	91431	NAD	NAD
402	91432	NAD	NAD
403	91433	NAD	NAD
404	91434	NAD	NAD
405	91435	NAD	NAD
406	91436	NAD	NAD
407	91437	NAD	NAD
408	91438	NAD	Left Eye: corneal dystrophy
409	91439	NAD	[b]
410	91440	NAD	NAD
411	91471	[c]	NAD

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN FEMALE MICE

GROUP: VI CONCENTRATION: 250 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
601	91441	NAD	NAD
602	91442	NAD	NAD
603	91443	NAD	NAD
604	91444	NAD	NAD
605	91445	NAD	NAD
606	91446	NAD	NAD
607	91447	NAD	b
608	91448	NAD	NAD
609	91449	NAD	NAD
610	91450	NAD	NAD

GROUP: VIII CONCENTRATION: 1000 ppm

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
801	91451	NAD	a
802	91452	NAD	a
803	91453	NAD	a
804	91454	NAD	a
805	91455	NAD	a
806	91456	NAD	NAD
807	91457	NAD	a
808	91458	NAD	NAD
809	91459	NAD	a
810	91460	NAD	a

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

INDIVIDUAL OPHTHALMOLOGICAL OBSERVATIONS IN FEMALE MICE

GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

TATTOO NUMBER	ANIMAL NUMBER	PRETEST EXAM (Test Day -1)	FINAL EXAM (Test Day 88)
1001	91461	NAD	NAD
1002	91462	NAD	NAD
1003	91463	NAD	a
1004	91464	NAD	NAD
1005	91465	NAD	NAD
1006	91466	NAD	Left Eye: enucleated
1007	91467	NAD	NAD
1008	91468	NAD	NAD
1009	91469	NAD	a
1010	91470	NAD	NAD

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PREVENTION AND TOXICS
94 APR 22 PM 2:40

Study Title

Subchronic Toxicity Study by the Inhalation Route in Rats and Mice with 4-Vinylcyclohexene

Volume 2 of 2

Laboratory Project ID

Haskell Laboratory Report No. 442-93

Author

Judith C. Stadler, Ph.D., D.A.B.T.

Study Completed on

February 14, 1994

Performing Laboratory

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Chemical Manufacturers Association Study Number

BUT-21.0-COT-DHL-03

Medical Research Project No. 9523-001

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX L

Individual Clinical Pathology Data of Rats

APPENDIX - EXPLANATORY NOTES

TERMS AND CRITERIA USED IN URINALYSES

Abbreviations for Descriptive Terms Normally Used for Gross Evaluation of Urine (other Abbreviations and Descriptive Terms may be used if they are more applicable)

Y = Yellow
LY = Light Yellow
DY = Dark Yellow
A = Amber
DA = Dark Amber
G = Green
BL = Bloody
R = Red
Br = Brown
C = Clear
CL = Cloudy
P/PPT = Precipitate
F = Feed
FEC = Feces
QNS = Quantity not sufficient

Abbreviations for Descriptive Terms Used for Microscopic Evaluation of Urine

Epith = Epithelial Cells
hpf = high power field
lpf = low power field
RBC = Red Blood Cells
WBC = White Blood Cells
TNTC = Too Numerous to Count

Definition of "Normal" and "Abnormal" Designations Used for Gross and Microscopic Appearance of Urine

Appearance	Normal	Abnormal
Color	Light to dark yellow or amber	Color other than yellow or amber
Microscopic		
Red Blood Cells	Average of 0 to 4 red blood cells per high power field	Average of more than 4 red blood cells per high power field
Casts	None observed	Average of more than 0 per low power field
Epithelial Cells	Average of 0 to 9 per high power field	Average of more than 9 per high power field
White Blood Cells	Average of 0 to 9 per high power field	Average of more than 9 per high power field

APPENDIX - EXPLANATORY NOTES (continued)

ANIMALS DYING PRIOR TO CLINICAL PATHOLOGY EVALUATION

Animal #	Group	Reason
531499	VI	Removed from study

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/29/93

CONCENTRATION: 0 ppm

ANIMAL#:	RBC x10^6/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT x10^3/ul
531423	8.41	15.7	43.	51.	19.	37.	1023.
531424	9.16	16.4	47.	51.	18.	35.	1189.
531425	8.72	16.7	48.	55.	19.	35.	1017.
531426	8.18	16.0	42.	52.	20.	38.	874.
531427	8.08	16.0	43.	53.	20.	38.	1056.
531428	7.40	14.5	41.	55.	20.	36.	973.
531429	7.86	15.4	40.	51.	20.	38.	1369.
531430	7.33	13.8	36.	49.	19.	38.	754.
531431	8.20	16.1	42.	52.	20.	38.	1158.
531432	8.09	15.5	39.	49.	19.	39.	935.
AVG.	8.14	15.6	42.	52.	19.	37.	1035.
S. D.	0.55	0.9	4.	2.	1.	1.	173.
S. E.	0.17	0.3	1.	1.	0.	0.	55.

GROUP: III
SAMPLE DATE: 04/29/93

CONCENTRATION: 250 ppm

ANIMAL#:	RBC x10^6/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT x10^3/ul
531433	8.87	16.4	48.	54.	19.	35.	648.
531434	7.22	14.4	40.	55.	20.	36.	1110.
531435	8.36	15.7	43.	52.	19.	36.	1177.
531436	8.13	15.4	42.	52.	19.	37.	1331.
531437	8.40	14.8	41.	49.	18.	36.	1243.
531438	8.64	16.0	45.	52.	19.	36.	1210.
531439	7.65	14.9	41.	53.	20.	37.	1066.
531440	7.51	15.5	41.	55.	21.	38.	1035.
531441	8.04	15.2	40.	50.	19.	38.	979.
531442	7.79	15.5	41.	52.	20.	38.	976.
AVG.	8.06	15.4	42.	52.	19.	37.	1078.
S. D.	0.52	0.6	3.	2.	1.	1.	190.
S. E.	0.17	0.2	1.	1.	0.	0.	60.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
531443	8.62	16.2	46.	53.	19.	36.	1088.
531444	8.23	16.0	43.	52.	19.	37.	1136.
531445	8.05	15.6	42.	52.	19.	37.	1076.
531446	8.25	15.2	42.	51.	18.	36.	1287.
531447	8.36	15.5	43.	52.	19.	36.	1265.
531448	8.60	15.4	42.	49.	18.	37.	1262.
531449	7.88	15.2	41.	52.	19.	37.	799.
531450	7.63	15.4	41.	54.	20.	37.	1227.
531451	8.15	15.9	44.	54.	20.	36.	983.
531452	7.36	13.9	37.	51.	19.	37.	1143.
AVG.	8.11	15.4	42.	52.	19.	37.	1127.
S. D.	0.40	0.6	2.	1.	1.	1.	151.
S. E.	0.13	0.2	1.	0.	0.	0.	48.

GROUP: VII
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
531453	7.99	15.7	41.	52.	20.	38.	1305.
531454	8.32	16.2	43.	52.	20.	37.	1155.
531455	7.50	15.5	41.	55.	21.	38.	1023.
531456	7.71	15.1	40.	52.	20.	38.	1027.
531457	7.86	15.5	41.	53.	20.	38.	1107.
531458	8.41	16.0	44.	52.	19.	36.	1174.
531459	7.76	15.5	42.	54.	20.	37.	1219.
531460	8.49	15.4	43.	50.	18.	36.	1197.
531461	7.92	15.2	40.	51.	19.	38.	1092.
531462	7.88	15.5	42.	53.	20.	37.	1240.
AVG.	7.98	15.6	42.	52.	20.	37.	1154.
S. D.	0.32	0.3	1.	1.	1.	1.	92.
S. E.	0.10	0.1	0.	0.	0.	0.	29.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: IX
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	RBC x10^6/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT x10^3/ul
531463	8.11	15.2	41.	51.	19.	37.	1020.
531464	7.97	16.4	42.	53.	21.	39.	772.
531465	7.81	14.4	40.	51.	18.	36.	1108.
531466	7.81	14.9	40.	52.	19.	37.	1138.
531467	7.84	15.5	42.	54.	20.	37.	965.
531468	8.57	15.7	43.	50.	18.	36.	1242.
531469	7.55	15.8	42.	55.	21.	38.	1029.
531470	9.21	16.0	45.	48.	17.	36.	1278.
531471	7.99	16.0	43.	54.	20.	37.	870.
531472	8.61	15.2	42.	48.	18.	37.	1275.
AVG.	8.15	15.5	42.	52.	19.	37.	1070.
S. D.	0.50	0.6	1.	2.	1.	1.	172.
S. E.	0.16	0.2	0.	1.	0.	0.	54.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/29/93

CONCENTRATION: 0 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531423	19.4	2716.	0.	15908.	0.	582.	194.	0.
531424	13.3	1197.	0.	11571.	0.	532.	0.	0.
531425	12.2	1464.	0.	9272.	0.	1342.	122.	0.
531426	14.6	1606.	0.	11680.	0.	1168.	146.	0.
531427	19.2	1728.	0.	15168.	0.	2112.	192.	0.
531428	28.7	8897.	0.	17794.	0.	1722.	287.	0.
531429	22.2	4218.	0.	15096.	0.	1998.	888.	0.
531430	13.1	1048.	0.	11397.	0.	655.	0.	0.
531431	15.4	2156.	0.	12166.	0.	1078.	0.	0.
531432	13.0	2860.	0.	9100.	0.	910.	130.	0.
AVG.	17.1	2789.	0.	12915.	0.	1210.	196.	0.
S. D.	5.3	2348.	0.	2918.	0.	576.	261.	0.
S. E.	1.7	742.	0.	923.	0.	182.	83.	0.

GROUP: III
SAMPLE DATE: 04/29/93

CONCENTRATION: 250 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531433	15.6	1872.	0.	12168.	0.	1560.	0.	0.
531434	25.5	2040.	0.	20655.	0.	2550.	255.	0.
531435	16.5	4290.	0.	11220.	0.	990.	0.	0.
531436	18.8	1504.	0.	15416.	0.	1880.	0.	0.
531437	17.7	1947.	0.	14160.	0.	1416.	177.	0.
531438	13.5	1485.	0.	10260.	0.	1350.	405.	0.
531439	17.6	1760.	0.	14608.	0.	1232.	0.	0.
531440	32.7	5559.	0.	23871.	0.	2943.	327.	0.
531441	21.6	2160.	0.	17064.	0.	2160.	216.	0.
531442	20.8	1040.	0.	18720.	0.	832.	208.	0.
AVG.	20.0	2366.	0.	15814.	0.	1691.	159.	0.
S. D.	5.6	1419.	0.	4320.	0.	684.	151.	0.
S. E.	1.8	449.	0.	1366.	0.	216.	48.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	WBC x10³/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531443	17.2	2236.	0.	13588.	0.	1204.	172.	0.
531444	16.4	1312.	0.	13612.	0.	1312.	164.	0.
531445	13.9	2363.	0.	10286.	0.	973.	278.	0.
531446	15.4	1694.	0.	12166.	0.	1386.	154.	0.
531447	26.3	2367.	0.	21303.	0.	1841.	789.	0.
531448	14.5	1305.	0.	12035.	0.	1015.	145.	0.
531449	11.9	1428.	0.	9163.	0.	1190.	119.	0.
531450	18.5	1295.	0.	16280.	0.	925.	0.	0.
531451	24.8	2976.	0.	19096.	0.	2728:	0.	0.
531452	19.0	1140.	0.	16340.	.0.	1520.	0.	0.
AVG.	17.8	1812.	0.	14387.	0.	1409.	182.	0.
S. D.	4.6	627.	0.	3847.	0.	539.	232.	0.
S. E.	1.5	198.	0.	1217.	0.	171.	73.	0.

GROUP: VII
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	WBC x10³/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531453	16.4	3116.	0.	11316.	0.	1804.	164.	0.
531454	9.6	768.	0.	8256.	0.	576.	0.	0.
531455	18.7	1683.	0.	14773.	0.	2057.	187.	0.
531456	20.5	3690.	0.	15375.	0.	1435.	0.	0.
531457	11.2	896.	0.	8624.	0.	1568.	112.	0.
531458	18.4	1472.	0.	14904.	0.	1840.	184.	0.
531459	16.5	2475.	0.	12210.	0.	1485.	330.	0.
531460	14.4	1872.	0.	10944.	0.	1584.	0.	0.
531461	16.8	1344.	0.	14280.	0.	1008.	168.	0.
531462	23.9	2390.	0.	18403.	0.	3107.	0.	0.
AVG.	16.6	1971.	0.	12909.	0.	1646.	115.	0.
S. D.	4.2	945.	0.	3206.	0.	667.	113.	0.
S. E.	1.3	299.	0.	1014.	0.	211.	36.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: IX
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531463	9.9	1089.	0.	8217.	0.	594.	0.	0.
531464	19.2	1344.	0.	15936.	0.	1920.	0.	0.
531465	18.0	1260.	0.	15120.	0.	1620.	0.	0.
531466	23.6	2832.	0.	19352.	0.	1416.	0.	0.
531467	17.6	1760.	0.	14256.	0.	1584.	0.	0.
531468	21.9	1971.	0.	15549.	0.	4161.	219.	0.
531469	13.5	1350.	0.	10395.	0.	1755.	0.	0.
531470	20.4	2856.	0.	15504.	0.	2040.	0.	0.
531471	18.7	2244.	0.	14773.	0.	1496.	187.	0.
531472	22.6	1356.	0.	19888.	0.	1356.	0.	0.
AVG.	18.5	1806.	0.	14899.	0.	1794.	41.	0.
S. D.	4.2	651.	0.	3528.	0.	920.	86.	0.
S. E.	1.3	206.	0.	1116.	0.	291.	27.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/29/93

CONCENTRATION: 0 ppm

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531423	71.	39.	122.	94.	18.	10.4	8.10
531424	60.	25.	117.	100.	17.	10.4	8.00
531425	55.	33.	71.	119.	15.	10.1	6.90
531426	153.	30.	74.	102.	17.	10.8	8.80
531427	135.	37.	74.	106.	15.	10.6	8.70
531428	93.	38.	78.	97.	15.	10.3	7.50
531429	76.	33.	66.	110.	19.	10.9	8.00
531430	115.	28.	73.	105.	14.	10.7	7.80
531431	123.	30.	78.	92.	16.	10.4	8.00
531432	88.	35.	86.	98.	17.	10.5	7.40
AVG.	97.	33.	84.	102.	16.	10.5	7.92
S. D.	33.	5.	20.	8.	2.	0.2	0.57
S. E.	11.	1.	6.	3.	0.	0.1	0.18

GROUP: III
SAMPLE DATE: 04/29/93

CONCENTRATION: 250 ppm

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531433	139.	33.	107.	103.	15.	10.7	8.10
531434	126.	34.	60.	115.	16.	11.0	7.70
531435	64.	33.	69.	88.	13.	10.7	7.60
531436	75.	28.	77.	103.	16.	10.3	7.60
531437	66.	25.	61.	108.	16.	10.8	7.90
531438	94.	37.	62.	98.	13.	10.5	7.20
531439	78.	33.	65.	106.	16.	10.7	8.30
531440	98.	30.	55.	107.	16.	10.9	7.50
531441	112.	36.	82.	113.	19.	10.9	8.30
531442	132.	27.	67.	108.	17.	10.2	8.40
AVG.	98.	32.	71.	105.	16.	10.7	7.86
S. D.	28.	4.	15.	8.	2.	0.3	0.40
S. E.	9.	1.	5.	2.	1.	0.1	0.13

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531443	89.	36.	89.	101.	16.	10.5	7.60
531444	101.	25.	52.	105.	14.	11.1	7.20
531445	103.	23.	65.	107.	17.	10.7	7.50
531446	75.	32.	65.	98.	16.	10.9	7.80
531447	95.	35.	65.	114.	14.	11.1	8.20
531448	91.	34.	73.	100.	17.	10.6	6.60
531449	77.	41.	87.	93.	14.	11.0	7.40
531450	112.	33.	79.	101.	17.	10.9	7.40
531451	90.	40.	75.	87.	16.	11.2	7.60
531452	77.	33.	75.	97.	21.	11.2	8.60
AVG.	91.	33.	73.	100.	16.	10.9	7.59
S. D.	12.	6.	11.	7.	2.	0.2	0.54
S. E.	4.	2.	4.	2.	1.	0.1	0.17

GROUP: VII
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531453	100.	36.	60.	94.	13.	10.9	7.60
531454	92.	38.	55.	144.	20.	11.6	10.00
531455	127.	30.	58.	110.	16.	10.8	8.30
531456	100.	32.	67.	115.	15.	10.5	6.70
531457	64.	27.	52.	96.	15.	10.7	7.30
531458	137.	35.	79.	93.	16.	10.9	7.90
531459	101.	30.	84.	103.	18.	11.2	7.70
531460	65.	29.	72.	86.	16.	11.4	7.70
531461	70.	39.	69.	93.	23.	10.9	7.30
531462	129.	41.	64.	93.	16.	10.6	7.00
AVG.	99.	34.	66.	103.	17.	10.9	7.75
S. D.	27.	5.	10.	17.	3.	0.4	0.91
S. E.	8.	2.	3.	5.	1.	0.1	0.29

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

CONCENTRATION: 1,000 ppm 1,3-butadiene

: IX
E DATE: 04/29/93

L#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
463	93.	24.	54.	107.	14.	10.5	6.30
.464	66.	33.	81.	104.	13.	11.1	7.00
.465	114.	31.	80.	102.	15.	11.1	8.00
1466	98.	44.	75.	105.	17.	10.5	7.80
1467	81.	28.	61.	103.	13.	10.7	6.70
1468	73.	43.	82.	124.	17.	11.4	8.20
1469	90.	48.	70.	115.	18.	10.4	6.80
1470	80.	37.	87.	123.	15.	10.8	7.90
1471	108.	33.	81.	113.	15.	11.0	8.50
1472	84.	36.	83.	116.	17.	10.9	8.20
AVG.	89.	36.	75.	111.	15.	10.8	7.54
S. D.	15.	8.	11.	8.	2.	0.3	0.77
S. E.	5.	2.	3.	3.	1.	0.1	0.24

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: I CONCENTRATION: 0 ppm
SAMPLE DATE: 04/29/93

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531423	0.16	57.	0.69	6.6	4.3	2.3
531424	0.16	74.	0.73	7.0	4.6	2.4
531425	0.11	62.	0.66	6.7	4.4	2.3
531426	0.16	59.	0.59	6.6	4.1	2.5
531427	0.15	60.	0.62	6.5	4.1	2.4
531428	0.13	64.	0.61	6.9	4.3	2.6
531429	0.14	68.	0.64	6.7	4.2	2.5
531430	0.16	83.	0.49	6.4	4.1	2.3
531431	0.13	50.	0.67	6.3	4.1	2.2
531432	0.13	68.	0.61	7.0	4.4	2.6
AVG.	0.14	65.	0.63	6.7	4.3	2.4
S. D.	0.02	9.	0.07	0.2	0.2	0.1
S. E.	0.01	3.	0.02	0.1	0.1	0.0

GROUP: III CONCENTRATION: 250 ppm
SAMPLE DATE: 04/29/93

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531433	0.09	42.	0.60	6.6	4.2	2.4
531434	0.12	82.	0.57	6.9	4.4	2.5
531435	0.16	72.	0.56	6.8	4.2	2.6
531436	0.16	60.	0.57	6.1	4.1	2.0
531437	0.16	70.	0.62	7.2	4.7	2.5
531438	0.30	43.	0.61	6.4	4.4	2.0
531439	0.13	79.	0.58	6.4	4.3	2.1
531440	0.12	65.	0.57	6.8	4.1	2.7
531441	0.16	60.	0.62	6.4	4.3	2.1
531442	0.13	46.	0.59	6.2	4.0	2.2
AVG.	0.15	62.	0.59	6.6	4.3	2.3
S. D.	0.06	14.	0.02	0.3	0.2	0.3
S. E.	0.02	5.	0.01	0.1	0.1	0.1

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531443	0.16	57.	0.66	6.9	4.8	2.1
531444	0.16	80.	0.68	7.1	4.8	2.3
531445	0.15	66.	0.66	7.1	4.7	2.4
531446	0.15	96.	0.63	6.9	4.6	2.3
531447	0.17	98.	0.51	6.7	4.4	2.3
531448	0.10	69.	0.58	7.0	4.6	2.4
531449	0.18	61.	0.64	7.4	5.0	2.4
531450	0.13	79.	0.66	6.8	4.5	2.3
531451	0.14	75.	0.60	7.0	4.5	2.5
531452	0.14	56.	0.71	6.4	4.3	2.1
AVG.	0.15	74.	0.63	6.9	4.6	2.3
S. D.	0.02	15.	0.06	0.3	0.2	0.1
S. E.	0.01	5.	0.02	0.1	0.1	0.0

GROUP: VII
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531453	0.13	74.	0.54	6.6	4.6	2.0
531454	0.16	66.	0.67	7.1	4.9	2.2
531455	0.14	42.	0.54	6.3	4.5	1.8
531456	0.11	66.	0.64	7.4	4.4	3.0
531457	0.10	78.	0.66	7.1	4.7	2.4
531458	0.11	48.	0.56	6.6	4.5	2.1
531459	0.16	75.	0.70	7.3	4.9	2.4
531460	0.12	89.	0.64	7.3	4.7	2.6
531461	0.20	66.	0.74	6.3	4.4	1.9
531462	0.14	60.	0.63	6.8	4.2	2.6
AVG.	0.14	66.	0.63	6.9	4.6	2.3
S. D.	0.03	14.	0.07	0.4	0.2	0.4
S. E.	0.01	4.	0.02	0.1	0.1	0.1

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: IX
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531463	0.17	54.	0.61	6.7	4.6	2.1
531464	0.10	57.	0.62	6.9	4.5	2.4
531465	0.13	43.	0.53	6.2	4.1	2.1
531466	0.17	43.	0.59	6.5	4.5	2.0
531467	0.13	52.	0.59	7.2	4.7	2.5
531468	0.13	67.	0.62	6.9	4.4	2.5
531469	0.16	64.	0.55	6.6	4.3	2.3
531470	0.09	57.	0.60	6.5	4.2	2.3
531471	0.14	44.	0.54	5.9	4.0	1.9
531472	0.13	45.	0.58	6.3	4.3	2.0
AVG.	0.14	53.	0.58	6.6	4.4	2.2
S. D.	0.03	9.	0.03	0.4	0.2	0.2
S. E.	0.01	3.	0.01	0.1	0.1	0.1

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/29/93

CONCENTRATION: 0 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531423	146.	6.4	98.
531424	143.	6.2	98.
531425	147.	6.0	99.
531426	144.	6.2	98.
531427	145.	6.3	96.
531428	146.	6.0	99.
531429	144.	6.2	97.
531430	145.	5.7	99.
531431	147.	6.1	99.
531432	148.	5.9	101.
AVG.	146.	6.1	98.
S. D.	2.	0.2	1.
S. E.	1.	0.1	0.

GROUP: III
SAMPLE DATE: 04/29/93

CONCENTRATION: 250 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531433	143.	6.9	97.
531434	144.	6.1	98.
531435	147.	6.0	97.
531436	146.	5.7	100.
531437	145.	5.9	97.
531438	147.	6.1	101.
531439	146.	6.2	100.
531440	145.	5.8	97.
531441	145.	6.4	99.
531442	144.	6.2	98.
AVG.	145.	6.1	98.
S. D.	1.	0.3	2.
S. E.	0.	0.1	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531443	143.	5.9	101.
531444	145.	5.6	98.
531445	145.	5.9	98.
531446	144.	6.3	97.
531447	145.	6.4	97.
531448	142.	6.2	98.
531449	143.	6.2	96.
531450	143.	6.3	97.
531451	145.	6.9	98.
531452	145.	6.1	96.
AVG.	144.	6.2	98.
S. D.	1.	0.3	1.
S. E.	0.	0.1	0.

GROUP: VII
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531453	145.	6.2	98.
531454	148.	7.0	101.
531455	146.	6.2	98.
531456	145.	5.6	97.
531457	145.	5.6	97.
531458	144.	6.1	96.
531459	146.	5.9	99.
531460	147.	5.9	99.
531461	146.	6.2	98.
531462	145.	6.0	97.
AVG.	146.	6.1	98.
S. D.	1.	0.4	1.
S. E.	0.	0.1	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: IX
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531463	146.	5.7	100.
531464	146.	5.5	99.
531465	147.	6.3	100.
531466	147.	5.4	99.
531467	146.	5.5	99.
531468	146.	5.9	97.
531469	144.	5.9	100.
531470	143.	5.9	99.
531471	146.	5.9	99.
531472	146.	5.8	98.
AVG.	146.	5.8	99.
S. D.	1.	0.3	1.
S. E.	0.	0.1	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/29/93

CONCENTRATION: 0 ppm

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531423	6.6	1529.	-	-	+2	6.5	-	0.2	+1
531424	7.0	1442.	-	-	+1	5.5	+1	0.2	-
531425	5.0	1334.	-	-	+1	6.5	-	0.2	+1
531426	6.8	1264.	-	-	+1	7.0	-	0.2	+
531427	7.4	1316.	-	-	+1	7.0	-	0.2	+1
531428	8.4	1155.	-	-	+1	7.0	-	0.2	+1
531429	5.0	1455.	-	-	+1	6.0	-	0.2	-
531430	7.0	1387.	-	-	+2	7.0	-	0.2	+1
531431	8.8	1047.	-	-	+1	7.0	-	0.2	+
531432	3.4	1698.	+	-	+2	6.0	-	0.2	+1
AVG.	6.5	1363.				6.6		0.2	
S. D.	1.6	186.				0.6		0.0	
S. E.	0.5	59.				0.2		0.0	

GROUP: III
SAMPLE DATE: 04/29/93

CONCENTRATION: 250 ppm

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531433	24.0	601.	-	-	+	7.0	-	0.2	+1
531434	11.6	1124.	-	-	+1	7.0	-	0.2	+1
531435	18.0	561.	-	-	+	7.0	-	0.2	-
531436	6.8	1129.	-	-	+1	7.0	-	0.2	+
531437	11.0	824.	-	-	+1	6.5	-	0.2	+
531438	11.2	916.	-	-	+1	6.5	-	0.2	+1
531439	7.8	1163.	-	-	+1	7.0	-	0.2	+1
531440	13.0	781.	-	-	+1	6.5	-	0.2	+1
531441	10.0	1272.	-	-	+2	6.5	-	0.2	+1
531442	9.8	1279.	+1	-	+2	7.5	-	0.2	+1
AVG.	12.3	965.				6.8		0.2	
S. D.	5.1	266.				0.3		0.0	
S. E.	1.6	84.				0.1		0.0	

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531443	9.8	1214.	-	-	+1	6.0	-	0.2	+1
531444	9.6	1111.	-	-	+1	6.0	-	0.2	+1
531445	7.0	1301.	-	-	+2	6.0	-	0.2	+1
531446	5.0	1868.	-	-	+2	6.0	-	0.2	+
531447	9.2	865.	-	-	+2	6.5	-	0.2	+
531448	6.0	1711.	-	-	+2	5.5	-	0.2	+1
531449	11.6	1041.	-	-	+1	6.5	-	0.2	+
531450	8.8	1068.	-	-	+1	6.0	-	0.2	+1
531451	5.6	1639.	-	-	+2	5.5	-	0.2	+1
531452	33.0	475.	-	-	+1	6.5	-	0.2	+
AVG.	10.6	1229.				6.1		0.2	
S. D.	8.2	420.				0.4		0.0	
S. E.	2.6	133.				0.1		0.0	

GROUP: VII
SAMPLE DATE: 04/29/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531453	20.0	626.	-	-	+1	7.0	-	0.2	+1
531454	9.6	1375.	-	-	+1	6.0	-	0.2	+1
531455	6.8	1658.	-	-	+2	6.5	-	0.2	+1
531456	14.4	926.	-	-	+	7.0	-	0.2	+1
531457	15.0	955.	-	-	+1	6.0	-	0.2	+
531458	7.2	1433.	-	-	+1	6.0	-	0.2	+1
531459	7.6	1158.	-	-	+2	6.0	-	0.2	+1
531460	8.6	1158.	-	-	+2	6.0	-	0.2	+1
531461	7.6	1512.	-	-	+2	6.0	-	0.2	+
531462	13.6	788.	-	-	+1	6.0	-	0.2	+1
AVG.	11.0	1159.				6.3		0.2	
S. D.	4.5	335.				0.4		0.0	
S. E.	1.4	106.				0.1		0.0	

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: IX CONCENTRATION: 1,000 ppm 1,3-butadiene
SAMPLE DATE: 04/29/93

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531463	10.6	1015.	-	-	+1	7.0	-	0.2	-
531464	11.8	931.	-	-	+1	6.0	-	0.2	+
531465	8.2	713.	-	-	+1	6.5	-	0.2	+
531466	4.6	1875.	-	-	+3	7.0	+1	0.2	+1
531467	10.2	860.	-	-	+1	7.5	-	0.2	+1
531468	11.6	1031.	-	-	+1	6.5	-	0.2	+1
531469	10.6	1083.	-	-	+1	6.5	-	0.2	+1
531470	6.0	1806.	-	-	+2	6.5	+1	0.2	+1
531471	6.2	1788.	-	-	+2	6.5	+1	0.2	+1
531472	9.0	1253.	-	-	+2	6.5	-	0.2	+1
AVG.	8.9	1236.				6.7		0.2	
S. D.	2.5	430.				0.4		0.0	
S. E.	0.8	136.				0.1		0.0	

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/29/93

CONCENTRATION: 0 ppm

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531423	0-0	1-2	1-2	0-0	Y CL PPT
531424	0-0	0-1	0-1	0-0	Y C
531425	0-0	0-0	0-1	0-0	Y CL PPT
531426	0-0	0-0	0-1	0-0	Y C
531427	1-2	0-2	1-2	0-0	Y CL PPT FEED
531428	0-1	1-2	1-2	0-0	Y CL PPT
531429	0-1	0-1	0-1	0-0	Y CL PPT
531430	0-2	0-0	0-1	0-0	Y CL PPT
531431	0-0	0-1	0-1	0-0	Y CL PPT
531432	3-4	0-0	0-2	0-0	Y CL PPT FEED

GROUP: III
SAMPLE DATE: 04/29/93

CONCENTRATION: 250 ppm

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531433	0-0	0-1	2-3	0-0	LY C
531434	0-0	0-1	1-2	0-0	Y CL PPT
531435	0-1	0-0	0-2	0-0	LY C
531436	0-2	0-0	1-2	0-0	Y CL PPT
531437	0-0	0-0	3-4	0-1	Y CL PPT
531438	0-0	0-1	0-2	0-0	Y C
531439	0-0	0-0	0-2	0-0	Y CL PPT
531440	0-0	0-2	1-2	0-0	Y C
531441	0-0	0-1	10-12	0-0	Y CL PPT
531442	4-5	0-0	6-8	0-0	DY CL PPT FEED FECES

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: V CONCENTRATION: 1,000 ppm
SAMPLE DATE: 04/29/93

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531443	0-0	0-1	6-8	0-0	Y CL PPT FEED
531444	0-1	0-0	1-3	0-0	Y C
531445	0-0	1-2	4-5	0-0	Y C
531446	0-0	0-1	1-2	0-1	Y C
531447	0-0	0-0	2-3	0-0	Y CL PPT
531448	0-0	0-1	5-6	0-2	Y CL PPT
531449	0-0	1-2	2-4	0-0	Y CL PPT
531450	0-0	1-2	1-3	0-0	Y C
531451	0-0	0-2	1-2	0-0	Y CL PPT
531452	0-1	0-0	5-6	0-0	LY C

GROUP: VII CONCENTRATION: 1,500 ppm
SAMPLE DATE: 04/29/93

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531453	0-0	0-1	1-3	0-0	LY CL PPT
531454	0-1	0-0	4-5	0-1	Y CL PPT FEED
531455	0-0	0-0	3-4	0-0	Y CL PPT
531456	0-0	0-1	1-2	0-0	LY CL PPT
531457	0-1	0-1	0-2	0-0	Y CL PPT FEED
531458	0-0	0-1	1-2	0-0	Y CL PPT FEED
531459	0-0	3-4	8-10	0-0	Y CL PPT
531460	0-2	0-0	3-4	0-0	Y CL PPT
531461	0-0	0-0	5-6	0-0	Y C
531462	0-0	0-2	0-2	0-0	Y C

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR MALE RATS
AT 90-DAY

GROUP: IX CONCENTRATION: 1,000 ppm 1,3-butadiene
SAMPLE DATE: 04/29/93

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531463	0-0	1-2	1-2	0-0	Y CL PPT
531464	0-0	0-1	0-2	0-0	Y C
531465	0-1	0-1	0-2	0-0	Y C
531466	0-0	1-2	0-2	0-0	Y CL PPT
531467	0-1	0-1	1-2	0-0	Y CL PPT
531468	0-1	0-1	2-4	0-0	Y C
531469	0-0	0-1	0-2	0-0	Y CL PPT
531470	0-0	0-0	1-3	0-0	Y CL PPT
531471	0-0	0-0	0-2	0-1	Y CL PPT
531472	0-0	1-2	6-8	0-0	Y CL PPT

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: II CONCENTRATION: 0 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
531478	7.75	16.0	43.	56.	21.	37.	1140.
531479	7.93	16.4	44.	55.	21.	38.	1054.
531480	7.93	16.2	44.	56.	20.	37.	1141.
531481	7.26	15.6	42.	57.	22.	38.	1176.
531482	7.08	15.0	41.	58.	21.	37.	916.
531483	7.98	16.5	45.	57.	21.	36.	1043.
531484	7.25	15.3	42.	57.	21.	37.	868.
531485	7.06	15.5	42.	59.	22.	37.	975.
531486	7.34	15.5	43.	59.	21.	36.	1013.
531487	7.36	15.3	42.	57.	21.	37.	1164.
AVG.	7.49	15.7	43.	57.	21.	37.	1049.
S. D.	0.36	0.5	1.	1.	1.	1.	107.
S. E.	0.12	0.2	0.	0.	0.	0.	34.

GROUP: IV CONCENTRATION: 250 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
531488	8.01	15.6	43.	54.	20.	36.	898.
531489	7.69	16.4	45.	58.	21.	37.	1032.
531490	7.71	16.2	44.	57.	21.	37.	990.
531491	7.76	15.9	44.	56.	21.	36.	985.
531492	7.64	15.5	43.	56.	20.	36.	1088.
531493	7.26	15.3	42.	57.	21.	37.	1107.
531494	7.39	15.8	43.	58.	21.	37.	1013.
531495	7.02	15.0	40.	57.	21.	38.	1071.
531496	7.64	15.6	42.	55.	20.	37.	1015.
531497	7.66	15.4	42.	55.	20.	36.	944.
AVG.	7.58	15.7	43.	56.	21.	37.	1014.
S. D.	0.28	0.4	1.	1.	1.	1.	65.
S. E.	0.09	0.1	0.	0.	0.	0.	20.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: VI
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
531498	7.28	15.9	42.	58.	22.	38.	858.
531500	7.68	15.5	42.	55.	20.	37.	908.
531501	7.75	16.3	44.	57.	21.	37.	1062.
531502	7.81	16.0	44.	56.	21.	37.	1261.
531503	7.46	15.1	42.	56.	20.	36.	997.
531504	7.75	16.0	44.	57.	21.	36.	1030.
531505	7.42	15.3	42.	56.	21.	37.	1114.
531506	7.25	15.0	40.	56.	21.	37.	1019.
531507	7.82	16.2	44.	57.	21.	37.	1264.
AVG.	7.58	15.7	43.	56.	21.	37.	1057.
S. D.	0.23	0.5	1.	1.	1.	1.	139.
S. E.	0.08	0.2	0.	0.	0.	0.	46.

GROUP: VIII
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
531508	6.98	14.4	39.	56.	21.	37.	1410.
531509	8.50	17.3	48.	56.	20.	36.	1157.
531510	7.28	15.7	43.	59.	22.	37.	1321.
531511	7.42	15.6	42.	56.	21.	38.	1309.
531512	7.22	15.2	41.	57.	21.	37.	1101.
531513	7.55	15.7	43.	57.	21.	37.	939.
531514	7.87	16.3	45.	57.	21.	37.	1294.
531515	6.86	15.0	41.	60.	22.	37.	1111.
531516	7.08	14.7	41.	57.	21.	36.	1087.
531517	7.66	15.5	43.	56.	20.	37.	1088.
AVG.	7.44	15.5	43.	57.	21.	37.	1182.
S. D.	0.49	0.8	3.	1.	1.	1.	145.
S. E.	0.15	0.3	1.	0.	0.	0.	46.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE RATS
AT 90-DAY

ROUP: X
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
531518	7.39	15.0	41.	55.	20.	37.	1196.
531519	7.20	15.1	41.	56.	21.	37.	1093.
531520	7.45	15.6	43.	57.	21.	36.	1167.
531521	7.54	15.0	41.	54.	20.	37.	1043.
531522	7.93	16.2	44.	55.	20.	37.	1122.
531523	7.55	15.1	42.	55.	20.	36.	1107.
531524	7.52	15.3	42.	56.	20.	36.	1049.
531525	7.32	15.4	43.	58.	21.	36.	1021.
531526	7.05	15.0	40.	57.	21.	37.	999.
531527	7.48	15.6	43.	57.	21.	36.	1095.
AVG.	7.44	15.3	42.	56.	21.	37.	1089.
S. D.	0.23	0.4	1.	1.	1.	1.	63.
S. E.	0.07	0.1	0.	0.	0.	0.	20.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: II
SAMPLE DATE: 04/30/93

CONCENTRATION: 0 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531478	10.4	832.	0.	8736.	0.	624.	208.	0.
531479	13.9	1807.	0.	10564.	0.	1390.	139.	0.
531480	11.0	660.	0.	9240.	0.	1100.	0.	0.
531481	10.1	606.	0.	8686.	0.	606.	202.	0.
531482	10.5	1155.	0.	8400.	0.	945.	0.	0.
531483	13.6	680.	0.	11968.	136.	816.	0.	0.
531484	17.2	688.	0.	14964.	0.	1376.	172.	0.
531485	11.9	595.	0.	10472.	238.	476.	119.	0.
531486	13.3	532.	0.	11704.	0.	1064.	0.	0.
531487	16.3	652.	0.	14833.	0.	489.	326.	0.
AVG.	12.8	821.	0.	10957.	37.	889.	117.	0.
S. D.	2.5	388.	0.	2418.	82.	342.	114.	0.
S. E.	0.8	123.	0.	765.	26.	108.	36.	0.

GROUP: IV
SAMPLE DATE: 04/30/93

CONCENTRATION: 250 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531488	9.6	192.	0.	8928.	0.	384.	96.	0.
531489	12.3	615.	0.	11070.	0.	492.	123.	0.
531490	14.1	705.	0.	12408.	141.	846.	0.	0.
531491	12.6	1890.	0.	9954.	0.	630.	126.	0.
531492	10.9	654.	0.	9592.	0.	654.	0.	0.
531493	12.4	1240.	0.	10416.	0.	620.	124.	0.
531494	13.6	544.	0.	11696.	0.	952.	408.	0.
531495	15.3	1224.	0.	12699.	0.	1224.	153.	0.
531496	11.5	1150.	0.	9430.	0.	805.	115.	0.
531497	9.9	594.	0.	8613.	0.	495.	198.	0.
AVG.	12.2	881.	0.	10481.	14.	710.	134.	0.
S. D.	1.8	490.	0.	1435.	45.	251.	114.	0.
S. E.	0.6	155.	0.	454.	14.	79.	36.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: VI CONCENTRATION: 1,000 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531498	9.1	637.	0.	7735.	0.	546.	182.	0.
531500	11.0	660.	0.	9240.	0.	1100.	0.	0.
531501	9.5	950.	0.	7790.	0.	665.	95.	0.
531502	11.3	339.	0.	10283.	0.	339.	339.	0.
531503	14.0	2660.	0.	10360.	420.	560.	0.	0.
531504	9.4	940.	0.	7614.	0.	846.	0.	0.
531505	9.1	1638.	0.	7007.	91.	273.	91.	0.
531506	17.3	2595.	0.	12802.	0.	1557.	346.	0.
531507	9.9	792.	0.	8217.	0.	792.	99.	0.
AVG.	11.2	1246.	0.	9005.	57.	742.	128.	0.
S. D.	2.8	859.	0.	1857.	139.	398.	136.	0.
S. E.	0.9	286.	0.	619.	46.	133.	45.	0.

GROUP: VIII CONCENTRATION: 1,500 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531508	13.4	2412.	0.	10318.	268.	402.	0.	0.
531509	10.1	808.	0.	7979.	0.	1212.	101.	0.
531510	14.1	3243.	0.	10152.	0.	705.	0.	0.
531511	16.4	4920.	0.	10660.	0.	820.	0.	0.
531512	8.0	640.	0.	6960.	0.	320.	80.	0.
531513	8.6	1118.	0.	6278.	0.	1032.	172.	0.
531514	10.2	1530.	0.	7344.	0.	1020.	306.	0.
531515	14.6	1022.	0.	12848.	0.	584.	146.	0.
531516	12.4	496.	0.	11036.	0.	744.	124.	0.
531517	16.3	2282.	0.	12714.	0.	1304.	0.	0.
AVG.	12.4	1847.	0.	9629.	27.	814.	93.	0.
S. D.	3.1	1397.	0.	2356.	85.	329.	100.	0.
S. E.	1.0	442.	0.	745.	27.	104.	32.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: X
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
531518	15.2	3648.	0.	9728.	0.	1672.	152.	0.
531519	8.4	1764.	0.	6216.	168.	84.	168.	0.
531520	14.8	2072.	0.	10656.	0.	1776.	296.	0.
531521	10.0	1000.	0.	8400.	0.	600.	0.	0.
531522	10.9	545.	0.	9701.	0.	654.	0.	0.
531523	11.4	342.	0.	10602.	0.	342.	114.	0.
531524	8.7	870.	0.	7221.	348.	174.	87.	0.
531525	15.5	620.	0.	14570.	0.	155.	155.	0.
531526	8.0	1120.	0.	6240.	0.	480.	160.	0.
531527	15.1	755.	0.	13137.	0.	1208.	0.	0.
AVG.	11.8	1274.	0.	9647.	52.	715.	113.	0.
S. D.	3.1	993.	0.	2772.	117.	624.	95.	0.
S. E.	1.0	314.	0.	877.	37.	197.	30.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: II
SAMPLE DATE: 04/30/93

CONCENTRATION: 0 ppm

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531478	40.	26.	64.	97.	16.	10.7	6.20
531479	74.	29.	59.	82.	16.	11.4	6.70
531480	81.	44.	90.	109.	18.	10.8	6.00
531481	47.	34.	71.	106.	17.	10.5	6.60
531482	59.	31.	59.	119.	13.	11.0	6.20
531483	83.	30.	79.	97.	12.	10.7	5.40
531484	44.	28.	63.	102.	12.	10.9	5.10
531485	39.	27.	66.	106.	18.	10.9	5.50
531486	55.	29.	56.	116.	12.	10.6	6.00
531487	59.	28.	56.	96.	16.	11.1	6.20
AVG.	58.	31.	66.	103.	15.	10.9	5.99
S. D.	16.	5.	11.	11.	2.	0.3	0.52
S. E.	5.	2.	3.	3.	1.	0.1	0.16

GROUP: IV
SAMPLE DATE: 04/30/93

CONCENTRATION: 250 ppm

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531488	47.	27.	74.	109.	19.	10.8	5.70
531489	56.	34.	58.	113.	16.	11.7	5.70
531490	68.	34.	70.	92.	18.	10.6	4.80
531491	76.	38.	76.	110.	14.	10.5	4.20
531492	56.	25.	63.	108.	12.	10.9	5.60
531493	62.	27.	72.	106.	14.	10.6	4.40
531494	32.	33.	60.	118.	16.	11.0	6.40
531495	47.	38.	81.	117.	18.	10.7	5.50
531496	46.	30.	55.	102.	16.	11.1	6.00
531497	64.	33.	88.	97.	14.	10.3	5.60
AVG.	55.	32.	70.	107.	16.	10.8	5.39
S. D.	13.	5.	11.	8.	2.	0.4	0.70
S. E.	4.	1.	3.	3.	1.	0.1	0.22

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: VI CONCENTRATION: 1,000 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531498	91.	36.	79.	118.	17.	10.7	5.50
531500	87.	43.	102.	107.	14.	10.3	5.60
531501	85.	34.	89.	108.	21.	10.7	5.00
531502	45.	20.	57.	107.	18.	10.7	6.10
531503	38.	35.	69.	100.	18.	10.4	3.70
531504	50.	43.	75.	101.	19.	11.0	5.50
531505	79.	27.	61.	107.	15.	10.9	5.50
531506	74.	31.	59.	101.	15.	11.0	6.20
531507	63.	34.	62.	93.	21.	11.0	.6.80
AVG.	68.	34.	73.	105.	18.	10.7	5.54
S. D.	20.	7.	15.	7.	3.	0.3	0.87
S. E.	7.	2.	5.	2.	1.	0.1	0.29

GROUP: VIII CONCENTRATION: 1,500 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531508	46.	38.	59.	105.	24.	10.8	6.50
531509	68.	28.	70.	99.	23.	10.9	6.70
531510	53.	45.	75.	107.	21.	10.8	6.20
531511	45.	37.	62.	95.	17.	10.2	5.90
531512	47.	34.	70.	106.	13.	10.3	4.40
531513	86.	28.	71.	93.	17.	10.8	6.00
531514	31.	20.	50.	108.	17.	11.0	5.80
531515	71.	38.	59.	108.	13.	11.3	5.70
531516	101.	48.	73.	103.	18.	10.8	5.50
531517	106.	47.	85.	100.	14.	10.4	6.10
AVG.	65.	36.	67.	102.	18.	10.7	5.88
S. D.	25.	9.	10.	5.	4.	0.3	0.63
S. E.	8.	3.	3.	2.	1.	0.1	0.20

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: X
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	ALP U/L	ALT U/L	AST U/L	GLUCO mg/dl	BUN mg/dl	CALC mg/dl	PHOS mg/dl
531518	54.	34.	62.	100.	16.	10.8	6.80
531519	56.	33.	75.	112.	12.	11.1	5.90
531520	51.	24.	49.	121.	21.	11.2	6.80
531521	25.	21.	54.	100.	12.	11.1	4.50
531522	78.	32.	86.	100.	12.	10.5	5.20
531523	74.	27.	54.	108.	14.	11.1	6.50
531524	109.	35.	73.	114.	19.	10.7	5.80
531525	87.	42.	61.	101.	17.	11.1	6.10
531526	43.	36.	75.	104.	16.	11.0	4.80
531527	93.	25.	52.	114.	15.	11.4	7.20
AVG.	67.	31.	64.	107.	15.	11.0	5.96
S. D.	26.	6.	12.	8.	3.	0.3	0.90
S. E.	8.	2.	4.	2.	1.	0.1	0.29

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: II CONCENTRATION: 0 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531478	0.13	76.	0.71	7.2	4.8	2.4
531479	0.23	52.	0.67	7.6	5.5	2.1
531480	0.18	52.	0.80	7.7	5.2	2.5
531481	0.16	59.	0.75	7.0	4.7	2.3
531482	0.18	37.	0.75	6.8	4.9	1.9
531483	0.16	79.	0.72	7.8	4.9	2.9
531484	0.16	102.	0.65	7.5	5.3	2.2
531485	0.14	96.	0.79	7.7	5.3	2.4
531486	0.23	91.	0.63	6.5	4.6	1.9
531487	0.20	64.	0.72	7.2	5.1	2.1
AVG.	0.18	71.	0.72	7.3	5.0	2.3
S. D.	0.03	21.	0.06	0.4	0.3	0.3
S. E.	0.01	7.	0.02	0.1	0.1	0.1

GROUP: IV CONCENTRATION: 250 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531488	0.16	58.	0.74	7.1	5.2	1.9
531489	0.29	112.	0.72	8.0	5.8	2.2
531490	0.19	76.	0.73	7.0	4.9	2.1
531491	0.15	79.	0.76	7.6	5.0	2.6
531492	0.14	67.	0.70	7.2	4.9	2.3
531493	0.28	30.	0.74	7.3	5.3	2.0
531494	0.22	92.	0.71	6.7	4.6	2.1
531495	0.24	75.	0.77	7.5	5.6	1.9
531496	0.16	55.	0.71	7.6	5.3	2.3
531497	0.14	97.	0.74	7.0	4.6	2.4
AVG.	0.20	74.	0.73	7.3	5.1	2.2
S. D.	0.06	23.	0.02	0.4	0.4	0.2
S. E.	0.02	7.	0.01	0.1	0.1	0.1

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: VI CONCENTRATION: 1,000 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531498	0.16	52.	0.74	7.8	5.4	2.4
531500	0.13	52.	0.67	7.3	4.9	2.4
531501	0.13	79.	0.95	7.1	4.7	2.4
531502	0.17	89.	0.73	6.9	4.7	2.2
531503	0.16	60.	0.68	7.1	4.9	2.2
531504	0.18	92.	0.68	7.6	5.6	2.0
531505	0.18	63.	0.78	7.8	5.3	2.5
531506	0.13	80.	0.69	7.2	5.0	2.2
531507	0.20	102.	0.74	7.5	5.1	2.4
AVG.	0.16	74.	0.74	7.4	5.1	2.3
S. D.	0.03	18.	0.09	0.3	0.3	0.2
S. E.	0.01	6.	0.03	0.1	0.1	0.1

GROUP: VIII CONCENTRATION: 1,500 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531508	0.18	101.	0.74	7.6	5.2	2.4
531509	0.13	64.	0.80	7.3	4.9	2.4
531510	0.16	106.	0.72	7.4	5.0	2.4
531511	0.16	69.	0.61	6.8	4.6	2.2
531512	0.13	80.	0.69	7.4	5.1	2.3
531513	0.19	80.	0.70	7.7	5.3	2.4
531514	0.13	124.	0.66	7.3	5.0	2.3
531515	0.20	144.	0.63	7.5	5.6	1.9
531516	0.16	84.	0.69	7.1	4.9	2.2
531517	0.16	76.	0.68	6.9	4.4	2.5
AVG.	0.16	93.	0.69	7.3	5.0	2.3
S. D.	0.02	26.	0.05	0.3	0.3	0.2
S. E.	0.01	8.	0.02	0.1	0.1	0.1

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: X
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	BILRN mg/dl	CHOL mg/dl	CREAT mg/dl	TPROT g/dl	ALBMN g/dl	GLOBN g/dl
531518	0.15	83.	0.64	6.6	4.6	2.0
531519	0.25	108.	0.67	7.7	5.9	1.8
531520	0.16	77.	0.72	7.0	4.5	2.5
531521	0.16	113.	0.69	7.7	5.5	2.2
531522	0.15	66.	0.73	7.2	4.9	2.3
531523	0.16	58.	0.65	7.1	5.2	1.9
531524	0.15	50.	0.74	6.9	4.9	2.0
531525	0.22	74.	0.74	7.7	5.8	1.9
531526	0.13	75.	0.69	7.2	5.2	2.0
531527	0.13	68.	0.68	7.1	4.8	2.3
AVG.	0.17	77.	0.69	7.2	5.1	2.1
S. D.	0.04	20.	0.04	0.4	0.5	0.2
S. E.	0.01	6.	0.01	0.1	0.2	0.1

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: II
SAMPLE DATE: 04/30/93

CONCENTRATION: 0 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531478	146.	5.8	100.
531479	145.	5.7	98.
531480	144.	5.4	97.
531481	144.	6.0	98.
531482	146.	5.6	100.
531483	145.	5.5	97.
531484	142.	5.6	98.
531485	143.	5.1	96.
531486	147.	5.6	101.
531487	141.	5.5	96.
AVG.	144.	5.6	98.
S. D.	2.	0.2	2.
S. E.	1.	0.1	1.

GROUP: IV
SAMPLE DATE: 04/30/93

CONCENTRATION: 250 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531488	146.	5.9	101.
531489	142.	5.1	97.
531490	145.	5.6	99.
531491	144.	5.2	100.
531492	145.	5.3	98.
531493	145.	5.0	99.
531494	143.	5.7	98.
531495	143.	5.0	99.
531496	139.	5.7	97.
531497	143.	5.4	98.
AVG.	144.	5.4	99.
S. D.	2.	0.3	1.
S. E.	1.	0.1	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: VI
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531498	146.	5.3	101.
531500	142.	5.7	99.
531501	143.	5.3	99.
531502	141.	5.7	98.
531503	143.	5.3	98.
531504	145.	5.8	99.
531505	142.	4.9	97.
531506	142.	5.1	96.
531507	141.	5.5	94.
AVG.	143.	5.4	98.
S. D.	2.	0.3	2.
S. E.	1.	0.1	1.

GROUP: VIII
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,500 ppm

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531508	143.	5.6	98.
531509	141.	6.0	95.
531510	143.	5.6	99.
531511	144.	5.8	102.
531512	143.	5.8	103.
531513	141.	5.4	97.
531514	147.	5.8	102.
531515	143.	4.9	97.
531516	143.	5.3	98.
531517	143.	5.4	99.
AVG.	143.	5.6	99.
S. D.	2.	0.3	3.
S. E.	1.	0.1	1.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL CHEMICAL FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: X
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	Na mmol/L	K mmol/L	Cl mmol/L
531518	144.	5.4	98.
531519	144.	5.1	98.
531520	143.	5.5	98.
531521	141.	5.4	97.
531522	143.	5.7	98.
531523	142.	6.1	98.
531524	145.	5.5	100.
531525	143.	5.5	97.
531526	147.	5.5	102.
531527	144.	5.6	97.
AVG.	144.	5.5	98.
S. D.	2.	0.3	2.
S. E.	1.	0.1	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: II CONCENTRATION: 0 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531478	9.8	592.	-	-	-	6.0	-	0.2	-
531479	8.8	965.	-	-	-	6.5	-	0.2	-
531480	2.2	1466.	-	-	+1	6.0	-	0.2	-
531481	7.0	899.	-	-	+	6.5	-	0.2	-
531482	19.5	436.	+	-	-	7.0	-	0.2	-
531483	9.6	714.	-	-	-	7.0	-	0.2	-
531484	7.6	730.	-	-	-	6.5	-	0.2	-
531485	7.8	962.	-	-	+	6.5	-	0.2	-
531486	10.6	743.	-	-	-	7.0	-	0.2	-
531487	8.4	782.	-	-	+	7.0	-	0.2	-
AVG.	9.1	829.				6.6		0.2	
S. D.	4.3	277.				0.4		0.0	
S. E.	1.4	88.				0.1		0.0	

GROUP: IV CONCENTRATION: 250 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531488	8.6	1007.	-	-	-	6.5	-	0.2	-
531489	4.4	887.	-	-	+1	6.0	-	0.2	+
531490	7.8	990.	-	-	+	6.0	-	0.2	-
531491	7.8	761.	-	-	-	6.5	-	0.2	-
531492	21.0	506.	-	-	-	6.5	-	0.2	-
531493	11.0	642.	-	-	-	6.5	-	0.2	-
531494	6.2	969.	-	-	+	5.5	-	0.2	+
531495	3.4	1645.	-	-	+1	6.0	-	0.2	+
531496	8.2	953.	-	-	+	6.0	-	0.2	-
531497	5.0	1098.	-	-	+1	6.0	-	0.2	+
AVG.	8.3	946.				6.2		0.2	
S. D.	5.0	306.				0.3		0.0	
S. E.	1.6	97.				0.1		0.0	

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: VI CONCENTRATION: 1,000 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531498	13.0	609.	-	-	-	7.0	-	0.2	-
531500	8.2	705.	-	-	+	6.5	-	0.2	+
531501	3.6	1345.	-	-	+2	6.5	-	0.2	+
531502	3.4	1664.	-	-	+2	5.5	-	0.2	+
531503	3.0	2244.	-	-	+2	6.0	-	1.0	-
531504	2.0	2523.	-	-	+2	5.5	-	0.2	-
531505	4.8	1540.	-	-	+1	6.0	-	0.2	-
531506	8.4	1268.	-	-	+1	6.0	-	0.2	+
531507	10.0	829.	-	-	-	6.0	-	0.2	+
AVG.	6.3	1414.				6.1		0.3	
S. D.	3.8	663.				0.5		0.3	
S. E.	1.3	221.				0.2		0.1	

GROUP: VIII CONCENTRATION: 1,500 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531508	4.0	1257.	-	-	+	6.0	-	0.2	+
531509	1.4	2900.	+2	-	+3	5.0	-	1.0	-
531510	13.0	884.	-	-	-	6.5	-	0.2	+
531511	4.2	1755.	-	-	+1	6.0	-	0.2	+
531512	18.0	581.	-	-	-	6.5	-	0.2	-
531513	4.8	1659.	-	-	+1	5.5	-	0.2	+
531514	2.2	2050.	-	-	+2	5.5	-	0.2	+
531515	9.0	978.	-	-	+1	5.0	-	0.2	-
531516	14.2	902.	-	-	+	6.5	-	0.2	+
531517	4.8	1640.	-	-	+1	5.5	-	0.2	+
AVG.	7.6	1461.				5.8		0.3	
S. D.	5.7	689.				0.6		0.3	
S. E.	1.8	218.				0.2		0.1	

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: X CONCENTRATION: 1,000 ppm 1,3-butadiene
SAMPLE DATE: 04/30/93

ANIMAL#:	VOL ml	OSMOL mOs	BLOOD	GLUCOSE	PRO-TEIN	pH	BILI-RUBIN	UROBL mg/dl	Ke-tone
531518	2.2	2456.	-	-	+2	5.0	+1	1.0	-
531519	13.6	720.	-	-	+1	6.0	-	0.2	-
531520	7.6	987.	-	-	+	6.5	-	0.2	-
531521	5.6	1168.	-	-	+1	6.0	-	0.2	-
531522	9.6	552.	+1	+	+	7.0	-	0.2	-
531523	2.6	1644.	+	-	+2	6.0	+1	0.2	-
531524	5.8	1403.	+1	-	+1	6.5	-	0.2	-
531525	4.0	1578.	-	-	+1	6.5	-	0.2	-
531526	2.8	2234.	-	-	+2	5.5	+1	1.0	-
531527	7.6	1178.	-	-	+1	6.5	-	0.2	-
AVG.	6.1	1392.				6.2		0.4	
S. D.	3.6	611.				0.6		0.3	
S. E.	1.1	193.				0.2		0.1	

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: II CONCENTRATION: 0 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531478	0-0	0-0	0-0	0-0	Y C
531479	0-1	0-1	1-2	0-0	Y C
531480	0-0	0-0	0-1	0-0	Y C
531481	0-0	0-1	0-1	0-0	Y C
531482	1-3	0-0	0-1	0-0	Y CL PPT FEED
531483	0-0	0-1	0-2	0-0	Y CL PPT FEED
531484	0-1	0-0	0-1	0-0	Y C
531485	0-0	0-1	0-1	0-0	Y C
531486	0-0	0-0	0-1	0-0	Y C
531487	0-1	0-1	0-1	0-0	Y C

GROUP: IV CONCENTRATION: 250 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531488	0-0	0-1	1-2	0-1	Y C
531489	0-1	1-2	4-5	0-0	Y CL PPT FEED
531490	0-1	0-1	2-3	0-0	Y C
531491	0-0	0-1	0-0	0-0	Y C
531492	0-0	0-1	0-1	0-0	LY C
531493	0-1	0-1	0-1	0-0	Y CL PPT FEED
531494	0-0	0-0	0-1	0-0	Y C
531495	0-0	1-2	0-2	0-0	Y CL PPT FEED
531496	0-1	0-2	1-2	0-0	Y C
531497	0-0	1-2	1-2	0-0	Y C

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: VI CONCENTRATION: 1,000 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531498	0-0	1-2	0-2	0-0	Y C
531500	0-1	0-1	0-2	0-0	Y C
531501	2-3	3-4	5-6	0-0	Y CL PPT FEED
531502	0-0	2-4	0-2	0-0	Y C
531503	0-1	0-0	0-1	0-0	Y C
531504	0-0	0-2	0-2	0-0	Y C
531505	0-0	0-0	0-2	0-0	Y C
531506	2-3	0-1	2-3	0-0	Y CL PPT FEED
531507	0-0	0-2	0-2	0-0	Y C FEED

GROUP: VIII CONCENTRATION: 1,500 ppm
SAMPLE DATE: 04/30/93

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531508	0-1	1-2	0-1	0-0	Y C
531509	12-15	0-2	3-4	0-0	Y C
531510	0-1	1-2	0-2	0-0	Y CL PPT FEED
531511	0-0	0-1	0-1	0-0	Y CL PPT
531512	0-0	0-0	0-2	0-1	LY C
531513	0-0	0-1	0-0	0-1	Y C
531514	0-1	0-1	0-1	0-1	Y C
531515	0-0	0-2	0-2	0-0	Y C
531516	0-2	0-0	0-2	0-0	Y CL PPT
531517	1-3	4-6	1-2	0-0	Y C

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL CLINICAL URINALYSIS FINDINGS FOR FEMALE RATS
AT 90-DAY

GROUP: X
SAMPLE DATE: 04/30/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	RBC /hpf	WBC /hpf	Epith /hpf	Cast /lpf	APPEARANCE
531518	0-0	0-2	0-1	0-0	Y C
531519	0-1	0-1	0-1	0-0	Y C
531520	0-1	0-0	0-1	0-0	Y CL PPT
531521	0-0	1-3	1-2	0-0	Y CL PPT
531522	6-8	3-4	5-6	0-0	DY CL PPT FEED FECES
531523	0-1	0-2	1-2	0-0	Y CL PPT
531524	4-6	1-2	4-5	0-0	Y CL PPT FEED
531525	0-1	0-1	0-1	0-0	Y C
531526	2-3	0-0	1-2	0-0	Y CL PPT
531527	0-0	0-2	0-2	0-1	Y C

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX M

Individual Clinical Pathology Data of Mice

APPENDIX - EXPLANATORY NOTES

ANIMALS DYING PRIOR TO CLINICAL PATHOLOGY EVALUATION

Animal #	Group	Reason
91375	I	sacrificed in extremis
91401	VII	found dead
91402	VII	found dead
91403	VII	found dead
91404	VII	found dead
91405	VII	found dead
91406	VII	found dead
91407	VII	found dead
91408	VII	found dead
91409	VII	found dead
91410	VII	found dead
91447	VI	sacrificed in extremis
91451	VIII	found dead
91452	VIII	found dead
91453	VIII	found dead
91454	VIII	found dead
91455	VIII	found dead
91457	VIII	found dead
91459	VIII	found dead
91460	VIII	found dead
91463	X	found dead
91469	X	found dead

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE MICE
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/27/93

CONCENTRATION: 0.0 ppm

ANIMAL#:	RBC x10^6/ul	Hb g/dl	Ht %	Retic x10^3/ul	MCV fl	MCH pg	MCHC g/dl	PLAT x10^3/ul
91371	10.93	17.9	53.	120.	49.	16.	34.	1318.
91372	10.77	17.3	50.	258.	47.	16.	34.	1180.
91373	9.99	16.2	49.	335.	49.	16.	33.	1136.
91374	10.84	18.4	53.	233.	49.	17.	35.	916.
91376	11.07	18.3	54.	194.	49.	17.	34.	590.
91377	10.48	17.5	53.	189.	50.	17.	33.	1044.
91378	10.15	16.6	49.	401.	48.	16.	34.	1010.
91379	10.54	17.3	51.	385.	48.	16.	34.	978.
91380	9.98	16.6	49.	235.	49.	17.	34.	950.
AVG.	10.53	17.3	51.	261.	49.	16.	34.	1014.
S. D.	0.41	0.8	2.	95.	1.	1.	1.	203.
S. E.	0.14	0.3	1.	32.	0.	0.	0.	68.

GROUP: III
SAMPLE DATE: 04/27/93

CONCENTRATION: 50 ppm

ANIMAL#:	RBC x10^6/ul	Hb g/dl	Ht %	Retic x10^3/ul	MCV fl	MCH pg	MCHC g/dl	PLAT x10^3/ul
91381	9.97	17.1	49.		49.	17.	35.	1159.
91382	10.78	17.3	51.		47.	16.	34.	892.
91383	6.70	12.3	34.		51.	18.	36.	1312.
91384	10.73	17.9	52.		48.	17.	35.	1173.
91385	10.84	18.0	51.		47.	17.	35.	781.
91386	11.07	18.7	54.		49.	17.	35.	993.
91387	10.71	17.1	51.		48.	16.	34.	985.
91388	10.96	17.3	51.		47.	16.	34.	872.
91389	10.30	16.2	48.		46.	16.	34.	1201.
91390	10.54	18.1	56.		53.	17.	33.	614.
AVG.	10.26	17.0	50.		49.	17.	35.	998.
S. D.	1.29	1.8	6.		2.	1.	1.	216.
S. E.	0.41	0.6	2.		1.	0.	0.	68.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE MICE
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/27/93

CONCENTRATION: 250 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	Retic $x10^3$/ul	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
91391	10.27	17.0	50.	329.	49.	17.	34.	953.
91392	10.52	17.6	51.	121.	49.	17.	34.	891.
91393	10.71	18.1	52.	236.	49.	17.	35.	745.
91394	10.33	17.0	50.	129.	49.	17.	34.	887.
91395	10.49	17.4	50.	157.	47.	17.	35.	905.
91396	10.71	18.1	52.	471.	49.	17.	35.	704.
91397	10.75	17.9	51.	355.	48.	17.	35.	860.
91398	10.42	18.0	52.	516.	50.	17.	35.	724.
91399	11.02	18.4	54.	259.	49.	17.	34.	866.
91400	10.64	17.9	51.	186.	48.	17.	35.	892.
AVG.	10.59	17.7	51.	276.	49.	17.	35.	843.
S. D.	0.22	0.5	1.	139.	1.	0.	1.	86.
S. E.	0.07	0.2	0.	44.	0.	0.	0.	27.

GROUP: IX
SAMPLE DATE: 04/27/93

CONCENTRATION: 1,000 ppm 1,3 butadiene

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	Retic $x10^3$/ul	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
91411	8.27	15.4	45.	538.	54.	19.	35.	729.
91412	8.17	15.7	44.	286.	54.	19.	36.	699.
91413	8.30	16.1	45.	187.	54.	19.	36.	685.
91414	8.08	15.6	44.	360.	54.	19.	36.	752.
91415	8.71	16.2	47.	322.	54.	19.	35.	844.
91416	8.55	16.2	46.	479.	54.	19.	35.	761.
91417	7.80	15.6	42.	402.	54.	20.	37.	696.
91418	8.45	16.0	45.	549.	53.	19.	36.	1020.
91419	8.37	15.9	46.	431.	55.	19.	34.	752.
91420	7.91	14.9	43.	324.	54.	19.	35.	816.
AVG.	8.26	15.8	45.	388.	54.	19.	36.	775.
S. D.	0.28	0.4	1.	115.	0.	0.	1.	100.
S. E.	0.09	0.1	0.	36.	0.	0.	0.	32.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE MICE
AT 90-DAY

GROUP: I
SAMPLE DATE: 04/27/93

CONCENTRATION: 0.0 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91371	2.4	792.	0.	1608.	0.	0.	0.	0.
91372	3.1	620.	0.	2387.	0.	62.	31.	0.
91373	3.0	1020.	0.	1950.	0.	30.	0.	0.
91374	2.7	486.	0.	2187.	0.	0.	27.	0.
91376	1.4	112.	0.	1274.	14.	0.	0.	0.
91377	2.8	784.	0.	1960.	0.	56.	0.	0.
91378	1.2	264.	0.	912.	0.	24.	0.	0.
91379	2.2	704.	0.	1452.	0.	22.	22.	0.
91380	2.5	325.	0.	2125.	0.	50.	0.	0.
AVG.	2.4	567.	0.	1762.	2.	27.	9.	0.
S. D.	0.7	293.	0.	482.	5.	25.	14.	0.
S. E.	0.2	98.	0.	161.	2.	8.	5.	0.

GROUP: III
SAMPLE DATE: 04/27/93

CONCENTRATION: 50 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91381	3.5	315.	0.	3185.	0.	0.	0.	0.
91382	3.4	442.	0.	2822.	0.	102.	34.	0.
91383	3.6	504.	0.	3096.	0.	0.	0.	0.
91384	3.0	300.	0.	2580.	0.	90.	30.	0.
91385	1.3	650.	0.	637.	0.	13.	0.	0.
91386	1.1	154.	0.	880.	0.	55.	11.	0.
91387	2.5	150.	0.	2350.	0.	0.	0.	0.
91388	3.9	507.	0.	3198.	0.	195.	0.	0.
91389	2.5	625.	0.	1800.	0.	75.	0.	0.
91390	1.6	128.	16.	1360.	0.	64.	32.	0.
AVG.	2.6	378.	2.	2191.	0.	59.	11.	0.
S. D.	1.0	196.	5.	965.	0.	62.	15.	0.
S. E.	0.3	62.	2.	305.	0.	19.	5.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR MALE MICE
AT 90-DAY

GROUP: V
SAMPLE DATE: 04/27/93

CONCENTRATION: 250 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91391	1.3	468.	0.	806.	0.	26.	0.	0.
91392	1.3	195.	0.	1079.	0.	26.	0.	0.
91393	2.3	782.	0.	1495.	0.	23.	0.	0.
91394	2.0	540.	20.	1440.	0.	0.	0.	0.
91395	2.4	240.	0.	2064.	0.	72.	24.	0.
91396	1.0	60.	0.	930.	0.	10.	0.	0.
91397	2.0	540.	0.	1460.	0.	0.	0.	0.
91398	1.1	66.	0.	1034.	0.	0.	0.	0.
91399	1.3	78.	0.	1222.	0.	0.	0.	0.
91400	1.8	126.	0.	1674.	0.	0.	0.	0.
AVG.	1.6	310.	2.	1320.	0.	16.	2.	0.
S. D.	0.5	254.	6.	381.	0.	23.	8.	0.
S. E.	0.2	80.	2.	121.	0.	7.	2.	0.

GROUP: IX
SAMPLE DATE: 04/27/93

CONCENTRATION: 1,000 ppm 1,3 butadiene

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91411	3.2	544.	0.	2592.	0.	64.	0.	0.
91412	2.7	405.	0.	2214.	0.	54.	27.	0.
91413	2.7	270.	0.	2376.	0.	54.	0.	0.
91414	3.1	496.	0.	2573.	0.	0.	31.	0.
91415	3.7	555.	0.	2923.	0.	185.	37.	0.
91416	2.7	216.	0.	2376.	0.	81.	27.	0.
91417	2.8	140.	0.	2660.	0.	0.	0.	0.
91418	2.7	405.	0.	2268.	0.	27.	0.	0.
91419	2.2	396.	0.	1782.	0.	0.	22.	0.
91420	2.7	378.	0.	2268.	0.	54.	0.	0.
AVG.	2.9	381.	0.	2403.	0.	52.	14.	0.
S. D.	0.4	137.	0.	309.	0.	55.	16.	0.
S. E.	0.1	43.	0.	98.	0.	17.	5.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE MICE
AT 90-DAY

GROUP: II
SAMPLE DATE: 04/28/93

CONCENTRATION: 0 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	Retic $x10^3$/ul	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
91421	10.69	17.5	51.	347.	48.	16.	34.	905.
91422	10.36	16.9	50.	186.	48.	16.	34.	1012.
91423	10.55	17.4	50.	16.	47.	17.	35.	1172.
91424	10.70	17.3	51.	70.	47.	16.	34.	1030.
91425	10.58	17.6	50.	48.	48.	17.	35.	862.
91426	10.48	16.9	49.	435.	47.	16.	34.	879.
91427	10.24	16.2	49.	287.	48.	16.	33.	782.
91428	10.60	18.1	51.	170.	48.	17.	36.	548.
91429	10.76	17.3	51.	269.	47.	16.	34.	827.
91430	10.86	17.0	52.	261.	48.	16.	33.	845.
AVG.	10.58	17.2	50.	209.	48.	16.	34.	886.
S. D.	0.19	0.5	1.	136.	1.	0.	1.	167.
S. E.	0.06	0.2	0.	43.	0.	0.	0.	53.

GROUP: IV
SAMPLE DATE: 04/28/93

CONCENTRATION: 50 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	Retic $x10^3$/ul	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
91431	10.93	18.0	51.		47.	17.	35.	786.
91432	10.61	17.7	51.		48.	17.	35.	761.
91433	11.04	18.3	52.		48.	17.	35.	587.
91434	10.86	17.7	51.		47.	16.	35.	705.
91435	10.46	17.0	49.		46.	16.	35.	786.
91436	11.07	18.1	52.		47.	16.	35.	651.
91437	10.72	17.2	51.		48.	16.	34.	784.
91438	10.79	17.4	51.		47.	16.	34.	806.
91440	10.79	17.3	52.		48.	16.	34.	764.
91471	10.84	17.0	52.		48.	16.	33.	1158.
AVG.	10.81	17.6	51.		47.	16.	35.	779.
S. D.	0.19	0.5	1.		1.	0.	1.	150.
S. E.	0.06	0.1	0.		0.	0.	0.	48.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE MICE
AT 90-DAY

GROUP: VI
SAMPLE DATE: 04/28/93

CONCENTRATION: 250 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	Retic $x10^3$/ul	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
91441	11.10	18.0	53.		48.	16.	34.	1022.
91442	10.65	17.1	51.		48.	16.	34.	890.
91443	10.67	17.0	50.		47.	16.	34.	830.
91444	10.24	16.8	49.		48.	16.	34.	949.
91445	10.82	16.9	52.		48.	16.	33.	1095.
91446	10.48	17.1	50.		47.	16.	35.	979.
91448	10.52	16.8	49.		46.	16.	35.	956.
91449	10.70	16.6	50.		47.	16.	33.	1006.
91450	10.81	17.3	52.		48.	16.	34.	946.
AVG.	10.67	17.1	51.		47.	16.	34.	964.
S. D.	0.24	0.4	1.		1.	0.	1.	76.
S. E.	0.08	0.1	0.		0.	0.	0.	25.

GROUP: VIII
SAMPLE DATE: 04/28/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	Retic $x10^3$/ul	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
91456	10.78	16.9	54.	194.	50.	16.	31.	1109.
91458	10.33	17.1	51.	491.	50.	17.	33.	1162.
AVG.	10.56	17.0	53.	343.	50.	17.	32.	1136.
S. D.	0.32	0.1	2.	210.	0.	1.	1.	37.
S. E.	0.22	0.1	2.	149.	0.	1.	1.	27.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE MICE
AT 90-DAY

GROUP: X
SAMPLE DATE: 04/28/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	RBC $x10^6$/ul	Hb g/dl	Ht %	Retic $x10^3$/ul	MCV fl	MCH pg	MCHC g/dl	PLAT $x10^3$/ul
91461	9.13	16.2	48.	301.	52.	18.	34.	739.
91462	9.80	16.7	51.	353.	52.	17.	33.	846.
91464	9.09	16.2	47.	373.	51.	18.	35.	765.
91465	9.47	16.9	50.	189.	52.	18.	34.	522.
91466	9.13	16.2	48.	561.	53.	18.	34.	701.
91467	9.51	16.8	50.	533.	53.	18.	34.	648.
91468	9.13	16.2	48.	324.	52.	18.	34.	738.
91470	8.76	16.2	45.	53.	52.	19.	36.	773.
AVG.	9.25	16.4	48.	336.	52.	18.	34.	717.
S. D.	0.32	0.3	2.	167.	1.	1.	1.	97.
S. E.	0.11	0.1	1.	59.	0.	0.	0.	34.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE MICE
AT 90-DAY

GROUP: II CONCENTRATION: 0 ppm
SAMPLE DATE: 04/28/93

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91421	5.5	440.	0.	4620.	0.	330.	110.	0.
91422	2.8	420.	0.	2324.	0.	56.	0.	0.
91423	1.6	576.	0.	992.	0.	32.	0.	0.
91424	3.1	1302.	0.	1798.	0.	0.	0.	0.
91425	2.7	702.	0.	1998.	0.	0.	0.	0.
91426	3.6	216.	0.	3312.	0.	0.	72.	0.
91427	1.2	156.	0.	1020.	0.	24.	0.	0.
91428	2.2	242.	0.	1936.	0.	22.	0.	0.
91429	3.8	1292.	0.	2508.	0.	0.	0.	0.
91430	2.0	160.	0.	1820.	0.	20.	0.	0.
AVG.	2.8	551.	0.	2233.	0.	48.	18.	0.
S. D.	1.2	432.	0.	1078.	0.	101.	39.	0.
S. E.	0.4	137.	0.	341.	0.	32.	12.	0.

GROUP: IV CONCENTRATION: 50 ppm
SAMPLE DATE: 04/28/93

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91431	1.6	352.	0.	1248.	0.	0.	0.	0.
91432	1.6	272.	0.	1312.	0.	16.	0.	0.
91433	2.6	416.	0.	2158.	0.	26.	0.	0.
91434	2.2	374.	0.	1826.	0.	0.	0.	0.
91435	2.5	850.	0.	1650.	0.	0.	0.	0.
91436	2.4	264.	0.	2088.	0.	48.	0.	0.
91437	2.1	294.	0.	1806.	0.	0.	0.	0.
91438	1.1	165.	0.	924.	0.	11.	0.	0.
91440	1.6	256.	0.	1328.	0.	16.	0.	0.
91471	3.7	1776.	0.	1924.	0.	0.	0.	0.
AVG.	2.1	502.	0.	1626.	0.	12.	0.	0.
S. D.	0.7	485.	0.	406.	0.	16.	0.	0.
S. E.	0.2	153.	0.	128.	0.	5.	0.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE MICE
AT 90-DAY

GROUP: VI
SAMPLE DATE: 04/28/93

CONCENTRATION: 250 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91441	3.2	1024.	0.	2176.	0.	0.	0.	0.
91442	3.6	648.	0.	2916.	0.	36.	0.	0.
91443	2.8	812.	0.	1960.	0.	28.	0.	0.
91444	3.0	180.	0.	2790.	0.	0.	30.	0.
91445	3.0	1620.	0.	1350.	0.	30.	0.	0.
91446	2.7	162.	0.	2511.	0.	27.	0.	0.
91448	1.8	630.	0.	1116.	0.	54.	0.	0.
91449	1.1	374.	0.	715.	0.	11.	0.	0.
91450	1.3	169.	0.	1131.	0.	0.	0.	0.
AVG.	2.5	624.	0.	1852.	0.	21.	3.	0.
S. D.	0.9	483.	0.	804.	0.	19.	10.	0.
S. E.	0.3	161.	0.	268.	0.	6.	3.	0.

GROUP: VIII
SAMPLE DATE: 04/28/93

CONCENTRATION: 1,000 ppm

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91456	4.5	585.	0.	3915.	0.	0.	0.	0.
91458	2.0	260.	0.	1740.	0.	0.	0.	0.
AVG.	3.3	423.	0.	2828.	0.	0.	0.	0.
S. D.	1.8	230.	0.	1538.	0.	0.	0.	0.
S. E.	1.3	163.	0.	1088.	0.	0.	0.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (continued)

INDIVIDUAL HEMATOLOGIC FINDINGS FOR FEMALE MICE
AT 90-DAY

GROUP: X
SAMPLE DATE: 04/28/93

CONCENTRATION: 1,000 ppm 1,3-butadiene

ANIMAL#:	WBC x10^3/ul	Neut WBCx%	Band WBCx%	Lymph WBCx%	Alym WBCx%	Mono WBCx%	Eosin WBCx%	Baso WBCx%
91461	3.2	160.	0.	3040.	0.	0.	0.	0.
91462	3.9	507.	0.	3393.	0.	0.	0.	0.
91464	4.8	576.	0.	4080.	0.	96.	48.	0.
91465	2.0	160.	0.	1840.	0.	0.	0.	0.
91466	2.7	243.	0.	2457.	0.	0.	0.	0.
91467	2.7	243.	0.	2430.	0.	27.	0.	0.
91468	3.8	380.	0.	3382.	0.	38.	0.	0.
91470	1.7	289.	0.	1394.	0.	17.	0.	0.
AVG.	3.1	320.	0.	2752.	0.	22.	6.	0.
S. D.	1.0	155.	0.	888.	0.	33.	17.	0.
S. E.	0.4	55.	0.	314.	0.	12.	6.	0.

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX N

Individual Animal Final Body and Organ Weights of Rats

APPENDIX
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE RATS

GROUP : I - 0 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531423	553.4	15.098	4.323	2.162	1.614	0.771	0.081	3.562	2.011
531424	542.7	15.610	3.950	2.017	1.836	0.780	0.066	3.793	2.173
531425	469.3	12.787	3.295	1.812	1.645	0.632	0.062	3.250	2.210
531426	509.1	13.487	3.585	1.959	1.683	0.833	0.065	3.967	2.171
531427	519.9	14.987	3.973	1.880	1.728	0.854	0.062	3.847	2.243
531428	564.5	16.006	4.447	2.230	1.756	1.319	0.070	3.535	2.645
531429	546.6	15.293	3.842	2.380	2.023	0.840	0.079	3.457	2.201
531430	593.2	18.189	4.345	1.951	2.027	0.848	0.067	3.762	2.266
531431	452.4	11.038	2.955	1.833	1.313	0.654	0.064	3.602	2.101
531432	482.4	15.018	3.714	1.815	1.692	0.605	0.074	3.356	2.091
GROUP MEAN	523.3	14.751	3.843	2.004	1.732	0.814	0.069	3.613	2.211
STD. DEV.	45.0	1.941	0.477	0.194	0.207	0.201	0.007	0.227	0.171

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE RATS

GROUP : III - 250 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531433	529.7	14.745	4.342	2.147	1.870	0.858	0.073	4.204	1.987
531434	583.1	17.302	4.275	2.168	1.741	0.963	0.062	4.055	2.222
531435	493.4	14.010	3.680	2.422	1.846	0.827	0.086	3.423	1.976
531436	508.9	16.956	4.225	2.414	1.686	0.892	0.073	3.783	2.264
531437	559.8	18.601	4.507	2.064	1.912	0.816	0.071	3.646	2.154
531438	447.9	11.884	3.323	1.542	1.406	0.581	0.053	3.780	2.200
531439	536.2	16.336	4.304	2.213	1.739	0.815	0.071	3.287	2.183
531440	531.5	14.169	4.035	1.959	1.751	0.962	0.084	3.795	2.131
531441	546.6	14.331	4.673	2.176	2.023	0.801	0.063	3.905	2.219
531442	526.5	14.461	4.247	2.066	1.970	0.882	0.071	3.936	2.157
GROUP MEAN	526.4	15.280	4.161	2.117	1.794	0.840	0.071	3.781	2.149
STD. DEV.	37.2	1.980	0.396	0.249	0.175	0.108	0.010	0.276	0.096

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE RATS

GROUP : V - 1000 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531443	512.7	15.723	4.160	2.004	1.662	0.849	0.060	3.689	2.101
531444	470.3	16.036	3.913	1.890	1.572	0.538	0.062	3.351	2.034
531445	492.6	15.441	3.647	1.913	1.520	0.679	0.052	3.503	2.086
531446	491.7	17.609	3.907	1.978	1.572	0.719	0.062	3.404	2.122
531447	539.3	16.947	4.139	2.101	1.572	0.889	0.073	3.661	2.055
531448	494.0	16.816	4.612	1.948	1.849	0.840	0.058	3.385	2.118
531449	467.1	15.966	4.401	1.825	1.633	0.702	0.067	3.549	2.330
531450	534.2	17.036	4.050	2.153	1.720	0.970	0.062	4.228	2.169
531451	461.7	15.815	4.328	2.167	1.651	0.874	0.062	3.739	2.136
531452	537.0	16.485	5.172	1.888	1.703	0.861	0.060	3.016	2.143
GROUP MEAN	500.1	16.387	4.233	1.987	1.645	0.792	0.062	3.553	2.129
STD. DEV.	29.5	0.698	0.430	0.118	0.096	0.129	0.005	0.317	0.081

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE RATS

GROUP : VII - 1500 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531453	466.7	15.308	3.815	2.028	1.516	0.692	0.069	3.401	2.024
531454	506.2	20.054	5.157	2.317	1.841	0.794	0.078	3.769	2.108
531455	478.9	16.059	4.276	1.938	1.858	0.951	0.075	3.875	2.102
531456	482.7	17.404	4.357	2.128	1.668	0.865	0.073	3.926	2.152
531457	506.9	17.137	4.059	1.915	1.733	0.616	0.073	3.523	2.217
531458	422.2	15.758	3.925	2.035	1.545	0.631	0.068	3.734	2.098
531459	472.7	16.448	4.232	1.816	1.537	0.654	0.062	3.493	2.070
531460	463.2	16.421	4.186	1.974	1.396	0.707	0.064	3.705	2.015
531461	505.7	19.904	4.503	2.227	1.492	0.852	0.063	3.471	2.139
531462	441.5	14.803	3.377	1.789	1.451	0.809	0.053	4.000	2.101
GROUP MEAN	474.7	16.930	4.189	2.017	1.604	0.757	0.068	3.690	2.103
STD. DEV.	28.1	1.784	0.467	0.169	0.162	0.113	0.007	0.209	0.059

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE RATS

GROUP : IX - 1000 ppm - 1,3-BUTADIENE

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531463	472.3	15.365	3.821	1.920	1.594	0.585	0.062	3.735	2.162
531464	465.5	13.828	3.406	1.936	1.505	0.718	0.060	3.492	2.073
531465	468.6	13.090	3.820	1.875	1.700	0.874	0.064	3.395	2.234
531466	429.2	13.283	3.462	1.568	1.472	0.662	0.067	2.818	2.122
531467	491.8	14.957	4.263	1.904	1.804	0.756	0.072	3.414	2.105
531468	606.2	18.622	4.862	2.002	1.988	0.962	0.064	3.882	2.323
531469	568.1	16.701	4.806	2.018	1.783	0.927	0.075	4.503	2.603
531470	529.7	16.558	4.308	1.930	1.807	0.825	0.057	3.604	2.323
531471	546.3	15.691	4.746	2.023	1.692	0.778	0.086	3.926	2.086
531472	562.8	18.231	4.932	2.108	1.703	0.972	0.071	3.719	2.372
GROUP MEAN	514.0	15.633	4.243	1.928	1.705	0.806	0.068	3.649	2.240
STD. DEV.	56.7	1.928	0.587	0.145	0.154	0.130	0.009	0.436	0.168

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE RATS

GROUP : 1 - 0 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531423	2.7282	0.7812	0.3907	0.2917	0.1393	0.0146	0.6437	0.3634
531424	2.8764	0.7278	0.3717	0.3383	0.1437	0.0122	0.6989	0.4004
531425	2.7247	0.7021	0.3861	0.3505	0.1347	0.0132	0.6925	0.4709
531426	2.6492	0.7042	0.3848	0.3306	0.1636	0.0128	0.7792	0.4264
531427	2.8827	0.7642	0.3616	0.3324	0.1643	0.0119	0.7399	0.4314
531428	2.8354	0.7878	0.3950	0.3111	0.2337	0.0124	0.6262	0.4686
531429	2.7978	0.7029	0.4354	0.3701	0.1537	0.0145	0.6325	0.4027
531430	3.0663	0.7325	0.3289	0.3417	0.1430	0.0113	0.6342	0.3820
531431	2.4399	0.6532	0.4052	0.2902	0.1446	0.0141	0.7962	0.4644
531432	3.1132	0.7699	0.3762	0.3507	0.1254	0.0153	0.6957	0.4335
GROUP MEAN	2.8114	0.7326	0.3836	0.3307	0.1546	0.0132	0.6939	0.4244
STD. DEV.	0.1957	0.0431	0.0279	0.0260	0.0303	0.0013	0.0618	0.0371

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE RATS

GROUP : III - 250 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531433	2.7837	0.8197	0.4053	0.3530	0.1620	0.0138	0.7937	0.3751
531434	2.9672	0.7332	0.3718	0.2986	0.1652	0.0106	0.6954	0.3811
531435	2.8395	0.7458	0.4909	0.3741	0.1676	0.0174	0.6938	0.4005
531436	3.3319	0.8302	0.4744	0.3313	0.1753	0.0143	0.7434	0.4449
531437	3.3228	0.8051	0.3687	0.3416	0.1458	0.0127	0.6513	0.3848
531438	2.6533	0.7419	0.3443	0.3139	0.1297	0.0118	0.8439	0.4912
531439	3.0466	0.8027	0.4127	0.3243	0.1520	0.0132	0.6130	0.4071
531440	2.6659	0.7592	0.3686	0.3294	0.1810	0.0158	0.7140	0.4009
531441	2.6218	0.8549	0.3981	0.3701	0.1465	0.0115	0.7144	0.4060
531442	2.7466	0.8066	0.3924	0.3742	0.1675	0.0135	0.7476	0.4097
GROUP MEAN	2.8979	0.7899	0.4027	0.3411	0.1593	0.0135	0.7210	0.4101
STD. DEV.	0.2635	0.0419	0.0469	0.0263	0.0156	0.0020	0.0663	0.0345

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE RATS

GROUP : V - 1000 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531443	3.0667	0.8114	0.3909	0.3242	0.1656	0.0117	0.7195	0.4098
531444	3.4097	0.8320	0.4019	0.3343	0.1144	0.0132	0.7125	0.4325
531445	3.1346	0.7404	0.3883	0.3086	0.1378	0.0106	0.7111	0.4235
531446	3.5812	0.7946	0.4023	0.3197	0.1462	0.0126	0.6923	0.4316
531447	3.1424	0.7675	0.3896	0.2915	0.1648	0.0135	0.6788	0.3810
531448	3.4040	0.9336	0.3943	0.3743	0.1700	0.0117	0.6852	0.4287
531449	3.4181	0.9422	0.3907	0.3496	0.1503	0.0143	0.7598	0.4988
531450	3.1891	0.7581	0.4030	0.3220	0.1816	0.0116	0.7915	0.4060
531451	3.4254	0.9374	0.4694	0.3576	0.1893	0.0134	0.8098	0.4626
531452	3.0698	0.9631	0.3516	0.3171	0.1603	0.0112	0.5616	0.3991
GROUP MEAN	3.2841	0.8480	0.3982	0.3299	0.1580	0.0124	0.7122	0.4274
STD. DEV.	0.1829	0.0869	0.0290	0.0246	0.0218	0.0012	0.0692	0.0335

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE RATS

GROUP : VII - 1500 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531453	3.2801	0.8174	0.4345	0.3248	0.1483	0.0148	0.7287	0.4337
531454	3.9617	1.0188	0.4577	0.3637	0.1569	0.0154	0.7446	0.4164
531455	3.3533	0.8929	0.4047	0.3880	0.1986	0.0157	0.8091	0.4389
531456	3.6056	0.9026	0.4409	0.3456	0.1792	0.0151	0.8133	0.4458
531457	3.3807	0.8007	0.3778	0.3419	0.1215	0.0144	0.6950	0.4374
531458	3.7324	0.9297	0.4820	0.3659	0.1495	0.0161	0.8844	0.4969
531459	3.4796	0.8953	0.3842	0.3252	0.1384	0.0131	0.7389	0.4379
531460	3.5451	0.9037	0.4262	0.3014	0.1526	0.0138	0.7999	0.4350
531461	3.9359	0.8904	0.4404	0.2950	0.1685	0.0125	0.6864	0.4230
531462	3.3529	0.7649	0.4052	0.3287	0.1832	0.0120	0.9060	0.4759
GROUP MEAN	3.5627	0.8816	0.4254	0.3380	0.1597	0.0143	0.7806	0.4441
STD. DEV.	0.2443	0.0721	0.0327	0.0291	0.0229	0.0014	0.0750	0.0243

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE RATS

GROUP : IX - 1000 ppm - 1,3-BUTADIENE

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	TESTES	BRAIN
531463	3.2532	0.8090	0.4065	0.3375	0.1239	0.0131	0.7908	0.4578
531464	2.9706	0.7317	0.4159	0.3233	0.1542	0.0129	0.7502	0.4453
531465	2.7934	0.8152	0.4001	0.3628	0.1865	0.0137	0.7245	0.4767
531466	3.0948	0.8066	0.3653	0.3430	0.1542	0.0156	0.6566	0.4944
531467	3.0413	0.8668	0.3871	0.3668	0.1537	0.0146	0.6942	0.4280
531468	3.0719	0.8020	0.3303	0.3279	0.1587	0.0106	0.6404	0.3832
531469	2.9398	0.8460	0.3552	0.3139	0.1632	0.0132	0.7926	0.4582
531470	3.1259	0.8133	0.3644	0.3411	0.1557	0.0108	0.6804	0.4386
531471	2.8722	0.8688	0.3703	0.3097	0.1424	0.0157	0.7187	0.3818
531472	3.2393	0.8763	0.3746	0.3026	0.1727	0.0126	0.6608	0.4215
GROUP MEAN	3.0403	0.8236	0.3770	0.3329	0.1565	0.0133	0.7109	0.4386
STD. DEV.	0.1492	0.0432	0.0259	0.0215	0.0167	0.0018	0.0543	0.0366

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE RATS

GROUP : II - 0 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531478	299.5	8.208	1.957	1.533	1.162	0.462	0.069	0.171	2.046
531479	380.3	9.886	2.648	2.128	1.115	0.652	0.087	0.124	2.346
531480	252.3	7.817	2.226	1.534	1.108	0.402	0.102	0.170	1.826
531481	312.5	8.367	2.372	1.535	1.156	0.521	0.084	0.157	1.952
531482	350.8	10.045	2.154	1.847	1.052	0.663	0.101	0.155	1.977
531483	317.5	8.694	2.185	1.517	1.027	0.633	0.081	0.160	1.918
531484	349.2	9.309	2.589	1.793	1.219	0.692	0.082	0.110	2.438
531485	330.8	8.797	2.339	2.066	1.222	0.703	0.072	0.145	1.969
531486	306.7	8.781	2.458	1.592	1.012	0.550	0.081	0.124	2.053
531487	330.1	8.183	2.102	1.521	1.103	0.625	0.075	0.142	1.945
GROUP MEAN	323.0	8.809	2.303	1.707	1.118	0.590	0.083	0.146	2.047
STD. DEV.	34.7	0.737	0.219	0.237	0.073	0.102	0.011	0.021	0.194

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE RATS

GROUP : IV - 250 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531488	280.4	8.270	1.975	1.686	1.034	0.498	0.083	0.179	1.867
531489	294.0	8.790	2.423	1.479	1.065	0.523	0.101	0.089	2.019
531490	284.2	8.083	2.053	2.540	1.157	0.546	0.108	0.177	1.915
531491	296.1	8.705	1.885	1.206	1.043	0.468	0.068	0.169	1.918
531492	379.1	10.608	2.524	1.749	1.225	0.656	0.090	0.125	2.022
531493	319.8	8.834	2.387	1.445	1.146	0.665	0.075	0.150	2.016
531494	332.0	9.949	2.567	1.625	1.062	0.575	0.092	0.120	1.951
531495	276.1	8.378	2.112	1.500	1.056	0.610	0.080	0.164	1.917
531496	314.0	8.919	2.264	1.732	1.132	0.506	0.082	0.148	1.930
531497	290.0	8.276	2.358	1.747	1.064	0.603	0.102	0.176	1.889
GROUP MEAN	306.6	8.881	2.255	1.671	1.098	0.565	0.088	0.150	1.944
STD. DEV.	31.3	0.802	0.236	0.351	0.063	0.068	0.013	0.030	0.056

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE RATS

GROUP : VI - 1000 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531498	321.7	10.608	2.426	1.636	0.967	0.499	0.102	0.126	1.889
531500	277.0	7.893	1.987	1.432	1.098	0.560	0.089	0.157	1.963
531501	301.5	9.069	1.757	1.484	1.040	0.546	0.090	0.144	1.874
531502	279.4	8.166	2.290	1.782	0.955	0.599	0.083	0.133	1.864
531503	287.4	8.656	2.354	1.663	0.952	0.603	0.082	0.148	1.920
531504	260.4	8.069	2.264	1.522	1.142	0.504	0.080	0.125	1.986
531505	372.1	11.075	2.711	1.858	1.263	0.569	0.091	0.094	2.015
531506	305.6	10.268	2.361	1.533	1.094	0.602	0.077	0.135	2.025
531507	290.6	7.917	2.063	1.498	1.034	0.529	0.084	0.121	2.008
GROUP MEAN	299.5	9.080	2.246	1.601	1.061	0.557	0.086	0.131	1.949
STD. DEV.	32.5	1.251	0.277	0.145	0.102	0.041	0.008	0.018	0.064

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE RATS

GROUP : VIII - 1500 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531508	249.5	8.989	2.101	1.310	1.121	0.542	0.076	0.085	1.797
531509	262.7	8.072	2.030	1.527	0.961	0.514	0.078	0.141	1.827
531510	275.6	9.477	2.111	1.549	1.247	0.659	0.077	0.121	1.984
531511	255.4	7.459	2.084	1.544	0.981	0.543	0.069	0.144	1.914
531512	328.1	10.355	2.841	1.581	1.120	0.697	0.087	0.115	2.033
531513	341.2	12.226	3.312	2.173	1.512	0.767	0.104	0.181	2.566
531514	247.6	8.513	2.150	1.547	0.931	0.455	0.062	0.106	1.893
531515	321.7	11.559	2.771	1.835	1.185	0.758	0.089	0.070	1.821
531516	311.8	10.104	2.578	1.613	1.076	0.668	0.082	0.183	1.860
531517	285.7	9.250	2.487	1.681	1.030	0.594	0.081	0.191	2.002
GROUP MEAN	287.9	9.600	2.447	1.636	1.116	0.620	0.080	0.134	1.970
STD. DEV.	35.1	1.497	0.429	0.230	0.171	0.106	0.011	0.042	0.225

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE RATS

GROUP : X - 1000 ppm - 1,3-BUTADIENE

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531518	300.6	9.185	2.347	1.546	1.068	0.529	0.080	0.175	1.914
531519	293.2	9.536	2.367	1.521	1.213	0.413	0.084	0.102	1.947
531520	294.4	8.981	2.331	1.496	1.098	0.591	0.081	0.154	1.920
531521	307.6	9.450	2.527	1.539	1.165	0.492	0.093	0.113	2.031
531522	285.9	8.220	2.019	1.445	1.064	0.585	0.085	0.144	1.901
531523	325.9	10.074	2.641	1.490	1.288	0.612	0.087	0.143	2.001
531524	292.9	8.407	2.157	1.409	1.031	0.547	0.081	0.140	1.911
531525	321.5	9.797	2.622	1.535	1.225	0.540	0.101	0.123	2.460
531526	317.0	9.503	2.480	1.558	1.014	0.574	0.085	0.173	2.107
531527	356.5	9.549	2.498	1.759	1.156	0.590	0.080	0.168	2.223
GROUP MEAN	309.5	9.270	2.399	1.530	1.132	0.547	0.086	0.144	2.042
STD. DEV.	21.3	0.587	0.198	0.093	0.091	0.059	0.007	0.025	0.180

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE RATS

GROUP : II - 0 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531478	2.7406	0.6534	0.5119	0.3880	0.1543	0.0230	0.0571	0.6831
531479	2.5995	0.6963	0.5596	0.2932	0.1714	0.0229	0.0326	0.6169
531480	3.0983	0.8823	0.6080	0.4392	0.1593	0.0404	0.0674	0.7237
531481	2.6774	0.7590	0.4912	0.3699	0.1667	0.0269	0.0502	0.6246
531482	2.8635	0.6140	0.5265	0.2999	0.1890	0.0288	0.0442	0.5636
531483	2.7383	0.6882	0.4778	0.3235	0.1994	0.0255	0.0504	0.6041
531484	2.6658	0.7414	0.5135	0.3491	0.1982	0.0235	0.0315	0.6982
531485	2.6593	0.7071	0.6245	0.3694	0.2125	0.0218	0.0438	0.5952
531486	2.8631	0.8014	0.5191	0.3300	0.1793	0.0264	0.0404	0.6694
531487	2.4789	0.6368	0.4608	0.3341	0.1893	0.0227	0.0430	0.5892
GROUP MEAN	2.7385	0.7180	0.5293	0.3496	0.1819	0.0262	0.0461	0.6368
STD. DEV.	0.1708	0.0811	0.0534	0.0438	0.0190	0.0055	0.0108	0.0532

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE RATS

GROUP : IV - 250 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531488	2.9494	0.7044	0.6013	0.3688	0.1776	0.0296	0.0638	0.6658
531489	2.9898	0.8241	0.5031	0.3622	0.1779	0.0344	0.0303	0.6867
531490	2.8441	0.7224	0.8937	0.4071	0.1921	0.0380	0.0623	0.6738
531491	2.9399	0.6366	0.4073	0.3522	0.1581	0.0230	0.0571	0.6478
531492	2.7982	0.6658	0.4614	0.3231	0.1730	0.0237	0.0330	0.5334
531493	2.7624	0.7464	0.4518	0.3583	0.2079	0.0235	0.0469	0.6304
531494	2.9967	0.7732	0.4895	0.3199	0.1732	0.0277	0.0361	0.5877
531495	3.0344	0.7649	0.5433	0.3825	0.2209	0.0290	0.0594	0.6943
531496	2.8404	0.7210	0.5516	0.3605	0.1611	0.0261	0.0471	0.6146
531497	2.8538	0.8131	0.6024	0.3669	0.2079	0.0352	0.0607	0.6514
GROUP MEAN	2.9009	0.7372	0.5505	0.3602	0.1850	0.0290	0.0497	0.6386
STD. DEV.	0.0929	0.0599	0.1363	0.0256	0.0213	0.0053	0.0128	0.0494

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE RATS

GROUP : VI - 1000 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531498	3.2975	0.7541	0.5085	0.3006	0.1551	0.0317	0.0392	0.5872
531500	2.8495	0.7173	0.5170	0.3964	0.2022	0.0321	0.0567	0.7087
531501	3.0080	0.5828	0.4922	0.3449	0.1811	0.0299	0.0478	0.6216
531502	2.9227	0.8196	0.6378	0.3418	0.2144	0.0297	0.0476	0.6671
531503	3.0118	0.8191	0.5786	0.3312	0.2098	0.0285	0.0515	0.6681
531504	3.0987	0.8694	0.5845	0.4386	0.1935	0.0307	0.0480	0.7627
531505	2.9764	0.7286	0.4993	0.3394	0.1529	0.0245	0.0253	0.5415
531506	3.3599	0.7726	0.5016	0.3580	0.1970	0.0252	0.0442	0.6626
531507	2.7244	0.7099	0.5155	0.3558	0.1820	0.0289	0.0416	0.6910
GROUP MEAN	3.0276	0.7526	0.5372	0.3563	0.1876	0.0290	0.0446	0.6567
STD. DEV.	0.2020	0.0833	0.0506	0.0399	0.0220	0.0027	0.0089	0.0659

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE RATS

GROUP : VIII - 1500 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531508	3.6028	0.8421	0.5251	0.4493	0.2172	0.0305	0.0341	0.7202
531509	3.0727	0.7727	0.5813	0.3658	0.1957	0.0297	0.0537	0.6955
531510	3.4387	0.7660	0.5620	0.4525	0.2391	0.0279	0.0439	0.7199
531511	2.9205	0.8160	0.6045	0.3841	0.2126	0.0270	0.0564	0.7494
531512	3.1560	0.8659	0.4819	0.3414	0.2124	0.0265	0.0351	0.6196
531513	3.5832	0.9707	0.6369	0.4431	0.2248	0.0305	0.0530	0.7521
531514	3.4382	0.8683	0.6248	0.3760	0.1838	0.0250	0.0428	0.7645
531515	3.5931	0.8614	0.5704	0.3684	0.2356	0.0277	0.0218	0.5661
531516	3.2405	0.8268	0.5173	0.3451	0.2142	0.0263	0.0587	0.5965
531517	3.2377	0.8705	0.5884	0.3605	0.2079	0.0284	0.0669	0.7007
GROUP MEAN	3.3284	0.8460	0.5693	0.3886	0.2143	0.0279	0.0466	0.6885
STD. DEV.	0.2385	0.0581	0.0492	0.0432	0.0167	0.0018	0.0136	0.0699

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE RATS

GROUP : X - 1000 ppm - 1,3-BUTADIENE

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	ADRENAL GLANDS	OVARIES	BRAIN
531518	3.0556	0.7808	0.5143	0.3553	0.1760	0.0266	0.0582	0.6367
531519	3.2524	0.8073	0.5188	0.4137	0.1409	0.0286	0.0348	0.6641
531520	3.0506	0.7918	0.5082	0.3730	0.2007	0.0275	0.0523	0.6522
531521	3.0722	0.8215	0.5003	0.3787	0.1599	0.0302	0.0367	0.6603
531522	2.8751	0.7062	0.5054	0.3722	0.2046	0.0297	0.0504	0.6649
531523	3.0911	0.8104	0.4572	0.3952	0.1878	0.0267	0.0439	0.6140
531524	2.8703	0.7364	0.4811	0.3520	0.1868	0.0277	0.0478	0.6524
531525	3.0473	0.8156	0.4774	0.3810	0.1680	0.0314	0.0383	0.7652
531526	2.9978	0.7823	0.4915	0.3199	0.1811	0.0268	0.0546	0.6647
531527	2.6785	0.7007	0.4934	0.3243	0.1655	0.0224	0.0471	0.6236
GROUP MEAN	2.9991	0.7753	0.4948	0.3665	0.1771	0.0278	0.0464	0.6598
STD. DEV.	0.1566	0.0450	0.0188	0.0294	0.0193	0.0025	0.0079	0.0411

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX O

Individual Animal Final Body and Organ Weights of Mice

APPENDIX
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE MICE

GROUP : 1 - 0 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91371	26.2	1.229	0.574	0.192	0.163	0.053	0.232	0.478
91372	26.7	1.124	0.520	0.216	0.168	0.053	0.227	0.474
91373	29.2	1.289	0.653	0.240	0.160	0.091	0.230	0.462
91374	29.9	1.423	0.718	0.313	0.235	0.066	0.238	0.518
91376	27.6	1.416	0.632	0.338	0.207	0.062	0.195	0.482
91377	26.9	1.290	0.558	0.224	0.189	0.045	0.236	0.455
91378	26.1	1.252	0.582	0.259	0.203	0.058	0.248	0.480
91380	28.7	1.239	0.650	0.210	0.179	0.053	0.248	0.498
GROUP MEAN	27.7	1.283	0.611	0.249	0.188	0.060	0.232	0.481
STD. DEV.	1.4	0.099	0.064	0.052	0.026	0.014	0.017	0.020

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE MICE

GROUP : III - 50 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91381	28.4	1.371	0.645	0.217	0.210	0.050	0.241	0.483
91382	27.9	1.227	0.597	0.244	0.177	0.054	0.233	0.500
91383	29.5	1.489	0.743	0.232	0.173	0.143	0.240	0.455
91384	33.2	1.721	0.793	0.310	0.182	0.073	0.258	0.506
91385	24.9	1.064	0.509	0.205	0.157	0.037	0.220	0.476
91387	28.9	1.310	0.632	0.233	0.198	0.057	0.257	0.486
91388	28.2	1.245	0.584	0.258	0.179	0.062	0.227	0.474
91389	26.6	1.356	0.648	0.210	0.177	0.057	0.240	0.469
91390	25.4	1.125	0.618	0.227	0.173	0.042	0.226	0.464
GROUP MEAN	28.1	1.323	0.641	0.237	0.181	0.064	0.238	0.479
STD. DEV.	2.5	0.197	0.084	0.032	0.015	0.031	0.013	0.016

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE MICE

GROUP : V - 250 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91391	27.2	1.442	0.635	0.270	0.190	0.082	0.224	0.470
91392	27.3	1.259	0.570	0.209	0.182	0.047	0.221	0.475
91393	26.4	1.066	0.522	0.228	0.163	0.045	0.235	0.456
91394	27.5	1.271	0.656	0.340	0.172	0.049	0.249	0.452
91395	28.0	1.320	0.562	0.261	0.157	0.064	0.252	0.481
91396	25.1	1.231	0.616	0.273	0.179	0.046	0.220	0.489
91397	26.7	1.233	0.551	0.222	0.172	0.049	0.246	0.487
91398	27.1	0.979	0.554	0.224	0.152	0.047	0.226	0.473
91399	32.1	1.457	0.707	0.326	0.195	0.056	0.262	0.492
91400	27.3	1.248	0.505	0.249	0.167	0.051	0.231	0.476
GROUP MEAN	27.5	1.251	0.588	0.260	0.173	0.054	0.237	0.475
STD. DEV.	1.8	0.146	0.064	0.044	0.014	0.011	0.015	0.013

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM MALE MICE

GROUP : IX - 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91411	30.1	1.456	0.662	0.254	0.199	0.068	0.163	0.486
91412	29.6	1.490	0.786	0.269	0.181	0.069	0.159	0.484
91413	27.9	1.306	0.571	0.242	0.168	0.059	0.147	0.491
91414	30.0	1.358	0.578	0.246	0.153	0.061	0.155	0.446
91415	27.2	1.316	0.605	0.239	0.157	0.062	0.149	0.446
91416	26.7	1.203	0.582	0.267	0.159	0.053	0.142	0.465
91417	26.4	1.311	0.522	0.236	0.145	0.071	0.141	0.449
91418	28.3	1.362	0.618	0.280	0.215	0.054	0.144	0.475
91419	29.6	1.415	0.637	0.276	0.211	0.065	0.159	0.469
91420	31.4	1.431	0.683	0.319	0.225	0.070	0.175	0.500
GROUP MEAN	28.7	1.365	0.624	0.263	0.181	0.063	0.153	0.471
STD. DEV.	1.7	0.086	0.074	0.025	0.029	0.006	0.011	0.020

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE MICE

GROUP : I - 0 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91371	4.6908	2.1908	0.7328	0.6221	0.2023	0.8855	1.8244
91372	4.2097	1.9476	0.8090	0.6292	0.1985	0.8502	1.7753
91373	4.4144	2.2363	0.8219	0.5479	0.3116	0.7877	1.5822
91374	4.7592	2.4013	1.0468	0.7860	0.2207	0.7960	1.7324
91376	5.1304	2.2899	1.2246	0.7500	0.2246	0.7065	1.7464
91377	4.7955	2.0743	0.8327	0.7026	0.1673	0.8773	1.6914
91378	4.7969	2.2299	0.9923	0.7778	0.2222	0.9502	1.8391
91380	4.3171	2.2648	0.7317	0.6237	0.1847	0.8641	1.7352
GROUP MEAN	4.6393	2.2044	0.8990	0.6799	0.2165	0.8397	1.7408
STD. DEV.	0.3039	0.1386	0.1735	0.0868	0.0433	0.0744	0.0807

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE MICE

GROUP : III - 50 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91381	4.8275	2.2711	0.7641	0.7394	0.1761	0.8486	1.7007
91382	4.3978	2.1398	0.8746	0.6344	0.1935	0.8351	1.7921
91383	5.0475	2.5186	0.7864	0.5864	0.4847	0.8136	1.5424
91384	5.1837	2.3886	0.9337	0.5482	0.2199	0.7771	1.5241
91385	4.2731	2.0442	0.8233	0.6305	0.1486	0.8835	1.9116
91387	4.5329	2.1869	0.8062	0.6851	0.1972	0.8893	1.6817
91388	4.4149	2.0709	0.9149	0.6348	0.2199	0.8050	1.6809
91389	5.0977	2.4361	0.7895	0.6654	0.2143	0.9023	1.7632
91390	4.4291	2.4331	0.8937	0.6811	0.1654	0.8898	1.8268
GROUP MEAN	4.6894	2.2766	0.8429	0.6450	0.2244	0.8494	1.7137
STD. DEV.	0.3508	0.1747	0.0622	0.0564	0.1007	0.0445	0.1266

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE MICE

GROUP : V - 250 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91391	5.3015	2.3346	0.9926	0.6985	0.3015	0.8235	1.7279
91392	4.6117	2.0879	0.7656	0.6667	0.1722	0.8095	1.7399
91393	4.0379	1.9773	0.8636	0.6174	0.1705	0.8902	1.7273
91394	4.6218	2.3855	1.2364	0.6255	0.1782	0.9055	1.6436
91395	4.7143	2.0071	0.9321	0.5607	0.2286	0.9000	1.7179
91396	4.9044	2.4542	1.0876	0.7131	0.1833	0.8765	1.9482
91397	4.6180	2.0637	0.8315	0.6442	0.1835	0.9213	1.8240
91398	3.6125	2.0443	0.8266	0.5609	0.1734	0.8339	1.7454
91399	4.5389	2.2025	1.0156	0.6075	0.1745	0.8162	1.5327
91400	4.5714	1.8498	0.9121	0.6117	0.1868	0.8462	1.7436
GROUP MEAN	4.5532	2.1407	0.9464	0.6306	0.1952	0.8623	1.7350
STD. DEV.	0.4561	0.1963	0.1412	0.0514	0.0410	0.0411	0.1071

SUBCHRONIC TOXICITY STUDY BY THE INHALATION ROUTE WITH 4-VINYLCYCLOHEXENE

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM MALE MICE

GROUP : IX - 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	TESTES	BRAIN
91411	4.8372	2.1993	0.8439	0.6611	0.2259	0.5415	1.6146
91412	5.0338	2.6554	0.9088	0.6115	0.2331	0.5372	1.6351
91413	4.6810	2.0466	0.8674	0.6022	0.2115	0.5269	1.7599
91414	4.5267	1.9267	0.8200	0.5100	0.2033	0.5167	1.4867
91415	4.8382	2.2243	0.8787	0.5772	0.2279	0.5478	1.6397
91416	4.5056	2.1798	1.0000	0.5955	0.1985	0.5318	1.7416
91417	4.9659	1.9773	0.8939	0.5492	0.2689	0.5341	1.7008
91418	4.8127	2.1837	0.9894	0.7597	0.1908	0.5088	1.6784
91419	4.7804	2.1520	0.9324	0.7128	0.2196	0.5372	1.5845
91420	4.5573	2.1752	1.0159	0.7166	0.2229	0.5573	1.5924
GROUP MEAN	4.7539	2.1720	0.9150	0.6296	0.2203	0.5339	1.6434
STD. DEV.	0.1822	0.1977	0.0678	0.0804	0.0220	0.0141	0.0812

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE MICE

GROUP : II - 0 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91421	23.9	1.100	0.372	0.359	0.221	0.091	0.482
91422	23.8	1.078	0.392	0.193	0.149	0.072	0.483
91423	22.0	0.910	0.384	0.178	0.118	0.056	0.477
91424	20.6	1.042	0.360	0.181	0.122	0.056	0.507
91425	22.9	1.102	0.433	0.222	0.150	0.059	0.485
91426	22.9	0.956	0.199@	0.205	0.138	0.072	0.477
91427	23.8	1.117	0.413	0.212	0.154	0.076	0.493
91428	24.0	1.145	0.423	0.334	0.167	0.076	0.502
91429	20.3	0.949	0.327	0.215	0.138	0.063	0.479
91430	21.7	0.917	0.413	0.196	0.131	0.067	0.464
GROUP MEAN	22.6	1.032	0.391	0.229	0.149	0.069	0.485
STD. DEV.	1.4	0.090	0.034	0.064	0.029	0.011	0.013

@ = WEIGHING ERROR AND NOT INCLUDED IN CALCULATIONS.

