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Microfiche No.	OTS0574013		
New Doc ID	89000000174	Old Doc ID	8EHQ-0300-14662
Date Produced	03/13/00	Date Received	03/31/00
		TSCA Section	8E
Submitting Organization	EASTMAN CHEM CO		
Contractor	EASTMAN CHEM CO		
Document Title	SUPPORT: FINAL REPORT, CYCLOPROPYL METHYL KETONE - A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT, WITH COVER LETTER DATED 03/24/00		
Chemical Category	CYCLOPROPYL METHYL KETONE		

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P. O. Box 431
Kingsport, Tennessee 37662

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Ladies and Gentlemen:

Eastman Chemical Company submits the following reports as required under TSCA §8(e) for your consideration.

Cyclopropyl Methyl Ketone - A Four-week Inhalation Toxicity Study in the Rat

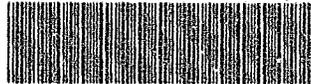
The 8(e) reference number for this substance is 8EHQ-00-14662. A preliminary report on this study was submitted by Eastman Chemical Company on February 15, 2000.

If you have questions, you may contact me by telephone at (423) 229-1654.

Very truly yours,

Karen R. Miller

Karen R. Miller
Principal Technical Representative
Product Safety & Stewardship



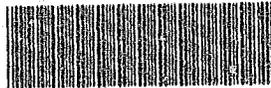
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Page 1 of 111

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FINAL REPORT

CYCLOPROPYL METHYL KETONE
SYNONYM: 1-CYCLOPROPYLETHANONE,
METHYL CYCLOPROPYLKETONE, MCPK, CPMK

HAEL No.: 1999-0215

EAN: 905571

CAS No.: 000765-43-5

PM No.: 20644-00

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

GUIDELINE

OECD: TG-412
EEC: Annex V., Test B.8

AUTHOR

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TESTING FACILITY

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

LABORATORY PROJECT ID

99021511

STUDY SPONSOR

Eastman Chemical Company
P.O. Box 431
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STUDY COMPLETION DATE

March 13, 2000

QUALITY ASSURANCE INSPECTION STATEMENT

(21CFR58.35(B)(7), 40CFR792.35(B)(7), and 40CFR160.35(B)(7))

Study: 99-0215-1
Accession Number: 905571
Study Director: David, R.M..Page 1
2/1/2000

Study Type: Repeated Inhalation (4-week)

M. James
(Auditor, Quality Assurance Unit)2/1/2000
Date

This study was inspected by one or more persons of the Quality Assurance Unit. Written status reports were submitted on the following dates:

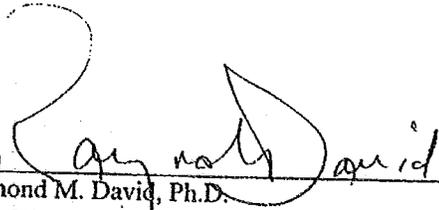
<u>Inspection Dates</u>	<u>Phase(s) Inspection</u>	<u>Status Report Dates</u>
10/1/1999	Protocol Submission	
10/8/1999	Clinical Signs During Dose Chamber Airflow/Temperature/Relative Humidity Readings 5 Hr Clinical	
11/3/1999	Bleeding Necropsy Specimen/Sample Weight Specimen Collection	11/3/1999
12/20/1999	Records Review Chamber Concentration Analysis Temperature, Humidity, Airflow, and Organ Weights	12/21/1999
1/26/1999	Records Review Gross Pathology, Histopathology, Pathology Report Clinical Chemistry, Hematology, Cell Morphology	1/26/1999
2/1/2000	Final Report Review	2/1/2000

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted according to:

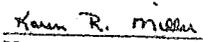
United States Environmental Protection Agency, Toxic Substances Control Act,
Good Laboratory Practice Standards, 40 CFR Part 792;

Organisation for Economic Cooperation and Development, Principles of Good
Laboratory Practice (as revised in 1997) [(C97)186/Final].



Raymond M. David, Ph.D.
Study Director

3/13/2000
Month/Day/Year



Karen R. Miller, Ph.D.
Sponsor's Representative

3/09/2000
Month/Day/Year

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ABSTRACT**CYCLOPROPYL METHYL KETONE
SYNONYM: 1-CYCLOPROPYLETHANONE,
METHYL CYCLOPROPYLKETONE, MCPK, CPMK**HAEL No.: 1999-0215
CAS No.: 000765-43-5EAN: 905571
PM No.: 20644-00**A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT**

Groups of five male and five female Sprague-Dawley rats were exposed to target vapor concentrations of 1.00, 0.30, 0.05, or 0.00 mg/L of the test substance 6 hours per day, 5 days per week, for 22 exposures. The daily time-weighted average (MIRAN) concentrations and the weekly analytical (GC/FID) concentrations were within 10% of the target concentrations for all groups. The temperature and relative humidity inside the chambers during exposure were 19.3 ± 0.4 and $70.0 \pm 1.5\%$, respectively.

All animals survived to study termination. Animals were observed for clinical signs of toxicity prior to exposure, once per hour during exposure, and 30 minutes to one hour after exposure. For the 1.00 mg/L group, reduced amounts of feces observed for one male rat on two days and for all female rats on two to thirteen days, and soft feces observed for one male and one female rat on one day each during the study