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE MICE

GROUP : IV - 50 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91431	21.9	1.070	0.373	0.251	0.134	0.068	0.463
91432	20.5	0.971	0.363	0.173	0.123	0.071	0.433
91433	22.7	1.051	0.357	0.253	0.131	0.061	0.474
91434	21.3	1.056	0.359	0.261	0.169	0.065	0.451
91435	21.9	1.112	0.371	0.217	0.151	0.079	0.487
91436	23.6	1.066	0.354	0.224	0.122	0.063	0.439
91437	21.2	1.018	0.385	0.270	0.130	0.083	0.446
91438	21.2	1.053	0.413	0.237	0.136	0.047	0.434
91440	22.2	0.716	0.352	0.187	0.127	0.040	0.467
91471	20.7	1.126	0.409	0.234	0.138	0.071	0.479
GROUP MEAN	21.7	1.024	0.374	0.231	0.136	0.065	0.457
STD. DEV.	0.9	0.117	0.022	0.031	0.014	0.013	0.019

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE MICE

GROUP : VI - 250 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91441	22.7	1.169	0.416	0.199	0.158	0.067	0.483
91442	23.0	1.113	0.380	0.217	0.131	0.070	0.471
91443	20.8	1.025	0.353	0.227	0.126	0.066	0.484
91444	22.6	1.117	0.390	0.258	0.148	0.074	0.465
91445	18.8	0.732	0.350	0.183	0.112	0.044	0.471
91446	20.4	1.004	0.334	0.172	0.117	0.077	0.441
91448	18.3	0.884	0.344	0.192	0.121	0.041	0.451
91449	20.1	0.698	0.346	0.192	0.121	0.045	0.445
91450	22.8	1.180	0.398	0.269	0.141	0.078	0.503
GROUP MEAN	21.1	0.991	0.368	0.212	0.131	0.062	0.468
STD. DEV.	1.8	0.182	0.029	0.034	0.015	0.015	0.020

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE MICE

GROUP : VIII - 1000 ppm

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91456	20.0	1.043	0.373	0.178	0.116	0.047	0.425
91458	22.2	1.247	0.426	0.206	0.135	0.052	0.442
GROUP MEAN	21.1	1.145	0.400	0.192	0.126	0.049	0.434
STD. DEV.	1.6	0.144	0.037	0.020	0.013	0.004	0.012

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

FINAL BODY AND ABSOLUTE ORGAN WEIGHTS (grams) FROM FEMALE MICE

GROUP : X - 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	FINAL BODY	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91461	23.5	1.107	0.419	0.291	0.162	0.057	0.494
91462	21.5	1.079	0.369	0.234	0.155	0.054	0.478
91464	22.2	1.071	0.397	0.213	0.144	0.052	0.498
91465	22.8	1.184	0.405	0.258	0.146	0.060	0.490
91466	22.5	1.059	0.377	0.219	0.135	0.071	0.431
91467	23.6	1.138	0.418	0.230	0.132	0.064	0.470
91468	20.7	1.034	0.374	0.246	0.157	0.058	0.472
91470	23.8	1.271	0.421	0.233	0.146	0.067	0.489
GROUP MEAN	22.6	1.118	0.398	0.241	0.147	0.060	0.478
STD. DEV.	1.1	0.078	0.022	0.025	0.010	0.007	0.021

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE MICE

GROUP : II - 0 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91421	4.6025	1.5565	1.5021	0.9247	0.3808	2.0167
91422	4.5294	1.6471	0.8109	0.6261	0.3025	2.0294
91423	4.1364	1.7455	0.8091	0.5364	0.2545	2.1682
91424	5.0583	1.7476	0.8786	0.5922	0.2718	2.4612
91425	4.8122	1.8908	0.9694	0.6550	0.2576	2.1179
91426	4.1747	0.8690@	0.8952	0.6026	0.3144	2.0830
91427	4.6933	1.7353	0.8908	0.6471	0.3193	2.0714
91428	4.7708	1.7625	1.3917	0.6958	0.3167	2.0917
91429	4.6749	1.6108	1.0591	0.6798	0.3103	2.3596
91430	4.2258	1.9032	0.9032	0.6037	0.3088	2.1382
GROUP MEAN	4.5678	1.7333	1.0110	0.6563	0.3037	2.1537
STD. DEV.	0.3035	0.1165	0.2422	0.1050	0.0366	0.1447

@ = WEIGHING ERROR AND NOT INCLUDED IN CALCULATIONS.

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE MICE

GROUP : IV - 50 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91431	4.8858	1.7032	1.1461	0.6119	0.3105	2.1142
91432	4.7366	1.7707	0.8439	0.6000	0.3463	2.1122
91433	4.6300	1.5727	1.1145	0.5771	0.2687	2.0881
91434	4.9577	1.6854	1.2254	0.7934	0.3052	2.1174
91435	5.0776	1.6941	0.9909	0.6895	0.3607	2.2237
91436	4.5169	1.5000	0.9492	0.5169	0.2669	1.8602
91437	4.8019	1.8160	1.2736	0.6132	0.3915	2.1038
91438	4.9670	1.9481	1.1179	0.6415	0.2217	2.0472
91440	3.2252	1.5856	0.8423	0.5721	0.1802	2.1036
91471	5.4396	1.9758	1.1304	0.6667	0.3430	2.3140
GROUP MEAN	4.7238	1.7252	1.0634	0.6282	0.2995	2.1084
STD. DEV.	0.5851	0.1561	0.1501	0.0761	0.0656	0.1162

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE MICE

GROUP : V1 - 250 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91441	5.1498	1.8326	0.8767	0.6960	0.2952	2.1278
91442	4.8391	1.6522	0.9435	0.5696	0.3043	2.0478
91443	4.9279	1.6971	1.0913	0.6058	0.3173	2.3269
91444	4.9425	1.7257	1.1416	0.6549	0.3274	2.0575
91445	3.8936	1.8617	0.9734	0.5957	0.2340	2.5053
91446	4.9216	1.6373	0.8431	0.5735	0.3775	2.1618
91448	4.8306	1.8798	1.0492	0.6612	0.2240	2.4645
91449	3.4726	1.7214	0.9552	0.6020	0.2239	2.2139
91450	5.1754	1.7456	1.1798	0.6184	0.3421	2.2061
GROUP MEAN	4.6837	1.7504	1.0060	0.6197	0.2940	2:2346
STD. DEV.	0.5894	0.0885	0.1165	0.0426	0.0553	0.1654

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE MICE

GROUP : VIII - 1000 ppm

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91456	5.2150	1.8650	0.8900	0.5800	0.2350	2.1250
91458	5.6171	1.9189	0.9279	0.6081	0.2342	1.9910
GROUP MEAN	5.4161	1.8920	0.9090	0.5941	0.2346	2.0580
STD. DEV.	0.2843	0.0381	0.0268	0.0199	0.0005	0.0948

APPENDIX (Continued)
INDIVIDUAL ANIMAL FINAL BODY AND ORGAN WEIGHTS

RELATIVE ORGAN WEIGHTS (% of body weight) FROM FEMALE MICE

GROUP : X - 1000 ppm 1,3-BUTADIENE

ANIMAL NUMBER	LIVER	KIDNEYS	LUNGS	HEART	SPLEEN	BRAIN
91461	4.7106	1.7830	1.2383	0.6894	0.2426	2.1021
91462	5.0186	1.7163	1.0884	0.7209	0.2512	2.2233
91464	4.8243	1.7883	0.9595	0.6486	0.2342	2.2432
91465	5.1930	1.7763	1.1316	0.6404	0.2632	2.1491
91466	4.7067	1.6756	0.9733	0.6000	0.3156	1.9156
91467	4.8220	1.7712	0.9746	0.5593	0.2712	1.9915
91468	4.9952	1.8068	1.1884	0.7585	0.2802	2.2802
91470	5.3403	1.7689	0.9790	0.6134	0.2815	2.0546
GROUP MEAN	4.9513	1.7608	1.0666	0.6538	0.2674	2.1200
STD. DEV.	0.2289	0.0431	0.1104	0.0659	0.0259	0.1283

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX P

Individual Animal Pathology Data of Rats

KEY TO APPENDIX
INDIVIDUAL ANIMAL PATHOLOGY DATA

MODE OF DEATH

SD: SACRIFICED BY DESIGN
SE: SACRIFICED IN EXTREMIS
FD: FOUND DEAD
AK: ACCIDENTALLY KILLED

LESION GRADING

HISTOPATHOLOGICAL CHANGES ARE DESCRIBED ACCORDING TO THEIR MORPHOLOGIC CHARACTER, DISTRIBUTION AND SEVERITY. THE DISTRIBUTION (EXTENT OF TISSUE INVOLVEMENT) IS INDICATED, WHERE APPROPRIATE, BY MODIFIERS SUCH AS FOCAL, MULTIFOCAL, DIFFUSE, UNILATERAL, BILATERAL, ETC.; A SEVERITY SCORE, IF APPROPRIATE, IS ALSO ASSIGNED AS FOLLOWS:

PRESENT: LESION PRESENT, SEVERITY NOT GRADED.
MINIMAL: THE AMOUNT OF CHANGE PRESENT BARELY EXCEEDS THAT WHICH IS CONSIDERED TO BE WITHIN NORMAL LIMITS.
MILD: IN GENERAL, THE LESION IS EASILY IDENTIFIED BUT OF LIMITED SEVERITY. THE LESION PROBABLY DOES NOT PRODUCE ANY FUNCTIONAL IMPAIRMENT.
MODERATE: THE LESION IS PROMINENT BUT THERE IS SIGNIFICANT POTENTIAL FOR INCREASED SEVERITY. LIMITED TISSUE OR ORGAN DYSFUNCTION IS POSSIBLE.
SEVERE: THE DEGREE OF CHANGE IS EITHER AS COMPLETE AS CONSIDERED POSSIBLE OR GREAT ENOUGH IN INTENSITY OR EXTENT TO EXPECT SIGNIFICANT TISSUE OR ORGAN DYSFUNCTION.

COMMENT:

GRADES MINIMAL THROUGH SEVERE REPRESENT PROGRESSIVE INVOLVEMENT/SEVERITY ALONG A CONTINUUM WITH MINIMAL LESIONS BEING THE LEAST SEVERE AND SEVERE LESIONS BEING THE MOST SEVERE. WHILE THE GRADES REFER TO THE MORPHOLOGICAL CHARACTERISTICS OF LESIONS, THEY ALSO INDICATE THEIR RELATIVE BIOLOGICAL SIGNIFICANCE.

MICROSCOPIC CORRELATE (selected lesions)

THE MICROSCOPIC CORRELATE FOR SELECTED GROSS OBSERVATIONS, PRIMARILY MASSES AND NODULES, IS PROVIDED TO THE RIGHT OF THE RESPECTIVE GROSS OBSERVATION.

NEOPLASM DESIGNATIONS

*B: BENIGN NEOPLASM, PRIMARY SITE
*M: MALIGNANT NEOPLASM, PRIMARY SITE
*X: MALIGNANT NEOPLASM, METASTATIC OR SECONDARY SITE. SITE OF ORIGIN IS INDICATED IN PARENTHESES.

APPENDIX
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531423 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED, FEW; 5MM DIA

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
- INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS - INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531424 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
HEART - INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531425 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
HEART - INFLAMMATION/DEGENERATION, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
LUNGS - INFLAMMATION, SUBACUTE/CHRONIC, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531426 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
PITUITARY GLAND - DEFORMITY, WHITE, RAISED

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
HEART - INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531427 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:		MICROSCOPIC CORRELATE (selected lesions):
LUNGS	- DISCOLORATION, RED, CAUDAL; 7MM DIA	- NOT CONFIRMED
THYMUS	- DISCOLORATION, RED, MOTTLED	- CONGESTION (WITHIN NORMAL LIMITS)

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS	- ATROPHY, ACINAR, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MINIMAL
TESTES	- DEGENERATION, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531428 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
MANDIBULAR LYMPH NODE	- LARGE, MILD	- NOT CONFIRMED
TESTES	- SMALL, SOFT, LEFT	

MICROSCOPIC OBSERVATIONS:

EPIDIDYMIDES	- OLIGOSPERMIA/GERM CELL DEBRIS, UNILATERAL, MODERATE
LIVER	- FIBROSIS, CAPSULE, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS	- INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MINIMAL
TESTES	- DEGENERATION, UNILATERAL, SEVERE
	- GRANULOMA, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531429 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
MANDIBULAR LYMPH NODE - LARGE, MILD, BILATERAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
HEART - INFLAMMATION/DEGENERATION, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
NOSE - HYPERPLASIA/SQUAMOUS METAPLASIA, RESP EPITH, MINIMAL
- INFLAMMATION, SUPPURATIVE, MINIMAL
OTHER - MESENTERY: INFLAMMATION, PERIVASCULAR, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531430 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531431 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
PITUITARY	- CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 531432 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE - ATROPHY/FIBROSIS, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531433 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531434 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531435 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
MANDIBULAR LYMPH NODE - LARGE, MILD, BILATERAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, MANDIBULAR LYMPH NODES, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531436 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
MANDIBULAR LYMPH NODE - DISCOLORATION, RED, LEFT

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
- INFLAMMATION, SUBACUTE, MINIMAL
MANDIBULAR LYMPH NODES - CONGESTION/HEMORRHAGE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531437 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

KIDNEYS	- DISCOLORATION, PALE, MOTTLED, BILATERAL	MICROSCOPIC CORRELATE (selected lesions): - NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531438 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531439 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531440 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531441 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: III CONCENTRATION: 250 ppm

ANIMAL #: 531442 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531443 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
STOMACH	- ULCER/EROSION, SINGLE, GLANDULAR; 1MM DIA	

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
STOMACH	- EROSION/ULCERATION, GLANDULAR MUCOSA, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE.

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531444 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531445 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
KIDNEYS	- DISCOLORATION, BILATERAL, MOTTLED	- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531446 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
KIDNEYS	- DISCOLORATION, PALE, MOTTLED, BILATERAL	- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531447 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531448 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531449 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
KIDNEYS - DILATATION, MODERATE, LEFT

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - HYALINE DROPLETS, INCREASED, MILD
- HYDRONEPHROSIS, UNILATERAL, MILD
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531450 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - HYALINE DROPLETS, INCREASED, MILD
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531451 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
THYMUS	- DISCOLORATION, RED, MOTTLED	- CONGESTION (WITHIN NORMAL LIMITS)

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE, THYMUS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: V CONCENTRATION: 1000 ppm

ANIMAL #: 531452 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531453 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MILD
PROSTATE	- INFLAMMATION, SUBACUTE, MILD
RETINA	- DEGENERATION/ATROPHY, UNILATERAL, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531454 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MINIMAL
TESTES	- DEGENERATION, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531455 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SKIN - CHROMODACRYORRHEA, LEFT, PERIOCULAR

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - HYALINE DROPLETS, INCREASED, MILD
- NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
- NECROSIS, FOCAL, MINIMAL
NOSE - DEGENERATION, OLFACTORY EPITHELIUM, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531456 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
MANDIBULAR LYMPH NODE - LARGE, BILATERAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
KIDNEYS - HYALINE DROPLETS, INCREASED, MILD
- NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531457 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL
PITUITARY	- CYST(S), MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531458 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531459 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MILD
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531460 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
KIDNEYS - DISCOLORATION, PALE, BILATERAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
KIDNEYS - HYALINE DROPLETS, INCREASED, MILD
- NEPHROPATHY, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531461 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS	- HYALINE DROPLETS, INCREASED, MILD
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: VII CONCENTRATION: 1500 ppm

ANIMAL #: 531462 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - HYALINE DROPLETS, INCREASED, MILD
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531463 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
STOMACH	- DISCOLORATION, RED, NONGLANDULAR; 3MM DIA	

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
STOMACH	- HEMORRHAGE, NONGLANDULAR MUCOSA, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531464 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531465 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
EPIDIDYMIDES	- MASS, TAN, SOFT, LEFT, HEAD; 4MM DIA	- SPERM GRANULOMA

MICROSCOPIC OBSERVATIONS:

EPIDIDYMIDES	- SPERM GRANULOMA
HEART	- INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531466 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL
PANCREAS	- ATROPHY, ACINAR, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531467 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531468 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE	- INFLAMMATION, SUBACUTE, MILD
RETINA	- DEGENERATION/ATROPHY, UNILATERAL, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531469 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
TESTES - HEMORRHAGE, RIGHT; 6MM DIA MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PROSTATE - INFLAMMATION, SUBACUTE, MINIMAL
TESTES - HEMORRHAGE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531470 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531471 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE RATS GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531472 DAYS ON TEST: 94 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MILD
KIDNEYS	- HYALINE DROPLETS, INCREASED, MINIMAL
	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MODERATE
	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- INFLAMMATION, SUBACUTE/CHRONIC, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531478 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MILD
	- FOCUS/FOCI, EOSINOPHILIC, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531479 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531480 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531481 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531482 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
KIDNEYS - DISCOLORATION, PALE, MOTTLED, BILATERAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: CECUM, COLON, DUODENUM, ILEUM, JEJUNUM, MESENTERIC LYMPH NODE, STOMACH

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531483 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS - ATROPHY, ACINAR, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531484 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
SPLEEN	- CYST, CLEAR; 2MM DIA	- NOT CONFIRMED
THYMUS	- DISCOLORATION, RED, MOTTLED	- CONGESTION (WITHIN NORMAL LIMITS)

MICROSCOPIC OBSERVATIONS:

LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
RETINA	- ROSETTES, MINIMAL
THYMUS	- HYPERPLASIA, EPITHELIAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531485 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531486 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
LIVER - DISCOLORATION, PALE, MIDDLE; 3MM DIA, CLEFT

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MILD
- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: COLON

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: II CONCENTRATION: 0 ppm

ANIMAL #: 531487 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- MEDULLA NOT PRESENT
KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL
RETINA	- DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531488 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531489 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531490 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531491 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - INFLAMMATION, SUBACUTE, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531492 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- INFLAMMATION, SUBACUTE, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
OVARIES	- CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531493 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
OVARIES	- CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531494 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- INFLAMMATION, SUBACUTE, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531495 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531496 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- MINERALIZATION, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: IV CONCENTRATION: 250 ppm

ANIMAL #: 531497 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531498 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:		MICROSCOPIC CORRELATE (selected lesions):
KIDNEYS	- DISCOLORATION, PALE, BILATERAL	- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
LUNGS	- HISTIOCYTOSIS, ALVEOLAR, MINIMAL
OVARIES	- CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531500 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531501 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
KIDNEYS - MISSING, LEFT

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
- ONE OF PAIR PRESENT
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531502 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
THYMUS - DISCOLORATION, RED, MOTTLED

MICROSCOPIC CORRELATE (selected lesions):
- CONGESTION (WITHIN NORMAL LIMITS)

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES, THYMUS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531503 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531504 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531505 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531506 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
LUNGS - HISTIOCYTOSIS, ALVEOLAR, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VI CONCENTRATION: 1000 ppm

ANIMAL #: 531507 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
PITUITARY GLAND - LARGE, CLEAR; 3MM DIA

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PITUITARY - CYST(S), MODERATE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531508 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
LIVER - DISCOLORATION, TAN, MIDDLE; CLEFT, 2MM DIA

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - ATROPHY, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531509 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
THYMUS - DISCOLORATION, RED

MICROSCOPIC CORRELATE (selected lesions):
- CONGESTION (WITHIN NORMAL LIMITS)

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531510 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
SPLEEN - CYST, FEW, CLEAR; < 2MM DIA

MICROSCOPIC OBSERVATIONS:
EXORBITAL LACRIMAL GLANDS - ATROPHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
SPLEEN - CYST(S), CAPSULE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531511 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:		MICROSCOPIC CORRELATE (selected lesions):
THYMUS	- DISCOLORATION, RED, MOTTLED	- CONGESTION (WITHIN NORMAL LIMITS)

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531512 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
OVARIES	- CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531513 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- FATTY CHANGE, FOCAL, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
NOSE	- INFLAMMATION, SUPPURATIVE, MINIMAL
SALIVARY GLANDS	- INFLAMMATION, SUBACUTE, MINIMAL
VAGINA	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531514 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531515 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
NO ABNORMALITIES DETECTED

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - ATROPHY, MINIMAL
- CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531516 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
LUNGS - HISTIOCYTOSIS, ALVEOLAR, MINIMAL
OVARIES - CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: VIII CONCENTRATION: 1500 ppm

ANIMAL #: 531517 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
MANDIBULAR LYMPH NODE - LARGE, BILATERAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, MICROSCOPIC COMMENTS, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531518 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531519 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
OVARIES - CYST(S), MINIMAL
RETINA - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531520 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - INFLAMMATION, SUBACUTE, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531521 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
OVARIES	- CYST(S), MINIMAL
SALIVARY GLANDS	- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531522 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531523 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531524 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531525 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531526 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

HEART	- INFLAMMATION/DEGENERATION, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
PANCREAS	- FOCUS, BASOPHILIC, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE RATS GROUP: X CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 531527 DAYS ON TEST: 95 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX Q

Individual Animal Pathology Data of Mice

KEY TO APPENDIX
INDIVIDUAL ANIMAL PATHOLOGY DATA

MODE OF DEATH

SD: SACRIFICED BY DESIGN
SE: SACRIFICED IN EXTREMIS
FD: FOUND DEAD
AK: ACCIDENTALLY KILLED

LESION GRADING

HISTOPATHOLOGICAL CHANGES ARE DESCRIBED ACCORDING TO THEIR MORPHOLOGIC CHARACTER, DISTRIBUTION AND SEVERITY. THE DISTRIBUTION (EXTENT OF TISSUE INVOLVEMENT) IS INDICATED, WHERE APPROPRIATE, BY MODIFIERS SUCH AS FOCAL, MULTIFOCAL, DIFFUSE, UNILATERAL, BILATERAL, ETC.; A SEVERITY SCORE, IF APPROPRIATE, IS ALSO ASSIGNED AS FOLLOWS:

PRESENT: LESION PRESENT, SEVERITY NOT GRADED.
MINIMAL: THE AMOUNT OF CHANGE PRESENT BARELY EXCEEDS THAT WHICH IS CONSIDERED TO BE WITHIN NORMAL LIMITS.
MILD: IN GENERAL, THE LESION IS EASILY IDENTIFIED BUT OF LIMITED SEVERITY. THE LESION PROBABLY DOES NOT PRODUCE ANY FUNCTIONAL IMPAIRMENT.
MODERATE: THE LESION IS PROMINENT BUT THERE IS SIGNIFICANT POTENTIAL FOR INCREASED SEVERITY. LIMITED TISSUE OR ORGAN DYSFUNCTION IS POSSIBLE.
SEVERE: THE DEGREE OF CHANGE IS EITHER AS COMPLETE AS CONSIDERED POSSIBLE OR GREAT ENOUGH IN INTENSITY OR EXTENT TO EXPECT SIGNIFICANT TISSUE OR ORGAN DYSFUNCTION.

COMMENT:

GRADES MINIMAL THROUGH SEVERE REPRESENT PROGRESSIVE INVOLVEMENT/SEVERITY ALONG A CONTINUUM WITH MINIMAL LESIONS BEING THE LEAST SEVERE AND SEVERE LESIONS BEING THE MOST SEVERE. WHILE THE GRADES REFER TO THE MORPHOLOGICAL CHARACTERISTICS OF LESIONS, THEY ALSO INDICATE THEIR RELATIVE BIOLOGICAL SIGNIFICANCE.

MICROSCOPIC CORRELATE (selected lesions)

THE MICROSCOPIC CORRELATE FOR SELECTED GROSS OBSERVATIONS, PRIMARILY MASSES AND NODULES, IS PROVIDED TO THE RIGHT OF THE RESPECTIVE GROSS OBSERVATION.

NEOPLASM DESIGNATIONS

*B: BENIGN NEOPLASM, PRIMARY SITE
*M: MALIGNANT NEOPLASM, PRIMARY SITE
*X: MALIGNANT NEOPLASM, METASTATIC OR SECONDARY SITE. SITE OF ORIGIN IS INDICATED IN PARENTHESES.

APPENDIX
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91371 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
TAIL - AMPUTATION

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91372 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: 1 CONCENTRATION: 0 ppm

ANIMAL #: 91373 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
BONE MARROW - MYELOID HYPERPLASIA, MINIMAL
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91374 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: 1 CONCENTRATION: 0 ppm

ANIMAL #: 91375 DAYS ON TEST: 84 MODE OF DEATH: SE

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):

SPLEEN	- SMALL
SKULL	- DEFORMITY, TAN, RAISED; NOSE
SKIN	- STAIN, BLACK, NOSE

MICROSCOPIC OBSERVATIONS:

MANDIBULAR LYMPH NODES	- FOAMY MACROPHAGE AGGREGATES, MILD
NOSE	- INFLAMMATION/SEPSIS, MILD
SPLEEN	- ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MESENTERIC LYMPH NODE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, TESTES, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: THYMUS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: NASAL INFLAMMATION

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91376 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91377 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SPLEEN - SMALL, PALE

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91378 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91379 DAYS ON TEST: 92 MODE OF DEATH: AK

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
LUNGS	- DISCOLORATION, RED	- POST MORTEM CONGESTION
TAIL	- AMPUTATION	

MICROSCOPIC OBSERVATIONS:

BONE MARROW	- MYELOID HYPERPLASIA, MINIMAL
SPLEEN	- EXTRAMEDULLARY HEMATOPOIESIS, INCREASED, MILD
TESTES	- DEGENERATION/ATROPHY, BILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: DUODENUM, JEJUNUM

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: MANDIBULAR LYMPH NODES

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: ACCIDENTAL DEATH

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: I CONCENTRATION: 0 ppm

ANIMAL #: 91380 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SPLEEN - SMALL, MINIMAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91381 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SPLEEN - SMALL, MINIMAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, SPLEEN

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91382 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91383 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
KIDNEYS - INFARCT, MINIMAL
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
- INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91384 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
LIVER - DISCOLORATION, TAN, MIDDLE; 2MM DIA

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91385 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SPLEEN - SMALL, MINIMAL, PALE

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, SPLEEN

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91386 DAYS ON TEST: 92 MODE OF DEATH: AK

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED

MICROSCOPIC CORRELATE (selected lesions):
- POST MORTEM CONGESTION

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: DUODENUM, GALLBLADDER, ILEUM

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: ACCIDENTAL DEATH

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91387 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SPLEEN - SMALL, PALE

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, SPLEEN

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91388 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91389 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: III CONCENTRATION: 50 ppm

ANIMAL #: 91390 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
SPLEEN - SMALL, PALE

MICROSCOPIC OBSERVATIONS:
SPLEEN - ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91391 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
TAIL - CHRONIC DERMATITIS

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
SPLEEN - EXTRAMEDULLARY HEMATOPOIESIS, INCREASED, MINIMAL
THYMUS - ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, TESTES, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91392 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SPLEEN - SMALL, MINIMAL, PALE

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
OTHER - TEETH: ODONTODYSPLASIA

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91393 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:		MICROSCOPIC CORRELATE (selected lesions):
LIVER	- DISCOLORATION, TAN, MIDDLE; 2MM DIA	- NOT CONFIRMED
SPLEEN	- SMALL, MINIMAL, PALE	- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91394 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91395 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91396 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91397 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91398 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:		MICROSCOPIC CORRELATE (selected lesions):
SPLEEN	- SMALL, MINIMAL, PALE	- NOT CONFIRMED
EYES	- DISCOLORATION, WHITE, LEFT	- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91399 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: V CONCENTRATION: 250 ppm

ANIMAL #: 91400 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91401 DAYS ON TEST: 12 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
EYES - ONE OF PAIR PRESENT

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, PROSTATE, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: CECUM, DUODENUM, JEJUNUM

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
RETINA

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91402 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
NO ABNORMALITIES DETECTED

MICROSCOPIC OBSERVATIONS:
EYES - ONE OF PAIR PRESENT
LIVER - MITOTIC FIGURES, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: DUODENUM, ILEUM, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: GALLBLADDER, PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91403 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):

SMALL INTESTINE	- LESION, OTHER, ; EMPTY OF INGESTA	
LARGE INTESTINE	- LESION, OTHER, ; EMPTY OF INGESTA	
LUNGS	- DISCOLORATION, DARK, RED; MOTTLING	- POST MORTEM CONGESTION

MICROSCOPIC OBSERVATIONS:

SPLEEN	- ATROPHY, LYMPHOID, MINIMAL
THYMUS	- ATROPHY, LYMPHOID, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, TESTES, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: GALLBLADDER

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: RETINA

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
EYES

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91404 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
THYMUS - ATROPHY, LYMPHOID, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: DUODENUM, GALLBLADDER, JEJUNUM, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS, PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91405 DAYS ON TEST: 12 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
THYMUS - ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: CECUM, DUODENUM, JEJUNUM, RETINA, URINARY BLADDER

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91406 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS: NONE

MICROSCOPIC OBSERVATIONS:
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL
THYMUS - ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: RETINA, URINARY BLADDER

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS, PITUITARY, PROSTATE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91407 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED; BRIGHT

MICROSCOPIC CORRELATE (selected lesions):
- POST MORTEM CONGESTION

MICROSCOPIC OBSERVATIONS:
LIVER - MITOTIC FIGURES, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: GALLBLADDER, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS, PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91408 DAYS ON TEST: 12 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
THYMUS - ATROPHY, LYMPHOID, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, COLON, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, TESTES, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: ILEUM, JEJUNUM

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: CECUM, DUODENUM, MANDIBULAR LYMPH NODES

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: SPLEEN

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91409 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED; BRIGHT

MICROSCOPIC CORRELATE (selected lesions):
- POST MORTEM CONGESTION

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: GALLBLADDER

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: VII CONCENTRATION: 1000 ppm

ANIMAL #: 91410 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED; BRIGHT

MICROSCOPIC CORRELATE (selected lesions):
- POST MORTEM CONGESTION

MICROSCOPIC OBSERVATIONS:
THYMUS - ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, TESTES, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: GALLBLADDER, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: DUODENUM

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: THYROID GLAND

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91411 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91412 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91413 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

KIDNEYS	- NEPHROPATHY, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
SPLEEN	- EXTRAMEDULLARY HEMATOPOIESIS, INCREASED, MINIMAL
TESTES	- DEGENERATION/ATROPHY, BILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91414 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: MANDIBULAR LYMPH NODES, PARATHYROID GLANDS, PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91415 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
KIDNEYS - NEPHROPATHY, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91416 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
STOMACH	- EROSION/ULCERATION, NONGLANDULAR MUCOSA, MINIMAL
TESTES	- DEGENERATION/ATROPHY, BILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, THYMUS, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS, PITUITARY, THYROID GLAND

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91417 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
EXORBITAL LACRIMAL GLANDS - LYMPHOID AGGREGATES, INCREASED, MINIMAL
SALIVARY GLANDS - INFLAMMATION, SUBACUTE, MINIMAL
TESTES - DEGENERATION/ATROPHY, UNILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91418 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
TESTES	- DEGENERATION/ATROPHY, BILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91419 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LUNGS - INFLAMMATION/TYPE II CELL HYPERPLASIA, MINIMAL
TESTES - DEGENERATION/ATROPHY, BILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, EPIDIDYMIDES, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, PROSTATE, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

MALE MICE GROUP: IX CONCENTRATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91420 DAYS ON TEST: 92 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

EPIDIDYMIDES	- GERM CELL DEBRIS, INCREASED, MINIMAL
EYES	- LENS NOT PRESENT
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
SALIVARY GLANDS	- INFLAMMATION, SUBACUTE, MINIMAL
TESTES	- DEGENERATION/ATROPHY, BILATERAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PROSTATE, RECTUM, RETINA, SCIATIC NERVE, SEMINAL VESICLES, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91421 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
NOSE	- DEGENERATION, TRIGEMINAL NERVE, MINIMAL
UTERUS	- CYST/CYSTIC HYPERPLASIA, ENDOMETRIAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, OVARIES, PANCREAS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91422 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91423 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
OVARIES	- CYST(S), MINIMAL
THYMUS	- ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91424 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
UTERUS - CYST/CYSTIC HYPERPLASIA, ENDOMETRIAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91425 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
EYES - LENS NOT PRESENT
UTERUS - CYST/CYSTIC HYPERPLASIA, ENDOMETRIAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91426 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN.

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91427 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
UTERUS - CYST/CYSTIC HYPERPLASIA, ENDOMETRIAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN .

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91428 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN.

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91429 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
EXORBITAL LACRIMAL GLANDS - ATROPHY, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN.

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: II CONCENTATION: 0 ppm

ANIMAL #: 91430 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
NOSE - HEMORRHAGE, NASOTURBINATE, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN.

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91431 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
SKIN - ALOPECIA, SEVERE, LEFT, REAR, LIMB

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
SKIN - ALOPECIA, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91432 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91433 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91434 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91435 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91436 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91437 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED, MINIMAL

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91438 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - NECROSIS, FOCAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91440 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
SPLEEN - SMALL, MINIMAL

MICROSCOPIC OBSERVATIONS:
LIVER - NECROSIS, FOCAL, MINIMAL
OVARIES - CYST(S), MINIMAL
SPLEEN - ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: IV CONCENTATION: 50 ppm

ANIMAL #: 91471 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
OVARIES - *B TERATOMA

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91441 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91442 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91443 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91444 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: V1 CONCENTATION: 250 ppm

ANIMAL #: 91445 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91446 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91447 DAYS ON TEST: 70 MODE OF DEATH: SE

GROSS OBSERVATIONS:

		MICROSCOPIC CORRELATE (selected lesions):
LUNGS	- DISCOLORATION, RED, DIFFUSE; BRIGHT	- NOT CONFIRMED
KIDNEYS	- LARGE, MILD, RIGHT	
	- DILATATION, BILATERAL, SEVERE, PELVIS	
	- DEFORMITY, CLEAR, LEFT; MISSHAPEN	
	- DEFORMITY, WHITE, RAISED, FIRM, RIGHT; 5MM DIA	
SPLEEN	- LARGE, MINIMAL	

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
	- MEDULLA NOT PRESENT
	- ONE OF PAIR PRESENT
HEART	- INFLAMMATION/DEGENERATION, MINIMAL
KIDNEYS	- PYELONEPHRITIS, SEVERE
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
SPLEEN	- ATROPHY, LYMPHOID, MODERATE
	- EXTRAMEDULLARY HEMATOPOIESIS, INCREASED, SEVERE
THYMUS	- ATROPHY, LYMPHOID, MILD
URINARY BLADDER	- HYPERPLASIA, MUCOSA, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYROID GLAND, TRACHEA, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: DUODENUM, GALLBLADDER, ILEUM, JEJUNUM

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: RETINA

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: PYELONEPHRITIS

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91448 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
SPLEEN - SMALL, PALE

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS: NONE

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, LUNGS, NOSE, OVARIES, SPLEEN

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91449 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LUNGS - INFLAMMATION, SUBACUTE/CHRONIC, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LIVER, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VI CONCENTATION: 250 ppm

ANIMAL #: 91450 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: KIDNEYS, LUNGS, NOSE, OVARIES

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91451 DAYS ON TEST: 88 MODE OF DEATH: FD

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED

MICROSCOPIC CORRELATE (selected lesions):
- POST MORTEM CONGESTION

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
OVARIES - ATROPHY, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: GALLBLADDER, JEJUNUM

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: RETINA

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: PITUITARY

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91452 DAYS ON TEST: 70 MODE OF DEATH: FD

GROSS OBSERVATIONS:
LUNGS - DISCOLORATION, RED, DIFFUSE; BRIGHT

MICROSCOPIC CORRELATE (selected lesions):
- NOT CONFIRMED

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
OVARIES - ATROPHY, MILD
VAGINA - ATROPHY, MUCOSA, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PITUITARY, RECTUM, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: DUODENUM, JEJUNUM

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
RETINA

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: ILEUM, PARATHYROID GLANDS

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91453 DAYS ON TEST: 88 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
OVARIES - ATROPHY, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: CECUM, DUODENUM, GALLBLADDER, ILEUM, JEJUNUM, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91454 DAYS ON TEST: 12 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
LIVER - MITOTIC FIGURES, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: ADRENAL GLANDS, AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: CECUM, JEJUNUM, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91455 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS:		MICROSCOPIC CORRELATE (selected lesions):
LUNGS	- DISCOLORATION, RED; BRIGHT	- POST MORTEM CONGESTION

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91456 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
OVARIES	- ATROPHY, MILD
	- ONE OF PAIR PRESENT
SPLEEN	- PIGMENT, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
MAMMARY GLAND

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

SUBCHRONIC INHALATION STUDY WITH 4-VINYLCYCLOHEXENE

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91457 DAYS ON TEST: 12 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - MITOTIC FIGURES, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: CECUM, DUODENUM, ILEUM, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: GALLBLADDER, RECTUM

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
PITUITARY, URINARY BLADDER

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91458 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER	- INFLAMMATION, SUBACUTE, MINIMAL
OVARIES	- ATROPHY, MILD
SPLEEN	- PIGMENT, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: MANDIBULAR LYMPH NODES

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91459 DAYS ON TEST: 12 MODE OF DEATH: FD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
- MITOTIC FIGURES, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: CECUM, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: VIII CONCENTATION: 1000 ppm

ANIMAL #: 91460 DAYS ON TEST: 11 MODE OF DEATH: FD

GROSS OBSERVATIONS: NONE

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - MITOTIC FIGURES, INCREASED, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: CECUM, DUODENUM, JEJUNUM, RETINA

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: PITUITARY

CAUSE OF DEATH/MORIBUND CONDITION: UNDETERMINED

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91461 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - ATROPHY, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: GALLBLADDER, PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91462 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
OVARIES - ATROPHY, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: SKIN

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91463 DAYS ON TEST: 64 MODE OF DEATH: FD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):

NOSE	- FRACTURE, SEVERE

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
BONE MARROW	- MYELOID HYPERPLASIA, MINIMAL
NOSE	- INFLAMMATION, SUPPURATIVE, MODERATE
OTHER	- ORAL MUCOSA: INFLAMMATION/ULCERATION, MODERATE
SPLEEN	- ATROPHY, LYMPHOID, MODERATE
THYMUS	- ATROPHY, LYMPHOID, MILD

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: DUODENUM, JEJUNUM, RETINA, STOMACH

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES: NONE

CAUSE OF DEATH/MORIBUND CONDITION: NASAL INFLAMMATION/TRAUMA

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91464 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:

ADRENAL GLANDS	- HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
KIDNEYS	- INFLAMMATION, CAPSULE, MINIMAL
LIVER	- FATTY CHANGE, MEDIAN CLEFT, MINIMAL
	- INFLAMMATION, SUBACUTE, MINIMAL
OVARIES	- ATROPHY, MINIMAL
	- CYST(S), MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91465 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - INFLAMMATION, SUBACUTE, MINIMAL
OVARIES - ATROPHY, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: SCIATIC NERVE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91466 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS: MICROSCOPIC CORRELATE (selected lesions):
EYES - MISSING, LEFT

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
EYES - ONE OF PAIR PRESENT
OVARIES - ATROPHY, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: PITUITARY

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91467 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
OTHER - ORAL MUCOSA: INFLAMMATION/ULCERATION, MINIMAL
SPLEEN - ATROPHY, LYMPHOID, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LIVER, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, OVARIES, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, UTERUS, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: GALLBLADDER

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

APPENDIX (Continued)
INDIVIDUAL ANIMAL PATHOLOGY DATA

FEMALE MICE GROUP: X CONCENTATION: 1000 ppm 1,3-BUTADIENE

ANIMAL #: 91470 DAYS ON TEST: 93 MODE OF DEATH: SD

GROSS OBSERVATIONS:
NO ABNORMALITIES DETECTED

MICROSCOPIC CORRELATE (selected lesions):

MICROSCOPIC OBSERVATIONS:
ADRENAL GLANDS - HYPERPLASIA, SUBCAPSULAR CELLS, MINIMAL
LIVER - FATTY CHANGE, MEDIAN CLEFT, MINIMAL
OVARIES - ATROPHY, MILD
UTERUS - CYST/CYSTIC HYPERPLASIA, ENDOMETRIAL, MINIMAL

THE FOLLOWING TISSUES WERE UNREMARKABLE MICROSCOPICALLY: AORTA, BONE MARROW, BRAIN, CECUM, COLON, DUODENUM, ESOPHAGUS, EXORBITAL LACRIMAL GLANDS, EYES, FEMUR/KNEE JOINT, GALLBLADDER, HARDERIAN GLANDS, HEART, ILEUM, JEJUNUM, KIDNEYS, LARYNX/PHARYNX, LUNGS, MAMMARY GLAND, MANDIBULAR LYMPH NODES, MESENTERIC LYMPH NODE, NOSE, PANCREAS, PARATHYROID GLANDS, PITUITARY, RECTUM, RETINA, SALIVARY GLANDS, SCIATIC NERVE, SKELETAL MUSCLE, SKIN, SPINAL CORD, SPLEEN, STERNUM, STOMACH, THYMUS, THYROID GLAND, TRACHEA, URINARY BLADDER, VAGINA, ZYMBAL'S GLANDS

THE FOLLOWING TISSUES WERE AUTOLYZED; DEGREE OF AUTOLYSIS DID NOT PRECLUDE MICROSCOPIC EXAMINATION: NONE

THE FOLLOWING TISSUES WERE SEVERELY AUTOLYZED; MICROSCOPIC DIAGNOSES NOT MADE, ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

SECTIONS OF THE FOLLOWING TISSUES WERE NOT PRESENT FOR MICROSCOPIC EXAMINATION: NONE

SECTIONS OF THE FOLLOWING TISSUES WERE INSUFFICIENT FOR MICROSCOPIC EXAMINATION; ORGAN NOT COUNTED IN INCIDENCE TABLES:
NONE

CAUSE OF DEATH/MORIBUND CONDITION: SACRIFICED BY DESIGN

Section No 001

Study Title

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE REVISION NO. 1

Author

Karin S. Bentley, Ph.D

RECD
OFFICE OF POLLUTION
PREVENTION AND TOXICS
94 APR 22 PM 2:41

Study Completed on

September 14, 1993

Revision No. 1 Completed on

February 22, 1994

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, Delaware 19714

Sponsor Study No.

BUT-21.0-COT-DHL-04

Medical Research No. 9523-001

Laboratory Project ID

Haskell Laboratory Report No. 506-93

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to EPA Good Laboratory Practice Standards (40 CFR 792) except for the deviations documented and explained below.

1. In some instances, standard forms were printed with initials prior to recording data; raw data entered on those forms were not, therefore, initialed and dated at the time of entry.

2. At the time of the study, the Standard Operating Procedure (SOP) for the automated environmental monitoring system used for inhalation chambers in this laboratory did not include the specific instrument used in the study. Although this instrument is similar in design to the others, the requirement for an SOP was not fully satisfied. The SOP has subsequently been revised and the instrument number added to the maintenance logs.

These deviations did not affect the integrity of the study.

Submitter: E. I. du Pont de Nemours and Company

Sponsor: Chemical Manufacturers Association, Washington, D.C.

Date

Study Director: Karin S. Bentley 2-15-94
Karin S. Bentley, Ph.D.
Senior Research Toxicologist

Sponsor Representative: 2/16/94
Elizabeth J. Moran, Ph.D., D.A.B.T.
Chemical Manufacturers Association

GENERAL INFORMATION

Material Tested:	Cyclohexene, 4-ethenyl-
Haskell No.:	H-19353
Synonyms and Codes:	· 4-ethenylcyclohexene · Vinylcyclohexene (Vinyl cyclohexene) · 4-Vinylcyclohexene-1 · 4-Vinylcyclohexene · VCH · VN MK 6A · ID# IN210G308
C.A.S. Registry No.:	100-40-3
Purity:	99.8% (Supplied); 99.6 - 99.7% (Reanalysis)
Contaminants:	t-butylcatechol as a stabilizer
Sponsor:	Chemical Manufacturers Association 2501 M Street, NW Washington, DC 20037
Study Initiated/Completed:	December 3, 1992 / September 14, 1993
Experimental Phase Initiated/Completed:	January 26, 1993 / May 28, 1993

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

SUMMARY

A micronucleus assay was conducted in rats and mice to determine whether 4-vinylcyclohexene (VCH) induces an increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow following subchronic (90 day) inhalation exposure. In this study, groups of male and female Crl:CD®BR rats and B6C3F1/CrlBR mice were placed in inhalation exposure chambers and exposed whole-body to VCH at target concentrations of 0, 250, 1000, and 1500 ppm (rats) and 0, 50, 250, and 1000 ppm (mice) for six hours/day, five days/week for 13 weeks. Bone marrow smears were prepared approximately 22 - 26 hours after the final exposure, and one thousand polychromatic erythrocytes per animal were evaluated for the presence of micronuclei.

No statistically significant increases in micronucleated polychromatic erythrocytes were observed in either species at any VCH concentration tested. In addition, no significant decrease in the ratio of young polychromatic erythrocytes to mature normochromatic erythrocytes were observed in either rats or mice. Under the conditions of this study, VCH is negative.

Reported by:

Kathy M. Gerber, B.A.
Toxicologist

Anthony S. Panepinto, M.S.
Research Toxicologist

Approved by:

Karin S. Bentley, Ph.D.
Senior Research Toxicologist
Study Director

Awni M. Sarrif, Ph.D.
Manager
Developmental, Genetic, and
Reproductive Toxicology

Reviewed and Approved for Issue by:

Karin S. Bentley, Ph.D.
Senior Research Toxicologist
Study Director

9-14-93
Date

QUALITY ASSURANCE DOCUMENTATION

(H-19353)

Dates of Inspection:

Conduct - 4/28/93

Records, Report(s) - 7/6-10,12,13/93

Revision No. 1 - 12/9/93; 2/21/94

Findings reported to:

Study Director - 7/29/93; 2/1,21/94

Management - 7/29/93; 2/1,21/94

Reported by: Deborah A. Vick — 2/2/94
Deborah A. Vick, Date
Quality Assurance Auditor

EXPLANATION OF REVISION

This report was revised in the following manner:

1. An additional deviation was added to the Good Laboratory Practice Compliance Statement (page 3).
2. The Sponsor's GLP Compliance Statement was removed from the Good Laboratory Practice Compliance Statement (page 3).
3. The size of the drum that the test material was supplied in was changed from a 50-gallon drum to a 55-gallon drum in Section C.1.
4. Section E.1 was edited to include an additional female mouse that was accidentally killed one day prior to study start.
5. The second paragraph of Section H.1 was edited to correct the airflow rate. The rate was changed from 35 - 65 L/min to 30 - 40 L/min for the 150-L chamber. In addition, the airflow rate for the 300-L chamber was added. In the same paragraph, the frequency of airflow recordings was changed from five-minute intervals to once per exposure for the negative control chamber.
6. In the first paragraph of Section H.3, the Displacement Pump model was changed from QG6-OSSY to QG6.
7. The third paragraph of Section H.3 was edited to clarify that adequate distribution of the test material for the 150-L chamber was confirmed in previous studies.
8. The model number for the Hewlett-Packard gas chromatograph was changed from 5880A to 5890A in fifth complete paragraph of Section H.3.
9. In the Results Section C (Chamber Temperature, Humidity, Airflow, and Oxygen Concentration) the mean temperature and relative humidity ranges were corrected. Daily mean temperature and humidity ranges were also added.
10. The footnotes on the Explanatory Notes for Appendices B - E have been edited. Original footnotes were clarified and additional footnotes have been added.
11. Appendices A - E were revised to reflect the updated footnotes. In addition, errors in calculations were corrected in Appendices B - D.

These changes did not affect the interpretation of the study results.

Approved by: Karin S. Bentley — Date: 2-22-94

Karin S. Bentley, Ph.D.
Senior Research Toxicologist

INTRODUCTION

The purpose of this study was to evaluate the ability of 4-vinylcyclohexene (VCH) to induce micronuclei in rat and mouse bone marrow polychromatic erythrocytes (PCEs) following subchronic (90 day) inhalation exposure. Micronuclei are small particles consisting of chromosome fragments or entire chromosomes which lag behind at the anaphase stage of cell division. They may arise from damage to chromosomes or the mitotic apparatus. As the erythroblast matures into an erythrocyte, the main nucleus is extruded, while micronuclei remain in the cytoplasm. The youngest erythrocytes, PCEs, are identified by uniform staining of the residual RNA which remains in the newly-formed cells for about two days after enucleation.

Micronucleated PCEs occur at low frequency under normal conditions in bone marrow. An increase in micronucleus frequency relative to the negative control group indicates that a test substance induced chromosomal and/or spindle damage in the nucleated erythroblast cells (Schmid, 1975; Salamone et al., 1980). The PCE matures into a normochromatic erythrocyte (NCE) and both cell types comprise the total erythrocyte population. A significant decrease in the proportion of PCEs relative to the control group is an indication of possible physiologic or toxic effects on the bone marrow.

MATERIALS AND METHODS

A. PROTOCOL

This study was performed according to the protocol for RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE (issued 12/3/92) and the accompanying protocol amendments. The study was conducted according to the Standard Operating Procedures for Chronic and Inhalation Toxicology and the Standard Operating Procedures for the Bone Marrow Micronucleus Assay in Rodents, which have been filed with Haskell Laboratory Quality Assurance.

B. REGULATORY COMPLIANCE

The study was conducted in compliance with the Testing Consent Order for 4-Vinylcyclohexene which was signed by the U.S. Environmental Protection Agency (EPA) and the CMA Butadiene Panel member companies (Docket No. OPTS-42116). The Micronucleus Assay procedures comply with published guidelines of the U.S. Environmental Protection Agency (40 CFR 798.5395). The inhalation phases of the study comply with the U.S. EPA guidelines for Subchronic Inhalation Toxicity Testing (40 CFR 798.2450).

C. TEST MATERIALS

1. Test Substance

The test substance, VCH, is a colorless liquid with a molecular weight of 108. The test substance was supplied in a 55-gallon drum by the Union Carbide Corporation, Institute, West Virginia (shipped 12/10/91, identified as Batch #1). Analytical purity and stability of a sample of the test material was determined by gas chromatography by Haskell Laboratory (see Appendix A).

2. Negative Control and Positive Indicators

Conditioned, filtered, house-line air was the negative control material for all the inhalation exposures.

The positive indicator material for rats was cyclophosphamide (CP; Cytoxan for Injection, Mead Johnson, Lot No. MBE93). A 20 mg/mL solution of CP was prepared in Sterile Water for Injection, U.S.P. (Baxter, Lot No. C207522) and further diluted for a final concentration of 8.0 mg/mL with 0.9% Sterile Sodium Chloride for Injection, U.S.P. (Baxter, Lot No. C209643). The CP was administered by intraperitoneal injection in a 5 mL/kg dosing volume yielding an effective dose of 40 mg/kg.

The positive indicator material for mice was 1,3-butadiene, (1,3-BD, H-19999, supplied in a DOT 4BW240 cylinder by Exxon Chemical Americas, Baton Rouge, LA., and shipped 11/30/92), which was administered by inhalation at a concentration of approximately 1000 ppm. The purity and stability of the 1,3-BD was determined by gas chromatography by Haskell Laboratory (see Appendix A).

D. TEST SYSTEM

The assessment of micronuclei was carried out on a subset of animals from the subchronic inhalation toxicity study of VCH in rats and mice (DuPont HLR 442-93).

Male and female Crl:CD®BR rats (approximately 22 days old) were received from Charles River Laboratories, Inc., Kingston, New York on 1/12/93. On 1/14/93, males weighed 47 - 73 grams while females weighed 35 - 73 grams.

Male and female B6C3F1/CrlBR mice (approximately 36 days old) were received from Charles River Laboratories, Inc., Raleigh, North Carolina on 1/12/93. On 1/14/93, males weighed 16 - 25 grams while females weighed 13 - 20 grams.

These strains were selected because NTP oral studies with VCH used B6C3F1 mice and inhalation studies with VCH used the CD rat and B6C3F1 mouse. At study start, rats were approximately 36 days old and mice were approximately 50 days old.

E. ANIMAL HUSBANDRY

1. Quarantine

Upon arrival at Haskell Laboratory, the animals were isolated for a quarantine period of two weeks. Animals were weighed three times (rats) or four times (mice) during this period and observed daily for any gross signs of disease or injury. During quarantine, three female mice died and were necropsied to check for the presence of disease; none was evident. An additional female mouse was accidentally killed one day prior to the study start date. It was replaced with another female mouse and assigned a new identification number. On 1/25/93 all surviving animals were examined by a veterinary ophthalmologist. One male mouse was found to have ophthalmic abnormalities and was removed from use in the study. On the basis of body weight gain and clinical observations, all remaining rats and mice were released from quarantine by the laboratory veterinarian.

2. Housing

During the quarantine period, rats and mice were housed in separate animal rooms. During the subchronic study, rats and mice were housed together in a single Bioclean® room (laminar flow air circulation pattern) in close proximity to the inhalation chambers. The animal rooms were maintained on a 12-hour light/dark cycle (fluorescent light) and targeted at a temperature of 23 ± 2°C and a relative humidity of 55 ± 15%. Excursions outside the target ranges were considered of insufficient magnitude or duration to have affected the outcome of the study.

3. Caging

Except during exposure, all animals were housed in stainless steel, wire-mesh cages suspended above cage boards; separate cage racks were used for the two species and sexes. Rack position was rotated in the room weekly, and cages were repositioned on the racks every two weeks. During the quarantine period, rats were housed individually and mice were housed individually and/or two to a cage. During the subchronic study, all rats and mice were individually housed.

4. Food and Water

Mice and rats were fed Purina® Certified Rodent Chow (chunk) #5002. Water was supplied by the Wilmington Suburban Water Corporation. Except during exposure, food and water were available ad libitum.

As part of an animal health monitoring program, food and water analyses for contaminants were conducted approximately every month. This program is monitored and administered by the laboratory veterinarian; data are maintained separately from study records. Feed analyses indicated that feed was within recommended limits set by the National Center for Toxicological Research for bacteria, bacterial spores, and filamentous fungi. Pathogenic bacteria were not detected in water samples collected during the course of the study.

5. Identification

During quarantine, rats and mice were assigned temporary identification numbers and/or symbols that were applied to the animals and cages. Prior to study start, each animal was assigned a unique five- or six-digit animal identification number and given a four-digit identification number that was tattooed on the tail.

6. Grouping

Near the end of the quarantine period, rats and mice were grouped using a computerized, stratified randomization program so that the mean body weight of each test group (within each species) was approximately equal.

F. STUDY START

For the purposes of in-life data collection, the study start date for all groups was 1/26/93. However, the initiation of inhalation exposures for different species and sexes was spread over a four-day period (1/26/93 - 1/29/93) in order to accommodate in-life study functions and necropsy. Rats and mice that were not assigned to a test group were removed from the animal rooms and sacrificed after the initiation of the exposures.

G. SELECTION OF EXPOSURE CONCENTRATIONS

In a two-week rangefinding study, four groups of five male and five female rats and five male and five female mice were exposed for two weeks by inhalation to nominal VCH concentrations of 0, 250, 750, or 1500 ppm. Actual mean concentrations were 0, 240, 720, and 1500 ppm. Exposures were for six hours/day, five days/week with only one day of rest separating the two weeks.

One female rat in the 720 ppm exposure group was found dead on study day 2, and 5/5 male mice and 4/5 female mice were found dead prior to exposure on test day 4 in the 1500 ppm exposure group. The fifth female mouse at 1500 ppm was moribund and was sacrificed in extremis without further inhalation exposure. There were no other mortalities, and the clinical

signs were unremarkable in the animals that survived the study. Significant body weight effects were observed in male rats exposed to 1500 ppm. The mean body weight gain of this group during the exposure period was lower (approximately 11%) than the mean weight gain of control males. All male and female mice that died had low mean body weights one to two days prior to death. The body weight effects were considered to be related to the exposure of VCH.

Based on these results, three dose levels of VCH were selected. The high level selected for rats was 1500 ppm with intermediate and low concentrations of 1000 and 250 ppm, respectively. For mice, the high level was 1000 ppm with intermediate and low concentrations of 250 and 50 ppm, respectively.

H. EXPOSURE SYSTEM AND GENERATION OF TEST ATMOSPHERES

1. Test Chambers

Six chambers (approximately 150 - 300 L) were dedicated to the study throughout the duration of the exposure period. The chambers and all surfaces that the test material contacted prior to exhaust from the chamber were constructed of stainless steel or glass and were thoroughly cleaned prior to introduction of the test substance.

The chambers were operated in a one-pass, flow-through mode with an airflow rate targeted at 30 - 40 L/min for the 150 L chambers and 60 - 70 L/min for the 300 L chambers. The chamber temperature and relative humidity were targeted at conditions similar to housing conditions (i.e., temperature of 22 $\pm$ 3°C, relative humidity of 40 - 60%). However, temperature and humidity variations outside of the ranges are commonly encountered with the type of generation procedures used in the conduct of this study. The variations that did occur are considered to have not affected the outcome of the study. Chamber temperature and relative humidity were monitored continuously and recorded at approximately five-minute intervals for the VCH and 1,3-BD chambers and at approximately thirty-minute intervals for the negative control chamber. Airflow was recorded once per exposure for the negative control and chamber oxygen concentration was measured at least two times per exposure.

Chamber exhaust was passed through a carbon bed prior to being vented through the roof exhaust system; emission was permitted under a lab-wide permit from the state of Delaware Department of Natural Resources and Environmental Control.

2. Exposures

Groups of ten male and ten female rats and ten male and ten female mice were exposed to one of three target VCH concentrations or one target 1,3-BD concentration. Exposures were for six hours/day, approximately

five days/week, for a minimum of 65 exposures over a 13-week period (weekends and holidays excluded, except where noted). A negative control group of ten male and ten female rats and ten male and ten female mice were exposed to air alone. A positive indicator group of five male and five female rats was excluded from inhalation exposure entirely and maintained throughout the 13-week period prior to administration of a single i.p. dose of CP (see MATERIALS AND METHODS, I. TREATMENT AND SAMPLING SCHEDULE).

Exposures were conducted during the same eight-hour period of the day to minimize diurnal physiological variations. During exposure, rats and mice were housed individually in stainless steel cages. At approximately one-hour intervals throughout the daily exposure periods, animals visible through the chamber window were observed for well-being, and once during each exposure, the animals' response to sound was determined by tapping on the chamber window. Before transfer to housing facilities at the end of each exposure, test groups were left in their respective exposure chamber for a period sufficient to allow clearance of VCH or 1,3-BD as determined by atmospheric analysis. Negative control animals were treated exactly as the test animals, except that the control chamber atmosphere contained air only.

3. Atmosphere Generation and Analysis

Atmospheres of VCH were generated by metering the liquid test substance (using either a Harvard Apparatus model 22 Syringe Infusion Pump or a Fluid Metering Incorporated model QG6-ISSY or QG6 Displacement Pump) into a heated, glass Instatherm flask. Vapor was diluted with filtered air to the desired concentration for each of the four VCH test chambers. Chamber concentrations of VCH were controlled by varying the test substance flow rate into the Instatherm flask. A baffle located at the inlet of the exposure chamber helped evenly disperse the vapor. Filtered air alone was metered in an identical manner into the negative control chamber. The Instatherm flask and connecting glass tubing were maintained at a constant temperature of approximately 28 - 30°C by controlling variacs.

An atmosphere of 1,3-BD was generated by metering the gaseous positive indicator substance through a Brooks model 1355 Sho-Rate Flowmeter. Vapor was diluted with filtered air to the desired concentration. The concentration of 1,3-BD was controlled by varying the flow rate into the exposure chamber. A baffle located at the inlet of the exposure chamber helped evenly disperse the vapor.

Chamber atmospheres were analyzed by gas chromatography at approximately 30-minute intervals during each six-hour exposure period. Samples were drawn by vacuum pump from representative areas of the chambers where animals were exposed. During prestudy method development, the distribution of VCH and 1,3-BD within the 300-L chambers was determined by collecting samples from the top, middle, and bottom of each test chamber. Adequate distribution of the test material in the 150-L chambers was confirmed in previous studies. The coefficient of variation between the sampling

points was less than 10% indicating that VCH and 1,3-BD were distributed homogeneously within the test chambers.

Samples of VCH atmospheres (approximately 0.5 mL) were analyzed using a Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector. The temperature of the injection port was 100°C, and the temperature of the flame ionization detector was 250°C. Nitrogen was used as the carrier gas at a flow rate of 15 mL/min. Samples were chromatographed isothermally at 75°C on a 15m x 0.53mm (inside diameter) SPB-S column. The detector was supplied with air (350 mL/min) and hydrogen (30 mL/min).

Samples of the 1,3-BD atmosphere (approximately 0.5 mL) were analyzed using a Hewlett-Packard 5890A gas chromatograph equipped with a flame ionization detector. The temperature of the injection port was 50°C, and the temperature of the flame ionization detector was 250°C. Nitrogen was utilized as the carrier gas at a flow rate of 12 mL/min. Samples were chromatographed isothermally at 35°C on a 10m x 0.53mm (inside diameter) HP-5 or HP-17 column. The detector was supplied with air (350 mL/min) and hydrogen (30 mL/min).

The atmospheric concentrations of VCH and 1,3-BD were determined by comparing detector responses of the chamber samples to that of gas standards with the use of standard curves. The gas standards were prepared prior to each exposure by injecting known volumes of VCH or 1,3-BD into gas bags which contained known volumes of air. The methodology used for preparing standards was documented in the study records.

I. TREATMENT AND SAMPLING SCHEDULE

The initial and final exposure dates and the dates of sacrifice for the VCH and negative control-treated rats and mice are given below. Numbers in parentheses are the days relative to the study start date of 1/26/93 (Day 1).

Species	Sex	Initial Exposure	Final Exposure	Sacrifice
Mouse	Male	1/26/93 (Day 1)	4/26/93 (Day 91)	4/27/93 (Day 92)
Mouse	Female	1/27/93 (Day 2)	4/27/93 (Day 92)	4/28/93 (Day 93)
Rat	Male	1/28/93 (Day 3)	4/28/93 (Day 93)	4/29/93 (Day 94)
Rat	Female	1/29/93 (Day 4)	4/29/93 (Day 94)	4/30/93 (Day 95)

For the 1,3-BD-exposed male and female mice, the first day of exposure was 1/27/93 (Day 2); final exposure and sacrifice dates were the same as the VCH-treated male and female mice, respectively. Because the exposures for male mice to 1,3-BD started one day later than that of the VCH-treated males, a make-up exposure day for the 1,3-BD-treated male mice was provided on 3/20/93.

For the purposes of the micronucleus evaluation, VCH-, air-, and 1,3-BD-treated animals were sacrificed approximately 22 - 26 hours following the final inhalation exposure. Samples of bone marrow were obtained from five randomly selected male and female rats and mice from each treatment group. The following exceptions occurred: bone marrow samples were obtained from two surviving female mice in the 1000 ppm VCH exposure group (see RESULTS, D. CLINICAL SIGNS OF TOXICITY); bone marrow was not sampled from the 1,3-BD-treated rats. The positive indicator group for rats consisted of five males and five females that were administered a single i.p dose of 40 mg CP/kg body weight 22 - 26 hours prior to sacrifice. The CP administration and sacrifices were scheduled to coincide with the final exposure and sacrifice dates of the VCH-treated male and female rats.

J. CLINICAL OBSERVATIONS AND BODY WEIGHTS

Cage-site examinations to detect moribund or dead animals, as well as abnormal behavior and appearance, were conducted twice daily on weekdays and at least once daily on weekends and holidays. On exposure days, the first observation was conducted while loading the chambers; the second observation was upon removal of the animals from the chamber.

Moribund animals were sacrificed. Moribund and dead animals were given a pathological examination. Body weights were taken prior to the first exposure, at weekly intervals during the exposure phase, and prior to sacrifice. At each weighing, animals were individually handled and examined for abnormal behavior, appearance, and palpable masses.

K. PREPARATION OF BONE MARROW SMEARS

On the day following the final exposure, animals were sacrificed by CO_2 asphyxiation and the femurs removed. The marrow from one femur of each animal was aspirated and flushed into approximately 2 - 3 mL prewarmed (37°C) fetal bovine serum. The marrow button was collected by centrifugation at approximately 200 x g for five minutes. Most of the supernatant was removed, and the cells were resuspended in the remaining 1-2 drops of serum. A Miniprep® automatic blood smearing instrument was used to make the bone marrow smears. At least two slides per animal were prepared and fixed in absolute methanol for eight minutes.

The slides were stained for three minutes in acridine orange (Sigma, Lot No. 73F-3673) at a concentration of 0.0125 mg/mL in phosphate buffer (pH 7.4). Prior to scoring, a coverslip was floated on each slide using phosphate buffer.

L. CYTOGENETIC EVALUATION

Representative slides from each animal were examined in a blind manner using incident light fluorescence microscopy (Leitz Dialux 20 microscope with Ploemopak® and H-2 filter cube). Only cells showing good morphology and staining were selected for scoring. PCEs were identified by their characteristic reddish staining; NCEs appeared dark green. One thousand PCEs per animal were scored for the presence of micronuclei, which are typically round, bright yellow-green fluorescent bodies. Cellular inclusions that were irregularly shaped or stained, or were not in the focal plane of the cell were considered artifacts. The unit of scoring was the micronucleated cell; PCEs containing more than one micronucleus were counted as a single micronucleated cell. The number of micronucleated NCEs seen in the optic fields scored to obtain 1000 PCEs was also recorded. The proportion of PCEs among 1000 erythrocytes (reported as the PCE:NCE ratio) was determined for each animal.

M. STATISTICAL ANALYSIS

Data for the proportion of PCEs among 1000 erythrocytes (PCE frequency) and the proportion of micronucleated PCEs (MN PCEs) among 1000 PCEs (MN PCE frequency) were transformed prior to statistical analysis using the arcsin square root transformation. This transformation is appropriate for percentages and proportions since the transformed data more closely approximate a normal distribution than do the nontransformed proportions (Sokal and Rohlf, 1969). Transformed data for each variable (PCE or MN PCE frequency) were analyzed separately for normality of distribution using the Shapiro-Wilkes test. If the results of this test indicated that the transformed values for each variable were normally distributed in both sexes, parametric methods (e.g., Analysis of Variance, Dunnett's test) were used. If there was evidence of nonnormality in either sex, nonparametric methods (e.g., Kruskal-Wallis test, Mann-Whitney U test) were employed for that variable using nontransformed proportions. Positive indicator data were not included in the evaluation of normality of distribution. Weight gain data were assumed to be normally distributed and were analyzed using Analysis of Variance (ANOVA).

Data from each sex were analyzed separately, and individual comparisons to the control were made using each animal as the experimental unit. All analyses were conducted at a significance level of 5%. Positive indicator data were analyzed separately.

Data from mice exposed to 1000 ppm VCH were excluded from statistical analysis due to the small size of the data set (see RESULTS, D. CLINICAL SIGNS OF TOXICITY).

N. RETENTION OF RECORDS AND DATA

All Haskell generated study records, microscope slides and raw data, along with the original final report, are retained at Haskell Laboratory or at the E. I. du Pont de Nemours and Company Records Management Center, Wilmington, Delaware.

RESULTS

A. PURITY AND STABILITY ANALYSIS

Samples of VCH and 1,3-BD were taken for purity and stability analysis prior to study start (1/13/93) and after the completion of the exposure phase (5/10/93). Results of the analysis indicated that the composition of VCH and 1,3-BD were essentially unchanged over the course of the study. The purity of VCH was 99.44 - 99.75%, and the purity of 1,3-BD was 99.9% (See Appendix A).

B. EXPOSURE CHAMBER CONCENTRATIONS

Mean VCH and 1,3-BD chamber concentrations are summarized in Table 1 and Appendix B. For simplicity, all exposure concentrations are referenced by the target concentrations.

The overall mean concentrations of VCH in the exposure chambers for the subchronic (90 day) test period were 0, 53, 250, 1,000, and 1,500 ppm corresponding to target concentrations of 0, 50, 250, 1,000, and 1,500 ppm, respectively. The overall mean concentration of 1,3-BD in the exposure chamber for the 90-day test period was 980 ppm.

C. CHAMBER TEMPERATURE, HUMIDITY, AIRFLOW, AND OXYGEN CONCENTRATION

The mean temperatures in the exposure chambers for all exposure days were between 23 - 27°C (Table 1), and the daily mean temperatures ranged from 21 - 29°C (Appendix C). The mean relative humidity over the course of the study was between 43 - 63% for the six chambers. The daily mean humidity readings generally ranged from 40 - 70%; however, there were a few excursions outside these readings (Appendix D). Airflow rates were targeted at either 35 L/min or 65 L/min and remained at those approximate levels throughout the study. Chamber oxygen concentrations were approximately 21% and did not vary during the study. Excursions outside the targeted ranges were considered not to have adversely affected the study outcome.

D. CLINICAL SIGNS OF TOXICITY

With the exception of the mice exposed to 1000 ppm VCH, clinical observations are for only those animals evaluated for micronuclei.

1. Rats

Clinical signs of toxicity were evident in male and female rats at all VCH concentrations evaluated. The most prevalent signs were lethargy, clear discharge from the mouth, and stained fur. Clinical signs were not evident in any of the air-exposed animals at any time during the study. All male and female rats survived until the scheduled sacrifice dates.

2. Mice

All male mice and one-half of the female mice exposed to 1000 ppm VCH were found dead by study day 12. At the end of the study, only two female mice at that concentration survived. No clinical signs of toxicity were observed in the 250 ppm-treated male and female mice. However, at 50 ppm, tremors and lethargy were observed in one male and two females. With the exception of ruffled fur, clinical signs were not evident in any of the negative control animals at any time during the study. All of the negative control, 50 ppm-, and 250 ppm-exposed mice survived until the scheduled sacrifice dates.

E. WEIGHT GAIN

Group weight gain comparisons were analyzed over the interval from the day of first exposure to the day of sacrifice. Weekly data are reported, however, no statistics were performed on those intervals. Only those animals evaluated for micronuclei were included in the statistical analysis.

Mean body weight gain data for male and female rats and mice are presented in Tables 2 - 5. A statistically significant decrease in weight gain was observed in male rats exposed to 1000 and 1500 ppm VCH and in female mice exposed to 250 ppm VCH.

F. FOOD CONSUMPTION AND FOOD EFFICIENCY

Compound-related effects on food consumption and food efficiency will be addressed in the subchronic report (HLR 442-93).

G. CYTOGENETIC EVALUATION

1. Rats

a. PCE Frequency

The arcsin square root transformations for PCE frequency were found to be normally distributed. Therefore, these data were analyzed using ANOVA. The VMS program "Dunnettstats" was used for all ANOVA calculations and for making individual comparisons to the control.

b. MN PCE Frequency

Results of the Shapiro-Wilkes test indicated that the transformed variables for MN PCE frequency were not normally distributed. Therefore, the Kruskal-Wallis test was employed for MN PCE frequency data using the PC statistical package "STATXACT".

2. Mice

The arcsin square root transformations for PCE and MN PCE frequencies were found to be normally distributed. Therefore, these data were analyzed using ANOVA. The VMS program "Dunnettstats" was used for all ANOVA calculations and for making individual comparisons to the control.

There were no statistically significant increases in MN PCEs among VCH-treated rats and mice at any exposure level (Tables 6 and 7). No significant depression in the proportion of PCEs among 1000 erythrocytes was observed in any VCH-treated group (Tables 8 and 9). 1,3-BD-treated mice and CP-treated rats showed significantly more MN PCEs than the concurrent negative controls. Individual animal data for rats and mice are given in Appendices F and G.

CONCLUSIONS

Under the conditions of this assay, VCH did not induce micronuclei in bone marrow cells of rats and mice; the compound is negative.

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ABBREVIATIONS

The following abbreviations are used in the data summary tables and the appendices:

VCH = 4-vinylcyclohexene
1,3-BD = 1,3-Butadiene
CP = cyclophosphamide
Eryth = erythrocytes
MN PCE = micronucleated polychromatic erythrocyte
MN NCE = micronucleated normochromatic erythrocyte
N = number of observations
NCE = normochromatic erythrocyte
PCE = polychromatic erythrocyte

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 1

SUMMARY OF CHAMBER CONCENTRATION, TEMPERATURE, RELATIVE HUMIDITY, AND AIRFLOW

TARGET CONCENTRATION (ppm):	0 VCH	50 VCH	250 VCH	1000 VCH	1500 VCH	1000 1,3-BD
PARAMETER MEASURED [a]						
Chamber Concentration (ppm):	0 (0)	53 (9.7)	250 (27)	1000 (80)	1500 (79)	980 (140)
Chamber Temperature (°C):	27 (0.91)	23 (0.88)	26 (1.3)	26 (1.2)	26 (1.2)	25 (0.75)
Chamber Relative Humidity (%):	43 (3.5)	57 (4.0)	57 (7.0)	44 (4.8)	55 (12)	63 (8.3)
Chamber Airflow (L/min):	60 (0)	33 (1.3)	64 (3.4)	66 (2.1)	65 (6.8)	65 (4.2)

[a] Each value presented is the mean of the daily means calculated over the 90-day test period, and the standard deviation is reported in parentheses.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 2

MEAN BODY WEIGHT GAINS OF MALE RATS

	MEAN BODY WEIGHT GAINS (g) [a]				
CONCENTRATION (PPM):	0 VCH	250 VCH	1000 VCH	1500 VCH	40 mg/kg CP
DAYS ON TEST					
3-10	55.5(1.0)	52.4(2.9)	47.1(3.1)	40.4(3.9)	59.4(7.2)
10-17	57.6(1.4)	58.3(3.8)	51.0(6.4)	53.7(2.6)	41.0(5.4)
17-24	53.4(2.5)	50.6(2.1)	52.1(4.7)	53.6(8.3)	77.8(9.2)
24-31	41.4(2.4)	50.8(3.0)	37.3(4.1)	29.6(6.8)	52.8(5.8)
31-38	36.8(4.8)	33.0(5.2)	34.3(2.5)	35.2(2.4)	50.6(7.4)
38-45	35.0(5.1)	29.5(3.2)	31.3(3.1)	26.4(2.3)	37.4(4.7)
45-52	25.8(2.7)	28.3(3.2)	12.6(13)	23.2(4.2)	37.2(3.1)
52-59	20.6(1.9)	28.3(2.1)	27.0(8.5)	18.6(1.9)	32.4(4.6)
59-66	15.3(2.1)	11.8(2.6)	16.2(2.4)	10.8(1.9)	22.1(3.1)
66-73	16.2(2.9)	15.3(2.3)	19.5(2.6)	19.1(3.6)	29.5(2.3)
73-80	18.7(4.6)	19.9(2.3)	2.0(3.6)	0.9(9.4)	20.2(2.0)
80-87	15.7(3.9)	8.4(1.8)	14.9(4.5)	18.5(11)	4.0(12)
87-94	-30.4(3.0)	-30.5(1.7)	-27.3(1.4)	-22.6(4.2)	-18.5(9.9)
3-94	361.5(8.4)	356.2(7.0)	317.8(18)*	307.4(5.7)*	445.8(20)*

Statistical methods: One-way ANOVA and Dunnett's tests were performed on summary data for the interval of Day 3 - 94.

* Statistically significant difference from control at alpha = 0.05.

[a] Standard error is reported in parentheses.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 3

MEAN BODY WEIGHT GAINS OF FEMALE RATS

	MEAN BODY WEIGHT GAINS (g) *				
CONCENTRATION (PPM):	0 VCH	250 VCH	1000 VCH	1500 VCH	40 mg/kg CP
DAYS ON TEST					
4-11	26.6(0.6)	28.8(3.6)	21.2(2.8)	22.0(1.2)	31.4(3.1)
11-18	29.5(2.9)	33.7(6.3)	22.9(2.1)	21.9(2.4)	18.8(3.1)
18-25	28.8(3.2)	17.9(4.2)	24.2(4.2)	17.0(3.1)	25.4(3.1)
25-32	16.0(1.2)	16.0(4.9)	18.8(2.7)	12.0(4.9)	17.8(4.0)
32-39	20.8(4.1)	16.4(2.0)	19.9(1.1)	12.7(2.0)	20.5(1.0)
39-46	6.3(6.2)	8.8(1.9)	6.7(2.2)	10.3(3.0)	15.9(3.5)
46-53	13.4(7.7)	9.3(3.0)	6.4(2.5)	5.7(2.6)	3.2(2.4)
53-60	5.9(4.3)	6.3(4.6)	12.9(2.0)	7.9(3.2)	15.3(3.6)
60-67	5.6(5.2)	11.6(3.2)	1.2(1.5)	7.5(3.3)	3.2(5.6)
67-74	15.9(3.5)	4.1(1.2)	6.5(2.2)	8.9(2.6)	13.9(2.1)
74-81	3.6(5.2)	1.3(2.2)	12.6(2.0)	5.8(2.8)	15.7(3.1)
81-88	7.4(4.5)	13.9(3.5)	-0.1(3.4)	5.7(2.8)	3.8(1.6)
88-95	-11.8(2.2)	-18.5(1.2)	-17.3(2.3)	-11.4(3.8)	-7.1(2.0)
4-95	168.1(14)	149.7(8.2)	136.0(15)	125.9(8.4)	177.7(8.6)

Statistical methods: One-way ANOVA and Dunnett's tests were performed on summary data for the interval of Day 4 - 95.

There were no statistically significant differences at alpha = 0.05.

* Standard error is reported in parentheses.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 4

MEAN BODY WEIGHT GAINS OF MALE MICE

	MEAN BODY WEIGHT GAINS (g) *			
CONCENTRATION (PPM):	0 VCH	50 VCH	250 VCH	1000 1,3-BD
DAYS ON TEST				
1- 8	0.7(0.4)	0.7(0.2)	0.1(0.9)	0.9(0.4)
8-15	1.5(0.7)	-0.3(0.8)	2.0(0.7)	0.9(0.6)
15-22	0.3(0.5)	-3.6(1.7)	-4.1(0.3)	1.3(0.4)
22-29	2.2(0.5)	6.7(1.9)	6.3(0.6)	1.5(0.6)
29-36	0.8(0.4)	0.0(1.0)	0.2(1.6)	0.7(1.2)
36-43	0.6(0.7)	1.4(0.4)	2.2(1.7)	-0.3(0.5)
43-50	0.1(0.5)	1.0(0.5)	0.2(0.2)	0.4(0.7)
50-57	0.7(1.1)	1.2(0.5)	1.5(0.6)	0.8(0.6)
57-64	-0.1(1.1)	0.6(0.8)	-0.4(0.4)	1.0(0.7)
64-71	0.5(0.4)	0.5(0.7)	0.1(0.5)	0.4(0.3)
71-78	0.4(0.3)	1.3(0.3)	0.7(0.1)	-0.3(0.5)
78-85	0.3(0.3)	-0.6(0.8)	1.1(0.4)	2.8(1.3)
85-92	-5.6(0.3)	-6.7(0.6)	-7.8(0.8)	-6.2(0.0)
1-92	2.2(0.7)	2.2(0.6)	2.1(0.7)	3.9(0.8)

Statistical methods: One-way ANOVA and Dunnett's tests were performed on summary data for the interval of Day 1 - 92.

There were no statistically significant differences at alpha = 0.05.

* Standard error is reported in parentheses.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 5

MEAN BODY WEIGHT GAINS OF FEMALE MICE

	MEAN BODY WEIGHT GAINS (g) [a]				
CONCENTRATION (PPM): DAYS ON TEST	0 VCH	50 VCH	250 VCH	1000 VCH	1000 1,3-BD
2- 9	0.1(0.4)	0.4(0.1)	0.5(0.4)	0.4(0.7)	1.0(0.5)
9-16	2.2(0.8)	-0.3(1.4)	-0.8(1.1)	0.8(1.4)	0.4(1.1)
16-23	1.1(0.3)	2.1(1.9)	3.0(1.2)	1.8(0.2)	2.7(1.3)
23-30	-0.4(0.8)	0.9(1.0)	-0.3(0.4)	0.3(0.8)	0.2(0.3)
30-37	2.7(0.3)	1.4(0.5)	1.0(0.4)	0.6(0.6)	0.8(0.9)
37-44	-0.4(0.5)	0.0(0.6)	1.1(0.4)	1.1(0.2)	-0.2(0.3)
44-51	0.5(0.8)	0.3(0.4)	-0.7(0.6)	-0.7(0.6)	0.6(1.0)
51-58	0.0(0.3)	0.0(0.7)	0.6(0.8)	1.1(0.3)	0.4(0.5)
58-65	1.3(0.6)	0.7(0.6)	0.5(0.5)	1.5(0.2)	0.1(0.6)
65-72	-0.4(0.5)	1.2(1.0)	0.9(0.5)	0.0(0.0)	0.8(0.7)
72-79	0.8(0.7)	0.1(0.8)	-0.4(0.6)	-0.4(0.1)	0.3(0.4)
79-86	0.4(0.4)	0.2(0.6)	-0.2(0.4)	-0.5(0.3)	-0.1(0.9)
86-93	-5.0(0.6)	-5.4(0.5)	-4.8(0.3)	-5.3(2.8)	-4.3(0.9)
2-93	2.8(0.3)	1.7(0.5)	0.5(0.7)*	0.8(1.2)	2.8(0.4)

Statistical methods: One-way ANOVA and Dunnett's tests were performed on summary data for the interval of Day 2 - 93.

* Statistically significant difference from control at alpha = 0.05.

[a] Standard error is reported in parentheses.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 6

DATA SUMMARY - MN PCE FREQUENCY

RATS

CONCENTRATION (PPM)	Sex	N	% MN PCE Mean [a]	% MN PCE Median [b]
0 VCH	M	5	0.08 (0.05)	0.00 (0.20)
0 VCH	F	5	0.20 (0.10)	0.10 (0.45)
250 VCH	M	5	0.08 (0.04)	0.10 (0.15)
250 VCH	F	5	0.16 (0.06)	0.10 (0.15)
1000 VCH	M	5	0.16 (0.09)	0.10 (0.35)
1000 VCH	F	5	0.12 (0.06)	0.10 (0.25)
1500 VCH	M	5	0.06 (0.04)	0.00 (0.15)
1500 VCH	F	5	0.10 (0.08)	0.00 (0.25)
40 mg/kg CP	M	5	0.96 (0.20)	0.90 (0.85)*
40 mg/kg CP	F	5	0.78 (0.12)	0.80 (0.55)*

Statistical methods: The Kruskal-Wallis test for equal medians was used for MN PCE frequency data.

* Statistically significant difference from control at alpha = 0.05.

[a] Standard error is reported in parentheses.

[b] Intraquantrile range is reported in parentheses.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 7

DATA SUMMARY - MN PCE FREQUENCY

MICE

CONCENTRATION (PPM)	Sex	N	Mean % MN PCE [a]
0 VCH	M	5	0.14 (0.00, 0.50)
0 VCH	F	5	0.10 (0.00, 0.35)
50 VCH	M	4 [b]	0.24 (0.12, 0.41)
50 VCH	F	5	0.14 (0.01, 0.43)
250 VCH	M	5	0.33 (0.12, 0.66)
250 VCH	F	5	0.23 (0.11, 0.39)
1000 VCH	F	2 [c]	0.19 (0.00, 3.58)
1000 1,3-BD	M	4 [b]	1.56 (0.24, 3.98)*
1000 1,3-BD	F	5	1.67 (1.29, 2.10)*

Statistical methods: One-way ANOVA and Donnett's tests were performed on MN PCE frequency data.

* Statistically significant difference from control at alpha = 0.05.

[a] The 95% confidence limits are reported in parentheses.

[b] Animal(s) removed from micronucleus study due to technical error.

[c] Only 2 animals survived until sacrifice; excluded from statistical analysis.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 8

DATA SUMMARY - PCE FREQUENCY

RATS

CONCENTRATION (PPM)	Sex	N	Mean % PCE [a]	Mean PCE:NCE Ratio [b]
0 VCH	M	5	43.5 (29.7, 57.9)	0.85 (0.20)
0 VCH	F	5	41.3 (24.3, 59.4)	0.80 (0.20)
250 VCH	M	5	49.1 (42.7, 55.6)	0.98 (0.10)
250 VCH	F	5	42.7 (35.3, 50.4)	0.76 (0.09)
1000 VCH	M	5	47.2 (35.4, 59.3)	0.96 (0.18)
1000 VCH	F	5	49.7 (38.6, 60.8)	1.04 (0.17)
1500 VCH	M	5	51.6 (45.7, 57.5)	1.08 (0.09)
1500 VCH	F	5	48.1 (41.2, 55.0)	0.94 (0.09)
40 mg/kg CP	M	5	25.2 (8.90, 46.3)	0.41 (0.13)
40 mg/kg CP	F	5	25.6 (18.1, 33.9)	0.36 (0.05)

Statistical methods: One-way ANOVA and Dunnett's tests were performed on PCE frequency data.

There were no statistically significant differences from control at alpha = 0.05.

[a] The 95% confidence limits are reported in parentheses.

[b] Standard error is reported in parentheses.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

TABLE 9

DATA SUMMARY - PCE FREQUENCY

MICE

CONCENTRATION (PPM)	Sex	N	Mean % PCE [a]	Mean PCE:NCE Ratio [b]
0 VCH	M	5	55.1 (49.8, 60.3)	1.24 (0.09)
0 VCH	F	5	59.8 (48.5, 70.6)	1.60 (0.31)
50 VCH	M	4 [c]	57.5 (47.8, 66.9)	1.39 (0.19)
50 VCH	F	5	59.4 (50.4, 68.1)	1.52 (0.21)
250 VCH	M	5	54.9 (49.6, 60.1)	1.23 (0.10)
250 VCH	F	5	60.2 (53.2, 67.1)	1.55 (0.16)
1000 VCH	F	2 [d]	63.2 (47.5, 77.5)	1.72 (0.09)
1000 1,3-BD	M	4 [c]	61.1 (48.1, 73.4)	1.64 (0.25)
1000 1,3-BD	F	5	72.3 (64.8, 79.2)	2.71 (0.32)

Statistical methods: One-way ANOVA and Dunnett's test were performed on MN PCE frequency data.

There were no statistically significant differences from control at alpha = 0.05.

[a] The 95% confidence limits are reported in parentheses.

[b] Standard error is reported in parentheses.

[c] Animal(s) removed from micronucleus study due to technical error

[d] Only 2 animals survived until sacrifice; excluded from statistical analysis

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

APPENDIX A

ANALYTICAL REPORT

SUBCHRONIC INHALATION TOXICITY: 90 DAY STUDY WITH 4-VINYLCYCLOHEXENE IN RATS AND MICE

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

ANALYSIS OF 4-VINYLCYCLOHEXENE AND 1,3-BUTADIENE

Medical Research Project Number:	9523-001
Haskell Sample Numbers:	19353 (VCH) and 19999 (1,3-BD)
Analytical Test Code:	999
Analytical Report Number:	HA-93-070

SUMMARY

Compounds 4-vinylcyclohexene (VCH, H-19353) and 1,3-butadiene (1,3-BD, H-19999) were analyzed by a method using gas chromatography with flame ionization detection.

Analysis of the VCH sample submitted on January 13, 1993 indicated that the major component comprised an average of 99.59% of the total sample with a range of 99.59 to 99.60% for an average of six replicate analyses. Analysis of the compound submitted on May 10, 1993 found the composition to be essentially unchanged at an average of 99.67% with a range of 99.44 to 99.75%.

Analysis of the 1,3-BD sample submitted on January 13, 1993 indicated that the major component comprised greater than 99.9% for an average of six replicate analyses. Analysis of the compound submitted on May 10, 1993 found the composition to be essentially unchanged at 99.9%.

HA-93-070p2

METHODS

Compounds VCH and 1,3-BD were analyzed using the following chromatographic method.

CHROMATOGRAPHIC CONDITIONS

Instrument	Hewlett-Packard Model 5890 Gas Chromatograph (NX2406)
Column	15m x 0.53 i.d. Nukol (SUPELCO Inc.) 0.5µm film thickness Helium carrier, 1 psi (6.5 mL/min)
Detector	Flame Ionization, 250°C Air, 300 mL/min Hydrogen, 32 mL/min Helium Make Up, 20.7 mL/min
Injector	250°C, spilt flow, 30 mL/min. split ratio: 4.6 to 1
Temperature Program . .	Initial Temperature: 40°C Initial Time: 5.00 min Program Rate: 20°C/min Final Temperature (VCH): 250°C Final Temperature (1,3-BD): 100°C Final Time (VCH): 5.00 min Final Time (1,3-BD): 0.00 min
Data Analysis	Hewlett-Packard 3365 GC Chemstation Area percent report option enabled

DETERMINATION OF COMPOSITION

External reference standards were available for this study. Compounds from Aldrich Chemical, VCH (Aldrich V220-8) and 1,3-BD (Aldrich 29,503-5), were used as retention time standards. Identification of the major component of the test materials, VCH (H-19,353) and 1,3-BD (H-19,999), was done by comparing retention times with those of the Aldrich materials.

The area of all eluted peaks was summed and the area percent of each peak was calculated using the area percent option in the Chemstation software.

The polymerization inhibitor, 4-tertbutylcatechol, present in both compounds was not eluted from the column. Therefore, the area percent was not corrected for this component.

HA-93-070p3

SAMPLE PREPARATION

All analyses were done with undiluted (neat) compound. VCH was sampled using a 10 µL syringe with the syringe plunger at zero. A manual injection with the plunger at zero gives an injection volume of 0.1 µL. The injection volume arises from the liquid remaining in the needle.

1,3-BD was sampled by removing 5 µL of gas from a bag containing 1 atmosphere of the compound. This compound was also analyzed using manual injections.

RESULTS

The results of the analyses are shown in Table I of this Analytical Report HA-93-070.

HA-93-070 p4

Report and Analyses by: Dennis A. Redder, Chemistry Associate — 8/20/93 Date

Approval by: Charlotte H. Lattin, Research Chemist — 8/20/93 Date

Date issued: August 20, 1993

Analytical Report Number: HA-93-070

Notebook References: E68447: 36, 38, 40, 48 - 56, 86 - 92

Number of Pages in Report: 5

HA-93-070p5

TABLE I

Area Percent of VCH

Submitted on: 13-Jan-93	Submitted on: 10-May-93
99.59	99.44
99.59	99.69
99.59	99.75
99.60	99.73
99.60	99.74
99.59	99.64
99.59 average	99.67 average

Area percent of 1,3-BD

Submitted on: 13-Jan-93	Submitted on: 10-May-93
99.99	99.95
99.99	99.99
99.99	99.95
99.99	99.97
99.99	99.97
99.99	99.97
>99.9 average	>99.9 average

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

APPENDIX B

MEAN DAILY CHAMBER CONCENTRATION

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER CONCENTRATION

EXPLANATORY NOTES

FOOTNOTES:

[a] Daily mean and standard deviation of 23 to 28 measurements, except as noted when there were fewer samples due to equipment failure:

Exposure 16: All 4-Vinylcyclohexene chambers 20 to 22 measurements.
Exposure 35: 250, 1000, and 1500 ppm chambers 21 to 22 measurements.
Exposure 41: All 4-Vinylcyclohexene chambers 20 to 22 measurements.
Exposure 60: All 4-Vinylcyclohexene chambers 15 to 16 measurements.

[b] Daily mean and standard deviation of 13 to 18 measurements, except as noted when there were more or fewer samples due to equipment failure:

Exposure 15: 8 measurements.
Exposure 24: 23 measurements.
Exposure 50: 7 measurements.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Make-up exposure for male mice exposed to 1,3-Butadiene. The number of days of exposure was subsequently the same for all male mice.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
1	1	61.0	(17.1)[c]	231.4	(51.5)	982.9	(81)	[d]		[d]	
2	2	42.6	(8.4)	283.4	(56)	1090.0	(54)	[d]		1142.6	(127.4)
3	3	33.4	(18.7)	232.3	(45.8)	918.4	(210.1)	1528.5	(317.7)	1235.0	(377.3)
4	4	45.1	(26.6)	248.3	(14.5)	923.5	(110.9)	1485.1	(258.7)	994.8	(486.7)
WEEK 1 MEAN•		45.5	(11.5)	248.9	(24.3)	978.7	(79.8)	1506.8	(30.7)	1124.1	(121.1)
7	5	61.3	(6.9)	205.8	(98.5)	976.0	(27.5)	1548.0	(43.9)	924.3	(116.7)
8	6	64.6	(11.1)	258.2	(24.9)	1042.4	(79.5)	1523.5	(126.3)	1033.6	(97.3)
9	7	54.7	(7.6)	227.3	(9.6)	920.5	(75.2)	1411.5	(206.3)	1193.1	(189)
10	8	59.4	(7.8)	238.7	(63.5)	1104.7	(40.7)	1592.3	(71)	1026.2	(37.2)
11	9	68.8	(9.3)	279.7	(16.8)	984.0	(46.5)	1459.4	(25.8)	1000.6	(552.4)
WEEK 2 MEAN		61.8	(5.3)	241.9	(28.4)	1005.5	(70.3)	1506.9	(71.8)	1035.6	(98.1)
14	10	40.6	(35.4)	237.9	(50.9)	960.2	(36.4)	1391.5	(443.4)	1233.9	(126.7)
15	11	63.5	(6.2)	257.0	(10)	993.7	(79.7)	1613.8	(110.5)	1064.7	(116.8)
16	12	59.8	(7.5)	261.0	(14.7)	953.3	(61.1)	1506.0	(231.9)	954.3	(124.9)
17	13	58.2	(6.2)	255.6	(9.8)	941.3	(88.9)	1635.2	(73.9)	1017.0	(59.4)
18	14	59.1	(5.8)	267.4	(22.2)	957.1	(48.4)	1423.1	(109.1)	942.6	(125.2)
WEEK 3 MEAN		56.2	(9)	255.8	(11)	961.1	(19.6)	1513.9	(109.5)	1042.5	(117.8)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
21	15	46.0	(16.2)	186.1	(59.2)	795.8	(66.3)	1387.5	(191.1)	853.5	(210.6)
22	16	40.3	(10.4)	234.6	(76.8)	922.8	(126.5)	1444.7	(138.6)	932.7	(182.7)
23	17	67.3	(9.6)	293.4	(7.5)	983.1	(46.7)	1337.8	(412.3)	849.6	(54)
24	18	66.9	(27)	198.5	(67.7)	998.5	(67.3)	1563.8	(205.8)	1142.7	(1044.1)
25	19	65.8	(25.4)	210.7	(60.5)	1040.7	(72.2)	1486.2	(80.9)	633.2	(146.5)
WEEK 4 MEAN		57.3	(13)	224.7	(42.4)	948.2	(95.1)	1444.0	(87.5)	882.3	(183.2)
28	20	61.7	(10.3)	263.1	(7.5)	1060.2	(23.8)	1541.0	(46.9)	1111.1	(178)
29	21	50.4	(11.1)	253.6	(32.4)	1033.4	(37.1)	1526.8	(41.8)	1052.9	(82)
30	22	39.8	(6.9)	247.9	(8)	905.4	(37.4)	1421.3	(304.5)	982.8	(445.8)
31	23	65.8	(24.4)	264.9	(25.9)	1026.4	(88)	1541.9	(83.6)	868.6	(338.5)
32	24	60.8	(10.9)	264.8	(38)	843.3	(316)	1555.1	(294.7)	695.0	(537.6)
WEEK 5 MEAN		55.7	(10.5)	258.9	(7.7)	973.7	(94.2)	1517.2	(54.5)	942.1	(165)
35	25	62.0	(7.3)	252.0	(22.1)	959.0	(82.1)	1630.6	(146.4)	748.3	(842)
36	26	64.7	(4.5)	303.6	(7.8)	1139.6	(13.9)	1582.5	(66.5)	878.6	(149.6)
37	27	50.2	(13.8)	234.4	(73.4)	1016.7	(20.5)	1523.3	(107.1)	1026.6	(294.4)
38	28	62.5	(8.2)	286.6	(17.9)	1052.5	(89.9)	1363.5	(555.7)	951.4	(145)
39	29	58.0	(19.7)	206.5	(52.9)	919.7	(53.7)	1346.1	(381.7)	752.3	(480.3)
WEEK 6 MEAN		59.5	(5.7)	256.6	(39.2)	1017.5	(85.3)	1489.2	(128.6)	871.4	(122.3)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
42	30	37.0	(2.7)	229.4	(15.9)	1004.7	(62.5)	1528.3	(85.6)	1159.5	(94.7)
43	31	33.7	(6)	233.7	(8.1)	1003.3	(18.4)	1519.8	(48.6)	998.3	(196)
44	32	48.1	(7.3)	263.0	(18.6)	998.8	(127)	1452.1	(140.2)	839.3	(327.6)
45	33	47.3	(4.5)	248.6	(4.1)	1029.7	(29.3)	1524.8	(47.3)	1083.3	(278.7)
46	34	60.5	(8.2)	282.6	(10.7)	1110.2	(36)	1486.7	(98.6)	966.2	(114.6)
WEEK 7 MEAN		45.3	(10.6)	251.5	(21.9)	1029.3	(46.8)	1502.3	(32.6)	1009.3	(121.4)
49	35	51.8	(11.1)	237.9	(17.8)	1000.8	(72.3)	1494.1	(89.5)	1144.6	(1126.0)
50	36	51.7	(21.5)	247.9	(55.3)	1083.7	(61.3)	1518.8	(43.3)	918.1	(265.7)
51	37	53.3	(4.2)	216.7	(9.7)	951.1	(44.9)	1476.0	(89.2)	966.8	(185.6)
52	38	52.1	(7.2)	253.1	(12.6)	1029.7	(82.6)	1448.0	(241)	891.6	(331.9)
53	39	49.1	(25.7)	114.4	(106.3)	683.6	(106.4)	1368.8	(242.4)	1080.3	(307.2)
54	[f]									988.3	(107)
WEEK 8 MEAN		51.6	(1.5)	214.0	(57.4)	949.8	(156.3)	1461.1	(57.7)	998.3	(97.0)
56	40	46.3	(10.6)	255.3	(8.8)	1101.3	(58.3)	1516.0	(86.9)	948.3	(316.9)
57	41	43.3	(5.7)	218.2	(7.6)	892.5	(104.2)	1425.8	(223.6)	943.3	(207.3)
58	42	48.6	(8.7)	260.9	(5.1)	1023.1	(30.5)	1588.4	(69.4)	1037.5	(57.3)
59	43	67.5	(10)	268.3	(3.6)	992.5	(43)	1521.2	(80)	1025.7	(192.8)
60	44	61.4	(6)	278.5	(7.5)	747.2	(35.8)	1400.5	(66.3)	1292.8	(110.3)
WEEK 9 MEAN		53.4	(10.5)	256.2	(23)	951.3	(136.4)	1490.4	(76.6)	1049.5	(142.7)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]		250[a]		1000[a]		1500[a]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #										
64	45	62.5	(19.3)	271.4	(17.4)	1098.8	(32.5)	1576.9	(343.1)	1117.0	(66.5)
65	46	57.3	(9.7)	264.4	(68.6)	939.3	(273)	1465.6	(174.2)	781.0	(478.6)
66	47	37.6	(9.4)	263.3	(5.6)	100[illegible].0	(30.5)	1480.1	(82.4)	987.0	(353.3)
67	48	59.9	(6.6)	250.3	(6.3)	998.1	(34.7)	1498.7	(82.1)	1148.9	(296.8)
68	49	61.6	(9.4)	256.3	(7.2)	987.0	(34.1)	1518.7	(59.1)	1182.5	(171)
WEEK 10 MEAN		55.8	(10.4)	261.1	(8.1)	1[illegible]05.6	(58.1)	1508.0	(43.4)	1043.3	(164.3)
70	50	63.6	(21.7)	241.1	(76.6)	1061.9	(51.8)	1554.3	(32.1)	1048.1	(37.6)
71	51	44.7	(5.1)	237.2	(8)	972.3	(46.7)	1512.9	(70.8)	962.7	(145.4)
72	52	49.0	(10)	243.9	(4)	975.9	(84.7)	1514.0	(54.9)	1112.6	(245.5)
73	53	58.5	(4.9)	244.4	(7.5)	963.1	(63.4)	1541.4	(55.1)	1027.3	(189.5)
WEEK 11 MEAN		54.0	(8.6)	241.7	(3.3)	993.3	(46)	1530.7	(20.6)	1037.7	(61.8)
77	54	47.0	(10)	249.9	(11.5)	995.6	(25)	1432.6	(47.3)	869.0	(81.5)
78	55	49.7	(9.2)	234.8	(6.5)	991.1	(63.3)	1478.2	(140.3)	836.8	(92.8)
79	56	43.3	(6.6)	243.6	(5.5)	1009.8	(30.4)	1480.6	(50.9)	859.4	(247.2)
80	57	47.3	(6)	256.3	(4.6)	1078.3	(48.9)	1224.2	(594.9)	934.2	(208.5)
81	58	46.9	(6.3)	255.7	(12.3)	1064.4	(20.1)	1566.0	(18.8)	819.1	(93.3)
82	59	46.7	(11)	224.6	(59.5)	1037.9	(27.3)	1507.8	(47.1)	731.5	(348.5)
WEEK 12 MEAN		46.8	(2)	244.2	(12.5)	1029.5	(36.6)	1448.2	(118.2)	841.7	(66.8)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER CONCENTRATION (ppm)

CONCENTRATION (ppm):		50[a]	250[a]	1000[a]	1500[a]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #					
84	60	52.4 (6.4)	262.6 (21.2)	1092.9 (63.3)	1552.1 (45.8)	1081.9 (129.2)
85	61	39.0 (6.9)	260.6 (3.4)	1070.8 (54.2)	1514.5 (66.1)	926.1 (101.2)
86	62	46.4 (14.8)	258.7 (19.5)	1041.4 (34.1)	1403.1 (404)	886.9 (252.5)
87	63	39.8 (6.1)	226.6 (6.6)	999.2 (70.9)	1382.7 (338.2)	868.3 (293.8)
88	64	55.9 (10.2)	260.0 (4.8)	1055.9 (15.8)	1571.8 (35.9)	1022.1 (119.3)
WEEK 13 MEAN		46.7 (7.5)	253.7 (15.2)	1052.0 (35.1)	1484.8 (86.7)	957.1 (91.6)
91	65	48.2 (9.1)	262.1 (11.1)	1048.4 (46.5)	1620.8 (28.4)	969.5 (77.8)
92	66	31.5 (10.2)	239.2 (6.5)	1010.1 (40.4)	1620.9 (27.7)	1135.6 (210)
93	67	[d]	247.9 (48)	1026.3 (40.9)	1532.8 (40.3)	1022.0 (108.7)
94	68	[d]	263.5 (110.1)	1050.0 (28.2)	1500.6 (47.2)	960.5 (190.3)
WEEK 14 MEAN		39.9 (11.8)	253.2 (11.7)	1033.7 (19.1)	1568.8 (61.6)	1021.9 (80.5)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

APPENDIX C

MEAN DAILY CHAMBER TEMPERATURE

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER TEMPERATURE

EXPLANATORY NOTES

FOOTNOTES:

[a] Daily mean and standard deviation of approximately 12 measurements.

[b] Daily mean and standard deviation of 60 to 77 measurements, except where noted.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Make-up exposure for male mice exposed to 1,3-Butadiene. Daily mean and standard deviation of 12 measurements. The number of days of exposure was subsequently the same for all male mice.

[g] One hour of data not collected due to technical error. Mean and standard deviation of 54 to 60 measurements.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
1	1	23.7 (0.6)[c]	22.0 (1)	22.0 (1)	22.0 (0)	[d]	[d]
2	2	23.3 (0.3)	23.0 (1)	22.0 (1)	23.0 (1)	[d]	23.0 (1)
3	3	23.9 (0.9)	23.0 (1)	24.0 (1)	24.0 (1)	24.0 (1)	24.0 (1)
4	4	25.1 (1)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
WEEK 1 MEAN[e]		24.0 (0.8)	22.8 (0.5)	23.5 (1.9)	23.5 (1.3)	24.5 (1)	24.0 (1)
7	5	26.6 (0.6)	23.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)
8	6	26.4 (0.6)	22.0 (1)	24.0 (1)	24.0 (1)	23.0 (1)	25.0 (1)
9	7	26.7 (1.1)	22.0 (1)	25.0 (1)	25.0 (1)	24.0 (1)	25.0 (1)
10	8	26.7 (0.9)	23.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)
11	9	26.4 (0.9)	23.0 (1)	25.0 (2)	25.0 (1)	25.0 (1)	25.0 (1)
WEEK 2 MEAN		26.6 (0.2)	22.6 (0.5)	25.2 (0.8)	25.2 (0.8)	24.4 (0.9)	25.0 (0)
14	10	26.3 (0.5)	23.0 (1)	26.0 (1)	25.0 (0)	25.0 (0)	24.0 (1)
15	11	26.4 (1.2)	23.0 (1)	25.0 (1)	25.0 (0)	25.0 (1)	25.0 (1)
16	12	26.7 (0.2)	22.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
17	13	26.9 (0.2)	23.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)
18	14	27.0 (0.4)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
WEEK 3 MEAN		26.7 (0.3)	22.8 (0.4)	25.8 (0.4)	25.2 (0.4)	25.0 (0)	24.8 (0.4)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
21	15	26.4 (0.5)	22.0 (1)[g]	25.0 (1)[g]	24.0 (1)[g]	24.0 (1)[g]	24.0 (1)[g]
22	16	26.2 (0.7)	23.0 (1)	26.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)
23	17	26.3 (0.8)	23.0 (1)	26.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)
24	18	26.9 (1)	22.0 (1)	26.0 (1)	25.0 (1)	25.0 (0)	25.0 (1)
25	19	26.0 (0.8)	21.0 (1)	24.0 (1)	24.0 (1)	24.0 (1)	25.0 (1)
WEEK 4 MEAN		26.4 (0.3)	22.2 (0.8)	25.4 (0.9)	25.0 (1)	25.0 (1)	24.8 (0.4)
28	20	27.2 (0.4)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
29	21	26.5 (1)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
30	22	26.9 (0.9)	22.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	25.0 (1)
31	23	27.5 (0.8)	22.0 (1)	25.0 (1)	25.0 (0)	25.0 (1)	26.0 (1)
32	24	26.5 (0.3)	22.0 (1)	26.0 (1)	25.0 (0)	25.0 (1)	25.0 (1)
WEEK 5 MEAN		26.9 (0.4)	22.4 (0.5)	25.8 (0.4)	25.0 (0)	25.0 (0)	25.2 (0.4)
35	25	27.0 (0.9)	22.0 (1)	26.0 (1)	25.0 (0)	25.0 (0)	25.0 (1)
36	26	26.7 (0.6)	23.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)	26.0 (1)
37	27	27.1 (0.6)	24.0 (1)	27.0 (1)	26.0 (1)	27.0 (0)	26.0 (1)
38	28	27.1 (0.3)	23.0 (1)	27.0 (1)	26.0 (0)	26.0 (0)	25.0 (1)
39	29	27.0 (0.5)	23.0 (1)	26.0 (1)	26.0 (0)	26.0 (0)	26.0 (1)
WEEK 6 MEAN		27.0 (0.2)	23.0 (0.7)	26.4 (0.5)	25.6 (0.5)	25.8 (0.8)	25.6 (0.5)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
42	30	27.6 (0.9)	24.0 (1)	27.0 (1)	26.0 (0)	26.0 (0)	26.0 (1)
43	31	27.2 (0.7)	24.0 (1)	27.0 (1)	26.0 (1)	26.0 (0)	26.0 (1)
44	32	26.8 (0.4)	23.0 (1)	27.0 (1)	26.0 (0)	26.0 (0)	25.0 (1)
45	33	26.8 (1.2)	23.0 (1)	27.0 (1)	26.0 (0)	26.0 (0)	26.0 (1)
46	34	27.1 (0.8)	23.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)	25.0 (1)
WEEK 7 MEAN		27.1 (0.3)	23.4 (0.5)	26.8 (0.4)	26.0 (0)	25.8 (0.4)	25.6 (0.5)
49	35	26.4 (0.7)	22.0 (1)	25.0 (1)	24.0 (1)	24.0 (1)	24.0 (1)
50	36	26.5 (0.9)	22.0 (1)	26.0 (1)	25.0 (1)	25.0 (0)	25.0 (1)
51	37	26.6 (0.9)	23.0 (1)	27.0 (1)	26.0 (1)	26.0 (0)	25.0 (1)
52	38	26.1 (0.5)	22.0 (1)	25.0 (1)	25.0 (0)	24.0 (1)	24.0 (1)
53	39	26.3 (0.4)	24.0 (1)	27.0 (1)	26.0 (1)	26.0 (1)	25.0 (1)
54	[c]						23.2 (1)
WEEK 8 MEAN		26.4 (0.2)	22.6 (0.9)	26.0 (1)	25.2 (0.8)	25.0 (1)	24.4 (0.8)
56	40	27.0 (1.2)	24.0 (1)	28.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
57	41	27.4 (0.4)	24.0 (1)	27.0 (1)	27.0 (1)	27.0 (0)	26.0 (1)
58	42	27.3 (0.6)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
59	43	27.1 (0.7)	24.0 (1)	27.0 (1)	27.0 (0)	26.0 (0)	26.0 (1)
60	44	27.1 (0.9)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
WEEK 9 MEAN		27.2 (0.2)	24.0 (0)	27.2 (0.4)	27.0 (0)	26.8 (0.4)	26.0 (0)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
64	45	28.0	(0.7)	24.0	(1)	28.0	(1)	27.0	(1)	27.0	(1)	26.0	(1)
65	46	27.2	(1)	24.0	(1)	27.0	(1)	27.0	(0)	27.0	(0)	26.0	(1)
66	47	27.1	(1.3)	23.0	(1)	27.0	(1)	27.0	(0)	26.0	(0)	26.0	(1)
67	48	26.8	(0.9)	24.0	(1)	27.0	(1)	26.0	(0)	26.0	(0)	25.0	(1)
68	49	26.5	(1)	23.0	(1)	27.0	(1)	25.0	(1)	25.0	(0)	26.0	(1)
WEEK 10 MEAN		27.1	(0.6)	23.6	(0.5)	27.2	(0.4)	26.4	(0.9)	26.2	(0.8)	25.8	(0.4)
70	50	27.4	(0.7)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
71	51	27.7	(0.4)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
72	52	27.1	(0.7)	25.0	(1)[g]	28.0	(1)[g]	28.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
73	53	27.3	(0.7)	25.0	(1)[g]	28.0	(1)[g]	28.0	(0)[g]	29.0	(0)[g]	26.0	(1)[g]
WEEK 11 MEAN		27.4	(0.3)	24.5	(0.6)	28.0	(0)	27.5	(0.6)	27.5	(1)	26.0	(0)
77	54	27.5	(1.1)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
78	55	27.5	(0.5)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
79	56	27.6	(0.7)	24.0	(1)[g]	28.0	(1)[g]	27.0	(0)[g]	27.0	(0)[g]	26.0	(1)[g]
80	57	27.7	(0.7)	24.0	(1)	27.0	(1)	27.0	(1)	27.0	(1)	26.0	(1)
81	58	27.7	(1.2)	25.0	(1)	28.0	(1)	28.0	(1)	27.0	(0)	26.0	(1)
82	59	27.0	(0.7)	24.0	(1)	27.0	(1)	27.0	(1)	27.0	(1)	26.0	(1)
WEEK 12 MEAN		27.5	(0.3)	24.2	(0.4)	27.7	(0.5)	27.2	(0.4)	27.0	(0)	26.0	(0)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER TEMPERATURE (°C)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
84	60	27.6 (0.9)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (1)	26.0 (1)
85	61	28.1 (1.3)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
86	62	27.8 (1.4)	24.0 (1)	28.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
87	63	27.8 (1.2)	24.0 (1)	27.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
88	64	27.0 (1.4)	24.0 (1)	27.0 (1)	27.0 (1)	27.0 (1)	26.0 (1)
WEEK 13 MEAN		27.7 (0.4)	24.0 (0)	27.2 (0.4)	27.0 (0)	27.0 (0)	26.0 (0)
91	65	27.5 (0.5)	24.0 (1)	28.0 (1)	27.0 (0)	27.0 (0)	26.0 (1)
92	66	27.0 (0.3)	23.0 (1)	27.0 (1)	26.0 (0)	27.0 (0)	25.0 (1)
93	67	26.4 (0.3)	[d]	26.0 (1)	26.0 (0)	26.0 (0)	25.0 (1)
94	68	24.5 (0.3)	[d]	24.0 (1)	24.0 (1)	24.0 (1)	24.0 (1)
WEEK 14 MEAN		26.4 (1.3)	23.5 (1)	26.3 (1.7)	25.8 (1.3)	26.0 (1.4)	25.0 (0.8)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

APPENDIX D

MEAN DAILY CHAMBER RELATIVE HUMIDITY

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER RELATIVE HUMIDITY

EXPLANATORY NOTES

FOOTNOTES:

[a] Daily mean and standard deviation of approximately 12 measurements.

[b] Daily mean and standard deviation of 60 to 77 measurements, except where noted.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Readings in error due to dry wet-bulb thermometer.

[g] Make-up exposure for male mice exposed to 1,3-Butadiene. Daily mean and standard deviation of 12 measurements. The number of days of exposure was subsequently the same for all male mice.

[h] One hour of data not collected due to technical error. Mean and standard deviation of 54 to 60 measurements.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
1	1	43.0 (1.8)[c]	50.0 (9)	40.0 (10)	40.0 (9)	[d]	[d]
2	2	43.0 (3)	53.0 (11)	45.0 (10)	39.0 (10)	[d]	50.0 (11)
3	3	41.8 (3.4)	54.0 (12)	52.0 (13)	50.0 (13)	47.0 (12)	56.0 (9)
4	4	39.9 (4)	50.0 (8)	56.0 (9)	49.0 (9)	59.0 (8)	55.0 (9)
WEEK 1 MEAN*		41.9 (1.5)	51.8 (2.1)	48.3 (7.1)	44.5 (5.8)	53.0 (8.5)	53.7 (3.2)
7	5	41.9 (3.3)	54.0 (9)	61.0 (9)	52.0 (12)	66.0 (10)	51.0 (12)
8	6	46.5 (1.5)	56.0 (10)	69.0 (11)	56.0 (12)	75.0 (11)	55.0 (10)
9	7	47.8 (2.8)	55.0 (11)	63.0 (12)	48.0 (11)	65.0 (16)	64.0 (11)
10	8	41.1 (2.4)	53.0 (11)	59.0 (11)	52.0 (14)	64.0 (12)	57.0 (12)
11	9	40.3 (0.9)	52.0 (11)	61.0 (11)	48.0 (12)	62.0 (13)	63.0 (11)
WEEK 2 MEAN		43.5 (3.4)	54.0 (1.6)	62.6 (3.8)	51.2 (3.3)	66.4 (5)	58.0 (5.5)
14	10	40.7 (1.5)	48.0 (7)	53.0 (9)	52.0 (10)	72.0 (10)	65.0 (12)
15	11	40.9 (0.7)	60.0 (13)	57.0 (8)	48.0 (6)	64.0 (18)	54.0 (11)
16	12	40.7 (0.8)	52.0 (8)	55.0 (6)	46.0 (5)	61.0 (12)	55.0 (9)
17	13	41.5 (1.3)	52.0 (10)	55.0 (10)	45.0 (9)	61.0 (14)	61.0 (12)
18	14	44.0 (2.4)	55.0 (10)	55.0 (10)	49.0 (10)	56.0 (16)	62.0 (11)
WEEK 3 MEAN		41.6 (1.4)	53.4 (4.4)	55.0 (1.4)	48.0 (2.7)	62.8 (5.9)	59.4 (4.7)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
21	15	42.2	(0.8)	60.0	(11)[h]	63.0	(17)[h]	61.0	(12)[h]	88.0	(13)[h]	70.0	(9)[h]
22	16	48.9	(0.4)	56.0	(8)	54.0	(8)	45.0	(8)	54.0	(10)	57.0	(8)
23	17	49.3	(0.4)	58.0	(6)	54.0	(6)	42.0	(7)	79.0	(7)	58.0	(9)
24	18	49.1	(0.4)	57.0	(10)	58.0	(7)	48.0	(11)	62.0	(14)	66.0	(16)
25	19	49.1	(0.3)	56.0	(8)	61.0	(6)	49.0	(10)	64.0	(13)	55.0	(10)
WEEK 4 MEAN		47.7	(3.1)	57.4	(1.7)	58.0	(4.1)	49.0	(7.2)	69.4	(13.8)	61.2	(6.5)
28	20	48.7	(0.5)	52.0	(8)	58.0	(8)	48.0	(12)	74.0	(17)	60.0	(8)
29	21	48.5	(0.4)	57.0	(13)	63.0	(11)	47.0	(14)	61.0	(10)	[f]	
30	22	48.6	(0.4)	59.0	(9)	62.0	(7)	45.0	(7)	59.0	(11)	62.0	(14)
31	23	49.0	(0.5)	57.0	(6)	64.0	(5)	45.0	(6)	50.0	(11)	58.0	(8)
32	24	49.1	(0.4)	62.0	(8)	61.0	(6)	43.0	(7)	51.0	(13)	70.0	(10)
WEEK 5 MEAN		48.8	(0.3)	57.4	(3.6)	61.6	(2.3)	45.6	(1.9)	59.0	(9.7)	62.5	(5.3)
35	25	45.9	(5.1)	56.0	(6)	60.0	(6)	45.0	(6)	59.0	(10)	64.0	(10)
36	26	41.3	(1.3)	56.0	(9)	59.0	(7)	42.0	(7)	50.0	(10)	61.0	(10)
37	27	46.3	(2.8)	58.0	(6)	61.0	(7)	44.0	(8)	48.0	(9)	59.0	(9)
38	28	49.1	(0.4)	59.0	(8)	57.0	(6)	42.0	(7)	51.0	(10)	61.0	(10)
39	29	42.8	(2.1)	61.0	(7)	59.0	(7)	43.0	(7)	49.0	(10)	57.0	(9)
WEEK 6 MEAN		45.1	(3.1)	58.0	(2.1)	59.2	(1.5)	43.2	(1.3)	51.4	(4.4)	60.4	(2.6)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
42	30	46.3 (3.5)	55.0 (8)	44.0 (5)	45.0 (7)	64.0 (14)	65.0 (11)
43	31	45.4 (1.1)	59.0 (8)	[f]	41.0 (7)	50.0 (13)	61.0 (8)
44	32	46.9 (1.2)	61.0 (10)	47.0 (15)	41.0 (5)	47.0 (8)	58.0 (9)
45	33	47.6 (0.4)	57.0 (7)	54.0 (6)	40.0 (7)	49.0 (14)	61.0 (8)
46	34	46.9 (1.6)	52.0 (7)	58.0 (5)	41.0 (7)	49.0 (11)	63.0 (9)
WEEK 7 MEAN		46.6 (0.8)	56.8 (3.5)	50.8 (6.4)	41.6 (1.9)	51.8 (6.9)	61.6 (2.6)
49	35	47.3 (1.3)	63.0 (9)	61.0 (9)	52.0 (14)	69.0 (18)	79.0 (13)
50	36	45.2 (1)	62.0 (7)	55.0 (4)	43.0 (5)	49.0 (8)	66.0 (8)
51	37	44.8 (1)	59.0 (10)	98.0 (6)	41.0 (7)	49.0 (11)	65.0 (9)
52	38	45.8 (1.3)	61.0 (6)	59.0 (4)	42.0 (7)	69.0 (9)	61.0 (14)
53	39	44.6 (0.8)	56.0 (8)	53.0 (7)	40.0 (7)	68.0 (11)	65.0 (12)
54	[g]						44.9 (13.7)
WEEK 8 MEAN		45.5 (1.1)	60.2 (2.8)	65.2 (18.6)	43.6 (4.8)	60.8 (10.8)	63.5 (11)
56	40	45.1 (0.6)	52.0 (8)	53.0 (3)	41.0 (7)	81.0 (12)	62.0 (7)
57	41	45.7 (1.2)	58.0 (9)	59.0 (5)	41.0 (8)	68.0 (15)	63.0 (12)
58	42	45.0 (2.2)	60.0 (9)	58.0 (6)	41.0 (8)	69.0 (13)	60.0 (10)
59	43	44.6 (1.3)	58.0 (8)	56.0 (5)	41.0 (7)	67.0 (12)	59.0 (11)
60	44	45.5 (2.1)	57.0 (8)	58.0 (7)	41.0 (8)	59.0 (18)	64.0 (10)
WEEK 9 MEAN		45.2 (0.4)	57.0 (3)	56.6 (2.4)	41.0 (0)	68.8 (7.9)	61.6 (2.1)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
64	45	44.2 (1.2)	61.0 (12)	60.0 (7)	50.0 (12)	53.0 (12)	75.0 (13)
65	46	44.1 (1.2)	56.0 (8)	59.0 (6)	43.0 (8)	42.0 (7)	68.0 (8)
66	47	43.5 (1.6)	66.0 (7)	53.0 (5)	39.0 (6)	47.0 (7)	71.0 (7)
67	48	41.7 (1.1)	56.0 (6)	59.0 (3)	38.0 (3)	44.0 (6)	69.0 (8)
68	49	37.9 (3.1)	59.0 (9)	61.0 (6)	40.0 (6)	46.0 (7)	66.0 (10)
WEEK 10 MEAN		42.3 (2.6)	59.6 (4.2)	58.4 (3.1)	42.0 (4.8)	46.4 (4.2)	69.8 (3.4)
70	50	41.7 (0.5)	56.0 (5)[h]	53.0 (2)[h]	39.0 (4)[h]	45.0 (4)[h]	69.0 (7)[h]
71	51	40.7 (1.4)	56.0 (6)[h]	50.0 (2)[h]	38.0 (2)[h]	40.0 (4)[h]	87.0 (17)[h]
72	52	40.5 (1.4)	51.0 (5)[h]	51.0 (2)[h]	36.0 (1)[h]	40.0 (3)[h]	67.0 (6)[h]
73	53	40.1 (1.5)	54.0 (4)[h]	53.0 (2)[h]	38.0 (2)[h]	41.0 (4)[h]	58.0 (6)[h]
WEEK 11 MEAN		40.8 (0.7)	54.3 (2.4)	51.8 (1.5)	37.8 (1.3)	41.5 (2.4)	70.3 (12.1)
77	54	40.6 (1.7)	58.0 (5)[h]	51.0 (3)[h]	42.0 (8)[h]	47.0 (8)[h]	73.0 (9)[h]
78	55	41.2 (2)	59.0 (6)[h]	55.0 (2)[h]	37.0 (2)[h]	45.0 (7)[h]	55.0 (6)[h]
79	56	36.5 (1)	58.0 (6)[h]	59.0 (3)[h]	37.0 (2)[h]	41.0 (3)[h]	58.0 (6)[h]
80	57	39.5 (1.8)	61.0 (7)	56.0 (7)	42.0 (9)	45.0 (8)	65.0 (9)
81	58	40.6 (1.7)	61.0 (8)	56.0 (9)	42.0 (11)	44.0 (8)	65.0 (10)
82	59	38.8 (0.9)	62.0 (9)	56.0 (9)	43.0 (11)	44.0 (8)	64.0 (12)
WEEK 12 MEAN		39.5 (1.7)	59.8 (1.7)	55.5 (2.6)	40.5 (2.7)	44.3 (2)	63.3 (6.3)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER RELATIVE HUMIDITY (%)

CONCENTRATION (ppm):		0[a]	50[b]	250[b]	1000[b]	1500[b]	1000 1,3-BUTADIENE[b]
TEST DAYS	EXPOSURE #						
84	60	40.5 (1.5)	55.0 (8)	54.0 (6)	41.0 (8)	48.0 (10)	74.0 (10)
85	61	38.8 (2.1)	60.0 (8)	54.0 (6)	41.0 (8)	41.0 (7)	65.0 (10)
86	62	39.2 (1.2)	64.0 (11)	56.0 (6)	40.0 (8)	44.0 (9)	72.0 (10)
87	63	39.1 (2.4)	63.0 (8)	55.0 (6)	40.0 (7)	41.0 (6)	68.0 (10)
88	64	37.4 (1.7)	63.0 (8)	55.0 (6)	41.0 (7)	44.0 (9)	64.0 (13)
WEEK 13 MEAN		39.0 (1.1)	61.0 (3.7)	54.8 (0.8)	40.6 (0.5)	43.6 (2.9)	68.6 (4.3)
91	65	42.5 (2.1)	68.0 (9)	59.0 (10)	45.0 (9)	51.0 (10)	73.0 (12)
92	66	39.9 (1.3)	58.0 (6)	52.0 (4)	42.0 (5)	46.0 (7)	67.0 (9)
93	67	41.4 (0.7)	[d]	51.0 (4)	40.0 (5)	46.0 (8)	63.0 (9)
94	68	39.9 (2)	[d]	51.0 (7)	39.0 (9)	40.0 (8)	51.0 (9)
WEEK 14 MEAN		40.9 (1.3)	63.0 (7.1)	53.3 (3.9)	41.5 (2.6)	45.8 (4.5)	63.5 (9.3)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

APPENDIX E

MEAN DAILY CHAMBER AIRFLOW

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER AIRFLOW

EXPLANATORY NOTES

FOOTNOTES:

[a] Represents one reading taken at start of exposure. Airflow was set and remained constant throughout exposure period.

[b] Daily mean and standard deviation of 60 to 77 measurements, except where noted.

[c] Standard deviation is reported in parenthesis.

[d] Exposure chamber not operated on a given day.

[e] Weekly means and standard deviations are calculated from the daily means.

[f] Make-up exposure for male mice exposed to 1,3-Butadiene. One airflow reading was taken at exposure start. The number of days of exposure was subsequently the same for all male mice.

[g] One hour of data not collected due to technical error. Mean and standard deviation of 54 to 60 measurements.

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
1	1	60	(0)[c]	35.0	(3)	58.0	(9)	61.0	(9)	[d]		[d]	
2	2	60	(0)	33.0	(3)	60.0	(10)	60.0	(9)	[d]		58.0	(9)
3	3	60	(0)	36.0	(4)	47.0	(10)	58.0	(13)	56.0	(11)	55.0	(10)
4	4	60	(0)	34.0	(2)	50.0	(5)	62.0	(4)	58.0	(3)	59.0	(6)
WEEK 1 MEAN•		60	(0)	34.5	(1.3)	53.8	(6.2)	60.3	(1.7)	57.0	(1.4)	57.3	(2.1)
7	5	60	(0)	34.0	(3)	58.0	(13)	64.0	(11)	62.0	(10)	60.0	(9)
8	6	60	(0)	34.0	(2)	65.0	(10)	65.0	(10)	65.0	(9)	62.0	(7)
9	7	60	(0)	34.0	(4)	67.0	(10)	65.0	(10)	66.0	(9)	61.0	(6)
10	8	60	(0)	37.0	(3)	66.0	(13)	63.0	(12)	65.0	(11)	60.0	(10)
11	9	60	(0)	37.0	(3)	67.0	(11)	64.0	(11)	66.0	(11)	58.0	(10)
WEEK 2 MEAN		60	(0)	35.2	(1.6)	64.6	(3.8)	64.2	(0.8)	64.8	(1.6)	60.2	(1.5)
14	10	60	(0)	37.0	(3)	67.0	(6)	66.0	(4)	68.0	(4)	60.0	(1)
15	11	60	(0)	35.0	(2)	67.0	(6)	66.0	(4)	68.0	(4)	60.0	(2)
16	12	60	(0)	36.0	(1)	68.0	(6)	65.0	(4)	68.0	(4)	61.0	(3)
17	13	60	(0)	33.0	(2)	67.0	(5)	66.0	(4)	68.0	(4)	59.0	(1)
18	14	60	(0)	33.0	(2)	65.0	(10)	64.0	(10)	66.0	(9)	59.0	(1)
WEEK 3 MEAN		60	(0)	34.8	(1.8)	66.8	(1.1)	65.4	(0.9)	67.6	(0.9)	59.8	(0.8)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
21	15	60	(0)	34.0	(2)[g]	64.0	(10)[g]	64.0	(11)[g]	67.0	(10)[g]	55.0	(2)[g]
22	16	60	(0)	34.0	(1)	64.0	(6)	66.0	(5)	68.0	(3)	54.0	(1)
23	17	60	(0)	34.0	(1)	63.0	(5)	66.0	(4)	64.0	(16)	58.0	(2)
24	18	60	(0)	34.0	(2)	62.0	(8)	64.0	(8)	66.0	(8)	63.0	(7)
25	19	60	(0)	33.0	(1)	62.0	(6)	65.0	(4)	68.0	(4)	71.0	(28)
WEEK 4 MEAN		60	(0)	33.8	(0.4)	63.0	(1)	65.0	(1)	66.6	(1.7)	60.2	(7)
28	20	60	(0)	33.0	(1)	64.0	(8)	67.0	(8)	65.0	(7)	65.0	(3)
29	21	60	(0)	32.0	(3)	58.0	(16)	59.0	(19)	59.0	(17)	63.0	(12)
30	22	60	(0)	33.0	(1)	64.0	(6)	67.0	(4)	65.0	(3)	65.0	(1)
31	23	60	(0)	33.0	(1)	64.0	(6)	67.0	(5)	67.0	(4)	65.0	(2)
32	24	60	(0)	33.0	(1)	64.0	(6)	67.0	(4)	67.0	(3)	63.0	(2)
WEEK 5 MEAN		60	(0)	32.8	(0.4)	62.8	(2.7)	65.4	(3.6)	64.6	(3.3)	64.2	(1.1)
35	25	60	(0)	32.0	(1)	65.0	(6)	66.0	(4)	66.0	(3)	63.0	(4)
36	26	60	(0)	33.0	(1)	65.0	(6)	66.0	(4)	65.0	(4)	61.0	(2)
37	27	60	(0)	33.0	(1)	66.0	(5)	66.0	(4)	66.0	(3)	63.0	(3)
38	28	60	(0)	33.0	(1)	66.0	(6)	66.0	(4)	65.0	(4)	65.0	(2)
39	29	60	(0)	33.0	(1)	66.0	(6)	66.0	(4)	66.0	(3)	64.0	(1)
WEEK 6 MEAN		60	(0)	32.8	(0.4)	65.6	(0.5)	66.0	(0)	65.6	(0.5)	63.2	(1.5)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
42	30	60	(0)	33.0	(1)	67.0	(6)	67.0	(4)	67.0	(4)	66.0	(3)
43	31	60	(0)	33.0	(1)	67.0	(5)	67.0	(4)	67.0	(3)	64.0	(2)
44	32	60	(0)	33.0	(1)	66.0	(6)	67.0	(4)	67.0	(3)	68.0	(2)
45	33	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	67.0	(4)	69.0	(2)
46	34	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	67.0	(4)	70.0	(2)
WEEK 7 MEAN		60	(0)	33.0	(0)	66.0	(1)	67.0	(0)	67.0	(0)	67.4	(2.4)
49	35	60	(0)	32.0	(2)	61.0	(13)	63.0	(15)	62.0	(13)	68.0	(11)
50	36	60	(0)	33.0	(1)	64.0	(10)	66.0	(10)	66.0	(9)	71.0	(2)
51	37	60	(0)	33.0	(1)	66.0	(5)	67.0	(4)	67.0	(6)	69.0	(2)
52	38	60	(0)	33.0	(1)	65.0	(6)	67.0	(5)	43.0	(2)	70.0	(2)
53	39	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	43.0	(2)	69.0	(1)
54	[c]											60.0	(0)
WEEK 8 MEAN		60	(0)	32.8	(0.4)	64.2	(1.9)	66.0	(1.7)	56.2	(12.2)	67.8	(4)
56	40	60	(0)	33.0	(1)	66.0	(5)	68.0	(4)	43.0	(2)	68.0	(2)
57	41	60	(0)	33.0	(1)	66.0	(5)	68.0	(4)	50.0	(3)	68.0	(1)
58	42	60	(0)	32.0	(1)	66.0	(5)	67.0	(4)	50.0	(3)	67.0	(1)
59	43	60	(0)	33.0	(1)	66.0	(5)	67.0	(4)	50.0	(3)	68.0	(1)
60	44	60	(0)	33.0	(1)	66.0	(5)	68.0	(4)	60.0	(11)	67.0	(1)
WEEK 9 MEAN		60	(0)	32.8	(0.4)	66.0	(0)	67.6	(0.5)	50.6	(6.1)	67.6	(0.5)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
64	45	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	68.0	(4)	66.0	(1)
65	46	60	(0)	33.0	(2)	65.0	(8)	66.0	(7)	67.0	(7)	67.0	(1)
66	47	60	(0)	32.0	(1)	66.0	(6)	68.0	(4)	68.0	(4)	66.0	(1)
67	48	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	69.0	(3)	66.0	(1)
68	49	60	(0)	33.0	(1)	64.0	(6)	67.0	(4)	70.0	(4)	67.0	(2)
WEEK 10 MEAN		60	(0)	32.8	(0.4)	65.0	(0.7)	67.0	(0.7)	68.4	(1.1)	66.4	(0.5)
70	50	60	(0)	33.0	(1)[g]	66.0	(5)[g]	67.0	(4)[g]	70.0	(4)[g]	67.0	(1)[g]
71	51	60	(0)	33.0	(1)[g]	68.0	(5)[g]	68.0	(3)[g]	70.0	(4)[g]	67.0	(1)[g]
72	52	60	(0)	33.0	(1)[g]	65.0	(5)[g]	67.0	(4)[g]	72.0	(4)[g]	67.0	(2)[g]
73	53	60	(0)	33.0	(1)[g]	64.0	(5)[g]	67.0	(4)[g]	71.0	(4)[g]	67.0	(1)[g]
WEEK 11 MEAN		60	(0)	33.0	(0)	65.8	(1.7)	67.3	(0.5)	70.8	(1)	67.0	(0)
77	54	60	(0)	33.0	(1)[g]	63.0	(8)[g]	66.0	(8)[g]	69.0	(7)[g]	66.0	(2)[g]
78	55	60	(0)	33.0	(1)[g]	64.0	(6)[g]	67.0	(4)[g]	70.0	(3)[g]	66.0	(1)[g]
79	56	60	(0)	29.0	(1)[g]	64.0	(6)[g]	67.0	(4)[g]	69.0	(4)[g]	67.0	(2)[g]
80	57	60	(0)	33.0	(1)	64.0	(5)	67.0	(4)	70.0	(4)	69.0	(1)
81	58	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	70.0	(4)	66.0	(3)
82	59	60	(0)	30.0	(2)	64.0	(8)	67.0	(8)	70.0	(8)	66.0	(7)
WEEK 12 MEAN		60	(0)	31.8	(1.8)	64.0	(0.6)	66.8	(0.4)	69.7	(0.5)	66.7	(1.2)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE
FOLLOWING SUBCHRONIC INHALATION EXPOSURE

MEAN DAILY CHAMBER AIRFLOW (L/min)

CONCENTRATION (ppm):		0[a]		50[b]		250[b]		1000[b]		1500[b]		1000 1,3-BUTADIENE[b]	
TEST DAYS	EXPOSURE #												
84	60	60	(0)	33.0	(1)	65.0	(6)	67.0	(4)	71.0	(4)	67.0	(2)
85	61	60	(0)	32.0	(1)	65.0	(6)	67.0	(4)	71.0	(4)	67.0	(2)
86	62	60	(0)	32.0	(1)	65.0	(6)	67.0	(4)	70.0	(4)	69.0	(2)
87	63	60	(0)	32.0	(1)	64.0	(5)	67.0	(5)	70.0	(4)	69.0	(1)
88	64	60	(0)	32.0	(1)	64.0	(6)	67.0	(4)	70.0	(4)	69.0	(1)
WEEK 13 MEAN		60	(0)	32.2	(0.4)	64.6	(0.5)	67.0	(0)	70.4	(0.5)	68.2	(1.1)
91	65	60	(0)	32.0	(1)	64.0	(6)	67.0	(4)	70.0	(4)	69.0	(1)
92	66	60	(0)	33.0	(1)	65.0	(5)	67.0	(4)	70.0	(3)	69.0	(1)
93	67	60	(0)	[d]		65.0	(6)	68.0	(4)	71.0	(4)	71.0	(2)
94	68	60	(0)	[d]		63.0	(9)	66.0	(10)	69.0	(10)	68.0	(10)
WEEK 14 MEAN		60	(0)	32.5	(1)	64.3	(1)	67.0	(0.8)	70.0	(0.8)	69.3	(1.3)

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

APPENDIX F

MICRONUCLEUS EVALUATION - INDIVIDUAL ANIMAL DATA

RATS

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

INDIVIDUAL ANIMAL DATA

RATS

CONCENTRATION (PPM)	Animal Number	Sex	PCE/ 1000 Eryth	PCE:NCE Ratio	MN PCEs/ 1000 PCEs	MN NCEs/ 1000 PCEs
0 VCH	531484	F	280	0.38889	4	0
	531482	F	401	0.66945	0	0
	531479	F	274	0.37741	1	0
	531480	F	559	1.26757	5	0
	531486	F	564	1.29358	0	0
	531427	M	328	0.48810	2	0
	531432	M	618	1.61780	0	0
	531423	M	456	0.83824	0	0
	531429	M	427	0.74520	0	0
	531428	M	352	0.54321	2	0
250 VCH	531490	F	404	0.67785	4	1
	531494	F	456	0.83824	1	0
	531496	F	512	1.04918	1	0
	531495	F	417	0.71527	1	1
	531493	F	349	0.53610	1	3
	531437	M	436	0.77305	0	0
	531440	M	569	1.32019	2	0
	531433	M	468	0.87970	0	0
	531441	M	467	0.87617	1	0
	531436	M	516	1.06612	1	0
1000 VCH	531504	F	505	1.02020	3	0
	531502	F	399	0.66389	0	0
	531501	F	534	1.14592	0	0
	531505	F	622	1.64550	1	0
	531500	F	424	0.73611	2	1
	531448	M	530	1.12766	0	0
	531443	M	404	0.67785	1	0
	531449	M	616	1.60417	5	0
	531451	M	402	0.67224	0	0
	531452	M	411	0.69779	2	2

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

INDIVIDUAL ANIMAL DATA

RATS

CONCENTRATION (PPM)	Animal Number	Sex	PCE/ 1000 Eryth	PCE:NCE Ratio	MN PCEs/ 1000 PCEs	MN NCEs/ 1000 PCEs
1500 VCH	531511	F	502	1.00803	1	0
	541510	F	429	0.75131	0	0
	531508	F	526	1.10970	0	0
	531516	F	534	1.14592	0	0
	531517	F	415	0.70940	4	0
	531453	M	548	1.21239	0	0
	531460	M	553	1.23714	0	0
	531455	M	475	0.90476	2	0
	531461	M	550	1.22222	1	0
	531456	M	454	0.83150	0	0
40 mg/kg CP	531528	F	327	0.48588	8	0
	531532	F	328	0.48810	5	0
	531529	F	223	0.28700	10	0
	531530	F	213	0.27065	5	1
	531531	F	197	0.24533	11	1
	531475	M	75	0.08108	4	0
	531474	M	164	0.19617	7	0
	531473	M	314	0.45773	12	0
	531477	M	452	0.82482	16	0
	531476	M	320	0.47059	9	0

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

APPENDIX G

MICRONUCLEUS EVALUATION - INDIVIDUAL ANIMAL DATA

MICE

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

INDIVIDUAL ANIMAL DATA

MICE

CONCENTRATION (PPM)	Animal Number	Sex	PCE/ 1000 Eryth	PCE:NCE Ratio	MN PCEs/ 1000 PCEs	MN NCEs/ 1000 PCEs
0 VCH	91423	F	501	1.00401	0	0
	91428	F	736	2.78788	4	1
	91421	F	559	1.26757	1	0
	91422	F	613	1.58398	1	1
	91426	F	574	1.34742	1	1
	91378	M	583	1.39808	4	2
	91371	M	581	1.38663	0	1
	91372	M	570	1.32558	4	0
	91380	M	482	0.93050	1	0
	91373	M	537	1.15983	1	1
50 VCH	91431	F	689	2.21543	2	0
	91434	F	637	1.75482	1	0
	91440	F	513	1.05339	3	1
	91436	F	581	1.38663	3	1
	91435	F	544	1.19298	0	0
	91381[a]	M	---	-------	-	-
	91382	M	548	1.21239	4	2
	91383	M	664	1.97619	2	0
	91390	M	540	1.17391	2	2
	91385	M	547	1.20751	2	3
250 VCH	91442	F	654	1.89017	2	0
	91446	F	657	1.91545	1	2
	91449	F	534	1.14592	3	3
	91448	F	556	1.25225	4	2
	91441	F	608	1.55102	2	3
	91398	M	589	1.43309	5	2
	91391	M	598	1.48756	2	6
	91392	M	524	1.10084	4	0
	91395	M	503	1.01207	6	2
	91393	M	529	1.12314	1	2

[a] Animal removed from micronucleus study due to technical error

RAT AND MOUSE BONE MARROW MICRONUCLEUS ASSAY OF 4-VINYLCYCLOHEXENE FOLLOWING SUBCHRONIC INHALATION EXPOSURE

INDIVIDUAL ANIMAL DATA

MICE

CONCENTRATION (PPM)	Animal Number	Sex	PCE/ 1000 Eryth	PCE:NCE Ratio	MN PCEs/ 1000 PCEs	MN NCEs/ 1000 PCEs
1000 VCH	91458	F	620	1.63158	1	0
	91456	F	644	1.80899	3	0
1000 1,3-BD	91462	F	780	3.54545	16	1
	91466	F	702	2.35570	14	7
	91467	F	629	1.69542	22	5
	91461	F	752	3.03226	18	0
	91465	F	745	2.92157	14	1
	91417	M	594	1.46305	19	2
	91411	M	677	2.09598	25	10
	91414	M	667	2.00300	22	5
	91415	M	503	1.01207	3	2
	91418[a]	M	---	-------	--	--

[a] Animal removed from micronucleus study due to technical error

Study Title

Two-Week Range-Finding Study
with 4-Vinylcyclohexene in Rats and Mice

Laboratory Project ID

Haskell Laboratory Report No. 759-93

RECD
OFFICE OF POLLUTION
PREVENTION AND TOXICS
94 APR 22 PM 2:41

Author

Judith C. Stadler, Ph.D., D.A.B.T.

Study Completed on

February 22, 1994

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, Delaware 19714

Medical Research Project No. 9300-001

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE IN RATS AND MICE

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with EPA TSCA Good Laboratory Practice Regulations (40 CFR 792) except for the deviation documented and explained below.

In some instances, standard forms were printed with initials prior to recording data; raw data entered on those forms were not, therefore, initialed and dated at the time of entry.

This deviation did not affect the integrity of the study.

Submitter: E. I. du Pont de Nemours and Company

Sponsor: Chemical Manufacturers Association
Washington, D.C.

Date

Study Director: Judith C. Stadler — 2/22/94
Judith C. Stadler, Ph.D., D.A.B.T.
Senior Research Toxicologist
Inhalation and Oral Toxicology

Sponsor Representative: — 2/16/94
Elizabeth J. Moran, Ph.D., D.A.B.T.
Chemical Manufacturers Association

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE IN RATS AND MICE

GENERAL INFORMATION

Substance Tested:	Cyclohexene, 4-ethenyl-
Haskell Numbers:	19,353
Synonyms and Other Codes:	4-Ethenylcyclohexene Vinylcyclohexene (Vinyl cyclohexene) 4-Vinylcyclohexene-1 4-Vinylcyclohexene VCH VN MK 6A ID# IN210G308
CAS Registry Number:	100-40-3
Physical Form:	Clear liquid
Purity:	99.8%
Contaminants:	t-Butylcatechol as a stabilizer
Sponsor:	Chemical Manufacturers Association Washington, D.C.
Substance Submitted by:	Union Carbide Corporation Institute, West Virginia
Study Initiated - Completed:	08/31/92 - 02/22/94
In-Life Phase Initiated - Completed:	09/08/92 - 09/21/92

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TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE IN RATS AND MICE

SUMMARY

Four groups of five male and five female rats and five male and five female mice each were exposed for two weeks by inhalation to concentrations of 4-vinylcyclohexene vapor targeted to 0, 250, 750, or 1500 ppm. The actual mean concentrations of 4-vinylcyclohexene were 0, 240, 720, and 1500 ppm. Exposures were for six hours a day, five days a week with one day of rest separating the two exposure weeks. The animals were weighed and observed for clinical signs of toxicity prior to each exposure. At the end of the exposure period, surviving animals were maintained for three days of recovery, then sacrificed without necropsy.

One rat in the 720 ppm exposure group and 9/10 mice in the 1500 ppm group died during the study. One female rat was found dead on the second day of the study. The death was not attributed to compound exposure. There were 5/5 male mice and 4/5 female mice found dead prior to exposure on test day 4. The fifth female mouse was moribund and was sacrificed *in extremis* without further inhalation exposure. Mortalities in mice were attributed to 4-vinylcyclohexene. Clinical signs of note included tremors in male and female mice following exposure on test day 3, preliminary to mortality on test day 4. Other clinical signs observed included rapid breathing, lethargy, hunched-over posture, and closed eyes.

There were significant body weight effects in male rats exposed to 1500 ppm 4-vinylcyclohexene. The mean body weight gain of this group during the exposure period was lower than the mean weight gain of male control rats. Also, male and female mice that died had low mean body weights one to two days prior to death. The low body weights observed in rats and mice exposed to 1500 ppm 4-vinylcyclohexene were considered compound-related effects.

The no-observable-adverse-effect level for parameters examined in this two-week range-finding study was 1500 ppm 4-vinylcyclohexene for female rats, and 720 ppm for male rats and male and female mice.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

SIGNATURE

Date

Authored, Reviewed, and Approved for Issue by Study Director:

Judith C. Stadler 2/22/94

Judith C. Stadler, Ph.D., D.A.B.T.
Senior Research Toxicologist
Inhalation and Oral Toxicology

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

QUALITY ASSURANCE DOCUMENTATION

Dates of Inspection:

Conduct - 9/9/92

Records, Report(s) - 11/11-12,15/93

Findings reported to:

Study Director - 11/15/93

Management - 12/2/93

Reported by: Deborah A. Vick — 2/17/94

Deborah A. Vick
Quality Assurance Auditor

Date

ACKNOWLEDGMENTS

The following individuals were responsible for the conduct of the study:

Manager:	Robert W. Rickard, Ph.D. (9/1/92 - present) Nancy C. Chromey, Ph.D. (8/31/92 - 9/1/92)
Study Director:	Judith C. Stadler, Ph.D.
Primary Technicians:	Scott M. Krenzel John A. Tedesco, B.S.
Report-Writing Supervisor:	Anne Marie Bathon, B.A.
Report Preparation:	Maryanne M. Wilford, B.A.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

INTRODUCTION

The test substance is an intermediate in the production of epoxy resins, and an off-gas in the production of synthetic rubber. This study was conducted to determine the effects of repeated exposure to 4-vinylcyclohexene on rats and mice. Inhalation was selected as a route which simulates expected human exposure, and the data from this study will be used to set appropriate exposure concentrations for a 90-day inhalation study.

MATERIALS AND METHODS

A. Test Substance

4-Vinylcyclohexene was supplied as a clear liquid by the Union Carbide Corporation, Institute, West Virginia. The purity of the test substance was 99.8%.

B. Animals

A total of 104 animals (26 male and 26 female Crl:CD®BR rats, and 26 male and 26 female B6C3F_1/CrlBR mice) were received from Charles River Breeding Laboratories, Kingston, New York. The animals were approximately 36 days old on the day of arrival (09/01/92).

Rats and mice have historically been used in safety evaluation studies for acute inhalation toxicity testing. The Crl:CD®BR rat and B6C3F_1/CrlBR mouse were selected based on the request of the study sponsor.

C. Animal Husbandry

Temporary Animal Identification and Quarantine: Upon arrival, each rat and mouse was assigned a temporary, one- to two-digit, identification number, which

was recorded on a card affixed to the cage. The rats and mice were quarantined for seven days prior to test initiation. During the quarantine period, they were observed for clinical signs of disease daily and weighed three times.

Housing: During all phases of the study, rats and mice were housed in suspended, stainless steel, wire-mesh cages. During quarantine, rats were housed individually in 5" x 11" x 7" cages, and mice were housed individually in 7" x 4" x 5" cages . After grouping and during the exposure and recovery periods, rats were housed individually and in pairs in 8" x 14" x 8" cages, and mice were housed individually in 7" x 4" x 5" cages.

Feed and Water: Except during exposure, Purina Certified Rodent Chow/ #5002 and tap water from the Wilmington Suburban Water Corporation were available ad libitum.

Animal Room Environment: Animal rooms were maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the rooms were targeted for temperatures of 23 ± 2°C and a relative humidity of 50 ± 10%. Excursions outside of these ranges were judged to have been of insufficient magnitude and/or duration to have adversely affected the validity of the study.

Animal Selection and Permanent Identification: Prior to the end of the quarantine period, animals were assigned to four groups of five male and female rats and five male and female mice each (20 animals per group). Prior to the start of the first exposure, the animals were divided into groups with the aid of a computer randomization program, so that mean body weights for each group were similar to one another. The animals were approximately 43 days old at the start of exposures. Male rats weighed between 149 and 180 grams and female rats weighed between 92 and 148 grams at the start of the exposures. Male mice weighed between 24 and 26 grams and female mice weighed between 19 and 22 grams at the start of the exposures.

After the rats and mice were placed in groups, each animal was assigned a one- to two-digit identification number, which was tattooed on the tail. Each rat was assigned a unique six-digit animal number, and each mouse was assigned

a unique five-digit animal number. Both identification and animal numbers were recorded on a card affixed to each cage.

D. Study Design

Four groups of five male rats, five female rats, five male mice and five female mice (20 animals per group, 80 animals total) were used to assess the toxic effects of repeated inhalation exposure to 4-vinylcyclohexene. Three test groups were exposed to concentrations targeted at 250, 750, or 1500 ppm 4-vinylcyclohexene vapor in air. A control group was exposed concurrently to air only. Rats and mice were exposed for six hours a day, five days a week for two weeks with one day of rest between the fifth and sixth exposure. All animals were retained for a three-day, postexposure recovery period. All animals were monitored throughout the study for mortality, clinical signs of toxicity, and body weight changes.

E. Inhalation Exposure Conditions

1. Chamber Construction and Design

The four groups of male and female rats and mice were exposed whole-body to vapors of 4-vinylcyclohexene or air. The rats and mice were placed in stainless steel wire-mesh baskets or cages. Baskets or cages were placed inside the stainless steel and glass, 150-L exposure chambers. The chambers were operated in a one-pass flow-through system with air flow rates set to a constant flow. Each chamber was fitted with a baffle to assist in the even dispersion of the test material. The exposure chamber atmosphere was exhausted through a condenser submerged in a mixture of ethylene glycol and water. The vapor was then directed into a scrubbing system using methanol as a trapping solvent and an MSA chemical and particulate filter, prior to discharge into the fume hood.

2. Chamber Environment

Chamber temperatures were targeted at 23 ± 2°C. Temperatures were measured continually with an Omega model HH-51 Digital Thermometer connected to an Omega Type K Thermocouple probe and recorded three to four times during each exposure. Relative humidity was targeted at 50 ± 10%. Humidity in the exposure chambers was measured one to two times during each exposure with an Omega model 5100 Micropsychrometer. Chamber oxygen concentration was targeted to at least 19% and measured one to two times during each exposure with a Biosystems model 3100R Oxygen Monitor.

F. Atmosphere Generation

Atmospheres of 4-vinylcyclohexene vapor were generated by metering the test substance into an Instatherm flask with a Harvard model 22 Infusion Pump. The Instatherm flasks were targeted to approximately 30°C for the 250 and 750 ppm target levels and 39°C for the 1500 ppm target level. The 4-vinylcyclohexene was diluted with conditioned, filtered air before it entered the exposure chamber. Desired atmospheric concentrations of 4-vinylcyclohexene vapor were produced by regulating the flow rate from the infusion pump, or the dilution airflow. The control group was exposed to conditioned, filtered air only.

The six-hour exposure period started when the generation system was turned on and ended when the system was shut off. Rats and mice remained in the chambers with continual flow of conditioned air until chamber concentrations of 4-vinylcyclohexene had reached less than 5 ppm.

G. Analysis of Test Atmospheres

Chamber atmospheres for each exposure group, including the control group, were monitored approximately every 30 minutes. Single samples of chamber atmosphere for the control chamber and duplicate samples of chamber atmosphere for the test material exposure chambers were automatically sampled and analyzed with a computer driven Hewlett Packard model 5880A Gas Chromatograph connected

to a Hewlett-Packard model 5880A Series Four integrator. The gas chromatograph was fitted with a stainless steel column packed with a 5% Supelcoport™ (mesh 100/120) and a flame ionization detector. The atmospheric concentrations of 4-vinylcyclohexene vapor were determined by comparing the chamber samples with standard curves prepared with 4-vinylcyclohexene. The standards were prepared each day prior to exposure by quantitatively diluting liquid 4-vinylcyclohexene in air.

H. Body Weights and Clinical Observations

During the exposure phase of the study, all male and female rats and mice were weighed and individually observed for clinical signs of toxicity before each six-hour exposure. Clinical observations were also made on groups of animals during exposures and immediately following each exposure. During the three-day recovery period, all male and female rats and mice were weighed and observed daily.

I. Statistical Analyses

Mean body weight and body weight gain data were statistically analyzed by a one-way analysis of variance. Exposure group values were compared to controls by the Dunnett's tests when the ratio of variance (F) indicated a significant among-to-within group variation. Significance was judged at the 0.05 probability level.

RECORDS AND SAMPLE RETENTION

All raw data and the final report will be stored in the archives of Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, or in the DuPont Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

RESULTS AND DISCUSSION

A. Test Substance and Exposure Conditions (Tables 1 and 2)

Purity checks on the test substance sample (H-19353) used in this study were conducted on 4/24/92 and 1/13/93, prior to and following the study. Analyzed purity was 99.4% and 99.6%; the compound was considered stable during the period of this range-finding study.

The mean concentrations of 4-vinylcyclohexene in the exposure chambers targeted at 250, 750, or 1500 ppm were 240 ± 6.4, 720 ± 22, and 1500 ± 16 ppm, respectively, over the duration of the study (Table 1). Excursions of 4-vinylcyclohexene concentrations (concentrations more than ± 20% of mean concentrations) occurred on several occasions; however, these excursions were considered not to have affected the outcome of the study. Future references to concentrations in this report will use actual mean concentrations obtained during the animal exposures.

In the chambers that contained test substance, the temperatures ranged from 22 to 26°C, relative humidity ranged from 23 to 40%, oxygen concentration was maintained at 21%, and airflow ranged from 34 to 35 L/min (Table 2). In the control chamber, the temperatures ranged from 21 to 25°C, relative humidity ranged from 22 to 60%, oxygen concentration was maintained at 21%, and airflow was maintained at 37 L/min (Table 2). Although the relative humidities were often below the targeted range of 40 to 60%, they were considered not to have affected the comfort of the animals nor the generation of the test atmosphere.

B. Body Weight Analysis (Tables 3 - 10; Figures 1 - 4)

There were several effects on mean body weight and/or body weight gain among animals exposed to 4-vinylcyclohexene during the study. The male rats in the 1500 ppm exposure group had a mean body weight that was approximately 11% lower than the mean body weight of the control group by the end of the exposure period on test day 11 (Table 3). Although male rats in the 1500 ppm exposure group gained weight each day (Figure 1), the overall mean body weight gain from

test days 1 to 11 (Table 5) was lower than the mean weight gain for male controls and the difference was statistically significant. There were no biologically or statistically significant effects on the mean body weight gains of males exposed to lower concentrations or of female rats.

All of the mice, including the controls, had mean body weights that were lower on test days 2 and 3 than their respective mean body weights prior to exposure on day 1 (Tables 7 and 8, Figures 3 and 4). On test day 3, the mean body weights of male and female mice in the 1500 ppm exposure group were significantly lower than the mean body weights of their respective control groups. These effects were considered compound-related. After day 3, mice in the 0, 240 and 720 ppm exposure groups began to gain weight; however, weight gains were inconsistent throughout the study period. The body weight parameters for male mice exposed to 240 ppm 4-vinylcyclohexene and for male and female mice exposed to 720 ppm were similar to controls. The mean body weight gain for female mice exposed to 240 ppm 4-vinylcyclohexene was lower than the mean body weight gain for female controls (Table 10) and statistically significant; however, the weight gain during the three day recovery period was higher than controls. The low mean body weight gain in females exposed to 240 ppm was considered not to have resulted from exposure to the test material.

C. Mortality and Clinical Observations

One female rat exposed to 720 ppm 4-vinylcyclohexene was found dead on test day 2 and was replaced with another rat. All male rats and the remaining female rats survived the 10-day exposure and 3-day recovery periods.

All male mice (5/5) and 4/5 female mice in the group exposed to 1500 ppm 4-vinylcyclohexene were found dead on test day 4. The fifth female mouse was sacrificed *in extremis* and not exposed to test material on that day.

A number of clinical signs of toxicity were observed in study animals. Transient signs were observed when animals were removed from the exposure chambers each day. Rapid breathing was frequently observed in both rats and mice exposed to all concentrations of test material. Lethargy was seen in rats

and mice exposed to 720 or 1500 ppm 4-vinylcyclohexene. Hunched-over posture and closed eyes were also seen on occasion. On test day 3, one rat in the 240 ppm group, three rats in the 1500 ppm group and seven mice in the 1500 ppm group had tremors following exposure. The tremors in the 1500 ppm groups of mice appeared to be a significant effect preceding death, and they were considered related to 4-vinylcyclohexene exposure. No tremors were observed the following day, or on any other day in rats. The only sign reported during pre-exposure daily observation was a mouse in the 1500 ppm exposure group with hunched-over posture on test day 4. This was the only mouse that was not found dead on that day, and the same one that was later killed in extremis.

CONCLUSIONS

Under the conditions of this study, the no-observable-adverse-effect levels were 720 ppm 4-vinylcyclohexene for male rats and 1500 ppm for female rats, based on the body weight effects seen in rats exposed to 1500 ppm of the test material. The no-observable-adverse effect level was 720 ppm for both male and female mice, based on the tremors and mortality that occurred in the groups exposed to 1500 ppm 4-vinylcyclohexene.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLES

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLES

EXPLANATORY NOTES

NOTES:

Tables 1 and 2

Values are reported to two significant figures.
Data are compiled from ten, six-hour exposures.

ABBREVIATIONS:

Tables 1 and 2

S.E.M. = Standard Error of the Mean

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 1

EXPOSURE CHAMBER ATMOSPHERIC CONCENTRATIONS

DESIGN CONCENTRATION (ppm)	ANALYZED CONCENTRATION (ppm)		
	MEAN[a]	S.E.M.[a]	DAILY MEAN RANGE
0	0	0	0 - 0
250	240	6.4	210 - 270
750	720	22	550 - 790
1500	1500	16	1400 - 1600

[a] Mean and S.E.M. of the daily means.

TABLE 2

EXPOSURE CHAMBER ENVIRONMENTAL CONDITIONS

4-VINYLCYCLOHEXENE CONCENTRATION (ppm)	TEMPERATURE (°C)	OXYGEN (%)	RELATIVE HUMIDITY (%)	TOTAL AIRFLOW (L/MIN)
0	21 - 25	21	22 - 60	37
240	22 - 24	21	25 - 40	35
720	22 - 25	21	24 - 35	34
1500	22 - 26	21	23 - 31	35

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 3

MEAN BODY WEIGHTS OF MALE RATS

	MEAN BODY WEIGHTS (g)			
GROUP:	I	III	V	VII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1	170.2(9.0)[a]	166.3(7.0)	165.4(5.8)	166.3(10.2)
2	179.9(9.4)	175.2(8.8)	169.6(5.7)	168.4(9.0)
3	187.3(10.4)	179.8(12.6)	176.5(5.5)	170.7(9.5)
4	195.2(13.1)	189.5(10.6)	184.5(3.4)	175.7(12.6)
5	202.8(16.0)	197.1(10.9)	193.5(3.8)	181.2(14.9)
7	217.2(19.3)	213.3(8.5)	208.7(4.9)	195.1(18.2)
8	227.5(20.2)	219.6(8.4)	215.6(4.1)	202.4(18.4)
9	234.2(20.7)	227.8(8.1)	221.6(7.8)	206.9(17.0)
10	240.7(23.1)	235.9(8.1)	231.1(8.9)	213.7(17.3)
11	249.3(22.8)	243.3(10.2)	237.2(10.2)	223.1(18.5)
12[b]	249.7(20.0)	245.6(8.6)	240.6(11.5)	222.5(19.3)
13	257.8(21.7)	250.3(8.4)	248.5(13.3)	232.6(23.0)
14	272.0(21.7)	267.3(9.7)	262.5(15.6)	248.1(21.7)

[a] Standard deviation is reported in parentheses

[b] Test day 12 was the beginning of the recovery period for male rats.

Statistical methods: One-way AOV and Dunnett's tests were performed on daily data. (See Statistical Analyses)

There were no statistically significant differences at alpha = 0.05.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 4

MEAN BODY WEIGHTS OF FEMALE RATS

	MEAN BODY WEIGHTS (g)			
GROUP:	II	IV	VI	VIII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1	133.9(5.9)[a]	133.3(9.0)	125.6(19.6)	132.3(4.1)
2	137.9(7.4)	138.4(8.2)	139.9(6.8)[c]	136.3(5.3)
3	143.6(7.0)	145.6(8.7)	146.5(6.8)	141.6(6.1)
4	153.1(8.5)	152.4(8.5)	151.5(5.3)	146.7(8.1)
5	158.9(9.5)	156.7(9.2)	157.6(6.0)	151.5(8.9)
7	167.7(8.2)	165.5(12.8)	163.7(7.5)	161.4(9.3)
8	173.2(11.8)	170.0(9.5)	171.2(5.4)	162.7(9.2)
9	175.5(13.0)	174.5(12.9)	173.6(6.7)	169.1(14.3)
10	178.5(13.8)	176.0(12.1)	178.0(6.4)	171.0(13.8)
11	182.9(10.7)	181.5(14.9)	178.8(6.1)	175.2(13.4)
12[b]	184.2(15.3)	183.0(15.4)	182.1(2.2)	172.8(10.1)
13	189.0(13.7)	185.3(12.6)	184.0(5.7)	181.4(12.7)
14	197.9(16.7)	192.6(16.0)	192.2(4.7)	189.1(11.7)

[a] Standard deviation is reported in parentheses

[b] Test day 12 was the beginning of the recovery period for female rats.

[c] One rat in group found dead on day 2 and was replaced.

Statistical methods: One-way AOV and Dunnett's tests were performed on daily data. (See Statistical Analyses)

There were no statistically significant differences at alpha = 0.05.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 5

MEAN BODY WEIGHT GAINS OF MALE RATS

	MEAN BODY WEIGHT GAINS (g)			
GROUP:	I	III	V	VII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1- 2	9.8(2.2)[a]	8.9(3.9)	4.2(1.1)*	2.1(3.4)*
2- 3	7.4(2.3)	4.6(4.2)	6.9(3.0)	2.3(2.5)
3- 4	7.9(3.9)	9.7(2.1)	7.9(2.7)	4.9(4.2)
4- 5	7.6(3.2)	7.6(1.9)	9.0(2.7)	5.5(2.6)
5- 7	14.4(4.5)	16.2(3.9)	15.2(3.0)	13.9(4.0)
7- 8	10.3(1.9)	6.3(1.0)*	6.9(1.6)*	7.3(2.7)
8- 9	6.7(1.8)	8.2(3.7)	6.0(4.0)	4.5(2.0)
9-10	6.4(2.8)	8.2(2.5)	9.5(2.3)	6.8(3.4)
10-11	8.6(1.4)	7.4(2.3)	6.1(2.0)	9.5(1.4)
11-12[b]	0.4(2.8)	2.3(2.2)	3.4(4.0)	-0.6(1.8)
12-13	8.1(1.9)	4.7(1.5)	7.9(4.2)	10.1(4.3)
13-14	14.2(1.2)	17.0(3.0)	14.0(2.5)	15.5(3.1)
1-11	79.1(14.0)	77.0(10.8)	71.8(11.0)	56.8(10.8)*
11-14	22.7(1.1)	24.0(1.4)	25.3(6.7)	25.0(5.5)
1-14	101.8(13.0)	101.0(11.1)	97.0(16.9)	81.8(15.0)

a Standard deviation is reported in parentheses

b Test day 12 was the beginning of the recovery period for male rats.

Statistical methods: One-way AOV and Dunnett's tests were performed on both daily and summary interval data. (See Statistical Analyses)

* Statistically significant difference from control at alpha = 0.05.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 6

MEAN BODY WEIGHT GAINS OF FEMALE RATS

	MEAN BODY WEIGHT GAINS (g)			
GROUP:	II	IV	VI	VIII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1- 2	4.0(2.9)[a]	5.0(2.6)	6.1(2.5)	4.0(4.0)
2- 3	5.7(4.0)	7.3(2.3)	6.6(1.7)	5.3(0.9)
3- 4	9.5(5.0)	6.7(2.9)	5.0(2.3)	5.0(2.5)
4- 5	5.7(1.8)	4.3(2.2)	6.1(1.3)	4.9(1.1)
5- 7	8.8(4.6)	8.8(5.7)	6.2(4.5)	9.9(1.3)
7- 8	5.5(5.0)	4.5(5.0)	7.4(4.3)	1.3(1.0)
8- 9	2.4(2.2)	4.5(4.2)	2.4(4.2)	6.4(5.6)
9-10	2.9(5.5)	1.5(2.9)	4.4(3.1)	1.9(1.1)
10-11	4.5(4.1)	5.5(3.6)	0.8(2.9)	4.2(1.2)
11-12[b]	1.3(5.1)	1.5(2.2)	3.3(4.1)	-2.4(3.8)
12-13	4.8(3.8)	2.3(3.8)	1.9(4.1)	8.6(3.3)
13-14	8.9(4.0)	7.4(4.4)	8.2(2.7)	7.6(2.6)
1-11	49.0(7.6)	48.1(10.9)	44.9(1.8)	42.9(12.2)
11-14	14.9(6.7)	11.2(2.0)	13.4(3.8)	13.8(2.7)
1-14	64.0(12.4)	59.3(12.6)	57.6(2.7)	56.8(11.0)

a Standard deviation is reported in parentheses

b Test day 12 was the beginning of the recovery period for female rats.

Statistical methods: One-way AOV and Dunnett's tests were performed on both daily and summary interval data. (See Statistical Analyses)

There were no statistically significant differences at alpha = 0.05.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 7

MEAN BODY WEIGHTS OF MALE MICE

	MEAN BODY WEIGHTS (g)			
GROUP:	I	III	V	VII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1	24.5(0.4)[a]	24.8(0.8)	24.6(1.0)	25.0(0.8)
2	23.7(0.4)	24.1(1.2)	23.3(0.8)	23.3(1.1)
3	23.6(0.4)	24.0(1.2)	23.2(0.3)	20.5(1.2)*
4	24.0(0.5)	24.0(0.8)	23.8(0.7)	[b]
5	23.6(0.5)	23.6(1.1)	24.5(0.7)	
7	24.1(0.8)	25.3(1.0)	24.7(0.7)	
8	24.4(0.4)	25.6(1.0)	24.9(0.8)	
9	24.8(0.9)	25.8(0.9)	25.1(0.5)	
10	25.4(0.7)	26.3(1.0)	25.9(0.6)	
11	26.2(1.1)	26.3(1.1)	26.2(0.2)	
12[c]	25.1(0.6)	25.5(1.4)	25.9(0.8)	
13	25.2(0.6)	25.5(1.0)	25.7(0.6)	
14	25.1(0.7)	26.0(1.2)	26.0(0.6)	

a Standard deviation is reported in parentheses

b Five of five male mice were found dead on test day 4.

c Test day 12 was the beginning of the recovery period for the male mice.

Statistical methods: One-way AOV and Dunnett's tests were performed on daily data. (See Statistical Analyses)

* Statistically significant difference from control at alpha = 0.05.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 8

MEAN BODY WEIGHTS OF FEMALE MICE

	MEAN BODY WEIGHTS (g)			
GROUP:	II	IV	VI	VIII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1	20.4(0.8)[a]	20.9(1.1)	21.0(0.9)	20.6(0.6)
2	20.1(0.8)	19.6(0.6)	20.2(0.9)	19.2(0.7)
3	20.0(0.9)	19.6(0.6)	20.2(1.0)	17.4(1.3)*
4	20.1(0.9)	19.8(0.9)	20.5(1.0)	16.6(0.0)*[b]
5	20.0(0.9)	20.0(1.2)	21.0(0.7)	
7	20.9(0.7)	20.5(0.9)	21.5(0.9)	
8	21.0(0.8)	21.1(0.8)	20.7(0.6)	
9	20.8(1.1)	20.8(1.2)	21.1(0.6)	
10	21.3(0.7)	21.1(1.4)	21.9(1.2)	
11	22.4(0.6)	20.8(0.6)*	22.5(1.0)	
12[c]	21.3(0.7)	20.9(0.9)	21.6(0.8)	
13	20.9(0.9)	21.1(0.8)	21.5(0.8)	
14	21.3(0.4)	21.5(1.3)	21.5(0.6)	

[a] Standard deviation is reported in parentheses

[b] Four of five female mice were found dead on test day 4. The remaining female mouse was sacrificed in extremis on test day 4 after it was weighed.

[c] Day 12 was the beginning of the recovery period for the female mice.

Statistical methods: One-way AOV and Dunnett's tests were performed on daily data. (See Statistical Analyses)

* Statistically significant difference from control at alpha = 0.05.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 9

MEAN BODY WEIGHT GAINS OF MALE MICE

	MEAN BODY WEIGHT GAINS (g)			
GROUP:	I	III	V	VII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1- 2	-0.8(0.6)[a]	-0.7(0.8)	-1.3(1.8)	-1.8(0.7)
2- 3	-0.1(0.1)	-0.1(0.4)	-0.1(0.7)	-2.8(0.6)*
3- 4	0.4(0.3)	0.0(0.8)	0.6(0.4)	[b]
4- 5	-0.4(0.3)	-0.4(1.4)	0.7(0.2)	
5- 7	0.5(0.5)	1.7(1.9)	0.2(0.5)	
7- 8	0.2(0.5)	0.3(0.5)	0.2(0.6)	
8- 9	0.5(1.0)	0.2(0.2)	0.1(0.5)	
9-10	0.6(0.2)	0.6(0.5)	0.8(0.2)	
10-11	0.7(0.6)	0.0(0.4)	0.3(0.4)	
11-12[c]	-1.1(0.5)	-0.8(0.6)	-0.3(0.6)	
12-13	0.2(0.4)	0.0(0.5)	-0.2(0.4)	
13-14	-0.1(0.9)	0.5(0.5)	0.3(0.4)	
1-11	1.7(1.3)	1.5(0.9)	1.6(1.2)	
11-14	-1.0(1.2)	-0.3(0.4)	-0.2(0.4)	
1- 3	-0.9(0.7)	-0.8(0.9)	-1.3(1.2)	-4.6(0.5)*
1-14	0.6(0.9)	1.2(1.1)	1.4(1.4)	

a Standard deviation is reported in parentheses

b Five of five male mice were found dead on test day 4.

c Test day 12 was the beginning of the recovery period for male mice.

Statistical methods: One-way AOV and Dunnett's tests were performed on both daily and summary interval data. (See Statistical Analyses)

* Statistically significant difference from control at alpha = 0.05.

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

TABLE 10

MEAN BODY WEIGHT GAINS OF FEMALE MICE

	MEAN BODY WEIGHT GAINS (g)			
GROUP:	II	IV	VI	VIII
CONCENTRATION (ppm):	0	240	720	1500
DAYS ON TEST				
1- 2	-0.4(0.2)[a]	-1.3(0.7)*	-0.7(0.7)	-1.4(0.4)*
2- 3	-0.1(0.5)	0.1(0.8)	0.0(0.3)	-1.8(1.1)*
3- 4	0.1(0.6)	0.1(0.7)	0.3(0.3)	[b]
4- 5	0.0(0.7)	0.2(1.0)	0.5(0.5)	
5- 7	0.9(0.7)	0.5(1.4)	0.4(1.4)	
7- 8	0.1(1.0)	0.5(0.4)	-0.7(0.7)	
8- 9	-0.1(0.9)	-0.2(0.9)	0.4(0.1)	
9-10	0.5(1.0)	0.3(1.1)	0.8(1.0)	
10-11	1.1(0.3)	-0.3(1.3)	0.5(0.5)	
11-12[c]	-1.1(0.7)	0.0(0.9)	-0.8(0.5)	
12-13	-0.3(0.4)	0.2(0.3)	-0.2(0.8)	
13-14	0.4(0.9)	0.5(0.7)	0.0(0.9)	
1-11	2.0(0.4)	0.0(0.6)*	1.5(1.4)	
11-14	-1.1(0.3)	0.7(1.3)*	-1.0(0.5)	
1- 3	-0.4(0.4)	-1.2(0.9)	-0.8(0.5)	-3.2(1.4)
1-14	0.9(0.5)	0.7(1.7)	0.5(1.1)	

[a] Standard deviation is reported in parentheses

[b] Four of five female mice were found dead on test day 4. The remaining female mouse was sacrificed in extremis on test day 4 after being weighed. Interval data was not reported.

[c] Test day 12 was the beginning of the recovery period for female mice.

Statistical methods: One-way AOV and Dunnett's tests were performed on both daily and summary interval data. (See Statistical Analyses)

* Statistically significant difference from control at alpha = 0.05.

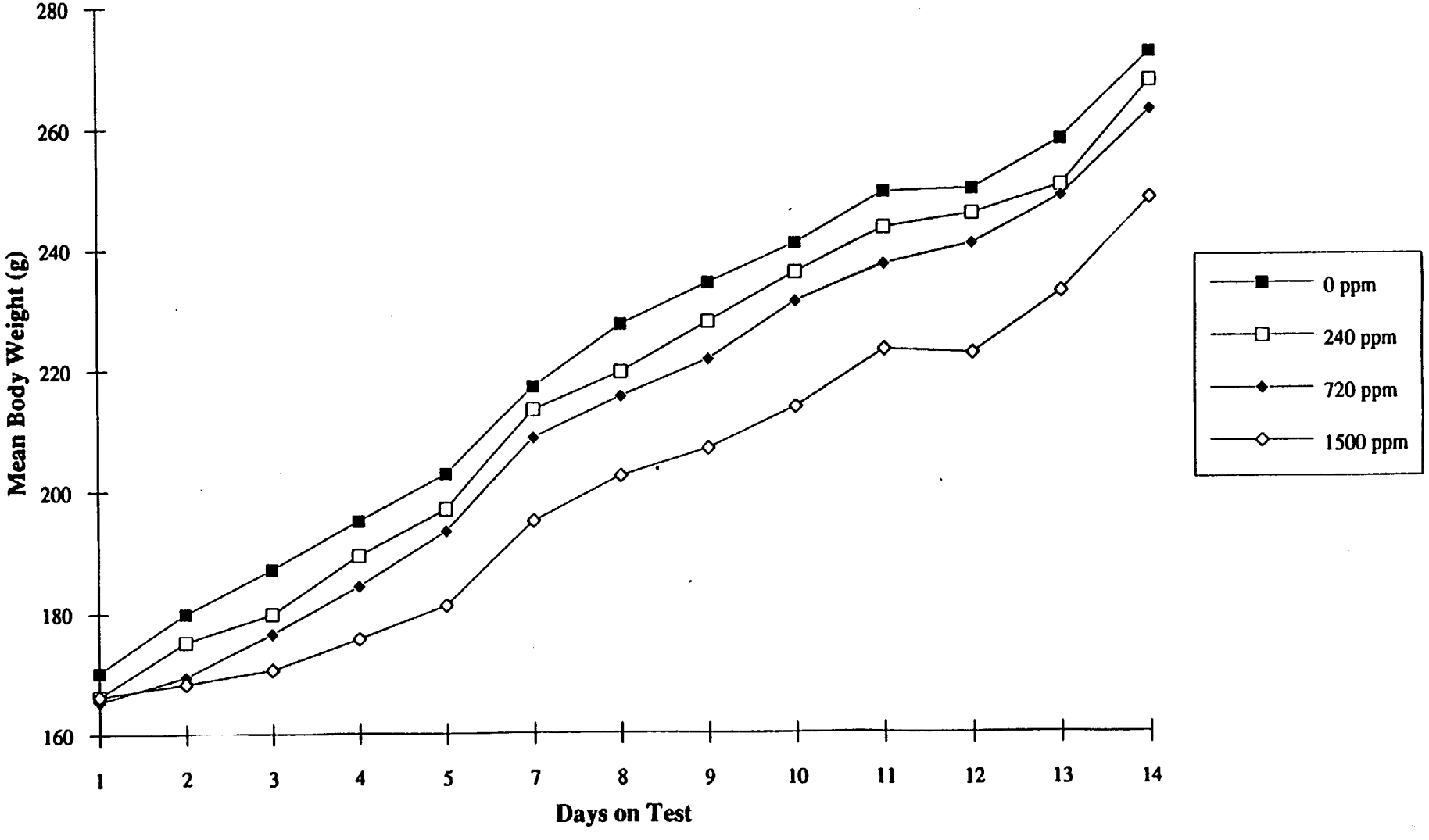
TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

FIGURES

TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 1

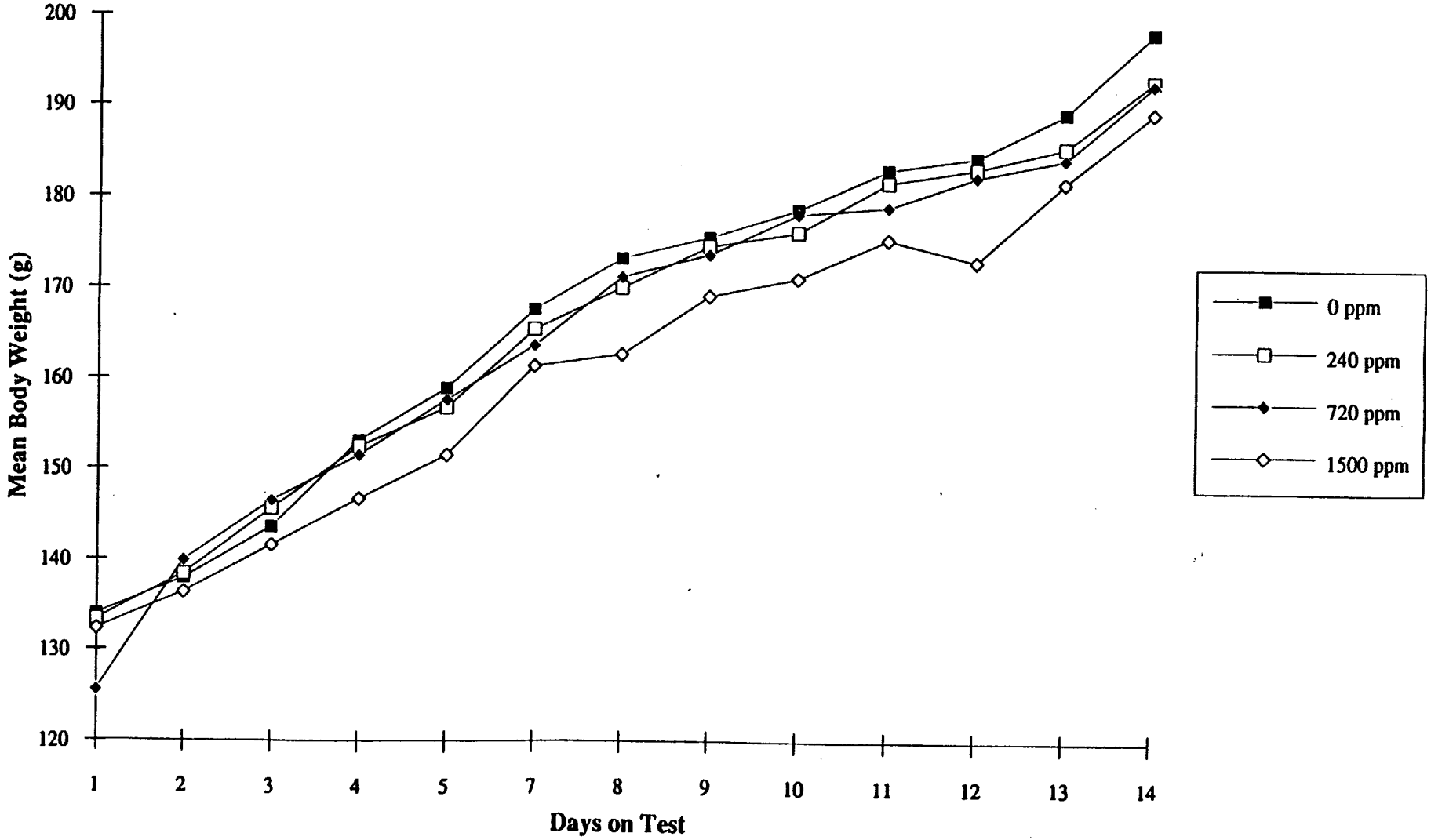
MEAN BODY WEIGHTS OF MALE RATS



TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 2

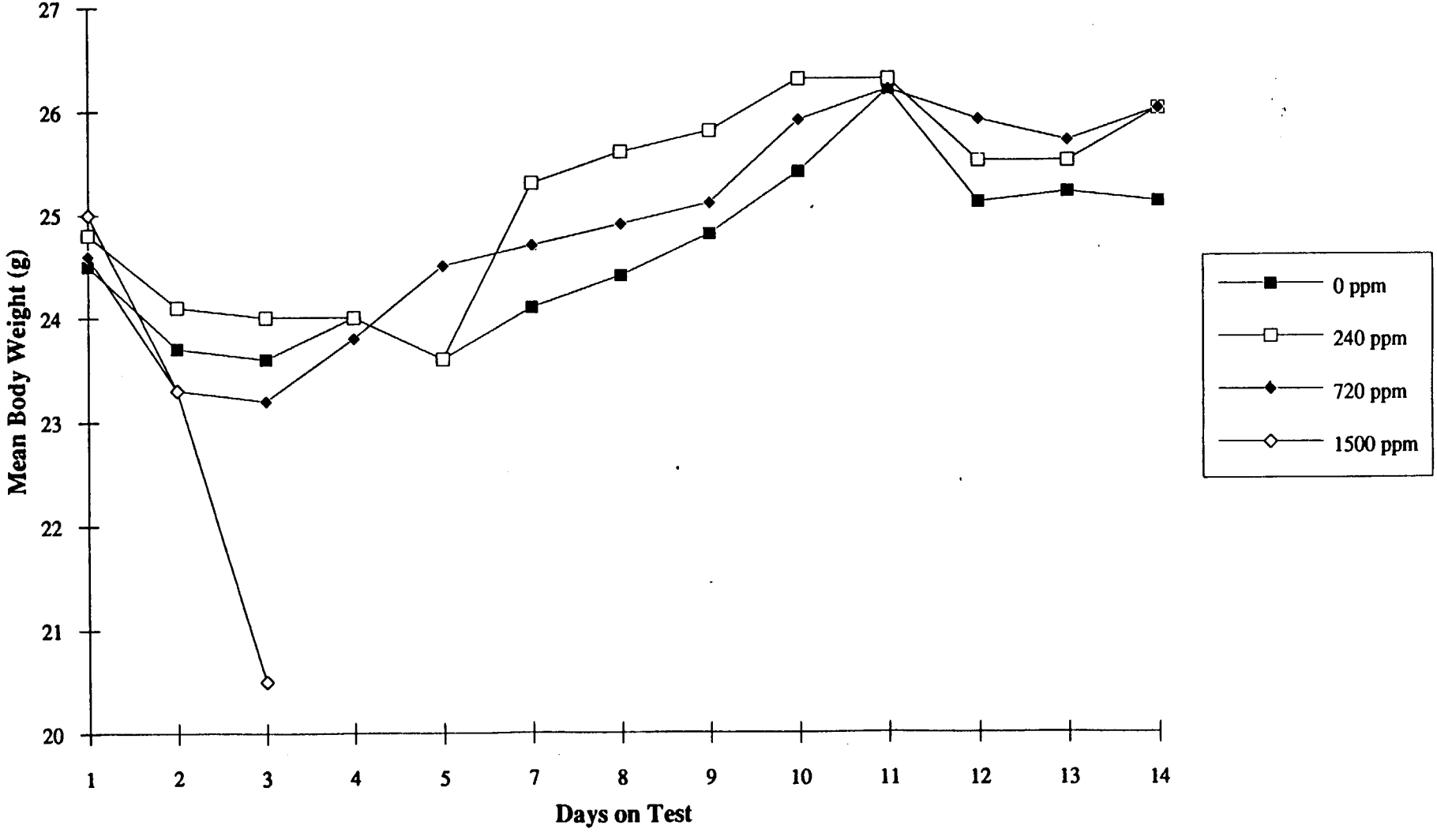
MEAN BODY WEIGHTS OF FEMALE RATS



TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 3

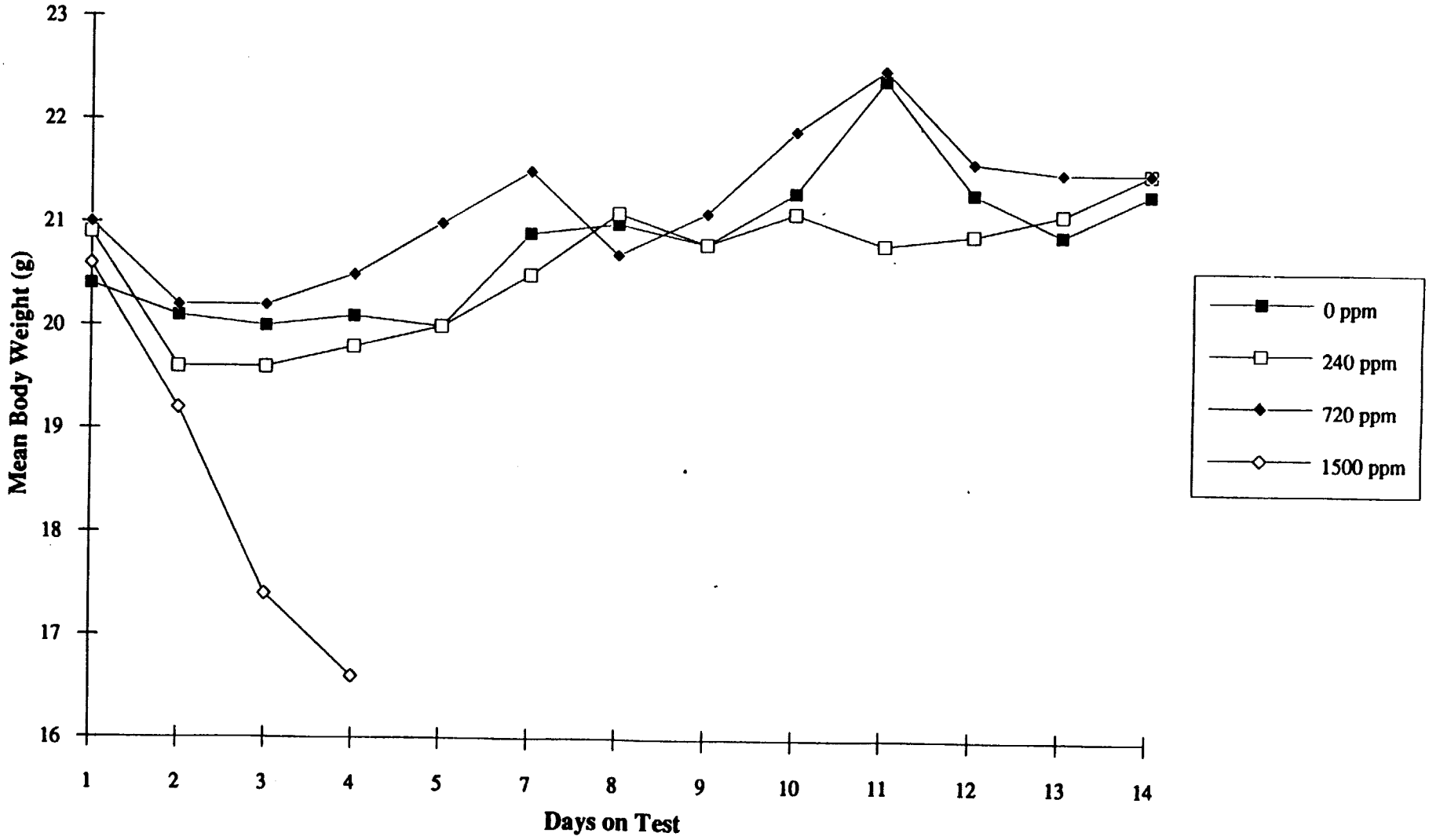
MEAN BODY WEIGHTS OF MALE MICE



TWO-WEEK RANGE-FINDING STUDY WITH 4-VINYLCYCLOHEXENE

FIGURE 4

MEAN BODY WEIGHTS OF FEMALE MICE



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In Vitro METABOLISM OF 4-VINYLCYCLOHEXENE AND METABOLITES

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PREVENTION AND TOXICS
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Study Completed on
September 21, 1993

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Medical Research Project No. 9298-001

Sponsor's Study No. BUT-21.0-COT-DHL-01

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Laboratory Project ID
Haskell Laboratory Report No. 600-93

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with EPA TSCA Good Laboratory Practice Standards (40 CFR 792).

Submitter: E. I. du Pont de Nemours and Company

Sponsor: Chemical Manufacturers Association

Study Director: Douglas A. Keller 9/21/93

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To the best of my knowledge, the study described in this final report was conducted in compliance with Good Laboratory Practice Standards (40 CFR 792), and no deviations existed that affected the validity of the study.

Sponsor Representative: 9/22/93

Elizabeth J. Moran, Ph.D., D.A.B.T. (date)
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GENERAL INFORMATION

Substances Tested	Lot Number	Purity	Haskell Number	CAS Registry Number
4-Vinylcyclohexene	1	99%	19353	100-40-3
4-Vinylcyclohexene-1,2-epoxide	00305TY	98%	19498	106-86-5
4-Vinylcyclohexene-7,8-epoxide	TMG-I-94C	98%	19646	5116-65-4
4-Vinylcyclohexene Diepoxide	03426LY	99%	19264	106-87-6
4-Vinylcyclohexene-1,2-diol	00305PY	95%	19383	31646-64-7
4-Vinylcyclohexene-7,8-diol	TMG-I-116A	98%	19778	64011-53-6
	TMG-I-113D	99%	19779	64011-53-6

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Study Start/End Dates:

Methods Development: 2-20-93 / 9-21-93
Experimental Phase: 5-03-93 / 9-21-93

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SUMMARY

Microsomal preparations from liver, lung and ovaries obtained from female Crl:CD®BR rats and female $B_6C_3F_1$ mice were tested for their ability to metabolize 4-vinylcyclohexene (4-VCH) and its epoxide metabolites. The reactions measured were metabolism of 4-VCH to 4-VCH-1,2-epoxide and 4-VCH-7,8-epoxide, 4-VCH-1,2-epoxide to 4-VCH diepoxide and 4-VCH-1,2-diol, 4-VCH-7,8-epoxide to 4-VCH diepoxide and 4-VCH-7,8-diol, and hydrolysis of 4-VCH diepoxide.

Samples of microsomes were incubated with the test chemical and necessary cofactors for 10 - 15 minutes at 37°C. Control reactions consisting of heat-treated microsomes were run concomitantly. Reactions were stopped by addition of ice-cold ethyl acetate and the reaction products were analyzed by gas chromatography. Reaction rates were calculated as nmol product/minute/mg microsomal protein. Reaction rates were also expressed per nmol cytochrome P450 if the product of the reaction was an epoxide. The K_m and V_{max} for each reaction was calculated from the reaction rates, if sufficient reaction product was detected.

Rat liver and lung microsomes and mouse liver and lung microsomes metabolized 4-VCH to 4-VCH-1,2-epoxide at detectable rates. Mouse liver had a V_{max} for the reaction that was 56-fold higher than for rat liver (11.1 and 0.20 nmol/min/mg protein for the mouse and rat, respectively). The V_{max} for mouse lung was 2 - 4-fold higher than for rat lung, depending on whether the activity was expressed on the basis of protein or cytochrome P-450. Formation of 4-VCH-1,2-epoxide was not detected in ovarian microsomes from rats or mice.

Metabolism of 4-VCH to 4-VCH-7,8-epoxide was detected in microsomes from rat liver and mouse liver and lung. Rat liver microsomes were very poor at forming 4-VCH-7,8-epoxide, with an estimated K_m of 1.1 mM and a V_{max} of 0.007 nmol/min/mg protein. Mouse liver and lung, in contrast, had K_m values for formation of the 7,8-epoxide that were equal to those for formation of the 1,2-epoxide (approximately 2 mM for liver and 0.7 mM for lung), although the V_{max} rates for 7,8-epoxide formation were 2- to 12-fold lower than for formation of the 1,2-epoxide. Mouse lung was more efficient at metabolism of 4-VCH to 4-VCH-7,8-epoxide than was mouse liver. The V_{max}/K_m ratio for mouse lung was 2.73 and for mouse liver was 0.43 (based on mg protein). No

metabolism of 4-VCH to 4-VCH-7,8-epoxide was detected in microsomes from rat lung or ovaries of either species.

Rat and mouse liver had very similar K_m and V_{max} values for metabolism of 4-VCH-1,2-epoxide to 4-VCH diepoxide, and the V_{max}/K_m ratios were similar for rat and mouse liver. The V_{max} for rat liver was 3.69 and for mouse liver was 5.35 nmol/min/mg protein. Lung tissue from rats and mice had lower K_m values than for liver. The lung activity was higher than for the liver when based on the amount of cytochrome P-450 in the tissue. When expressed per nmol P450, the V_{max}/K_m ratios were higher for the lung than for the liver, by a factor of 8 for the rat and a factor of 18 for the mouse. Rat and mouse ovary tissue did not have detectable capacity to metabolize 4-VCH-1,2-epoxide to the diepoxide.

As with the metabolism of the 1,2-epoxide to the diepoxide, rat and mouse liver and lung have very similar K_m and V_{max} values for metabolism of 4-VCH-7,8-epoxide to the diepoxide. The V_{max} values for mouse and rat liver are 8.83 and 9.45 nmol/min/mg protein, respectively. The V_{max}/K_m ratios are virtually identical for rat and mouse liver. Lung tissue from rats and mice had more efficient activity than the liver based on the amount of cytochrome P-450 present in the tissue, by a factor of 2 for rats and a factor of 30 for mice. Mouse lung was particularly active and had a low K_m. Rat and mouse ovaries did not have detectable rates of metabolism of 4-VCH-7,8-epoxide to the diepoxide.

Hydrolysis of 4-VCH-1,2-epoxide to 4-VCH-1,2-diol was detected only in rat and mouse liver microsomes. K_m and V_{max} values were virtually identical for the two species.

Hydrolysis of 4-VCH-7,8-epoxide to 4-VCH-7,8-diol was detected only in rat liver microsomes. The reaction had a very high V_{max} (135.8 nmol/min/mg protein), but is probably of little consequence since the rat forms 4-VCH-7,8-epoxide from 4-VCH very inefficiently.

Hydrolysis of 4-VCH diepoxide was detected in rat and mouse liver and lung, and in rat ovary microsomes. The V_{max} for rat liver was 9-fold greater than for mouse liver (5.51

and 0.63 nmol/min/mg protein, respectively, and lung and ovary tissues were not as active as rat liver.

The balance of activation versus detoxication reactions in rats and mice indicates that the mouse may be more susceptible to 4-VCH toxicity resulting from epoxide metabolites. In general, the mouse is more efficient at metabolism of 4-VCH to epoxides than is the rat, and has greater V_{max}/K_m ratio for epoxide formation. In contrast, the rat may be more efficient at hydrolysis of epoxides. Thus, the rat would tend to produce a lower concentration of epoxide metabolites than the mouse, given an equal dose of 4-VCH.

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QUALITY ASSURANCE DOCUMENTATION

Medical Research Project No. 9298-001

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Findings Reported to: Study Director - 9/17/93
Management - 9/21/93

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STUDY PERSONNEL

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Managers:	Robert W. Rickard, Ph.D. (01/22/92-05/06/93) Matthew S. Bogdanffy, Ph.D. (05/07/93-present)
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The following individuals were responsible for the Quality Assurance auditing of the study:

Auditor:	Kimberly B. Brebner, B.S.
Coordinator:	Joseph C. Hamill

INTRODUCTION

4-Vinylcyclohexene (4-VCH) is a dimer of 1,3-butadiene and is formed spontaneously during the manufacture of 1,3-butadiene. 4-VCH is also produced for use as an intermediate in polymer manufacturing.

4-VCH caused decreased body weights in mice and rats in subchronic oral studies, at doses of 400 mg/kg/day or greater in rats, and 600 mg/kg/day or greater in mice.[1] 4-VCH caused ovarian tumors in mice at 200 and 400 mg/kg/day in long-term oral studies, but mortality was too high in rats and in male mice to evaluate oncogenicity and chronic toxicity.[2]

4-VCH is known to be metabolized to mono- and di-epoxides (Figure 1), which are suspected to cause the ovarian toxicity in mice. Mice metabolize 4-VCH faster than rats. After oral dosing, the monoepoxide metabolite 4-VCH-1,2-epoxide was detected in mouse blood but not in rats. At high concentrations of 4-VCH, the mouse liver microsomes were found to metabolize 4-VCH faster than rat liver microsomes, while human liver microsomes metabolized 4-VCH at a slightly lower rate than did rat liver microsomes.[3,4]

OBJECTIVE

The objective of this study is to measure the kinetic constants for the metabolism of 4-VCH to epoxide metabolites, and the further metabolism of the epoxides. These data, along with the partition coefficients measured in an earlier study, will allow for prediction of concentrations of 4-VCH or its metabolites in tissues of rodents, and will allow construction of a physiologically-based pharmacokinetic model for metabolism of 4-VCH metabolism and disposition.

MATERIALS AND METHODS

A. Test Substances

4-Vinylcyclohexene, approximately 99% pure, was obtained from Union Carbide Corporation, Institute, West Virginia, and assigned Haskell sample number 19353. 4-Vinylcyclohexene-1,2-epoxide, approximately 98% pure, was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, and assigned Haskell sample number 19498. 4-Vinylcyclohexene diepoxide, approximately 99% pure, was obtained from Aldrich Chemical Company and assigned Haskell sample number 19264. 4-Vinylcyclohexene-7,8-epoxide, approximately 98% pure, was obtained from the University of Arizona and assigned Haskell sample number 19646. 4-Vinylcyclohexene-1,2-diol, approximately 95% pure, was obtained from Aldrich Chemical Company and assigned Haskell sample number 19383. 4-Vinylcyclohexene-7,8-diol, approximately 98% pure, was obtained from the University of Arizona and assigned Haskell sample numbers 19778 and 19779.

The purity of the test substances was assayed by gas chromatography at least once every three months after initiation of testing.

B. Test Species

Female Crl:CD®BR rats and female $B_6C_3F_1$ mice were obtained from Charles River Laboratories, Inc. in Kingston, New York and Raleigh, North Carolina, respectively. Rats and mice were quarantined for approximately one week before release for use on the study. The rats were 35-64 days of age when they were received and were sacrificed for use on the study when the body weights were between 200 and 300 grams. A total of 245 rats were used for this study. The mice were approximately 65 days of age when they were received, and were sacrificed for use on the study when the body weights were between 20 and 27.1 grams. A total of 280 mice were used for this study. The number of rats and mice used includes methods development experiments.

C. Animal Husbandry

After release from quarantine, all rats and mice were housed individually in stainless steel, wire-mesh cages suspended above absorbent cage boards. Animal rooms were kept between 21-25°C and 40-60% relative humidity. Animal rooms were artificially illuminated with a 12-hour light/dark cycle. All rats were fed Purina® Certified Rodent Chow® #5002 and tap water *ad libitum*. All rats and mice used for this study were free of any clinical signs of disease.

D. *In Vitro* Metabolism Testing

Tissue homogenates were prepared and centrifuged to yield microsomes. Rats and mice were anesthetized with CO_2 and sacrificed by exsanguination. Tissues were removed from animals and homogenized in an ice-cold buffer of 0.25 M sucrose with 4 mM $MgCl_2$, 2 mM glutathione, 1 mM EDTA, and 0.05 mM leupeptin, adjusted to pH 7.4 with NaOH. Homogenates were centrifuged at approximately 1,000 x g for 15 minutes, then the supernatant was removed and centrifuged at approximately 21,000 x g for 15 minutes. The supernatant from the 15,000 x g centrifugation was then centrifuged at approximately 193,000 x g for 1 hour and 10 minutes. The supernatant was then discarded and the pellet (containing microsomes) was resuspended in the homogenization buffer. All centrifugations were performed at approximately 4°C, and microsomal samples were stored at approximately -80°C until use.

The protein content of the microsomal samples was analyzed with the Bradford method.[5] Total cytochrome P-450 content was assayed by the method of Omura and Sato.[6]

Microsomes from rat and mouse liver, lung, and ovary were incubated with the test chemicals for 15 minutes at 37°C in sealed vials placed in a vortexer. The microsomes were diluted in 0.1 M phosphate buffer, pH 7.4, with 15 mM $MgCl_2$ and 0.1 mM EDTA. An NADPH regenerating system was used for reactions that were producing an epoxide metabolite. If the reaction was producing a diol metabolite, an NADPH regenerating system was not used since epoxide hydrolase does not require NADPH.[7] The NADPH regenerating system was composed of 0.5 mM $NADP^+$, 10 mM glucose-6-phosphate and

two units of glucose-6-phosphate dehydrogenase. Protein concentrations used in the reactions ranged from 0.3 mg/ml to 3.8 mg/ml. The concentration of protein used was in the linear range of the curve for activity versus protein concentration. The total volume of the incubation mixtures was approximately 2.0 ml. Control samples were run at the same time as the samples of interest. Control samples contained all of the reagents as the other samples, except that the microsomes had been boiled for ten minutes before use. For reactions in which an epoxide metabolite was expected, 1 mM 1,1,1-trichloropropene oxide (TCPO) was added as an inhibitor of epoxide hydrolase activity.

Tissue samples were tested for their ability to catalyze the following reactions: 4-VCH to 4-VCH-1,2-epoxide and 4-VCH-7,8-epoxide; 4-VCH-1,2-epoxide to 4-VCH diepoxide and 4-VCH-1,2-diol; 4-VCH-7,8-epoxide to 4-VCH diepoxide and 4-VCH-7,8-diol; and hydrolysis of 4-VCH diepoxide. Starting concentrations for each assay exceeded the limit of detection of the test chemical and the metabolite expected, and spanned at least one order of magnitude. At least three starting concentrations were tested for each reaction, and each reaction was run in duplicate, except for the mouse ovary samples.

After the incubation period, the sample was split into two 900 µl aliquots and cyclodecene added to a final concentration of 0.044 mM as an internal standard. Two hundred seventy microliters of ice-cold ethyl acetate was added to the sample to stop the reaction. The samples were mixed well, then centrifuged for ten minutes to pellet the denatured protein, and the organic layer was analyzed for products of metabolism. In the case of the hydrolysis of 4-VCH diepoxide, the product of metabolism was not analyzed for, but the rate of disappearance of the diepoxide was measured.

Analysis of metabolites was performed on a Hewlett-Packard 5890 gas chromatograph with a 0.53 mm x 30 m RSL-300 column and a flame ionization detector. The injection volume was 5 µl. The method was adapted from Smith *et al.*[3] The oven temperature was started at 80°C, then increased to 100°C over 2 minutes. Retention times for the analytes were approximately 0.8 minutes for 4-VCH, 1.7 minutes for 4-VCH-1,2-epoxide, 2.0 minutes for 4-VCH-7,8-epoxide, 2.4 minutes for cyclodecene, 5.4 minutes for 4-VCH-1,2-diol, and 7.9 minutes for 4-VCH-7,8-diol. 4-VCH diepoxide was detected as two peaks, one eluting with a retention time of 5.0 minutes and the other at 5.1 minutes.

These peaks were the optical isomers of the diepoxide, and were added together to determine total peak area.

E. Data Analysis

Each peak area measured for a metabolite was converted to a mM concentration in the incubation mixture, from which a rate of nmole product/min/mg microsomal protein was calculated. The concentration of metabolites was corrected for the percent recovery of the metabolite from ethyl acetate, if necessary. A rate based on the nmole of cytochrome P-450 in the sample was also calculated for reactions dependent upon cytochrome P-450 (reactions where the product was an epoxide). Since epoxide hydrolase is not a cytochrome P-450 containing enzyme, the rates of hydrolysis reactions were calculated only on the basis of microsomal protein. Where control reactions showed the formation of product, the rate for the controls was subtracted from the rate for the non-control samples. In the case of the reaction measuring the rate of hydrolysis of 4-VCH diepoxide, a control sample was run at the same concentration as each non-control sample, and the difference between the amount of diepoxide present in the non-control samples and the control samples was used to calculate rates of metabolism. All of the above calculatons, including linear regression, were performed by Microsoft Excel.

Reaction rates were analyzed with EZ-FIT, a computer program designed to calculate kinetic constants for metabolic reactions.[8] The best estimate and standard deviation for V_{max} and K_m are reported in the tables.

RESULTS

The purity of the test materials as assayed at Haskell Laboratory agreed with the analyses of the suppliers, within analytical variability. The purities of 4-VCH-7,8-epoxide and 4-VCH-7,8-diol were not reported by the supplier, but the compunds were analyzed when used. The purity of 4-VCH was 99% or greater at each time measured. The purity of 4-VCH-1,2-epoxide ranged from 95 - 99%, the purity of 4-VCH-7,8-epoxide ranged from 97 - 99%, and the purity of 4-VCH diepoxide ranged from 83 - 99%. The 83% value for the diepoxide was considered an anomaly, since the sample was analyzed again ten days

later and was found to be 98% pure. 4-VCH-1,2-diol was analyzed for purity twice, and was found to be 83% pure and 92% pure. Although the purity of the 4-VCH-1,2-diol may have been somewhat lower than the 95%+ purity stated by the supplier, the slightly lower purity does not affect the study results since the diol was used only as a standard and not as a starting material. One lot of the 4-VCH-7,8-diol was found to be 98% pure; the other lot was found to be 99% pure.

The reactions of 4-VCH metabolism were linear in the protein range of 0.3 - 4 mg of protein (Figures 2,3) and the reactions were linear for at least 15 minutes. The results for each reaction, including starting concentrations and results from blank reactions, are listed in Appendix A. The recoveries of the metabolites from ethyl acetate extracts of microsomal samples were approximately 96% for 4-VCH-1,2-epoxide, 89% for 4-VCH-7,8-epoxide, 71% for 4-VCH diepoxide, 59% for 4-VCH-1,2-diol, and 20% for 4-VCH-7,8-diol. Corrections were made for recovery of the diepoxide and the diols, but not for the monoepoxides.

The reaction of 4-VCH to 4-VCH-1,2-epoxide proceeded at a detectable rate in rat liver and lung, and in mouse liver and lung (Table 1). Figures 4-11 show Lineweaver-Burk plots of the data for the metabolism of 4-VCH to 4-VCH-1,2,-epoxide. No reaction product was detected in rat or mouse ovary. Mouse liver had a V_{max} value 56-fold higher than for the rat liver. Rat and mouse lung each had a greater V_{max} for metabolism of 4-VCH to 4-VCH-1,2-epoxide than did the liver of the same species (when expressed per nmol P450), and the K_m for lung tissue was lower than for liver tissue. In lung tissue, however, the reaction proceeded only 2.5 - 3.8-fold faster in mice than in rats. Thus, while lung tissue has a greater capacity to metabolize 4-VCH to the 1,2-epoxide (per unit weight) and has a lower K_m, the difference between rats and mice for lung is not as great as it is for liver tissue. The ratio of V_{max} to K_m indicates the efficiency of the reaction at sub-saturating concentrations. The V_{max}/K_m ratio for metabolism of 4-VCH to 4-VCH-1,2-epoxide is 32-fold higher for mouse liver than for rat liver, indicating higher efficiency of metabolism in the mouse at concentrations of 4-VCH that are likely to be encountered under bioassay conditions. In lung the species difference is less dramatic. The V_{max}/K_m ratio is 4- to 7-fold higher for mouse lung than for rat lung, depending on whether the rate is expressed per mg protein or per nmol P450.

The reaction of 4-VCH to 4-VCH-7,8-epoxide proceeded at a detectable rate in rat and mouse liver and in mouse lung (Table 2, Figures 12-17). No reaction product was detected in rat lung or in the ovaries from rats or mice. The reaction was extremely inefficient in rat liver, with a V_{max}/K_m ratio of 0.005. The V_{max} was very small, 0.007 nmol/min/mg protein. Mouse liver had a K_m for the reaction of 2.14 mM, approximately the same as for metabolism of 4-VCH to 4-VCH-1,2-epoxide. The V_{max} for metabolism to the 7,8-epoxide in mouse liver, however, was approximately 12-fold lower than for metabolism to the 1,2-epoxide. Mouse lung, on the other hand, produced 4-VCH-7,8-epoxide at rates approximately half of those for the production of the 1,2-epoxide. Thus, only the mouse has significant capacity to metabolize 4-VCH to the 7,8-epoxide.

Table 3 shows the kinetic constants for metabolism of 4-VCH-1,2-epoxide to 4-VCH diepoxide. Lineweaver-Burk plots are shown in Figures 18-25. Rat and mouse liver were very similar in their K_m and V_{max} values for this reaction, and the V_{max}/K_m ratios were similar for rat and mouse liver. Lung tissue from rats and mice had lower K_m values than for liver, and the lung activity was higher than for the liver when based on the amount of cytochrome P-450 in the tissue. The V_{max}/K_m ratios were higher for the lung than for the liver, with mouse lung having the highest ratio. Rat and mouse ovary tissue did not have detectable capacity to metabolize 4-VCH-1,2-epoxide to the diepoxide.

The kinetic constants for the metabolism of 4-VCH-7,8-epoxide to 4-VCH diepoxide are shown in Table 4 (Figures 26-33). As with the metabolism of the 1,2-epoxide to the diepoxide, rat and mouse liver have very similar K_m and V_{max} values. The V_{max}/K_m ratios are virtually identical for rat and mouse liver. Lung tissue from rats and mice had higher activity than the liver based on the amount of cytochrome P-450 present in the tissue. Mouse lung was particularly active and had a low K_m. Rat and mouse ovaries did not have detectable rates of metabolism of 4-VCH-7,8-epoxide to the diepoxide.

The metabolism of 4-VCH-1,2-epoxide to 4-VCH-1,2-diol was only detectable in liver tissue from rats and mice (Table 5, Figures 34-35). Rat and mouse liver had virtually identical K_m and V_{max} values for the reaction. Lung and ovary tissues from rats and mice had no detectable activity.

The hydrolysis of 4-VCH-7,8-epoxide to 4-VCH-7,8-diol was only detectable in rat liver (Table 6, Figure 36). Hydrolysis of the 7,8 epoxide was determined to be a high capacity process in the rat liver, with a V_{max} of 135.8 nmol/min/mg protein. Mouse tissues and rat lung and ovary did not have detectable activity.

Table 7 shows the kinetic constants for the hydrolysis of 4-VCH diepoxide, presumably to the tetrol metabolite. Lineweaver-Burk plots are shown in Figures 37 and 38. Hydrolysis of 4-VCH diepoxide was detectable in rat and mouse liver and lung and in rat ovary. The reaction rates appeared to increase with concentration when the substrate concentrations were low, but at high substrate concentrations (>0.24 mM) the reaction rates were lower than at low substrate concentrations. In liver tissue, at very low substrate concentrations ($\leq$0.06 mM for rat liver, 0.01 mM for mouse liver) there was no diepoxide detected after the 15 minute incubation in non-control samples, while controls had detectable diepoxide. Therefore, for purposes of calculating rates of reaction it was assumed that the diepoxide was present at the limit of detection. This assumption will underestimate the actual rate of reaction, but helps illustrate the differences in rat and mouse activity. Only in liver was the increase in rate with increasing substrate concentration large enough to calculate kinetic constants. Rat liver had a V_{max} nine-fold higher than did mouse liver, but mouse liver had a lower K_m. Caution should be used, however, in interpreting the K_m values, since the assumption made in the calculations (where no diepoxide was detected after 15 minutes at very low concentrations) will lead to an overestimate of the K_m, more so from rats than from mice. For lung and ovary, the reactions seemed to be saturated even at the lowest concentrations where substrate was detectable, and therefore only a V_{max} is reported with no standard deviation. Maximum activity is approximately 14-fold lower in rat lung than in rat liver, and is approximately equal in mouse liver and lung. There was not sufficient ovary tissue from mice to adequately test the hydrolysis of the diepoxide, since controls had to be run for each substrate concentration. Reactions run without controls present showed that the mouse ovary had activity less than or equal to the rat ovary, and in both species the ovarian activity was low.

DISCUSSION

The overall results from this study indicate that the mouse has significantly greater capacity to metabolize 4-VCH to reactive species than does the rat, and in many cases performs the reactions more efficiently than the rat. In particular, mouse lung tissue is very active in its ability to metabolize 4-VCH to epoxides and diepoxides. In contrast, the mouse does not hydrolyze epoxides well, while the rat hydrolyzes the epoxides to a greater extent than the mouse. This balance of activation reactions with detoxication reactions leads to the conclusion that the mouse may be more susceptible to the toxic effects of 4-VCH, if epoxide metabolites are the species that ultimately cause the toxic insult.

The data presented in this report indicate that 4-VCH is metabolized predominantly to the 1,2-epoxide, with the 7,8-epoxide being a minor metabolite. These results agree with the results of Watabe, *et al.*[9] *In vitro*, rat tissues are extremely poor metabolizers of 4-VCH to the 7,8-epoxide, while mouse liver produces the 1,2-epoxide at rates more than 10-fold greater than it produces the 7,8-epoxide. Only in mouse lung are the kinetic constants indicative of a potential for production of 4-VCH-7,8-epoxide in significant amounts under *in vivo* conditions.

The 1,2-epoxide of 4-VCH is readily formed in microsomes from rat liver and lung, and mouse liver and lung. Mouse lung had the greatest ratio of V_{max} to K_m of the tissues that metabolized 4-VCH to the 1,2-epoxide, and also had the lowest K_m of the tissues. Lung tissue in general is more efficient at metabolizing 4-VCH to the 1,2-epoxide than is liver tissue.

The V_{max} values for metabolism of 4-VCH to 4-VCH-1,2-epoxide agree well with the data of Smith *et al.*[3] In the study by Smith *et al.*, microsomes from rat and mouse livers were incubated with 1 mM 4-VCH, which is less than the K_m reported here. The rates of reaction were 1.4 and 9.1 nmole/min/mg protein for rat and mouse, respectively, compared to V_{max} values of 0.2 and 11.1 nmole/min/mg protein for rat and mouse in the current study. One reason for the slight difference may be that Smith *et al.* used F344 rats instead of Crl:CD®BR rats.

Further metabolism of 4-VCH-1,2-epoxide to 4-VCH diepoxide is a higher efficiency reaction in microsomes from rat liver and lung and in mouse liver and lung than is metabolism of 4-VCH to the 1,2-epoxide. The K_m values for formation of the diepoxide are all under 1 mM, and the V_{max}/K_m ratios are all higher than for formation of the 1,2-epoxide. Mouse lung is again the most efficient producer of 4-VCH diepoxide from the 1,2-epoxide particularly when expressed on the basis of cytochrome P-450 content, while rat lung is also very efficient when the rates are expressed on the basis of cytochrome P-450 content. Thus, when 4-VCH-1,2-epoxide is formed in significant quantities in rat or mouse liver or lung, it is likely that the diepoxide will also be formed.

Metabolism of 4-VCH-7,8-epoxide to 4-VCH diepoxide was more efficient than was the formation of the 7,8-epoxide from 4-VCH. For microsomes from rat liver and mouse liver and lung, metabolism to the diepoxide from the 7,8-epoxide was shown to be a slightly greater capacity reaction than metabolism to the diepoxide from the 1,2-epoxide. Rat lung microsomes were slightly better at forming the diepoxide from the 1,2-epoxide than from the 7,8-epoxide. Thus, when 4-VCH-7,8-epoxide is formed in significant quantities in rat or mouse liver or lung, it is likely that the diepoxide will also be formed. In practice, however, it is unlikely that the rat will produce 4-VCH diepoxide from the 7,8-epoxide after *in vivo* administration of 4-VCH, since the formation of the 7,8-epoxide from 4-VCH is not an efficient reaction in the rat.

Metabolism of the monoepoxides to their respective diols was detected in liver tissue only. Hydrolysis of the 1,2-epoxide was detected in rat and mouse liver, while hydrolysis of the 7,8-epoxide was detected only in rat liver. Compared to formation of the diepoxide from the 1,2-epoxide, hydrolysis appears to be a more efficient reaction in both rat and mouse liver, with K_m values less than half of those for diepoxide formation, and V_{max}/K_m ratios 4 - 6-fold higher. Thus, the bulk of the 4-VCH-1,2-epoxide formed in rat and mouse liver may be hydrolyzed to the diol, rather than being metabolized to the diepoxide. In contrast, diepoxide may be formed in the lung from the 1,2-epoxide in preference to hydrolysis, since hydrolysis of the 1,2-epoxide was not detected in lung tissue. Hydrolysis of the 7,8-epoxide was difficult to detect due to the low recovery of the 4-VCH-7,8-diol, and activity may be present in mouse tissues and in rat lung. However, since rats have little capacity to produce the 7,8-epoxide, the hydrolytic activity towards the 7,8-epoxide may be of little consequence. The apparent lack of hydrolytic activity towards the 7,8-

epoxide in the mouse may be of consequence to the mouse upon exposure to 4-VCH, particularly in the lung.

Hydrolysis of 4-VCH diepoxide *in vivo* was described by Salyers, *et al.*[10] The rat has a greater capacity to metabolize the diepoxide to the tetrol than does the mouse after oral administration of the diepoxide. The ability of the rat to hydrolyze the diepoxide is confirmed in the present study, as is the greater capacity of the rat to perform the hydrolysis, compared to the mouse. Given the striking ovarian toxicity of 4-VCH diepoxide,[11] hydrolysis rates for the diepoxide may be critical in determining species differences in toxicity of 4-VCH and 4-VCH diepoxide.

The lack of detection of metabolic activity in ovarian tissues of the rat or mouse may be due to the low amounts of cytochrome P-450 present in the ovary. The ovary certainly has cytochrome P-450 activity,[12] but, given the role of ovarian tissue in maintenance of hormonal balance, the specific forms of cytochrome P-450 present in the ovary may be selective for metabolism of steroids, and may not have much activity towards 4-VCH or its metabolites.

Smith *et al.*[3] detected epoxides of 4-VCH in blood of mice, but not rats, administered large gavage doses of 4-VCH. Given the apparent stability of epoxides of 4-VCH and the lack of detectable ovarian metabolic activity towards 4-VCH, it is likely that the observed ovarian toxicity in the mouse is due to circulating epoxides initially produced in the liver and lung, and not to site-specific metabolism in the ovary.

The balance of activation and detoxication of 4-VCH is likely to be a critical determinant in the toxicity of 4-VCH administered by any route. To date, only gavage studies have been performed in rats and mice with 4-VCH. Toxicity was observed in the ovary of female mice administered 4-VCH, but not in the ovary of female rats. No lung toxicity was described in these studies. Given the ability of lung tissue from rats and mice to metabolize 4-VCH, it is possible that the lung tissue may be a target of toxicity during inhalation studies. It is likely, however, that the mouse lung will be more susceptible to toxicity from 4-VCH than the rat lung, since the mouse lung produces epoxides and the diepoxide from 4-VCH more efficiently than does the rat lung.

This study confirms the species differences in metabolism of 4-VCH described by Smith *et al.*[3] The results in this report extend the knowledge of species differences in 4-VCH metabolism to lung tissue, and to metabolism of 4-VCH epoxides. This study also confirms that a second strain of rat, the female Crl:CD®BR rat, differs greatly from the female $B_6C_3F_1$ mouse in its metabolism of 4-VCH. In addition, kinetic constants have been determined for the reactions in many tissues, allowing a more detailed description of metabolism and disposition of 4-VCH to be developed than was possible earlier, particularly at the low concentrations expected during bioassays. Given the rate of metabolism of 4-VCH in human liver[4] in comparison to rat and mouse liver[3], it is likely that rats would be a better model for human metabolism of 4-VCH and toxicity from 4-VCH exposure. This relationship also appears to be true for 1,3-butadiene, as shown by the results of Csanady *et al.*[13]

RECORDS AND SAMPLE RETENTION

All original records are retained at Haskell Laboratory or at the Records Management Center, E. I. du Pont de Nemours and Company, Wilmington, Delaware. Archived samples of the test materials are retained at Haskell Laboratory.

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11. Smith, B.J., Mattison, D.R. and Sipes, I.G. (1990). The role of epoxidation in 4-vinylcyclohexene-induced ovarian toxicity. *Toxicol. Appl. Pharmacol.* **105**, 372-381.

12. Okey, A.B. (1989). Enzyme induction in the cytochrome P-450 system. *Pharmac. Ther.* **45**, 241-298.

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TABLES

Table 1

Kinetic Constants for Metabolism of 4-Vinylcyclohexene to 4-Vinylcyclohexene-1,2-Epoxide

Tissue	K_m* (mM)	V_{max} (nmol/min/mg protein)	V_{max} (nmol/min/nmol P450)	V_{max}/K_m (per mg protein)	V_{max}/K_m (per nmol P450)
Rat Liver	1.58 ± 0.51	0.20 ± 0.04	0.13 ± 0.02	0.13	0.08
Mouse Liver	2.71 ± 0.99	11.1 ± 2.78	7.36 ± 1.79	4.10	2.71
Rat Lung	1.06 ± 0.12	1.39 ± 0.07	7.64 ± 0.40	1.31	7.21
Mouse Lung	0.61 ± 0.06	3.49 ± 0.14	29.5 ± 1.16	5.72	48.4
Rat Ovary	--[a]	--	--	--	--
Mouse Ovary	--	--	--	--	--

Values for K_m and V_{max} are reported as means ± standard deviation.

* K_m values are based on analysis of data expressed as nmol/min/mg protein only. K_m values expressed as nmol/min/nmol P450 are within 2% of the reported K_m value.

a No reaction product detected.

Table 2

Kinetic Constants for Metabolism of 4-Vinylcyclohexene to 4-Vinylcyclohexene-7,8-Epoxide

Tissue	K_m* (mM)	V_{max} (nmol/min/mg protein)	V_{max} (nmol/min/nmol P450)	V_{max}/K_m (per mg protein)	V_{max}/K_m (per nmol P450)
Rat Liver	1.10 ± 1.03	0.007±0.003	0.005± 0.002	0.006	0.005
Mouse Liver	2.14 ± 0.53	0.91 ± 0.15	0.61 ± 0.10	0.43	0.29
Rat Lung	--[a]	--	--	--	--
Mouse Lung	0.67 ± 0.14	1.83 ± 0.15	15.5 ± 1.28	2.73	23.1
Rat Ovary	--	--	--	--	--
Mouse Ovary	--	--	--	--	--

Values for K_m and V_{max} are reported as means ± standard deviation.

* K_m values are based on analysis of data expressed as nmol/min/mg protein only. K_m values expressed as nmol/min/nmol P450 are within 2% of the reported K_m value.

a No reaction product detected.

Table 3

Kinetic Constants for Metabolism of 4-Vinylcyclohexene-1,2-Epoxide to 4-Vinylcyclohexene Diepoxide

Tissue	K_m* (mM)	V_{max} (nmol/min/mg protein)	V_{max} (nmol/min/nmol P450)	V_{max}/K_m (per mg protein)	V_{max}/K_m (per nmol P450)
Rat Liver	0.59 ± 0.22	3.69 ± 0.50	3.80 ± 0.52	6.25	6.44
Mouse Liver	0.51 ± 0.06	5.35 ± 0.20	3.64 ± 0.14	10.5	7.13
Rat Lung	0.29 ± 0.07	2.06 ± 0.15	14.4 ± 1.10	7.10	49.7
Mouse Lung	0.10 ± 0.009	2.70 ± 0.05	12.7 ± 0.25	27.0	127.0
Rat Ovary	--[a]	--	--	--	--
Mouse Ovary	--	--	--	--	--

Values for K_m and V_{max} are reported as means ± standard deviation.

* K_m values are based on analysis of data expressed as nmol/min/mg protein only. K_m values expressed as nmol/min/nmol P450 are within 2% of the reported K_m value.

a No reaction product detected.

Table 4

Kinetic Constants for Metabolism of 4-Vinylcyclohexene-7,8-Epoxide to 4-Vinylcyclohexene Diepoxide

Tissue	K_m* (mM)	V_{max} (nmol/min/mg protein)	V_{max} (nmol/min/nmol P450)	V_{max}/K_m (per mg protein)	V_{max}/K_m (per nmol P450)
Rat Liver	0.67 ± 0.07	9.45 ± 0.40	6.84 ± 0.29	14.1	10.2
Mouse Liver	0.57 ± 0.09	8.83 ± 0.53	5.94 ± 0.36	15.5	10.4
Rat Lung	0.60 ± 0.25	1.35 ± 0.24	11.2 ± 2.00	2.25	18.7
Mouse Lung	0.20 ± 0.08	11.8 ± 1.36	59.8 ± 6.89	59.0	299.0
Rat Ovary	--[a]	--	--	--	--
Mouse Ovary	--	--	--	--	--

Values for K_m and V_{max} are reported as means ± standard deviation.

* K_m values are based on analysis of data expressed as nmol/min/mg protein only. K_m values expressed as nmol/min/nmol P450 are within 2% of the reported K_m value.

[a] No reaction product detected.

Table 5

Kinetic Constants for Metabolism of 4-Vinylcyclohexene-1,2-Epoxide to 4-Vinylcyclohexene-1,2-Diol

Tissue	K_m (mM)	V_{max} (nmol/min/mg protein)	V_{max}/K_m
Rat Liver	0.19 ± 0.03	6.53 ± 0.51	34.4
Mouse Liver	0.14 ± 0.06	5.76 ± 0.45	41.1
Rat Lung	--[a]	--	--
Mouse Lung	--	--	--
Rat Ovary	--	--	--
Mouse Ovary	--	--	--

Values for K_m and V_{max} are reported as means ± standard deviation.

[a] No reaction product detected.

Table 6

Kinetic Constants for Metabolism of 4-Vinylcyclohexene-7,8-Epoxide to 4-Vinylcyclohexene-7,8-Diol

Tissue	K_m (mM)	V_{max} (nmol/min/mg protein)	V_{max}/K_m
Rat Liver	0.57 ± 0.11	135.8 ± 7.88	238
Mouse Liver	-- [a]	--	--
Rat Lung	--	--	--
Mouse Lung	--	--	--
Rat Ovary	--	--	--
Mouse Ovary	--	--	--

Values for K_m and V_{max} are reported as means ± standard deviation.

[a] No reaction product detected.

Table 7

Kinetic Constants for Metabolism of 4-Vinylcyclohexene Diepoxide

Tissue	K_m (mM)	V_{max} (nmol/min/mg protein)	V_{max}/K_m
Rat Liver	0.19 ± 0.11	5.51 ± 1.69	29.0
Mouse Liver	0.03 ± 0.002	0.63 ± 0.01	21.0
Rat Lung	-- [a]	0.39 [b]	--
Mouse Lung	--	1.06	--
Rat Ovary	--	0.90	--
Mouse Ovary[c]	--	--	--

Values for K_m and V_{max} are reported as means ± standard deviation.

a Insufficient data to calculate K_m.

b Insufficient data to calculate standard deviaton for V_{max}.

c Insufficient tissue present to conduct experiment.

FIGURES

Note

Figures 4 - 38 represent Lineweaver-Burk (double reciprocal) plots of the data from which the kinetic constants were calculated. The kinetic constants listed on the figures are the ones calculated by EZ-FIT and correspond to the constants in the tables. The kinetic constants estimated from the intercepts of the figures may not correspond exactly to the kinetic constants listed in the figure. This is due to the analysis that EZ-FIT performs being a more sophistocated analysis than taking kinetic constants from the intercepts.

Figure 1

Metabolic Pathway for 4-Vinylcyclohexene

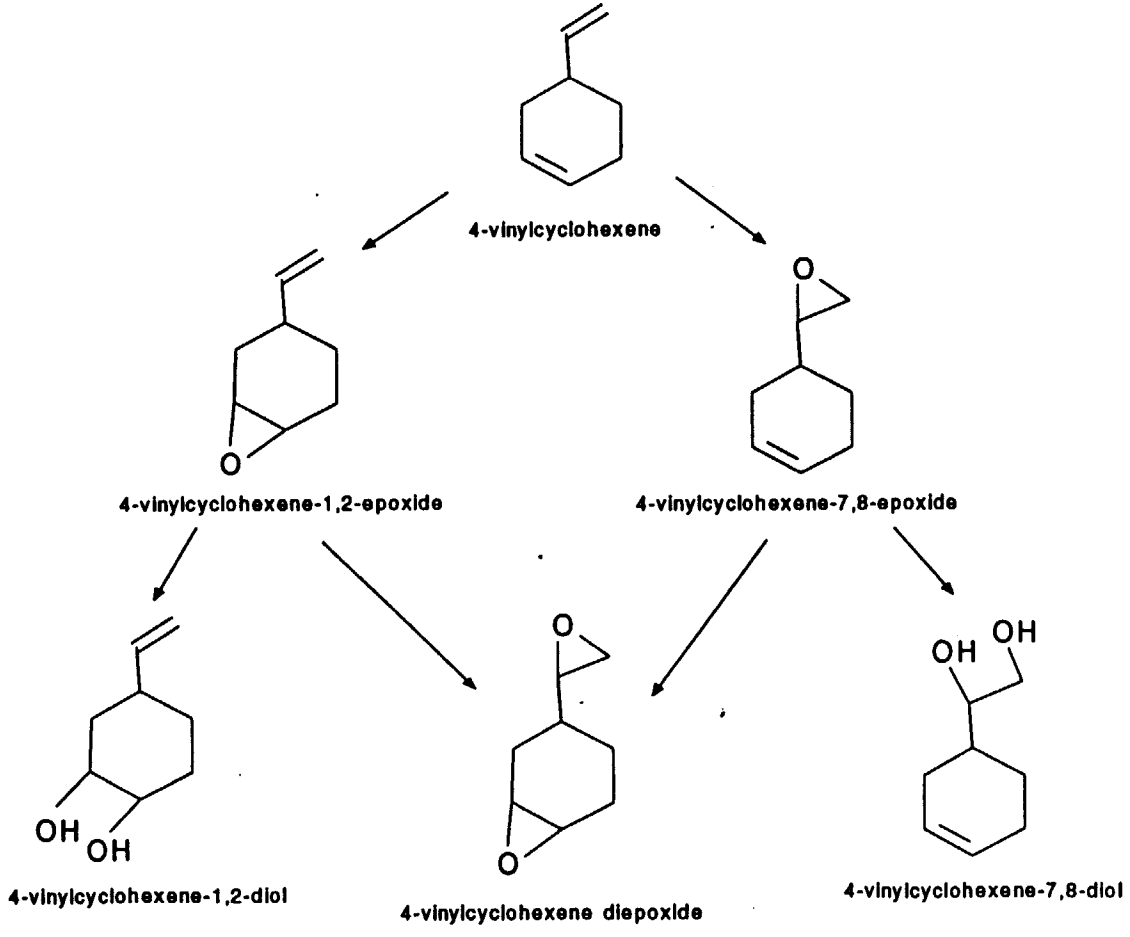


Figure 2

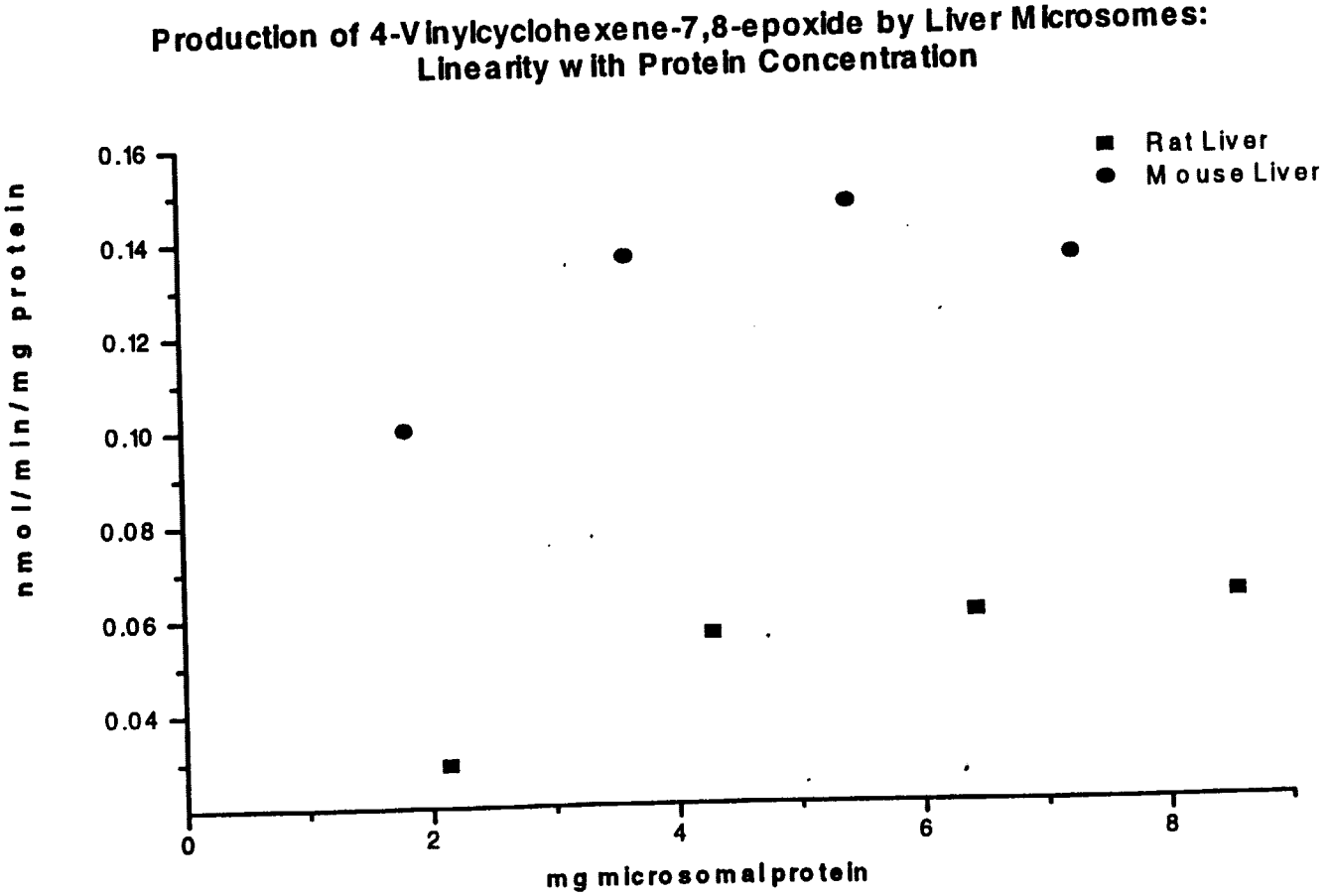

Production of 4-Vinylcyclohexene-7,8-epoxide by Liver Microsomes:
Linearity with Protein Concentration
Rat Liver
Mouse Liver
nmol/min/mg protein
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0
2
4
6
8
mg microsomal protein

Figure 3

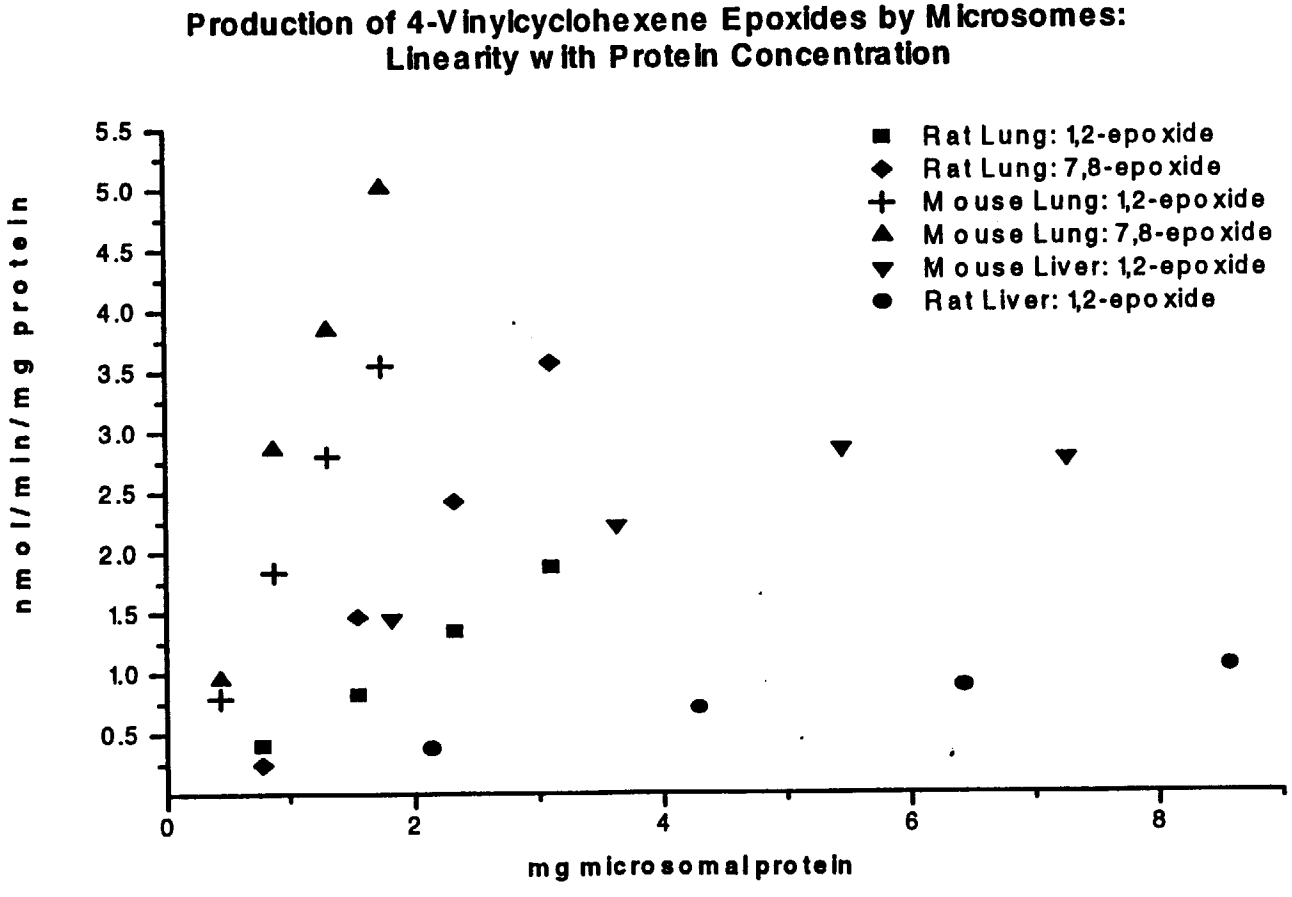

Production of 4-Vinylcyclohexene Epoxides by Microsomes:
Linearity with Protein Concentration
Rat Lung: 1,2-epoxide
Rat Lung: 7,8-epoxide
Mouse Lung: 1,2-epoxide
Mouse Lung: 7,8-epoxide
Mouse Liver: 1,2-epoxide
Rat Liver: 1,2-epoxide
nmol/min/mg protein
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
2
4
6
8
mg microsomal protein

Figure 4

Production of 4-Vinylcyclohexene-1,2-epoxide by Rat Liver Microsomes

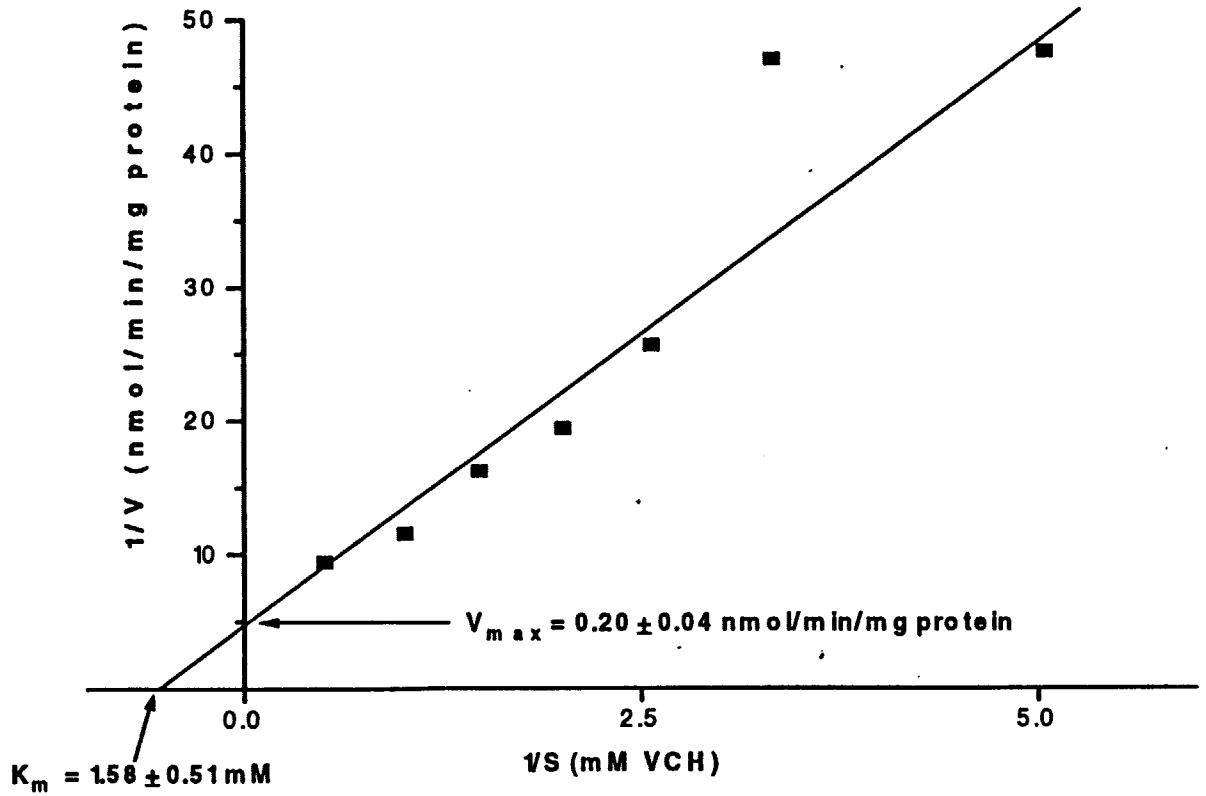


Figure 5

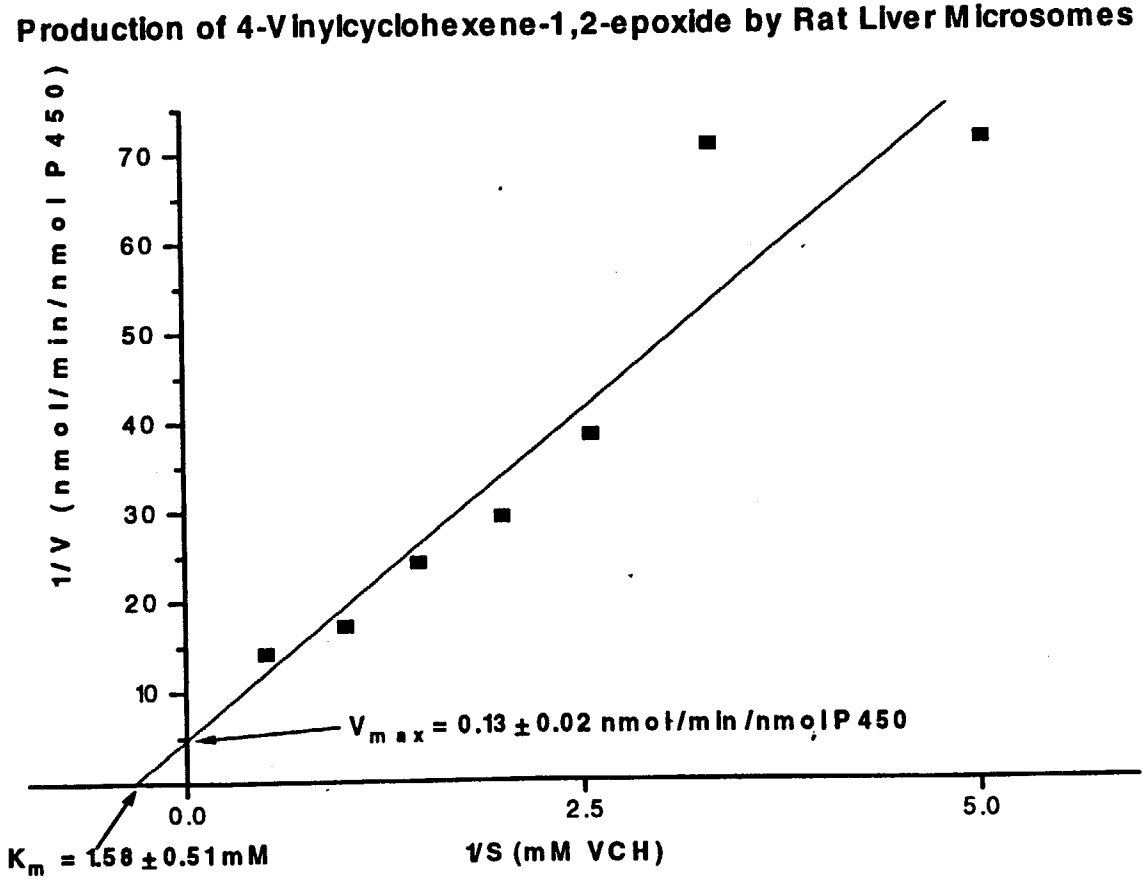

Production of 4-Vinylcyclohexene-1,2-epoxide by Rat Liver Microsomes
1/V (nmol/min/nmol P450)
70
60
50
40
30
20
10
0.0
2.5
5.0
1/S (mM VCH)
V_{max} = 0.13 ± 0.02 nmol/min /nmol P450
K_m = 1.58 ± 0.51 mM

Figure 6

Production of 4-Vinylcyclohexene-1,2-epoxide by Mouse Liver Microsomes

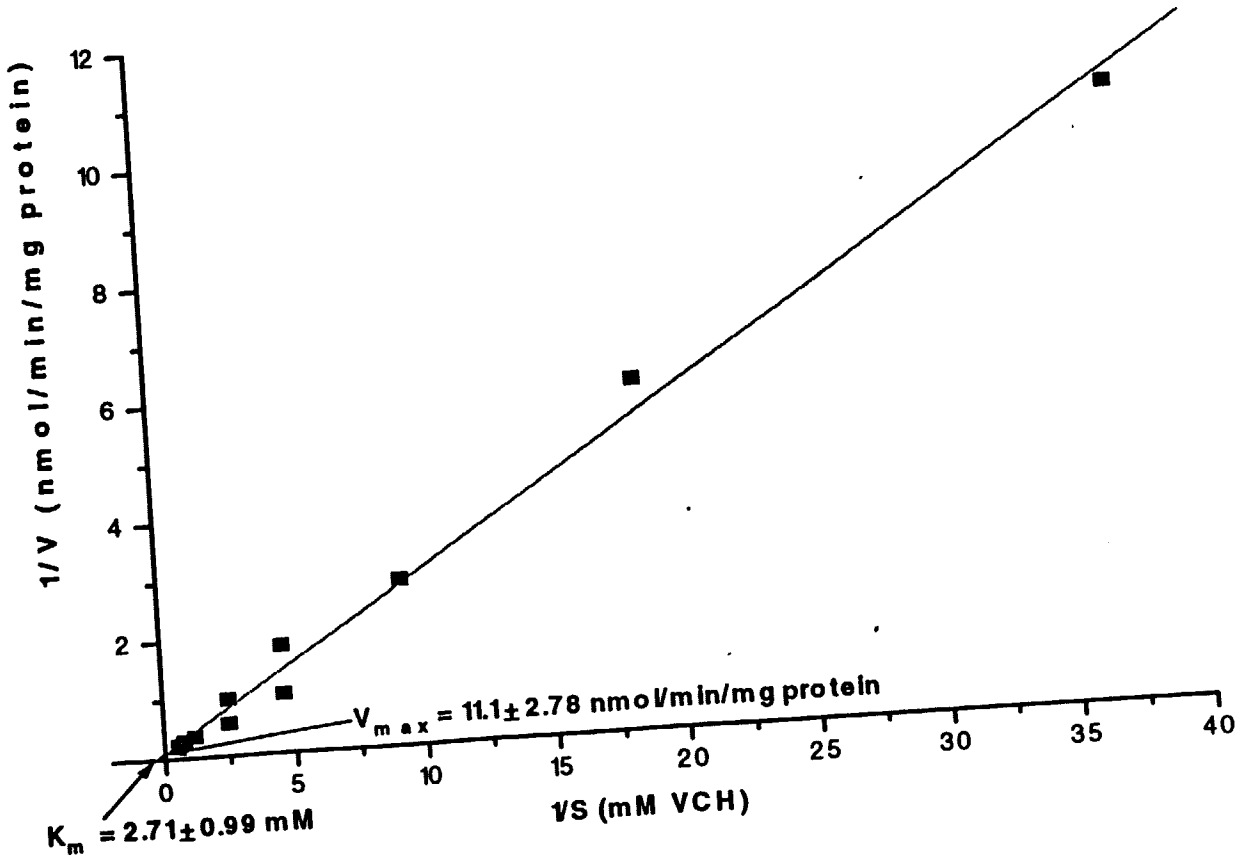


Figure 7

Production of 4-Vinylcyclohexene-1,2-epoxide by Mouse Liver Microsomes

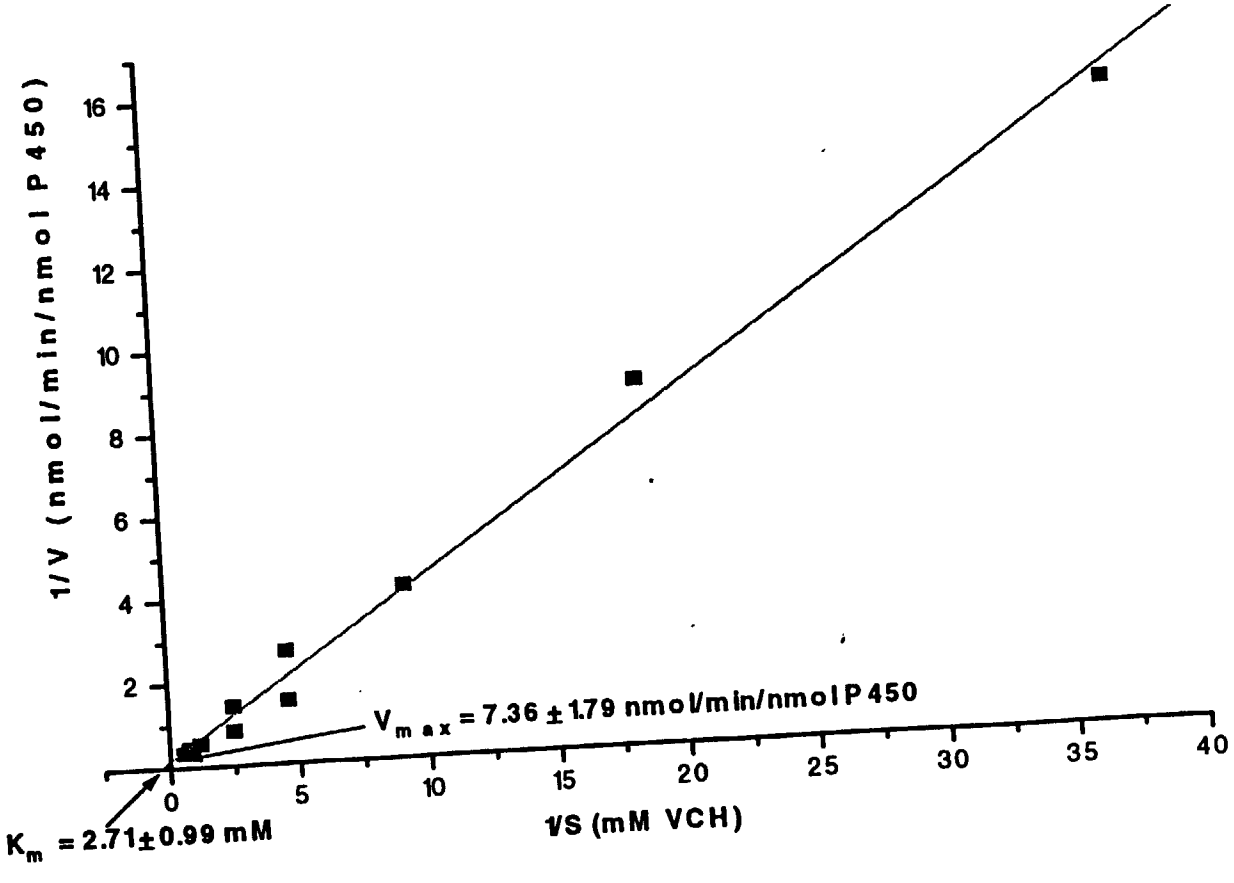


Figure 8

Production of 4-Vinylcyclohexene-1,2-epoxide by Rat Lung Microsomes

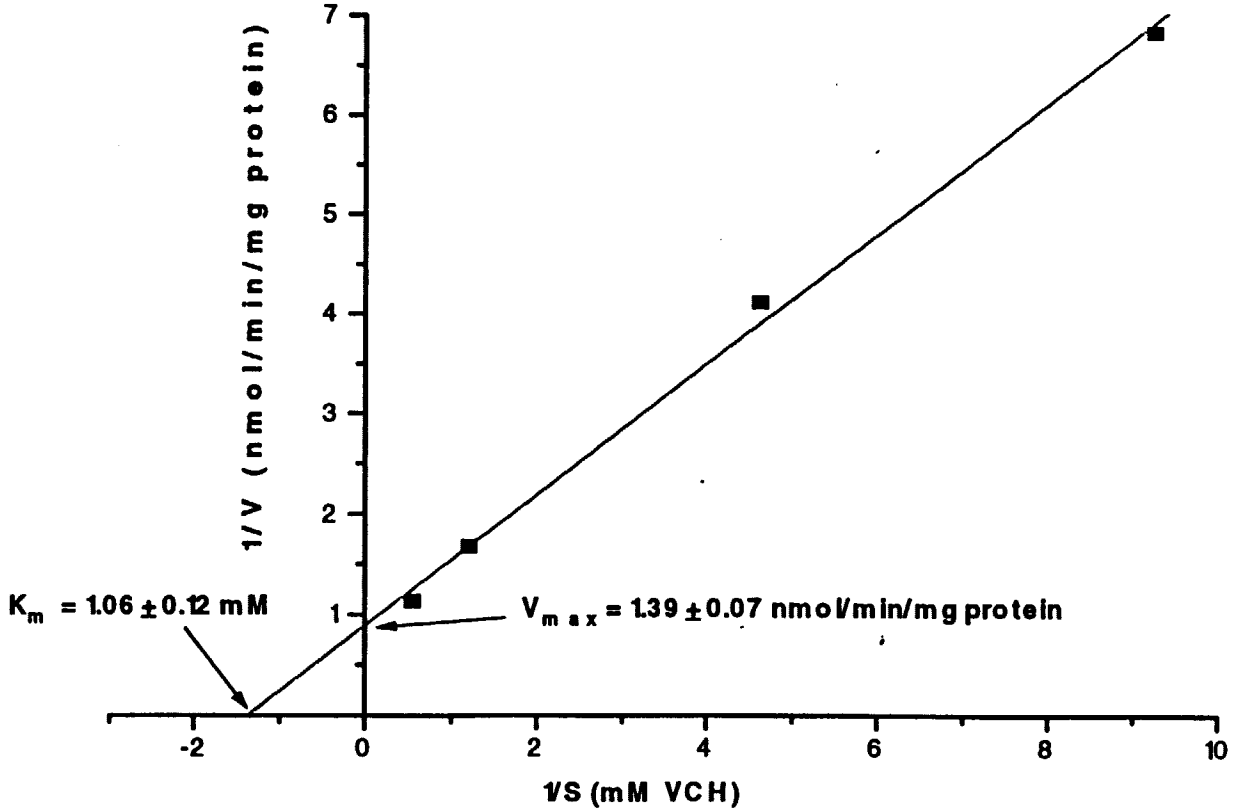


Figure 9

Production of 4-Vinylcyclohexene-1,2-epoxide by Rat Lung Microsomes

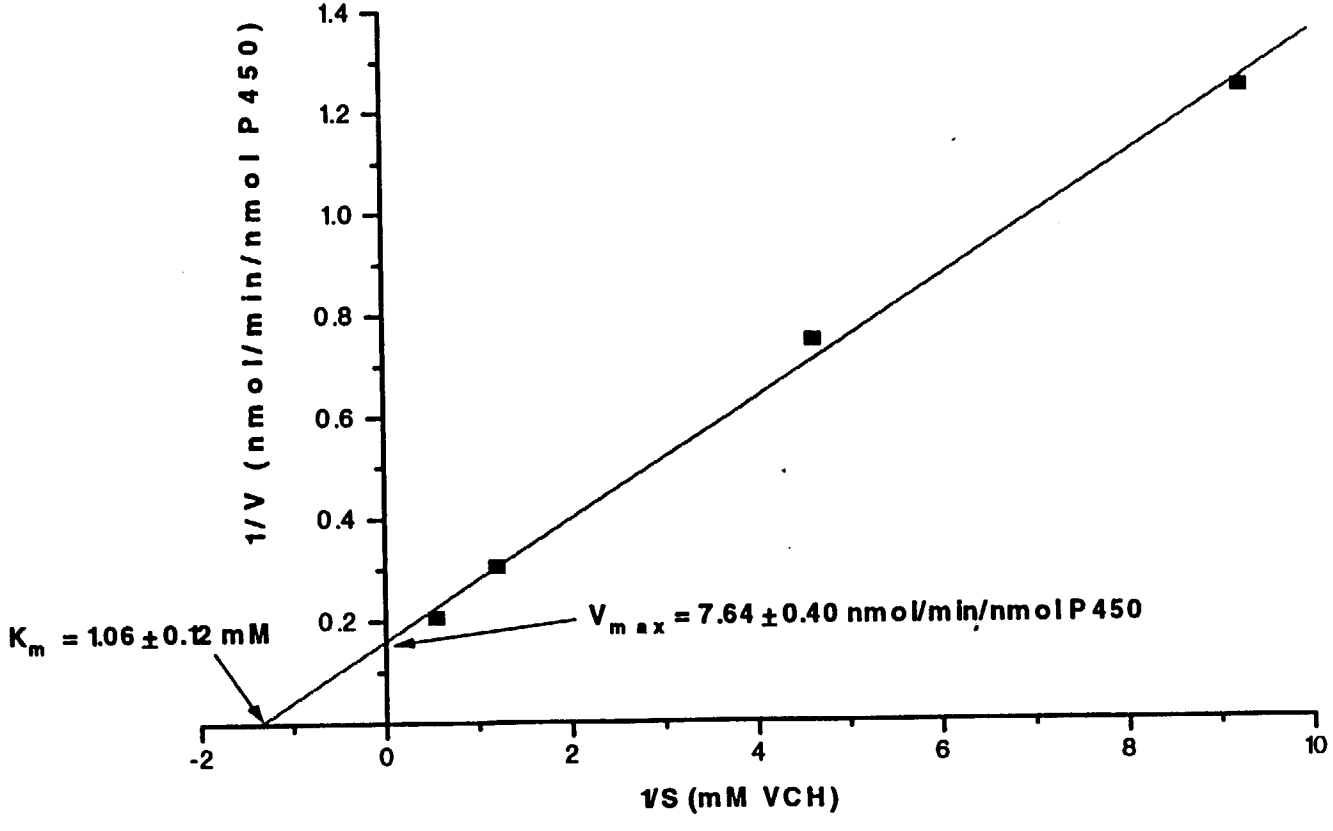


Figure 10

Production of 4-Vinylcycohexene-1,2-epoxide by Mouse Lung Microsomes

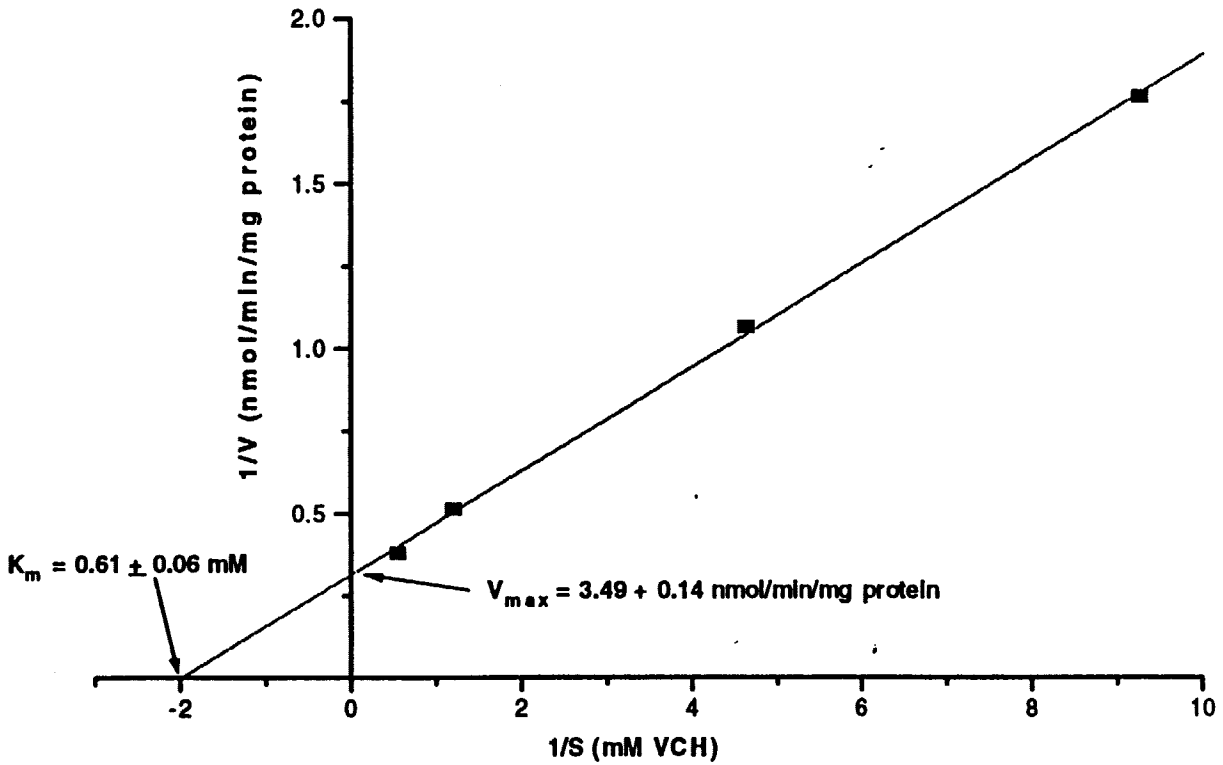


Figure 11

Production of 4-Vinylcyclohexene-1,2-epoxide by Mouse Lung Microsomes

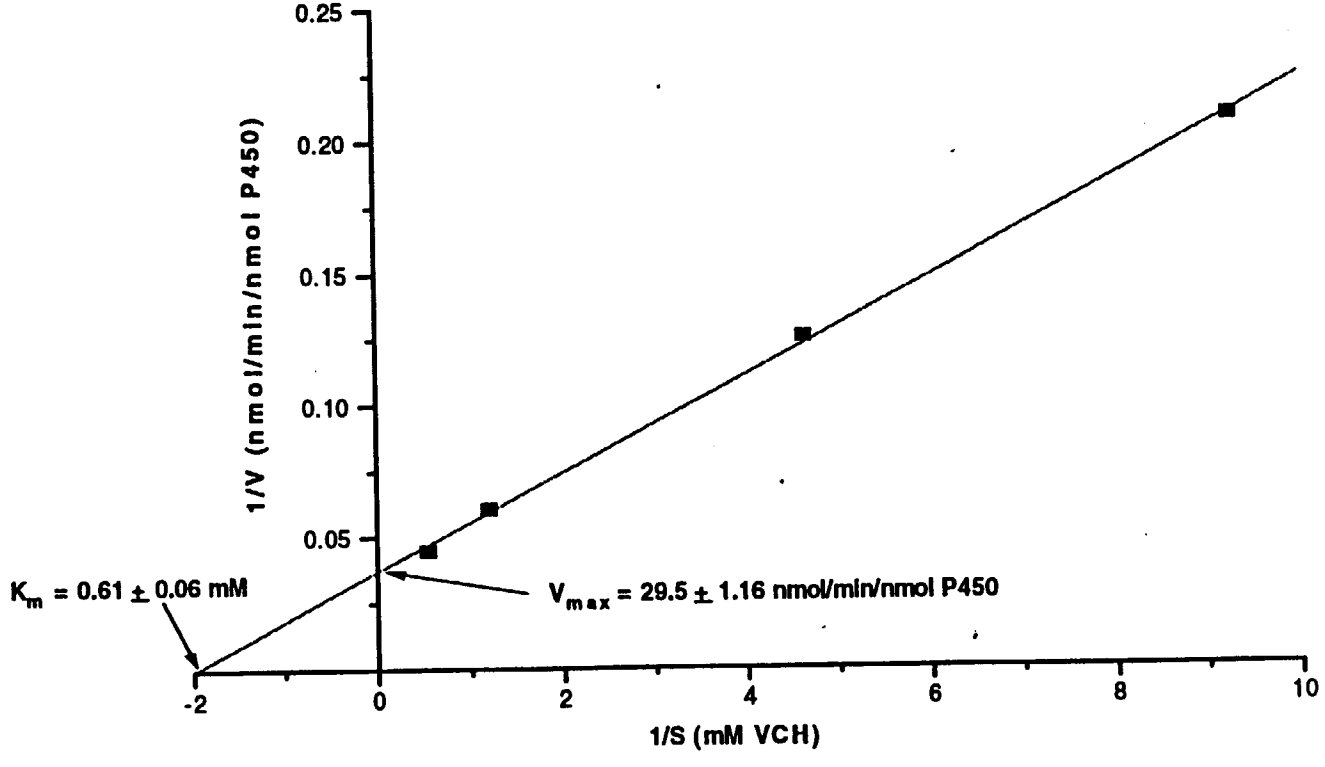


Figure 12

Production of 4-Vinylcyclohexene-7,8-epoxide by Rat Liver Microsomes

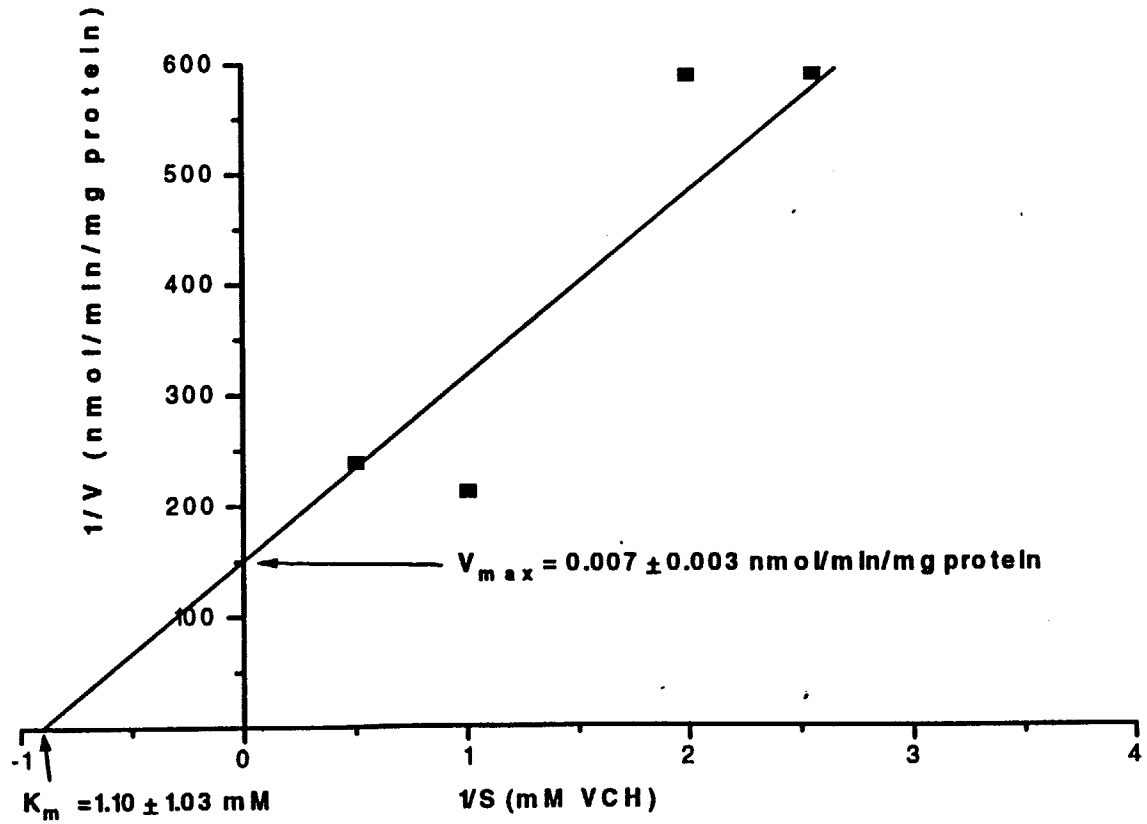


Figure 13

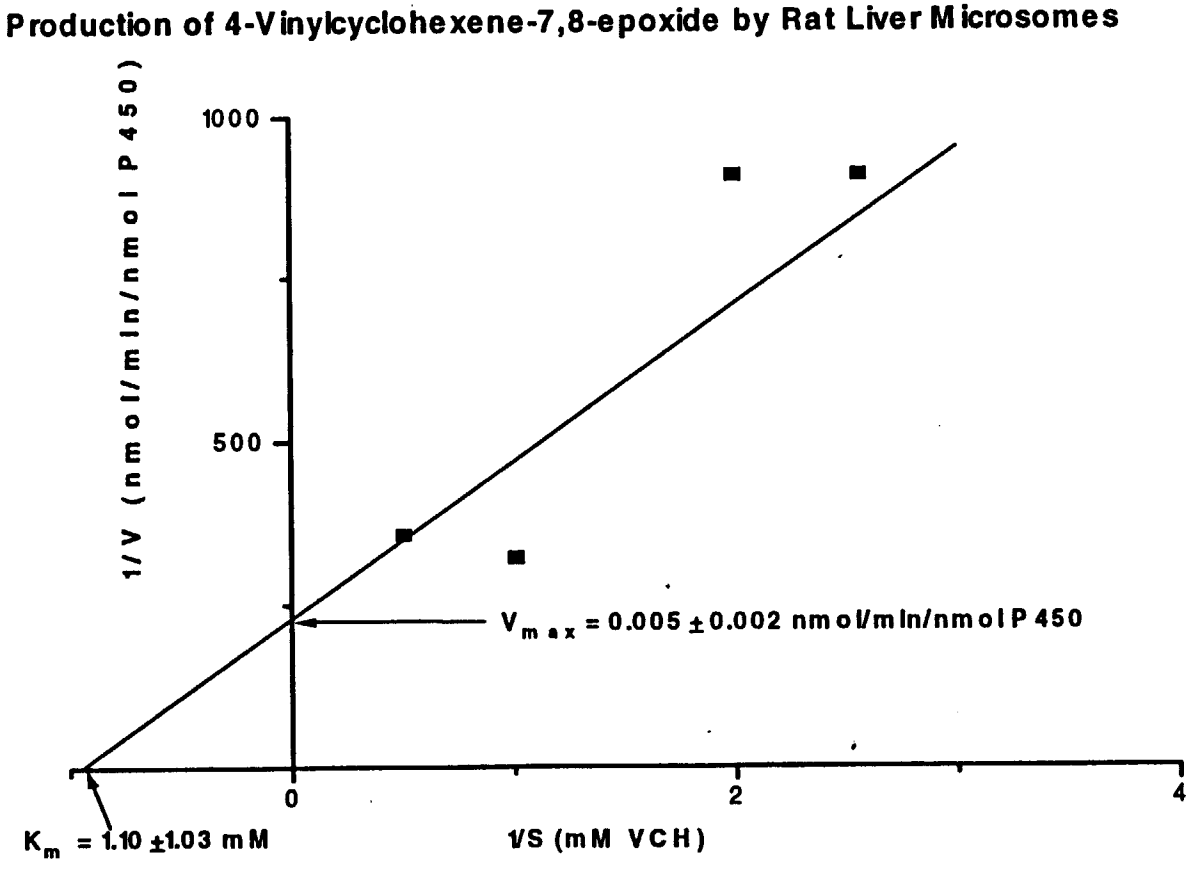

Production of 4-Vinylcyclohexene-7,8-epoxide by Rat Liver Microsomes
1/V (nmol/min/nmol P 450)
1000
500
0
2
4
V_{max} = 0.005 ±0.002 nmol/min/nmol P 450
K_m = 1.10 ±1.03 mM
1/S (mM VCH)

Figure 14

Production of 4-Vinylcyclohexene-7,8-epoxide by Mouse Liver Microsomes

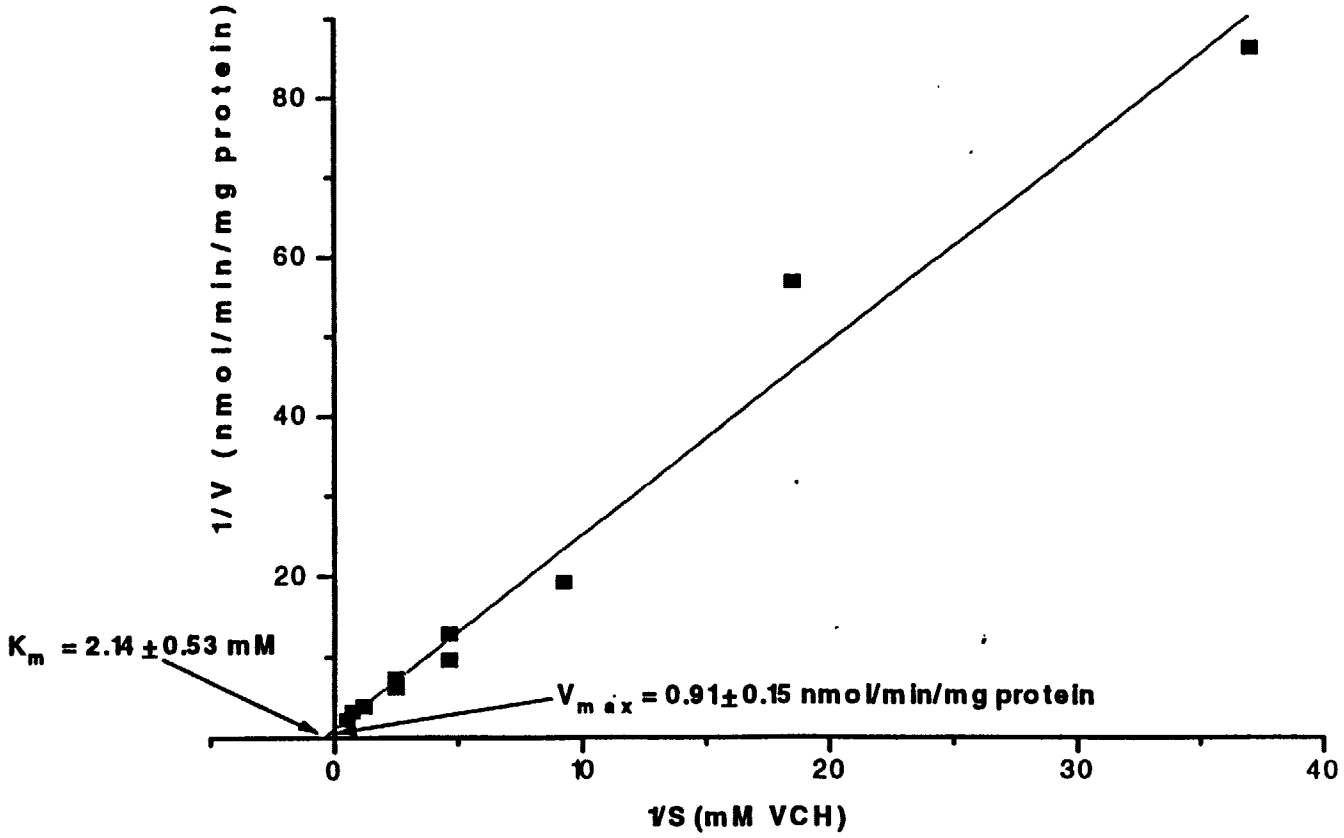


Figure 15

Production of 4-Vinylcyclohexene-7,8-epoxide by Mouse Liver Microsomes

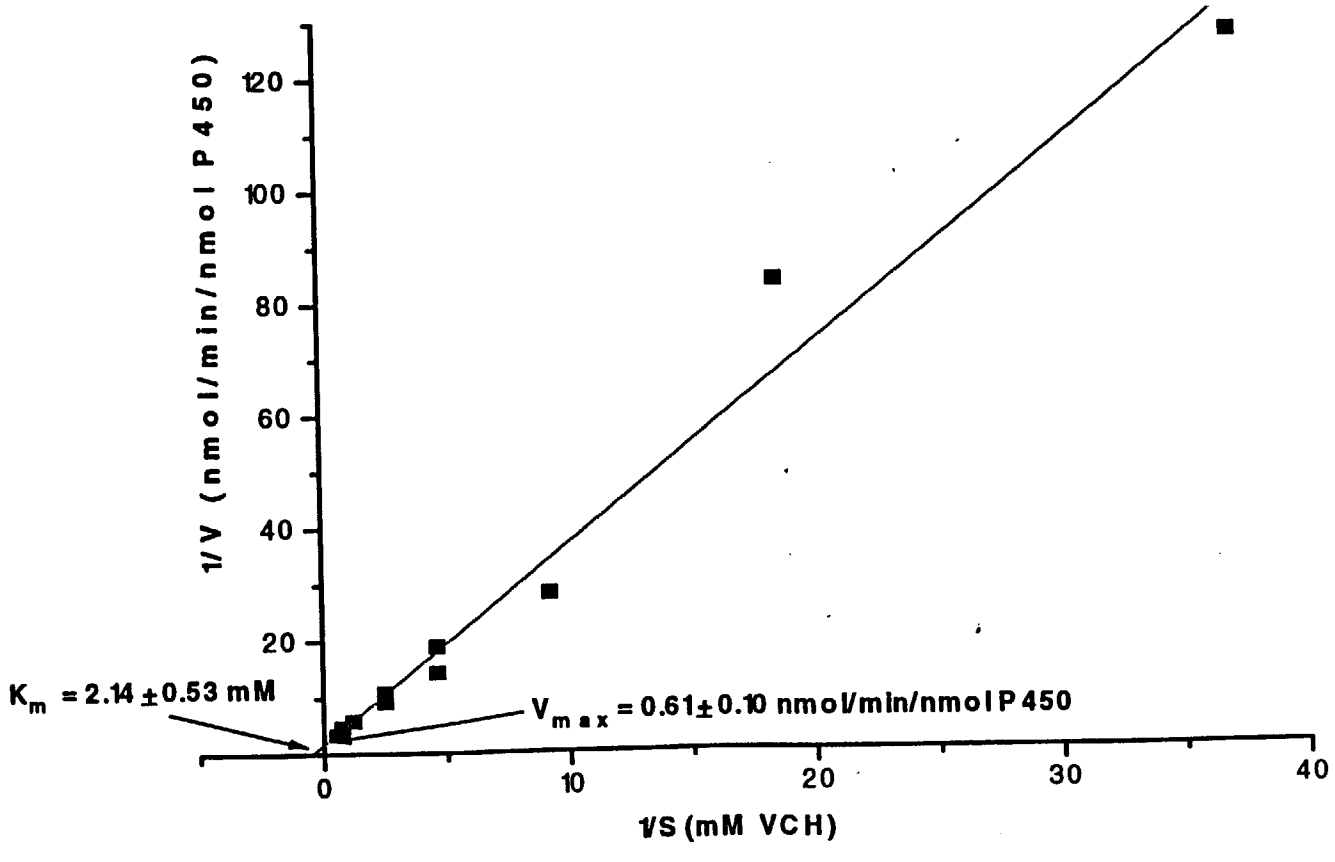


Figure 16

Production of 4-Vinylcyclohexene-7,8-epoxide by Mouse Lung Microsomes

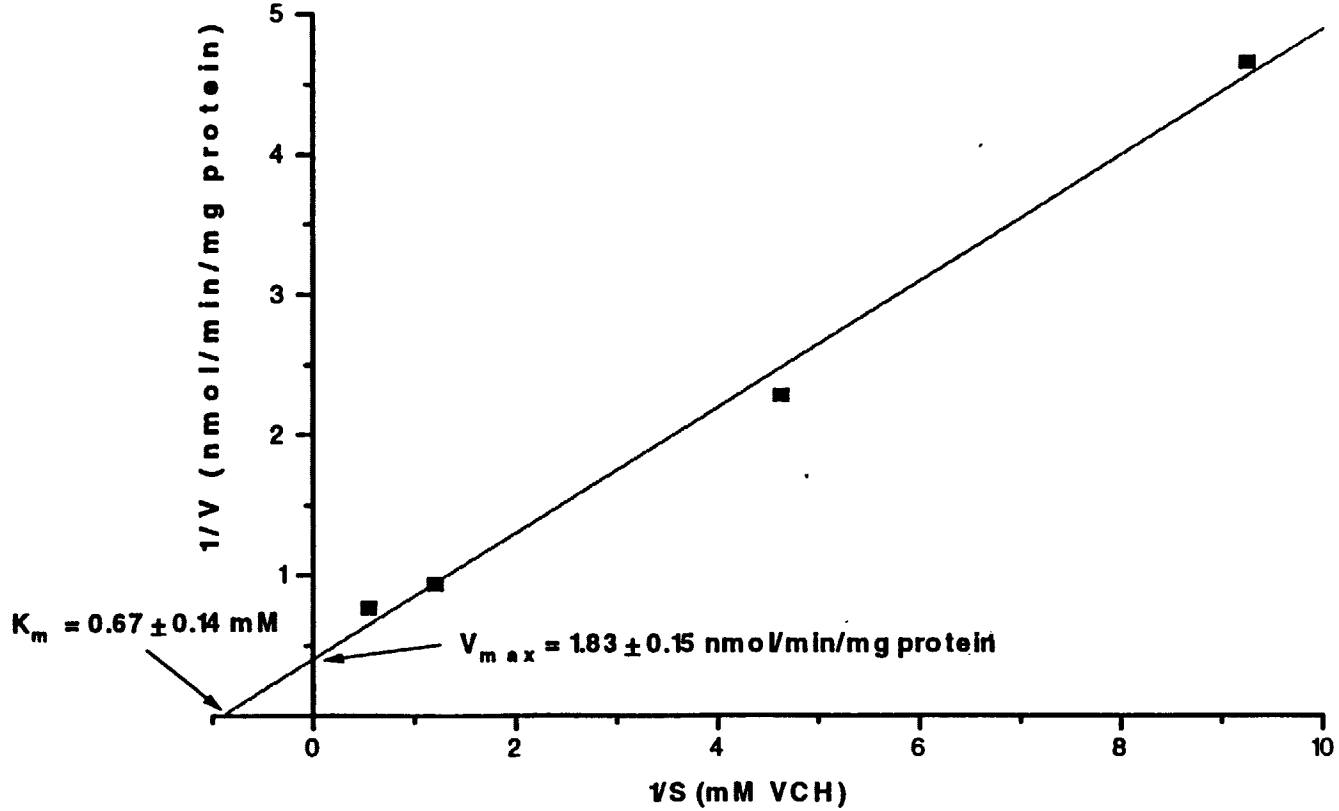


Figure 17

Production of 4-Vinylcyclohexene-7,8-epoxide by Mouse Lung Microsomes

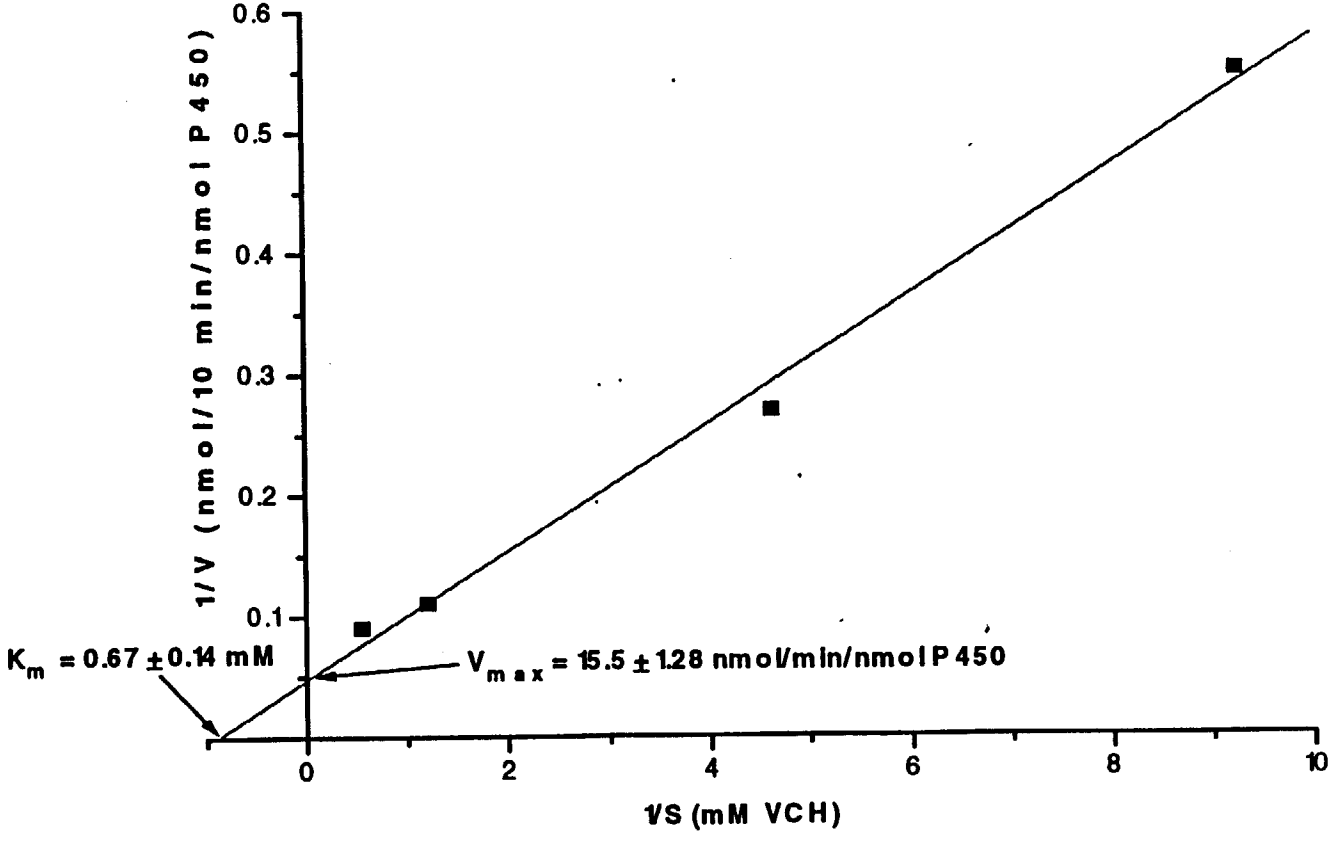


Figure 18

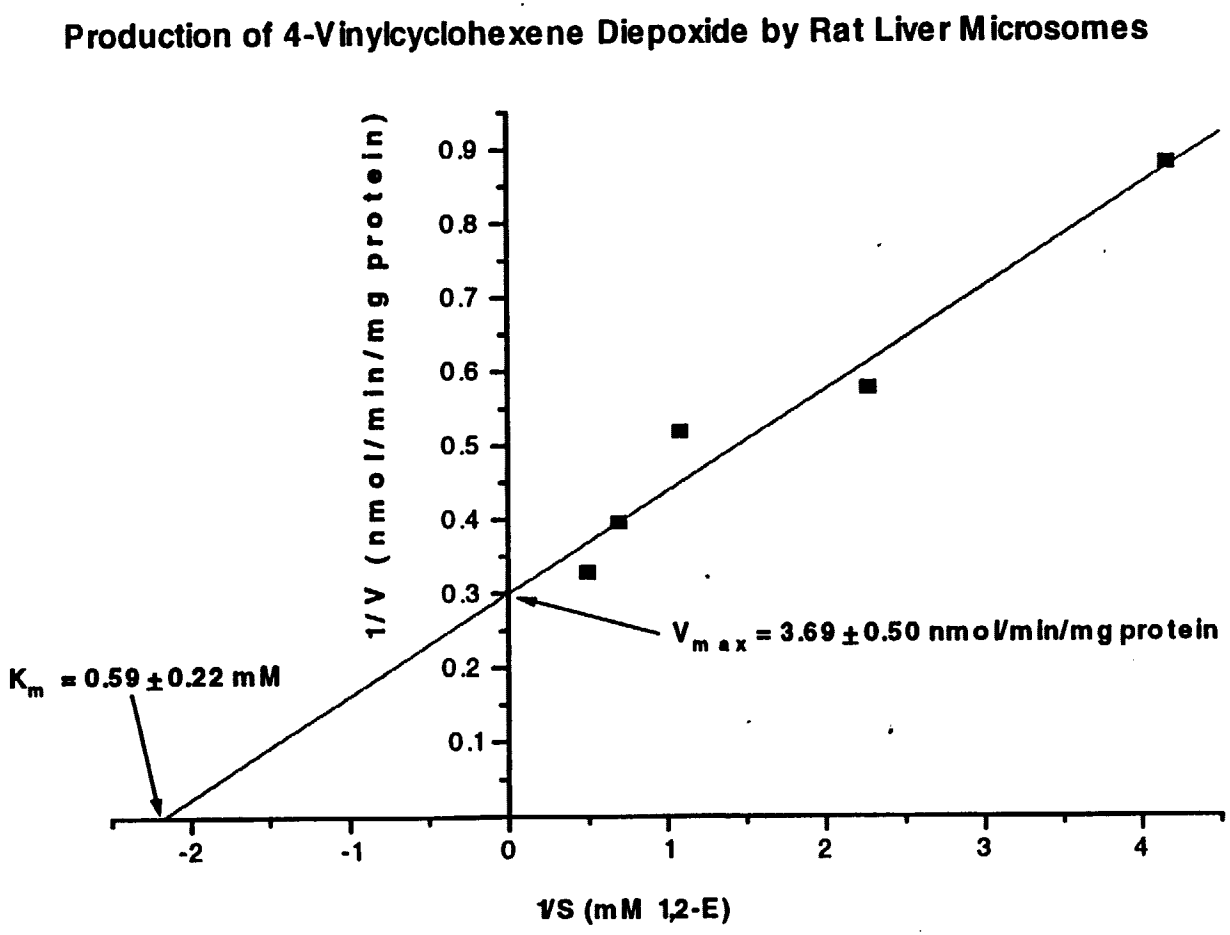

Production of 4-Vinylcyclohexene Diepoxide by Rat Liver Microsomes
1/V (nmol/min/mg protein)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
-2
-1
0
1
2
3
4
1/S (mM 1,2-E)
K_m = 0.59 ± 0.22 mM
V_{max} = 3.69 ± 0.50 nmol/min/mg protein

Figure 19

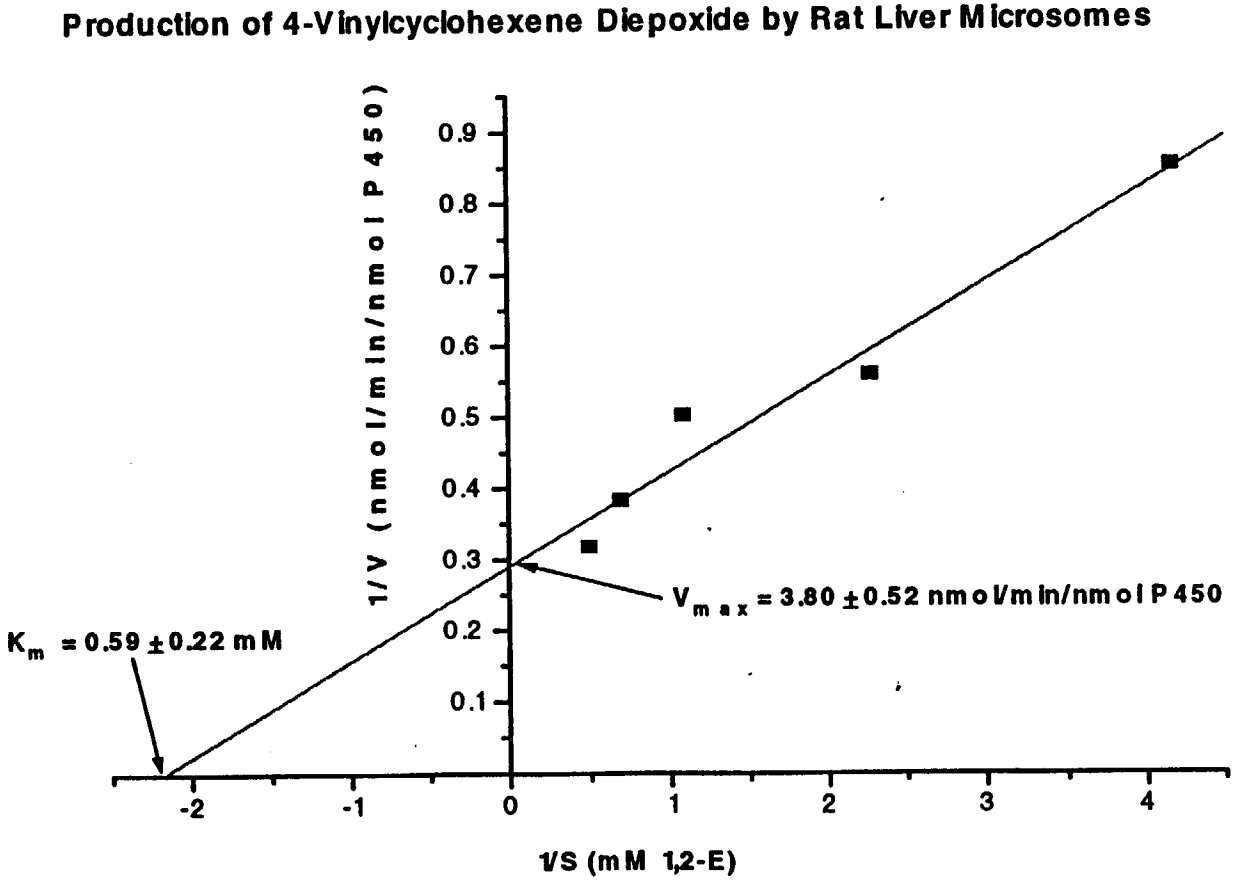

Production of 4-Vinylcyclohexene Diepoxide by Rat Liver Microsomes
1/V (nmol/min/nmol P450)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
-2
-1
0
1
2
3
4
1/S (mM 1,2-E)
K_m = 0.59 ± 0.22 mM
V_{max} = 3.80 ± 0.52 nmol/min/nmol P450

Figure 20

Production of 4-Vinylcyclohexene Diepoxide by Mouse Liver Microsomes

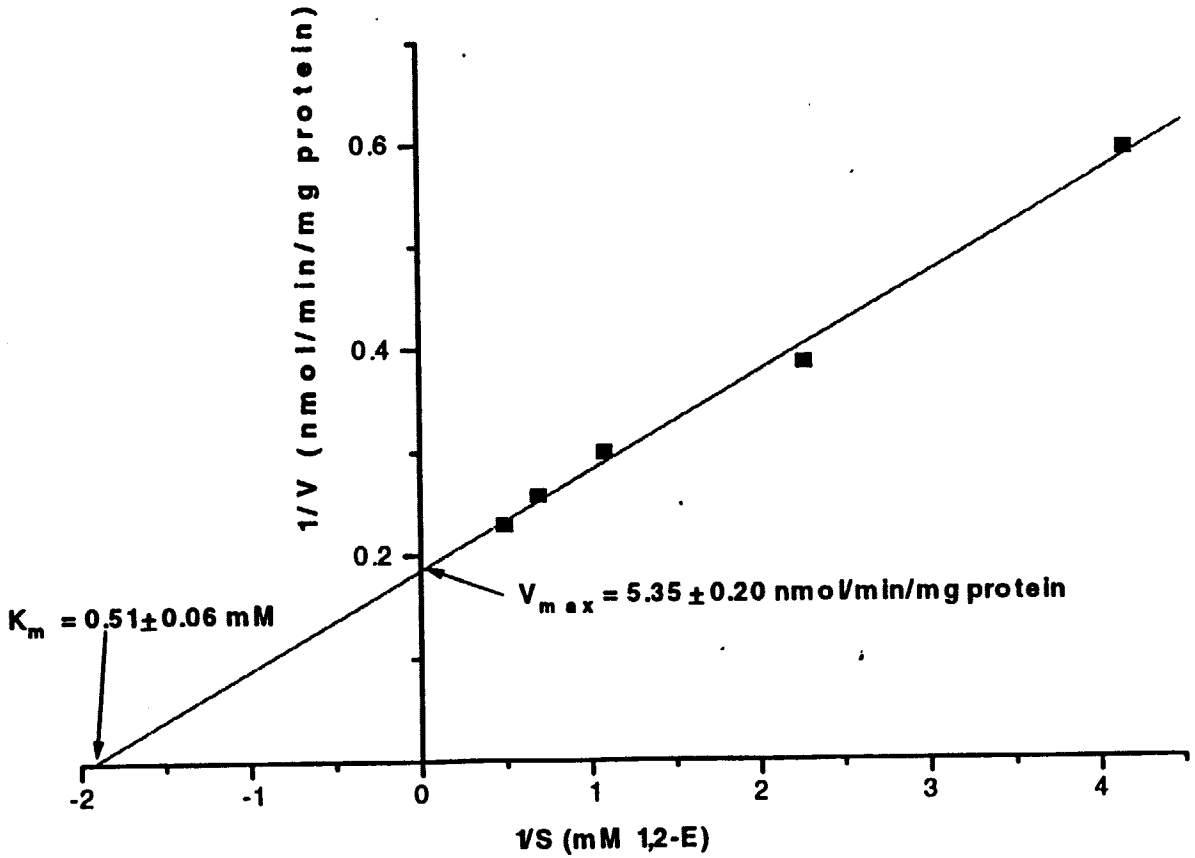


Figure 21

Production of 4-Vinylcyclohexene Diepoxide by Mouse Liver Microsomes

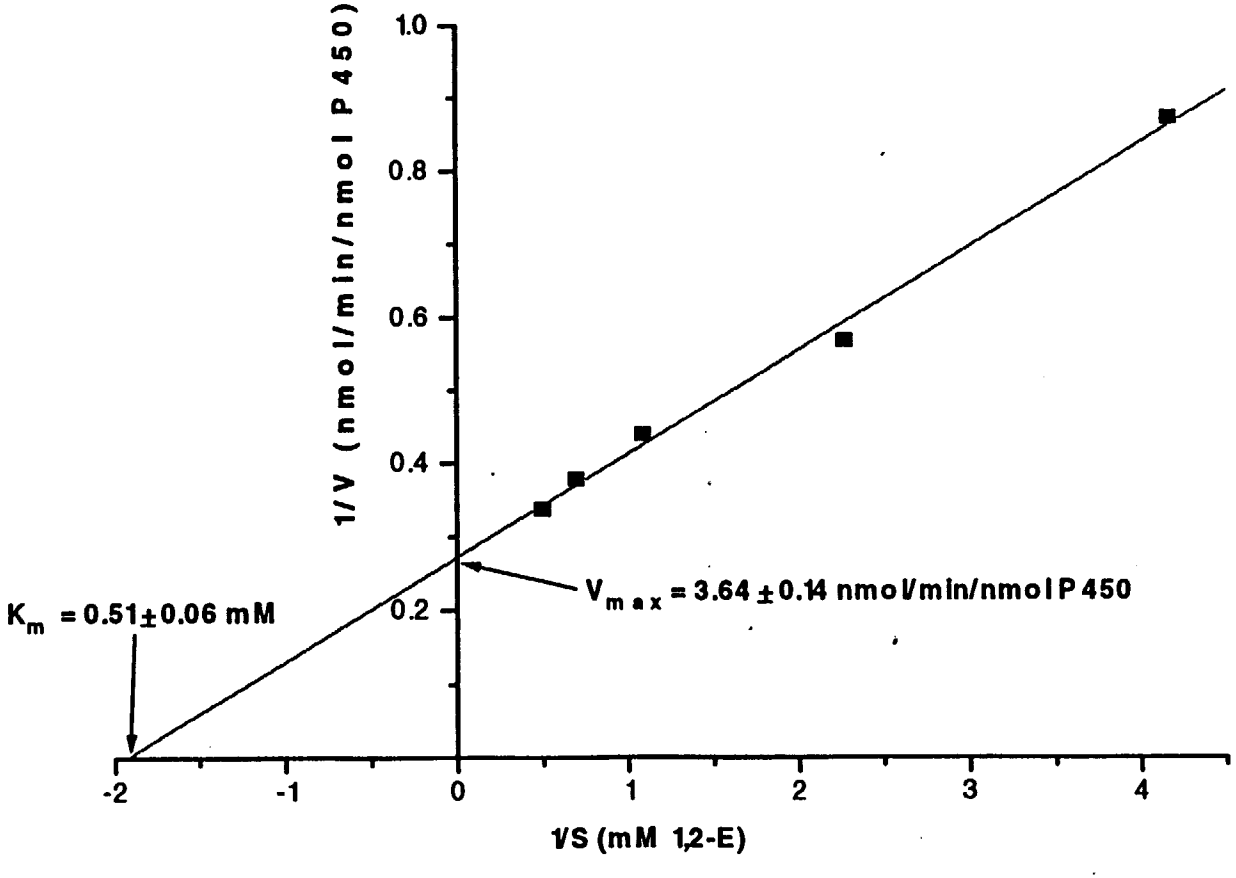


Figure 22

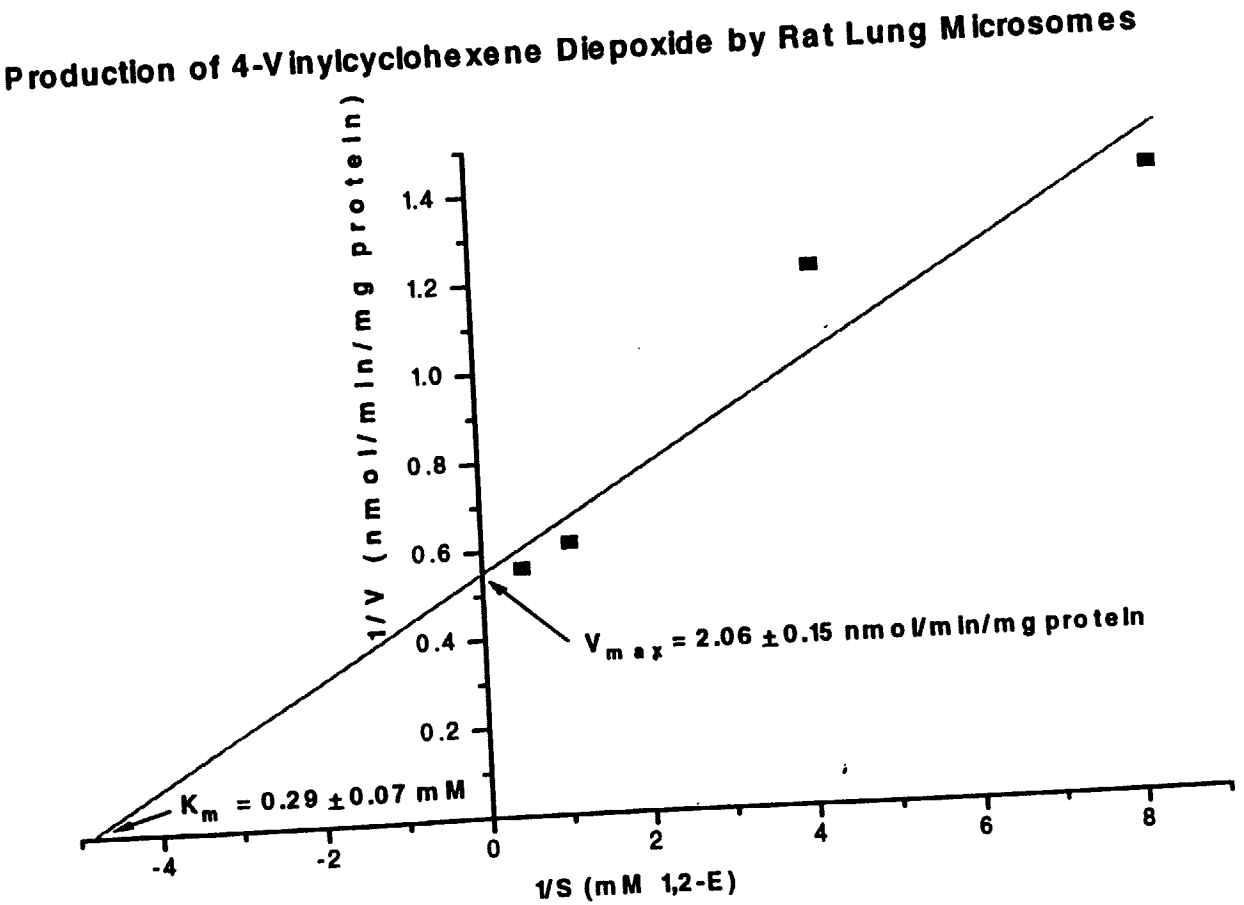

Production of 4-Vinylcyclohexene Diepoxide by Rat Lung Microsomes
1/V (nmol/min/mg protein)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
V_{max} = 2.06 ±0.15 nmol/min/mg protein
K_m = 0.29 ±0.07 mM
-4
-2
0
2
4
6
8
1/S (mM 1,2-E)

Figure 23

Production of 4-Vinylcyclohexene Diepoxide by Rat Lung Microsomes

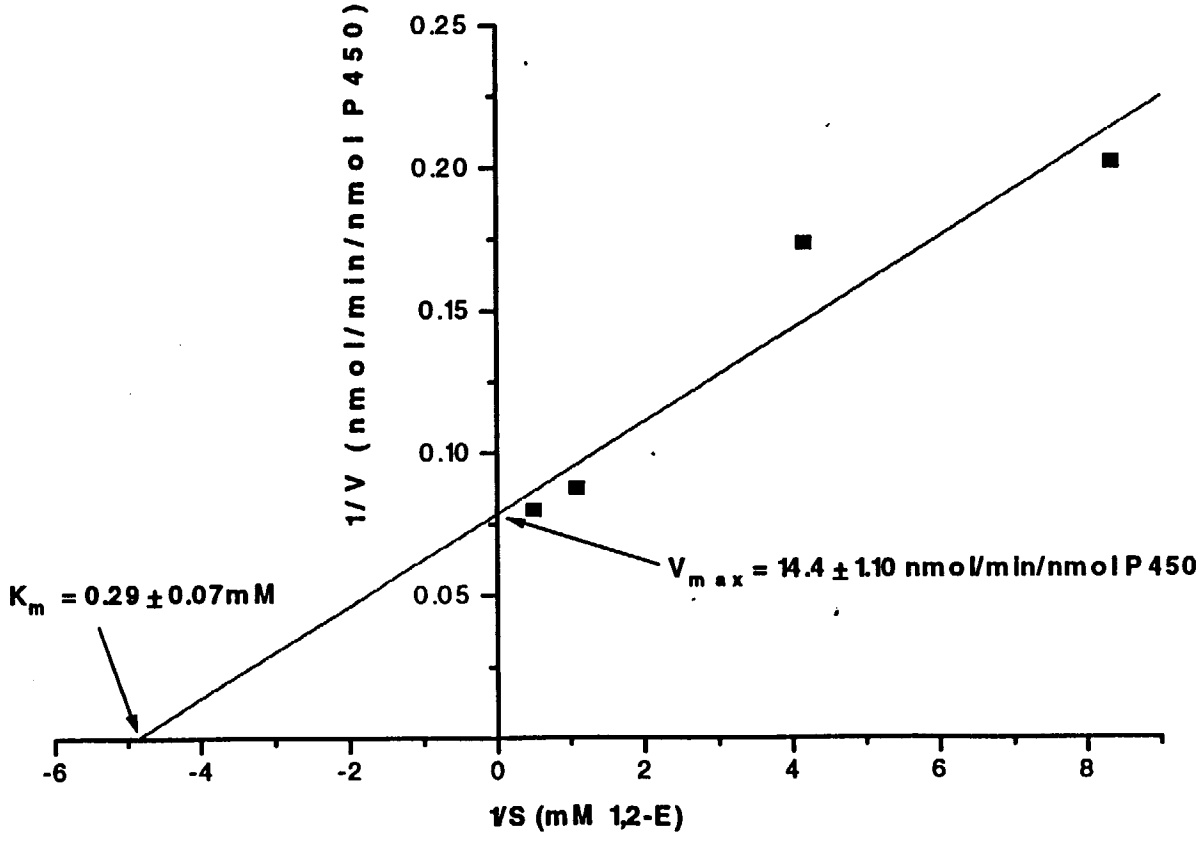


Figure 24

Production of 4-Vinylcyclohexene Diepoxide by Mouse Lung Microsomes

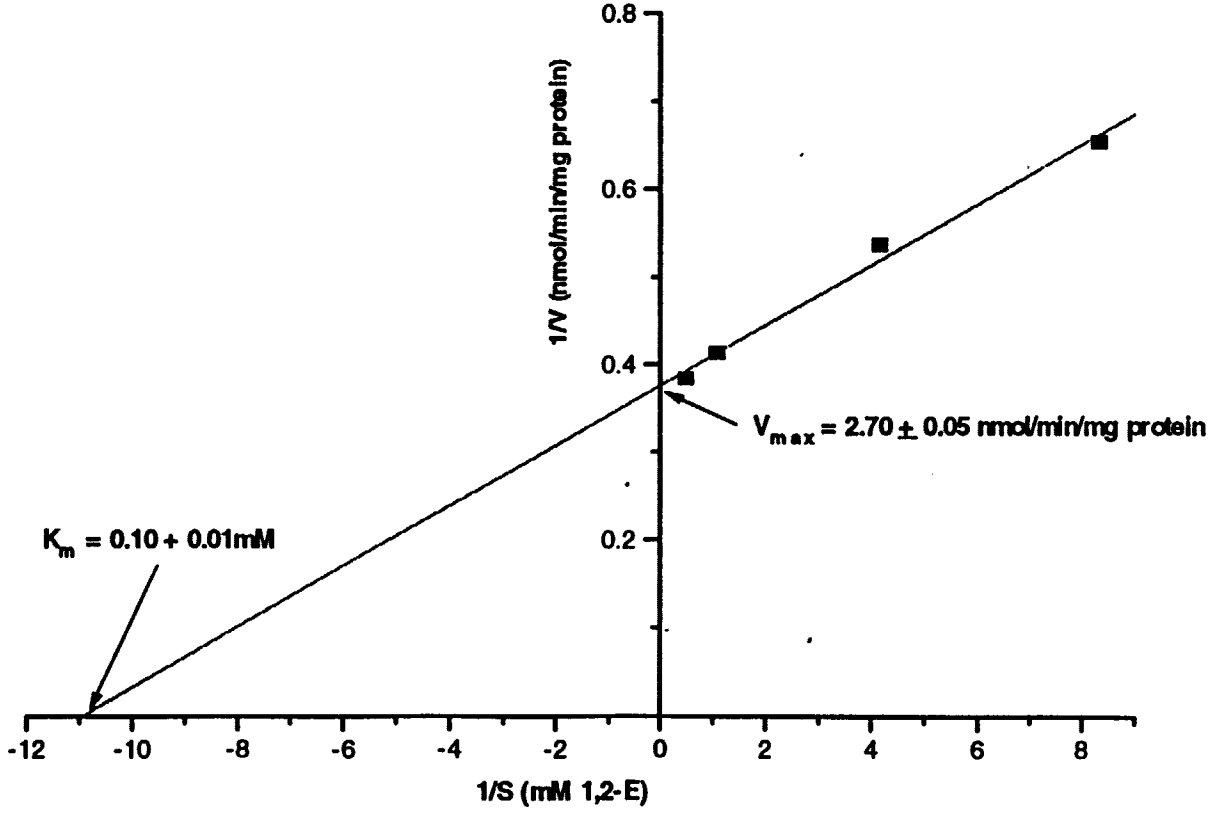


Figure 25

Production of 4-Vinylcyclohexene Diepoxide by Mouse Lung Microsomes

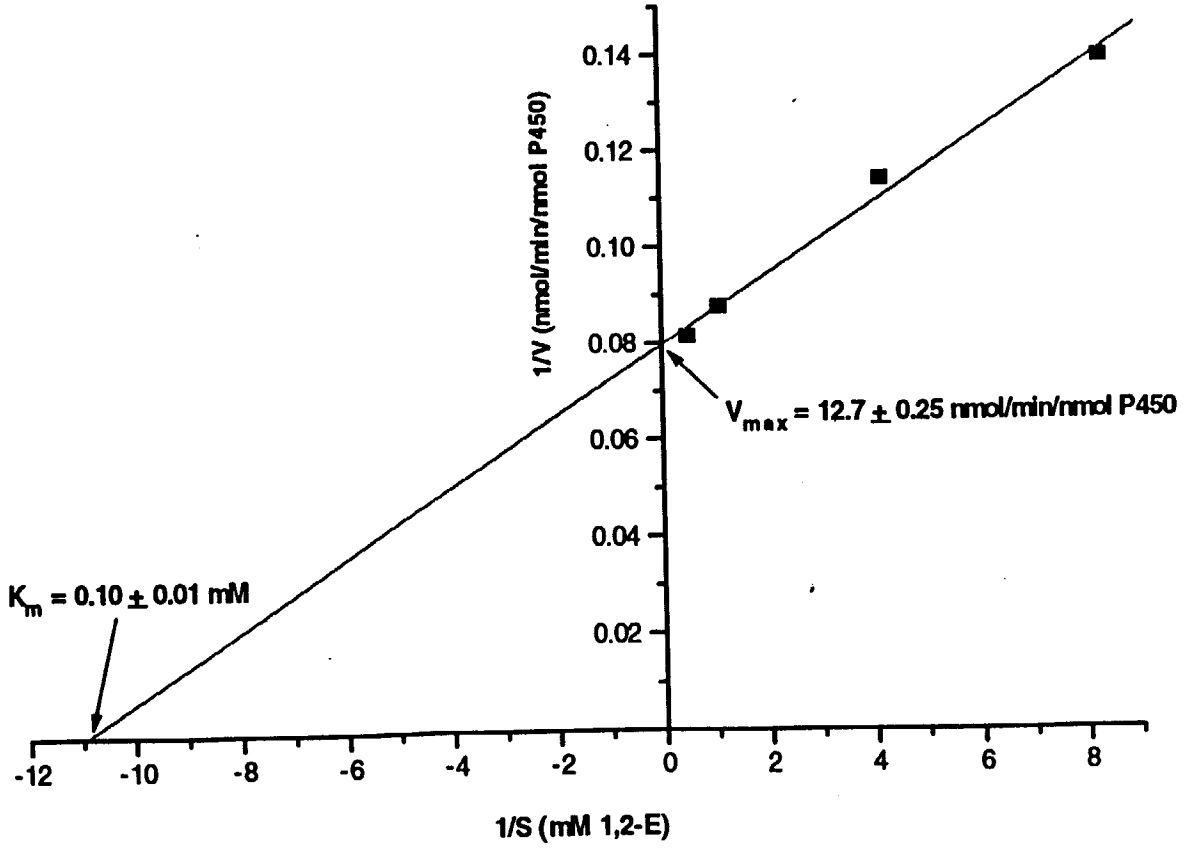


Figure 26

Production of 4-Vinylcyclohexene Diepoxide by Rat Liver Microsomes

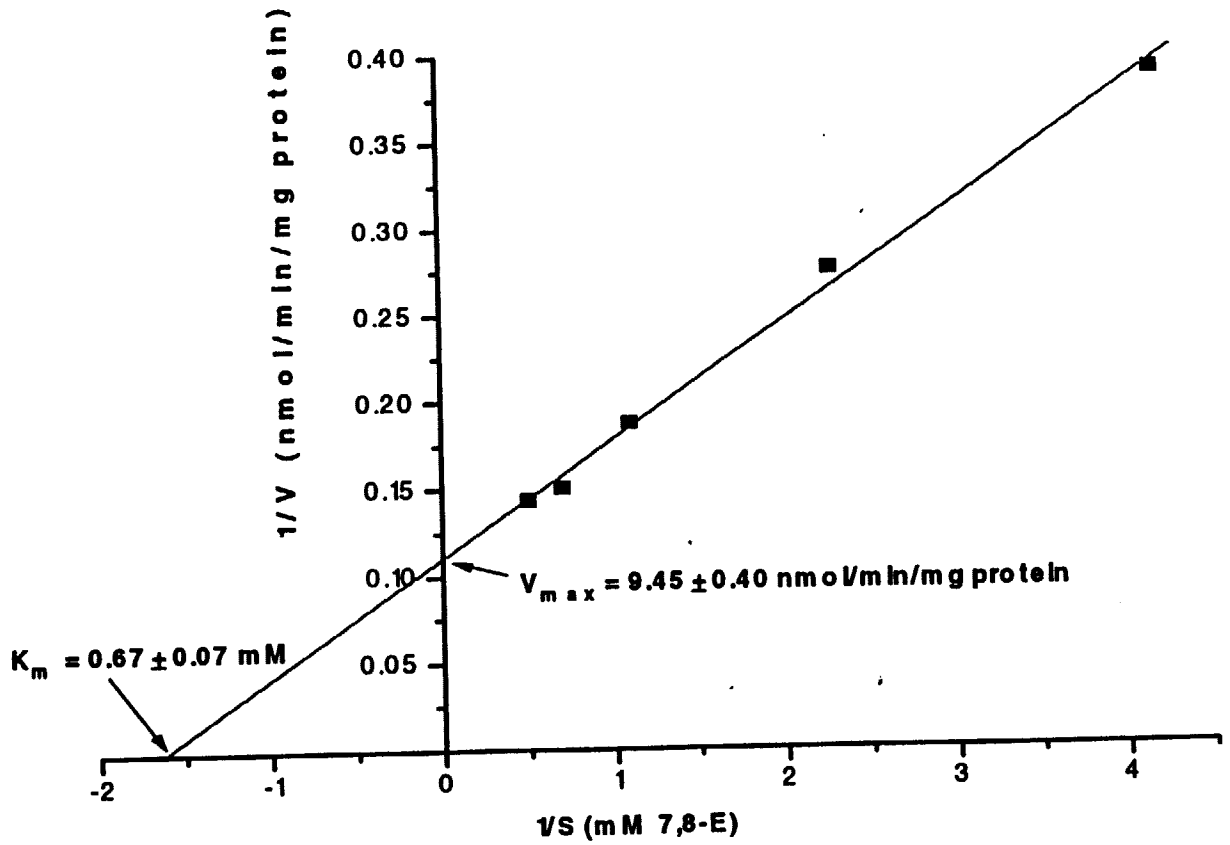


Figure 27

Production of 4-Vinylcyclohexene Diepoxide by Rat Liver Microsomes

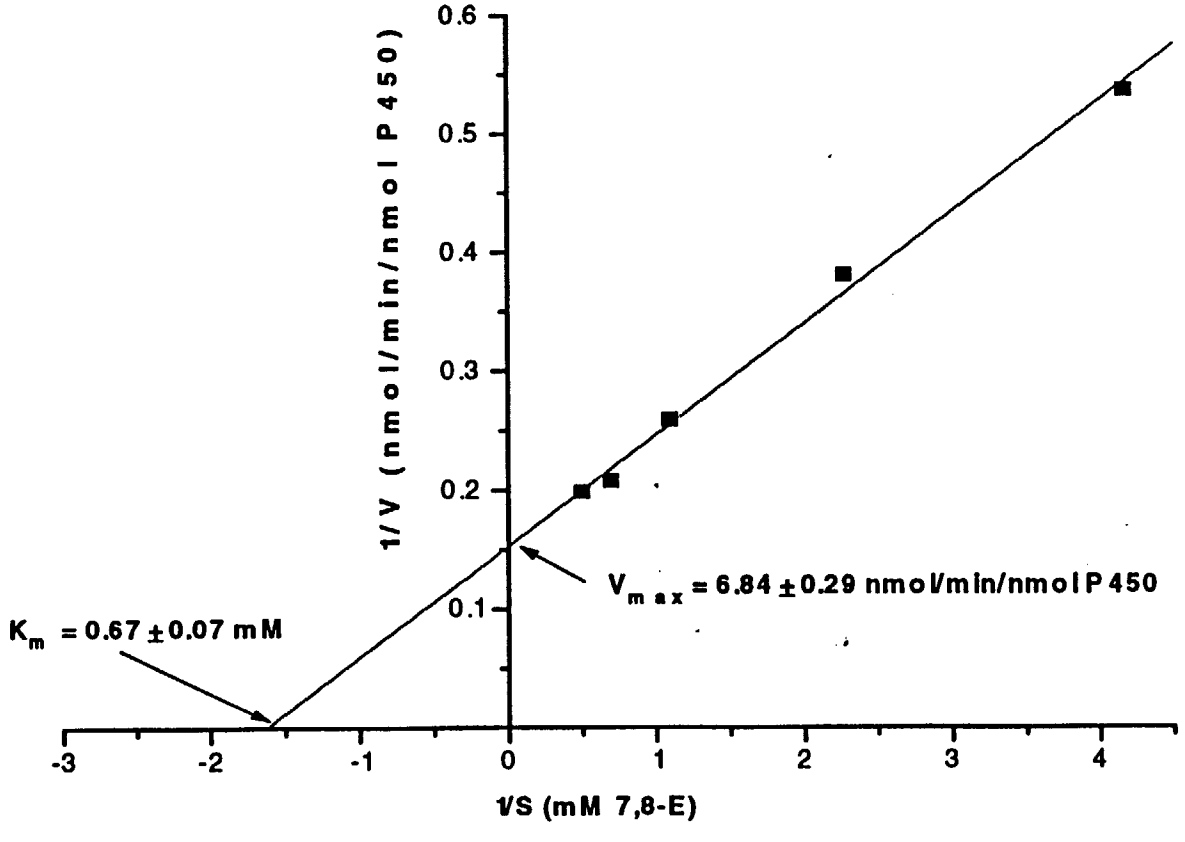


Figure 28

Production of 4-Vinylcyclohexene Diepoxide by Mouse Liver Microsomes

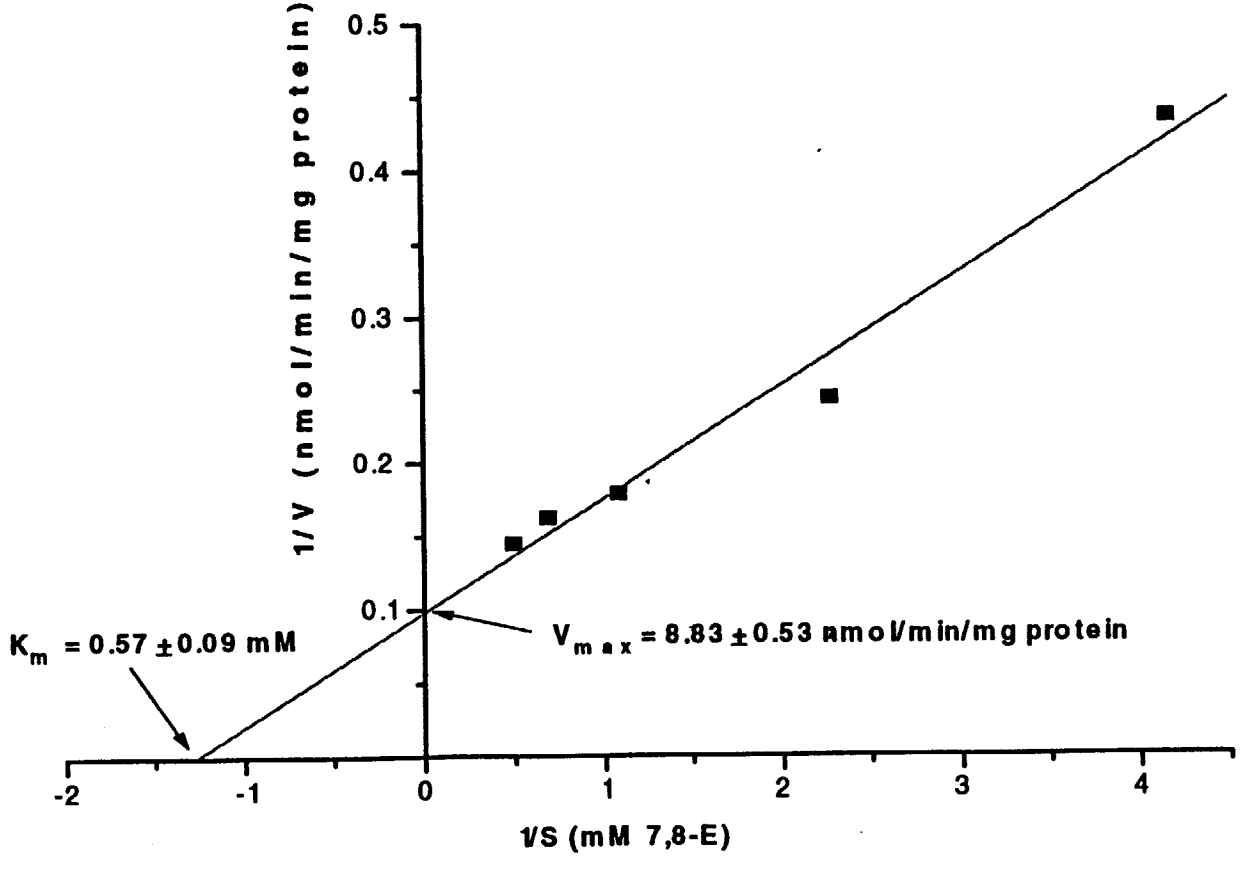


Figure 29

Production of 4-Vinylcyclohexene Diepoxide by Mouse Liver Microsomes

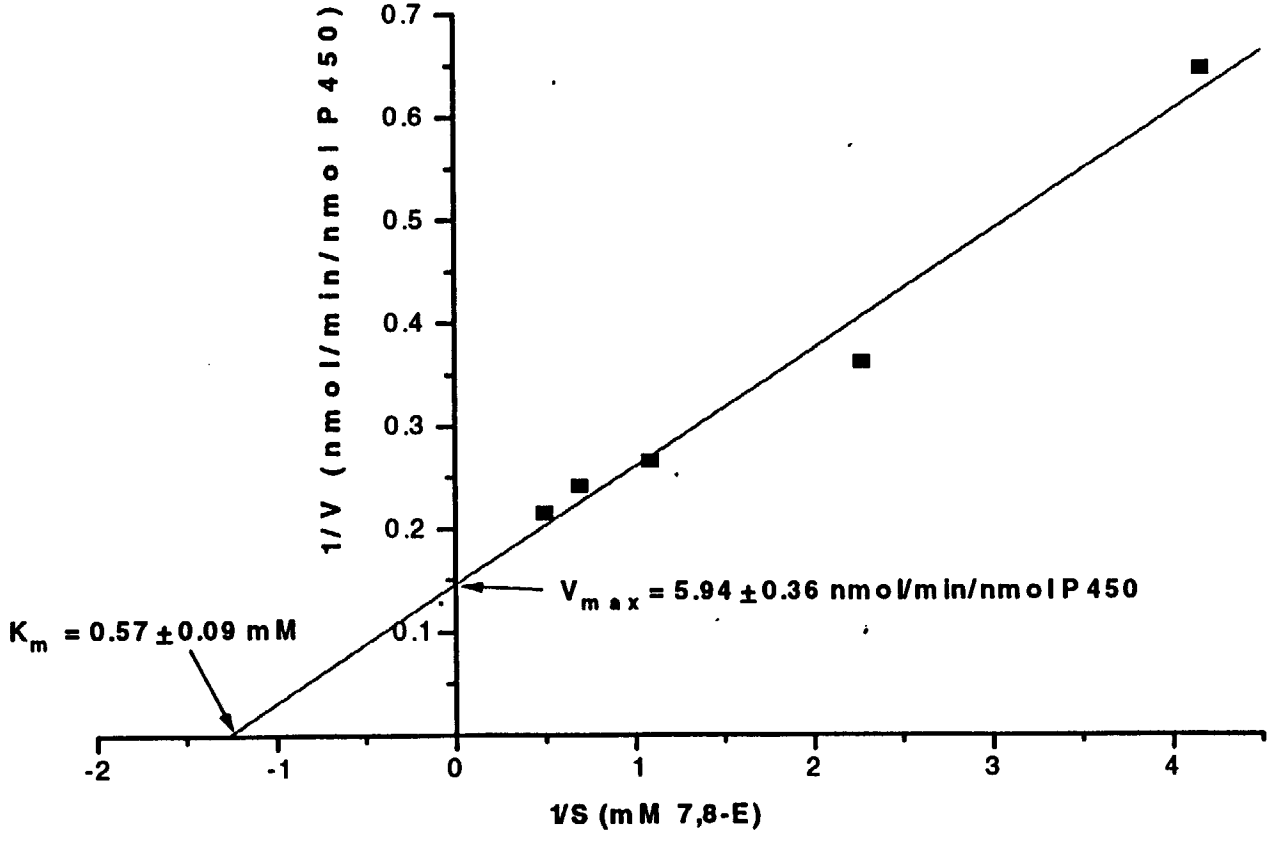


Figure 30

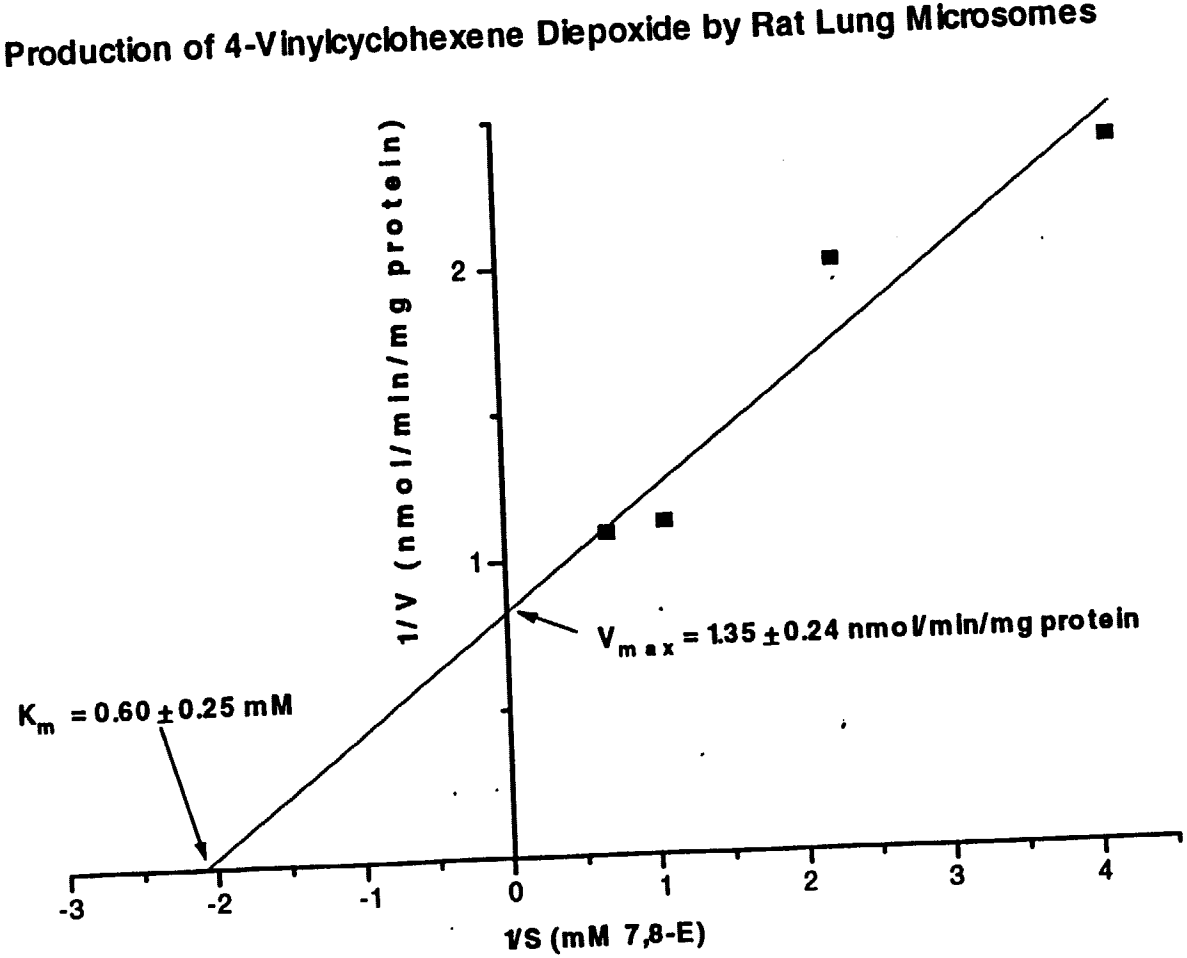

Production of 4-Vinylcyclohexene Diepoxide by Rat Lung Microsomes
1/V (nmol/min/mg protein)
2
1
V_{max} = 1.35 ± 0.24 nmol/min/mg protein
K_m = 0.60 ± 0.25 mM
-3
-2
-1
0
1
2
3
4
1/S (mM 7,8-E)

Figure 31

Production of 4-Vinylcyclohexene Diepoxide by Rat Lung Microsomes

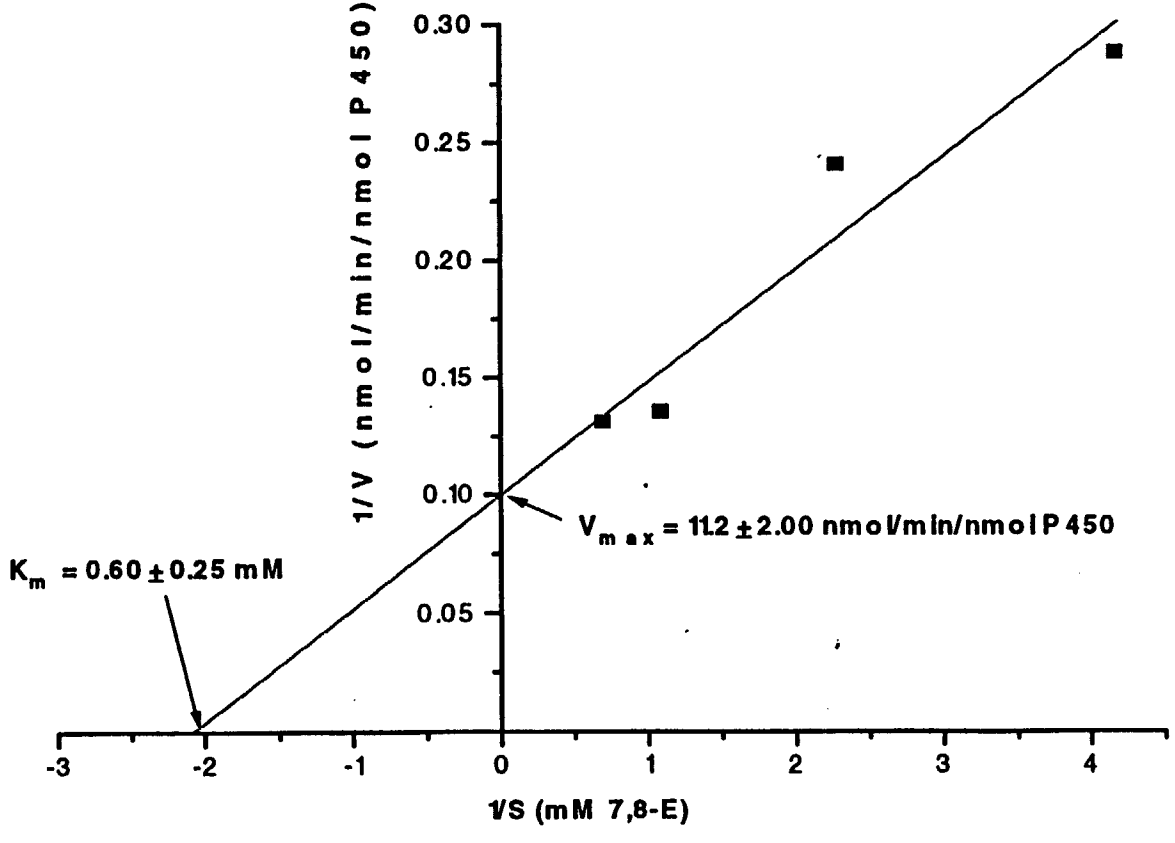


Figure 32

Production of 4-Vinylcyclohexene Diepoxide by Mouse Lung Microsomes

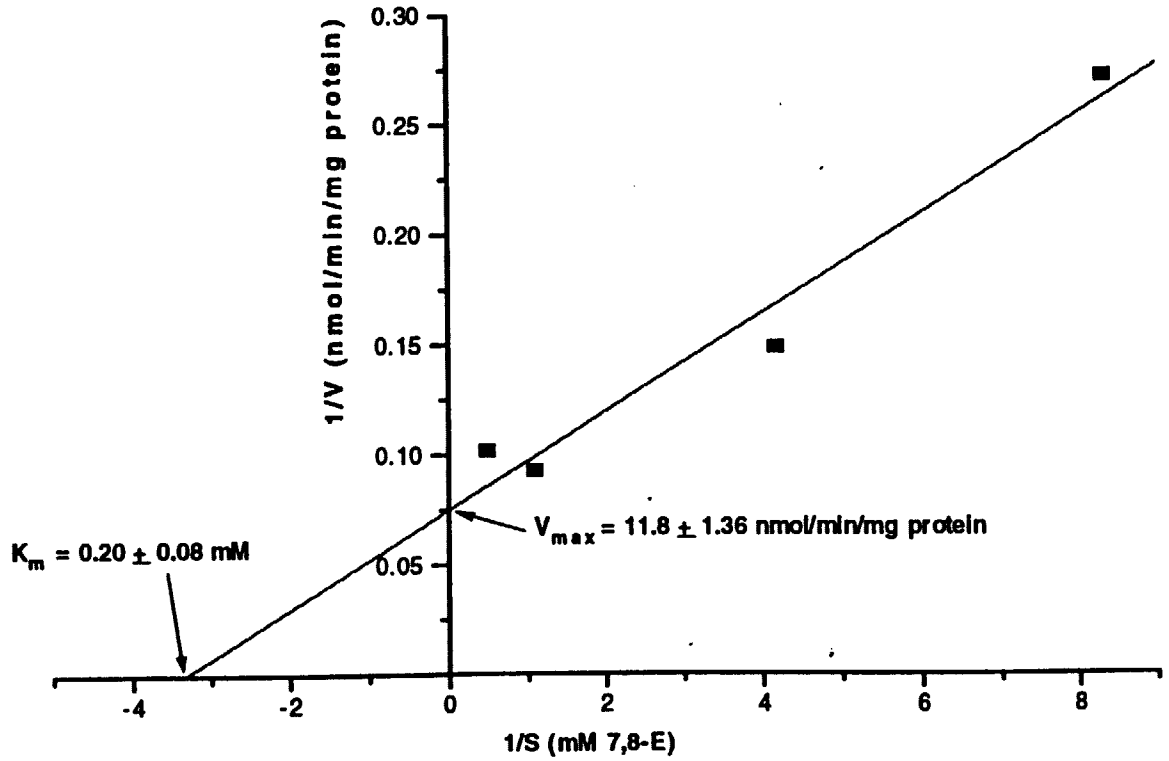


Figure 33

Production of 4-Vinylcyclohexene Diepoxide by Mouse Lung Microsomes

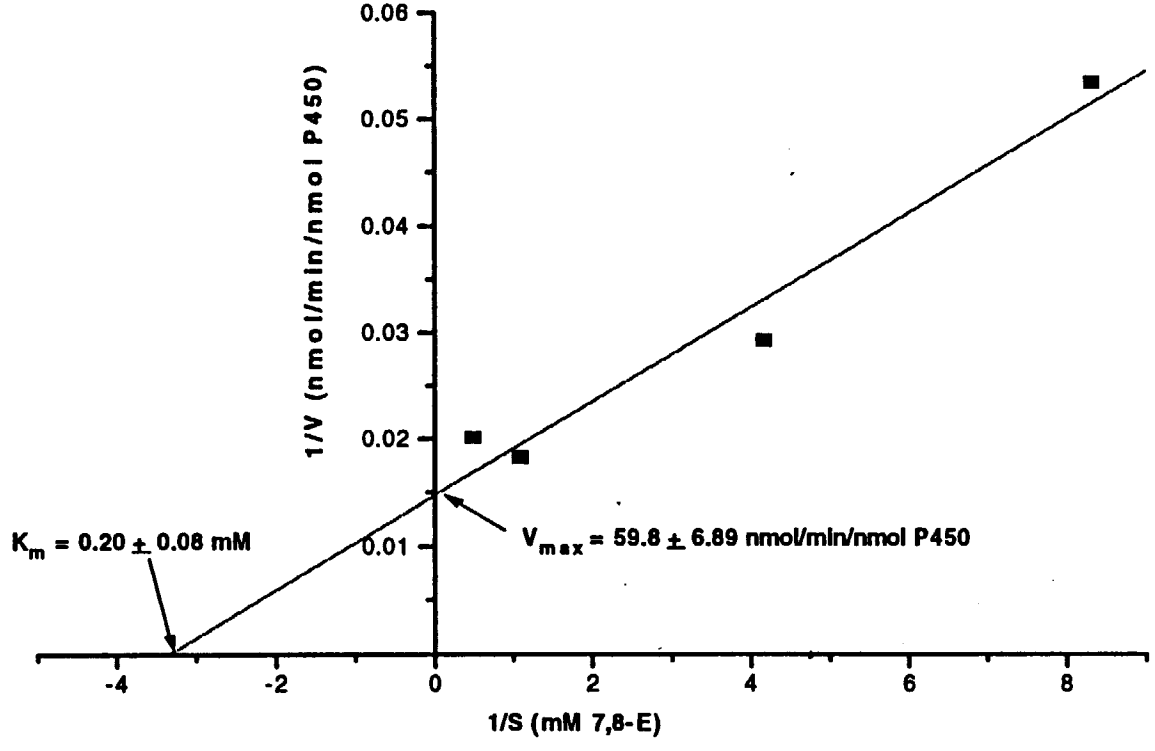


Figure 34

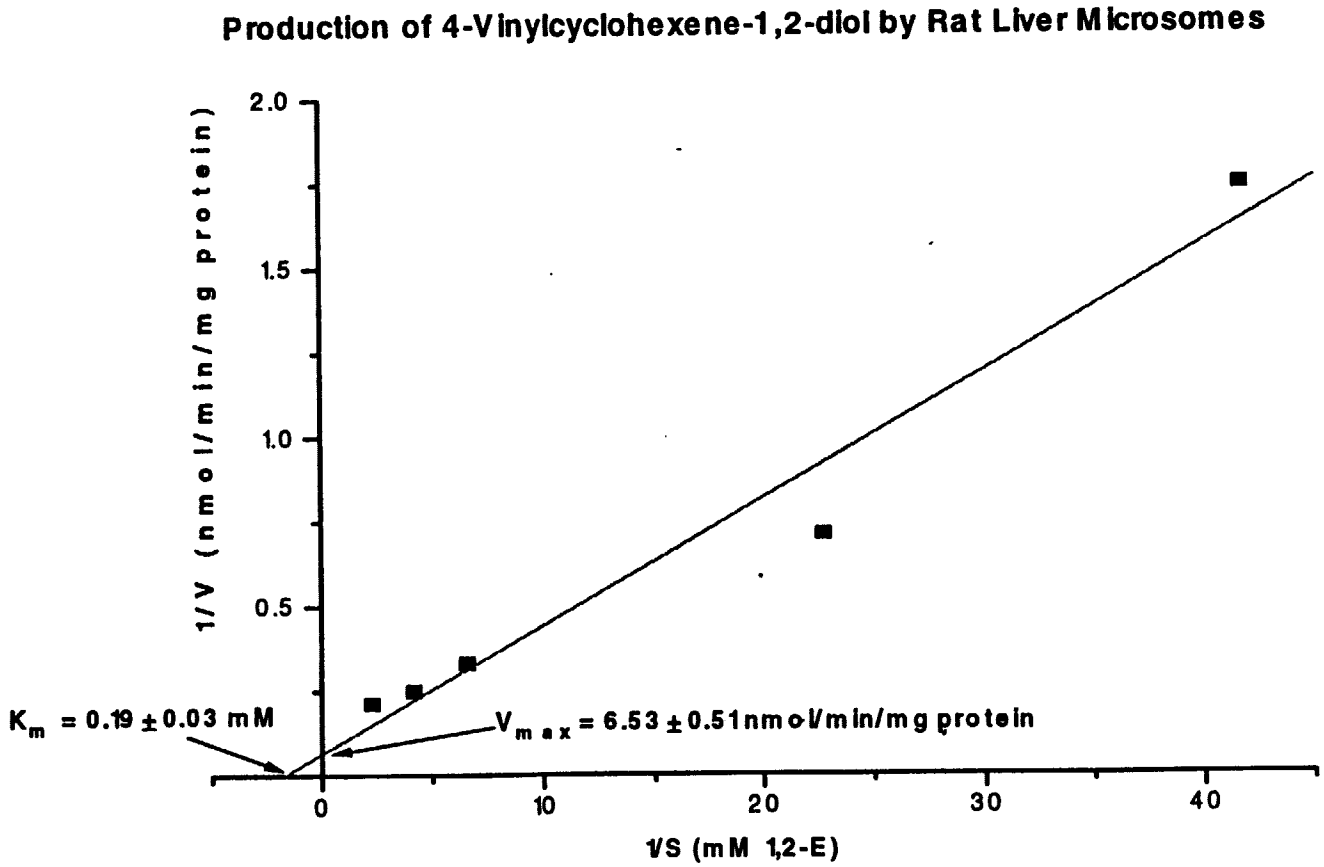

Production of 4-Vinylcyclohexene-1,2-diol by Rat Liver Microsomes
1/V (nmol/min/mg protein)
2.0
1.5
1.0
0.5
0
10
20
30
40
1/S (mM 1,2-E)
K_m = 0.19 ± 0.03 mM
V_{max} = 6.53 ± 0.51 nmol/min/mg protein

Figure 35

Production of 4-Vinylcyclohexene-1,2-diol by Mouse Liver Microsomes

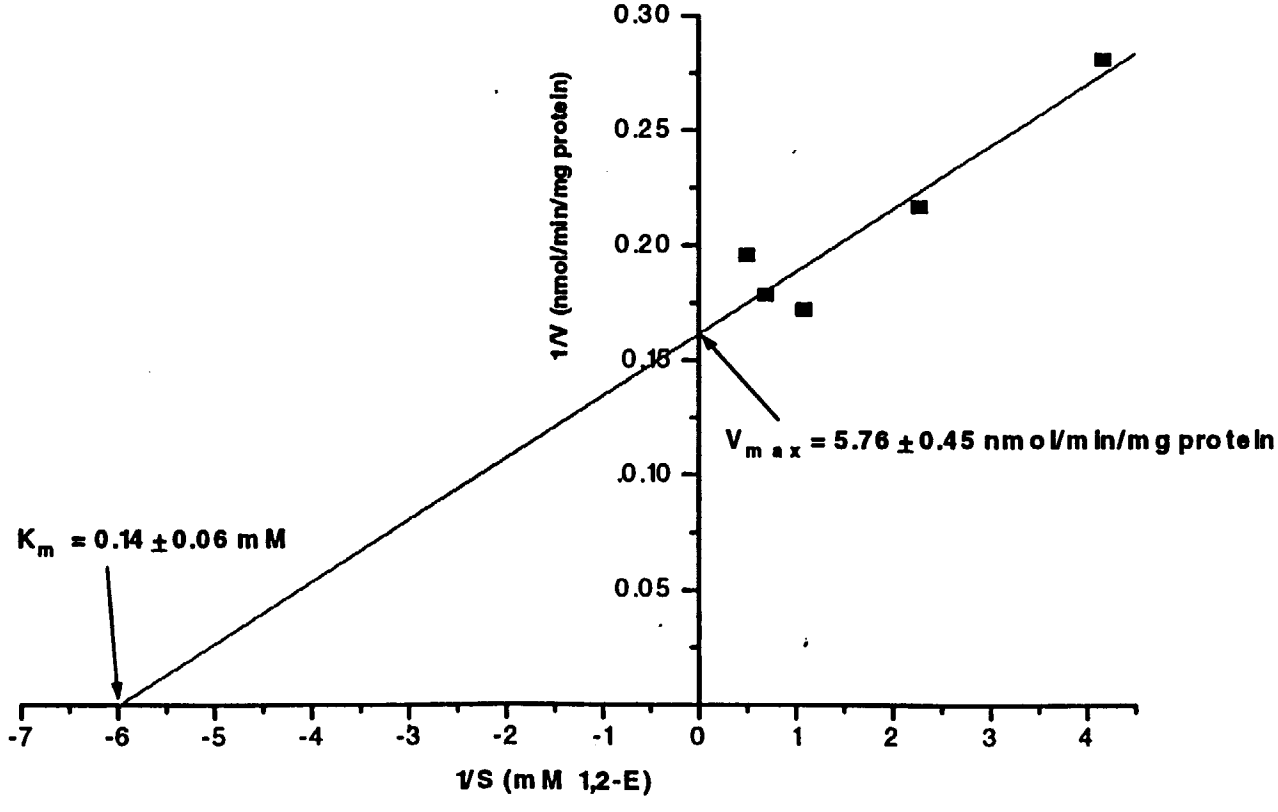


Figure 36

Production of 4-Vinylcyclohexene-7,8-diol by Rat Liver Microsomes

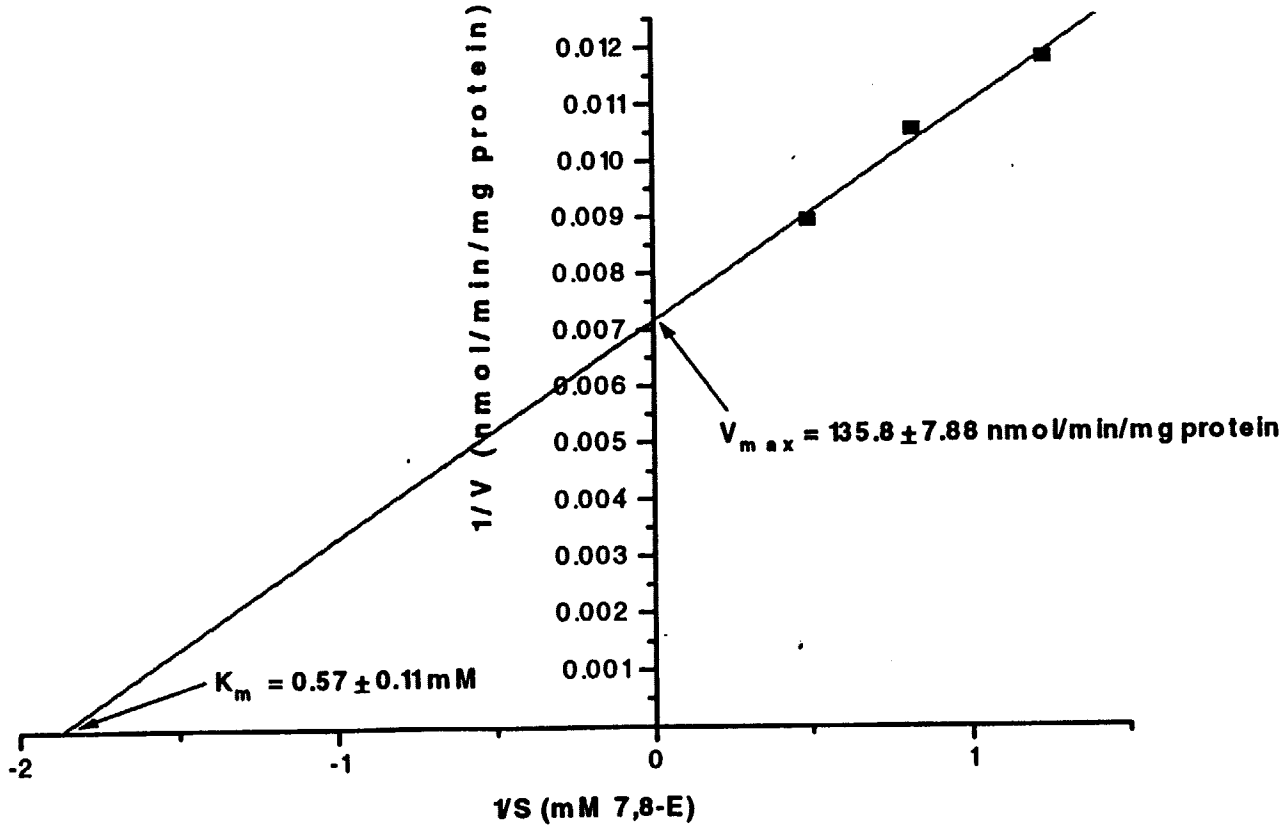


Figure 37

Hydrolysis of 4-Vinylcyclohexene Diepoxide by Rat Liver Microsomes

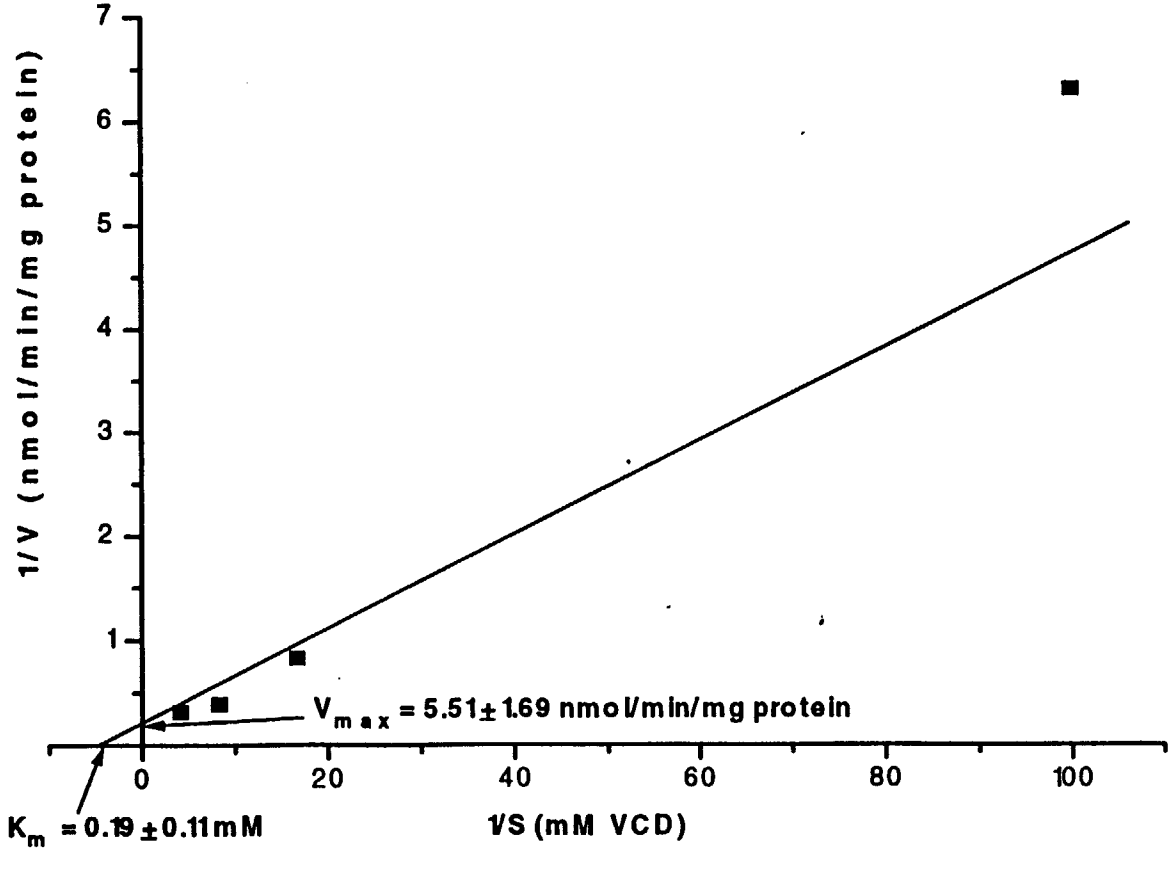
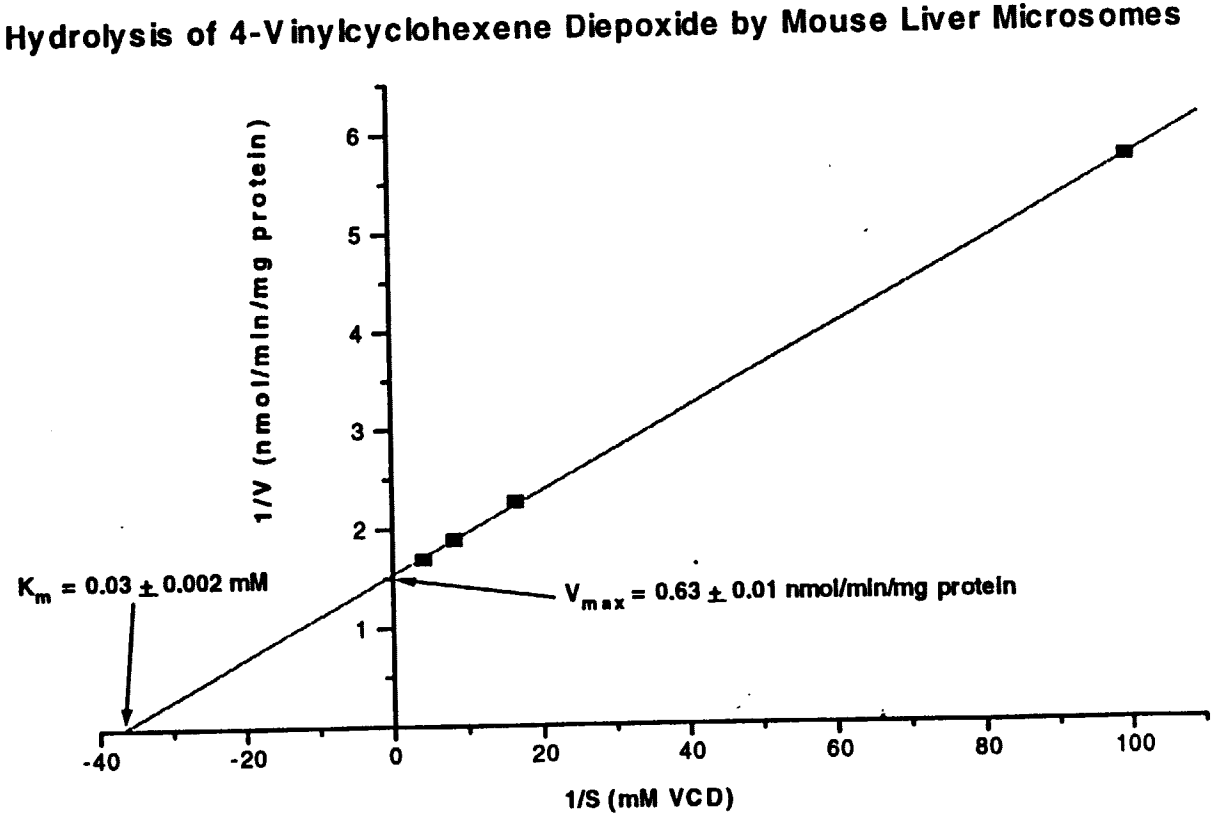


Figure 38


Hydrolysis of 4-Vinylcyclohexene Diepoxide by Mouse Liver Microsomes
1/V (nmol/min/mg protein)
6
5
4
3
2
1
K_m = 0.03 ± 0.002 mM
V_{max} = 0.63 ± 0.01 nmol/min/mg protein
-40
-20
0
20
40
60
80
100
1/S (mM VCD)

APPENDIX A

Rates of Metabolism of 4-Vinylcyclohexene and Metabolites

Explanatory Notes:

1. The first column for each reaction is the concentration of the substrate in the incubation mixture. The second (and third, if present) column is the rate of formation of the metabolite per minute.

2. Abbreviations:

   ND = No reaction product detected.
   1,2E = 4-VCH-1,2-epoxide
   7,8E = 4-VCH-7,8-epoxide
   VCD = 4-VCH diepoxide

3. Example Calculations.

   The example is taken from the reaction of 4-VCH to 4-VCH-1,2-epoxide, catalyzed by rat liver.

   The standard curve for 1,2E had a slope of 314930 and a y-intercept of 19.57.

   In the samples with a starting concentration of 1.98 mM 4-VCH, the mean peak area for the 1,2E was 2136. Using linear regression with the standard curve, the concentration of 1,2E in the ethyl acetate was calculated to be:

   (2136 - 19.57) / 314930 = 0.0067203 mM

   Since the ethyl acetate layer was only 0.311 times the volume of the amount of sample extracted, the concentration of 1,2E was corrected for the concentration during extraction, by multiplying the mM concentration calculated above by 0.311:

   0.0067203 mM x 0.311 = 0.00209 mM

Explanatory Notes (Continued):

The nmol 1,2E per sample was calculated by multiplying the mM concentration of 1,2E by the volume of the sample, and then by a correction factor to convert mmoles to nmoles:

0.00209 mM x 0.0021006 liters x 1000000 nmol/mmol = 4.390 nmol/sample

The nmol/min/mg protein was then calculated by dividing the nmol/sample by the minutes the incubation ran, and by the mg protein in the sample:

4.390 nmol/sample / 10 minutes / 3.4787 mg protein = 0.1262 nmol/min/mg protein

Since there was a measurable control rate for this reaction (0.0202 nmol/min/mg protein) this control rate was then subtracted from the rate for the 1.98 mM 4-VCH sample to get the final rate of the reaction:

0.1262 nmol/min/mg protein - 0.0202 nmol/min/mg protein = 0.1060 nmol/min/mg protein

To obtain a rate based on nmol P450 content, the protein-based rate was divided by the nmol P450 per mg protein:

0.1060 nmol/min/mg protein / 1.5055 nmol P450/mg protein = 0.0704 nmol/min/nmol P450

Production of 4-vinylcyclohexene-1,2-epoxide from 4-VCH in rat liver microsomes

mM 4-VCH	nmol/min/ mg protein*	nmol/min/ nmol P450*
0.05	ND	ND
0.10	0.0015	0.0010
0.20	0.021	0.014
0.30	0.021	0.014
0.39	0.039	0.026
0.50	0.051	0.034
0.68	0.062	0.041
0.99	0.087	0.058
1.98	0.106	0.070
Control 1.98	0.020	0.013

*Control rate subtracted out

Production of 4-vinylcyclohexene-1,2-epoxide from 4-VCH in mouse liver microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.03	0.09	0.062
0.05	0.16	0.11
0.11	0.36	0.24
0.22	0.55	0.38
	0.99	0.66
0.40	1.06	0.72
	1.81	1.21
0.83	2.79	1.87
1.30	3.44	2.31
1.82	4.49	3.01
Control 2.02	ND	ND

Data from experiments on two separate days were used.

Production of 4-vinylcyclohexene-1,2-epoxide from 4-VCH in rat lung microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.11	0.15	0.81
0.22	0.24	1.34
0.83	0.60	3.28
1.82	0.88	4.86
Control 1.82	0.03	0.19

Production of 4-vinylcyclohexene-1,2-epoxide from 4-VCH in rat ovary microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.22	0.03	0.13
0.83	0.04	0.18
1.82	0.10	0.41
Control 1.82	0.08	0.33

**No sample had a peak area above normal background.

Production of 4-vinylcyclohexene-1,2-epoxide from 4-VCH in mouse lung microsomes

mM 4-VCH	nmol/min/ mg protein*	nmol/min/ nmol P450*
0.11	0.57	4.82
0.22	0.94	7.97
0.83	1.95	16.53
1.82	2.64	22.36
Control 1.82	0.14	1.19

*Control rate subtracted out

Production of 4-vinylcyclohexene-1,2-epoxide from 4-VCH in mouse ovary microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.22	ND	ND
0.83	ND	ND
1.82	ND	ND

Production of 4-vinylcyclohexene-7,8-epoxide from 4-VCH in rat liver microsomes

mM 4-VCH	nmol/min/ mg protein*	nmol/min/ nmol P450*
0.05	ND	ND
0.10	0.0002	0.00015
0.20	ND	ND
0.30	ND	ND
0.39	0.0017	0.0011
0.50	0.0017	0.0011
0.68	ND	ND
0.99	0.0047	0.0031
1.98	0.0042	0.0028
Control 1.98	0.0038	0.0025

*Control rate subtracted out

Production of 4-vinylcyclohexene-7,8-epoxide from 4-VCH in mouse liver microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.03	0.01	0.0079
0.05	0.02	0.012
0.11	0.05	0.035
0.22	0.08	0.053
	0.10	0.070
0.40	0.14	0.093
	0.16	0.11
0.83	0.26	0.17
1.30	0.31	0.21
1.82	0.44	0.30
Control 2.02	ND	ND

Data from experiments on two separate days were used.

Production of 4-vinylcyclohexene-7,8-epoxide from 4-VCH in rat lung microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.11	ND	ND
0.22	0.18	0.97
0.83	0.34	1.86
1.82	ND	ND
Control 1.82	ND	ND

Production of 4-vinylcyclohexene-7,8-epoxide from 4-VCH in mouse lung microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.11	0.22	1.83
0.22	0.44	3.72
0.83	1.07	9.06
1.82	1.31	11.1
Control 1.82	ND	ND

Production of 4-vinylcyclohexene-7,8-epoxide from 4-VCH in rat ovary microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.22	ND	ND
0.83	ND	ND
1.82	ND	ND
Control 1.82	ND	ND

Production of 4-vinylcyclohexene-7,8-epoxide from 4-VCH in mouse ovary microsomes

mM 4-VCH	nmol/min/ mg protein	nmol/min/ nmol P450
0.22	ND	ND
0.83	ND	ND
1.82	ND	ND

Production of 4-vinylcyclohexene diepoxide from 4-VCH-1,2-epoxide in rat liver microsomes

mM 1,2-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	1.14	1.18
0.44	1.73	1.79
0.92	1.93	1.99
1.44	2.53	2.61
2.02	3.05	3.15
Control 0.92	ND	ND

Production of 4-vinylcyclohexene diepoxide from 4-VCH-1,2-epoxide in mouse liver microsomes

mM 1,2-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	1.69	1.15
0.44	2.59	1.76
0.92	3.34	2.28
1.44	3.89	2.65
2.02	4.36	2.97
Control 2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-1,2-epoxide in rat lung microsomes

mM 1,2-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.12	0.71	4.98
0.24	0.83	5.77
0.92	1.62	11.3
2.02	1.79	12.5
Control 2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-1,2-epoxide in mouse lung microsomes

mM 1,2-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.12	1.53	7.22
0.24	1.87	8.80
0.92	2.42	11.4
2.02	2.61	12.3
Control 2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-1,2-epoxide in rat ovary microsomes

mM 1,2-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	ND	ND
0.92	ND	ND
2.02	ND	ND
Control 2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-1,2-epoxide in mouse ovary microsomes

mM 1,2-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	ND	ND
0.92	ND	ND
2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-7,8-epoxide in rat liver microsomes

mM 7,8-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	2.58	1.87
0.44	3.63	2.63
0.91	5.34	3.86
1.43	6.64	4.81
2.00	6.96	5.04
Control 0.91	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-7,8-epoxide in mouse liver microsomes

mM 7,8-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	2.31	1.55
0.44	4.11	2.77
0.92	5.59	3.77
1.44	6.17	4.15
2.02	6.91	4.65
Control 2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-7,8-epoxide in rat lung microsomes

mM 7,8-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	0.42	3.48
0.44	0.50	4.16
0.92	0.89	7.39
1.44	0.92	7.64
Control 0.92	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-7,8-epoxide in mouse lung microsomes

mM 7,8-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.12	3.70	18.8
0.24	6.74	34.2
0.92	10.79	54.8
2.02	9.80	49.8
Control 2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-7,8-epoxide in rat ovary microsomes

mM 7,8-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	ND	ND
0.92	ND	ND
2.02	ND	ND
Control 2.02	ND	ND

Production of 4-vinylcyclohexene diepoxide
from 4-VCH-7,8-epoxide in mouse ovary microsomes

mM 7,8-E	nmol/min/ mg protein	nmol/min/ nmol P450
0.24	ND	ND
0.92	ND	ND
2.02	ND	ND

Production of 4-vinylcyclohexene-1,2-diol
from 4-VCH-1,2-epoxide in rat liver microsomes

mM 1,2-E	nmol/min/ mg protein
0.024	0.55
0.044	1.34
0.152	2.88
0.240	3.84
0.440	4.52
Control 0.152	ND

Production of 4-vinylcyclohexene-1,2-diol
from 4-VCH-1,2-epoxide in mouse liver microsomes

mM 1,2-E	nmol/min/ mg protein
0.24	3.40
0.44	4.40
0.92	5.54
1.44	5.34
2.02	4.87
Control 2.02	ND

Production of 4-vinylcyclohexene-1,2-diol
from 4-VCH-1,2-epoxide in rat lung microsomes

mM 1,2-E	nmol/min/ mg protein
0.12	ND
0.24	ND
0.92	ND
2.02	ND
Control 2.02	ND

Production of 4-vinylcyclohexene-1,2-diol
from 4-VCH-1,2-epoxide in mouse lung microsomes

mM 1,2-E	nmol/min/ mg protein
0.12	ND
0.24	ND
0.92	ND
2.02	ND
Control 2.02	ND

Production of 4-vinylcyclohexene-1,2-diol
from 4-VCH-1,2-epoxide in rat ovary microsomes

mM 1,2-E	nmol/min/ mg protein
0.24	ND
0.92	ND
2.02	ND
Control 2.02	ND

Production of 4-vinylcyclohexene-1,2-diol
from 4-VCH-1,2-epoxide in mouse ovary microsomes

mM 1,2-E	nmol/min/ mg protein
0.24	ND
0.92	ND
2.02	ND

Production of 4-vinylcyclohexene-7,8-diol
from 4-VCH-7,8-epoxide in rat liver microsomes

mM 7,8-E	nmol/min/ mg protein
0.404	ND
0.808	80.8
1.212	90.5
2.020	106.8
Control 0.808	ND

Production of 4-vinylcyclohexene-7,8-diol
from 4-VCH-7,8-epoxide in mouse liver microsomes

mM 7,8-E	nmol/min/ mg protein
0.24	ND
0.44	ND
0.92	ND
1.44	ND
2.02	ND
Control 2.02	10.9

Production of 4-vinylcyclohexene-7,8-diol
from 4-VCH-7,8-epoxide in rat lung microsomes

mM 7,8-E	nmol/min/ mg protein
0.24	ND
0.44	ND
0.92	ND
1.44	ND
2.02	ND
Control 0.92	ND

Production of 4-vinylcyclohexene-7,8-diol
from 4-VCH-7,8-epoxide in mouse lung microsomes

mM 7,8-E	nmol/min/ mg protein
0.12	ND
0.24	ND
0.92	ND
2.02	ND
Control 2.02	ND

Production of 4-vinylcyclohexene-7,8-diol
from 4-VCH-7,8-epoxide in rat ovary microsomes

mM 7,8-E	nmol/min/ mg protein
0.24	ND
0.92	ND
2.02	ND
Control 2.02	ND

Production of 4-vinylcyclohexene-7,8-diol
from 4-VCH-7,8-epoxide in mouse ovary microsomes

mM 7,8-E	nmol/min/ mg protein
0.24	ND
0.92	ND
2.02	ND

Hydrolysis of 4-vinylcyclohexene diepoxide by rat liver microsomes

mM VCD	nmol/min/ mg protein
0.01	0.15
0.06	1.15
0.12	2.44
0.24	3.01

Hydrolysis of 4-vinylcyclohexene diepoxide by mouse liver microsomes

mM VCD	nmol/min/ mg protein
0.01	0.17
0.06	0.42
0.12	0.51
0.24	0.57

Hydrolysis of 4-vinylcyclohexene diepoxide by rat lung microsomes

mM VCD	nmol/min/ mg protein
0.12	0.39
0.24	0.20
0.92	0.58
2.02	*

* Unable to calculate

Hydrolysis of 4-vinylcyclohexene diepoxide by mouse lung microsomes

mM VCD	nmol/min/ mg protein
0.12	1.36
0.24	*
0.92	*
2.02	*

* Unable to calculate

Hydrolysis of 4-vinylcyclohexene diepoxide by rat ovary microsomes

mM VCD	nmol/min/ mg protein
0.24	1.43
0.92	*
2.02	*

* Unable to calculate

APPENDIX B

Protocol and Protocol Amendments

METABOLISM AND PHARMACOKINETICS OF 4-VINYLCYCLOHEXENE IN RATS AND MICE

MEDICAL RESEARCH PROJECT NO. 9298-001

PROTOCOL

Haskell Laboratory for Toxicology and Industrial Medicine
Post Office Box 50, Elkton Road
Newark, DE 19714

Principal Investigator: Douglas A. Keller, Ph.D.

Metabolism and Pharmacokinetics of 4-Vinylcyclohexene in Rats and Mice

Medical Research Project No. 9298-001

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Metabolism and Pharmacokinetics of 4-Vinylcyclohexene in Rats and Mice

INTRODUCTION

4-Vinylcyclohexene (4-VCH, CAS No. 100-40-3) is a dimer of 1,3-butadiene and is formed spontaneously during the manufacture of 1,3-butadiene. 4-VCH is also produced for use as an intermediate in polymer manufacturing. Concentrations of 4-VCH in the workplace are generally below 0.1 ppm (1).

4-VCH was tested by the gavage route in rats and mice in subchronic and chronic toxicity test protocols. Doses for the subchronic studies (13 weeks) were 0, 50, 100, 200, 400, or 800 mg/kg/day for rats and 0, 75, 150, 300, 600, or 1200 mg/kg/day for mice. Body weights were decreased in male rats at 400 mg/kg/day or greater and in female rats at 800 mg/kg/day. Female mice receiving 600 mg/kg/day had decreased body weights, and mortality was high at 1200 mg/kg/day in mice. Male rats had hyaline droplet degeneration in the kidney at all doses, and female mice in the 1200 mg/kg/day group had decreased numbers of primary follicles in the ovary. Female mice in other groups were not examined for ovarian effects (2).

Long-term gavage studies with 4-VCH were conducted at 0, 200, or 400 mg/kg/day with both rats and mice. High mortality in the rats made the study inadequate for evaluation of carcinogenicity. Survival of the high dose female mice was reduced, and ovarian tumors were present in female mice at both doses (3).

Metabolism of 4-VCH has been studied *in vitro* and *in vivo* at saturating concentrations (4, 5, 6). Metabolism occurs via the cytochrome P-450 pathway, producing mono- and diepoxides. At saturating concentrations the mouse tissues metabolized 4-VCH faster than the rat tissues. The monoepoxide was detected in rats and mice, with mice producing more epoxide than rats. In mice, the diepoxide metabolite was detected in blood after a gavage dose of 4-VCH, while the diepoxide was not detectable in rats. The diepoxide was shown to be the metabolite responsible for the ovarian toxicity.

OBJECTIVE

The objective of these studies is to investigate, in rats and mice, the rates of metabolism of 4-VCH to epoxide metabolites and the rate of metabolism of the epoxides to diols. Metabolism at subsaturating concentrations is important in estimating toxicity at low concentrations, and will be investigated. Knowledge of the concentration at which

metabolism is saturated is important for setting dose levels for inhalation studies. Partition coefficients of 4-VCH and the epoxide metabolites into blood and tissues will be measured to allow construction of a physiologically-based pharmacokinetic model for 4-VCH distribution and metabolism in rats and mice. This model will allow estimation of delivered doses of 4-VCH and its metabolites to target organs in rats and mice at any concentration via inhalation or oral exposure.

The experiments outlined in this protocol are being conducted as part of a Consent Order between the Butadiene Panel of the Chemical Manufacturers Association and the U.S. Environmental Protection Agency. All experimental work will be conducted according to Good Laboratory Practices (7).

SPONSOR AND TEST FACILITY

This study is sponsored by the Butadiene Panel of the Chemical Manufacturers Association, 2501 M Street, N.W., Washington, D.C. 20037. The study will be conducted at Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware, 19714.

STUDY DESIGN

The experimental design is outlined in Appendices 3 and 4 of the *Testing Consent Order for 4-Vinylcyclohexene (4-VCH)* (Docket No. OPTS-42116), which are attached to this protocol as Appendices II and III. Two types of tesing will be conducted: Partition coefficient testing and *in vitro* metabolism testing.

Tissues for testing will be obtained from female rats and mice, since the ovary is a target tissue.

MATERIALS AND METHODS

A. Test Materials

The test materials will be purchased from Aldrich Chemical Company, Milwaukee, Wisconson. 4-VCH will be at least 98% pure. The purity of the monoepoxides and diepoxides will be dependent on the efficiency of synthetic methods. Diol metabolites of the epoxides will be used only as analytical standards. The purity of all test materials used will be determined at least every three months during the experimental phase of the study.

B. Test Species

Female Crl:CD®BR rats, approximately 28 days of age, with body weights ranging from 100 - 200 g will be acquired from Charles River Laboratories, Inc., Kingston, NY. Rats will be sacrificed for use on the study when they weigh between 200 and 300 g.

Female CD-1® mice, approximately 28 days of age, with body weights ranging from 20 - 28 g will be acquired from Charles River Laboratories, Inc., Raleigh, NC. Mice will be sacrificed for use on the study when they weigh between 25 and 35 g.

Other strains of rats and mice may be used at the discretion of the study director.

C. Animal Husbandry

Haskell Laboratory is accredited by the American Association for Accreditation of Laboratory Animal Care. All animals will be cared for according to National Institutes of Health guidelines.

All animals will be housed in stainless steel, wire-mesh cages suspended above absorbent cage boards. Animal rooms will be targeted at a temperature of 23 ± 2°C and a relative humidity of 50 ± 10%. Animal rooms will be artificially illuminated on a 12-hour light/dark cycle. All animals will be provided with tap water and fed Purina® Certified Rodent Chow #5002 *ad libitum*.

Haskell Laboratory has an animal health monitoring program which consists of periodic food and water analyses for contaminants, sampling freshly washed cages and cage racks for bacteria, and sampling animals housed in the colony for pathogens. This program is monitored and administered by the laboratory veterinarian. Data are maintained separately from the study records; any deviations from acceptable values will be maintained in the study records.

Rats and mice to be used for these studies will be quarantined for at least 1 week, and released by the laboratory veterinarian after demonstrating adequate weight gain and freedom from disease.

D. Partition Coefficient Determinations

Partition coefficients for 4-VCH, 4-VCH-7,8-epoxide, 4-VCH-1,2-epoxide, and 4-VCH-diepoxide will be determined, if possible, between air and the following tissues or liquids from both rats and mice: blood, liver, lung, ovary, fat, skeletal muscle. The testing for partition coefficients will follow the procedures outlined in reference (8). The guideline attached as Appendix II will be followed.

E. *In Vitro* Metabolism Determinations

Metabolic rate constants (V_{max}, K_m) will be determined for the following reactions:

4-VCH to 4-VCH-1,2-epoxide
4-VCH to 4-VCH-7,8-epoxide
4-VCH-1,2-epoxide to 4-VCH-diepoxide
4-VCH-1,2-epoxide to 4-VCH-1,2-dihydrodiol
4-VCH-7,8-epoxide to 4-VCH-diepoxide
4-VCH-7,8-epoxide to 4-VCH-7,8-dihydrodiol
4-VCH-diepoxide hydrolysis

Microsomal preparations of liver, lung, and ovary tissue from rats and mice will be used for these determinations, based on the guideline in Appendix III. Analysis of the data for V_{max} and K_m will be performed with EZ-FIT, a software program for the IBM PC (Perrella Scientific, Springfield, PA).

SAFETY AND HOUSEKEEPING

4-VCH and its epoxides will be handled as carcinogens. Specific handling procedures are contained in the Chemical Catalogue, which should be read before handling the chemicals. Good housekeeping practices will be followed to avoid contamination of the laboratory. Gloves will be worn while handling all compounds and solutions containing the compounds. Neat test materials will be handled in a chemical hood. Waste material will be incinerated.

RECORDS AND SAMPLE RETENTION

All original records will be retained at Haskell Laboratory or at the records management center, E. I. du Pont de Nemours and Company, Wilmington, Delaware.

A portion of the test chemicals will be collected for archive purposes prior to the outset of the study and retained at Haskell Laboratory.

Any change, addition, or deletion to the protocol will be documented, approved by the sponsor, and maintained in the study records.

REFERENCES

1. Notice of Intended Change - 4-Vinylcyclohexene. Appl. Occup. Environ. Hyg. 6(7):628-632 (1991).

2. Collins, J.J. and A.G. Manus. Toxicological Evaluation of 4-Vinylcyclohexene. I. Prechronic (14-Day) and Subchronic (13-Week) Gavage Studies in Fischer 344 Rats and B6C3F$_1$ Mice. J. Toxicol. Environ. Helath 21:493-505 (1987).

3. Collins, J.J., R.J. Montali, and A.G. Manus. Toxicological Evaluation of 4-Vinylcyclohexene. II. Induction of Ovarian Tumors in Female B6C3F$_1$ Mice by Chronic Oral Administration of 4-Vinylcyclohexene. J. Toxicol. Environ. Helath 21:507-524 (1987).

4. Gervasi, P.G., A. Abbondandolo, L. Citti, and G. Turchi. Microsomal 4-Vinylcyclohexene Mono-Oxygenase and Mutagenic Activity of Metabolic Intermediates. Ind. Environ. Xenobiotics, Proc. Int. Conf., 205-210 (1981).

5. Smith, B.J., D.E. Carter, and I.G. Sipes. Comparison of the Disposition and *in Vitro* Metabolism of 4-Vinylcyclohexene in the Female Mouse and Rat. Toxicol. Appl. Pharmacol. 105:364-371 (1990).

6. Smith, B.J., D.R. Mattison, and I.G. Sipes. The Role of Epoxidation in 4-Vinylcyclohexene-Induced Ovarian Toxicity. Toxicol. Appl. Pharmacol. 105:372-381 (1990).

7. Part III. Environmental Protection Agency, Toxic Substances Control Act; Good Laboratory Practice Standards 40 CFR Part 792. Published August 17, 1989.

8. Gargas, M.L., R.J. Burgess, D.E. Voisard, G.H. Cason, and M.E. Andersen. Partition Coefficients of Low-Molecular-Weight Volatile Chemicals in Various Liquids and Tissues. Toxicol. Appl. Pharmacol. 98:87-99 (1989).

PROTOCOL APPENDIX I

MEDICAL RESEARCH PROJECT NO. 9298-001

Metabolism and Pharmacokinetics of 4-Vinylcyclohexene in Rats and Mice

STUDY PERSONNEL AND STUDY DATES

A. Study Function	Study Personnel
1. Study Director:	D. A. Keller Research Toxicologist
2. Co-Investigators	M. S. Bogdanffy Senior Research Toxicologist
	D. R. Plowchalk Research Toxicologist

B. Study Dates	
1. Proposed Experiment Start	February, 1992
2. Proposed Experiment End	October, 1992
3. Draft Report Due	November, 1992
4. Final Report Due	January, 1993

PROTOCOL APPENDIX II

MEDICAL RESEARCH PROJECT NO. 9298-001

Partition Coefficient Testing with 4-Vinylcyclohexene

(a) *Purpose.* Pharmacokinetic parameters of 4-vinylcyclohexene (4-VCH) can be estimated by determination of partition coefficients for 4-VCH and its epoxide metabolites, and by measuring the rate of metabolism of the parent compound and the metabolites *in vitro*. Partition coefficients describe the ratio of chemical concentration between blood and air, and the ratio of concentrations between tissues and blood for various organs. When combined with knowledge of the rate of metabolism of the parent compound to metabolites and the rates of conversion of the metabolites to further metabolites, a mathematical model can be constructed to describe the movement of the chemical into and out of the animal and its tissues.

(b) *Definition.* (1) A partition coefficient for a given chemical is the ratio of concentrations achieved between two different media at equilibrium.

(c) *Test method.* (1) *Principle.* Tissue homogenates are incubated in sealed vials with the test chemical for a suitable period of time and sampled for test chemical concentration in the headspace. The test chemical will equilibrate with the tissue homogenate, and the concentration of the test chemical in the headspace when compared to the headspace concentration in a vial containing no homogenate yields the partition coefficient. Air:tissue partition coefficients divided by air:blood partition coefficients yield blood:tissue partition coefficients.

(2) *Description.* Tissue homogenates shall be incubated with a known concentration of test chemical vapor in a sealed vial at 37°C for a defined period of time. Reference vials containing the liquid used to homogenize the tissues, or empty vials for reference to blood, shall be incubated with test chemical vapors for an identical period of time. Vials shall be sampled for test chemical concentration in the headspace after the defined time periods, until there is no more decrease in the headspace concentration with time.

(3) *Tissues.* Tissues to be evaluated shall include blood, liver, lung, ovary, fat, and skeletal muscle. Tissues shall be evaluated from female mice and female rats.

(4) *Metabolism.* If equilibration of the test chemical and tissue is not reached within a reasonable period of time, metabolism of the test chemical may be interfering with the assay. In this case, appropriate metabolic inhibitors may be added to the homogenate or blood to eliminate the metabolic effect.

(5) *Test chemicals.* 4-VCH shall be tested. If technically feasible, 4-VCH-1,2-epoxide, 4-VCH-7,8-epoxide, and 4-VCH-diepoxide should also be tested. Starting concentrations in the headspace of the vials should exceed ten times the lower limit of detection for the analytical method, and should not exceed 500 ppm.

(d) *Test performance.* (1) Tissues should be removed from animals and homogenized in an appropriate diluent, usually 0.9% saline. If sufficient tissue for testing is not obtained from a single animal, tissues from several animals may be pooled. Blood is not diluted, but is heparinized to prevent clotting. The tissue homogenate, blood, or diluent is placed in a sealed vial with a septum. Reference vials shall contain the diluent used for homogenization or are empty for reference to blood. The vial should be incubated at 37°C for approximately 15 minutes, and the pressure released by insertion of a needle through the septum. A volume of gas equal to that of the test chemical to be introduced shall then be removed from the vial. The volume of test chemical at a known concentration is then introduced into the vial through the septum. The vial shall be incubated and agitated at 37°C for a suitable period of time, usually 1 and 3 hours.

(2) After the incubation period, the vial shall be sampled through the septum for concentration of the test chemical in the headspace. Samples shall be analyzed by procedures based on those by Sipes *et al.*, (2), or liquid scintillation counting if necessary, including construction of a standard curve.

(3) Results should be confirmed in an independent experiment, preferably the experiments should be performed in triplicate.

(e) *Data and report.* (1) *Treatment of results.* Data shall be presented in tabular form, listing the mean and standard deviation of the partition coefficient for each test medium (tissue homogenate, blood, or diluent). For blood or diluent, the partition coefficient should be calculated using the equation

$$P_i = \frac{C_{ref}(V_{vial}) - C_i(V_{vial} - V_i)}{C_i(V_i)} \quad (1)$$

where P_i is the partition coefficient (i = the liquid or tissue), C_{ref} = the chemical concentration in the headspace of the reference vial, C_i is the headspace chemical concentration of the test vial, and V_{vial} and V_i are the volumes of the empty vial and test liquid (or tissue), respectively.

For tissue homogenates, the partition coefficient should be calculated using the equation

$$P_j = \frac{C_{ref}(V_{vial} - V_{sal}) - C_j(V_{vial} - V_{sal} - V_j) + (C_{ref} - C_j)P_{sal}V_{sal}}{C_j(V_j)} \quad (2)$$

Where P_j is the partition coefficient (j = tissue), V_{sal} is the saline volume, P_{sal} is the diluent:air or blood:air partition coefficient calculated using equation (1), C_{ref} and C_j are the headspace concentrations in the reference and test vials, respectively, and V_{vial} and V_j are the volumes of the empty vial and tissue, respectively.

(2) *Test report.* The following specific information shall be reported.

(i) Mouse and rat stock used in the assay, age of animals, and number of animals used.

(ii) Test chemicals, headspace concentrations, reference vial results, and volumes of homogenates, blood, or diluent used.

(f) *References.* For additional information on this test guideline, the following references should be consulted:

1. Gargas, M. L., Burgess, R. J., Voisard, D. E., Carson, G. H., and Andersen, M. E. Partition coefficients of low-molecular weight volatile chemicals in various liquids and tissues. Toxicol. Appl. Pharmacol. 98:87-99 (1989).

2. Sipes, I. G., Carter, D. E., and Smith, B. J. Chemical disposition in mammals: Final report (Investigations into the role of disposition and metabolism in 4-vinylcyclohexene (VCH) induced ovarian tumors). NIEHS Contract No. NO1-ES-3-5031 (1989).

PROTOCOL APPENDIX III

MEDICAL RESEARCH PROJECT NO. 9298-001

In Vitro Metabolism Testing of 4-Vinylcyclohexene

(a) *Purpose.* Pharmacokinetic parameters of 4-vinylcyclohexene (4-VCH) can be estimated by determination of partition coefficients for 4-VCH and its epoxide metabolites, and by measuring the rate of metabolism of the parent compound and the metabolites *in vitro*. Metabolism rate constants describe the amount of compound metabolized per unit time at a specific concentration in a tissue. When combined with the partition coefficients for the compound, a mathematical model can be constructed to describe the movement of the chemical into and out of the animal and its tissues.

(b) *Definitions.* (1) K_m is the substrate concentration at half of the maximal velocity of the enzymatic reaction metabolizing a compound.

(2) V_{max} is the maximal velocity of an enzymatic reaction.

(3) S9 is the supernatant of a 9,000 x g centrifugation of a tissue homogenate.

(4) Microsomes are the vesicles pelleted when S9 is centrifuged at 100,000 x g for one hour at 4°C.

(5) A metabolite is a new form of a chemical produced via enzymatic catalysis.

(c) *Test method.* (1) *Principle.* Microsomal metabolism of a test chemical is measured by combining the test chemical with microsomes prepared from the tissues of interest and the cofactors required for metabolism. The test chemical will be changed to a new chemical form by the enzymes present in the microsomes, and the metabolites will be measured at several time intervals. Non-enzymatic conversion of the test chemical to its metabolites is measured by including heat-treated microsomes instead of active microsomes, and subtracted from the rates obtained using active microsomes. By using several starting concentrations of the test chemical, the kinetic constants K_m and V_{max} can be calculated.

(2) *Description.* Tissue homogenates shall be prepared and centrifuged in a two-step process to yield microsomes. Microsomes from tissues of interest shall be incubated with the test chemical and a system of cofactors for a defined period of time, with regular mixing, at 37°C. At regular time intervals, the system shall be sampled and analyzed for

metabolites. Several concentrations of the test chemical shall be used to allow for calculation of metabolic rate constants. Control reactions shall be run using heat-treated microsomes to eliminate enzymatic metabolism and allow calculation of non-enzymatic rates of conversion.

(3) *Tissues.* Tissues to be evaluated shall include liver, lung, and ovary. Tissues shall be evaluated from female mice and female rats.

(4) *Test chemicals.* 4-VCH shall be tested for metabolism to 4-VCH-1,2-epoxide and 4-VCH-7,8-epoxide. 4-VCH-1,2-epoxide, and 4-VCH-7,8-epoxide, shall be tested for metabolism to 4-VCH-diepoxide and for metabolism to their respective dihydrodiol metabolites. 4-VCH-diepoxide shall be tested for its rate of hydrolysis. Starting concentrations for the test chemicals should exceed the limit of detection of the analytical method for the metabolite anticipated, and should span at least one order of magnitude. At least three concentrations of test chemical shall be tested for each reaction.

(d) *Test performance.* (1) Tissues should be removed from animals and homogenized in an appropriate diluent, usually a Tris-KCl buffer. Tissues from at least three animals of each species shall be used. If sufficient tissue volume cannot be obtained from a single animal for conducting the test, tissues from several animals may be pooled to produce one sample. Microsomes shall be prepared by centrifugation of the homogenate at 9,000 x g and 4°C for 15 minutes, then by centrifuging the supernatant of the first centrifugation at 100,000 x g and 4°C for one hour. The supernatant from the second centrifugation shall be discarded and the microsomes purified by resuspending and centrifuging a second time at 100,000 x g and 4°C. Microsomal protein content and cytochrome P-450 content should be determined as described in the reference.

(2) Microsomal incubations shall contain microsomal protein at a concentration in the range of linearity from the reaction velocity versus protein content curve. An NADPH generating system shall be added as described in the reference. For reactions intending to analyze an epoxide metabolite, 2 mM trichloropropene oxide shall be added to the incubation mixture to prevent destruction of the epoxide before measurement. Blank reactions to measure non-enzymatic metabolism shall contain all necessary ingredients except that microsomes are boiled for 10 minutes before use. Reactions assaying epoxide metabolites should be terminated by removal of an aliquot of reaction mixture, and addition of 0.2 volumes of 5 mM NaOH. Reactions assaying dihydrodiol metabolites or other hydrolysis products should be terminated by addition of 2 ml ice cold ethyl acetate.

Samples should then be analyzed for metabolites by the methods described in the reference (1), or if necessary by liquid scintillation counting .

(3) Results should be confirmed in an independent experiment, preferably the experiments should be performed in triplicate.

(e) *Data and report.* (1) *Treatment of results.* Data should be presented in tabular form, listing the mean and standard deviation of each metabolic rate constant for each tissue and test chemical. Data shall also be presented describing the range of linearity of the reactions, and reaction rates described in terms of nmol/minute/mg protein for each test chemical at each concentration.

(2) *Test report.* The following specific information shall be reported.

(i) Mouse and rat stock used in the assay, age of animals, and number of animals used.

(ii) Test chemicals, concentrations used, blank reaction results, and volumes of microsomes and cofactors used.

(f) *Reference.*

1. Sipes, I. G., Carter, D. E., and Smith, B. J. Chemical disposition in mammals: Final report (Investigations into the role of disposition and metabolism in 4-vinylcyclohexene (VCH) induced ovarian tumors). NIEHS Contract No. NO1-ES-3-5031 (1989).

METABOLISM AND PHARMACOKINETICS OF 4-VINYLCYCLOHEXENE IN RATS AND MICE

MEDICAL RESEARCH PROJECT NO. 9298-001

PROTOCOL

		Date
Prepared and Approved by:	Douglas A. Keller Study Director	1/22/92
Approved by:	Matthew S. Bogdanffy Co-Investigator	1/22/92
	David R. Plowchalk Co-investigator	1/23/92
	Robert W. Rickard Manager Chronic and Biochemical Toxicology	1/27/92

DU PONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

November 23, 1992

TO: MEDICAL RESEARCH PROJECT NO. 9298-001

METABOLISM AND PHARMACOKINETICS OF 4-VINYLCYCLOHEXENE IN RATS AND MICE

PROTOCOL AMENDMENT #1

The protocol is amended as follows:

1. Page 4, MATERIALS AND METHODS, A. Test Materials. Replace the first three sentences with:

> The test materials will be purchased from Aldrich Chemical Company (4-VCH, 4-VCH-1,2-epoxide, 4-VCH-1,2-diol, 4-VCH-diepoxide), Sigma-Chemical Company (4-VCH-diepoxide), or from the University of Arizona (4-VCH-7,8-epoxide, 4-VCH-7,8-diol).

These changes are made to reflect the actual sources of the test materials, since Aldrich Chemical Company was unable to supply all test substances.

2. Page 5, MATERIALS AND METHODS, B. Test Species. Replace second paragraph with:

> Female $B_6C_3F_1$ mice, approximately 49 days of age, with body weights ranging from 13 - 22 g, will be acquired from Charles River Laboratories, Inc., Raleigh, NC. Mice will be sacrificed for use on the study when they weigh between 22 and 30 g.

This change is made to show the actual body weights of mice received for the study, and the body weight range of adult female $B_6C_3F_1$ mice. $B_6C_3F_1$ mice are being used since this strain was used in the National Toxicology Program bioassay on 4-VCH.

Date

Approved by: Douglas A. Keller 11/23/92
Douglas A. Keller
Study Director

11/23/92
Elizabeth J. Moran
Study Sponsor

DU PONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

March 8, 1993

TO: MEDICAL RESEARCH PROJECT NO. 9298-001

METABOLISM AND PHARMACOKINETICS OF 4-VINYLCYCLOHEXENE IN RATS AND MICE

PROTOCOL AMENDMENT #2

The protocol is amended as follows:

1. Page 4, MATERIALS AND METHODS, A. Test Materials, and Protocol Amendment #1, item 1:

Protocol Amendment #1 item 1. replaces only the first sentence in the protocol under A. Test Materials, not the first three sentences. The source of the 4-VCH is changed to Union Carbide Corporation, Institute, West Virginia. The test materials were assigned the following Haskell numbers:

4-VCH	19353
4-VCH-1,2-epoxide	19498
4-VCH-7,8-epoxide	19646
4-VCH diepoxide	19264

These changes are made to reflect the actual sources of the test materials, since Aldrich Chemical Company was unable to supply all test substances, and to list the Haskell identification numbers assigned to the test materials.

2. Page 5, MATERIALS AND METHODS, B. Test Species, and Protocol Amendment #1, item 2.:

The weight range of mice when sacrificed for use on the study is changed to 26 - 37 g.

This change is made to show the actual body weights of the mice used on the study for tissue collection during the partition coefficient portiton of the study.

3. Page 7, RECORDS AND SAMPLE RETENTION, first sentence. Change to:

The test chemicals will not be collected for archive purposes during the study.

This change is made to reflect the fact that test chemicals were not archived during the conduct of the study. All test chemicals were not received prior to the start of the study, and the amount of test compound available for the epoxide metabolites was too small to allow archiving.

MEDICAL RESEARCH PROJECT NO. 9298-001

PROTOCOL AMENDMENT #2 (Continued)

4. Page 8, Study Personnel and Study Dates, B., Study Dates, items 2., 3., and 4. are changed to:

2. Proposed Experiment End	September, 1993
3. Draft Report Due	
Partition Coefficient Determinations	February, 1993
Metabolism of 4-Vinylcyclohexene and Metabolites	August, 1993
4. Final Report Due	
Partition Coefficient Determinations	March, 1993
Metabolism of 4-Vinylcyclohexene and Metabolites	September, 1993

These changes are made to show that the results of the study are being reported in two separate reports. The first report, on the partition coefficient determinations, is entitled "Partition Coefficients of 4-Vinylcyclohexene and Metabolites". the second report, on *in vitro* metabolism, is to be entitled "*In Vitro* Metabolism of 4-Vinylcyclohexene and Metabolites". The separate reports are necessary to meet the conditions of the Testing Consent Order for 4-Vinylcyclohexene (CAS No. 100-40-3), Docket No. OPTS-42116.

Date

Approved by: Douglas A. Keller 3/8/93
Douglas A. Keller
Study Director

3/8/93
Elizabeth J. Moran
Study Sponsor

BUT-21.0-COT-DHL-01

DU PONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

March 12, 1993

TO: MEDICAL RESEARCH PROJECT NO. 9298-001

METABOLISM AND PHARMACOKINETICS OF 4-VINYLCYCLOHEXENE IN RATS AND MICE

PROTOCOL AMENDMENT #3

The protocol is amended as follows:

1. Page 6, E. *In Vitro* Metabolism Determinations, second paragraph. Change to:

Microsomal preparations of liver, lung, and ovary tissue from rats, and liver and lung from mice will be used for these determinations, based on the guideline in Appendix III. Microsomal preparations of ovary tissue from mice will be used if technically feasible. Analysis of the data for V_{max} and K_m will be performed with EZ-FIT, a software program for the IBM PC, or another suitable data analysis package.

This change is made to reflect the possibility that analysis of metabolic parameters from mouse ovaries may not be technically feasible, and that improved software programs may be available for data analysis.

2. Page 13, Appendix III, (c) *Test Method.* (3) *Tissues.* Change to:

Tissues to be evaluated from the rat shall include liver, lung, and ovary. Tissues to be evaluated from the mouse shall include liver and lung and should include ovary, if technically feasible. Tissues from female mice and female rats shall be used.

This change is made to reflect the possibility that analysis of metabolic parameters from mouse ovaries may not be technically feasible.

3. Page 13, Appendix III, (d) *Test Performance.* (2). Change first sentence to:

For rat liver, lung, and ovary, and mouse liver and lung, microsomal incubations shall contain microsomal protein at a concentration in the range of linearity from the reaction velocity versus protein concentration curve.

This change is made to reflect the possibility that analysis of metabolic parameters from mouse ovaries may not be technically feasible.

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4. Page 13, (d) *Test Performance*. (2). Delete fifth sentence. Change sixth sentence to:

Reactions should be terminated by addition of 2 ml of ice cold ethyl acetate.

This change is made to eliminate the use of NaOH to stop enzymatic reactions, since ethyl acetate was proven to provide superior recovery of products.

5. Page 14, Appendix III, (d) *Test Performance*. (3). Insert after:

(4) For mouse ovaries the following procedure should be used: (1) All incubations with mouse lung and liver tissues should be performed before any work is conducted in mouse ovaries; (2) Experiments for linearity with protein concentration and time should not be conducted in mouse ovaries; (3) Data obtained in experiments with other tissues should be used to estimate the assay conditions for determining rate constants for mouse ovaries; and (4) The ovarian microsomes from mice should be used for one set of incubations with each substrate, with varying substrate concentrations. If adequate data are not obtained from the single experiment, further experiments with mouse ovarian microsomes are not required.

This change is made to reflect the possibility that analysis of metabolic parameters from mouse ovaries may not be technically feasible, and to set the conditions for performing assays with mouse ovarian microsomes.

Date

Approved by: Douglas A. Keller 3/12/93
Douglas A. Keller
Study Director

3/17/93
Elizabeth J. Moran
Study Sponsor

BUT-21.0-COT-DHL-01

DU PONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

September 16, 1993

TO: MEDICAL RESEARCH PROJECT NO. 9298-001

METABOLISM AND PHARMACOKINETICS OF 4-VINYLCYCLOHEXENE IN RATS AND MICE

PROTOCOL AMENDMENT #4

The protocol is amended as follows:

1. Page 5, MATERIALS AND METHODS, B. Test Species, and Protocol Amendment #2, item 2.:

> The age of rats received for use on the metabolism portion of the study is changed to 35 - 64 days. The age of mice received for use on the metabolism portion of the study is changed to approximately 65 days. The weight range of mice when sacrificed for use on the metabolism portion of the study is changed to 20.0 - 27.1 g.

These changes are made to show the actual ages and body weights of the rats and mice used on the study for the *in vitro* metabolism portion of the study.

2. Page 13, Appendix III, (d) Test performance. Replace fourth and fifth sentences with:

Microsomes shall be prepared by centrifugation of the homogenate at approximately 1000 x g at 4°C for 15 minutes, then by centrifugation of the supernatant of the first centrifugation at approximately 21,000 x g at 4°C for 15 minutes. The supernatant from the second centrifugation shall then be centrifuged at approximately 193,000 x g at 4°C for 1 hour and ten minutes, yielding microsomes.

These changes are made to reflect an improved method of preparing microsomes by centrifugation at higher centrifugal forces.

3. Page 13, Appendix III, (d) Test performance (2), and Protocol Amendment #3, item 4.:

Reactions should be terminated by addition of 0.3 volumes of ice-cold ethyl acetate.

This change is made to reflect a method of terminating reactions that increases the sensitivity of metabolite analyses.

BUT-21.0-COT-DHL-01

MEDICAL RESEARCH PROJECT NO. 9298-001

PROTOCOL AMENDMENT #4 (Continued)

4. Page 13, Appendix III, (d) Test performance (2), third sentence:

Change 2 mM trichloropropene oxide to 1 mM trichloropropene oxide.

This change is made due to a very limited supply of trichloropropene oxide, and the fact that 1 mM trichloropropene oxide was as effective as 2 mM.

Date

Approved by: Douglas A. Keller 9/16/93
Douglas A. Keller, Ph.D.
Study Director

9/17/93
Elizabeth J. Moran, Ph.D.
Study Sponsor

Contains No CBI

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PREVENTION AND TOXICS
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STUDY TITLE

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

TEST SUBSTANCE

Ethylene

DATA REQUIREMENT

Not Applicable

AUTHORS

J. S. Vergnes and W. J. Kintigh

STUDY COMPLETION DATE

February 25, 1994

PERFORMING LABORATORY

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LABORATORY PROJECT ID

93N1296

SPONSOR

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Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The portions of this study conducted by BRRC meet the requirements of the following Good Laboratory Practice Standards: Toxic Substances Control Act (TSCA), 40 CFR Part 792; Organisation for Economic Co-operation and Development (OECD), C(81)30(Final).

Study Director: _____ _____

Jane S. Vergnes, Ph.D. Date

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Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

SUMMARY

Ethylene (CAS No. 74-85-1) was given to male Fischer 344 rats (10 animals/group) by whole body vapor exposure for 6 hours/day, 5 days/week for 4 weeks. The ethylene target concentrations were 0, 40, 1000, and 3000 ppm. An ethylene oxide (EO) control group was exposed under the same conditions at a target concentration of 200 ppm. Male B6C3F1 mice were exposed to ethylene and EO as previously described for rats, except that exposures were performed for only 4 days during the fourth week. Bone marrow was collected from both femurs of each animal approximately 24 hours after its final exposure.

There were no mortalities or exposure-related clinical signs observed during the study. The EO-exposed rats gained significantly less weight than the air-only control rats during the study. There were no consistent body weight trends in ethylene- or EO-exposed mice or in ethylene-exposed rats during the study.

No significant differences in the polychromatophilic erythrocyte (PCE) to normochromatophilic erythrocyte (NCE) ratios were observed in any treatment group. The mean PCE/1000 erythrocytes ranged from a low of 226.4 for the 40 ppm ethylene-exposed rats to a high of 303.6 for the EO-exposed rats. The mean PCE/1000 erythrocytes ranged from a low of 522.9 for the EO-exposed mice to a high of 552.4 for the air-only control mice.

The number of micronucleated PCE/2000 PCE ranged from 2 to 14 for air-only control rats, 1 to 18 for ethylene-exposed rats, and from 9 to 35 for EO-exposed rats. The mean percentages of micronucleated PCE in rats were 0.30, 0.26, 0.22, 0.24, and 0.79% for the air-only, low, mid, high, and EO exposure groups, respectively. The number of micronucleated PCE/2000 PCE ranged from 2 to 7 for air-only control mice, 2 to 8 for ethylene-exposed mice, and from 12 to 17 for EO-exposed mice. The mean percentages of micronucleated PCE in mice were 0.22, 0.30, 0.26, 0.26, and 0.72% for the air-only, low, mid, high, and EO exposure groups, respectively.

No statistically significant increases in the frequencies of micronucleated PCE were observed at 40, 1000, or 3000 ppm ethylene in either male rats or male mice. The EO control produced highly significant increases in the frequencies of micronucleated PCE in both rats and mice. Micronucleus frequencies in air-only control rats and mice were within acceptable ranges.

Ethylene did not produce significant, exposure-related increases in the frequency of micronucleated polychromatophilic erythrocytes in the bone marrow of male Fischer 344 rats or male B6C3F1 mice sampled at 24 hours after the final exposure to ethylene vapor. Therefore, ethylene was not considered to be an inducer of micronuclei under the conditions of this *in vivo* assay.

OBJECTIVE

The objective of this study was to assess the potential of ethylene to cause significant increases in the incidence of micronucleated polychromatophilic erythrocytes in the bone marrow of Fischer 344 rats and B6C3F1 mice which may have resulted from a 4-week repeated inhalation exposure regimen.

BACKGROUND INFORMATION

Ethylene is a highly flammable gas of extremely low toxicity. A 90-day inhalation study in Sprague Dawley® rats exposed to 0, 300, 1000, or 10,000 ppm ethylene 6 hours/day, 5 days/week, revealed no exposure-related toxic effects (Rhudy et al., 1978). No exposure-related increases in tumor incidence were observed in a cancer bioassay in Fischer 344 rats exposed at ethylene target concentrations of 0, 300, 1000, or 3000 ppm (Hamm et al., 1984). Pharmacokinetic studies have shown that metabolic saturation occurs at ethylene exposure concentrations of 1000 ppm or higher in rats (Bolt et al., 1984). Ethylene is known to be metabolized to EO in rats, mice, and humans (Walker et al., 1990). According to the calculations of Bolt and Filser (1987), exposure to approximately 40 ppm ethylene is theoretically equivalent to exposure to 1 ppm EO, the current Threshold Limit Value (TLV) for EO. Inhalation exposure to high concentrations of EO has been associated with cancer in rats and mice. Furthermore, EO is known to react with DNA in vivo as well as in vitro. However, the relevance of the EO data to ethylene exposure cannot be determined without additional studies designed to assess the genotoxic potential of ethylene. The micronucleus study described in this report was designed to assess the genotoxic potential of ethylene in vivo in male Fischer 344 rats and B6C3F1 mice.

TARGET CONCENTRATION SELECTION

Target ethylene vapor concentrations of 0 (control), 40, 1000, and 3000 ppm were selected by the Sponsor on the basis of the previously mentioned cancer bioassay and pharmacokinetic studies. The target exposure concentration of EO was 200 ppm, as selected by the Sponsor.

MATERIALS AND METHODS

The protocol detailing the design and conduct of this study is included in Appendix 5. Protocol deviations are also included in Appendix 5.

Test Substance and Control Substance

Two cylinders, each containing 30 pounds of ethylene, CAS No. 74-85-1, Lot E28-0001-A4, BRRC No. 56-319 A and B, were received from Matheson Gas Products (Twinsburg, OH) by way of Union Carbide Corporation (South Charleston, WV) on July 23, 1993. Only 1 cylinder, 56-319A, was used for the study. The test substance was a colorless, compressed gas. The test substance was stored outdoors in a locked chemical storage area. The compositional analyses were provided by the Sponsor. A summary of the test substance characterization report is presented in Table 2 of Appendix 1; the entire report is presented as Attachment 1 of Appendix 1. The prestudy and poststudy compositional analyses indicated that the test substance was 99.99% pure and had remained stable for the duration of the exposure regimen.

Fifty preanalyzed gas cylinders, each containing 143 cubic feet of ethylene oxide (EO) in nitrogen, Cylinder Lot No. 091402, BRRC Sample No. 55-143 A through XX, were received from Scott Medical Products (Plumsteadville, PA) on May 21, 1992. The control substance was stored outdoors in a locked chemical storage area. Cylinders 55-143 OO, PP, QQ, RR, SS, TT, UU, VV, WW, MM, NN, and II were used during the study either for preliminary work or animal exposures. The compositional analyses and the EO concentration were obtained from cylinder samples taken both before and after the exposure regimen. The prestudy gas chromatographic analysis indicated that the control substance was 7600 ppm EO in nitrogen and that the EO component was nearly 99.93% pure. A summary of the EO control substance analysis is presented in Table 3 of Appendix 1; the entire report is presented as Attachment 2 of Appendix 1.

Animals and Husbandry

One hundred sixty-six (1 was found dead) male Fischer 344 rats arrived on August 2, 1993, from Harlan Sprague Dawley Inc. (Indianapolis, IN). They were designated by the supplier to be approximately 43 days old (the birth date was recorded as June 21, 1993). Fifty rats were assigned to this study. The remaining animals were assigned to a separate but concurrent ethylene inhalation study for the same Sponsor or were euthanized.

One-hundred sixty-eight male B6C3F1 mice arrived on August 3, 1993, from Charles River Laboratories (Portage, MI). They were designated by the supplier to be approximately 42 days old (the birth date was recorded as June 21, 1993) and 21 to 24 g upon arrival. Fifty mice were assigned to this study. The remaining animals were assigned to a separate but concurrent ethylene inhalation study for the same Sponsor or were euthanized.

The rats were housed in Room 155 from arrival to termination of the study, except during exposures. The mice were housed in a Relocatable Containment System® unit (Hazleton Systems, Inc., Aberdeen, MD) in Room 164D from arrival until August 13, 1993 (11 days). The mice were then housed in Room 155 for the duration of the study, except during exposures. Within 2 days of receipt, the rats and mice were examined by a Clinical Veterinarian, and representative rats and mice were subjected to a pretest health screen including full necropsy, histologic examination of selected tissues, serum viral antibody analyses (mice only), and examinations for fecal parasites. The serum viral antibody analyses for rats were performed 9 days after the arrival of the animals. Based on the results of these data, the Clinical Veterinarian indicated that these animals were in good health and suitable for use.

All animals were assigned unique numbers and identified by cage tags. Rats considered available for the study were also identified by a tail tattooing procedure. Mice considered available for the study were identified by a toe-clipping procedure which was supplemented, when necessary, by an ear notching procedure. Animals selected for the pretest health screen were identified by a toe-clipping procedure after sacrifice, except for the rats selected for the serum viral antibody analyses. These animals were identified by tail tattoo.

The rats were individually housed in stainless steel, wire mesh cages (22.5 x 15.5 x 18.0 cm) for the duration of the study. The mice were housed 2/cage for approximately 5 days in stainless steel, wire mesh cages (22.5 x 10.0 x 12.5 cm). The purpose of the double housing was to help acclimate the mice to their new surroundings. DACB® (Deotized Animal Cage Board; Shepherd Specialty Papers, Inc.) was placed under each cage and changed at least 3 times each week. Cages were changed at least once every 2 weeks. An automatic timer was set to provide fluorescent lighting for a 12-hour photoperiod (approximately 0500 to 1700 hours for the light phase). Temperature and relative humidity were recorded (Cole-Parmer Hygrothermograph® Seven-Day Continuous Recorder, Model No. 8368-00, Cole-Parmer Instrument Co., Chicago, IL). Temperature was routinely maintained at 66-77°F; relative humidity was routinely maintained at 40-70%. Any minor exceptions to these specified ranges were noted in the raw data.

Tap water (Municipal Authority of Westmoreland County, Greensburg, PA) was available ad libitum, except during exposures, and was delivered by an automatic watering system with demand control valves mounted on each rack. Water analyses were provided by the supplier, Halliburton NUS Environmental Laboratories, Professional Service Industries, Inc., and Lancaster Laboratories, Inc., at regular intervals. EPA standards for maximum levels of contaminants were not exceeded. Pelleted, certified AGWAY® PROLAB® Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.) was available ad libitum except during exposures. Analyses for chemical composition and possible contaminants of each feed lot were performed by Agway Inc., and the results were included in the raw data.

Animal Acclimation

The acclimation period was approximately 2 weeks. During this period, the animals were weighed twice at scheduled intervals. Detailed clinical observations were conducted in conjunction with body weight measurements. Cage-side animal observations were conducted at least once daily, and mortality checks were conducted twice daily (morning and afternoon). The animals were examined just prior to the end of the acclimation period by a Clinical Veterinarian. Animals considered unacceptable for the study, based on the clinical signs, body weight, or body weight gain, were rejected. The fate of rejected animals and the reasons for rejection were documented in the raw data.

Study Organization

Following the second pretest body weight, the animals were assigned to 3 exposure groups, an air-only control group, and an EO control group using a nonstratified randomization procedure based on body weight. At the time of group assignment, only animals with body weight within ± 20% of the population mean were included. The body weight range on the day of first exposure was 135.4 to 186.6 g for male rats and 22.1 to 29.2 g for male mice. The following table summarizes the organization of the study.

Group	Number of Animals Male Rats	Male Mice	Target Concentration (ppm)
Air-Control	10	10	0
EO Control	10	10	200
Low	10	10	40
Intermediate	10	10	1000
High	10	10	3000

The exposures began on August 16, 1993 (Study Day 1). Rats and mice were exposed for 6 hours/day for 5 consecutive days/week for 4 weeks, except that the mice were exposed for 4 consecutive days during the fourth week of exposure so that bone marrow collection for rats and mice would not occur on the same day. The 6-hour exposure period was defined as the time when the generation system was turned on and subsequently turned off. All control animals were exposed to filtered air only using the same exposure regimen. All animals were sacrificed 24 hours after the last exposure. The rats were sacrificed on September 11, 1993, and the mice were sacrificed on September 10, 1993.

Administration of Test Substance

Inhalation Chamber Description and Operation

The inhalation chambers (Wahmann Manufacturing Company, Timonium, MD) used for this study were located in Room 137. The chambers, constructed from stainless steel with glass windows for animal observation, were rectangular (60 x 101 x 101 cm) in shape with pyramidal top and bottom. The volume of each chamber was approximately 900 l, and the airflow was approximately 200 l/min (13 air changes/hour) for the 0, 1000, and 3000 ppm ethylene, and the 200 ppm EO target exposure concentrations and 300 l/min (20 air changes/hour) for the 40 ppm ethylene target exposure concentration. A Dwyer Magnehelic® pressure gauge (Dwyer Instruments, Inc., Michigan City, IN) was used to monitor chamber airflow. The theoretically-derived time (t_{99}) required for each chamber to reach 99% of the target concentration was calculated to range from 13.8 to 20.7 minutes.

Vapor Generation

Ethylene vapor was metered by a pressure regulator and a flowmeter though stainless steel tubing into each ethylene exposure chamber. The flowmeters were calibrated prior to the study with a soap buret or mini-Buck Calibrator® (A. P. Buck, Inc., Orlando, FL). EO vapor was delivered to the exposure chambers using a Linde semi-automatic manifold system with check valves and line regulator in conjunction with calibrated flowmeters. Conditioned supply air was used for dilution of EO vapor. The flowmeters were calibrated prior to the study using a mini-Buck Calibrator® (A. P. Buck, Inc., Orlando, FL).

The oxygen content of the exposure chambers and air-only chamber was measured during preliminary level setting using an MSA® Oxygen Indicator Model 245R (Mine Safety Appliance Company, Pittsburgh, PA). The oxygen content of all chambers was 20.8%.

Chamber Atmosphere Measurements

Each exposure chamber atmosphere was analyzed for ethylene or EO approximately 7 times during each 6-hour exposure by flame ionization gas chromatography. The nominal concentrations of ethylene and EO were estimates calculated from the quantity of ethylene or EO delivered and the chamber airflow rate.

In addition, the EO content of the ethylene exposure chambers was checked weekly using flame ionization gas chromatography. Syringe samples were taken for these EO analyses. After the first exposure, the housing area (Room 155) was also checked for EO in the breathing zones of the ethylene-exposed and the EO-exposed animals using the same method.

The distribution of ethylene and EO vapor within each of the 4 exposure chambers was examined. Each chamber was tested once prior to the initiation of the exposure regimen. A description and results of the chamber distribution are presented in Appendix 1.

Chamber temperature and relative humidity were recorded using a Fisherbrand® dial type thermometer (Fisher Scientific, Pittsburgh, PA) and an Airguide humidity indicator (Airguide Instrument Co., Chicago, IL). Temperature and relative humidity measurements were recorded approximately 2 times each hour of exposure.

Observations and Measurements

In-life Evaluations

All animals were observed individually for signs of toxic effects except during the exposures. During the exposures, observations were recorded on a group basis. Preceding and following each exposure, observations were recorded for animals exhibiting overt clinical signs. At the time of body weight collection and just preceding sacrifice, detailed observations were performed on all animals.

Body weight data were collected for all animals on the morning prior to initiation of the first exposure (denoted as Study Day 1 in the tables) and on Study Days 5, 12, and 19, and preceding sacrifice.

Micronucleus Evaluations

Approximately 24 hours after the final exposure, the animals were sacrificed by carbon dioxide asphyxiation. Both femurs were removed and the bone marrow was aspirated with a syringe into fetal bovine serum (FBS) (Schmid, 1975). Nucleated cells were then removed from the rat bone marrow preparations using a cellulose column (Romagna and Staniforth, 1989). Bone marrow cells were pelleted by centrifugation of either the column eluate (rat) or the whole aspirated marrow sample (mouse). The cell pellet was resuspended gently and a drop of the bone marrow cell suspension was smeared on a microscope slide.

One or 2 smears were made for each animal. Slides were stained with Giemsa stain (Gollapudi and Kamra, 1979), coded with the randomly assigned animal numbers, and read without knowledge of the exposure group to prevent bias.

The polychromatophilic (PCE) to normochromatophilic (NCE) ratio for approximately 1000 total cells was calculated, recorded, and reported to provide an estimate of the cytotoxicity of the test agent. Excessive cytotoxicity in this test system was defined by a PCE to NCE ratio of 0.10 or lower.

Data Analyses

The data for quantitative continuous variables were intercompared for the 3 exposure groups, EO control group, and the air-only control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when necessary, by a separate variance t-test for pairwise comparisons (Dixon, 1990). For all tests the probability value of < 0.05 (two-tailed) was used as the critical level of significance.

Micronucleus data for the air-only and ethylene-exposed groups were statistically evaluated using the Mantel-Haenszel trend test (Mantel, 1963) and the large sample version of the distribution-free multiple comparison test described in Hollander and Wolfe (1973). The EO-exposed group was compared to the air-only group using the Mann-Whitney U-test (Mann and Whitney, 1947). The probability value of < 0.05 was used as the critical level of significance.

Various models of calculators, computers, and computer programs may have been used to analyze data for this study. Since various models round or truncate numbers differently, values in some tables may differ slightly from those in other tables or from independently calculated data. The integrity of the study and interpretation of the data were unaffected by these differences.

RETENTION OF RECORDS

All raw data, documentation, the protocol and any amendments, and a copy of the final report generated as a result of this study will be retained in the BRRC Archives for at least 10 years.

RESULTS AND DISCUSSION

All references of differences in group mean values in the following text refer to comparisons of statistically significant differences between the exposure group and the control group, unless otherwise noted. Repeated reference to the control and the statistical significance will not be made in order to simplify the text.

Chamber Atmosphere

Detailed results and discussion of the chamber atmosphere measurements are included in Appendix 1.

The means of daily mean (± standard deviation) ethylene chamber atmosphere concentrations were 39.1 (± 0.92), 966 (± 29), and 2995 (± 116) ppm for the target concentrations of 40, 1000, and 3000 ppm, respectively. No daily mean ethylene concentration above the estimated minimum detection limit of 4 ppm was detected in the air-only control chamber atmosphere.

The mean of daily mean (± standard deviation) EO chamber atmosphere concentrations was 202 (± 7.0) ppm for the EO target concentration of 200 ppm.

The uniformity of ethylene vapor concentration in each of 3 exposure chambers and the uniformity of EO vapor in 1 exposure chamber were examined. Each chamber was tested once prior to the start of the exposure regimen. Concentrations were measured at 5 positions for each individual distribution test. In each test, these concentrations were representative of a uniform vapor distribution as indicated by low coefficient of variation (CV) values.

Clinical Observations

Summaries of clinical observations are presented in Tables 1 and 2. Individual clinical observations are presented in Appendix 2. There were no mortalities or exposure-related clinical signs observed during this study. One mouse was observed with an ulcer of the genital area during the second week of exposure.

Body Weights

Summaries of body weights and body weight gains are presented in Tables 3 through 6. Individual body weights are included in Appendix 3. Body weights in EO-exposed rats were significantly lower than in air-only control rats for the third and fourth weeks of exposure. Body weight gains were significantly lower in EO-exposed rats throughout the study. Body weights in mice exposed to 3000 ppm ethylene or EO were significantly higher for the second week of exposure. Body weight gains for the second week of exposure were significantly higher for mice exposed to 1000 or 3000 ppm ethylene.

Micronucleus Observations

Summaries of the PCE to NCE ratios for each species at each exposure concentration are presented in Tables 7 and 9. Individual micronucleus data are included in Appendix 4. No significant differences in the PCE to NCE ratios were observed in any treatment group. The mean PCE/1000 erythrocytes ranged from a low of 226.4 for the 40 ppm ethylene-exposed rats to a high of 303.6 for the EO-exposed rats. The mean PCE/1000 erythrocytes ranged from a low of 522.9 for the EO-exposed mice to a high of 552.4 for the air-only control mice.

Tables 8 and 10 present the number of micronucleated PCE/2000 PCE for each animal evaluated in the study and the mean number of micronucleated PCE/2000 PCE for each species and exposure group. The number of micronucleated

PCE/2000 PCE ranged from 2 to 14 for air-only control rats, 1 to 18 for ethylene-exposed rats, and from 9 to 35 for EO-exposed rats. The mean percentages of micronucleated PCE in rats were 0.30, 0.26, 0.22, 0.24, and 0.79% for the air-only, low, mid, high, and EO exposure groups, respectively. The number of micronucleated PCE/2000 PCE ranged from 2 to 7 for air-only control mice, 2 to 8 for ethylene-exposed mice, and from 12 to 17 for EO-exposed mice. The mean percentages of micronucleated PCE in mice were 0.22, 0.30, 0.26, 0.26, and 0.72% for the air-only, low, mid, high, and EO exposure groups, respectively.

No statistically significant increases in the frequencies of micronucleated PCE were observed at 40, 1000, or 3000 ppm ethylene in either male rats or male mice. The EO control substance produced highly significant increases in the frequencies of micronucleated PCE in both rats and mice. Micronucleus frequencies in air-only control rats and mice were within acceptable ranges.

CONCLUSIONS

Ethylene did not produce significant, exposure-related increases in the frequency of micronucleated polychromatophilic erythrocytes in the bone marrow of male Fischer 344 rats or male B6C3F1 mice sampled at 24 hours after the final exposure to ethylene vapor. Therefore, ethylene was not considered to be an inducer of micronuclei under the conditions of this in vivo assay.

REVIEW AND APPROVAL

Study Director: Jane S. Vergnes, Ph.D. — Date: 2/25/94

Associate Director: James D. Sun, Ph.D. — Date: 2-25-94

Director: John P. Van Miller, Ph.D., DABT — Date: 2-25-94

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TABLE 1

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

RATS

SUMMARY OF CLINICAL OBSERVATIONS[a]

MALES

CATEGORY FINDING (LOCATION)	GRADE[b]	TARGET CONCENTRATION (PPM) 0 (DAYS)[c]	40 (DAYS)	1000 (DAYS)	3000 (DAYS)	200 (EO) (DAYS)
DEAD						
SCHEDULED SACRIFICE	P	10 (27)	10 (27)	10 (27)	10 (27)	10 (27)

[a]Number of animals exhibiting the finding at least once during the study.
[b]Grades: P = present, 1 = mild, 2 = moderate, 3 = severe.
[c]Earliest to latest day a finding of the specified grade was observed.

RPT_IN:M1296R.CLI 102693

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE
SUMMARY OF CLINICAL OBSERVATIONS[a]

CATEGORY FINDING (LOCATION)	GRADE[b]	MALES TARGET CONCENTRATION (PPM) 0 (DAYS)[c]	40 (DAYS)	1000 (DAYS)	3000 (DAYS)	200 (EO) (DAYS)
DEAD						
SCHEDULED SACRIFICE	P	10 (26)	10 (26)	10 (26)	10 (26)	10 (26)
SKIN						
ULCER(GENITAL)	P	0	0	0	0	1 (12)

[a]Number of animals exhibiting the finding at least once during the study.
[b]Grades: P = present, 1 = mild, 2 = moderate, 3 = severe.
[c]Earliest to latest day a finding of the specified grade was observed.

RPT_IN:M1296M.CLI 102693

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS
SUMMARY OF BODY WEIGHT (GRAMS)

MALES

GROUP: PPM	0	40	1000	3000	200 (EO)
DAY 1					
MEAN	154.3	157.3	154.1	153.9	162.0
S.D.	11.31	15.54	15.47	17.80	11.22
N	10	10	10	10	10
DAY 5					
MEAN	161.3	164.4	161.8	160.8	161.8
S.D.	11.26	17.46	15.66	17.80	10.49
N	10	10	10	10	10
DAY 12					
MEAN	175.3	179.9	178.1	175.3	166.8
S.D.	12.40	21.09	16.10	18.08	12.20
N	10	10	10	10	10
DAY 19					
MEAN	188.5	195.1	193.8	189.9	170.7*
S.D.	13.77	24.50	16.76	17.83	13.64
N	10	10	10	10	10
DAY 26					
MEAN	204.8	210.3	209.1	205.8	174.2**
S.D.	16.24	26.79	18.30	16.97	14.42
N	10	10	10	10	10

* Significantly different from control group ($p < .05$)
** Significantly different from control group ($p < .01$)

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS
SUMMARY OF BODY WEIGHT GAIN (GRAMS)

MALES

GROUP: PPM	0	40	1000	3000	200 (EO)
DAY 1 TO 5					
MEAN	6.9	7.1	7.7	6.9	-0.3**
S.D.	1.78	3.23	2.57	2.23	2.31
N	10	10	10	10	10
DAY 1 TO 12					
MEAN	21.0	22.6	24.0	21.3	4.7**
S.D.	4.60	7.10	3.58	2.69	3.35
N	10	10	10	10	10
DAY 1 TO 19					
MEAN	34.2	37.8	39.7	35.9	8.6**
S.D.	8.15	10.73	5.37	4.28	5.04
N	10	10	10	10	10
DAY 1 TO 26					
MEAN	50.5	52.9	55.0	51.8	12.1**
S.D.	11.96	14.23	8.82	6.76	6.96
N	10	10	10	10	10

** Significantly different from control group ($p < .01$)

TABLE 5
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE
SUMMARY OF BODY WEIGHT (GRAMS)

MALES

GROUP: PPM	0	40	1000	3000	200 (EO)
DAY 1					
MEAN	24.7	24.8	24.7	25.5	25.8
S.D.	1.57	1.31	1.15	2.05	1.20
N	10	10	10	10	10
DAY 5					
MEAN	25.5	25.6	25.4	26.6	26.2
S.D.	1.66	1.40	1.36	1.93	1.43
N	10	10	10	10	10
DAY 12					
MEAN	26.4	26.5	27.4	28.3*	27.9*
S.D.	1.65	1.48	1.35	2.11	1.25
N	10	10	10	10	10
DAY 19					
MEAN	26.9	27.4	27.6	28.5	28.1
S.D.	2.07	1.81	1.75	2.51	3.00
N	10	10	10	10	10
DAY 26					
MEAN	28.1	28.2	28.4	29.4	29.3
S.D.	2.11	1.62	1.64	2.59	1.39
N	10	10	10	10	10

* Significantly different from control group ($p < .05$)

TABLE 6
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE
SUMMARY OF BODY WEIGHT GAIN (GRAMS)

MALES

GROUP: PPM	0	40	1000	3000	200 (EO)
DAY 1 TO 5					
MEAN	0.8	0.8	0.7	1.0	0.4
S.D.	0.30	0.73	0.64	0.86	0.70
N	10	10	10	10	10
DAY 1 TO 12					
MEAN	1.8	1.7	2.7*	2.8**	2.1
S.D.	0.63	0.71	0.67	0.93	0.75
N	10	10	10	10	10
DAY 1 TO 19					
MEAN	2.2	2.6	2.9	3.0	2.3
S.D.	0.77	1.03	0.98	1.40	2.58
N	10	10	10	10	10
DAY 1 TO 26					
MEAN	3.4	3.4	3.7	3.8	3.5
S.D.	1.13	1.10	1.03	1.47	0.89
N	10	10	10	10	10

* Significantly different from control group ($p < .05$)
** Significantly different from control group ($p < .01$)

TABLE 7

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
SUMMARY OF POLYCHROMATOPHILIC ERYTHROCYTE TO NORMOCHROMATOPHILIC ERYTHROCYTE RATIOS
MALE RATS

			24 HOUR SAMPLE	
DOSE	SEX	N	MEAN PCE/1000 ERYTHROCYTES (S.D.)	% of CONTROL
Air (0 PPM)	M	10	233.1 (56.27)	----
40 PPM	M	10	226.4 (36.23)	97
1000 PPM	M	10	293.9 (87.26)	126
3000 PPM	M	10	236.9 (39.44)	102
ETHYLENE OXIDE (200 PPM)	M	10	303.6 (59.72)	130

Abbreviations: PCE - polychromatophilic erythrocytes; M - male; S.D. - standard deviation.

GMOD_RPT:M1296R.PCE
021794

TABLE 8

ETHTYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
SUMMARY OF MICRONUCLEATED PCE
MALE RATS

DOSE	SEX	N	MN-PCE/ 2000 PCE	TOTAL MN-PCE	MEAN MN-PCE/ 2000 PCE (S.D.)	MEAN % MN-PCE
			24 HOUR SAMPLE			
AIR (0 PPM)	M	10	4,7,7,7,2, 4,14,7,6,2	60	6.0 (3.46)	0.30
40 PPM	M	10	5,5,2,5,4, 1,6,18,4,2	52	5.2 (4.78)	0.26
1000 PPM	M	10	11,3,1,6,5, 3,4,4,3,5	45	4.5 (2.68)	0.22
3000 PPM	M	10	6,4,5,4,8, 6,1,4,2,8	48	4.8 (2.30)	0.24
ETHYLENE OXIDE (200 PPM)	M	10	9,19,10,35,16, 13,15,12,15,14	158	15.8 (7.34)	0.79 **

** = $p < 0.01$
Abbreviations: PCE - polychromatophilic erythrocyte; MN-PCE - micronucleated polychromatophilic erythrocyte; M - male; S.D. - standard deviation.

GMOD_RPT:M1296R.FMN
102693

TABLE 9

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
SUMMARY OF POLYCHROMATOPHILIC ERYTHROCYTE TO NORMOCHROMATOPHILIC ERYTHROCYTE RATIOS
MALE MICE

	24 HOUR SAMPLE			
DOSE	SEX	N	MEAN PCE/1000 ERYTHROCYTES (S.D.)	% of CONTROL
Air (0 PPM)	M	10	552.4 (47.38)	——
40 PPM	M	10	550.7 (47.60)	100
1000 PPM	M	10	532.4 (39.88)	96
3000 PPM	M	10	524.7 (41.08)	95
ETHYLENE OXIDE (200 PPM)	M	10	522.9 (36.40)	95

Abbreviations: PCE - polychromatophilic erythrocytes; M - male; S.D. - standard deviation.

GMOD_RPT:M1296M.PCE
021794

TABLE 10

ETHTYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
SUMMARY OF MICRONUCLEATED PCE
MALE MICE

DOSE	SEX	N	MN-PCE/ 2000 PCE	TOTAL MN-PCE	MEAN MN-PCE/ 2000 PCE (S.D.)	MEAN % MN-PCE
			24 HOUR SAMPLE			
AIR (0 PPM)	M	10	7,4,4,4,4, 6,2,3,7,2	43	4.3 (1.83)	0.22
40 PPM	M	10	6,7,7,7,5, 7,7,2,5,7	60	6.0 (1.63)	0.30 *
1000 PPM	M	10	2,4,7,2,7, 8,8,4,7,4	53	5.3 (2.36)	0.26
3000 PPM	M	10	4,4,8,2,5, 7,3,7,7,5	52	5.2 (1.99)	0.26
ETHYLENE OXIDE (200 PPM)	M	10	15,14,12,12,15, 14,17,16,14,15	144	14.4 (1.58)	0.72 **

* = $p < 0.05$, ** = $p < 0.01$
Abbreviations: PCE - polychromatophilic erythrocyte; MN-PCE - micronucleated polychromatophilic erythrocyte; M - male; S.D. - standard deviation.

GMOD_RPT:M1296M.FMN
102693

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

QUALITY ASSURANCE UNIT INSPECTION SUMMARY

Inspection Date(s)	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
07-30-93 to 08-02-93	PROTOCOL	08-02-93	08-02-93
08-06-93	EVENT-ANIMAL RECEIPT	08-06-93	09-07-93
09-10-93	EVENT-BONE MARROW HARVEST	09-15-93	09-15-93
11-04-93 to 11-08-93	ANALYTICAL CHEMISTRY DATA, REPORT	11-09-93	02-25-94
11-08-93 to 11-10-93	RAW DATA, REPORT	11-10-93	02-25-94
02-25-94	ARCHIVES	02-25-94	02-25-94

Linda J. Calisti 2/25/94

Linda J. Calisti, Manager — Date
Good Laboratory Practices/Quality Assurance

ARCH_DOC\93N1296\93N1296.QAU

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

Chamber Atmosphere Report

(54 Pages)

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SUMMARY

The concentration of ethylene and ethylene oxide vapor in the exposure chamber atmospheres was monitored throughout the 20 days of exposure by flame ionization gas chromatography.

The means of daily mean ethylene (± standard deviation) chamber atmosphere concentrations were 39.1 (± 0.92), 966 (± 29), and 2995 (± 116) ppm, for target concentrations of 40, 1000, and 3000 ppm, respectively. Ethylene was not detected in the air-only control chamber atmosphere. No ethylene was detected (estimated minimum detection limit of 4 ppm) in the control chamber atmosphere during the study.

The mean of daily mean ethylene oxide (± standard deviation) chamber atmosphere concentrations was 202 (± 7.0) ppm for the ethylene oxide target concentration of 200 ppm.

The ethylene test substance and the ethylene oxide control substance were analyzed before and after the exposure regimen. The ethylene remained 99.99% pure throughout the exposure regimen. The prestudy analysis indicated that the ethylene oxide-nitrogen mixture was approximately 7600 ppm ethylene oxide.

The uniformity of ethylene vapor concentration in each of 3 exposure chambers and the uniformity of ethylene oxide vapor in the one exposure chamber were examined. Each chamber was tested once prior to the start of the exposure regimen. Concentrations were measured at 5 positions for each individual distribution test. In each test, these concentrations were representative of a uniform vapor distribution as indicated by a low coefficient of variation (CV) value. CV values of less than 4% were found for each of the 4 distribution tests. Furthermore, the results indicate that the analytical sampling position for each chamber was properly located within the chamber, such that sampling results were representative of ethylene or ethylene oxide concentrations in the breathing zones of the animals.

MATERIALS AND METHODS

Test Substance

Two cylinders, each containing 30 pounds of ethylene, CAS No. 74-85-1, Lot E28-0001-A4, BRRC No. 56-319 A and B, were received from Matheson Gas Products (Twinsburg, OH) by way of Union Carbide Corporation (South Charleston, WV) on July 23, 1993. Only 1 cylinder, 56-319A, was used for the study. The chemical and physical properties of the test substance are described in Table 1. The compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. A summary of this report is presented in Table 2; the entire report is presented in Attachment 1. The prestudy and poststudy compositional analyses indicated that the test substance was 99.99% pure and had remained stable for the duration of the exposure regimen.

Control Substance

Fifty preanalyzed gas cylinders, each containing 143 cubic feet of ethylene oxide in nitrogen, Cylinder Lot No. 091402, BRRC Sample No. 55-143 A through XX, were received from Scott Medical Products (Plumsteadville, PA) on May 21, 1992. Cylinders 55-143 OO, PP, QQ, RR, SS, TT, UU, VV, WW, MM, NN, and II were used during the study either for preliminary or animal exposures. The compositional analyses and the ethylene oxide concentration were obtained from cylinder samples taken both before and after the exposure regimen. The compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. These analyses indicated that the positive control substance was approximately 7600 ppm ethylene oxide in nitrogen and that the ethylene oxide component was over 99.9% pure. A summary of the analyses is presented in Table 3; the entire report is presented as Attachment 2.

Test Substance and Control Substance Generation

The methods used to generate ethylene and ethylene oxide in the exposure chambers are described in the text of the main report.

Chamber Distribution Setup

The uniformity of ethylene vapor concentration in each of 3 exposure chambers and the uniformity of ethylene oxide vapor concentration in the 1 exposure chamber were examined. Each chamber was tested once prior to the start of the exposure regimen. Concentrations were measured at 5 positions for each individual distribution test. The distribution tests simulated actual animal exposures, including the use of similar animal cages, cage carriers with collection trays, and airflow rates. No animals were present in the exposure chambers. The positions of the sampling probes within the chambers are described in Table 11. One of these positions was the analytical probe position. The analytical sampling for ethylene oxide consisted of 2 cycles, each cycle requiring approximately 30 minutes to complete. The analytical cycle sampling for ethylene required approximately 15 minutes to complete. Since the chamber concentrations may vary slightly with time, the data from the 2 cycles (Table 12) were averaged to eliminate time-dependent concentration variations. Also, the sampling occurred after the equilibration of the chamber concentration (t_{99}) had taken place.

Analytical Instrumentation

A Perkin-Elmer Sigma 2000 gas chromatograph (GC) equipped with a flame ionization detector was used to analyze the ethylene or ethylene oxide exposure chamber atmospheres. The GC operating conditions are presented in Table 4. A Spectra-Physics ChromJet Integrator provided a record of the chromatograms and chromatographic analyses as well as peak integration. The data were captured using an IBM PS/2 Computer with Spectra-Physics Chromstation/2 software. In-house software was used to compute daily statistics and also to provide an alarm system which monitored chamber concentrations.

In addition, a second Sigma 2000 gas chromatograph equipped with a flame ionization detector was used to check the ethylene exposure chambers for ethylene oxide at concentrations as low as 1 ppm. Syringe samples were taken for these ethylene oxide analyses. The GC operating conditions are presented in Table 5.

Calibration

Calibration of the gas chromatograph was achieved by injecting gas standards, which were prepared by syringe injection of ethylene or ethylene oxide into Tedlar™ gas bags containing air. These standards were prepared using the mathematical relationship:

$$V_g = C \times V_h \times 10^{-6} \times 100/\%P$$

where:

V_g = required volume of ethylene or ethylene oxide gas standard, in milliliters
C = desired calibration concentration, in ppm
V_h = volume of gas bag, in liters
%P = percent purity = % ethylene or ethylene oxide in standard

Calibration curves (Figures 1, 2, and 3) were constructed by plotting peak areas versus the gas standard concentrations. The calibrations were checked once each week during the exposure regimen.

RESULTS AND DISCUSSION

Chamber Atmosphere Analysis

Each exposure chamber atmosphere was analyzed for ethylene or ethylene oxide approximately 7 times during each of the 20, 6-hour exposures by flame ionization gas chromatography. The daily mean analytical concentrations are listed in Tables 6 through 10.

The means of daily mean ethylene chamber atmosphere concentrations (± standard deviations) were 39.1 (± 0.92), 966 (± 29), and 2995 (± 116) ppm, for target concentrations of 40, 1000, and 3000 ppm, respectively. No ethylene was detected (estimated minimum detection limit of 4 ppm) in the control chamber atmosphere during the study.

The mean of daily mean ethylene oxide chamber atmosphere concentrations (± standard deviation) was 202 (± 7.0) for the target concentration of 200 ppm.

In addition, the ethylene oxide content of the ethylene exposure chambers was checked weekly using an analysis for ethylene oxide which had a minimum detection limit of 1 ppm. No ethylene oxide was detected. The housing area was also checked for ethylene oxide after the first exposure using this

sensitive method; however, ethylene oxide was not detected either in the breathing zones of the ethylene oxide-exposed animals or the ethylene-exposed animals.

Analytical/Nominal Concentration Ratio

The daily analytical/nominal (A/NOM) ethylene or ethylene oxide concentration ratios are given in Tables 7 through 10, the nominal concentration being an estimate calculated from the quantity of ethylene or ethylene oxide delivered and the chamber airflow rate. The overall mean A/NOM concentration ratios were 0.93, 0.88, and 0.91, for ethylene target concentrations of 40, 1000, and 3000 ppm, respectively. The overall mean ethylene oxide A/NOM concentration ratio was 0.87 for the ethylene oxide target concentration of 200 ppm.

Temperature and Relative Humidity

The daily mean temperature and relative humidity values for the exposure chambers are presented in Tables 6 through 10. The means of daily mean temperature values were 23, 22, 22, and 22°C, for ethylene target concentrations of 0, 40, 1000, and 3000 ppm, respectively. The mean of daily mean temperature values was 21°C, for the ethylene oxide target concentration of 200 ppm. The means of daily mean relative humidity values were 56, 48, 48, and 50%, for the ethylene target concentrations of 0, 40, 1000, and 3000 ppm, respectively. The mean of daily mean relative humidity values for the ethylene oxide target concentration of 200 ppm was 45%.

Chamber Distribution

The uniformity of ethylene and ethylene oxide vapor concentration distribution in 4 exposure chambers was examined. The results and the statistical summaries are presented in Table 12. In each test, the concentrations were representative of a uniform vapor distribution as indicated by low coefficient of variation (CV) values. CV values of 2.6, 3.4, 2.1, and 3.9% were obtained for exposure chambers 37-7 (40 ppm ethylene), 37-8 (1000 ppm ethylene), 37-9 (3000 ppm ethylene), and 37-10 (200 ppm ethylene oxide), respectively. Furthermore, the results indicate that the analytical sampling position for each chamber was properly located within the chamber such that sampling results were representative of ethylene concentrations in the breathing zones of the animals.

Analytical Chemist: Irvin Pritts 2-25-94

Irvin M. Pritts, Ph.D. Date

INHAL\ANALYT\AETH_4V
FEBRUARY 23, 1994

TABLE 1
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

CHEMICAL AND PHYSICAL PROPERTIES[1]

Synonyms:	Ethene, acetene
Molecular Weight:	28.05
Molecular Formula:	CH_2CH_2
Appearance and Odor:	Colorless compressed gas with a sweet odor and taste
Boiling Point, 760 mm Hg:	-104°C
Solubility in Water:	22.6%
Vapor density (air =1):	1.0

[1]Material Safety Data Sheet, Matheson Gas Products, Revised 9/12/90.

TABLE 2

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

ETHYLENE: TEST SUBSTANCE ANALYSIS[1]

Component	Prestudy Area%	Poststudy Area%
Ethylene	99.99	99.99
Ethane	0.0003	0.00405
Acetylene	0.0003	0.00055

[1]The capillary gas chromatographic compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. In addition, gas chromatography-mass spectrometry and infrared spectral data were independently used to confirm the sample's identity.

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST
IN RATS AND MICE

ETHYLENE OXIDE: CONTROL SUBSTANCE ANALYSIS[1]

Component	Prestudy Area%	Poststudy Area%
Ethylene oxide	99.93	99.99
Unknown	0.04	----
Unknown	0.03	----
Cylinder mixture ethylene oxide in N_2 (ppm)	7600	7880

[1]The capillary gas chromatographic compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. In addition, gas chromatography-mass spectrometry and infrared spectral data were independently used to confirm the sample's identity.

TABLE 4

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

GAS CHROMATOGRAPH OPERATING PARAMETERS: ETHYLENE AND ETHYLENE OXIDE (200 PPM)

Chromatograph:	Perkin-Elmer Sigma 2000
Detector:	Flame Ionization
Column:	Hay Sep D, 80 /100 mesh, 10 ft. x 1/8 in. stainless steel
Column temperature:	150°C
Injector temperature:	Ambient gas sample valve
Detector temperature:	300°C
Carrier flow rate:	25 mL/minute nitrogen
Sample size:	0.1 cc
Retention time:	1.7 minutes Ethylene 5.4 minutes Ethylene Oxide
GC attenuation:	Range = 100
Minimum detection limit:	Approximately 4 ppm ethylene
Integrator attenuation:	32

TABLE 5
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

GAS CHROMATOGRAPH OPERATING PARAMETERS: ETHYLENE OXIDE (1 PPM)

Chromatograph:	Perkin-Elmer Sigma 2000
Detector:	Flame Ionization
Column:	Hay Sep D, 80/100 mesh, 10 ft. x 1/8 in. stainless steel
Column temperature:	165°C
Injector temperature:	120°C
Detector temperature:	350°C
Carrier flow rate:	20 mL/minute nitrogen
Sample size:	0.5 cc syringe
Retention time:	3.9 minutes
GC attenuation:	Range = 1
Minimum detection limit:	1 ppm
Integrator attenuation:	1

TABLE 6

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

CHAMBER ATMOSPHERE DATA

0 PPM CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)
1	23.7	48.5	<MDL
2	22.9	64.8	<MDL
3	24.8	57.9	<MDL
4	25.0	58.9	<MDL
5	23.6	56.2	<MDL
6	23.3	54.2	<MDL
7	23.0	54.1	<MDL
8	24.4	55.8	<MDL
9	21.8	54.6	<MDL
10	22.0	53.1	<MDL
11	22.3	56.2	<MDL
12	23.8	54.9	<MDL
13	21.1	52.7	<MDL
14	21.3	53.0	<MDL
15	21.2	52.7	<MDL
16	23.0	53.7	<MDL
17	20.9	57.8	<MDL
18	21.9	54.2	<MDL
19	22.7	56.2	<MDL
20	23.6	61.9	<MDL
Mean:	22.8	55.6	<MDL
SD:	1.23	3.54	---

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
<MDL = less than the estimated minimum detection limit (4 ppm)

TABLE 7

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

CHAMBER ATMOSPHERE DATA

40 PPM ETHYLENE CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	22.3	40.3	39.6	1.6	41.3	0.96
2	22.0	45.7	39.5	0.7	42.0	0.94
3	21.8	44.2	39.7	1.4	42.0	0.95
4	23.1	46.2	41.5	1.0	42.0	0.99
5	21.0	45.7	39.6	0.9	42.0	0.94
6	21.4	50.7	39.4	1.5	42.0	0.94
7	22.2	44.9	38.4	3.2	42.0	0.91
8	22.4	45.8	39.5	1.8	42.0	0.94
9	21.4	52.2	38.4	4.2	42.0	0.91
10	21.0	48.4	39.6	2.7	42.0	0.94
11	22.6	48.2	38.7	3.9	42.0	0.92
12	23.5	46.9	40.6	1.5	42.0	0.97
13	22.3	45.7	38.2	1.1	42.0	0.91
14	21.3	47.4	39.4	2.0	42.0	0.94
15	23.0	45.2	39.1	1.7	42.0	0.93
16	22.3	51.3	38.1	1.4	42.0	0.91
17	22.0	52.1	38.5	0.9	42.0	0.92
18	21.3	49.3	37.6	1.0	42.0	0.90
19	22.8	47.3	39.2	1.2	42.0	0.93
20	22.0	53.4	38.2	1.5	42.0	0.91
Mean:	22.1	47.5	39.1	——	42.0	0.93
SD:	0.71	3.22	0.92	——	0.16	0.023

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 8

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

CHAMBER ATMOSPHERE DATA
1000 PPM ETHYLENE CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	22.4	42.1	988	44.6	1055	0.94
2	22.0	47.8	978	12.5	1080	0.91
3	22.0	46.5	992	9.2	1080	0.92
4	22.8	48.2	1009	11.0	1080	0.93
5	20.8	47.6	985	12.1	1080	0.91
6	21.2	51.1	1007	36.8	1080	0.93
7	23.0	48.1	953	14.9	1080	0.88
8	22.5	47.1	976	11.9	1080	0.90
9	20.8	49.2	984	13.5	1080	0.91
10	21.0	48.2	963	18.4	1080	0.89
11	22.2	48.7	949	9.4	1080	0.88
12	22.9	49.1	934	14.8	1080	0.86
13	21.7	47.6	945	14.2	1080	0.88
14	21.3	46.6	942	11.6	1080	0.87
15	23.0	43.8	936	24.7	1080	0.87
16	22.0	49.8	900	14.3	1080	0.83
17	20.8	50.6	942	22.9	1171	0.80
18	20.8	47.8	955	11.3	1190	0.80
19	22.4	47.4	1002	19.7	1190	0.84
20	22.0	62.4	984	12.5	1190	0.83
Mean:	21.9	48.5	966	——	1100	0.88
SD:	0.79	3.86	28.7	——	44.3	0.042

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 9

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

CHAMBER ATMOSPHERE DATA

3000 PPM ETHYLENE CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	23.0	44.3	3000	124	3178	0.94
2	23.0	49.8	3108	96	3265	0.95
3	23.1	48.2	3042	33	3265	0.93
4	24.5	49.7	3127	40	3265	0.96
5	21.6	49.6	3102	51	3265	0.95
6	22.7	50.8	3100	110	3265	0.95
7	23.2	49.8	3028	273	3265	0.93
8	21.2	49.2	3013	220	3265	0.92
9	19.5	51.8	3074	55	3265	0.94
10	21.0	53.8	3095	177	3265	0.95
11	23.4	50.2	3052	44	3265	0.93
12	23.4	48.8	2999	62	3265	0.92
13	21.0	48.5	2994	47	3265	0.92
14	20.4	49.5	2973	74	3265	0.91
15	22.8	47.5	2886	79	3265	0.88
16	22.5	49.2	2714	68	3265	0.83
17	20.0	51.6	2799	102	3381	0.83
18	20.0	49.4	2772	28	3465	0.80
19	22.7	50.2	3006	54	3465	0.87
20	19.0	60.5	3011	320	3465	0.87
Mean:	21.9	50.1	2995	----	3296	0.91
SD:	1.54	3.07	116	----	80	0.047

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 10

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

CHAMBER ATMOSPHERE DATA

200 PPM ETHYLENE OXIDE CONTROL CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	22.1	40.2	198	14.5	226	0.88
2	21.0	44.2	202	4.2	232	0.87
3	22.0	43.1	201	3.0	232	0.87
4	22.4	43.8	205	2.6	232	0.88
5	19.9	45.8	204	8.0	232	0.88
6	20.0	48.3	203	3.8	232	0.88
7	21.2	42.1	202	11.0	232	0.87
8	22.5	42.3	207	4.5	232	0.89
9	20.4	48.4	201	3.6	232	0.87
10	20.0	45.4	206	4.7	232	0.89
11	22.2	44.2	203	5.9	232	0.88
12	23.0	40.8	210	10.0	232	0.91
13	21.0	43.3	208	3.0	232	0.90
14	20.1	46.2	196	3.6	232	0.84
15	22.5	41.4	202	2.7	232	0.87
16	21.1	45.4	183	4.5	232	0.79
17	20.1	46.2	192	4.8	232	0.83
18	20.0	45.9	190	2.2	232	0.82
19	21.1	43.9	208	5.0	232	0.90
20	20.0	52.9	211	7.8	232	0.91
Mean:	21.1	44.7	202	----	232	0.87
SD:	1.05	2.97	7.0	----	1.3	0.030

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 11

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

CHAMBER DESCRIPTION AND CHAMBER PROBE POSITIONS

CHAMBER

Construction: Stainless steel and glass.
Manufacturer: Wahmann Manufacturing Company (Timonium, MD)
Shape: Rectangular with pyramidical top and bottom
Dimensions: Height: 60 cm
Width: 101 cm
Depth: 101 cm

Chamber #37-7 Probe Placement (40 ppm Target)

Probe Number	Location (x:y:z)* Units (cm)		
1 Analytical Probe	51	44	33
2	13	10	45
3	84	91	45
4	86	9	17
5	16	90	15

Chamber #37-8 Probe Placement (1000 ppm Target)

Probe Number	Location (x:y:z)* Units (cm)		
1	17	11	17
2 Analytical Probe	50	49	37
3	85	9	44
4	15	89	44
5	86	90	15

Chamber #37-9 Probe Placement (3000 ppm Target)

Probe Number	Location (x:y:z)* Units (cm)		
1	12	90	46
2	88	7	16
3 Analytical Probe	50	43	28
4	14	10	41
5	87	88	15

*Location described by a three-dimensional coordinate system: x = width coordinate; y = depth coordinate; z = height coordinate. The origin of the coordinate system (0:0:0) is the lower left front corner of the internal chamber.

TABLE 11 (CONTINUED)
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST
IN RATS AND MICE

CHAMBER DESCRIPTION AND CHAMBER PROBE POSITIONS

Chamber #37-10 Probe Placement (200 ppm EO Target)

Probe Number	Location (x:y:z)* Units (cm)				
1	82	:	86	:	43
2	83	:	22	:	19
3	18	:	89	:	19
4 Analytical Probe	47	:	68	:	29
5	17	:	19	:	48

*Location described by a three-dimensional coordinate system: x = width coordinate; y = depth coordinate; z = height coordinate. The origin of the coordinate system (0:0:0) is the lower left front corner of the internal chamber.

TABLE 12
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST
IN RATS AND MICE

CHAMBER DISTRIBUTION DATA

(Chamber 37-7)

	1	2	3	4	5
	37.1	37.7	37.3	35.7	38.5
	37.2	37.5	36.6	35.5	37.9
Mean:	37.1	37.6	37.0	35.6	38.2
SD:	0.03	0.13	0.49	0.18	0.44
		Grand mean = 37.1	SD = 0.96	% CV = 2.6	

(Chamber 37-8)

	1	2	3	4	5
	959	963	901	929	903
	961	959	887	922	902
Mean:	960	961	894	926	903
SD:	0.8	2.5	10.0	4.9	0.4
		Grand mean = 929	SD = 31.2	% CV.= 3.4	

(Chamber 37-9)

	1	2	3	4	5
	2804	2873	2878	2845	2738
	2726	2807	2848	2811	2705
Mean:	2765	2840	2863	2828	2721
SD:	55.5	46.9	21.0	23.6	23.0
		Grand mean = 2803	SD = 58.7	% CV = 2.1	

(Chamber 37-10)

	1	2	3	4	5
	173	195	183	182	185
	181	197	177	179	184
Mean:	177	196	180	180	185
SD:	5.5	1.4	4.8	1.5	0.7
		Grand mean = 184	SD = 7.2	% CV = 3.9	

FIGURE 1. ETHYLENE CALIBRATION CURVE

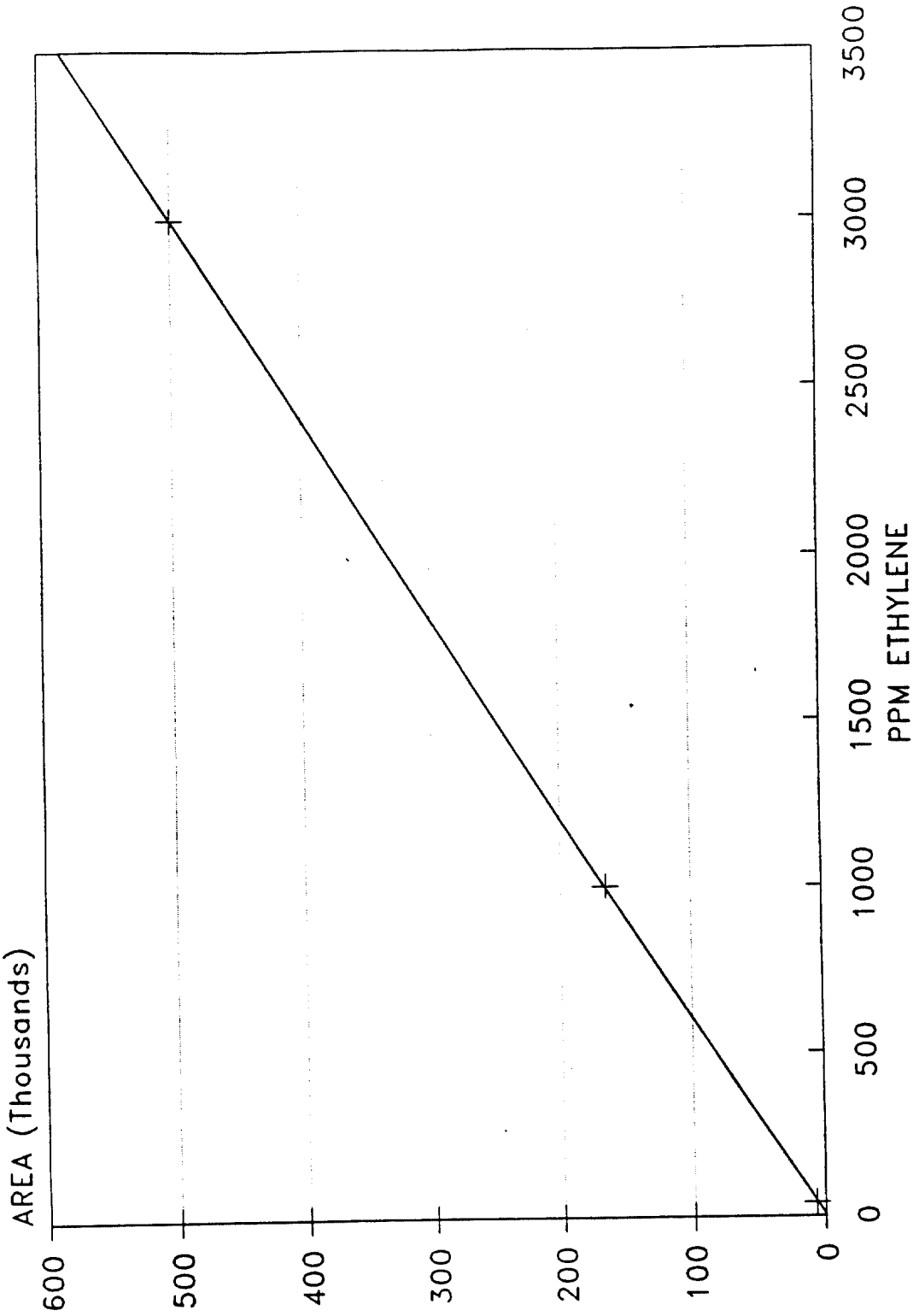


FIGURE 2. ETHYLENE OXIDE CALIBRATION CURVE

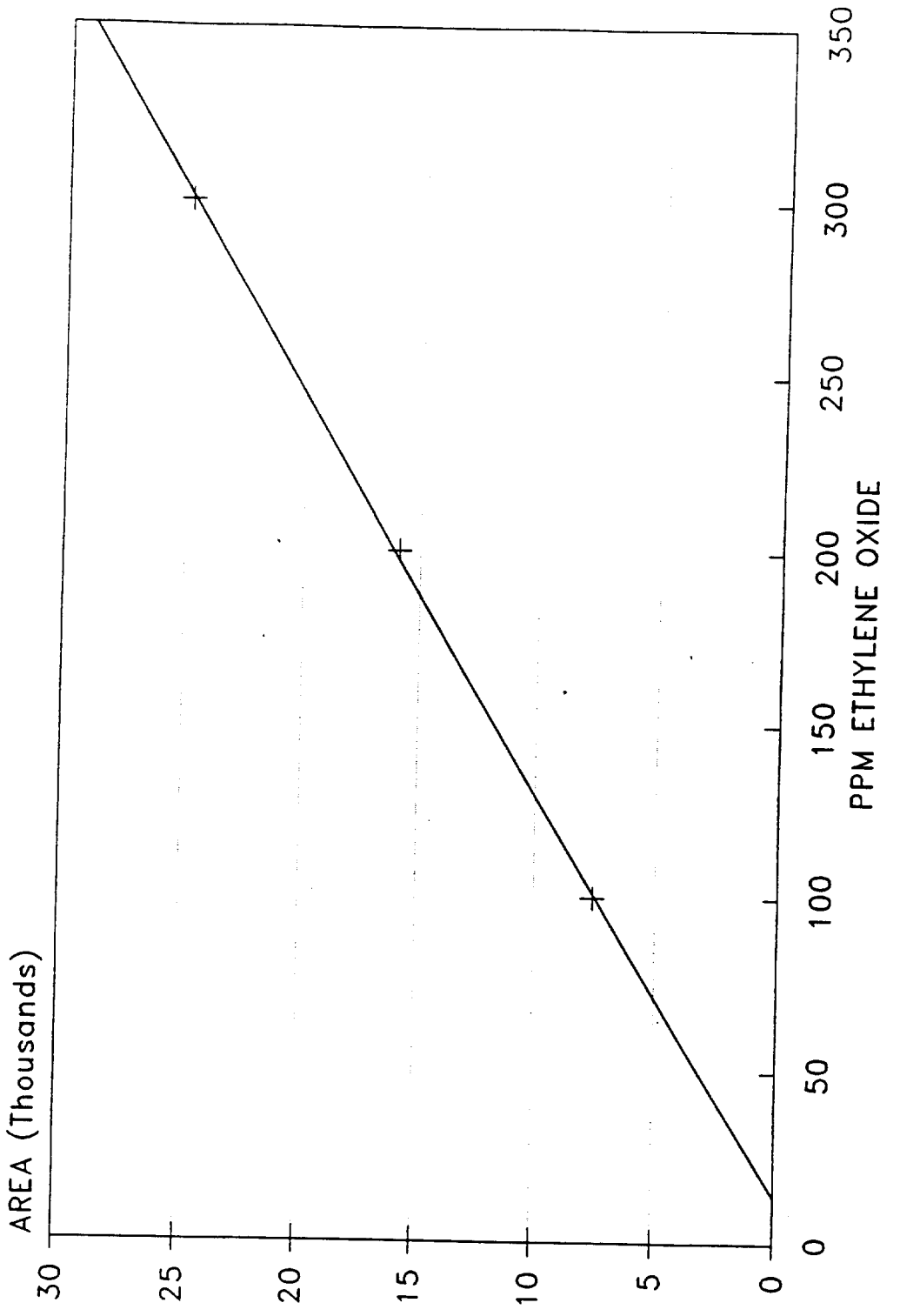
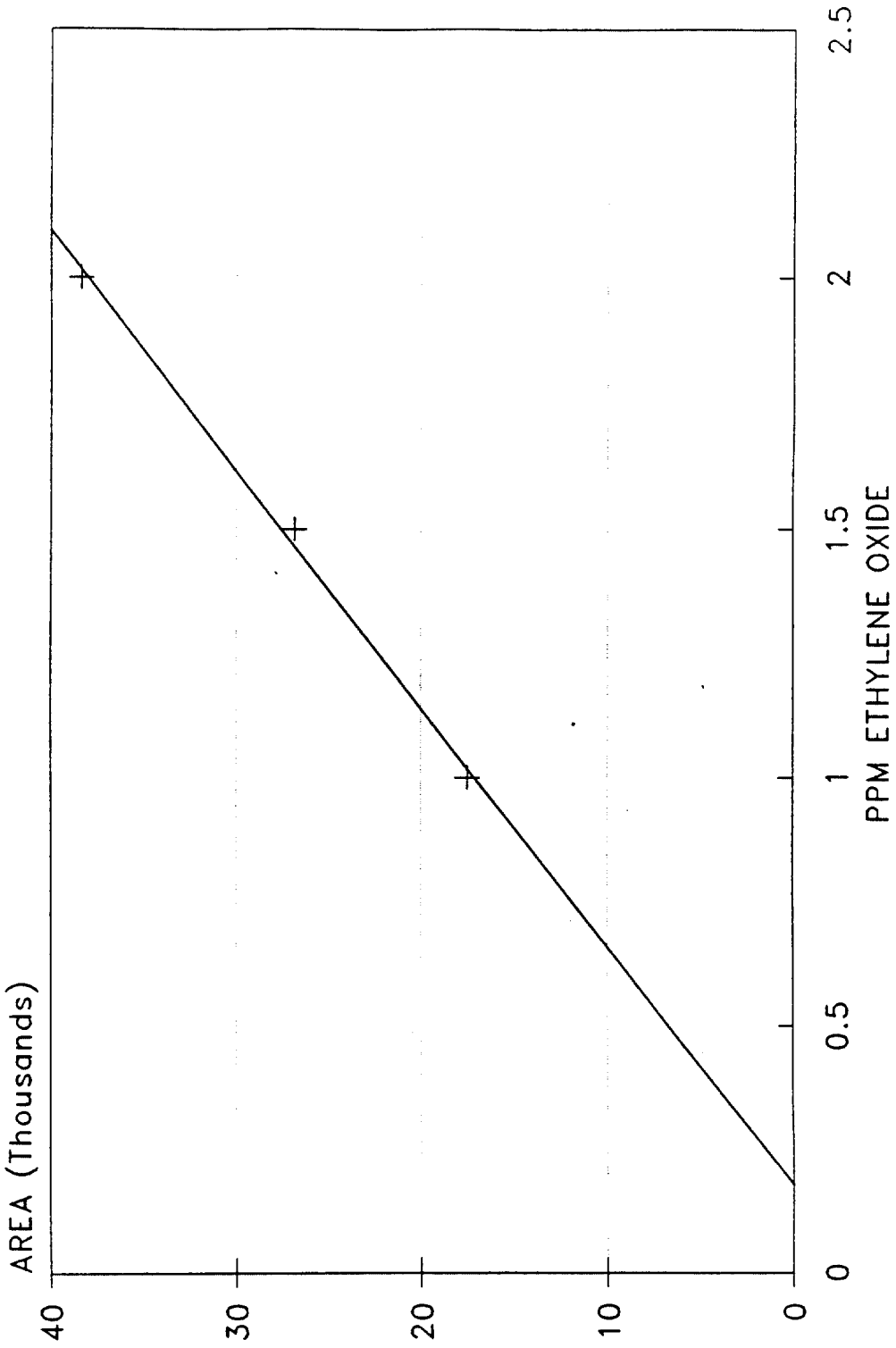


FIGURE 3. ETHYLENE OXIDE CALIBRATION CURVE



Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

Ethylene Test Substance Characterization Report

Union Carbide Corporation

STUDY TITLE

GLP Analysis-Final Report

TEST SUBSTANCE

Ethylene

DATA REQUIREMENT

U.S. FDA, 21 CFR 58
U.S. EPA TSCA, 40 CFR 792
U.S. EPA FIFRA 40 CFR 160

STUDY DIRECTOR

Alexander E. Gabany, Jr.

STUDY COMPLETED ON

November 19, 1993

PERFORMING LABORATORY

Union Carbide Corporation
PO Box 8361
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UCC R/D LABORATORY PROJECT ID

Study # 37-AEG-89

SPONSOR COMPANY

Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for Union Carbide Corporation (UCC) Study # 37-AEG-89, Ethylene Study for Chemical Manufacturers Association (CMA), Washington, D.C.

In accordance with UCC's intent that all tests conducted follow good laboratory practices, UCC's study director for the above study confirms that the study was conducted in compliance with the U.S. EPA Section 4 Good Laboratory Practice standards: TSCA 40 CFR 792 and FIFRA 40 CFR 160. All original raw data, records, protocols, and final reports are being retained at UCC&P's South Charleston, WV, Technical Center.

Alexander E. Gabany, Jr. 11/19/93
Study Director Date

PROTOCOL DEVIATION STATEMENT

Protocol Deviation Statement for Union Carbide Corporation (UCC) Study # 37-AEG-89, Ethylene study for Bushy Run Research Center.

In accordance with UCC's intent that all tests conducted by our facility follow good laboratory practices, UCC's study director for the above test confirms that there were no protocol deviations taken during the study. The study was conducted in compliance with the protocol established and signed on 7/19/93 by Alexander E. Gabany, GLP Study Director.

Alexander E. Gabany

Alexander E. Gabany, Jr. date 4/19/93
Study Director

SIGNATURE PAGE

Submitted by: Union Carbide Corporation
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Prepared by:

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(QAU) Representative

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Ethylene

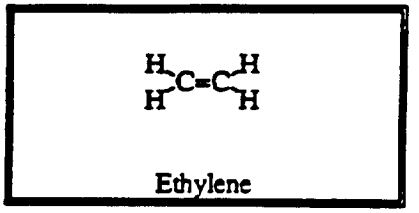
Ethylene
Study # 37-AEG-89

ABSTRACT Ethylene, was analyzed to provide analytical data as part of the toxicity study at Bushy Run Research Center. The analyses were performed in compliance with Good Laboratory Practice standards (GLP) to meet EPA Section 4 Test Rule requirements and FDA, 21 CFR 58. Gas chromatography-mass spectrometry (GC/MS) and infrared spectroscopy (IR) techniques were independently used to confirm the sample's identity. Purity, measured by capillary GC, is ≈99.99% for both the pre-study and the post-study sample. Ethylene oxide (EO) was not found by GC/MS analysis at a concentration of ≥ 10 ppb based upon the response of a 1 ppm EO in ethylene standard prepared by UCC's Gas Mixing Skill Center. The pre-study sample was received from Matheson Gas Products, Twinsburg, Ohio and subsequently sent to Bushy Run Research Center. The post-study sample was received from Bushy Run Research Center. All raw data, documentation, records, protocols, and final reports are being retained.

INTRODUCTION The CMA Olefins Panel of Chemical Manufacturers Association, this study's sponsor, requested that Bushy Run Research Center (BRRC) test ethylene, for toxicity. Such studies must follow GLP standards established by the EPA that require they be conducted with authentic material whose identity and purity are verified analytically.

Two high pressure cylinders of ethylene, (cylinder #'s 50884 and 55746T, 37-AEG-92) were received 7/15/93 from Matheson Gas Products, Twinsburg, Ohio, for analytical characterization. The cylinders (Lot # E28-0001-A4, BRRC# 56-319) were subsequently sent to Bushy Run Research Center for toxicological testing. A GLP protocol describing the analytical characterization of the sample was prepared (Appendix I). The protocol called for structural identification by GC/MS and IR and for the capillary GC measurement of any impurities identified by GC/MS. The detectability limit of ethylene oxide (EO) to be verfied by 1 ppm EO in ethylene standard by GC/MS. Note: The lot # E28-0001-A4 was not known at the time of the protocol preparation. The post-study sample (cylinder # 55746T, 37-AEG-92R) was received from Bushy Run Research Center on 9/27/93.

Shown at right is the structure of ethylene; its Chemical Abstracts Service Registry number (CAS #) is 74-85-1.



Ethylene

DISCUSSION The data from the analyses are summarized below.

IR spectroscopy Analyses An infrared spectrum of Matheson ethylene (Cylinder #50884, 37-AEG-92, IASC #3793, UCC Laboratory Notebook 43-REJ-13) was collected on a Mattson Galaxy 5020 Fourier Transform infrared (FTIR) spectrometer. Data collection parameters included 50 coadded scans at 4 cm^{-1} resolution with triangular apodization. The sample was run as a gas sample in the infrared gas cell (SOP IR-007). The spectrum obtained (Figure 1) compares well with reference spectra of ethylene found in The Sadtler Library of Infrared Spectra of Gases and Vapors, published by Sadtler Research Laboratories. The functional group assignments are as follows:

Absorbance Band Frequency, cm^{-1}	Functional Group Assignment
3123-2971	CH_2 antisymmetric and symmetric stretch
1912, 1865	CH_2 antisymmetric deformation
1464, 1419	CH_2 symmetric deformation
954	CH_2 wag

The spectrum is consistent with ethylene and shows no indication of contamination.

GC/MS Analysis Electron ionization (EI) mass spectral data were collected in the UCC MS Skill Center using a Finnigan TSQ-70 mass spectrometer interfaced to a Hewlett-Packard (HP) 5890 gas chromatograph. The sample, cylinder # 50884, 37-AEG-92, was analyzed by injecting 1 cc aliquots, via a VALCO gas sampling valve, onto a CP-Sil-5-CB capillary column held at -50°C for 2 minutes, and temperature programmed to 0°C at 8°/minute, then temperature programmed to 250°C at 15°/ min. Figure 2 shows the EI total ion current

chromatogram for the sample (scanned from m/z 10 to m/z 310 EI mode). This chromatogram is annotated with identifications based on the components' EI spectrum.

The ethylene oxide (EO) detection limit was determined using a 1 ppm EO in ethylene standard, prepared in UCC's Gas Mixing Skill Center, for reference. Aliquots (1 cc) of the standard were injected into the GC/MS system; the GC peak area for EO was determined by mass spectrometric analysis by using extracted ion current profiles for EO. Based on the area counts recorded for the EO peak (204,465) it was determined that the detection limit for EO in ethylene was ≥5 ppb.

Capillary GC — A HP 5890 gas chromatograph equipped with a flame ionization detector was used to analyze the sample. Aliquots (1 mL) were manually injected with a 100:1 split ratio onto an Al_2O_3/KCl capillary column held isothermally at 100°C for 20 minutes (Figures 3 and 4 show chromatograms of the pre-study and post-study samples, respectively). The average of triplicate analyses taken from the Al_2O_3/KCl column data are given below (normalized chromatogram area percent).

Component name	37-AEG-92	37-AEG-92R
Ethane	0.0003	0.00405
Ethylene	99.99	99.99
Acetylene	0.0003	0.00055

CONCLUSION — IR spectral data and mass spectral fragmentation data from the UCC Skill Centers show that this sample is ethylene. These independent methods satisfy the analytical requirements for structural identification, as defined in the sample protocol. Sample purity, measured by capillary GC, is ≈ 99.99% for both the pre-study and the post-study sample. Ethylene oxide (EO) was not found by GC/MS analysis at a concentration of ≥10 ppb based on the response of a 1 ppm EO in ethylene standard.

ARCHIVES — All raw data, records, protocols, and final reports are being retained at UCC's South Charleston, WV, Technical Center as follows:

- raw data from GC, IR and GC/MS studies are in 770-361, 770-223, and 770-123, respectively;
- protocols, notebook and other records are to be kept in the GLP archives.

ACKNOWLEDGEMENTS — We would like to thank Trudy Barker for sample handling, collecting the GC data, and preparing the bulk of the report, and Jim Richardson for collecting the IR data.

NOTEBOOK REFERENCE: 37-AEG-89 and related pages

Figure 1 — Infrared Spectrum of 37-AEG-92 (Ethylene)

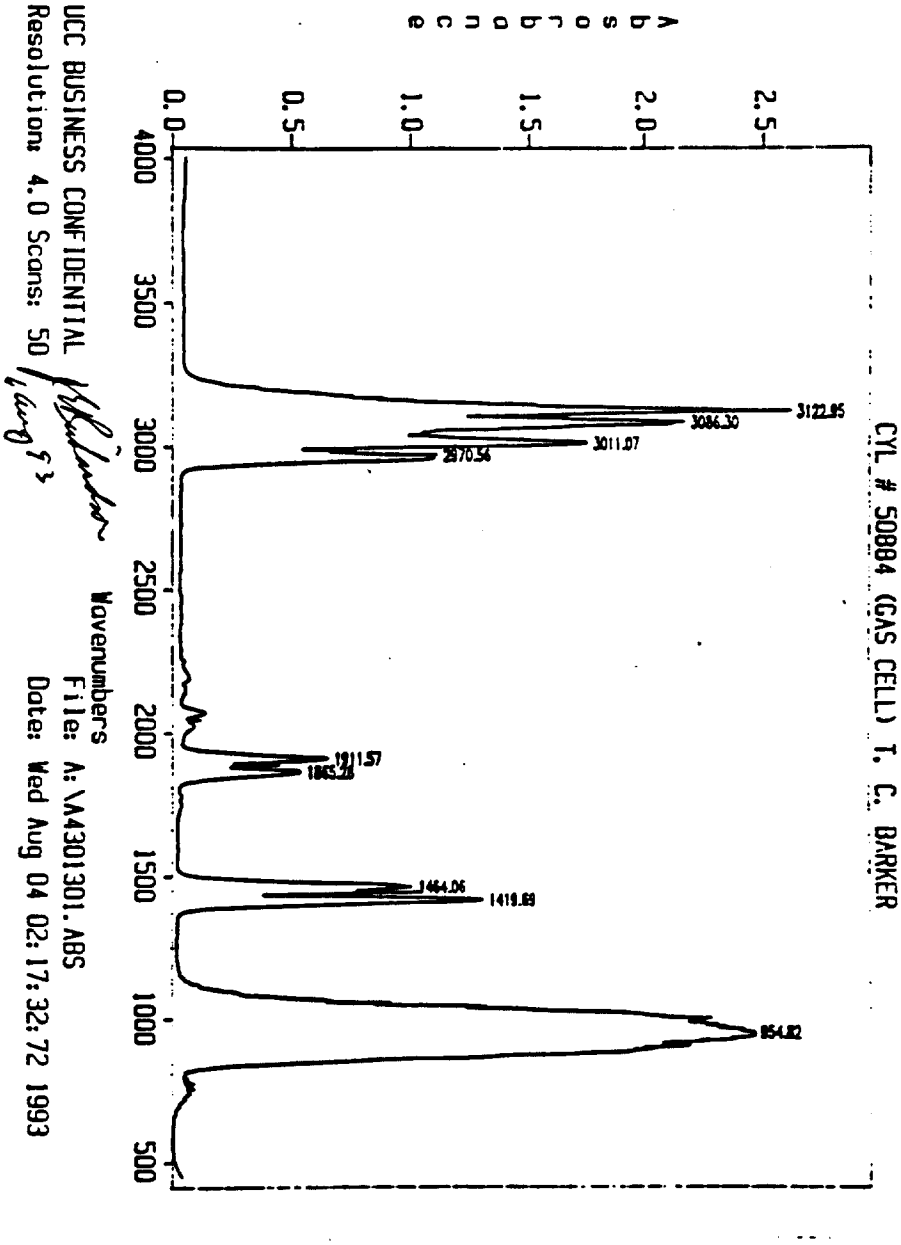


Figure 2 — Capillary GC/MS RIC of 37-AEG-92 (Ethylene)

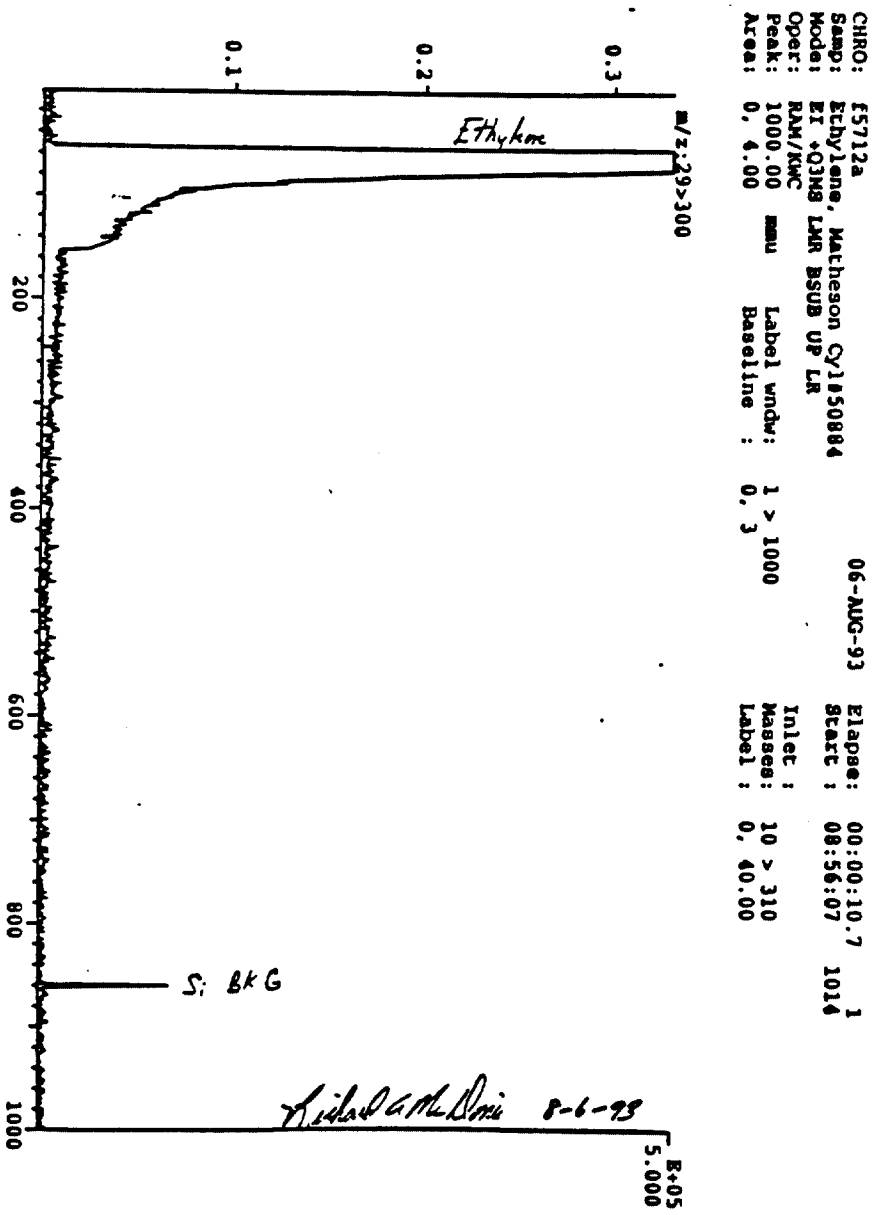


Figure 3— Capillary Gas Chromatogram of 37-AEG-92 (Ethylene)

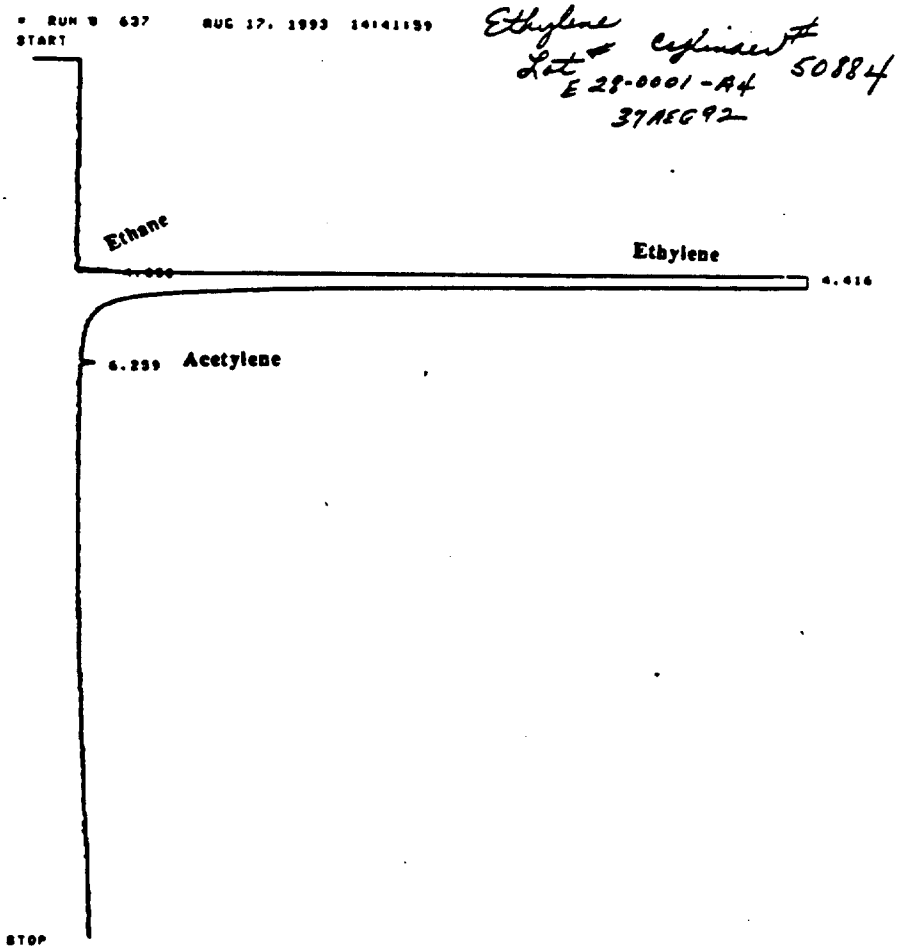
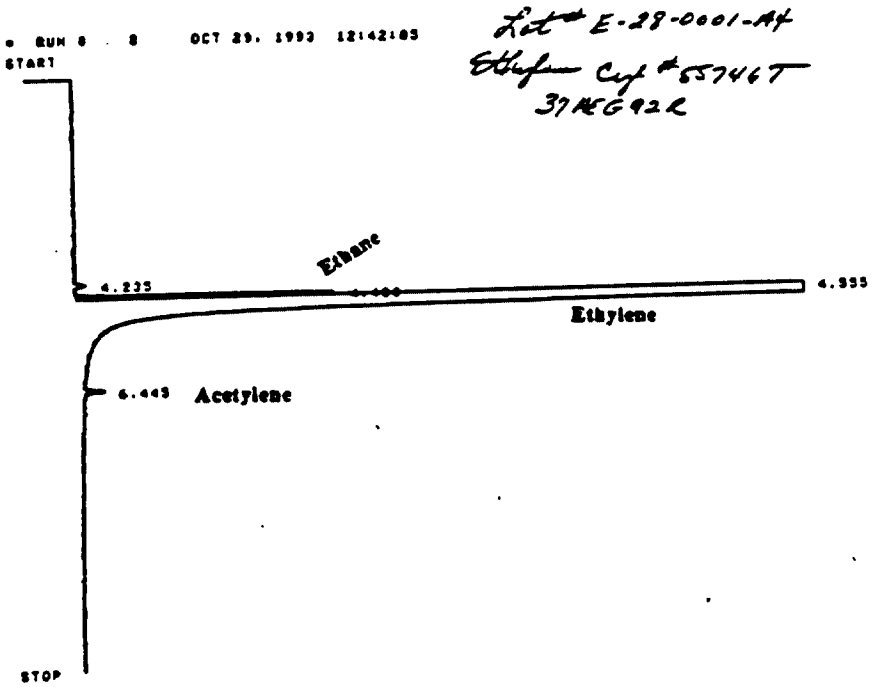


Figure.4— Capillary Gas Chromatogram of 37-AEG-92R (Ethylene)



RUN# 8 OCT 29, 1993 12:42:03

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4.483	1522	PP	.033	.00444
4.555	34278288	PB	.071	99.99448
6.445	252	PP	.078	.00074

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AUL FACTOR=1.0000E+00

JC Barker 10/29/93

APPENDIX I Protocol

PROTOCOL

GOOD LABORATORY PRACTICE (GLP) STUDY

title	Ethylene
purpose	Analytical Characterization of Sample(s) for Toxicology Studies at Bushy Run Research Center
study number	37-AEG-89
sponsor	CMA Olefins Panel Chemical Manufacturers Association 2501 M Street, NW Washington, DC 20037
testing facility	UCC Technical Center, South Charleston, WV 25303 (Location 511)
Proposed Starting Date:	Monday, July 19, 1993
Proposed Completion Date:	September 15, 1993
Estimated Date of Final Report:	October 15, 1993

Test Substance(s) 37-AEG-92

Name	Ethylene
Source	Lot #?; Matheson Gas Products, Seacaucus, NJ
CAS Registry No.	74-85-1
Description	colorless gas, sweet odor
Purity	≈99.9%
Health/Safety	highly toxic; stable. MSDS available upon request
Storage Conditions	ambient

Study Design

The test substance(s) will be characterized by:

- Detectability limit of ethylene oxide to be verified by 1 ppm EO in ethylene standard by GC/MS
- Verification of identity by GC/MS.
- Verification of identity by Infrared spectroscopy.
- Quantitation of the identified impurities by capillary GC.

Reviewed and Approved by:

Alexander E. Gabany	date	Denise L. Johnson	date	Rne S. Vergnes	date
GLP Study Director		GLP Quality Assurance Unit (QAU) Representative		Study Director Bushy Run Research Center	

Elizabeth J. Moran date
CMA Olefins Panel Manager,
Sponsor's Representative

This study will be performed in compliance with the following GLP standards: FDA, 21 CFR, Part 58; TSCA, 40 CFR, Part 792; and FIFRA, 40 CFR, Part 160. All changes of an approved protocol and the reasons therefore shall be documented, signed by the study director, dated, and maintained with the protocol. All raw data and reports from this study will be retained at Location 511 for at least 10 years after completion of the study. A comprehensive final report will be submitted to the Sponsor within one month after the completion of the analysis. The final report will be inspected by the QAU and will contain a signed quality assurance statement.

Quality Assurance Unit Study Inspection Summary

Test Substance: ETHYLENE

Study No.: 37-AEG-89

Study Director: A.E. Gabany, B.S.

The Quality Assurance Unit of the Union Carbide Technical Center conducted the inspections listed below and reported the results to the study director and management on the date indicated. It is the practice of this Quality Assurance Unit to report the results to both the study director and management.

Inspection Date	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
Feb. 10, 1992	Laboratory Compliance Review*	Feb. 10, 1992	May, 1992
July 19, 1993	Protocol Compliance Review	July 19, 1993	July 19, 1993
Nov. 22, 1993	Final Report Compliance Review	Nov. 22, 1993	Nov. 22, 1993

*The process of doing the GLP characterization studies is audited periodically to assure these studies comply with GLP requirements. The QA unit is exempted from performing in-life study inspections for studies designed to determine physical and chemical characteristics of a test substance as described in 40 CFR 792.135.

11/22/93

Denise L. Johnson, QAU Representative (Date)
Good Laboratory Practices/Quality Assurance

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

Ethylene Oxide Control Substance Characterization Report

Union Carbide Corporation

STUDY TITLE

GLP Analysis-Final Report

TEST SUBSTANCE

Ethylene Oxide (8000 ppm in nitrogen)

DATA REQUIREMENT

U.S. FDA, 21 CFR 58
U.S. EPA TSCA, 40 CFR 792
U.S. EPA FIFRA 40 CFR 160

STUDY DIRECTOR

Alexander E. Gabany, Jr.

STUDY COMPLETED ON

November 19, 1993

PERFORMING LABORATORY

Union Carbide Corporation
PO Box 8361
South Charleston, West Virginia 25303

UCC R/D LABORATORY PROJECT ID

Study # 37-AEG-88

SPONSOR COMPANY

Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for Union Carbide Corporation (UCC) Study # 37-AEG-88, Ethylene Oxide (8000 ppm in nitrogen) Study for Chemical Manufacturers Association (CMA), Washington, D.C.

In accordance with UCC's intent that all tests conducted follow good laboratory practices, UCC's study director for the above study confirms that the study was conducted in compliance with the U.S. EPA Section 4 Good Laboratory Practice standards: TSCA 40 CFR 792 and FIFRA 40 CFR 160. All original raw data, records, protocols, and final reports are being retained at UCC&P's South Charleston, WV, Technical Center.

Alexander E. Gabany, Jr. Date 11/4/93
Study Director

PROTOCOL DEVIATION STATEMENT

Protocol Deviation Statement for Union Carbide Corporation (UCC) Study # 37-AEG-88, Ethylene Oxide (8000 ppm in nitrogen) study for Chemical Manufacturers Association (CMA), Washington, D.C..

In accordance with UCC's intent that all tests conducted by our facility follow good laboratory practices, UCC's study director for the above test confirms that there were no protocol deviations taken during the study. The study was conducted in compliance with the protocol established and signed on 7/19/93 by Alexander E. Gabany, GLP Study Director.

Alexander E. Gabany, Jr. 11/19/93
Study Director date

SIGNATURE PAGE

Submitted by: Union Carbide Corporation
P.O. Box 8361
South Charleston, West Virginia 25303

Prepared by:

Alexander E. Gabany, Jr. — 11/19/93
Study Director — date

Richard A. McDonie — 11-19-93
GC/MS Skill Area Specialist — date

Russell A. Miller — 11/19/93
Infrared Skill Area Specialist — date

Quality Assurance Review by:

Denise L. Johnson — 11/22/93
GLP Quality Assurance Unit — date
(QAU) Representative

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Ethylene Oxide (8000 ppm in nitrogen)

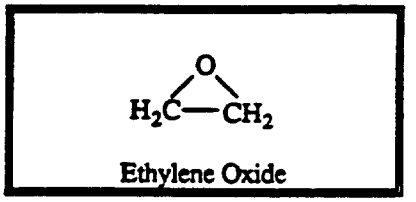
Ethylene Oxide (8000 ppm in nitrogen)
Study # 37-AEG-88

ABSTRACT Ethylene oxide (8000 ppm in nitrogen), was analyzed to provide analytical data as part of the toxicity study at Bushy Run Research Center. The analyses were performed in compliance with Good Laboratory Practice standards (GLP) to meet EPA Section 4 Test Rule requirements and FDA, 21 CFR 58. Gas chromatography-mass spectrometry (GC/MS) and infrared spectroscopy (IR) techniques were independently used to confirm the sample's identity. Purity, measured by capillary GC, is ~99.93% ethylene oxide, nitrogen-free basis, for the pre-study sample and ~99.99% ethylene oxide, nitrogen-free basis, for the post-study sample. GC/MS analyses confirmed the presence of ~7600 ppm ethylene oxide in the pre-study sample and ~7880 ppm ethylene oxide in the post-study sample. The pre-study sample was received from Bushy Run Research Center and subsequently returned to Bushy Run Research Center. The post-study sample was received from Bushy Run Research Center. All raw data, documentation, records, protocols, and final reports are being retained.

INTRODUCTION The CMA Olefins Panel of Chemical Manufacturers Association, this study's sponsor, requested that Bushy Run Research Center (BRRC) test ethylene oxide (8000 ppm in nitrogen), for toxicity. Such studies must follow GLP standards established by the EPA that require they be conducted with authentic material whose identity and purity are verified analytically.

A high pressure cylinder of ethylene oxide (8000 ppm in nitrogen), (cylinder # ALM-030222, 37-AEG-90) was received 7/15/93 from Bushy Run Research Center for analytical characterization. The cylinder of ethylene oxide (8000 ppm in nitrogen), (Lot # 091402, BRRC# 55-143) was subsequently returned to Bushy Run Research Center for toxicological testing. A GLP protocol describing the analytical characterization of the sample was prepared (Appendix I). The protocol called for structural identification by GC/MS and IR analyses; for the capillary GC measurement of any impurities identified by GC/MS; and the ethylene oxide concentration to be verified by GC/MS. Note: Scott Medical Products, Plumsteadville, PA was the source of the ethylene oxide in nitrogen mixture characterized in this study. Linde, East Chicago, IN, supplied the ethylene oxide used to prepare the mixture by Scott Medical Products. The post-study sample (cylinder # ALM-030208, 37-AEG-90R) was received from Bushy Run Research Center on 9/27/93.

Shown at right is the structure of Ethylene Oxide; its Chemical Abstracts Service Registry number (CAS #) is 75-21-8.



Ethylene Oxide

DISCUSSION The data from the analyses are summarized below.

IR spectroscopy Analyses An infrared spectrum of the sample (8000 ppm ethylene oxide, with the balance composed of nitrogen) (Cylinder # ALM-030222, 37-AEG-90, IASC # 3794, UCC Laboratory Notebook 43-REJ-14) was collected on a Mattson Galaxy 5020 Fourier Transform Infrared (FTIR) spectrometer. Data collection parameters included 50 coadded scans at 4 cm^{-1} resolution with triangular apodization. The sample was run as a gas sample in the infrared gas cell (SOP IR-007). The spectrum obtained (Figure 1) compares well with reference spectra of ethylene oxide found in The Sadtler Library of Infrared Spectra of Gases and Vapors, published by Sadtler Research Laboratories. The functional group assignments are as follows:

Absorbance Band Frequency, cm^{-1}	Functional Group Assignment
3092-2930	CH_2 antisymmetric and symmetric stretch
871	C-O-C stretch

The spectrum is consistent with ethylene oxide and shows no indication of contamination. The nitrogen is invisible to IR analysis.

GC/MS Analysis Electron ionization (EI) mass spectral data were collected in the UCC MS Skill Center using a Finnigan TSQ-70 mass spectrometer interfaced to a Hewlett-Packard (HP) 5890 gas chromatograph. The sample, cylinder # ALM-030222, 37-AEG-90, was analyzed by injecting 1 cc aliquots, via a VALCO gas sampling valve, onto a CP-Sil-5-CB capillary column held at -20°C for 2 minutes, and temperature programmed to 0°C at 8°/minute,

then temperature programmed to 250°C at 15°/min. Figure 2 shows the EI total ion current chromatogram for the sample (scanned from m/z 10 to m/z 310 EI mode). This chromatogram is annotated with identifications based on the components' EI spectrum.

The ethylene oxide concentration in the sample was determined using a 8000 ppm ethylene oxide in nitrogen standard, prepared in UCC's gas mixing skill center, for reference. Aliquots of the sample and reference material were injected into the GC/MS system; the GC peak area for ethylene oxide were determined by mass spectrometric analysis by using extracted ion current profiles for ethylene oxide. The concentration of ethylene oxide in the sample was determined by comparison of the two areas. The pre-study sample was determined to be ≈7600 ppm ethylene oxide in nitrogen and the post-study sample contained ≈7880 ppm ethylene oxide in nitrogen.

Capillary GC A HP 5890 gas chromatograph equipped with a flame ionization detector was used to analyze the sample. Aliquots (1 mL) were injected manually with a 100:1 split ratio onto a DB-1 (5μ film thickness) capillary column maintained isothermally at 50°C (see Figure 3). The average of triplicate analyses are given below (normalized chromatogram area percent), nitrogen-free basis. The gas chromatogram for the post-sample (37-AEG-90R) is shown in Figure 4.

Component name	37-AEG-90	37-AEG-90R
Unknown impurity	0.04	
Unknown impurity	0.03	
Ethylene Oxide	99.93	99.99

CONCLUSION IR spectral data from the UCC Skill Center shows that the sample is ethylene oxide; the nitrogen is invisible to IR analysis. The mass spectral fragmentation data from the UCC Skill Center show that the sample is ethylene oxide in nitrogen. These independent methods satisfy the analytical requirements for structural identification, as defined in the sample protocol. Sample purity, measured by capillary GC, is ≈99.93 % and ≈99.99% ethylene oxide, nitrogen-free basis, respectively for the pre-study sample and the post-study sample. The pre-study sample was analyzed by GC/MS to be ≈7600 ppm ethylene oxide in nitrogen, whereas the post-study sample was ≈7880 ppm ethylene oxide in nitrogen.

ARCHIVES All raw data, records, protocols, and final reports are being retained at UCC's South Charleston, WV, Technical Center as follows:

- raw data from GC, IR and GC/MS studies are in 770-361, 770-223, and 770-123, respectively;
- protocols, notebook and other records are to be kept in the GLP archives.

ACKNOWLEDGEMENTS We would like to thank Trudy Barker for sample handling, collecting the GC data, and preparing the bulk of the report, and Jim Richardson for collecting the IR data.

NOTEBOOK REFERENCE: 37-AEG-88 and related pages

Figure 1 — Infrared Spectrum of 37-AEG-90 (Ethylene Oxide (8000 ppm in nitrogen))

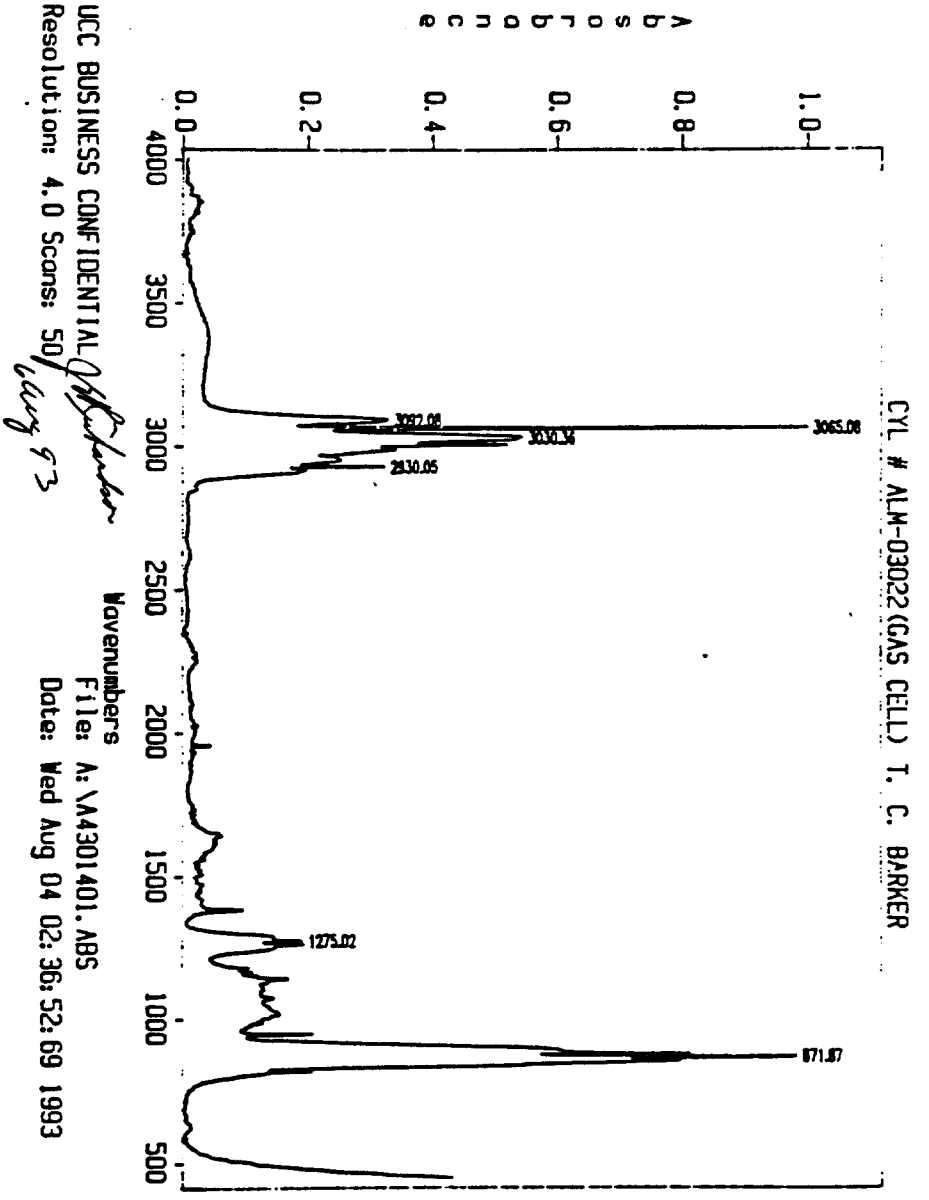


Figure 2 — Capillary GC/MS RIC of 37-AEG-90 (Ethylene Oxide @000 ppm in nitrogen))

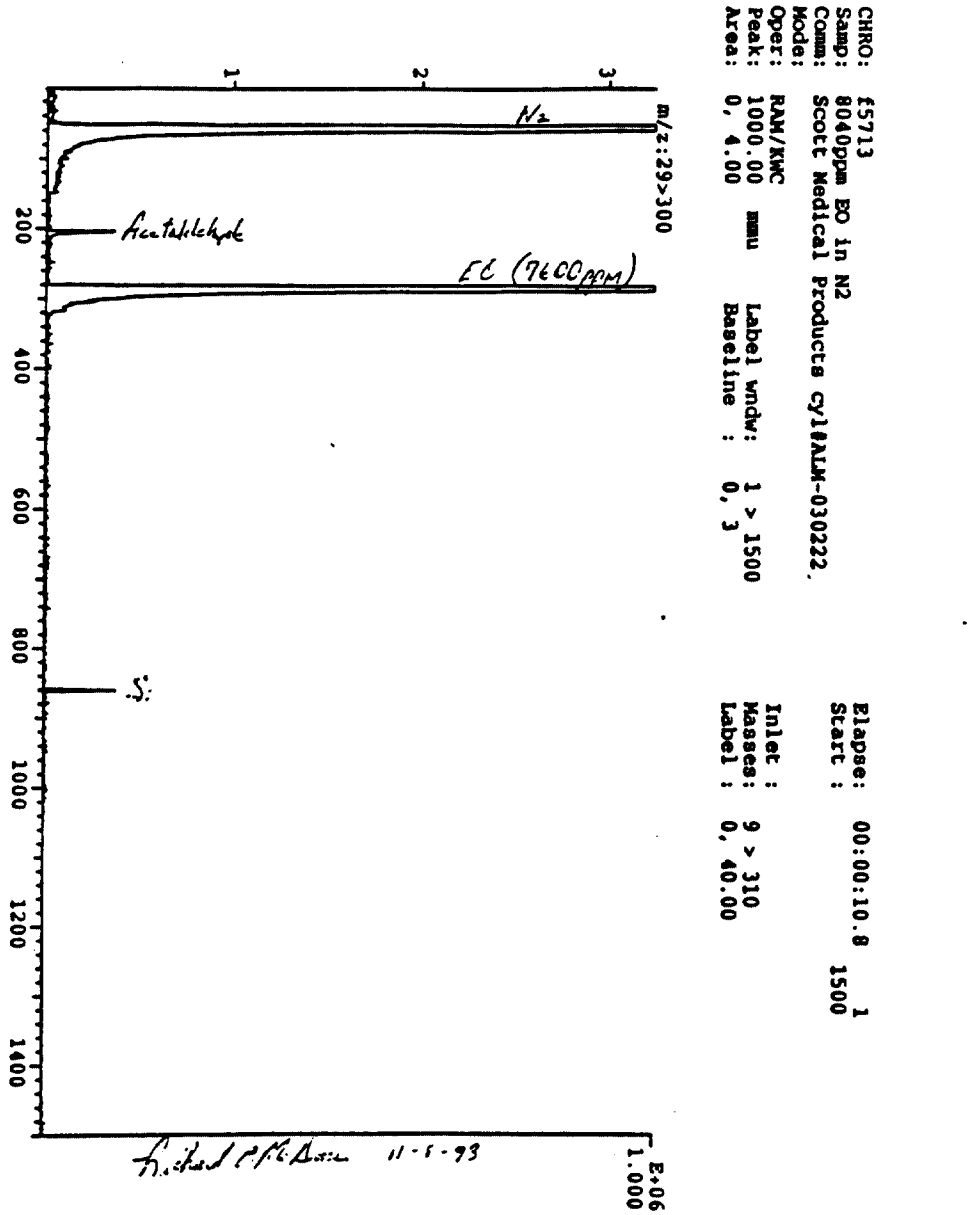
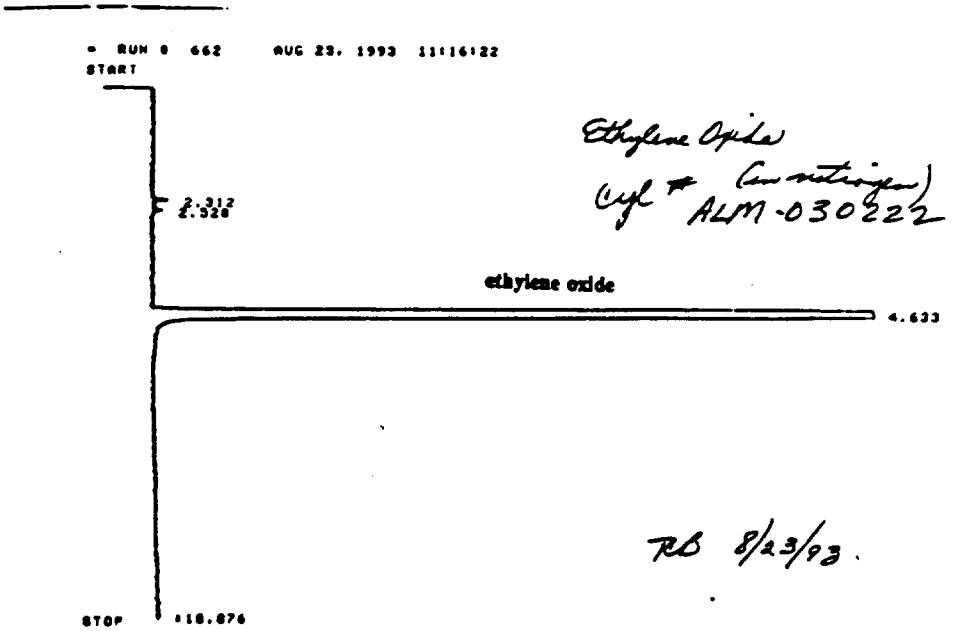


Figure 3— Capillary Gas Chromatogram of 37-AEG-90 (Ethylene Oxide (8000 ppm in nitrogen))



Ethylene Oxide
Cyl # ALM-030222 (in nitrogen)

RB 8/23/93.

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2.528	87	VV	.048	.02788
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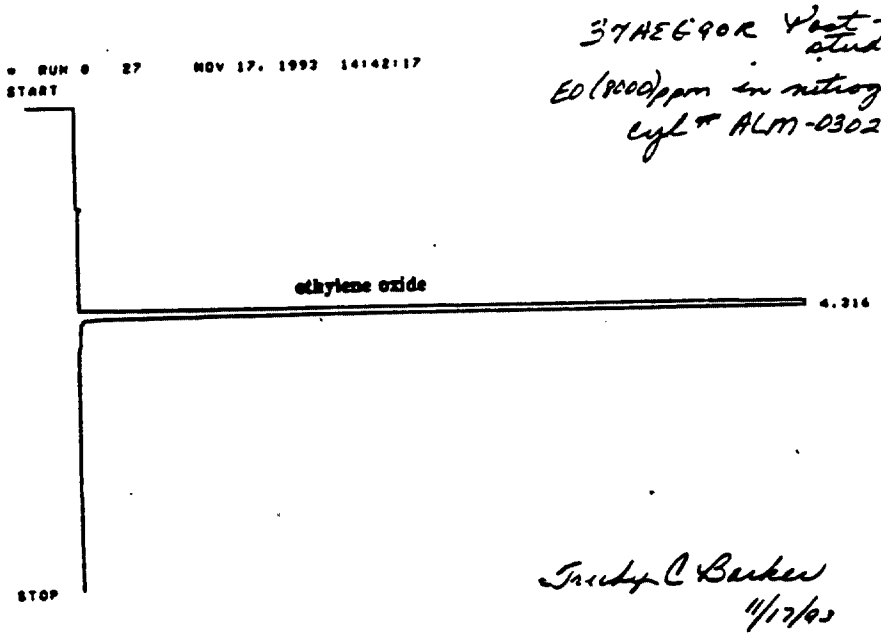
AUL FACTOR=1.0000E+00

Figure 4— Capillary Gas Chromatogram of 37-AEG-90R (Ethylene Oxide (8000 ppm in nitrogen))



APPENDIX I Protocol

PROTOCOL

GOOD LABORATORY PRACTICE (GLP) STUDY

title	Ethylene Oxide (8000 ppm in nitrogen)
purpose	Analytical Characterization of Sample(s) for Toxicology Studies at Bushy Run Research Center
study number	37-AEG-88
sponsor	CMA Olefins Panel Chemical Manufacturers Association 2501 M Street, NW Washington, DC 20037
testing facility	UCC Technical Center, South Charleston, WV 25303 (Location 511)
Proposed Starting Date:	Monday, July 19, 1993
Proposed Completion Date:	September 15, 1993
Estimated Date of Final Report:	October 15, 1993

Test Substance(s) 37-AEG-90

Name	Ethylene Oxide
Source	Lot # 091402; Linde, East Chicago, IN 46312
CAS Registry No.	75-21-8
Description	colorless gas, ether-like odor above 500 ppm
Purity	≈99.9%
Health/Safety	highly toxic; stable. MSDS available upon request
Storage Conditions	ambient

Study Design

The test substance(s) will be characterized by:

- Concentration verified by an 8000 ppm EO in nitrogen standard by GC/MS
- Verification of identity by GC/MS.
- Verification of identity by Infrared spectroscopy.
- Quantitation of the identified impurities by capillary GC.

Reviewed and Approved by:

Alexander E. Gabany	date 7/14/93	Denise L. Johnston	date 7/19/93	Jane S. Vergnes	date 7/21/93
GLP Study Director		GLP Quality Assurance Unit (QAU) Representative		Study Director Bushy Run Research Center	

Elizabeth J. Moran date 7/21/93
CMA Olefins Panel Manager,
Sponsor's Representative

This study will be performed in compliance with the following GLP standards: FDA, 21 CFR, Part 58; TSCA, 40 CFR, Part 792; and FIFRA, 40 CFR, Part 160. All changes of an approved protocol and the reasons therefore shall be documented, signed by the study director, dated, and maintained with the protocol. All raw data and reports and from this study will be retained at Location 511 for at least 10 years after completion of the study. A comprehensive final report will be submitted to the Sponsor within one month after the completion of the analysis. The final report will be inspected by the QAU and will contain a signed quality assurance statement.

Quality Assurance Unit Study Inspection Summary

Test Substance: ETHYLENE OXIDE (8000 ppm in nitrogen)

Study No.: 37-AEG-88

Study Director: A.E. Gabany, B.S.

The Quality Assurance Unit of the Union Carbide Technical Center conducted the inspections listed below and reported the results to the study director and management on the date indicated. It is the practice of this Quality Assurance Unit to report the results to both the study director and management.

Inspection Date	Type	Date QAU Report Issued To Study Director	To Management
Feb. 10, 1992	Laboratory Compliance Review*	Feb. 10, 1992	May, 1992
July 19, 1993	Protocol Compliance Review	July 19, 1993	July 19, 1993
Nov. 22, 1993	Final Report Compliance Review	Nov. 22, 1993	Nov. 22, 1993

*The process of doing the GLP characterization studies is audited periodically to assure these studies comply with GLP requirements. The QA unit is exempted from performing in-life study inspections for studies designed to determine physical and chemical characteristics of a test substance as described in 40 CFR 792.135.

Denise L. Johnson 11/22/93

Denise L. Johnson, QAU Representative (Date)
Good Laboratory Practices/Quality Assurance

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

Individual Clinical Observation Data

(9 Pages)

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TABLE 1

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

INDIVIDUAL CLINICAL OBSERVATION DATA

ABBREVIATIONS

The following is a list of three letter abbreviations for locations of clinical signs that may appear in this appendix.

ABD ABDOMEN
ANS ANUS
AXB AXILLA-BOTH
AXL AXILLA-LEFT
AXR AXILLA-RIGHT
BCK BACK
BDY ENTIRE BODY
CHS CHEST
EAB EAR-BOTH
EAL EAR-LEFT
EAR EAR-RIGHT
ELB EYELID-BOTH
ELL EYELID-LEFT
ELR EYELID-RIGHT
EYB EYE-BOTH
EYL EYE-LEFT
EYR EYE-RIGHT
FAC FACE
GEN GENITAL
HED HEAD
HPB HIP-BOTH
HPL HIP-LEFT
HPR HIP-RIGHT
INB INGUINAL-BOTH
INL INGUINAL-LEFT
INR INGUINAL-RIGHT
LAL LEGS-ALL
LFB LEG-FORE-BOTH
LFL LEG-FORE-LEFT
LFR LEG-FORE-RIGHT
LHB LEG-HIND-BOTH
LHL LEG-HIND-LEFT
LHR LEG-HIND-RIGHT
LNS LOCATION NOT SPECIFIED
MTH MOUTH
MUL MULTIPLE AREAS,NOS*
NCK NECK
NSE NOSE
PAL PAWS-ALL
PFB PAW-FORE-BOTH
PFL PAW-FORE-LEFT
PFR PAW-FORE-RIGHT
PHB PAW-HIND-BOTH
PHL PAW-HIND-LEFT
PHR PAW-HIND-RIGHT
PNS PENIS
SCR SCROTUM
SDB SIDE-BOTH.
SDL SIDE-LEFT
SDR SIDE-RIGHT
SHB SHOULDER-BOTH
SHL SHOULDER-LEFT
SHR SHOULDER-RIGHT
TAL TAIL
TEE TEETH
TRA TREATMENT AREA
TSB TESTIS-BOTH
TSL TESTIS-LEFT
TSR TESTIS-RIGHT
VAG VAGINA
*NOS NOT OTHERWISE SPECIFIED

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
0 PPM					
	6561	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6579	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6583	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6588	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6615	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6620	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6633	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6640	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6682	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6684	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
40 PPM					
	6566	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6569	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6594	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6605	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6609	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6632	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6667	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6678	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6696	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6711	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
1000 PPM					
	6578	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
1000 PPM					
	6578	FATE	1	27	SCHEDULED SACRIFICE
	6592	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6613	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6619	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6627	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6666	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6669	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6679	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6680	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6705	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
3000 PPM					
	6595	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6599	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6608	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6614	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6639	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6655	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6659	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6660	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6700	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6709	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
200 (EO) PPM					
	6571	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
200 (EO) PPM	6606	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6618	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6621	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6631	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6641	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6645	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6653	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6664	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE
	6670	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	27	SCHEDULED SACRIFICE

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
0 PPM					
	6735	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6739	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6741	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6765	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6779	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6780	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6788	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6840	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6846	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6852	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
40 PPM					
	6725	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6726	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6731	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6732	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6801	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6814	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6830	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6836	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6844	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6851	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
1000 PPM					
	6728	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
1000 PPM					
	6728	FATE	1	26	SCHEDULED SACRIFICE
	6733	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6745	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6751	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6760	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6761	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6825	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6826	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6855	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6864	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
3000 PPM					
	6740	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6756	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6757	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6790	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6820	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6828	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6858	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6871	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6872	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6873	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
200 (EO) PPM					
	6754	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
200 (EO) PPM					
	6759	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6773	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6799	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6803	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6813	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6833	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6850	NORMAL	4	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
		SKIN	1	12	ULCER (GEN 1)
	6877	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE
	6883	NORMAL	5	1- 26	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	26	SCHEDULED SACRIFICE

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

Individual Body Weight Data

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TABLE 1

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

INDIVIDUAL BODY WEIGHT DATA

ABBREVIATIONS

The following is a list of abbreviations or words that may appear in this appendix.

dead = indicates that the animal died prior to the period in which this word appears.

sacr = indicates that the animal was a scheduled sacrifice prior to the period in which this abbreviation appears.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 0 PPM

DAY	1	5	12	19	26
ANIMAL					
6561	161.8	169.7	186.6	203.1	219.3
6579	163.7	166.5	173.5	180.7	189.3
6583	161.6	168.1	181.7	193.4	209.0
6588	144.5	153.1	168.1	189.4	213.0
6615	140.4	146.3	159.1	167.3	179.4
6620	160.6	168.2	184.5	202.3	222.2
6633	144.3	150.8	163.8	177.4	194.8
6640	140.5	149.1	163.2	176.2	189.3
6682	172.6	181.1	198.5	210.4	228.2
6684	153.5	160.0	174.4	184.9	203.6
MEAN	154.3	161.3	175.3	188.5	204.8
S.D.	11.31	11.26	12.40	13.77	16.24
N	10	10	10	10	10

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 40 PPM

DAY	1	5	12	19	26
ANIMAL					
6566	138.2	141.8	154.3	165.0	177.8
6569	157.7	161.4	171.2	187.0	191.7
6594	165.2	170.0	189.6	199.3	213.2
6605	139.5	149.4	166.4	184.3	204.6
6609	165.0	173.4	188.8	205.7	227.9
6632	184.5	196.5	220.8	244.0	262.0
6667	152.6	159.3	169.0	186.6	202.6
6678	173.9	185.4	202.2	220.9	236.9
6696	139.0	144.7	155.0	162.6	174.7
6711	157.9	162.0	182.1	195.7	211.1
MEAN	157.3	164.4	179.9	195.1	210.3
S.D.	15.54	17.46	21.09	24.50	26.79
N	10	10	10	10	10

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 1000 PPM

DAY	1	5	12	19	26
ANIMAL					
6578	156.2	160.8	176.3	192.4	203.7
6592	184.8	192.9	210.1	224.9	243.5
6613	148.9	157.3	175.7	190.6	209.1
6619	152.3	160.9	171.7	180.0	186.9
6627	155.2	168.6	186.8	203.0	211.4
6666	137.6	145.9	159.8	173.8	188.3
6669	142.8	147.5	164.6	185.0	203.5
6679	141.8	150.6	167.4	184.7	207.5
6680	144.4	150.6	168.7	184.8	200.1
6705	177.1	183.3	199.6	218.9	236.7
MEAN	154.1	161.8	178.1	193.8	209.1
S.D.	15.47	15.66	16.10	16.76	18.30
N	10	10	10	10	10

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 3000 PPM

DAY	1	5	12	19	26
ANIMAL					
6595	157.1	160.4	176.2	191.4	202.1
6599	148.4	153.8	166.6	181.5	197.0
6608	135.4	144.8	155.5	164.8	179.3
6614	138.7	145.1	158.1	175.6	193.4
6639	135.9	144.5	162.7	176.4	190.3
6655	186.6	194.7	208.3	221.7	235.3
6659	153.0	156.3	173.3	185.9	213.3
6660	150.2	158.1	170.8	189.3	208.0
6700	182.7	190.2	205.4	216.7	226.3
6709	151.4	160.3	176.1	195.4	212.7
MEAN	153.9	160.8	175.3	189.9	205.8
S.D.	17.80	17.80	18.08	17.83	16.97
N	10	10	10	10	10

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 200 (EO) PPM

DAY	1	5	12	19	26
ANIMAL					
6571	170.1	164.1	171.9	172.4	171.1
6606	156.1	157.7	164.4	168.4	172.8
6618	152.4	151.4	155.5	158.6	162.1
6621	152.8	153.8	160.8	162.6	166.2
6631	183.0	181.9	189.6	194.8	198.0
6641	173.6	174.7	179.9	191.3	197.1
6645	167.8	166.6	169.3	171.4	174.2
6653	148.1	148.6	146.4	150.2	151.2
6664	161.9	162.7	168.3	171.4	178.6
6670	154.6	156.4	161.6	165.5	170.3
MEAN	162.0	161.8	166.8	170.7	174.2
S.D.	11.22	10.49	12.20	13.64	14.42
N	10	10	10	10	10

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 0 PPM

DAY	1	5	12	19	26
ANIMAL					
6735	22.7	23.7	24.6	24.8	25.9
6739	25.4	26.1	26.7	27.1	28.4
6741	25.4	25.9	27.1	27.3	28.3
6765	27.6	28.9	29.5	31.2	31.8
6779	25.5	25.9	26.3	28.1	28.1
6780	24.1	24.8	25.7	25.0	25.1
6788	22.5	23.2	24.1	24.6	26.1
6840	25.0	25.8	26.4	26.9	29.3
6846	25.4	26.6	28.5	28.7	30.6
6852	23.4	24.2	25.5	25.4	27.0
MEAN	24.7	25.5	26.4	26.9	28.1
S.D.	1.57	1.66	1.65	2.07	2.11
N	10	10	10	10	10

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 40 PPM

DAY	1	5	12	19	26
ANIMAL					
6725	27.2	27.2	27.5	28.9	29.2
6726	25.6	27.1	27.2	28.5	28.8
6731	23.5	23.9	24.8	25.4	26.5
6732	25.5	26.0	27.8	28.7	29.4
6801	23.4	23.5	24.6	24.5	25.2
6814	25.7	25.8	27.7	28.8	29.4
6830	23.2	24.8	24.7	26.1	27.6
6836	23.7	24.8	25.5	26.8	28.1
6844	24.9	25.3	26.4	26.3	27.4
6851	25.5	27.6	28.6	30.0	30.7
MEAN	24.8	25.6	26.5	27.4	28.2
S.D.	1.31	1.40	1.48	1.81	1.62
N	10	10	10	10	10

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 1000 PPM

DAY	1	5	12	19	26
ANIMAL					
6728	26.0	26.5	28.2	29.1	29.3
6733	24.8	25.5	26.5	27.6	28.2
6745	22.1	22.3	24.0	23.5	24.6
6751	24.6	25.3	27.0	25.7	26.4
6760	25.3	24.7	28.2	28.4	29.5
6761	25.0	26.1	28.5	29.3	29.9
6825	24.8	25.5	27.6	28.1	29.3
6826	23.5	24.6	27.3	27.5	28.6
6855	25.5	26.0	27.8	28.0	28.8
6864	25.6	27.4	28.6	28.8	29.4
MEAN	24.7	25.4	27.4	27.6	28.4
S.D.	1.15	1.36	1.35	1.75	1.64
N	10	10	10	10	10

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 3000 PPM

DAY	1	5	12	19	26
ANIMAL					
6740	24.5	25.2	27.7	28.3	28.3
6756	24.1	24.8	26.5	25.5	26.1
6757	27.6	29.4	31.5	32.6	33.9
6790	24.4	24.9	26.0	25.3	26.0
6820	23.9	25.7	27.4	27.4	28.3
6828	22.6	24.7	25.8	26.8	28.0
6858	29.2	28.7	30.4	31.2	32.3
6871	26.6	28.5	30.5	31.1	31.5
6872	25.2	25.3	27.4	27.5	29.2
6873	27.3	28.5	30.2	29.4	30.3
MEAN	25.5	26.6	28.3	28.5	29.4
S.D.	2.05	1.93	2.11	2.51	2.59
N	10	10	10	10	10

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 200 (EO) PPM

DAY	1	5	12	19	26
ANIMAL					
6754	25.9	26.5	27.9	29.5	30.0
6759	24.8	24.8	26.3	27.4	28.2
6773	25.1	26.1	28.5	20.4	29.5
6799	26.6	26.8	28.7	29.7	30.8
6803	28.4	28.7	29.7	30.6	31.1
6813	24.1	25.1	26.6	27.6	27.9
6833	25.9	25.5	28.2	28.0	27.9
6850	24.8	24.0	26.0	27.3	27.4
6877	26.5	27.6	27.9	29.9	29.9
6883	26.0	27.3	29.1	30.5	30.8
MEAN	25.8	26.2	27.9	28.1	29.3
S.D.	1.20	1.43	1.25	3.00	1.39
N	10	10	10	10	10

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

Individual Micronucleus Data

(5 Pages)

TABLE 1
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST
IN RATS AND MICE
INDIVIDUAL MICRONUCLEUS DATA
24 HOUR MALE RATS

CONCENTRATION	ANIMAL	NCE/1000 ERYTHROCYTES	PCE/1000 ERYTHROCYTES	MN-PCE/2000 ERYTHROCYTES
0 PPM	6561	828	172	4
	6579	756	244	7
	6583	676	324	7
	6588	814	186	7
	6615	735	265	2
	6620	742	258	4
	6633	784	216	14
	6640	776	224	7
	6682	702	298	6
	6684	856	144	2
40 PPM	6566	771	229	5
	6569	763	237	5
	6594	718	282	2
	6605	737	263	5
	6609	814	186	4
	6632	755	245	1
	6667	755	245	6
	6678	834	166	18
	6696	809	191	4
	6711	780	220	2
1000 PPM	6578	850	150	11
	6592	688	312	3
	6613	731	269	1
	6619	780	220	6
	6627	680	320	5
	6666	617	383	3
	6669	587	413	4
	6679	644	356	4
	6680	820	180	3
	6705	664	336	5

TABLE 1 (Continued)
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST
IN RATS AND MICE
INDIVIDUAL MICRONUCLEUS DATA
24 HOUR MALE RATS

CONCENTRATION	ANIMAL	NCE/1000 ERYTHROCYTES	PCE/1000 ERYTHROCYTES	MN-PCE/2000 ERYTHROCYTES
3000 PPM	6595	720	280	6
	6599	817	183	4
	6608	792	208	5
	6614	789	211	4
	6639	768	232	8
	6655	768	232	6
	6659	779	221	1
	6660	695	305	4
	6700	787	213	2
	6709	716	284	8
200 PPM EO	6571	712	288	9
	6606	757	243	19
	6618	723	277	10
	6621	551	449	35
	6631	683	317	16
	6641	692	308	13
	6645	763	237	15
	6653	714	286	12
	6664	708	292	15
	6670	661	339	14

Abbreviations: PCE - Polychromatophilic erythrocyte; MN-PCE - Micronucleated polychromatophilic erythrocyte; EO - Ethylene Oxide

TABLE 2

ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST IN RATS AND MICE

INDIVIDUAL MICRONUCLEUS DATA

24 HOUR MALE MICE

CONCENTRATION	ANIMAL	NCE/1000 ERYTHROCYTES	PCE/1000 ERYTHROCYTES	MN-PCE/2000 ERYTHROCYTES
0 PPM	6735	489	511	7
	6739	495	505	4
	6741	486	514	4
	6765	435	565	4
	6779	365	635	4
	6780	501	499	6
	6788	402	598	2
	6840	395	605	3
	6846	452	548	7
	6852	456	544	2
40 PPM	6725	455	545	6
	6726	421	579	7
	6731	513	487	7
	6732	512	488	7
	6801	450	550	5
	6814	472	528	7
	6830	444	556	7
	6836	426	574	2
	6844	347	653	5
	6851	453	547	7
1000 PPM	6728	532	468	2
	6733	490	510	4
	6745	446	554	7
	6751	502	498	2
	6760	452	548	7
	6761	450	550	8
	6825	400	600	8
	6826	426	574	4
	6855	500	500	7
	6864	478	522	4

TABLE 2 (Continued)
ETHYLENE: FOUR WEEK VAPOR INHALATION BONE MARROW MICRONUCLEUS TEST
IN RATS AND MICE
INDIVIDUAL MICRONUCLEUS DATA
24 HOUR MALE MICE

CONCENTRATION	ANIMAL	NCE/1000 ERYTHROCYTES	PCE/1000 ERYTHROCYTES	MN-PCE/2000 ERYTHROCYTES
3000 PPM	6740	517	483	4
	6756	509	491	4
	6757	483	517	8
	6790	495	505	2
	6820	498	502	5
	6828	465	535	7
	6858	486	514	3
	6871	372	628	7
	6872	454	546	7
	6873	474	526	5
200 PPM EO	6754	528	472	15
	6759	427	573	14
	6773	505	495	12
	6799	471	529	12
	6803	445	555	15
	6813	540	460	14
	6833	471	529	17
	6850	465	535	16
	6877	470	530	14
	6883	449	551	15

Abbreviations: PCE - Polychromatophilic erythrocyte; MN-PCE - Micronucleated polychromatophilic erythrocyte; EO - Ethylene Oxide

Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

Protocol and Protocol Deviations

(16 Pages)

UNION CARBIDE

BUSHY RUN RESEARCH CENTER

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PROTOCOL

TITLE: Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

BRRC PROJECT ID: 93N1296

SPONSOR:
CMA Olefins Panel
Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

TESTING FACILITY:
Bushy Run Research Center (BRRC)
Union Carbide Corporation
6702 Mellon Road
Export, PA 15632-8902

Reviewed and Approved by:

Bushy Run Research Center:

Jane S. Vergnes, Ph.D. — Date 8/2/93
Study Director

James D. Sun, Ph.D. — Date 8/2/93
Associate Director

Linda J. Calisti, B.S. — Date 8/2/93
Manager, Good Laboratory Practices/Quality Assurance

John P. Van Miller, Ph.D., DABT — Date 8/2/93
Director

Sponsor's Representative:

Elizabeth J. Moran, Ph.D. — Date 8/3/93
CMA Olefins Panel Manager

OBJECTIVE

The objective of this study is to assess the potential of ethylene to cause significant increases in the incidence of micronucleated polychromatophilic erythrocytes in the bone marrow of Fischer 344 rats and B6C3F1 mice which may result from a 4-week repeated inhalation exposure regimen.

Design and Basis for the Study

This study will consist of 3 exposure groups (40, 1000, and 3000 ppm ethylene), an air-exposed control group, and an ethylene oxide-exposed (EO, 200 ppm) control group. Each group will consist of 10 males/species. Animals will be exposed to ethylene vapor for 6 hours/day, 5 consecutive days/week, for 4 weeks, except that mice will receive their final exposure on Study Day 27. Bone marrow will be collected for the evaluation of polychromatophilic erythrocytes approximately 24 hours after the final exposure.

The portions of this study conducted at BRRC will be in compliance with the following guidelines and standards:

U.S. Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR Part 792.

Organisation for Economic Co-operation and Development (OECD) (1981). OECD Principles of Good Laboratory Practice, C(81)30(Final).

PERSONNEL

All personnel who participate in the conduct of the study will be documented in the raw data.

PROJECT DATES

Starting Date of Acclimation	August 3, 1993
Proposed Starting Date of Test Substance Administration	August 16, 1993
Proposed Date for Completion of In-Life Phase	September 11, 1993
Proposed Date for Submission of Draft Final Report	November 19, 1993

METHODS

Test Substance

Chemical Name	Ethylene
CAS Registry Number	74-85-1
Source	Matheson Gas Products, Twinsburg, OH, by way of Union Carbide Corporation, S. Charleston, WV

Sponsor Identification Number	E28-0001-A4
BRRC Sample Number	56-319
Description	Colorless compressed gas with a sweet odor and taste, according to the Material Safety Data Sheet (MSDS).
Purity	Purity information will be documented in the raw data and final report.
Stability	Stable
Storage Conditions	Outside, under cover, in a locked enclosure.
Quantity	2 steel cylinders
Reserve Sample	A reserve sample will not be retained at BRRC due to the volatile nature of the test substance.
Safety	An MSDS will be reviewed by all relevant personnel before their participation in the study. This review will be documented. Normal precautions for untested substances will be used. These procedures include the use of disposable Tyvek® or plastic coats or jumpsuits, hats, booties or shoe covers, and rubber gloves while in the animal rooms. Eye protection will include the use of safety glasses at all times. Disposable Tyvek® coats or smocks and appropriate gloves will be worn during administration of the test substance. In addition, monogoggles will be used when handling the test substance.

Test Animals

Species and Strain	Fischer 344 rats and B6C3F1 mice
Supplier	Rats - Harlan Sprague Dawley, Inc., Indianapolis, IN Mice - Charles River Laboratories, Portage, MI
Rationale	These species and strains were selected by the Sponsor based upon previous studies performed using ethylene and/or ethylene oxide.
Number and Sex	A total of 168 males of each species will be ordered from which 50 males of each species will be selected for the study. Ninety-eight males of each species will be assigned to a separate but concurrent ethylene inhalation study for the same sponsor.
Age	The animals will be requested to be approximately 5 to 6 weeks of age on the scheduled animal receipt date.

Acclimation and Pretest Evaluations

Shortly after their arrival at the laboratory, the animals will be transported to the room selected for the study. Once in the room, the animals will be removed from the shipping cartons and examined. All animals with evidence of disease or physical abnormalities will be discarded and the reason for rejection will be recorded. If an unusually large number of animals shows evidence of disease or physical abnormalities, the entire shipment of animals will be rejected for use in the study. A total of 10 animals (5/species) will be randomly selected for a pretest health screen as discussed below. Additional animals not selected for the study may be held for possible random serology testing.

All remaining animals will be housed 2 to a cage in order to acclimate the animals to the automatic watering system. The acclimation period will be approximately 2 weeks. At the time of group assignment, all animals will be housed individually.

During the acclimation period, animals will be fed the same diet that will be used during the study. Animals will be observed twice daily for any overt clinical signs of disease or abnormality. Individual detailed physical examinations will be conducted weekly. Animals showing abnormalities deemed by the Study Director or other appropriate personnel to render the animal unacceptable for placement on the study will be sacrificed and discarded on the day observed, and the reason for sacrifice will be recorded.

The animals will be weighed at least 2 times during the weeks prior to exposure. Any animal whose weight gain during the acclimation period is not considered normal for this age and strain of animal, or whose absolute body weight at the time of the second weighing is outside ± 20% of the population mean for each sex, will not be considered for use in the study.

Pretest Health Screen

A pretest health screen will be initiated within 2 days after the receipt of the animals. The pretest health screen will be performed on 5 animals/species selected directly from the shipping cartons with as many cartons as possible being represented.

Rats

The pretest health screen will consist of examinations for fecal parasites, necropsy, and histopathologic evaluations of selected tissues (3 animals). Serology testing for Sendai virus, rat coronavirus/ sialodacryoadenitis virus, and Mycoplasma pulmonis

will be performed approximately 2 weeks after receipt using animals not selected for the study. Serology results will be available prior to the start of the study.

Additional animals not selected for the study may be held for possible random serology testing. The following organisms will be included in serologic testing conducted periodically throughout the facility:

Sendai virus (SEND)
Pneumonia virus of mice (PVM)
Rat coronavirus/Sialodacryoadenitis virus (RCV/SDA)
Kilham rat virus (KRV)
Toolan's H-1 virus (H-1)
Reovirus type 3 (REO3)
Mycoplasma pulmonis (MPUL)
Mouse polio virus (GD-7)
Lymphocytic choriomeningitis virus (LCMV)
Mouse adenovirus FL/K87 (MAD)
Minute virus of mice (MVM)
Polyoma virus (POLY)

Mice

The pretest health screen will consist of a serology screen, examinations for fecal parasites, necropsy, and histopathologic evaluations of selected tissues (3 animals).

The following organisms will be included in serologic testing:

Sendai virus (SEND)
Pneumonia virus of mice (PVM)
Mouse hepatitis virus (MHV)
Minute virus of mice (MVM)
Mouse polio virus (GD-7)
Reovirus type 3 (REO-3)
Mycoplasma pulmonis (MPUL)
Lymphocytic choriomeningitis virus (LCMV)
Mouse adenovirus FL/K87 (MAD)
Ectromelia virus (ECTRO)
Mouse pneumonitis virus (K)
Polyoma virus (POLY)
Epizootic diarrhea of infant mice virus (EDIM)
Mouse cytomegalovirus (MCMV)
Hantann virus (HANT)
Encephalitozoon cuniculi (ECUN)

Fecal examination for parasites will be conducted using a cellophane tape test as a prestudy screen and by zinc sulfate flotation from cecal contents obtained at necropsy.

Histopathology will consist of examination of at least the following tissues: liver, kidneys, trachea, lungs, heart, spleen, salivary glands, submandibular lymph nodes, and nasal cavities.

The purpose of this screen is to determine the suitability of the population of animals proposed for this study. Therefore, the results of this screen will be available to the Study Director before the study begins.

All animals will be examined by a veterinarian shortly after their arrival and again prior to study start. The dates of the examinations will be documented in the raw data.

Identification

Each animal will be assigned a unique identification number prior to the initiation of the study. Rats will be identified by a cage tag and tail tattoo. Mice will be identified by a cage tag and toe-clip. Animals sacrificed for use in the pretest health screen will be identified by cage tags only until sacrifice and then toe-clipped for further identification. Records will be kept documenting the fate of all animals received for the study.

Husbandry

Conditions

All animals will be housed in an appropriate animal room at BRRC from arrival until termination of the in-life phase of the study. Stainless steel cages with wire mesh floors will be used throughout all phases of the study. Cages will be changed and sanitized at least once every 2 weeks. DACB® (Deotized Animal Cage Board; Shepherd Specialty Papers, Inc.) will be changed at least 3 times each week.

Cages housing EO-exposed animals will not be placed on the same carriers as cages housing air-only or ethylene-exposed animals. Carriers of EO-exposed animals will not be in close proximity to carriers of air-only or ethylene-exposed animals.

The animal room concentrations of EO will be determined by a gas chromatographic technique after exposure on Study Day 1. Probes for sampling will be placed in the breathing zones of the EO-exposed animals and the ethylene-exposed animals.

Temperature and humidity will be recorded continuously using an automatic recorder. Temperature will be maintained at 66-77°F and relative humidity will be maintained at 40-70%. The temperature and humidity will be checked by a technician at each room check and a record will be kept indicating that it was done. Appropriate corrective action will be taken whenever readings outside the specified limits are observed.

The accuracy of the temperature and humidity recording devices will be checked periodically and calibrated when necessary. The verification and calibration data will be recorded. In the event that continuous recording cannot be maintained, the temperature and humidity will be manually recorded at each room check.

An automatic timer will be set to provide fluorescent lighting for a 12-hour photoperiod (approximately 0500 to 1700 hours for the light phase). In the animal room, there will be at least 10 air changes each hour.

Diet — Pelleted, certified AGWAY® PROLAB® Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.) will be available ad libitum except during exposures. The analyses of chemical composition and possible contaminants of each batch of diet will be performed by Agway Inc., and the results of the analyses will be reviewed by the Study Director.

Water — Tap water (Municipal Authority of Westmoreland County, Greensburg, PA) will be available ad libitum, except during exposures, by an automatic watering system with demand control valves mounted on each rack. Water pressure and function of the individual cage rack systems will be checked at each room check, and a record will be kept indicating it was done. Drinking water contaminant levels will be measured at regular intervals according to EPA specifications, to include the "priority" pollutants, and will comply with human drinking water requirements. The results of the analyses will be reviewed by the Study Director.

Administration of Test Substance

Route and Justification — The route of administration will be by inhalation. This is considered to be a meaningful way to evaluate the toxicity of chemicals with the use pattern of the test substance. The inhalation route is also a potential route of human exposure.

Target Exposure Concentrations	Target exposure concentrations of the test substance will be 0 (filtered air only), 40, 1000, and 3000 ppm ethylene, as selected by the Sponsor. The target exposure concentration of the EO control will be 200 ppm, as selected by the Sponsor.
Test Substance Analysis	Prior to initiation of the study and following the study, samples of ethylene and EO will be drawn, and compositional analyses will be performed by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. Compositional analyses of ethylene will include levels of EO.
Exposure Chambers	Five stainless steel chambers with glass doors and windows for animal observation will be used. The total "volume" of the animals will not exceed 5% of the volume of the test chamber. Chambers will be provided with air at a flowrate of approximately 13-14 air changes each hour to ensure an adequate oxygen content of at least 19%. The rate of airflow will be monitored continuously and recorded approximately every 30 minutes. All chambers will be maintained at a slight negative pressure to prevent any test substance or EO from entering the room containing the chambers. The temperature and relative humidity of the exposure chambers will be monitored continuously and recorded approximately 12 times during each exposure. If possible, temperature will be maintained at 22 ± 2°C, and relative humidity will be maintained between 40 to 60%. In case of undetected differences in environment or test substance concentration within the chamber, all exposure cage positions will be rotated daily. A description of the rotation will be provided in the raw data. The oxygen content of the chambers will be monitored prior to initiation of the study.
Duration of Treatment	Animals will be exposed for 6 hours/day, 5 consecutive days/week, for 4 weeks, except that mice will receive their final exposure on Study Day 27. Animals will be acclimated to the exposure chamber (mock exposure) for 2 days during the week prior to initiation of exposures.
Generation of Test and EO Control Substances	The test and EO control substances will be generated by the dilution of rotameter-controlled ethylene or EO streams with chamber air to the desired concentration.

Chamber Atmosphere Sampling

Chamber concentrations of the test substance will be determined approximately every 60 minutes (contingent on analytical limitations) by a gas chromatographic technique. Chamber concentrations of EO will also be determined in air-only and ethylene chambers at least once weekly. The air-only and EO chamber atmospheres will also be analyzed approximately every 60 minutes. Chamber probes for sampling will be placed in the breathing zone of the animals. The daily nominal (estimated) chamber concentration will also be determined.

Chamber Distribution

The uniformity of the vapor concentration in each of the 3 exposure chambers and in the EO control chamber will be examined. Each chamber will be tested prior to initiation of the study. For each individual distribution test, vapor concentrations will be measured at 5 positions.

EO Control

At the Sponsor's request one group of animals will be exposed to EO (CAS No. 75-21-8) as previously described.

Study Design

Group Assignment

Based on the second pretest body weights, the animals will be selected for the study from the remaining population. These animals will be divided into 5 experimental groups. Each group size is shown below and will be based on a weight randomization procedure.

Animals not selected for the study will remain housed in the study room until the study begins and will be used as replacements in the event that any of the selected animals dies prior to the start of the study. After the selected animals have received their first exposure, all animals not selected for the study will be humanely sacrificed and discarded, used for training of BRRC staff, used for random serology testing, or used for methods development, and no further replacement of animals will be made. The fate of all animals not selected for use in this study will be documented in the raw data.

Following body weight measurement just prior to the first exposure, statistical evaluation of the body weights for all groups will be conducted, and statistical equivalence and homogeneity of variance will be examined. In the event that either criterion is not met, changes will be made prior to the initial exposure to ensure statistically equivalent body weights for all groups. Animals with any abnormal clinical signs will also be replaced prior to exposure and the statistical criterion reevaluated.

Organization

Group	Number of Animals	Exposure Concentrations (ppm)
Air-Only Control	10/species	0
EO Control	10/species	200
Low	10/species	40
Mid	10/species	1000
High	10/species	3000

Experimental Evaluations

Mortality Checks and Clinical Signs

All animals will be observed for mortality and overt signs of toxicity twice each day, 7 days a week. On exposure days, the animals will be observed just prior to exposure and shortly following the exposure. On nonexposure days, the first daily check will generally be conducted before 9:00 a.m., and the second mortality check will be conducted after noon. Animals will also be observed on a group basis during the 6-hour exposure. Should mortality and/or signs of overt toxicity be observed, it will be recorded on the day observed. Overt signs will also be recorded on subsequent days until the sign disappears or the animal dies. Detailed examinations for clinical signs of disease or abnormality, which involve animal handling, will be conducted once weekly (a.m.) during the study.

Sacrifice of Distressed Animals

If any animal shows signs of extreme distress or is moribund, it will be sacrificed for humane reasons before the scheduled date.

Body Weight

Individual body weights will be measured weekly. Body weight gains will be computed.

Micronucleus Evaluation

Approximately 24 hours after the final exposure, animals will be sacrificed by carbon dioxide asphyxiation (Study Day 28 for Mice and Study Day 29 for rats). Both femurs will be removed and the bone marrow will be aspirated with a syringe into fetal bovine serum (FBS). Bone marrow cells will be pelleted by centrifugation of the aspirated marrow sample. All but a small amount of the supernate will be removed. The cell pellet will be resuspended gently and a drop of the bone marrow cell suspension will be smeared on a microscope slide. One or two

smears will be made for each animal. Slides will be stained, coded with the randomly-assigned animal numbers, and read without knowledge of the exposure group to prevent bias.

The polychromatophilic (PCE) to normochromatophilic (NCE) ratio for approximately 1000 total cells will be calculated, recorded, and shown in the final report to provide an estimate of the cytotoxicity of the test agent. Excessive cytotoxicity in this test system will be defined by a PCE:NCE ratio of 0.10 or lower. Animals with an excessive reduction in the PCE:NCE ratio will be identified in the raw data and final report but micronuclei will not be scored. A minimum of 2000 PCE/animal will be scored for the presence of micronuclei unless the cytotoxicity of the test substance prevents this.

Evaluation of Test Results

Statistical analyses to determine the significance of increases in the frequencies of micronuclei will compare the frequency of cells with micronuclei at each concentration with the air-only control value using the Mann-Whitney U-test. The test will be considered positive if there is a statistically significant, concentration-related increase in micronucleated polychromatophilic erythrocytes as compared to the air-only control. The evaluation of a statistically significant response at a single concentration will be made by the Study Director on a case-by-case basis. The final evaluation of the results will be based on statistical significance, biological significance and the scientific judgment of the Study Director. The probability value of < 0.05 (2-tailed) will be used as the critical level of significance for all tests.

ALTERATION OF PROTOCOL

Alterations to this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such change will be honored. However, it then becomes the responsibility of the Sponsor to follow such verbal change with a written verification. BRRC reserves the right to revise the protocol or deviate therefrom solely at the discretion of the Study Director if prior approval of the Sponsor cannot be obtained and the integrity of the study is considered in jeopardy. In this event, the Sponsor will be notified of the alteration as soon as possible, and documentation of the change will be the responsibility of the Study Director.

RETENTION OF RECORDS

All raw data, documentation, the protocol and any amendments, and a copy of the final report generated as a result of this study will be retained in the BRRC Archives for at least 10 years.

Following the retention period specified above, the Sponsor will be contacted and given the option of taking receipt, destroying, or arranging for other storage of the data and materials. All data and materials mentioned above will remain the sole property of the Sponsor and can be removed from BRRC at the Sponsor's discretion.

REPORTS

Draft Final Report

An audited draft of the final report will be submitted to the Sponsor approximately 2 months after the completion of the terminal sacrifice. This report will be a comprehensive report which will include all information necessary to provide a complete and accurate description and evaluation of BRRC test procedures and results. It will include: a summary; appropriate text discussions of the experimental design, materials and methods, and results; and summary mean or incidence tables of in-life data. In addition, it will contain appendices with individual animal data and other pertinent information. One bound and 1 unbound copy of the draft report will be provided to the Sponsor.

Final Report

The draft final report will be reviewed by the Sponsor, and comments on the report will be provided to BRRC within 4 weeks from the date of submission of the draft version. BRRC will consider these comments in preparing the final report. Assuming the Sponsor's comments are received at the specified time and no major revisions are required, BRRC will submit a final report within 8 weeks of issuance of the draft report.

The final report will be audited by the Quality Assurance Unit and contain a signed quality assurance statement. It will conform to the formatting specifications of EPA PR notice 86-5. One bound and 1 unbound copy of the final report will be submitted to the Sponsor.

ANIMAL USE POLICY

It is the goal of BRRC, through the establishment and activities of the Institutional Animal Care and Use Committee, to comply with the U.S. Animal Welfare Act and the subsequent rules promulgated by the U.S. Department of Agriculture and in effect on the date of this protocol. It has been determined that the work described herein minimizes the number of animals used, is necessary, and uses the most appropriate species and strain in order to provide meaningful results and the most useful information for comparative purposes relative to previous studies. Furthermore, this study will be conducted humanely, and to the best of our knowledge, neither unnecessarily duplicates any previous work, nor can it be accomplished using currently available, validated nonanimal models.

GOOD LABORATORY PRACTICE COMPLIANCE

BRRC, through the administration of a quality assurance program by the Good Laboratory Practice Committee and Quality Assurance Unit, assures compliance of all phases of studies conducted at BRRC with existing regulations and generally accepted good laboratory practices.

The study will be subjected to periodic inspections and the final report will be reviewed by the BRRC Quality Assurance Unit.

PROTOCOL DEVIATIONS

TITLE: Ethylene: Four Week Vapor Inhalation Bone Marrow Micronucleus Test in Rats and Mice

BRRC PROJECT ID: 93N1296

Item 1

Location of Deviation	Page 4, Acclimation and Pretest Evaluations
Description of Deviation	Rats were housed individually during the acclimation period instead of 2 to a cage. Mice were also placed in individual housing on August 6 and 7, 1993.

Item 2

Location of Deviation	Page 4, Acclimation and Pretest Evaluations
Description of Deviation	No animal observations were recorded on August 4, 1993.

Item 3

Location of Deviation	Page 4, Acclimation and Pretest Evaluations
Description of Deviation	Mortality checks were conducted twice daily until the animals were identified. Then animals were observed once daily for overt signs of disease or abnormality and an afternoon mortality check was conducted daily during the acclimation period.

Item 4

Location of Deviation	Page 6, Identification
Description of Deviation	The toe-clipping procedure used to identify the mice was supplemented with ear notches when the mice could not be uniquely identified by toe-clipping alone.

Item 5

Location of Deviation	Page 6, Husbandry, Conditions
Description of Deviation	Cages were not changed and sanitized from August 13, 1993 to September 1, 1993.

Item 6

Location of Deviation	Page 7, Husbandry, Conditions
Description of Deviation	Several gaps in the continuous recording of temperature and humidity occurred during the study.

Item 7

Location of Deviation	Page 7, Husbandry, Conditions
Description of Deviation	Verification of temperature and humidity checks is not available for each room check.

Item 8

Location of Deviation	Page 8, Exposure Chambers
Description of Deviation	There were approximately 20 air changes each hour in the 40 ppm ethylene chamber.

Item 9

Location of Deviation	Page 8, Generation of Test and EO Control Substances
Description of Deviation	Flowmeters were used, rather than rotameters, to generate ethylene and EO.

Item 10

Location of Deviation	Page 10, Experimental Evaluations, Mortality Checks and Clinical Signs
Description of Deviation	Overt observations were not recorded on the afternoons during weekends.

Item 11

Location of Deviation	Page 11, Evaluation of Test Results
Description of Deviation	The Mantel-Haenszel trend test and a large sample distribution-free multiple comparison test were used to evaluate micronucleus frequencies in the ethylene-exposed and air-only groups.

These deviations are not expected to compromise the integrity of the results and conclusion of the study.

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RECD
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PREVENTION AND TOXICS
94 APR 22 PM 2:42

STUDY TITLE

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

TEST SUBSTANCE

Ethylene

DATA REQUIREMENT

Not Applicable

AUTHORS

J. S. Vergnes and W. J. Kintigh

STUDY COMPLETION DATE

February 25, 1994

PERFORMING LABORATORY

Bushy Run Research Center (BRRC)
Union Carbide Corporation (UCC)
6702 Mellon Road
Export, PA 15632-8902

LABORATORY PROJECT ID

93N1311

SPONSOR

CMA Olefins Panel
Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The portions of this study conducted by BRRC meet the requirements of the following Good Laboratory Practice Standards: Toxic Substances Control Act (TSCA), 40 CFR Part 792; Organisation for Economic Co-operation and Development (OECD), C(81)30(Final).

Study Director: _____ 5/25/94

Jane S. Vergnes, Ph.D. Date

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Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

SUMMARY

Ethylene (CAS No. 74-85-1) was given to male Fischer 344 rats and B6C3F1 mice (7 animals/group) by whole body vapor exposure for 6 hours/day, 5 days/week, as described below. The ethylene target concentrations were 0, 40, 1000, and 3000 ppm. An ethylene oxide (EO) control group was exposed under the same conditions at a target concentration of 200 ppm. Seven males/species/group were exposed to the following test or control concentrations for the designated time periods: air-only control and 3000 ppm ethylene for 1, 2, and 4 weeks; 40 and 1000 ppm ethylene and 200 ppm EO for 4 weeks. Animals were sacrificed approximately 2 to 4 hours after exposure and blood, brains, lungs, livers, and spleens were taken and processed according to instructions provided by the University of North Carolina. After processing, tissues were packed in dry ice and shipped to the University of North Carolina for determination of DNA and hemoglobin adducts and abasic sites under the direction of Dr. James A. Swenberg.

An additional 7 males/species/group were exposed for 4 weeks as previously described and then held for a recovery period of approximately 5 weeks for rats and 8 weeks for mice. Recovery animals were then shipped to the University of North Carolina for determination of HPRT mutations under the direction of Dr. James A. Swenberg. The results of the evaluations conducted by the University of North Carolina will be reported by Dr. Swenberg and his associates in a separate report.

There were no mortalities or clinical signs related to ethylene exposure observed during the study. The EO-exposed rats gained significantly less weight than the air-only control rats during the study. There were no consistent body weight trends in ethylene- or EO-exposed mice or in ethylene-exposed rats during the study.

OBJECTIVE

The objective of this study was to evaluate DNA and hemoglobin adducts, abasic sites, and HPRT mutations in Fisher 344 rats and B6C3F1 mice which may have resulted from a 4-week repeated inhalation exposure regimen.

BACKGROUND INFORMATION

Ethylene is a highly flammable gas of extremely low toxicity. A 90-day inhalation study in Sprague Dawley® rats exposed to 0, 300, 1000, or 10,000 ppm ethylene for 6 hours/day, 5 days/week, revealed no exposure-related toxic effects (Rhudy et al., 1978). No exposure-related increases in tumor incidence were observed in a cancer bioassay in Fischer 344 rats exposed at ethylene target concentrations of 0, 300, 1000, or 3000 ppm (Hamm et al., 1984). Pharmacokinetic studies have shown that metabolic saturation occurs at ethylene exposure concentrations of 1000 ppm or higher in rats (Bolt et al., 1984). Ethylene is known to be metabolized to EO in rats, mice, and humans (Walker et al., 1990). Exposure to the current Threshold Limit Value (TLV) for EO, 1 ppm, is equivalent to exposure to approximately 40 ppm ethylene (Bolt and Filser, 1987). Inhalation exposure to high concentrations of EO has been associated with cancer in rats and mice. Furthermore, EO is known to react with DNA in vivo as well as in vitro. However, the relevance of the EO data to ethylene exposure cannot be determined without additional studies. The study described in this report was designed to assess the genotoxic potential of ethylene in vivo in male Fischer 344 rats and B6C3F1 mice.

TARGET CONCENTRATION SELECTION

Target ethylene vapor concentrations of 0 (control), 40, 1000, and 3000 ppm were selected by the Sponsor on the basis of the previously mentioned cancer bioassay and pharmacokinetic studies. The target exposure concentration of EO was 200 ppm, as selected by the Sponsor.

MATERIALS AND METHODS

The protocol detailing the design and conduct of this study is included in Appendix 5. Protocol deviations are also included in Appendix 5.

Test Substance and Control Substance

Two cylinders, each containing 30 pounds of ethylene, CAS No. 74-85-1, Lot E28-0001-A4, BRRC No. 56-319 A and B, were received from Matheson Gas Products (Twinsburg, OH) by way of Union Carbide Corporation (South Charleston, WV) on July 23, 1993. Only 1 cylinder, 56-319A, was used for the study. The test substance was a colorless, compressed gas. The test substance was stored outdoors in a locked chemical storage area. The compositional analyses were provided by the Sponsor. A summary of the test substance characterization report is presented in Table 2 of Appendix 1; the entire report is presented as Attachment 1 of Appendix 1. The prestudy and poststudy compositional analyses indicated that the test substance was 99.99% pure and had remained stable for the duration of the exposure regimen.

Fifty preanalyzed gas cylinders, each containing 143 cubic feet of ethylene oxide (EO) in nitrogen, Cylinder Lot No. 091402, BRRC Sample No. 55-143 A through XX, were received from Scott Medical Products (Plumsteadville, PA) on May 21, 1992. The control substance was stored outdoors in a locked chemical storage area. Cylinders 55-143 OO, PP, QQ, RR, SS, TT, UU, VV, WW, MM, NN, and II were used during the study either for preliminary work or animal exposures. The compositional analyses and the EO concentration were obtained from cylinder samples taken both before and after the exposure regimen. The prestudy gas chromatographic analysis indicated that the control substance was 7600 ppm EO in nitrogen and that the EO component was nearly 99.93% pure. A summary of the EO control substance analysis is presented in Table 3 of Appendix 1; the entire report is presented as Attachment 2 of Appendix 1.

Animals and Husbandry

One hundred sixty-six (1 was found dead) male Fischer 344 rats arrived on August 2, 1993, from Harlan Sprague Dawley Inc. (Indianapolis, IN). They were designated by the supplier to be approximately 43 days old (the birth date was recorded as June 21, 1993). Ninety-eight rats were assigned to this study. The remaining animals were assigned to a separate but concurrent ethylene inhalation study for the same Sponsor or were euthanized.

One-hundred sixty-eight male B6C3F1 mice arrived on August 3, 1993, from Charles River Laboratories (Portage, MI). They were designated by the supplier to be approximately 42 days old (the birth date was recorded as June 21, 1993) and 21 to 24 g upon arrival. Ninety-eight mice were assigned to this study. The remaining animals were assigned to a separate but concurrent ethylene inhalation study for the same Sponsor or were euthanized.

The rats were housed in Room 155 from arrival until October 4, 1993. The rats were then housed in a Relocatable Containment System® unit (Hazleton Systems, Inc., Aberdeen, MD) in Room 164D and Room 164E from October 4, 1993 until October 13, 1993. The mice were housed in a Relocatable Containment System® unit (Hazleton Systems, Inc., Aberdeen, MD) in Room 164D from arrival until August 13, 1993 (11 days). The mice were then housed in Room 155 until October 4, 1993. The mice were then housed in a Relocatable Containment System® unit (Hazleton Systems, Inc., Aberdeen, MD) in Room 164D and Room 164E from October 4, 1993 until November 3, 1993. Within 2 days of receipt, the rats and mice were examined by a Clinical Veterinarian, and representative rats and mice were subjected to a pretest health screen including full necropsy, histologic examination of selected tissues, serum viral antibody analyses (mice only), and examinations for fecal parasites. The serum viral antibody analyses for rats were performed 9 days after the arrival of the animals. Based on the results of these data, the Clinical Veterinarian indicated that these animals were in good health and suitable for use.

All animals were assigned unique numbers and identified by cage tags. Rats considered available for the study were also identified by a tail tattooing procedure. Mice considered available for the study were identified by a toe-clipping procedure which was supplemented, when necessary, by an ear notching procedure. Animals selected for the pretest health screen were identified by a toe-clipping procedure after sacrifice, except for the rats selected for the serum viral antibody analyses. These animals were identified by tail tattoo.

The rats were individually housed in stainless steel, wire mesh cages (22.5 x 15.5 x 18.0 cm) for the duration of the study. The mice were housed 2/cage for approximately 5 days in stainless steel, wire mesh cages (22.5 x 10.0 x 12.5 cm), and then individually housed for the remainder of the study. The purpose of the double housing was to help acclimate the mice to their new surroundings. DACB® (Deotized Animal Cage Board; Shepherd Specialty Papers, Inc.) was placed under each cage and changed at least 3 times each week. Cages were changed at least once every 2 weeks. An automatic timer was set to provide fluorescent lighting for a 12-hour photoperiod (approximately 0500 to 1700 hours for the light phase). Temperature and relative humidity were recorded (Cole-Parmer Hygrothermograph® Seven-Day Continuous Recorder, Model No. 8368-00, Cole-Parmer Instrument Co., Chicago, IL). Temperature was routinely maintained at 66-77°F; relative humidity was routinely maintained at 40-70%. Any minor exceptions to these specified ranges were noted in the raw data.

Tap water (Municipal Authority of Westmoreland County, Greensburg, PA) was available ad libitum, except during exposures, and was delivered by an automatic watering system with demand control valves mounted on each rack. Water analyses were provided by the supplier, Halliburton NUS Environmental Laboratories, Professional Service Industries, Inc., and Lancaster Laboratories, Inc., at regular intervals. EPA standards for maximum levels of contaminants were not exceeded. Pelleted, certified AGWAY® PROLAB® Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.) was available ad libitum except during exposures. Analyses for chemical composition and possible contaminants of each feed lot were performed by Agway Inc., and the results were included in the raw data.

Animal Acclimation

The acclimation period was approximately 2 weeks. During this period, the animals were weighed twice at scheduled intervals. Detailed clinical observations were conducted in conjunction with body weight measurements. Cage-side animal observations were conducted at least once daily, and mortality checks were conducted twice daily (morning and afternoon). The animals were examined just prior to the end of the acclimation period by a Clinical Veterinarian. Animals considered unacceptable for the study, based on the clinical signs, body weight, or body weight gain, were rejected. The fate of rejected animals and the reasons for rejection were documented in the raw data.

Study Organization

Following the second pretest body weight, the animals were assigned to 3 exposure groups, an air-only control group, and an EO control group using a nonstratified randomization procedure based on body weight. At the time of group assignment, only animals with body weight within ± 20% of the population mean were included. The body weight range on the day of first exposure was 131.5 to 188.9 g for male rats and 21.2 to 28.1 g for male mice. The following table summarizes the organization of the study.

Group	Target Exposure Concentrations (ppm)	Number of Animals/Species/Week		
		1	2	4*
Air-Control	0	7	7	14
EO Control	200	0	0	14
Low	40	0	0	14
Intermediate	1000	0	0	14
High	3000	7	7	14

*7 animals/species for evaluation of adducts and abasic sites,
7 animals/species for evaluation of HPRT mutations.

The exposures began on August 16, 1993 (Study Day 1). Rats and mice were exposed for 6 hours/day for 5 consecutive days/week, as described below. Seven males/species/group were exposed to the following test or control concentrations for the designated time periods: air-only control and 3000 ppm ethylene for 1, 2, and 4 weeks; 40 and 1000 ppm ethylene and 200 ppm EO for 4 weeks. Animals were sacrificed approximately 2 to 4 hours after exposure and blood, brains, lungs, livers, and spleens were taken and processed according to instructions provided by the University of North Carolina. An additional 7 males/species/group were exposed for 4 weeks as previously described and then held for a recovery period of approximately 5 weeks for rats and 8 weeks for mice. Recovery group animals were then shipped live to the University of North Carolina for evaluation.

Administration of Test Substance

Inhalation Chamber Description and Operation

The inhalation chambers (Wahmann Manufacturing Company, Timonium, MD) used for this study were located in Room 137. The chambers, constructed from stainless steel with glass windows for animal observation, were rectangular (60 x 101 x 101 cm) in shape with pyramidal top and bottom. The volume of each chamber was approximately 900 l, and the airflow was approximately 200 l/min (13 air changes/hour) for the 0, 1000, and 3000 ppm ethylene, and the 200 ppm EO target exposure concentrations and 300 l/min (20 air changes/hour) for the 40 ppm ethylene target exposure concentration. A Dwyer Magnehelic® pressure gauge (Dwyer Instruments, Inc., Michigan City, IN) was used to monitor chamber airflow. The theoretically-derived time (t_{99}) required for each chamber to reach 99% of the target concentration was calculated to range from 13.8 to 20.7 minutes.

Vapor Generation

Ethylene vapor was metered by a pressure regulator and a flowmeter though stainless steel tubing into each ethylene exposure chamber. The flowmeters were calibrated prior to the study with a soap buret or mini-Buck Calibrator®

(A. P. Buck, Inc., Orlando, FL). EO vapor was delivered to the exposure chambers using a Linde semi-automatic manifold system with check valves and line regulator in conjunction with calibrated flowmeters. Conditioned supply air was used for dilution of EO vapor. The flowmeters were calibrated prior to the study using a mini-Buck Calibrator® (A. P. Buck, Inc., Orlando, FL).

The oxygen content of the exposure chambers and air-only chamber was measured during preliminary level setting using an MSA® Oxygen Indicator Model 245R (Mine Safety Appliance Company, Pittsburgh, PA). The oxygen content of all chambers was 20.8%.

Chamber Atmosphere Measurements

Each exposure chamber atmosphere was analyzed for ethylene or EO approximately 7 times during each 6-hour exposure by flame ionization gas chromatography. The nominal concentrations of ethylene and EO were estimates calculated from the quantity of ethylene or EO delivered and the chamber airflow rate.

In addition, the EO content of the ethylene exposure chambers was checked weekly using flame ionization gas chromatography. Syringe samples were taken for these EO analyses. After the first exposure, the housing area (Room 155) was also checked for EO in the breathing zones of the ethylene-exposed and the EO-exposed animals using the same method.

The distribution of ethylene and EO vapor within each of the 4 exposure chambers was examined. Each chamber was tested once prior to the initiation of the exposure regimen. A description and results of the chamber distribution are presented in Appendix 1.

Chamber temperature and relative humidity were recorded using a Fisherbrand® dial type thermometer (Fisher Scientific, Pittsburgh, PA) and an Airguide humidity indicator (Airguide Instrument Co., Chicago, IL). Temperature and relative humidity measurements were recorded approximately 2 times each hour of exposure.

Observations and Measurements

In-life Evaluations

All animals were observed individually for signs of toxic effects except during the exposures. During the exposures, observations were recorded on a group basis. Preceding and following each exposure, observations were recorded for animals exhibiting overt clinical signs. At the time of body weight collection and just preceding sacrifice, detailed observations were performed on all animals. Recovery animals were transported to the University of North Carolina by Frame's Animal Transport (Ridley Park, PA) on October 13, 1993 (rats) and November 3, 1993 (mice).

Body weight data were collected for all animals on the morning prior to initiation of the first exposure (denoted as Study Day 1 in the tables) and weekly during the exposure regimen. All animals were weighed weekly during the recovery period.

Special Evaluations

The animals designated for evaluation of DNA and hemoglobin adducts and abasic sites were anesthetized with methoxyflurane. Blood was collected by heart puncture. Packed red cells were prepared, rinsed in isotonic saline, and then frozen. The brain, liver, lungs, and spleen were removed, and each organ was individually wrapped in aluminum foil and frozen in liquid nitrogen. Frozen blood and tissue samples were packed in dry ice and shipped to the University of North Carolina for evaluation.

Recovery Phase

Rats designated for analysis of HPRT mutations were held for approximately 5 weeks after the termination of the exposure regimen. Mice designated for analysis of HPRT mutations were held for approximately 8 weeks after the termination of the exposure regimen. The recovery phase animals were placed into individual compartments in divided boxes and shipped live to the University of North Carolina for evaluation. Divided boxes were provided by Frame's Animal Transport (Ridley Park, PA).

Data Analyses

The data for quantitative continuous variables were intercompared for the 3 exposure groups, EO control group, and the air-only control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated similar variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when necessary, by a separate variance t-test for pairwise comparisons (Dixon, 1990). The t-tests were used when only one group was compared to the control group.

Various models of calculators, computers, and computer programs may have been used to analyze data for this study. Since various models round or truncate numbers differently, values in some tables may differ slightly from those in other tables or from independently calculated data. The integrity of the study and interpretation of the data were unaffected by these differences.

RETENTION OF RECORDS

All raw data, documentation, the protocol and any amendments, and a copy of the final report generated as a result of this study will be retained in the BRRC Archives for at least 10 years.

RESULTS AND DISCUSSION

All references of differences in group mean values in the following text refer to comparisons of statistically significant differences between the exposure group and the control group, unless otherwise noted. Repeated reference to the control and the statistical significance will not be made in order to simplify the text.

Chamber Atmosphere

Detailed results and discussion of the chamber atmosphere measurements are included in Appendix 1.

The means of daily mean ethylene (± standard deviation) chamber atmosphere concentrations were 39.1 (± 0.92), 966 (± 29), and 2995 (± 116) ppm for the target concentrations of 40, 1000, and 3000 ppm, respectively. No daily mean ethylene concentration above the estimated minimum detection limit of 4 ppm was detected in the air-only control chamber atmosphere.

The mean of daily mean EO (± standard deviation) chamber atmosphere concentrations was 202 (± 7.0) ppm for the EO target concentration of 200 ppm.

The uniformity of ethylene vapor concentration in each of 3 exposure chambers and the uniformity of EO vapor in 1 exposure chamber were examined. Each chamber was tested once prior to the start of the exposure regimen. Concentrations were measured at 5 positions for each individual distribution test. In each test, these concentrations were representative of a uniform vapor distribution as indicated by low coefficient of variation (CV) values.

Necropsy Observations

Individual anatomic pathology data are included in Appendix 4. There were no significant findings that could be attributed to ethylene or EO exposure.

Clinical Observations

Summaries of clinical observations are presented in Tables 1 and 2. Individual clinical observations are presented in Appendix 2. There were no mortalities or exposure-related clinical signs observed during this study.

Body Weights

Summaries of body weights and body weight gains are presented in Tables 3 through 6. Individual body weights are presented in Appendix 3. Body weights in EO-exposed rats were significantly lower than in air-only control rats during Study Weeks 2 through 9. Body weight gains were significantly lower in EO-exposed rats throughout the study. There were no significant differences in body weights between air-only control and ethylene- or EO-exposed mice. EO-exposed mice had significantly lower body weight gains at Week 5. There were no consistent body weight trends in ethylene- or EO-exposed mice or in ethylene-exposed rats during the study.

REVIEW AND APPROVAL

Study Director: Jane S. Vergnes, Ph.D. — Date: 2/25/94

Associate Director: James D. Sun, Ph.D. — Date: 2-25-94

Director: John P. Van Miller, Ph.D., DABT — Date: 2-25-94

KEY PERSONNEL

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Additional personnel are listed in the raw data.

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TABLE 1
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS
SUMMARY OF CLINICAL OBSERVATIONS[a]

CATEGORY / FINDING (LOCATION)	GRADE[b]	MALES TARGET CONCENTRATION (PPM) 0 (DAYS)[c]	40 (DAYS)	1000 (DAYS)	3000 (DAYS)	200 (EO) (DAYS)
DEAD						
SCHEDULED SACRIFICE	P	21(4- 25)	7 (25)	7 (25)	21(4- 25)	7 (25)
SHIPPED OFF SITE	P	7 (58)	7 (58)	7 (58)	7 (58)	7 (58)
BODY						
TRAUMATIZED(TAIL)	P	0	1 (25)	0	0	0
EYES/EARS/NOSE						
OCULAR DISCHARGE(EYE-RIGHT)	P	1(46- 53)	0	0	0	0
SWOLLEN PERIOCULAR TISSUE (EYE-RIGHT)	P	1(32- 58)	0	0	0	0
PERIOCULAR ENCRUSTATION(EYE-RIGHT)	P	1(39- 53)	0	0	0	0
SKIN						
ALOPECIA(LEG-FRONT-BOTH)	P	0	0	0	1(39- 57)	0

[a]Number of animals exhibiting the finding at least once during the study.
[b]Grades: P = present, 1 = mild, 2 = moderate, 3 = severe.
[c]Earliest to latest day a finding of the specified grade was observed.

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TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE
SUMMARY OF CLINICAL OBSERVATIONS[a]

CATEGORY / FINDING (LOCATION)	GRADE[b]	MALES TARGET CONCENTRATION (PPM) 0 (DAYS)[c]	40 (DAYS)	1000 (DAYS)	3000 (DAYS)	200 (EO) (DAYS)
DEAD						
SCHEDULED SACRIFICE	P	21(4- 25)	7 (25)	7 (25)	21(4- 25)	7 (25)
SHIPPED OFF SITE	P	7 (79)	7 (79)	7 (79)	7 (79)	7 (79)
BODY						
SWELLING(GENITAL)	P	0	1(67- 79)	1(57- 79)	0	0
SKIN						
ALOPECIA		2	0	4	2	0
(CHEST)	P	0	0	3(53- 79)	1 (25)	0
(GENITAL)	P	1(74- 79)	0	0	0	0
(HIP-LEFT)	P	1(74- 79)	0	0	0	0
(MULTIPLE AREAS-NOS)	P	0	0	1(53- 79)	1(25- 79)	0
(SIDE-LEFT)	P	0	0	1 (78)	0	0

[a]Number of animals exhibiting the finding at least once during the study.
[b]Grades: P = present, 1 = mild, 2 = moderate, 3 = severe.
[c]Earliest to latest day a finding of the specified grade was observed.

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TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS
SUMMARY OF BODY WEIGHT (GRAMS)

	MALES				
GROUP: PPM	0	40	1000	3000	200 (EO)
WEEK 0					
MEAN	158.9	162.9	157.0	158.6	161.7
S.D.	15.03	12.08	15.66	11.41	14.63
N	28	14	14	28	14
WEEK 1					
MEAN	165.9	172.1	163.2	165.2	160.3
S.D.	15.31	11.47	16.66	11.25	15.10
N	28	14	14	28	14
WEEK 2					
MEAN	183.0	189.4	180.5	180.9	165.2**
S.D.	16.05	13.79	18.07	12.99	15.02
N	21	14	14	21	14
WEEK 3					
MEAN	194.5	204.9	196.0	197.9	169.0**
S.D.	16.87	14.55	19.28	16.52	14.92
N	14	14	14	14	14
WEEK 4					
MEAN	209.6	219.9	212.3	210.3	172.0**
S.D.	18.92	14.04	20.87	18.80	14.58
N	14	14	14	14	14
WEEK 5 (RECOVERY)					
MEAN	215.0	224.8	221.5	222.4	186.4*
S.D.	23.35	10.74	25.03	17.22	21.52
N	7	7	7	7	7
WEEK 6 (RECOVERY)					
MEAN	225.1	234.5	231.5	232.1	192.9**
S.D.	24.19	13.59	25.49	18.66	22.22
N	7	7	7	7	7
WEEK 7 (RECOVERY)					
MEAN	231.9	242.3	237.7	244.3	200.7*
S.D.	24.47	15.31	23.47	21.06	23.20
N	7	7	7	7	7
WEEK 8 (RECOVERY)					
MEAN	242.6	251.5	246.0	254.2	212.7*
S.D.	25.75	14.84	25.19	22.53	27.90
N	7	7	7	7	7
WEEK 9 (RECOVERY)					
MEAN	250.4	258.7	254.4	262.4	218.3*
S.D.	26.09	17.39	25.43	23.17	29.50
N	7	7	7	7	7

* Significantly different from control group ($p < .05$)
** Significantly different from control group ($p < .01$)

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS
SUMMARY OF BODY WEIGHT GAIN (GRAMS)

MALES

GROUP: PPM	0	40	1000	3000	200 (EO)
WEEK 0 TO 1					
MEAN	7.0	9.2*	6.2	6.6	-1.4**
S.D.	2.49	2.67	2.53	3.36	3.10
N	28	14	14	28	14
WEEK 0 TO 2					
MEAN	22.6	26.4*	23.5	21.4	3.5**
S.D.	4.56	4.50	5.30	7.21	3.28
N	21	14	14	21	14
WEEK 0 TO 3					
MEAN	37.0	42.0	39.0	35.2	7.3**
S.D.	8.35	6.71	7.73	11.98	5.98
N	14	14	14	14	14
WEEK 0 TO 4					
MEAN	52.0	57.0	55.3	47.5	10.3**
S.D.	11.05	6.39	10.27	14.89	7.71
N	14	14	14	14	14
WEEK 0 TO 5 (RECOVERY)					
MEAN	60.6	63.5	65.5	59.5	25.6**
S.D.	12.90	7.53	12.88	15.59	11.52
N	7	7	7	7	7
WEEK 0 TO 6 (RECOVERY)					
MEAN	70.7	73.2	75.5	69.2	32.2**
S.D.	14.30	10.90	13.41	17.02	10.95
N	7	7	7	7	7
WEEK 0 TO 7 (RECOVERY)					
MEAN	77.5	80.9	81.7	81.4	40.0**
S.D.	14.47	13.40	11.91	19.50	12.11
N	7	7	7	7	7
WEEK 0 TO 8 (RECOVERY)					
MEAN	88.2	90.2	90.0	91.3	52.0**
S.D.	14.82	13.97	15.04	20.86	16.51
N	7	7	7	7	7
WEEK 0 TO 9 (RECOVERY)					
MEAN	96.0	97.4	98.4	99.6	57.6**
S.D.	15.87	15.50	14.16	21.83	17.98
N	7	7	7	7	7

* Significantly different from control group ($p < .05$)
** Significantly different from control group ($p < .01$)

TABLE 5
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE
SUMMARY OF BODY WEIGHT (GRAMS)

MALES

GROUP: PPM	0	40	1000	3000	200 (EO)
WEEK 0					
MEAN	25.5	24.9	25.3	25.3	25.4
S.D.	1.11	1.72	1.38	1.35	1.35
N	28	14	14	28	14
WEEK 1					
MEAN	26.2	25.4	26.2	25.9	26.4
S.D.	1.16	1.87	1.32	1.42	1.29
N	28	14	14	28	14
WEEK 2					
MEAN	27.0	26.4	27.1	26.3	27.2
S.D.	1.80	1.99	1.56	1.40	1.27
N	21	14	14	21	14
WEEK 3					
MEAN	28.2	27.1	27.9	26.6	28.1
S.D.	1.69	2.11	1.73	1.57	1.45
N	14	14	14	14	14
WEEK 4					
MEAN	29.1	27.7	28.7	27.5	28.2
S.D.	1.92	2.17	1.94	1.68	1.74
N	14	14	14	14	14
WEEK 5 (RECOVERY)					
MEAN	28.8	27.2	28.5	27.9	26.8
S.D.	1.40	1.75	1.53	1.53	1.43
N	7	7	7	7	7
WEEK 6 (RECOVERY)					
MEAN	29.3	27.6	29.3	28.1	28.5
S.D.	1.71	1.69	1.78	1.39	1.40
N	7	7	7	7	7
WEEK 7 (RECOVERY)					
MEAN	29.4	27.8	29.6	28.2	29.1
S.D.	1.93	1.78	1.70	1.42	1.66
N	7	7	7	7	7
WEEK 8 (RECOVERY)					
MEAN	30.7	28.8	30.4	28.8	29.6
S.D.	1.68	2.39	1.80	1.36	2.08
N	7	7	7	7	7
WEEK 9 (RECOVERY)					
MEAN	30.9	28.7	31.3	29.3	29.9
S.D.	2.05	2.62	2.30	0.87	2.01
N	7	7	7	7	7
WEEK 10 (RECOVERY)					
MEAN	31.1	29.1	31.9	30.0	30.4
S.D.	2.17	2.40	2.60	0.89	1.72
N	7	7	7	7	7
WEEK 11 (RECOVERY)					
MEAN	30.8	28.9	31.7	30.0	31.2
S.D.	2.28	2.66	1.95	0.86	2.01
N	7	7	7	7	7
WEEK 12 (RECOVERY)					
MEAN	31.1	29.0	31.8	30.2	30.6
S.D.	2.23	2.58	1.72	0.67	1.95
N	7	7	7	7	7

None significantly different from control group

TABLE 6
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE
SUMMARY OF BODY WEIGHT GAIN (GRAMS)

MALES

GROUP: PPM	0	40	1000	3000	200 (EO)
WEEK 0 TO 1					
MEAN	0.8	0.4	0.8	0.6	1.0
S.D.	0.61	0.89	0.88	0.77	0.58
N	28	14	14	28	14
WEEK 0 TO 2					
MEAN	1.7	1.4	1.8	1.1	1.8
S.D.	1.13	1.07	1.00	0.88	0.83
N	21	14	14	21	14
WEEK 0 TO 3					
MEAN	2.4	2.2	2.6	1.5	2.7
S.D.	1.22	1.13	1.29	0.93	1.20
N	14	14	14	14	14
WEEK 0 TO 4					
MEAN	3.4	2.8	3.3	2.5	2.8
S.D.	1.47	1.16	1.53	1.07	1.54
N	14	14	14	14	14
WEEK 0 TO 5 (RECOVERY)					
MEAN	3.0	3.4	3.5	3.4	1.8*
S.D.	1.03	0.68	1.17	1.12	0.95
N	7	7	7	7	7
WEEK 0 TO 6 (RECOVERY)					
MEAN	3.5	3.7	4.3	3.6	3.5
S.D.	1.12	0.57	0.84	1.02	1.06
N	7	7	7	7	7
WEEK 0 TO 7 (RECOVERY)					
MEAN	3.6	4.0	4.7	3.7	4.1
S.D.	1.26	0.52	0.95	1.36	1.25
N	7	7	7	7	7
WEEK 0 TO 8 (RECOVERY)					
MEAN	4.9	4.9	5.4	4.2	4.5
S.D.	0.98	1.22	1.30	1.12	1.24
N	7	7	7	7	7
WEEK 0 TO 9 (RECOVERY)					
MEAN	5.1	4.9	6.3	4.8	4.9
S.D.	1.50	1.33	1.43	0.90	1.25
N	7	7	7	7	7
WEEK 0 TO 10 (RECOVERY)					
MEAN	5.3	5.2	7.0	5.5	5.4
S.D.	1.56	1.21	1.62	1.18	1.08
N	7	7	7	7	7
WEEK 0 TO 11 (RECOVERY)					
MEAN	5.0	5.0	6.7	5.4	6.2
S.D.	1.75	1.49	1.56	1.35	1.48
N	7	7	7	7	7
WEEK 0 TO 12 (RECOVERY)					
MEAN	5.2	5.2	6.8	5.7	5.6
S.D.	1.82	1.50	1.41	1.26	1.53
N	7	7	7	7	7

* Significantly different from control group ($p < .05$)

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

QUALITY ASSURANCE UNIT INSPECTION SUMMARY

Inspection Date(s)	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
07-30-93 to 08-02-93	PROTOCOL	08-02-93	08-02-93
08-06-93	EVENT-ANIMAL RECEIPT	08-06-93	09-07-93
08-16-93	EVENT-EXPOSURE	08-16-93	09-07-93
08-20-93	EVENT-SACRIFICE	09-03-93	02-25-94
11-04-93 to 11-11-93	ANALYTICAL CHEMISTRY DATA, REPORT	11-11-93	02-25-94
11-10-93 to 11-11-93	RAW DATA, REPORT	11-12-93	02-25-94
02-25-94	ARCHIVES	02-25-94	02-25-94

Linda J. Calisti, Manager — 2/25/94 Date
Good Laboratory Practices/Quality Assurance

ARCH_DOC\93N1311\93N1311.QAU

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

Chamber Atmosphere Report

(54 Pages)

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SUMMARY

The concentration of ethylene and ethylene oxide vapor in the exposure chamber atmospheres was monitored throughout the 20 days of exposure by flame ionization gas chromatography.

The means of daily mean ethylene (± standard deviation) chamber atmosphere concentrations were 39.1 (± 0.92), 966 (± 29), and 2995 (± 116) ppm, for target concentrations of 40, 1000, and 3000 ppm, respectively. Ethylene was not detected in the air-only control chamber atmosphere. No ethylene was detected (estimated minimum detection limit of 4 ppm) in the control chamber atmosphere during the study.

The mean of daily mean ethylene oxide (± standard deviation) chamber atmosphere concentrations was 202 (± 7.0) ppm for the ethylene oxide target concentration of 200 ppm.

The ethylene test substance and the ethylene oxide control substance were analyzed before and after the exposure regimen. The ethylene remained 99.99% pure throughout the exposure regimen. The prestudy analysis indicated that the ethylene oxide-nitrogen mixture was approximately 7600 ppm ethylene oxide.

The uniformity of ethylene vapor concentration in each of 3 exposure chambers and the uniformity of ethylene oxide vapor in the one exposure chamber were examined. Each chamber was tested once prior to the start of the exposure regimen. Concentrations were measured at 5 positions for each individual distribution test. In each test, these concentrations were representative of a uniform vapor distribution as indicated by a low coefficient of variation (CV) value. CV values of less than 4% were found for each of the 4 distribution tests. Furthermore, the results indicate that the analytical sampling position for each chamber was properly located within the chamber, such that sampling results were representative of ethylene or ethylene oxide concentrations in the breathing zones of the animals.

MATERIALS AND METHODS

Test Substance

Two cylinders, each containing 30 pounds of ethylene, CAS No. 74-85-1, Lot E28-0001-A4, BRRC No. 56-319 A and B, were received from Matheson Gas Products (Twinsburg, OH) by way of Union Carbide Corporation (S. Charleston, WV) on July 23, 1993. Only 1 cylinder, 56-319A, was used for the study. The chemical and physical properties of the test substance are described in Table 1. The compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. A summary of this report is presented in Table 2; the entire report is presented as Attachment 1. The prestudy and poststudy compositional analyses indicated that the test substance was 99.99% pure and had remained stable for the duration of the exposure regimen.

Control Substance

Fifty preanalyzed gas cylinders, each containing 143 cubic feet of ethylene oxide in nitrogen, Cylinder Lot No. 091402, BRRC Sample No. 55-143 A through XX, were received from Scott Medical Products (Plumsteadville, PA) on May 21, 1992. Cylinders 55-143 OO, PP, QQ, RR, SS, TT, UU, VV, WW, MM, NN, and II were used during the study either for preliminary or animal exposures. The compositional analyses and the ethylene oxide concentration were obtained from cylinder samples taken both before and after the exposure regimen. The compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. These analyses indicated that the positive control substance was approximately 7600 ppm ethylene oxide in nitrogen and that the ethylene oxide component was over 99.9% pure. A summary of the analyses is presented in Table 3; the entire report is presented as Attachment 2.

Test Substance and Control Substance Generation

The methods used to generate ethylene and ethylene oxide in the exposure chambers are described in the text of the main report.

Chamber Distribution Setup

The uniformity of ethylene vapor concentration in each of 3 exposure chambers and the uniformity of ethylene oxide vapor concentration in the 1 exposure chamber were examined. Each chamber was tested once prior to the start of the exposure regimen. Concentrations were measured at 5 positions for each individual distribution test. The distribution tests simulated actual animal exposures, including the use of similar animal cages, cage carriers with collection trays, and airflow rates. No animals were present in the exposure chambers. The positions of the sampling probes within the chambers are described in Table 11. One of these positions was the analytical probe position. The analytical sampling for ethylene oxide consisted of 2 cycles, each cycle requiring approximately 30 minutes to complete. The analytical cycle sampling for ethylene required approximately 15 minutes to complete. Since the chamber concentrations may vary slightly with time, the data from the 2 cycles (Table 12) were averaged to eliminate time-dependent concentration variations. Also, the sampling occurred after the equilibration of the chamber concentration (t_{99}) had taken place.

Analytical Instrumentation

A Perkin-Elmer Sigma 2000 gas chromatograph (GC) equipped with a flame ionization detector was used to analyze the ethylene or ethylene oxide exposure chamber atmospheres. The GC operating conditions are presented in Table 4. A Spectra-Physics ChromJet Integrator provided a record of the chromatograms and chromatographic analyses as well as peak integration. The data were captured using an IBM PS/2 Computer with Spectra-Physics Chromstation/2 software. In-house software was used to compute daily statistics and also to provide an alarm system which monitored chamber concentrations.

In addition, a second Sigma 2000 gas chromatograph equipped with a flame ionization detector was used to check the ethylene exposure chambers for ethylene oxide at concentrations as low as 1 ppm. Syringe samples were taken for these ethylene oxide analyses. The GC operating conditions are presented in Table 5.

Calibration

Calibration of the gas chromatograph was achieved by injecting gas standards, which were prepared by syringe injection of ethylene or ethylene oxide into Tedlar™ gas bags containing air. These standards were prepared using the mathematical relationship:

$$V_g = C \times V_h \times 10^{-6} \times 100/\%P$$

where:

V_g = required volume of ethylene or ethylene oxide gas standard, in milliliters
C = desired calibration concentration, in ppm
V_h = volume of gas bag, in liters
%P = percent purity = % ethylene or ethylene oxide in standard

Calibration curves (Figures 1, 2, and 3) were constructed by plotting peak areas versus the gas standard concentrations. The calibrations were checked once each week during the exposure regimen.

RESULTS AND DISCUSSION

Chamber Atmosphere Analysis

Each exposure chamber atmosphere was analyzed for ethylene or ethylene oxide approximately 7 times during each of the 20, 6-hour exposures by flame ionization gas chromatography. The daily mean analytical concentrations are listed in Tables 6 through 10.

The means of daily mean ethylene chamber atmosphere concentrations (± standard deviations) were 39.1 (± 0.92), 966 (± 29), and 2995 (± 116) ppm, for target concentrations of 40, 1000, and 3000 ppm, respectively. No ethylene was detected (estimated minimum detection limit of 4 ppm) in the control chamber atmosphere during the study.

The mean of daily mean ethylene oxide chamber atmosphere concentrations (± standard deviation) was 202 (± 7.0) for the target concentration of 200 ppm.

In addition, the ethylene oxide content of the ethylene exposure chambers was checked weekly using an analysis for ethylene oxide which had a minimum detection limit of 1 ppm. No ethylene oxide was detected. The housing area was also checked for ethylene oxide after the first exposure using this sensitive method; however, ethylene oxide was not detected either in the breathing zones of the ethylene oxide-exposed animals or the ethylene-exposed animals.

Analytical/Nominal Concentration Ratio

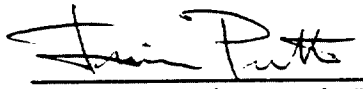
The daily analytical/nominal (A/NOM) ethylene or ethylene oxide concentration ratios are given in Tables 7 through 10, the nominal concentration being an estimate calculated from the quantity of ethylene or ethylene oxide delivered and the chamber airflow rate. The overall mean A/NOM concentration ratios were 0.93, 0.88, and 0.91, for ethylene target concentrations of 40, 1000, and 3000 ppm, respectively. The overall mean ethylene oxide A/NOM concentration ratio was 0.87 for the ethylene oxide target concentration of 200 ppm.

Temperature and Relative Humidity

The daily mean temperature and relative humidity values for the exposure chambers are presented in Tables 6 through 10. The means of daily mean temperature values were 23, 22, 22, and 22°C, for ethylene target concentrations of 0, 40, 1000, and 3000 ppm, respectively. The mean of daily mean temperature values was 21°C, for the ethylene oxide target concentration of 200 ppm. The means of daily mean relative humidity values were 56, 48, 48, and 50%, for the ethylene target concentrations of 0, 40, 1000, and 3000 ppm, respectively. The mean of daily mean relative humidity values for the ethylene oxide target concentration of 200 ppm was 45%.

Chamber Distribution

The uniformity of ethylene and ethylene oxide vapor concentration distribution in 4 exposure chambers was examined. The results and the statistical summaries are presented in Table 12. In each test, the concentrations were representative of a uniform vapor distribution as indicated by low coefficient of variation (CV) values. CV values of 2.6, 3.4, 2.1, and 3.9% were obtained for exposure chambers 37-7 (40 ppm ethylene), 37-8 (1000 ppm ethylene), 37-9 (3000 ppm ethylene), and 37-10 (200 ppm ethylene oxide), respectively. Furthermore, the results indicate that the analytical sampling position for each chamber was properly located within the chamber such that sampling results were representative of ethylene concentrations in the breathing zones of the animals.

Analytical Chemist:  2-25-94

Irvin M. Pritts, Ph.D. Date

INHAL\ANALYT\AENCP4V
FEBRUARY 24, 1994

TABLE 1
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHEMICAL AND PHYSICAL PROPERTIES[1]

Synonyms:	Ethene, acetene
Molecular Weight:	28.05
Molecular Formula:	CH_2CH_2
Appearance and Odor:	Colorless compressed gas with a sweet odor and taste
Boiling Point, 760 mm Hg:	-104°C
Solubility in Water:	22.6%
Vapor density (air =1):	1.0

[1]Material Safety Data Sheet, Matheson Gas Products, Revised 9/12/90.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

ETHYLENE: TEST SUBSTANCE ANALYSIS[1]

Component	Prestudy Area%	Poststudy Area%
Ethylene	99.99	99.99
Ethane	0.0003	0.00405
Acetylene	0.0003	0.00055

[1]The capillary gas chromatographic compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. In addition, gas chromatography-mass spectrometry and infrared spectral data were independently used to confirm the sample's identity.

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

ETHYLENE OXIDE: CONTROL SUBSTANCE ANALYSIS[1]

Component	Prestudy Area%	Poststudy Area%
Ethylene oxide	99.93	99.99
Unknown	0.04	----
Unknown	0.03	----
Cylinder mixture ethylene oxide in N_2 (ppm)	7600	7880

[1]The capillary gas chromatographic compositional analyses were provided by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. In addition, gas chromatography-mass spectrometry and infrared spectral data were independently used to confirm the sample's identity.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

GAS CHROMATOGRAPH OPERATING PARAMETERS: ETHYLENE AND ETHYLENE OXIDE (200 PPM)

Chromatograph:	Perkin-Elmer Sigma 2000
Detector:	Flame Ionization
Column:	Hay Sep D, 80 /100 mesh, 10 ft. x 1/8 in. stainless steel
Column temperature:	150°C
Injector temperature:	Ambient gas sample valve
Detector temperature:	300°C
Carrier flow rate:	25 mL/minute nitrogen
Sample size:	0.1 cc
Retention time:	1.7 minutes Ethylene 5.4 minutes Ethylene Oxide
GC attenuation:	Range = 100
Minimum detection limit:	Approximately 4 ppm ethylene
Integrator attenuation:	32

TABLE 5

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

GAS CHROMATOGRAPH OPERATING PARAMETERS: ETHYLENE OXIDE (1 PPM)

Chromatograph:	Perkin-Elmer Sigma 2000
Detector:	Flame Ionization
Column:	Hay Sep D, 80/100 mesh, 10 ft. x 1/8 in. stainless steel
Column temperature:	165°C
Injector temperature:	120°C
Detector temperature:	350°C
Carrier flow rate:	20 mL/minute nitrogen
Sample size:	0.5 cc syringe
Retention time:	3.9 minutes
GC attenuation:	Range = 1
Minimum detection limit:	1 ppm
Integrator attenuation:	1

TABLE 6

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER ATMOSPHERE DATA

0 PPM CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)
1	23.7	48.5	<MDL
2	22.9	64.8	<MDL
3	24.8	57.9	<MDL
4	25.0	58.9	<MDL
5	23.6	56.2	<MDL
6	23.3	54.2	<MDL
7	23.0	54.1	<MDL
8	24.4	55.8	<MDL
9	21.8	54.6	<MDL
10	22.0	53.1	<MDL
11	22.3	56.2	<MDL
12	23.8	54.9	<MDL
13	21.1	52.7	<MDL
14	21.3	53.0	<MDL
15	21.2	52.7	<MDL
16	23.0	53.7	<MDL
17	20.9	57.8	<MDL
18	21.9	54.2	<MDL
19	22.7	56.2	<MDL
20	23.6	61.9	<MDL
Mean:	22.8	55.6	<MDL
SD:	1.23	3.54	---

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
<MDL = less than the estimated minimum detection limit (4 ppm)

TABLE 7

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER ATMOSPHERE DATA

40 PPM ETHYLENE CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	22.3	40.3	39.6	1.6	41.3	0.96
2	22.0	45.7	39.5	0.7	42.0	0.94
3	21.8	44.2	39.7	1.4	42.0	0.95
4	23.1	46.2	41.5	1.0	42.0	0.99
5	21.0	45.7	39.6	0.9	42.0	0.94
6	21.4	50.7	39.4	1.5	42.0	0.94
7	22.2	44.9	38.4	3.2	42.0	0.91
8	22.4	45.8	39.5	1.8	42.0	0.94
9	21.4	52.2	38.4	4.2	42.0	0.91
10	21.0	48.4	39.6	2.7	42.0	0.94
11	22.6	48.2	38.7	3.9	42.0	0.92
12	23.5	46.9	40.6	1.5	42.0	0.97
13	22.3	45.7	38.2	1.1	42.0	0.91
14	21.3	47.4	39.4	2.0	42.0	0.94
15	23.0	45.2	39.1	1.7	42.0	0.93
16	22.3	51.3	38.1	1.4	42.0	0.91
17	22.0	52.1	38.5	0.9	42.0	0.92
18	21.3	49.3	37.6	1.0	42.0	0.90
19	22.8	47.3	39.2	1.2	42.0	0.93
20	22.0	53.4	38.2	1.5	42.0	0.91
Mean:	22.1	47.5	39.1	----	42.0	0.93
SD:	0.71	3.22	0.92	----	0.16	0.023

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 8

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER ATMOSPHERE DATA
1000 PPM ETHYLENE CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	22.4	42.1	988	44.6	1055	0.94
2	22.0	47.8	978	12.5	1080	0.91
3	22.0	46.5	992	9.2	1080	0.92
4	22.8	48.2	1009	11.0	1080	0.93
5	20.8	47.6	985	12.1	1080	0.91
6	21.2	51.1	1007	36.8	1080	0.93
7	23.0	48.1	953	14.9	1080	0.88
8	22.5	47.1	976	11.9	1080	0.90
9	20.8	49.2	984	13.5	1080	0.91
10	21.0	48.2	963	18.4	1080	0.89
11	22.2	48.7	949	9.4	1080	0.88
12	22.9	49.1	934	14.8	1080	0.86
13	21.7	47.6	945	14.2	1080	0.88
14	21.3	46.6	942	11.6	1080	0.87
15	23.0	43.8	936	24.7	1080	0.87
16	22.0	49.8	900	14.3	1080	0.83
17	20.8	50.6	942	22.9	1171	0.80
18	20.8	47.8	955	11.3	1190	0.80
19	22.4	47.4	1002	19.7	1190	0.84
20	22.0	62.4	984	12.5	1190	0.83
Mean:	21.9	48.5	966	——	1100	0.88
SD:	0.79	3.86	28.7	——	44.3	0.042

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 9
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER ATMOSPHERE DATA
3000 PPM ETHYLENE CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	23.0	44.3	3000	124	3178	0.94
2	23.0	49.8	3108	96	3265	0.95
3	23.1	48.2	3042	33	3265	0.93
4	24.5	49.7	3127	40	3265	0.96
5	21.6	49.6	3102	51	3265	0.95
6	22.7	50.8	3100	110	3265	0.95
7	23.2	49.8	3028	273	3265	0.93
8	21.2	49.2	3013	220	3265	0.92
9	19.5	51.8	3074	55	3265	0.94
10	21.0	53.8	3095	177	3265	0.95
11	23.4	50.2	3052	44	3265	0.93
12	23.4	48.8	2999	62	3265	0.92
13	21.0	48.5	2994	47	3265	0.92
14	20.4	49.5	2973	74	3265	0.91
15	22.8	47.5	2886	79	3265	0.88
16	22.5	49.2	2714	68	3265	0.83
17	20.0	51.6	2799	102	3381	0.83
18	20.0	49.4	2772	28	3465	0.80
19	22.7	50.2	3006	54	3465	0.87
20	19.0	60.5	3011	320	3465	0.87
Mean:	21.9	50.1	2995	----	3296	0.91
SD:	1.54	3.07	116	----	80	0.047

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 10

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER ATMOSPHERE DATA
200 PPM ETHYLENE OXIDE CONTROL CHAMBER

Exposure Day	TEMP (°C)	RH (%)	A (ppm)	±SD	NOM (ppm)	A/NOM
1	22.1	40.2	198	14.5	226	0.88
2	21.0	44.2	202	4.2	232	0.87
3	22.0	43.1	201	3.0	232	0.87
4	22.4	43.8	205	2.6	232	0.88
5	19.9	45.8	204	8.0	232	0.88
6	20.0	48.3	203	3.8	232	0.88
7	21.2	42.1	202	11.0	232	0.87
8	22.5	42.3	207	4.5	232	0.89
9	20.4	48.4	201	3.6	232	0.87
10	20.0	45.4	206	4.7	232	0.89
11	22.2	44.2	203	5.9	232	0.88
12	23.0	40.8	210	10.0	232	0.91
13	21.0	43.3	208	3.0	232	0.90
14	20.1	46.2	196	3.6	232	0.84
15	22.5	41.4	202	2.7	232	0.87
16	21.1	45.4	183	4.5	232	0.79
17	20.1	46.2	192	4.8	232	0.83
18	20.0	45.9	190	2.2	232	0.82
19	21.1	43.9	208	5.0	232	0.90
20	20.0	52.9	211	7.8	232	0.91
Mean:	21.1	44.7	202	—	232	0.87
SD:	1.05	2.97	7.0	—	1.3	0.030

TEMP = temperature (daily mean)
RH = relative humidity (daily mean)
A = analytical concentration (daily mean)
SD = standard deviation
NOM = nominal concentration
A/NOM = analytical concentration/nominal concentration

TABLE 11

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER DESCRIPTION AND CHAMBER PROBE POSITIONS

CHAMBER

Construction: Stainless steel and glass.
Manufacturer: Wahmann Manufacturing Company (Timonium, MD)
Shape: Rectangular with pyramidical top and bottom
Dimensions: Height: 60 cm
Width: 101 cm
Depth: 101 cm

Chamber #37-7 Probe Placement (40 ppm Target)

Probe Number	Location (x:y:z)* Units (cm)				
1 Analytical Probe	51	:	44	:	33
2	13	:	10	:	45
3	84	:	91	:	45
4	86	:	9	:	17
5	16	:	90	:	15

Chamber #37-8 Probe Placement (1000 ppm Target)

Probe Number	Location (x:y:z)* Units (cm)				
1	17	:	11	:	17
2 Analytical Probe	50	:	49	:	37
3	85	:	9	:	44
4	15	:	89	:	44
5	86	:	90	:	15

Chamber #37-9 Probe Placement (3000 ppm Target)

Probe Number	Location (x:y:z)* Units (cm)				
1	12	:	90	:	46
2	88	:	7	:	16
3 Analytical Probe	50	:	43	:	28
4	14	:	10	:	41
5	87	:	88	:	15

*Location described by a three-dimensional coordinate system: x = width coordinate; y = depth coordinate; z = height coordinate. The origin of the coordinate system (0:0:0) is the lower left front corner of the internal chamber.

TABLE 11 (Continued)
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER DESCRIPTION AND CHAMBER PROBE POSITIONS

Chamber #37-10 Probe Placement (200 ppm EO Target)

Probe Number	Location (x:y:z)* Units (cm)		
1	82	86	43
2	83	22	19
3	18	89	19
4 Analytical Probe	47	68	29
5	17	19	48

*Location described by a three-dimensional coordinate system: x = width coordinate; y = depth coordinate; z = height coordinate. The origin of the coordinate system (0:0:0) is the lower left front corner of the internal chamber.

TABLE 12

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

CHAMBER DISTRIBUTION DATA

(Chamber 37-7)

	1	2	3	4	5
	37.1	37.7	37.3	35.7	38.5
	37.2	37.5	36.6	35.5	37.9
Mean:	37.1	37.6	37.0	35.6	38.2
SD:	0.03	0.13	0.49	0.18	0.44
		Grand mean = 37.1	SD = 0.96	% CV = 2.6	

(Chamber 37-8)

	1	2	3	4	5
	959	963	901	929	903
	961	959	887	922	902
Mean:	960	961	894	926	903
SD:	0.8	2.5	10.0	4.9	0.4
		Grand mean = 929	SD = 31.2	% CV = 3.4	

(Chamber 37-9)

	1	2	3	4	5
	2804	2873	2878	2845	2738
	2726	2807	2848	2811	2705
Mean:	2765	2840	2863	2828	2721
SD:	55.5	46.9	21.0	23.6	23.0
		Grand mean = 2803	SD = 58.7	% CV = 2.1	

(Chamber 37-10)

	1	2	3	4	5
	173	195	183	182	185
	181	197	177	179	184
Mean:	177	196	180	180	185
SD:	5.5	1.4	4.8	1.5	0.7
		Grand mean = 184	SD = 7.2	% CV = 3.9	

FIGURE 1. ETHYLENE CALIBRATION CURVE

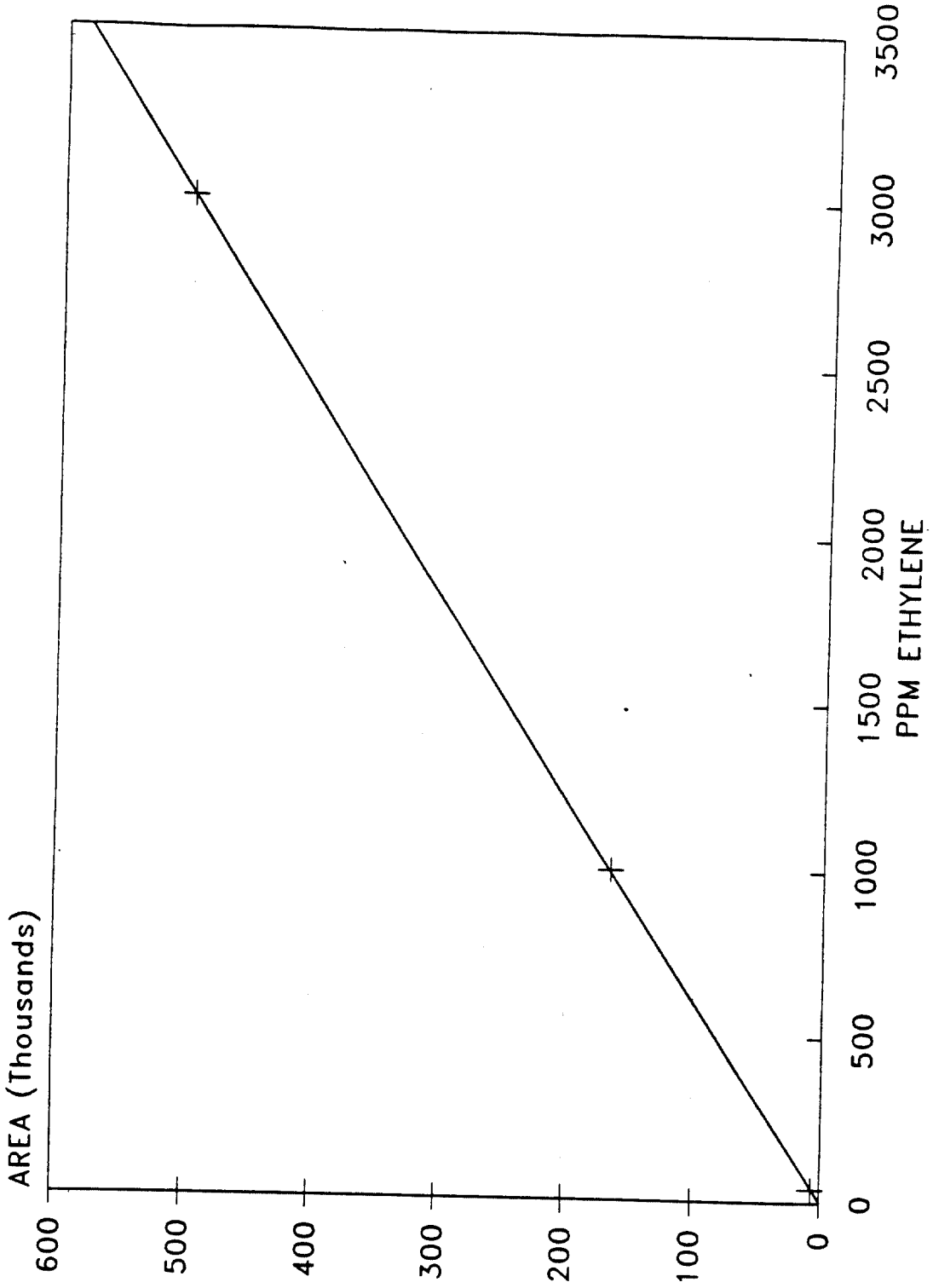


FIGURE 2. ETHYLENE OXIDE CALIBRATION CURVE

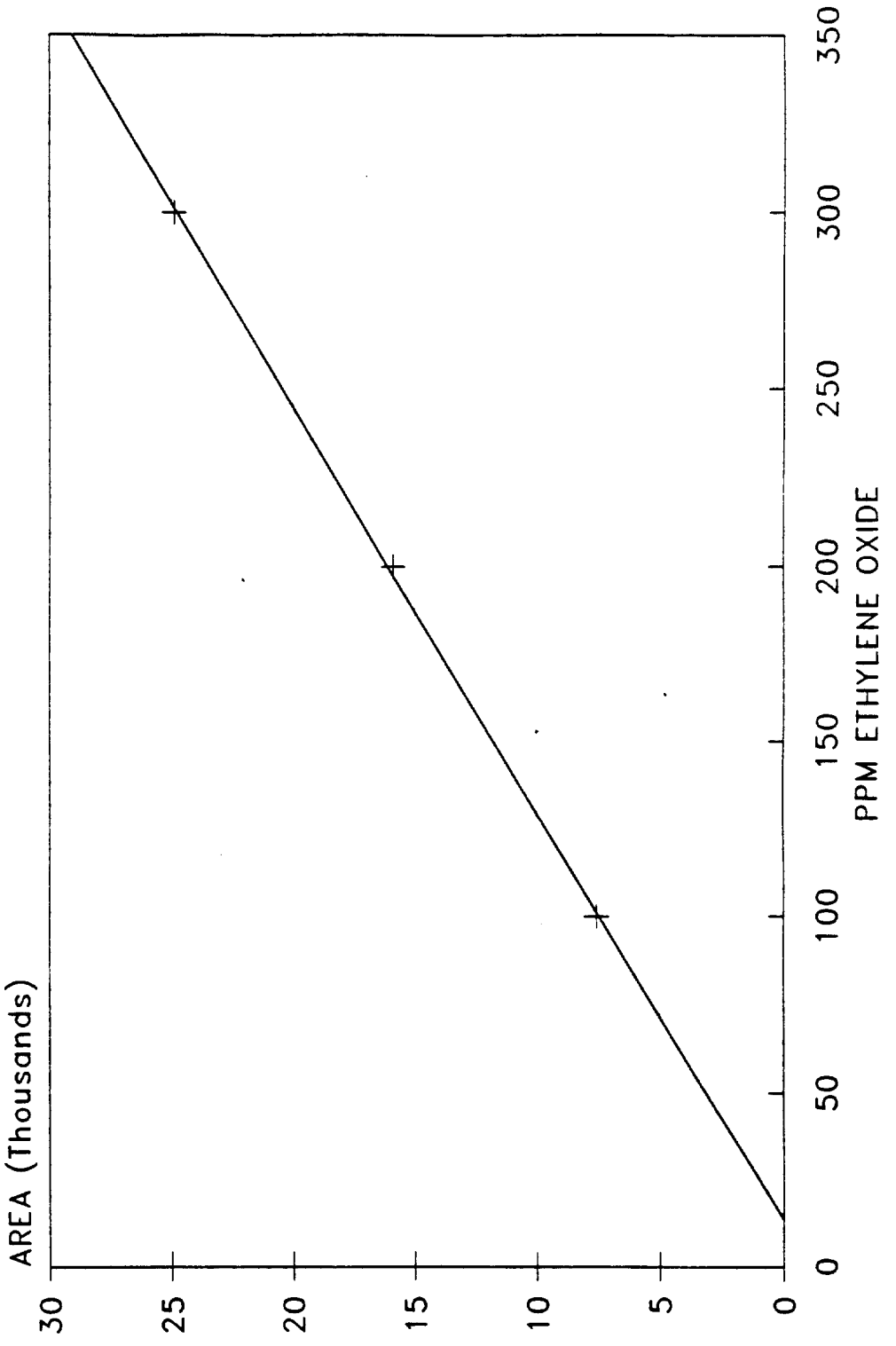
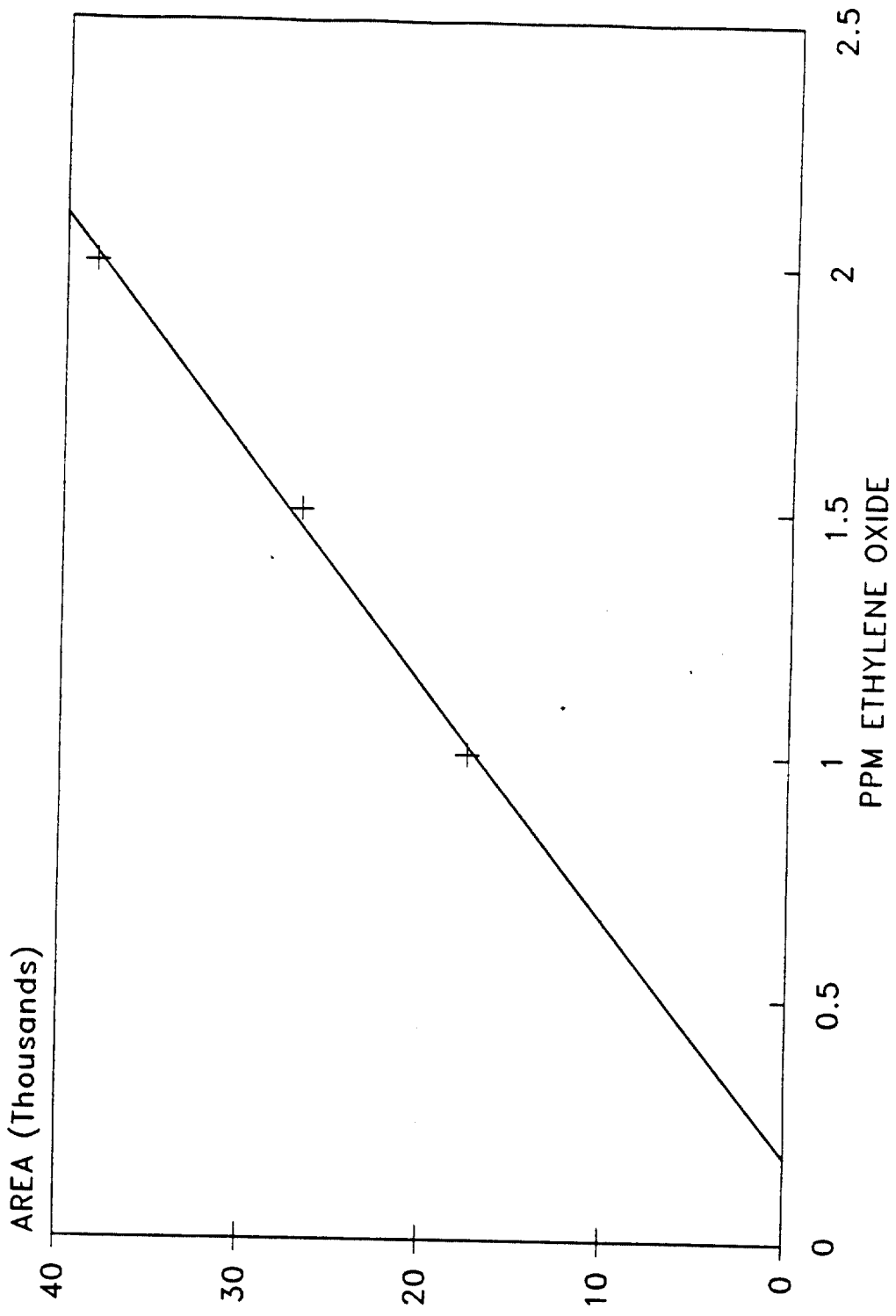


FIGURE 3. ETHYLENE OXIDE CALIBRATION CURVE



Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

Ethylene Test Substance Characterization Report

Union Carbide Corporation

STUDY TITLE

GLP Analysis-Final Report

TEST SUBSTANCE

Ethylene

DATA REQUIREMENT

U.S. FDA, 21 CFR 58
U.S. EPA TSCA, 40 CFR 792
U.S. EPA FIFRA 40 CFR 160

STUDY DIRECTOR

Alexander E. Gabany, Jr.

STUDY COMPLETED ON

November 19, 1993

PERFORMING LABORATORY

Union Carbide Corporation
PO Box 8361
South Charleston, West Virginia 25303

UCC R/D LABORATORY PROJECT ID

Study # 37-AEG-89

SPONSOR COMPANY

Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for Union Carbide Corporation (UCC) Study # 37-AEG-89, Ethylene Study for Chemical Manufacturers Association (CMA), Washington, D.C.

In accordance with UCC's intent that all tests conducted follow good laboratory practices, UCC's study director for the above study confirms that the study was conducted in compliance with the U.S. EPA Section 4 Good Laboratory Practice standards: TSCA 40 CFR 792 and FIFRA 40 CFR 160. All original raw data, records, protocols, and final reports are being retained at UCC&P's South Charleston, WV, Technical Center.

Alexander E. Gabany, Jr. 11/19/93
Study Director Date

PROTOCOL DEVIATION STATEMENT

Protocol Deviation Statement for Union Carbide Corporation (UCC) Study # 37-AEG-89, Ethylene study for Bushy Run Research Center.

In accordance with UCC's intent that all tests conducted by our facility follow good laboratory practices, UCC's study director for the above test confirms that there were no protocol deviations taken during the study. The study was conducted in compliance with the protocol established and signed on 7/19/93 by Alexander E. Gabany, GLP Study Director.

Alexander E. Gabany, Jr. date 4/19/93
Study Director

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

TABLE 3

MICE

INDIVIDUAL BODY WEIGHT (GRAMS)

MALES GROUP: 0 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6727	25.0	25.4	25.4	26.2	26.8	27.5	27.5	27.4	29.0	28.4
6730	24.9	24.6	25.5	26.3	26.4	sacr				
6747	24.8	25.7	26.6	26.7	28.0	27.8	28.1	28.2	29.2	29.4
6750	24.4	26.0	27.7	27.9	29.3	sacr				
6755	23.6	24.1	23.9	sacr						
6762	25.2	26.5	27.8	28.3	29.7	29.2	29.0	28.7	30.0	30.0
6764	26.1	25.4	26.5	27.4	28.8	sacr				
6766	25.3	26.9	28.1	28.4	29.1	sacr				
6767	25.1	25.5	sacr							
6771	24.8	25.7	sacr							
6772	25.7	26.6	27.8	28.4	29.7	28.5	29.5	29.5	30.7	32.6
6781	26.3	26.7	sacr							
6789	26.1	27.1	27.5	27.4	27.5	27.4	28.0	28.0	29.7	29.3
6791	25.3	26.2	26.7	27.2	27.3	sacr				
6806	25.9	26.5	sacr							
6816	24.2	25.6	25.5	sacr						
6817	26.5	28.0	sacr							
6834	23.7	24.5	24.7	sacr						
6837	25.0	26.4	sacr							
6838	24.3	24.8	24.7	sacr						
6849	27.6	29.0	31.0	31.9	32.8	sacr				
6857	26.0	26.5	27.5	sacr						
6863	24.4	25.3	26.6	sacr						
6868	26.7	28.0	30.4	31.4	32.6	31.1	32.0	32.2	33.2	33.4
6870	27.2	26.9	27.4	28.2	29.6	30.0	31.3	32.1	32.8	32.9
6876	26.1	27.3	29.4	29.0	29.9	sacr				
6881	28.1	27.9	sacr							
6882	24.9	25.6	26.0	sacr						
MEAN	25.5	26.2	27.0	28.2	29.1	28.8	29.3	29.4	30.7	30.9
S.D.	1.11	1.16	1.80	1.69	1.92	1.40	1.71	1.93	1.68	2.05
N	28	28	21	14	14	7	7	7	7	7

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TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 3000 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6557	162.0	174.1	sacr							
6558	151.0	161.7	179.8	sacr						
6562	150.6	155.2	170.9	sacr						
6564	162.2	164.5	170.8	175.0	180.7	191.2	198.1	205.8	211.8	218.2
6565	167.4	168.9	175.3	186.3	195.6	sacr				
6567	154.6	162.8	173.3	188.3	199.8	207.6	217.6	228.5	239.2	246.7
6573	166.9	172.9	189.7	208.8	214.4	230.6	242.3	254.6	265.6	274.4
6577	173.9	175.3	189.6	sacr						
6593	162.6	170.4	sacr							
6600	158.2	166.2	176.6	sacr						
6610	135.0	140.2	150.8	159.7	170.2	sacr				
6612	148.8	159.1	179.8	194.9	210.5	220.7	228.2	240.1	250.4	260.9
6622	166.3	174.3	189.5	204.3	215.2	sacr				
6635	175.5	185.7	202.7	217.8	231.0	sacr				
6636	158.2	169.9	192.7	213.6	233.5	sacr				
6642	169.4	174.9	sacr							
6647	167.0	169.8	183.1	195.5	209.6	sacr				
6650	179.6	184.2	198.4	210.8	224.4	235.5	243.9	255.2	265.7	273.5
6654	156.1	155.6	171.1	sacr						
6656	141.1	150.4	sacr							
6662	168.6	172.8	186.7	196.2	209.1	sacr				
6668	156.1	160.4	sacr							
6673	143.8	151.9	171.3	sacr						
6685	148.6	153.5	sacr							
6686	159.7	171.1	190.8	204.9	216.3	234.5	247.2	262.3	270.3	279.7
6687	137.2	144.4	159.6	sacr						
6688	152.8	159.1	sacr							
6697	168.5	177.0	196.0	215.3	233.5	236.5	247.6	263.4	276.2	283.7
MEAN	158.6	165.2	180.9	197.9	210.3	222.4	232.1	244.3	254.2	262.4
S.D.	11.41	11.25	12.99	16.52	18.80	17.22	18.66	21.06	22.53	23.17
N	28	28	21	14	14	7	7	7	7	7

Ethylene

Ethylene
Study # 37-AEG-89

ABSTRACT Ethylene, was analyzed to provide analytical data as part of the toxicity study at Bushy Run Research Center. The analyses were performed in compliance with Good Laboratory Practice standards (GLP) to meet EPA Section 4 Test Rule requirements and FDA, 21 CFR 58. Gas chromatography-mass spectrometry (GC/MS) and infrared spectroscopy (IR) techniques were independently used to confirm the sample's identity. Purity, measured by capillary GC, is ≈99.99% for both the pre-study and the post-study sample. Ethylene oxide (EO) was not found by GC/MS analysis at a concentration of ≥ 10 ppb based upon the response of a 1 ppm EO in ethylene standard prepared by UCC's Gas Mixing Skill Center. The pre-study sample was received from Matheson Gas Products, Twinsburg, Ohio and subsequently sent to Bushy Run Research Center. The post-study sample was received from Bushy Run Research Center. All raw data, documentation, records, protocols, and final reports are being retained.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 40 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6559	164.1	178.5	198.1	215.2	225.4	sacr				
6570	158.7	168.6	186.9	199.6	211.0	224.1	234.2	240.8	252.0	256.1
6575	164.7	174.6	196.4	214.9	233.9	sacr				
6580	179.4	190.3	214.2	232.4	239.9	sacr				
6585	170.2	177.1	192.0	202.3	217.1	225.9	233.3	246.3	253.3	263.1
6590	166.7	175.7	192.6	213.3	228.5	241.3	257.6	264.5	274.4	283.1
6602	136.9	148.5	164.0	177.5	192.1	sacr				
6617	159.3	171.0	183.9	203.7	215.4	229.1	242.0	257.6	265.8	278.3
6644	177.0	184.4	200.2	208.7	222.1	230.6	236.7	237.6	244.7	251.9
6674	151.5	159.1	173.6	188.3	204.4	211.6	222.0	226.5	236.2	242.2
6675	145.9	154.4	166.8	182.3	201.6	210.9	215.7	222.6	233.9	236.5
6690	160.4	170.9	188.2	204.4	219.9	sacr				
6692	170.6	176.1	193.1	212.3	231.9	sacr				
6713	175.9	180.3	201.3	213.7	236.0	sacr				
MEAN	162.9	172.1	189.4	204.9	219.9	224.8	234.5	242.3	251.5	258.7
S.D.	12.08	11.47	13.79	14.55	14.04	10.74	13.59	15.31	14.84	17.39
N	14	14	14	14	14	7	7	7	7	7

chromatogram for the sample (scanned from m/z 10 to m/z 310 EI mode). This chromatogram is annotated with identifications based on the components' EI spectrum.

The ethylene oxide (EO) detection limit was determined using a 1 ppm EO in ethylene standard, prepared in UCC's Gas Mixing Skill Center, for reference. Aliquots (1 cc) of the standard were injected into the GC/MS system; the GC peak area for EO was determined by mass spectrometric analysis by using extracted ion current profiles for EO. Based on the area counts recorded for the EO peak (204,465) it was determined that the detection limit for EO in ethylene was ≥5 ppb.

Capillary GC A HP 5890 gas chromatograph equipped with a flame ionization detector was used to analyze the sample. Aliquots (1 mL) were manually injected with a 100:1 split ratio onto an Al_2O_3/KCl capillary column held isothermally at 100°C for 20 minutes (Figures 3 and 4 show chromatograms of the pre-study and post-study samples, respectively). The average of triplicate analyses taken from the Al_2O_3/KCl column data are given below (normalized chromatogram area percent).

Component name	37-AEG-92	37-AEG-92R
Ethane	0.0003	0.00405
Ethylene	99.99	99.99
Acetylene	0.0003	0.00055

CONCLUSION IR spectral data and mass spectral fragmentation data from the UCC Skill Centers show that this sample is ethylene. These independent methods satisfy the analytical requirements for structural identification, as defined in the sample protocol. Sample purity, measured by capillary GC, is ≈ 99.99% for both the pre-study and the post-study sample. Ethylene oxide (EO) was not found by GC/MS analysis at a concentration of ≥10 ppb based on the response of a 1 ppm EO in ethylene standard.

ARCHIVES All raw data, records, protocols, and final reports are being retained at UCC's South Charleston, WV, Technical Center as follows:

- raw data from GC, IR and GC/MS studies are in 770-361, 770-223, and 770-123, respectively;
- protocols, notebook and other records are to be kept in the GLP archives.

ACKNOWLEDGEMENTS We would like to thank Trudy Barker for sample handling, collecting the GC data, and preparing the bulk of the report, and Jim Richardson for collecting the IR data.

NOTEBOOK REFERENCE: 37-AEG-89 and related pages

TABLE 1

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

INDIVIDUAL BODY WEIGHT DATA

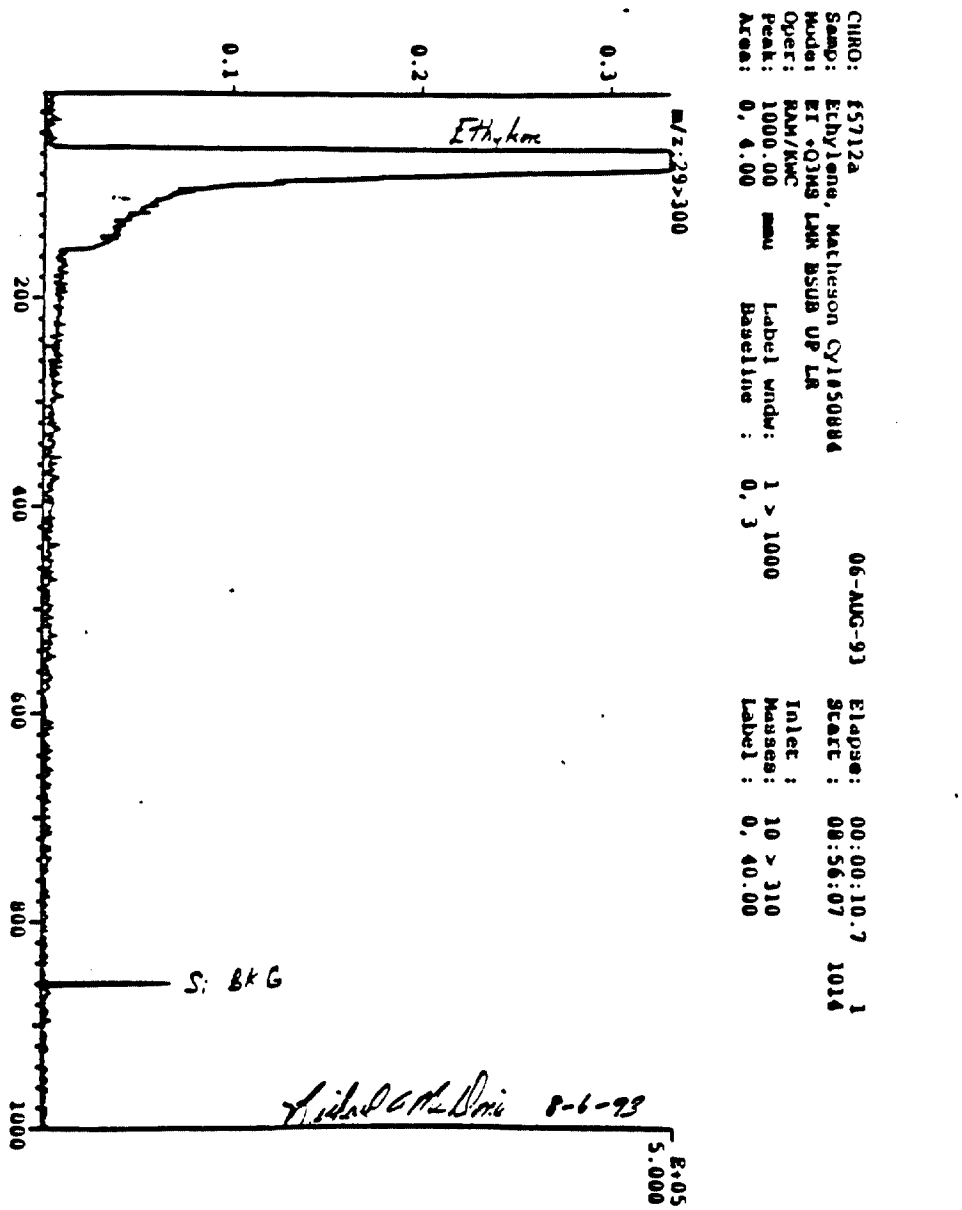
ABBREVIATIONS

The following is a list of abbreviations or words that may appear in this appendix.

dead = indicates that the animal died prior to the period in which this word appears.

sacr = indicates that the animal was a scheduled sacrifice prior to the period in which this abbreviation appears.

Figure 2 — Capillary GC/MS RIC of 37-AEG-92 (Ethylene)

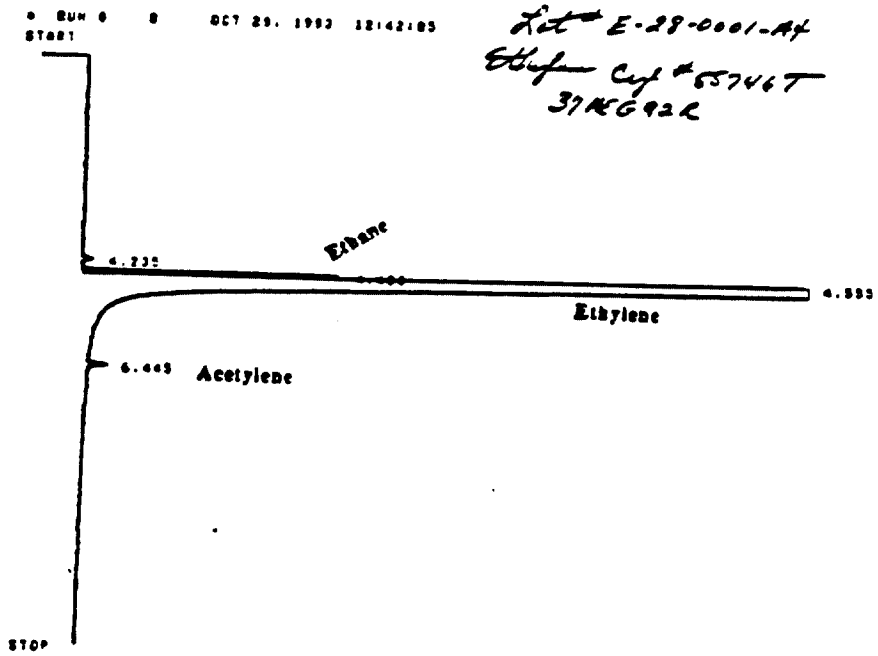


Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

Individual Body Weight Data

(15 Pages)

Figure.4— Capillary Gas Chromatogram of 37-AEG-92R (Ethylene)



RUN# 8 OCT 29, 1993 12:42:05

AREA%

RT	AREA	TYPE	WIDTH	AREA%
4.235	168	PP	.074	.00047
4.483	1522	PP	.033	.00444
4.933	34278208	PB	.071	99.99448
6.443	232	PP	.070	.00074

TOTAL AREA=3.4298E+07
RUN FACTOR=1.0000E+00

JC Barker 10/29/93

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
3000 PPM					
	6743	SKIN	1	25	ALOPECIA (CHS 1)
	6744	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6749	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6752	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6758	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6769	NORMAL	4	0- 18	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	11	25- 79	ALOPECIA (MUL 11)
	6775	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6777	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6782	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6802	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6804	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6805	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6807	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6808	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6815	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6818	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6823	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6835	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6842	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6843	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6856	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6869	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS

Quality Assurance Unit Study Inspection Summary

Test Substance: ETHYLENE

Study No.: 37-AEG-89

Study Director: A.E. Gabany, B.S.

The Quality Assurance Unit of the Union Carbide Technical Center conducted the inspections listed below and reported the results to the study director and management on the date indicated. It is the practice of this Quality Assurance Unit to report the results to both the study director and management.

Inspection Date	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
Feb. 10, 1992	Laboratory Compliance Review*	Feb. 10, 1992	May, 1992
July 19, 1993	Protocol Compliance Review	July 19, 1993	July 19, 1993
Nov. 22, 1993	Final Report Compliance Review	Nov. 22, 1993	Nov. 22, 1993

*The process of doing the GLP characterization studies is audited periodically to assure these studies comply with GLP requirements. The QA unit is exempted from performing in-life study inspections for studies designed to determine physical and chemical characteristics of a test substance as described in 40 CFR 792.135.

11/22/93

Denise L. Johnson, QAU Representative (Date)
Good Laboratory Practices/Quality Assurance

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
0 PPM					
	6849	FATE	1	25	SCHEDULED SACRIFICE
	6857	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6863	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6868	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6870	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6876	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6881	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6882	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
40 PPM					
	6724	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6734	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6742	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6768	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6794	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6810	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6822	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6824	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6832	NORMAL	12	0- 74	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		BODY	3	67- 79	SWELLING (GEN 3)
	6839	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6847	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6854	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6860	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE

Union Carbide Corporation

STUDY TITLE

GLP Analysis-Final Report

TEST SUBSTANCE

Ethylene Oxide (8000 ppm in nitrogen)

DATA REQUIREMENT

U.S. FDA, 21 CFR 58
U.S. EPA TSCA, 40 CFR 792
U.S. EPA FIFRA 40 CFR 160

STUDY DIRECTOR

Alexander E. Gabany, Jr.

STUDY COMPLETED ON

November 19, 1993

PERFORMING LABORATORY

Union Carbide Corporation
PO Box 8361
South Charleston, West Virginia 25303

UCC R/D LABORATORY PROJECT ID

Study # 37-AEG-88

SPONSOR COMPANY

Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

STUDY COMPLIANCE STATEMENT

Study Compliance Statement for Union Carbide Corporation (UCC) Study # 37-AEG-88, Ethylene Oxide (8000 ppm in nitrogen) Study for Chemical Manufacturers Association (CMA), Washington, D.C.

In accordance with UCC's intent that all tests conducted follow good laboratory practices, UCC's study director for the above study confirms that the study was conducted in compliance with the U.S. EPA Section 4 Good Laboratory Practice standards: TSCA 40 CFR 792 and FIFRA 40 CFR 160. All original raw data, records, protocols, and final reports are being retained at UCC&P's South Charleston, WV, Technical Center.

Alexander E. Gabany, Jr. 11/19/93
Study Director Date

PROTOCOL DEVIATION STATEMENT

Protocol Deviation Statement for Union Carbide Corporation (UCC) Study # 37-AEG-88, Ethylene Oxide (8000 ppm in nitrogen) study for Chemical Manufacturers Association (CMA), Washington, D.C..

In accordance with UCC's intent that all tests conducted by our facility follow good laboratory practices, UCC's study director for the above test confirms that there were no protocol deviations taken during the study. The study was conducted in compliance with the protocol established and signed on 7/19/93 by Alexander E. Gabany, GLP Study Director.

Alexander E. Gabany, Jr. 11/19/93
Study Director date

SIGNATURE PAGE

Submitted by: Union Carbide Corporation
P.O. Box 8361
South Charleston, West Virginia 25303

Prepared by:

Alexander E. Gabany, Jr. — 11/19/93
Study Director — date

Richard A. McDonie — 11-19-93
GC/MS Skill Area Specialist — date

Russell A. Miller — 11/19/93
Infrared Skill Area Specialist — date

Quality Assurance Review by:

Denise L. Johnson — 11/22/93
GLP Quality Assurance Unit — date
(QAU) Representative

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Ethylene Oxide (8000 ppm in nitrogen)

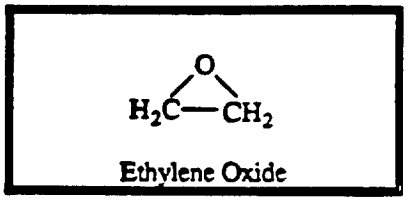
Ethylene Oxide (8000 ppm in nitrogen)
Study # 37-AEG-88

ABSTRACT Ethylene oxide (8000 ppm in nitrogen), was analyzed to provide analytical data as part of the toxicity study at Bushy Run Research Center. The analyses were performed in compliance with Good Laboratory Practice standards (GLP) to meet EPA Section 4 Test Rule requirements and FDA, 21 CFR 58. Gas chromatography-mass spectrometry (GC/MS) and infrared spectroscopy (IR) techniques were independently used to confirm the sample's identity. Purity, measured by capillary GC, is ≈99.93% ethylene oxide, nitrogen-free basis, for the pre-study sample and ≈99.99% ethylene oxide, nitrogen-free basis, for the post-study sample. GC/MS analyses confirmed the presence of ≈7600 ppm ethylene oxide in the pre-study sample and ≈7880 ppm ethylene oxide in the post-study sample. The pre-study sample was received from Bushy Run Research Center and subsequently returned to Bushy Run Research Center. The post-study sample was received from Bushy Run Research Center. All raw data, documentation, records, protocols, and final reports are being retained.

INTRODUCTION The CMA Olefins Panel of Chemical Manufacturers Association, this study's sponsor, requested that Bushy Run Research Center (BRRC) test ethylene oxide (8000 ppm in nitrogen), for toxicity. Such studies must follow GLP standards established by the EPA that require they be conducted with authentic material whose identity and purity are verified analytically.

A high pressure cylinder of ethylene oxide (8000 ppm in nitrogen), (cylinder # ALM-030222, 37-AEG-90) was received 7/15/93 from Bushy Run Research Center for analytical characterization. The cylinder of ethylene oxide (8000 ppm in nitrogen), (Lot # 091402, BRRC# 55-143) was subsequently returned to Bushy Run Research Center for toxicological testing. A GLP protocol describing the analytical characterization of the sample was prepared (Appendix I). The protocol called for structural identification by GC/MS and IR analyses; for the capillary GC measurement of any impurities identified by GC/MS; and the ethylene oxide concentration to be verified by GC/MS. Note: Scott Medical Products, Plumsteadville, PA was the source of the ethylene oxide in nitrogen mixture characterized in this study. Linde, East Chicago, IN, supplied the ethylene oxide used to prepare the mixture by Scott Medical Products. The post-study sample (cylinder # ALM-030208, 37-AEG-90R) was received from Bushy Run Research Center on 9/27/93.

Shown at right is the structure of Ethylene Oxide; its Chemical Abstracts Service Registry number (CAS #) is 75-21-8.



Ethylene Oxide

DISCUSSION The data from the analyses are summarized below.

IR spectroscopy Analyses An infrared spectrum of the sample (8000 ppm ethylene oxide, with the balance composed of nitrogen) (Cylinder # ALM-030222, 37-AEG-90, IASC # 3794, UCC Laboratory Notebook 43-REJ-14) was collected on a Mattson Galaxy 5020 Fourier Transform Infrared (FTIR) spectrometer. Data collection parameters included 50 coadded scans at 4 cm^{-1} resolution with triangular apodization. The sample was run as a gas sample in the infrared gas cell (SOP IR-007). The spectrum obtained (Figure 1) compares well with reference spectra of ethylene oxide found in The Sadtler Library of Infrared Spectra of Gases and Vapors, published by Sadtler Research Laboratories. The functional group assignments are as follows:

Absorbance Band Frequency, cm^{-1}	Functional Group Assignment
3092-2930	CH_2 antisymmetric and symmetric stretch
871	C-O-C stretch

The spectrum is consistent with ethylene oxide and shows no indication of contamination. The nitrogen is invisible to IR analysis.

GC/MS Analysis Electron ionization (EI) mass spectral data were collected in the UCC MS Skill Center using a Finnigan TSQ-70 mass spectrometer interfaced to a Hewlett-Packard (HP) 5890 gas chromatograph. The sample, cylinder # ALM-030222, 37-AEG-90, was analyzed by injecting 1 cc aliquots, via a VALCO gas sampling valve, onto a CP-Sil-5-CB capillary column held at -20°C for 2 minutes, and temperature programmed to 0°C at 8°/minute,

then temperature programmed to 250°C at 15°/min. Figure 2 shows the EI total ion current chromatogram for the sample (scanned from m/z 10 to m/z 310 EI mode). This chromatogram is annotated with identifications based on the components' EI spectrum.

The ethylene oxide concentration in the sample was determined using a 8000 ppm ethylene oxide in nitrogen standard, prepared in UCC's gas mixing skill center, for reference. Aliquots of the sample and reference material were injected into the GC/MS system; the GC peak area for ethylene oxide were determined by mass spectrometric analysis by using extracted ion current profiles for ethylene oxide. The concentration of ethylene oxide in the sample was determined by comparison of the two areas. The pre-study sample was determined to be ≈7600 ppm ethylene oxide in nitrogen and the post-study sample contained ≈7880 ppm ethylene oxide in nitrogen.

Capillary GC A HP 5890 gas chromatograph equipped with a flame ionization detector was used to analyze the sample. Aliquots (1 mL) were injected manually with a 100:1 split ratio onto a DB-1 (5μ film thickness) capillary column maintained isothermally at 50°C (see Figure 3). The average of triplicate analyses are given below (normalized chromatogram area percent), nitrogen-free basis. The gas chromatogram for the post-sample (37-AEG-90R) is shown in Figure 4.

Component name	37-AEG-90	37-AEG-90R
Unknown impurity	0.04	
Unknown impurity	0.03	
Ethylene Oxide	99.93	99.99

CONCLUSION IR spectral data from the UCC Skill Center shows that the sample is ethylene oxide; the nitrogen is invisible to IR analysis. The mass spectral fragmentation data from the UCC Skill Center show that the sample is ethylene oxide in nitrogen. These independent methods satisfy the analytical requirements for structural identification, as defined in the sample protocol. Sample purity, measured by capillary GC, is ≈99.93 % and ≈99.99% ethylene oxide, nitrogen-free basis, respectively for the pre-study sample and the post-study sample. The pre-study sample was analyzed by GC/MS to be ≈7600 ppm ethylene oxide in nitrogen, whereas the post-study sample was ≈7880 ppm ethylene oxide in nitrogen.

ARCHIVES All raw data, records, protocols, and final reports are being retained at UCC's South Charleston, WV, Technical Center as follows:

- raw data from GC, IR and GC/MS studies are in 770-361, 770-223, and 770-123, respectively;
- protocols, notebook and other records are to be kept in the GLP archives.

ACKNOWLEDGEMENTS We would like to thank Trudy Barker for sample handling, collecting the GC data, and preparing the bulk of the report, and Jim Richardson for collecting the IR data.

NOTEBOOK REFERENCE: 37-AEG-88 and related pages

Figure 1 — Infrared Spectrum of 37-AEG-90 (Ethylene Oxide (8000 ppm in nitrogen))

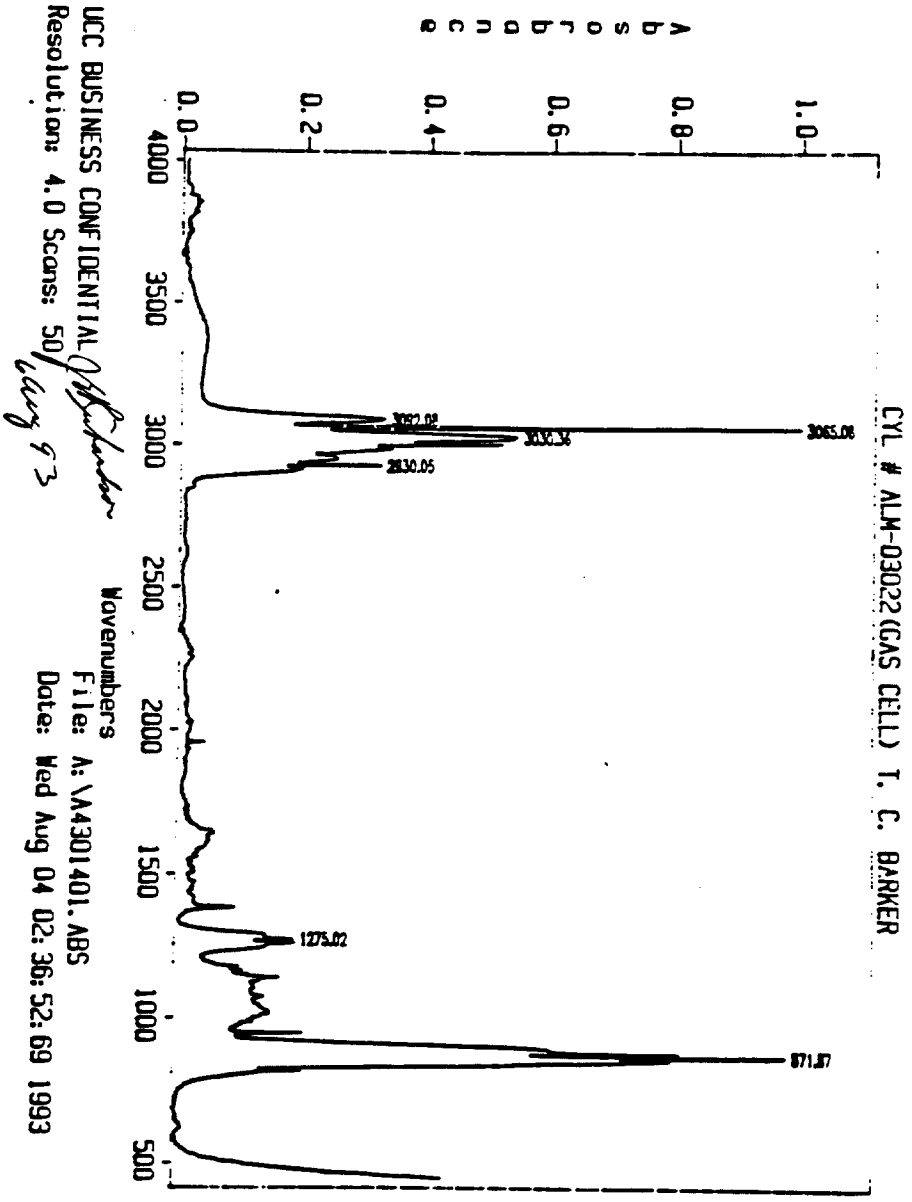
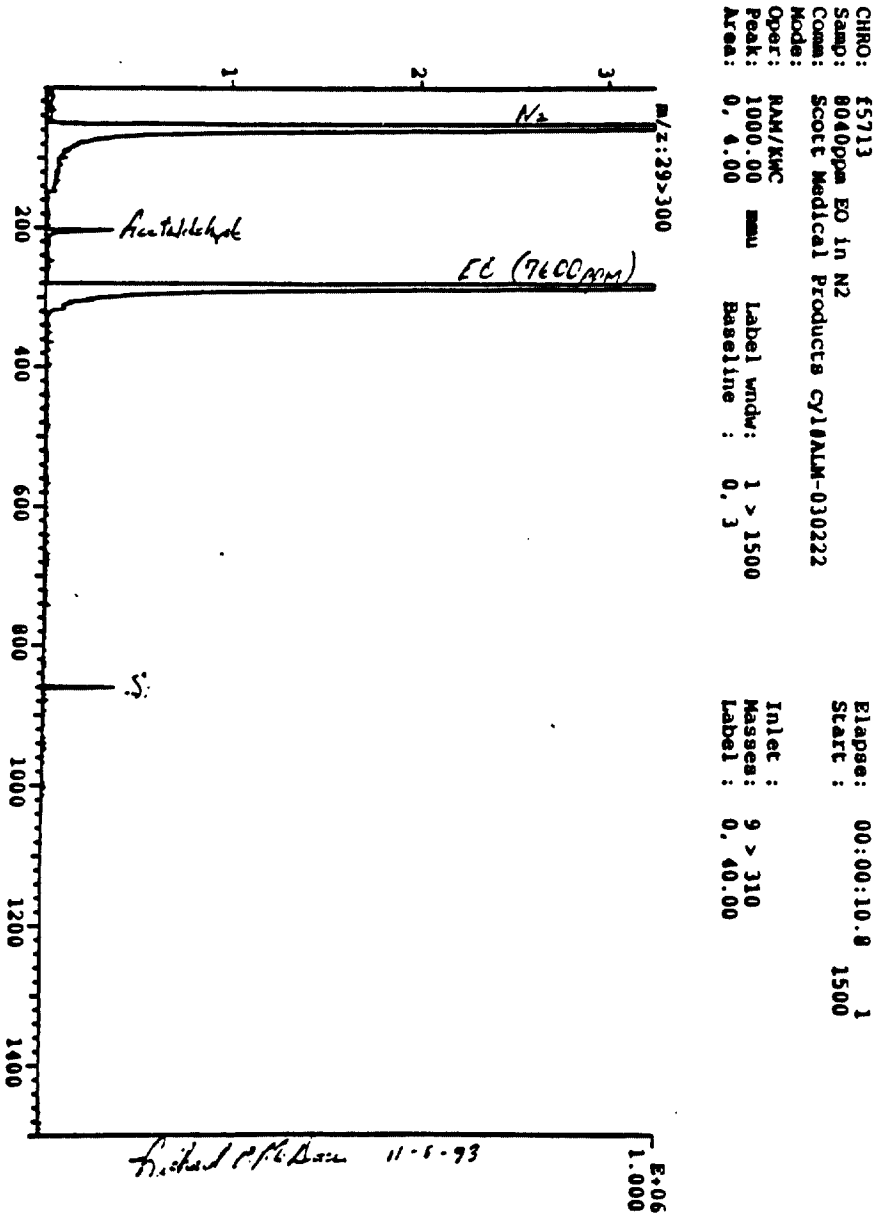


Figure 2 — Capillary GC/MS RIC of 37-AEG-90 (Ethylene Oxide @000 ppm in nitrogen))



Study # 37-AEG-88

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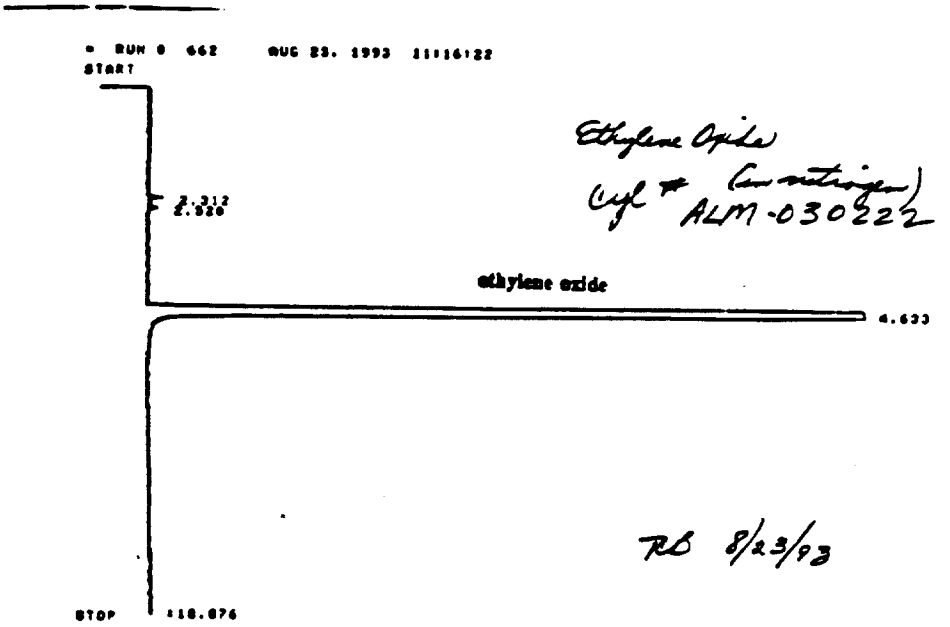
Figure 3— Capillary Gas Chromatogram of 37-AEG-90 (Ethylene Oxide (8000 ppm in nitrogen))



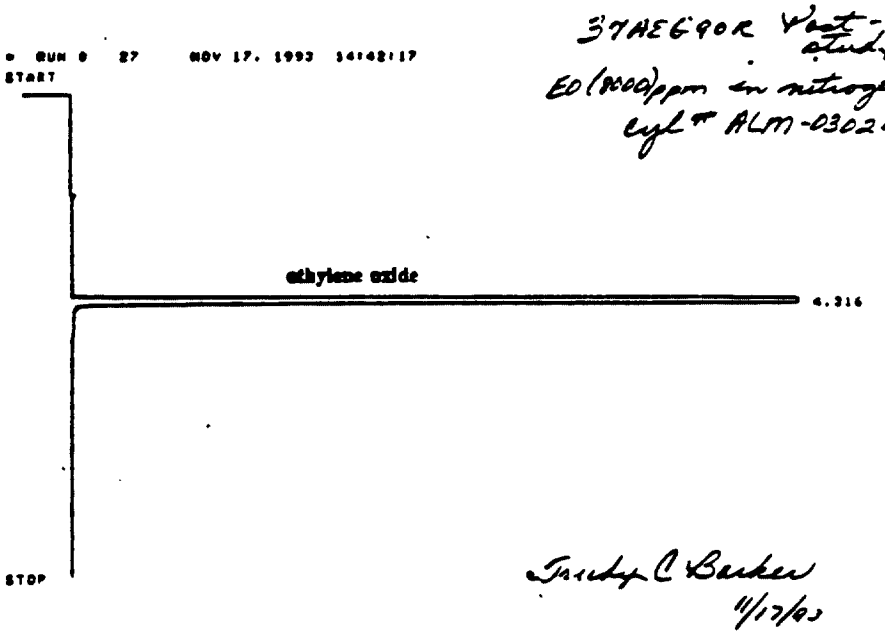
RUN# 662 AUG 23. 1993 11:16:22

AREA%

RT	AREA	TYPE	WIDTH	AREA%
2.312	113	PV	.044	.03621
2.528	87	VV	.048	.02788
4.633	311844	PB	.074	99.93590

TOTAL AREA= 312044
AUL FACTOR=1.00000E+00

Figure 4— Capillary Gas Chromatogram of 37-AEG-90R (Ethylene Oxide (8000 ppm in nitrogen))



RUN# 27 NOV 17, 1993 14:42:17

AREA%

RT	AREA	TYPE	WIDTH	AREA%
4.316	581524	PB	.067	100.00000

TOTAL AREA= 581524
AUL FACTOR=1.00000E+00

APPENDIX I Protocol

PROTOCOL

GOOD LABORATORY PRACTICE (GLP) STUDY

title	Ethylene Oxide (8000 ppm in nitrogen)
purpose	Analytical Characterization of Sample(s) for Toxicology Studies at Bushy Run Research Center
study number	37-AEG-88
sponsor	CMA Olefins Panel Chemical Manufacturers Association 2501 M Street, NW Washington, DC 20037
testing facility	UCC Technical Center, South Charleston, WV 25303 (Location 511)
Proposed Starting Date:	Monday, July 19, 1993
Proposed Completion Date:	September 15, 1993
Estimated Date of Final Report:	October 15, 1993

Test Substance(s) 37-AEG-90

Name	Ethylene Oxide
Source	Lot # 091402; Linde, East Chicago, IN 46312
CAS Registry No.	75-21-8
Description	colorless gas, ether-like odor above 500 ppm
Purity	≈99.9%
Health/Safety	highly toxic; stable. MSDS available upon request
Storage Conditions	ambient

Study Design

The test substance(s) will be characterized by:

- Concentration verified by an 8000 ppm EO in nitrogen standard by GC/MS
- Verification of identity by GC/MS.
- Verification of identity by Infrared spectroscopy.
- Quantitation of the identified impurities by capillary GC.

Reviewed and Approved by:

Alexander E. Gabany	date	Denise L. Johnston	date	Rene S. Vergnes	date
GLP Study Director		GLP Quality Assurance Unit (QAU) Representative		Study Director, Bushy Run Research Center	

Elizabeth J. Morlin date
CMA Olefins Panel Manager,
Sponsor's Representative

This study will be performed in compliance with the following GLP standards: FDA, 21 CFR, Part 58, TSCA, 40 CFR, Part 792; and FIFRA, 40 CFR, Part 160. All changes of an approved protocol and the reasons therefore shall be documented, signed by the study director, dated, and maintained with the protocol. All raw data and reports and from this study will be retained at Location 511 for at least 10 years after completion of the study. A comprehensive final report will be submitted to the Sponsor within one month after the completion of the analysis. The final report will be inspected by the QAU and will contain a signed quality assurance statement.

Quality Assurance Unit Study Inspection Summary

Test Substance: ETHYLENE OXIDE (8000 ppm in nitrogen)

Study No.: 37-AEG-88

Study Director: A.E. Gabany, B.S.

The Quality Assurance Unit of the Union Carbide Technical Center conducted the inspections listed below and reported the results to the study director and management on the date indicated. It is the practice of this Quality Assurance Unit to report the results to both the study director and management.

Inspection Date	Inspection Type	Date QAU Report Issued To Study Director	Date QAU Report Issued To Management
Feb. 10, 1992	Laboratory Compliance Review*	Feb. 10, 1992	May, 1992
July 19, 1993	Protocol Compliance Review	July 19, 1993	July 19, 1993
Nov. 22, 1993	Final Report Compliance Review	Nov. 22, 1993	Nov. 22, 1993

*The process of doing the GLP characterization studies is audited periodically to assure these studies comply with GLP requirements. The QA unit is exempted from performing in-life study inspections for studies designed to determine physical and chemical characteristics of a test substance as described in 40 CFR 792.135.

Denise L. Johnson 11/22/93

Denise L. Johnson, QAU Representative (Date)
Good Laboratory Practices/Quality Assurance

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

Individual Clinical Observation Data

(13 Pages)

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TABLE 1
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

INDIVIDUAL CLINICAL OBSERVATION DATA

ABBREVIATIONS

The following is a list of three letter abbreviations for locations of clinical signs that may appear in this appendix.

ABD ABDOMEN
ANS ANUS
AXB AXILLA-BOTH
AXL AXILLA-LEFT
AXR AXILLA-RIGHT
BCK BACK
BDY ENTIRE BODY
CHS CHEST
EAB EAR-BOTH
EAL EAR-LEFT
EAR EAR-RIGHT
ELB EYELID-BOTH
ELL EYELID-LEFT
ELR EYELID-RIGHT
EYB EYE-BOTH
EYL EYE-LEFT
EYR EYE-RIGHT
FAC FACE
GEN GENITAL
HED HEAD
HPB HIP-BOTH
HPL HIP-LEFT
HPR HIP-RIGHT
INB INGUINAL-BOTH
INL INGUINAL-LEFT
INR INGUINAL-RIGHT
LAL LEGS-ALL
LFB LEG-FORE-BOTH
LFL LEG-FORE-LEFT
LFR LEG-FORE-RIGHT
LHB LEG-HIND-BOTH
LHL LEG-HIND-LEFT
LHR LEG-HIND-RIGHT
LNS LOCATION NOT SPECIFIED
MTH MOUTH
MUL MULTIPLE AREAS,NOS*
NCK NECK
NSE NOSE
PAL PAWS-ALL
PFB PAW-FORE-BOTH
PFL PAW-FORE-LEFT
PFR PAW-FORE-RIGHT
PHB PAW-HIND-BOTH
PHL PAW-HIND-LEFT
PHR PAW-HIND-RIGHT
PNS PENIS
SCR SCROTUM
SDB SIDE-BOTH
SDL SIDE-LEFT
SDR SIDE-RIGHT
SHB SHOULDER-BOTH
SHL SHOULDER-LEFT
SHR SHOULDER-RIGHT
TAL TAIL
TEE TEETH
TRA TREATMENT AREA
TSB TESTIS-BOTH
TSL TESTIS-LEFT
TSR TESTIS-RIGHT
VAG VAGINA
*NOS NOT OTHERWISE SPECIFIED

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
0 PPM					
	6581	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6582	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6586	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6589	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6591	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6596	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6603	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6604	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6607	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6611	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
		EYES/EARS/NOSE	6	32- 58	SWOLLEN PERIOCULAR TISSUE (EYR 6)
			3	39- 53	PERIOCULAR ENCRUSTATION (EYR 3)
			2	46- 53	OCULAR DISCHARGE (EYR 2)
	6623	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6626	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6628	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6630	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6634	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6646	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6649	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6665	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6671	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6676	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
0 PPM					
	6683	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6691	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6699	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6703	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6704	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6708	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6712	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6714	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
40 PPM					
	6559	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6570	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6575	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6580	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
		BODY	1	25	TRAUMATIZED (TAL 1)
	6585	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6590	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6602	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6617	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6644	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6674	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6675	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6690	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6692	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
40 PPM					
	6692	FATE	1	25	SCHEDULED SACRIFICE
	6713	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
1000 PPM					
	6560	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6574	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6576	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6625	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6638	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6643	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6648	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6651	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6663	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6672	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6677	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6694	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6701	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6710	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
3000 PPM					
	6557	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6558	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6562	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6564	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6565	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
3000 PPM					
	6567	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6573	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6577	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6593	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6600	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6610	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6612	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6622	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6635	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6636	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6642	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6647	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6650	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6654	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6656	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6662	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6668	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6673	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6685	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6686	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6687	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6688	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS

TABLE 2

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

RATS

INDIVIDUAL CLINICAL OBSERVATIONS

MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
3000 PPM					
	6688	FATE	1	4	SCHEDULED SACRIFICE
	6697	NORMAL	7	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
		SKIN	4	39- 57	ALOPECIA (LFB 4)
200 PPM EO					
	6556	NORMAL	6	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6568	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6584	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6616	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6624	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6629	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6637	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6661	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6681	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6695	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6698	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6706	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6707	NORMAL	11	0- 58	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	58	SHIPPED OFF SITE
	6715	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
0 PPM					
	6727	NORMAL	12	0- 67	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	3	74- 79	ALOPECIA (GEN 3)
	6730	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6747	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6750	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6755	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6762	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6764	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6766	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6767	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6771	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6772	NORMAL	12	0- 67	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	3	74- 79	ALOPECIA (HPL 3)
	6781	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6789	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6791	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6806	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6816	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6817	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6834	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6837	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6838	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6849	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
0 PPM					
	6849	FATE	1	25	SCHEDULED SACRIFICE
	6857	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6863	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6868	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6870	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6876	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6881	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6882	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
40 PPM					
	6724	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6734	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6742	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6768	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6794	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6810	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6822	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6824	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6832	NORMAL	12	0- 74	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		BODY	3	67- 79	SWELLING (GEN 3)
	6839	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6847	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6854	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6860	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
40 PPM					
	6878	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
1000 PPM					
	6723	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6738	NORMAL	14	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	1	53	ALOPECIA (CHS 1)
	6746	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6748	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6753	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6763	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6770	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6774	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6792	NORMAL	13	0- 74	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	2	78- 79	ALOPECIA (CHS 2)
	6793	NORMAL	9	0- 53	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		BODY	6	57- 79	SWELLING (GEN 6)
	6821	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6845	NORMAL	8	0- 46	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	7	53- 79	ALOPECIA (MUL 6, SDL 1)
	6853	NORMAL	13	0- 74	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	2	78- 79	ALOPECIA (CHS 2)
	6865	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
3000 PPM					
	6729	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6736	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6743	NORMAL	4	0- 18	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL CLINICAL OBSERVATIONS
MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
3000 PPM					
	6743	SKIN	1	25	ALOPECIA (CHS 1)
	6744	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6749	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6752	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6758	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6769	NORMAL	4	0- 18	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
		SKIN	11	25- 79	ALOPECIA (MUL 11)
	6775	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6777	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6782	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6802	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6804	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6805	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6807	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6808	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6815	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6818	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6823	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6835	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6842	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6843	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6856	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6869	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS

TABLE 3

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

MICE

INDIVIDUAL CLINICAL OBSERVATIONS

MALES

DOSAGE GROUP	ANIMAL	CATEGORY	#	STUDY DAYS	FINDING
3000 PPM					
	6869	FATE	1	25	SCHEDULED SACRIFICE
	6874	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6879	NORMAL	3	0- 11	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	11	SCHEDULED SACRIFICE
	6880	NORMAL	2	0- 4	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	4	SCHEDULED SACRIFICE
	6884	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
200 PPM EO					
	6737	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6776	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6783	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6786	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6795	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6796	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6800	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6809	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6811	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6819	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6829	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6831	NORMAL	15	0- 79	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	79	SHIPPED OFF SITE
	6848	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE
	6859	NORMAL	5	0- 25	NO SIGNIFICANT CLINICAL OBSERVATIONS
		FATE	1	25	SCHEDULED SACRIFICE

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

Individual Body Weight Data

(15 Pages)

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TABLE 1

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

INDIVIDUAL BODY WEIGHT DATA

ABBREVIATIONS

The following is a list of abbreviations or words that may appear in this appendix.

dead = indicates that the animal died prior to the period in which this word appears.

sacr = indicates that the animal was a scheduled sacrifice prior to the period in which this abbreviation appears.

TABLE 2

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

RATS

INDIVIDUAL BODY WEIGHT (GRAMS)

MALES GROUP: 0 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6581	187.1	197.3	210.9	sacr						
6582	179.4	183.6	193.6	201.3	215.2	sacr				
6586	149.5	159.6	176.4	186.8	194.8	209.2	218.3	217.0	234.2	246.0
6589	157.5	163.7	178.3	194.8	207.5	215.4	225.9	228.3	232.9	238.9
6591	131.5	135.9	143.1	151.4	158.8	166.6	174.5	186.0	193.9	199.8
6596	188.9	197.2	213.3	224.7	243.4	sacr				
6603	148.6	151.6	170.9	191.4	212.4	sacr				
6604	135.5	143.6	sacr							
6607	166.9	174.1	183.9	sacr						
6611	149.3	160.7	177.7	194.4	211.1	225.8	234.0	240.1	248.5	257.4
6623	147.7	154.7	173.6	sacr						
6626	172.5	176.0	sacr							
6628	148.8	153.4	170.1	186.5	204.6	sacr				
6630	179.0	184.8	201.5	214.1	226.5	240.1	247.1	258.0	273.8	279.5
6634	174.4	182.9	199.4	sacr						
6646	158.6	163.4	sacr							
6649	174.9	179.5	sacr							
6665	143.2	145.5	sacr							
6671	170.6	178.9	193.8	sacr						
6676	150.2	159.0	168.3	sacr						
6683	146.8	155.4	168.3	180.3	199.9	sacr				
6691	159.0	164.9	180.7	193.7	207.0	223.4	235.9	242.7	256.4	263.4
6699	167.4	175.4	191.4	sacr						
6703	152.4	158.8	sacr							
6704	142.5	151.8	sacr							
6708	157.6	163.4	181.2	200.0	215.6	sacr				
6712	154.9	165.8	185.6	198.5	213.1	sacr				
6714	154.9	164.9	182.1	205.6	224.2	224.2	240.2	251.5	258.2	268.1
MEAN	158.9	165.9	183.0	194.5	209.6	215.0	225.1	231.9	242.6	250.4
S.D.	15.03	15.31	16.05	16.87	18.92	23.35	24.19	24.47	25.75	26.09
N	28	28	21	14	14	7	7	7	7	7

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 40 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6559	164.1	178.5	198.1	215.2	225.4	sacr				
6570	158.7	168.6	186.9	199.6	211.0	224.1	234.2	240.8	252.0	256.1
6575	164.7	174.6	196.4	214.9	233.9	sacr				
6580	179.4	190.3	214.2	232.4	239.9	sacr				
6585	170.2	177.1	192.0	202.3	217.1	225.9	233.3	246.3	253.3	263.1
6590	166.7	175.7	192.6	213.3	228.5	241.3	257.6	264.5	274.4	283.1
6602	136.9	148.5	164.0	177.5	192.1	sacr				
6617	159.3	171.0	183.9	203.7	215.4	229.1	242.0	257.6	265.8	278.3
6644	177.0	184.4	200.2	208.7	222.1	230.6	236.7	237.6	244.7	251.9
6674	151.5	159.1	173.6	188.3	204.4	211.6	222.0	226.5	236.2	242.2
6675	145.9	154.4	166.8	182.3	201.6	210.9	215.7	222.6	233.9	236.5
6690	160.4	170.9	188.2	204.4	219.9	sacr				
6692	170.6	176.1	193.1	212.3	231.9	sacr				
6713	175.9	180.3	201.3	213.7	236.0	sacr				
MEAN	162.9	172.1	189.4	204.9	219.9	224.8	234.5	242.3	251.5	258.7
S.D.	12.08	11.47	13.79	14.55	14.04	10.74	13.59	15.31	14.84	17.39
N	14	14	14	14	14	7	7	7	7	7

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 1000 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6560	158.7	161.9	181.6	198.7	218.5	sacr				
6574	182.9	192.6	210.9	227.6	246.4	259.2	270.0	271.0	283.0	292.8
6576	170.5	173.3	187.4	201.1	214.0	223.7	237.6	247.9	249.9	261.2
6625	164.8	170.8	191.5	215.8	237.3	sacr				
6638	147.3	156.4	175.6	187.5	203.7	sacr				
6643	139.8	143.1	156.2	169.0	186.1	195.6	205.6	213.1	219.6	226.4
6648	177.9	184.5	197.1	209.7	224.6	sacr				
6651	143.2	148.0	165.8	183.0	197.5	203.8	210.4	221.4	224.5	233.4
6663	149.1	156.5	175.3	193.4	213.1	sacr				
6672	147.5	155.8	171.9	190.1	210.7	226.4	242.9	249.6	265.1	270.7
6677	143.0	146.5	163.3	175.2	187.8	195.2	203.6	208.4	219.6	228.2
6694	165.1	175.4	200.0	218.1	233.8	246.5	250.3	252.5	260.5	268.0
6701	174.5	181.6	199.9	211.1	224.7	sacr				
6710	133.9	139.0	150.7	163.4	173.8	sacr				
MEAN	157.0	163.2	180.5	196.0	212.3	221.5	231.5	237.7	246.0	254.4
S.D.	15.66	16.66	18.07	19.28	20.87	25.03	25.49	23.47	25.19	25.43
N	14	14	14	14	14	7	7	7	7	7

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 3000 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6557	162.0	174.1	sacr							
6558	151.0	161.7	179.8	sacr						
6562	150.6	155.2	170.9	sacr						
6564	162.2	164.5	170.8	175.0	180.7	191.2	198.1	205.8	211.8	218.2
6565	167.4	168.9	175.3	186.3	195.6	sacr				
6567	154.6	162.8	173.3	188.3	199.8	207.6	217.6	228.5	239.2	246.7
6573	166.9	172.9	189.7	208.8	214.4	230.6	242.3	254.6	265.6	274.4
6577	173.9	175.3	189.6	sacr						
6593	162.6	170.4	sacr							
6600	158.2	166.2	176.6	sacr						
6610	135.0	140.2	150.8	159.7	170.2	sacr				
6612	148.8	159.1	179.8	194.9	210.5	220.7	228.2	240.1	250.4	260.9
6622	166.3	174.3	189.5	204.3	215.2	sacr				
6635	175.5	185.7	202.7	217.8	231.0	sacr				
6636	158.2	169.9	192.7	213.6	233.5	sacr				
6642	169.4	174.9	sacr							
6647	167.0	169.8	183.1	195.5	209.6	sacr				
6650	179.6	184.2	198.4	210.8	224.4	235.5	243.9	255.2	265.7	273.5
6654	156.1	155.6	171.1	sacr						
6656	141.1	150.4	sacr							
6662	168.6	172.8	186.7	196.2	209.1	sacr				
6668	156.1	160.4	sacr							
6673	143.8	151.9	171.3	sacr						
6685	148.6	153.5	sacr							
6686	159.7	171.1	190.8	204.9	216.3	234.5	247.2	262.3	270.3	279.7
6687	137.2	144.4	159.6	sacr						
6688	152.8	159.1	sacr							
6697	168.5	177.0	196.0	215.3	233.5	236.5	247.6	263.4	276.2	283.7
MEAN	158.6	165.2	180.9	197.9	210.3	222.4	232.1	244.3	254.2	262.4
S.D.	11.41	11.25	12.99	16.52	18.80	17.22	18.66	21.06	22.53	23.17
N	28	28	21	14	14	7	7	7	7	7

TABLE 2

ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS

RATS

INDIVIDUAL BODY WEIGHT (GRAMS)

MALES GROUP: 200 PPM EO

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6556	160.6	161.9	167.5	170.2	174.3	sacr				
6568	154.6	153.8	154.7	163.1	162.7	sacr				
6584	184.5	178.5	182.8	183.1	183.1	sacr				
6616	165.3	164.4	171.8	177.3	182.9	204.5	213.2	216.2	231.0	235.4
6624	154.6	153.4	161.5	170.5	174.1	sacr				
6629	152.8	144.9	151.0	153.9	153.8	162.8	169.9	174.7	183.1	185.2
6637	137.4	135.5	139.7	148.3	158.8	sacr				
6661	161.4	162.0	164.3	167.0	170.2	188.0	196.5	205.7	216.1	221.5
6681	152.8	152.8	157.7	159.2	162.2	187.2	185.6	201.6	215.0	222.7
6695	178.4	176.8	183.3	180.5	181.2	sacr				
6698	138.0	135.2	140.6	138.4	141.1	152.4	159.3	163.4	167.3	171.2
6706	180.8	177.4	181.1	181.7	184.7	199.4	209.6	218.6	231.8	240.7
6707	173.9	178.1	181.3	189.1	192.2	210.2	216.2	224.7	244.6	251.2
6715	168.6	170.2	175.1	183.2	186.5	sacr				
MEAN	161.7	160.3	165.2	169.0	172.0	186.4	192.9	200.7	212.7	218.3
S.D.	14.63	15.10	15.02	14.92	14.58	21.52	22.22	23.20	27.90	29.50
N	14	14	14	14	14	7	7	7	7	7

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 0 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6727	25.0	25.4	25.4	26.2	26.8	27.5	27.5	27.4	29.0	28.4
6730	24.9	24.6	25.5	26.3	26.4	sacr				
6747	24.8	25.7	26.6	26.7	28.0	27.8	28.1	28.2	29.2	29.4
6750	24.4	26.0	27.7	27.9	29.3	sacr				
6755	23.6	24.1	23.9	sacr						
6762	25.2	26.5	27.8	28.3	29.7	29.2	29.0	28.7	30.0	30.0
6764	26.1	25.4	26.5	27.4	28.8	sacr				
6766	25.3	26.9	28.1	28.4	29.1	sacr				
6767	25.1	25.5	sacr							
6771	24.8	25.7	sacr							
6772	25.7	26.6	27.8	28.4	29.7	28.5	29.5	29.5	30.7	32.6
6781	26.3	26.7	sacr							
6789	26.1	27.1	27.5	27.4	27.5	27.4	28.0	28.0	29.7	29.3
6791	25.3	26.2	26.7	27.2	27.3	sacr				
6806	25.9	26.5	sacr							
6816	24.2	25.6	25.5	sacr						
6817	26.5	28.0	sacr							
6834	23.7	24.5	24.7	sacr						
6837	25.0	26.4	sacr							
6838	24.3	24.8	24.7	sacr						
6849	27.6	29.0	31.0	31.9	32.8	sacr				
6857	26.0	26.5	27.5	sacr						
6863	24.4	25.3	26.6	sacr						
6868	26.7	28.0	30.4	31.4	32.6	31.1	32.0	32.2	33.2	33.4
6870	27.2	26.9	27.4	28.2	29.6	30.0	31.3	32.1	32.8	32.9
6876	26.1	27.3	29.4	29.0	29.9	sacr				
6881	28.1	27.9	sacr							
6882	24.9	25.6	26.0	sacr						
MEAN	25.5	26.2	27.0	28.2	29.1	28.8	29.3	29.4	30.7	30.9
S.D.	1.11	1.16	1.80	1.69	1.92	1.40	1.71	1.93	1.68	2.05
N	28	28	21	14	14	7	7	7	7	7

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 0 PPM

WEEK	10	11	12
ANIMAL			
6727	28.5	28.9	29.6
6747	29.7	29.2	29.8
6762	30.1	30.1	30.4
6772	32.3	31.5	32.0
6789	29.3	28.5	28.2
6868	34.0	34.7	34.8
6870	33.6	32.7	32.7
MEAN	31.1	30.8	31.1
S.D.	2.17	2.28	2.23
N	7	7	7

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 40 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6724	25.7	25.8	26.5	28.0	28.3	29.9	30.1	30.0	32.6	33.3
6734	21.2	21.1	22.6	22.9	23.3	24.4	24.9	25.1	26.6	25.9
6742	23.6	23.3	24.4	25.8	26.4	26.8	27.0	27.1	27.6	27.4
6768	25.2	25.1	25.7	26.8	27.0	sacr				
6794	25.1	25.1	26.8	28.3	28.1	sacr				
6810	25.3	24.9	26.3	26.8	26.5	28.0	28.0	28.7	30.5	30.4
6822	26.2	26.8	28.1	29.5	30.1	sacr				
6824	25.1	25.6	28.3	27.5	29.4	sacr				
6832	23.7	26.3	27.0	27.9	28.2	28.0	27.7	27.9	29.0	28.2
6839	24.7	25.7	26.1	26.3	27.1	27.3	28.8	29.4	29.5	29.5
6847	22.6	23.1	23.1	23.2	24.7	25.8	26.4	26.2	25.6	26.3
6854	26.5	26.5	27.1	28.4	28.2	sacr				
6860	26.9	28.6	29.8	30.5	31.7	sacr				
6878	27.6	27.0	27.7	27.8	29.5	sacr				
MEAN	24.9	25.4	26.4	27.1	27.7	27.2	27.6	27.8	28.8	28.7
S.D.	1.72	1.87	1.99	2.11	2.17	1.75	1.69	1.78	2.39	2.62
N	14	14	14	14	14	7	7	7	7	7

WEEK	10	11	12
6724	33.5	33.4	33.7
6734	26.4	25.7	26.5
6742	28.0	28.2	27.8
6810	30.2	29.0	29.2
6832	28.3	28.3	28.6
6839	30.0	31.0	30.8
6847	27.0	26.4	26.4
MEAN	29.1	28.9	29.0
S.D.	2.40	2.66	2.58
N	7	7	7

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 1000 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6723	23.0	24.1	25.2	26.4	27.6	28.2	28.6	29.2	30.7	31.9
6738	24.1	25.6	26.4	26.9	28.5	28.0	28.1	27.6	28.4	29.1
6746	25.2	27.1	27.0	27.6	28.1	sacr				
6748	26.6	26.3	27.4	28.5	28.8	sacr				
6753	24.8	24.7	25.1	26.1	26.3	28.5	29.3	28.9	30.6	30.7
6763	27.3	28.7	30.2	31.8	32.4	sacr				
6770	25.3	26.2	27.1	27.7	28.1	sacr				
6774	25.4	26.8	27.9	29.5	29.9	sacr				
6792	26.3	26.8	27.3	27.2	29.4	29.2	30.3	30.8	31.2	32.4
6793	27.9	28.1	29.9	30.4	31.5	31.4	32.8	32.9	33.7	35.6
6821	25.4	25.0	25.5	25.8	25.4	sacr				
6845	25.2	24.7	25.9	26.6	27.0	26.6	28.1	29.1	29.0	30.6
6853	23.4	25.5	26.4	27.4	27.6	27.5	27.6	28.8	29.0	28.7
6865	25.0	26.5	28.2	29.0	30.4	sacr				
MEAN	25.3	26.2	27.1	27.9	28.7	28.5	29.3	29.6	30.4	31.3
S.D.	1.38	1.32	1.56	1.73	1.94	1.53	1.78	1.70	1.80	2.30
N	14	14	14	14	14	7	7	7	7	7

WEEK	10	11	12
6723	32.5	33.0	32.8
6738	29.8	29.5	29.9
6753	31.0	31.5	31.7
6792	32.6	32.4	32.8
6793	36.9	34.6	34.4
6845	31.7	31.8	31.0
6853	28.9	29.0	29.6
MEAN	31.9	31.7	31.8
S.D.	2.60	1.95	1.72
N	7	7	7

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 3000 PPM

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6729	24.2	25.2	25.4	sacr						
6736	25.6	27.0	27.8	29.4	30.3	sacr				
6743	23.3	24.4	24.1	24.9	25.9	sacr				
6744	23.5	23.9	24.6	24.7	25.5	25.8	26.4	26.1	27.4	28.3
6749	25.3	25.0	26.8	26.5	27.0	sacr				
6752	23.2	24.2	26.1	sacr						
6758	25.9	27.6	28.2	sacr						
6769	24.9	24.1	25.4	25.7	26.4	27.7	28.1	28.1	28.5	29.0
6775	25.5	26.4	26.9	27.0	27.9	28.8	29.5	29.1	29.6	29.7
6777	26.6	26.0	26.2	26.5	27.8	sacr				
6782	27.7	27.1	27.0	sacr						
6802	24.5	25.2	sacr							
6804	25.3	26.9	sacr							
6805	26.9	26.4	sacr							
6807	25.1	25.1	sacr							
6808	24.0	24.3	25.2	sacr						
6815	25.3	25.6	26.0	26.1	27.1	27.2	27.3	26.9	27.7	29.0
6818	26.3	25.6	26.0	27.2	27.4	sacr				
6823	24.9	25.9	26.3	27.0	28.5	29.3	30.0	30.0	30.8	30.5
6835	22.2	22.8	23.3	24.2	24.6	26.6	26.7	27.7	27.4	28.5
6842	24.8	25.6	sacr							
6843	26.3	28.5	sacr							
6856	27.5	28.3	28.6	sacr						
6869	27.4	28.4	28.1	29.5	30.3	sacr				
6874	25.3	25.8	26.7	27.7	29.3	30.0	28.9	29.5	29.9	30.3
6879	26.1	26.7	28.0	sacr						
6880	25.9	26.9	sacr							
6884	24.4	25.3	25.4	25.9	27.5	sacr				
MEAN	25.3	25.9	26.3	26.6	27.5	27.9	28.1	28.2	28.8	29.3
S.D.	1.35	1.42	1.40	1.57	1.68	1.53	1.39	1.42	1.36	0.87
N	28	28	21	14	14	7	7	7	7	7

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 3000 PPM

WEEK	10	11	12
ANIMAL			
6744	29.5	28.5	29.1
6769	30.2	29.9	30.0
6775	30.1	30.4	30.3
6815	28.9	29.1	29.7
6823	30.6	30.6	30.8
6835	29.5	30.4	30.5
6874	31.6	30.9	31.1
MEAN	30.0	30.0	30.2
S.D.	0.89	0.86	0.67
N	7	7	7

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL BODY WEIGHT (GRAMS)
MALES GROUP: 200 PPM EO

WEEK	0	1	2	3	4	5	6	7	8	9
ANIMAL										
6737	26.9	27.2	27.6	29.0	28.6	27.5	28.6	28.9	30.4	30.2
6776	27.7	27.6	28.9	28.5	28.3	sacr				
6783	25.0	25.6	25.8	26.5	27.0	sacr				
6786	27.1	28.3	28.5	29.5	29.6	28.5	30.4	31.2	32.0	33.1
6795	25.3	26.6	27.8	28.3	29.6	27.1	28.6	29.9	29.9	29.8
6796	23.4	25.1	25.7	26.7	25.7	24.4	26.5	27.2	26.7	26.9
6800	26.3	28.4	28.8	29.6	29.9	sacr				
6809	24.9	25.8	28.5	30.9	31.5	28.1	30.1	31.1	31.9	31.6
6811	24.5	25.6	27.5	27.9	28.4	sacr				
6819	23.8	25.3	25.5	26.4	26.6	25.9	27.5	27.4	27.9	29.4
6829	25.7	26.8	27.1	28.9	29.0	sacr				
6831	23.7	24.2	25.1	25.9	26.0	26.5	27.9	28.1	28.1	28.5
6848	25.0	25.5	26.6	27.0	25.8	sacr				
6859	26.6	27.5	27.8	28.8	28.5	sacr				
MEAN	25.4	26.4	27.2	28.1	28.2	26.8	28.5	29.1	29.6	29.9
S.D.	1.35	1.29	1.27	1.45	1.74	1.43	1.40	1.66	2.08	2.01
N	14	14	14	14	14	7	7	7	7	7

WEEK	10	11	12
6737	31.5	31.9	30.9
6786	32.3	33.3	32.7
6795	30.0	30.5	30.0
6796	27.8	28.3	27.6
6809	32.5	34.1	33.4
6819	29.6	30.3	29.6
6831	29.4	30.3	29.8
MEAN	30.4	31.2	30.6
S.D.	1.72	2.01	1.95
N	7	7	7

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

Individual Anatomic Pathology Data

(28 Pages)

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TABLE 1
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

NECROPSY PROTOCOL

MALES

The following tissues were examined at necropsy with no significant lesions observed unless specified on individual animal page:

LIVER	SPLEEN	BRAIN	LUNGS	BLOOD

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 0 PPM MALE

ANIMAL 6581 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6582 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6586 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6589 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6591 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6596 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6603 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6604 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6607 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6611 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 0 PPM MALE

ANIMAL 6623 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6626 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
LIVER
GROSS: ANOMALY
LEFT LATERAL LOBE, 4X3X2 MM
LUNGS
GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
LEFT LOBE, DARK RED FOCUS
MICRO: N O T E X A M I N E D

ANIMAL 6628 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6630 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6634 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6646 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6649 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6665 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6671 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
LUNGS
GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
TWO 1X1 MM DARK RED FOCI, LEFT LOBE
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 0 PPM MALE

ANIMAL 6676 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6683 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6691 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6699 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6703 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6704 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
LUNGS
GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
ONE PUNCTATE FOCI ON EACH, RIGHT APICAL AND LEFT LOBES
MICRO: N O T E X A M I N E D

ANIMAL 6708 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6712 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6714 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 40 PPM MALE

ANIMAL 6559 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6570 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6575 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6580 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
TAIL
GROSS: TRAUMATIZED
TIP
MICRO: N O T E X A M I N E D

ANIMAL 6585 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6590 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6602 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6617 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6644 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6674 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 40 PPM MALE

ANIMAL 6674 (CONTINUED)
MICRO: N O T E X A M I N E D

ANIMAL 6675 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6690 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6692 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6713 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 1000 PPM MALE

ANIMAL 6560 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6574 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6576 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6625 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6638 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
THYMIC REGION
GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
MOTTLED RED AND TAN, RIGHT SIDE
MICRO: N O T E X A M I N E D

ANIMAL 6643 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6648 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6651 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6663 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6672 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 1000 PPM MALE

ANIMAL 6677 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6694 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6701 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6710 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 3000 PPM MALE

ANIMAL 6557 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
LIVER
GROSS: ANOMALY
5X4X4 MM, HILUS REGION
MICRO: N O T E X A M I N E D

ANIMAL 6558 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6562 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6564 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6565 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6567 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6573 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6577 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6593 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6600 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 3000 PPM MALE

ANIMAL 6610 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6612 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6622 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6635 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6636 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6642 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6647 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6650 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6654 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6656 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6662 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 3000 PPM MALE

ANIMAL 6668 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
LUNGS
GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
LEFT LOBE, TWO, DARK RED FOCI
MICRO: N O T E X A M I N E D

ANIMAL 6673 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
LUNGS
GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
DARK RED FOCUS, LEFT LOBE
MICRO: N O T E X A M I N E D

ANIMAL 6685 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6686 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6687 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6688 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6697 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 200 PPM EO MALE

ANIMAL 6556 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6568 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6584 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6616 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6624 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6629 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6637 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6661 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6681 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6695 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 2
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
RATS

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 200 PPM EO MALE

ANIMAL 6698 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6706 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6707 13-OCT-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6715 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 3
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

NECROPSY PROTOCOL

MALES

The following tissues were examined at necropsy with no significant lesions observed unless specified on individual animal page:

LIVER	SPLEEN	BRAIN	LUNGS	BLOOD

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 0 PPM MALE

ANIMAL 6727 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6730 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6747 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6750 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6755 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
KIDNEYS
GROSS: DILATED PELVIS
SEVERE, RIGHT
KIDNEYS
GROSS: COLOR CHANGE, FOCAL/MULTIFOCAL
RIGHT, 3/4 TAN, 1/4 RED
MICRO: N O T E X A M I N E D

ANIMAL 6762 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6764 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6766 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6767 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 0 PPM MALE

ANIMAL 6771 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6772 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6781 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6789 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6791 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6806 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6816 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6817 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6834 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6837 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6838 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 0 PPM MALE

ANIMAL 6849 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6857 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6863 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6868 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6870 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6876 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6881 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6882 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 40 PPM MALE

ANIMAL 6724 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6734 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6742 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6768 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6794 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6810 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6822 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6824 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6832 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6839 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 40 PPM MALE

ANIMAL 6847 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6854 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6860 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6878 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 1000 PPM MALE

ANIMAL 6723 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6738 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6746 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6748 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6753 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6763 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6770 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6774 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6792 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6793 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 1000 PPM MALE

ANIMAL 6821 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6845 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6853 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6865 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 3000 PPM MALE

ANIMAL 6729 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6736 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6743 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
SKIN
GROSS: ALOPECIA
CHEST AREA, 10X5 MM
MICRO: N O T E X A M I N E D

ANIMAL 6744 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6749 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6752 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6758 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6769 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6775 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6777 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 3000 PPM MALE

ANIMAL 6782 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6802 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6804 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6805 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6807 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6808 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6815 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6818 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6823 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6835 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6842 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 3000 PPM MALE

ANIMAL 6843 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6856 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6869 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6874 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6879 27-AUG-93 STUDY DAY 11
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6880 20-AUG-93 STUDY DAY 4
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6884 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 200 PPM EO MALE

ANIMAL 6737 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6776 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6783 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6786 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6795 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6796 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6800 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6809 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6811 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6819 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

TABLE 4
ETHYLENE: FOUR WEEK VAPOR INHALATION EXPOSURE OF RATS AND MICE FOR GENETIC TOXICOLOGY ENDPOINTS
MICE

INDIVIDUAL NECROPSY OBSERVATIONS AND/OR MICROSCOPIC DIAGNOSES

GROUP: 200 PPM EO MALE

ANIMAL 6829 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6831 3-NOV-93
TYPE OF DEATH: SHIPPED OFF SITE
GROSS: N O T E X A M I N E D
MICRO: N O T E X A M I N E D

ANIMAL 6848 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

ANIMAL 6859 10-SEP-93 STUDY DAY 25
TYPE OF DEATH: SCHEDULED SACRIFICE
GROSS: EXAMINED - NO SIGNIFICANT LESIONS
MICRO: N O T E X A M I N E D

See necropsy protocol page for list of tissues examined grossly and for explanation of grades.

Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

Protocol and Protocol Deviations

(16 Pages)

UNION CARBIDE

BUSHY RUN RESEARCH CENTER

6702 Mellon Road, Export, Pennsylvania 15632-8902

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PROTOCOL

TITLE: Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

BRRC PROJECT ID: 93N1311

SPONSOR:
CMA Olefins Panel
Chemical Manufacturers Association
2501 M Street, NW
Washington, DC 20037

TESTING FACILITY:
Bushy Run Research Center (BRRC)
Union Carbide Corporation
6702 Mellon Road
Export, PA 15632-8902

Reviewed and Approved by:

Bushy Run Research Center:

Jane S. Vergnes, Ph.D. — Date 8/2/93
Study Director

James D. Sun, Ph.D. — Date 8/2/93
Associate Director

Linda J. Caliguri, B.S. — Date 8/2/93
Manager, Good Laboratory Practices/Quality Assurance

John P. Van-Miller, Ph.D., DABT — Date 8/2/93
Director

Sponsor's Representative:

Elizabeth J. Moran, Ph.D. — Date 8/3/93
CMA Olefins Panel Manager

OBJECTIVE

The objective of this study is to evaluate DNA and hemoglobin adducts, abasic sites, and HPRT mutations in Fischer 344 rats and in B6C3F1 mice which may result from inhalation exposure to ethylene.

Design and Basis for the Study

This study will consist of 3 exposure groups (40, 1000, and 3000 ppm ethylene), an air-exposed control group, and an ethylene oxide-exposed (EO, 200 ppm) control group. Animals will be exposed for 6 hours/day, 5 consecutive days/week as described below.

Seven males/species/group will be exposed to the following test or control concentrations for the designated time periods: air-only control and 3000 ppm ethylene for 1, 2, and 4 weeks; 40 and 1000 ppm ethylene and 200 ppm EO for 4 weeks. Animals will be sacrificed approximately 2 to 4 hours after exposure and selected tissues will be taken and processed according to instructions provided by the University of North Carolina. After processing, tissues will be shipped to the University of North Carolina for determination of DNA and hemoglobin adducts and abasic sites under the direction of Dr. James A. Swenberg.

An additional 7 males/species/group will be exposed for 4 weeks and then held for a recovery period of approximately 5 weeks for rats and 8 weeks for mice. Recovery animals will then be shipped to Dr. Swenberg's research team at the University of North Carolina for evaluation of mutations in the hypoxanthine guanine phosphoribosyltransferase (HPRT) gene.

The determination of DNA and hemoglobin adducts, abasic sites, and HPRT mutations, and any specimen preparation required after collection of specified tissues at necropsy so that such determinations can be made, are not included in this protocol.

The portions of this study conducted at BRRC will be in compliance with the following guidelines and standards, with the exception of those procedures provided by University of North Carolina personnel:

> U.S. Environmental Protection Agency (EPA), Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards, 40 CFR Part 792.
>
> Organisation for Economic Co-operation and Development (OECD) (1981). OECD Principles of Good Laboratory Practice, C(81)30(Final).

The portions of this study conducted at the University of North Carolina are research activities and will not be performed in compliance with existing regulations or generally accepted good laboratory practices.

PERSONNEL

All personnel who participate in the conduct of the study will be documented in the raw data.

PROJECT DATES

Starting Date of Acclimation	August 3, 1993
Proposed Starting Date of Test Substance Administration	August 16, 1993
Proposed Date for Completion of In-Life Phase	November 5, 1993
Proposed Date for Submission of Draft Final Report	November 19, 1993

METHODS

Test Substance

Chemical Name	Ethylene
CAS Registry Number	74-85-1
Source	Matheson Gas Products, Twinsburg, OH, by way of Union Carbide Corporation, S. Charleston, WV
Sponsor Identification Number	E28-0001-A4
BRRC Sample Number	56-319
Description	Colorless compressed gas with a sweet odor and taste, according to the Material Safety Data Sheet (MSDS).
Purity	Purity information will be documented in the raw data and final report.
Stability	Stable
Storage Conditions	Outside, under cover, in a locked enclosure.
Quantity	2 steel cylinders
Reserve Sample	A reserve sample will not be retained at BRRC due to the volatile nature of the test substance.
Safety	An MSDS will be reviewed by all relevant personnel before their participation in the study. This review will be documented. Normal precautions for untested substances will be used. These procedures include the use of disposable Tyvek® or plastic coats or jumpsuits, hats, booties or shoe covers, and rubber gloves while in the animal rooms. Eye protection will include the use of safety glasses at all times.

Disposable Tyvek® coats or smocks and appropriate gloves will be worn during administration of the test substance. In addition, monogoggles will be used when handling the test substance.

Test Animals

Species and Strain	Fischer 344 rats and B6C3F1 mice
Supplier	Rats - Harlan Sprague Dawley, Inc., Indianapolis, IN Mice - Charles River Laboratories, Portage, MI
Rationale	These species and strains were selected by the Sponsor based upon previous studies performed using ethylene and/or ethylene oxide.
Number and Sex	A total of 168 males of each species will be ordered from which 98 males of each species will be selected for the study. Fifty males of each species will be assigned to a separate but concurrent ethylene inhalation study for the same sponsor.
Age	The animals will be requested to be approximately 5 to 6 weeks of age on the scheduled animal receipt date.
Acclimation and Pretest Evaluations	Shortly after their arrival at the laboratory, the animals will be transported to the room selected for the study. Once in the room, the animals will be removed from the shipping cartons and examined. All animals with evidence of disease or physical abnormalities will be discarded and the reason for rejection will be recorded. If an unusually large number of animals shows evidence of disease or physical abnormalities, the entire shipment of animals will be rejected for use in the study. A total of 10 animals (5/species) will be randomly selected for a pretest health screen as discussed below. Additional animals not selected for the study may be held for possible random serology testing. All remaining animals will be housed 2 to a cage in order to acclimate the animals to the automatic watering system. The acclimation period will be approximately 2 weeks. At the time of group assignment, all animals will be housed individually. During the acclimation period, animals will be fed the same diet that will be used during the study. Animals will be observed twice daily for any overt clinical signs of disease or abnormality. Individual detailed physical examinations will be conducted weekly.

Animals showing abnormalities deemed by the Study Director or other appropriate personnel to render the animal unacceptable for placement on the study will be sacrificed and discarded on the day observed, and the reason for sacrifice will be recorded.

The animals will be weighed at least 2 times during the weeks prior to exposure. Any animal whose weight gain during the acclimation period is not considered normal for this age and strain of animal, or whose absolute body weight at the time of the second weighing is outside ± 20% of the population mean for each sex, will not be considered for use in the study.

Pretest Health Screen

A pretest health screen will be initiated within 2 days after the receipt of the animals. The pretest health screen will be performed on 5 animals/species selected directly from the shipping cartons with as many cartons as possible being represented.

Rats

The pretest health screen will consist of examinations for fecal parasites, necropsy, and histopathologic evaluations of selected tissues (3 animals). Serology testing for Sendai virus, rat coronavirus/ sialodacryoadenitis virus, and Mycoplasma pulmonis will be performed approximately 2 weeks after receipt using animals not selected for the study. Serology results will be available prior to the start of the study.

Additional animals not selected for the study may be held for possible random serology testing. The following organisms will be included in serologic testing conducted periodically throughout the facility:

Sendai virus (SEND)
Pneumonia virus of mice (PVM)
Rat coronavirus/Sialodacryoadenitis virus (RCV/SDA)
Kilham rat virus (KRV)
Toolan's H-1 virus (H-1)
Reovirus type 3 (REO3)
Mycoplasma pulmonis (MPUL)
Mouse polio virus (GD-7)
Lymphocytic choriomeningitis virus (LCMV)
Mouse adenovirus FL/K87 (MAD)
Minute virus of mice (MVM)
Polyoma virus (POLY)

Mice

The pretest health screen will consist of a serology screen, examinations for fecal parasites, necropsy, and histopathologic evaluations of selected tissues (3 animals).

The following organisms will be included in serologic testing:

Sendai virus (SEND)
Pneumonia virus of mice (PVM)
Mouse hepatitis virus (MHV)
Minute virus of mice (MVM)
Mouse polio virus (GD-7)
Reovirus type 3 (REO-3)
Mycoplasma pulmonis (MPUL)
Lymphocytic choriomeningitis virus (LCMV)
Mouse adenovirus FL/K87 (MAD)
Ectromelia virus (ECTRO)
Mouse pneumonitis virus (K)
Polyoma virus (POLY)
Epizootic diarrhea of infant mice virus (EDIM)
Mouse cytomegalovirus (MCMV)
Hantann virus (HANT)
Encephalitozoon cuniculi (ECUN)

Fecal examination for parasites will be conducted using a cellophane tape test as a prestudy screen and by zinc sulfate flotation from cecal contents obtained at necropsy.

Histopathology will consist of examination of at least the following tissues: liver, kidneys, trachea, lungs, heart, spleen, salivary glands, submandibular lymph nodes, and nasal cavities.

The purpose of this screen is to determine the suitability of the population of animals proposed for this study. Therefore, the results of this screen will be available to the Study Director before the study begins.

All animals will be examined by a veterinarian shortly after their arrival and again prior to study start. The dates of the examinations will be documented in the raw data.

Identification

Each animal will be assigned a unique identification number prior to the initiation of the study. Rats will be identified by a cage tag and tail tattoo. Mice will be identified by a cage tag and toe-clip. Animals sacrificed for use in the pretest health screen will be identified by cage tags only until sacrifice and then toe-clipped for further identification. Records will be kept documenting the fate of all animals received for the study.

Husbandry

Conditions

All animals will be housed in an appropriate animal room at BRRC from arrival until termination of the in-life phase of the study. Stainless steel cages with wire mesh floors will be used throughout all phases of the study. Cages will be changed and sanitized at least once every 2 weeks. DACB® (Deotized Animal Cage Board; Shepherd Specialty Papers, Inc.) will be changed at least 3 times each week.

Cages housing EO-exposed animals will not be placed on the same carriers as cages housing air-only or ethylene-exposed animals. Carriers of EO-exposed animals will not be in close proximity to carriers of air-only or ethylene-exposed animals.

The animal room concentrations of EO will be determined by a gas chromatographic technique after exposure on Study Day 1. Samples will be taken in the breathing zones of the EO-exposed animals and the ethylene-exposed animals.

Temperature and humidity will be recorded continuously using an automatic recorder. Temperature will be maintained at 66-77°F and relative humidity will be maintained at 40-70%. The temperature and humidity will be checked by a technician at each room check and a record will be kept indicating that it was done. Appropriate corrective action will be taken whenever readings outside the specified limits are observed.

The accuracy of the temperature and humidity recording devices will be checked periodically and calibrated when necessary. The verification and calibration data will be recorded. In the event that continuous recording cannot be maintained, the temperature and humidity will be manually recorded at each room check.

An automatic timer will be set to provide fluorescent lighting for a 12-hour photoperiod (approximately 0500 to 1700 hours for the light phase). In the animal room, there will be at least 10 air changes each hour.

Diet

Pelleted, certified AGWAY® PROLAB® Animal Diet Rat, Mouse, Hamster 3000 (Agway Inc.) will be available ad libitum except during exposures. The analyses of chemical composition and possible contaminants of each batch of diet will be performed by Agway Inc., and the results of the analyses will be reviewed by the Study Director.

Water	Tap water (Municipal Authority of Westmoreland County, Greensburg, PA) will be available ad libitum, except during exposures, by an automatic watering system with demand control valves mounted on each rack. Water pressure and function of the individual cage rack systems will be checked at each room check, and a record will be kept indicating it was done. Drinking water contaminant levels will be measured at regular intervals according to EPA specifications, to include the "priority" pollutants, and will comply with human drinking water requirements. The results of the analyses will be reviewed by the Study Director.

Administration of Test Substance

Route and Justification	The route of administration will be by inhalation. This is considered to be a meaningful way to evaluate the toxicity of chemicals with the use pattern of the test substance. The inhalation route is also a potential route of human exposure.
Target Exposure Concentrations	Target exposure concentrations of the test substance will be 0 (filtered air only), 40, 1000, and 3000 ppm ethylene, as selected by the Sponsor. The target exposure concentration of the EO control will be 200 ppm, as selected by the Sponsor.
Test Substance Analysis	Prior to initiation of the study and following the study, samples of ethylene and EO will be drawn, and compositional analyses will be performed by the GLP Analytical Skills Center at the UCC South Charleston, WV, Technical Center. Compositional analyses of ethylene will include levels of EO.
Exposure Chambers	Five stainless steel chambers with glass doors and windows for animal observation will be used. The total "volume" of the animals will not exceed 5% of the volume of the test chamber. Chambers will be provided with air at a flowrate of approximately 13-14 air changes each hour to ensure an adequate oxygen content of at least 19%. The rate of airflow will be monitored continuously and recorded approximately every 30 minutes. All chambers will be maintained at a slight negative pressure to prevent any test substance or EO from entering the room containing the chambers. The temperature and relative humidity of the exposure chambers will be monitored continuously and recorded approximately 12 times during each exposure. If possible, temperature will be maintained at 22 ± 2°C, and relative humidity will be maintained between 40 to 60%. In case of undetected differences in environment

or test substance concentration within the chamber, all exposure cage positions will be rotated daily. A description of the rotation will be provided in the raw data.

The oxygen content of the chambers will be monitored prior to initiation of the study.

Duration of Treatment

Animals will be exposed for 6 hours/day, 5 consecutive days/week, for 4 weeks, with the following exceptions. Seven males/species/group in the air-only and high concentration groups designated for analysis of DNA and hemoglobin adducts and abasic sites will receive their final exposures on Study Days 5 and 12.

Animals will be acclimated to the exposure chamber (mock exposure) for 2 days during the week prior to initiation of exposures.

Generation of Test and EO Control Substances

The test and EO control substances will be generated by the dilution of rotameter-controlled ethylene or EO streams with chamber air to the desired concentration.

Chamber Atmosphere Sampling

Chamber concentrations of the test substance will be determined approximately every 60 minutes (contingent on analytical limitations) by a gas chromatographic technique. Chamber concentrations of EO will also be determined in air-only and ethylene chambers at least once weekly. The air-only and EO chamber atmospheres will also be analyzed approximately every 60 minutes. Chamber probes for sampling will be placed in the breathing zone of the animals. The daily nominal (estimated) chamber concentration will also be determined.

Chamber Distribution

The uniformity of the vapor concentration in each of the 3 exposure chambers and in the EO control chamber will be examined. Each chamber will be tested prior to initiation of the study. For each individual distribution test, vapor concentrations will be measured at 5 positions.

EO Control

At the Sponsor's request, one group of animals will be exposed to EO (CAS No. 75-21-8) as previously described.

Study Design

Group Assignment

Based on the second pretest body weights, the animals will be selected for the study from the remaining population. These animals will be divided into 5 experimental groups. Each group size is shown below and will be based on a weight randomization procedure.

Animals not selected for the studies will remain housed in the study room until the studies begin and will be used as replacements in the event that any of the selected animals dies prior to the start of the studies. After the selected animals have received their first exposure, all animals not selected for the studies will be humanely sacrificed and discarded, used for training of BRRC staff, used for random serology testing, or used for methods development, and no further replacement of animals will be made. The fate of all animals not selected for use in the studies will be documented in the raw data.

Following body weight measurement just prior to the first exposure, statistical evaluation of the body weights for all groups will be conducted, and statistical equivalence and homogeneity of variance will be examined. In the event that either criterion is not met, changes will be made prior to the initial exposure to ensure statistically equivalent body weights for all groups. Animals with any abnormal clinical signs will also be replaced prior to exposure and the statistical criterion reevaluated.

Organization

Group	Exposure Concentrations (ppm)	Number of Animals/ Species/ Week		
		1	2	4*
Air-Only Control	0	7	7	14
EO Control	200	0	0	14
Low	40	0	0	14
Mid	1000	0	0	14
High	3000	7	7	14

*7 animals/sex are for adducts/abasic sites, 7 animals/sex are for HPRT mutation analysis.

Experimental Evaluations

Mortality Checks and Clinical Signs

All animals will be observed for mortality and overt signs of toxicity twice each day, 7 days a week. On exposure days, the animals will be observed just prior to exposure and shortly following the exposure. On nonexposure days, the first daily check will generally be conducted before 9:00 a.m., and the second mortality check will be conducted after noon. Animals will also be observed on a group basis during the 6-hour exposure. Should mortality and/or signs of overt toxicity be observed, it will be recorded on the

day observed. Overt signs will also be recorded on subsequent days until the sign disappears or the animal dies. Detailed examinations for clinical signs of disease or abnormality, which involve animal handling, will be conducted once weekly (a.m.) during the study.

Sacrifice of Distressed Animals

If any animal shows signs of extreme distress or is moribund, it will be sacrificed for humane reasons before the scheduled date.

Body Weight

Individual body weights will be measured weekly. Body weight gains will be computed.

Statistical Evaluations

The data for quantitative continuous variables will be intercompared for the dose and control groups by Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests will be used following a significant ANOVA to delineate which groups differ from the control group. If Levene's test indicates homogeneous variances, the groups will be compared by an ANOVA for equal variances followed, when appropriate, by pooled variance t-tests. If Levene's test indicates heterogeneous variances, the groups will be compared by an ANOVA for unequal variances followed, when appropriate, by separate variance t-tests. For nonparametric data, the Kruskal-Wallis test followed, when appropriate, by Mann-Whitney U-tests, will be used. Incidence data will be compared using the appropriate statistical test, generally Fisher's Exact Test. Statistical analyses will be performed using either BMDP Statistical Software or other statistical programs, as deemed appropriate (Dixon, 1990; Sokal and Rohlf, 1981). The probability value of less than 0.05 (2-tailed) will be used as the critical level of significance for all tests.

Special Evaluations

At the request of the Sponsor, animals designated for evaluation of DNA and hemoglobin adducts and abasic sites will be sacrificed by BRRC personnel and the following tissues will be taken: brain, liver, lung, spleen, and blood. After processing according to procedures provided by University of North Carolina personnel, the tissues will be shipped to the University of North Carolina for evaluation.

Recovery Phase

Rats designated for analysis of HPRT mutations will be housed at BRRC for approximately 5 weeks after termination of the exposure regimen. Mice designated for analysis of HPRT mutations will be housed at BRRC for approximately 8 weeks after termination of the

exposure regimen. At the request of the Sponsor, recovery phase animals will be shipped to the University of North Carolina at the end of the recovery phase.

ALTERATION OF PROTOCOL

Alterations to this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such change will be honored. However, it then becomes the responsibility of the Sponsor to follow such verbal change with a written verification. BRRC reserves the right to revise the protocol or deviate therefrom solely at the discretion of the Study Director if prior approval of the Sponsor cannot be obtained and the integrity of the study is considered in jeopardy. In this event, the Sponsor will be notified of the alteration as soon as possible, and documentation of the change will be the responsibility of the Study Director.

RETENTION OF RECORDS

All raw data, documentation, the protocol and any amendments, and a copy of the final report generated by BRRC as a result of this study will be retained in the BRRC Archives for at least 10 years.

Following the retention period specified above, the Sponsor will be contacted and given the option of taking receipt, destroying, or arranging for other storage of the data and materials. All data and materials mentioned above will remain the sole property of the Sponsor and can be removed from BRRC at the Sponsor's discretion.

REPORTS

Draft Final Report

An audited draft of the final report will be submitted to the Sponsor approximately 2 months after the completion of the terminal sacrifice. This report will be a comprehensive report which will include all information necessary to provide a complete and accurate description and evaluation of BRRC test procedures and results. It will include: a summary; appropriate text discussions of the experimental design, materials and methods, and results; and summary mean or incidence tables of in-life data. In addition, it will contain appendices with individual animal data and other pertinent information. One bound and 1 unbound copy of the draft report will be provided to the Sponsor.

Final Report

The draft final report will be reviewed by the Sponsor, and comments on the report will be provided to BRRC within 4 weeks from the date of submission of the draft version. BRRC will consider these comments in preparing the final report. Assuming the Sponsor's comments are received at the specified time and no major revisions are required, BRRC will submit a final report within 8 weeks of issuance of the draft report.

The final report will be audited by the Quality Assurance Unit and contain a signed quality assurance statement. It will conform to the formatting specifications of EPA PR notice 86-5. One bound and 1 unbound copy of the final report will be submitted to the Sponsor.

ANIMAL USE POLICY

It is the goal of BRRC, through the establishment and activities of the Institutional Animal Care and Use Committee, to comply with the U.S. Animal Welfare Act and the subsequent rules promulgated by the U.S. Department of Agriculture and in effect on the date of this protocol. It has been determined that the work described herein minimizes the number of animals used, is necessary, and uses the most appropriate species and strain in order to provide meaningful results and the most useful information for comparative purposes relative to previous studies. Furthermore, this study will be conducted humanely, and to the best of our knowledge, neither unnecessarily duplicates any previous work, nor can it be accomplished using currently available, validated nonanimal models.

GOOD LABORATORY PRACTICE COMPLIANCE

BRRC, through the administration of a quality assurance program by the Good Laboratory Practice Committee and Quality Assurance Unit, assures compliance of all phases of studies conducted at BRRC with existing regulations and generally accepted good laboratory practices.

The study will be subjected to periodic inspections and the final report will be reviewed by the BRRC Quality Assurance Unit.

REFERENCES

Dixon, W. J. (1990). BMDP Statistical Software. University of California Press, Berkeley, CA.

Sokal, R. R. and Rohlf, F. J. (1981). Biometry, 2nd Edition, W. H. Freeman and Co., San Francisco, CA.

PROTOCOL DEVIATIONS

TITLE: Ethylene: Four Week Vapor Inhalation Exposure of Rats and Mice for Genetic Toxicology Endpoints

BRRC PROJECT ID: 93N1311

Item 1

Location of Deviation	Page 4, Acclimation and Pretest Evaluations
Description of Deviation	Rats were housed individually during the acclimation period instead of 2 to a cage. Mice were also placed in individual housing on August 6 and 7, 1993.

Item 2

Location of Deviation	Page 4, Acclimation and Pretest Evaluations
Description of Deviation	No animal observations were recorded on August 4, 1993.

Item 3

Location of Deviation	Page 4, Acclimation and Pretest Evaluations
Description of Deviation	Mortality checks were conducted twice daily until the animals were identified. Then animals were observed once daily for overt signs of disease or abnormality and an afternoon mortality check was conducted daily during the acclimation period.

Item 4

Location of Deviation	Page 6, Identification
Description of Deviation	The toe-clipping procedure used to identify the mice was supplemented with ear notches when the mice could not be uniquely identified by toe-clipping alone.

Item 5

Location of Deviation	Page 7, Husbandry, Conditions
Description of Deviation	Cages were not changed and sanitized from August 13, 1993 to September 1, 1993.

Item 6

Location of Deviation	Page 7, Husbandry, Conditions
Description of Deviation	Several gaps in the continuous recording of temperature and humidity occurred during the study.

Item 7

Location of Deviation	Page 7, Husbandry, Conditions
Description of Deviation	Verification of temperature and humidity checks is not available for each room check.

Item 8

Location of Deviation	Page 8, Exposure Chambers
Description of Deviation	There were approximately 20 air changes each hour in the 40 ppm ethylene chamber.

Item 9

Location of Deviation	Page 9, Generation of Test and EO Control Substances
Description of Deviation	Flowmeters were used, rather than rotameters, to generate ethylene and EO.

Item 10

Location of Deviation	Page 10, Experimental Evaluations, Mortality Checks and Clinical Signs
Description of Deviation	Overt observations were not recorded on the afternoons during weekends. Overt observations were not recorded on the afternoon of October 4, 1993 for several animals of each species. Overt observations were not recorded on the afternoon of October 8, 1993 for any of the animals.

These deviations are not expected to compromise the integrity of the results and conclusion of the study.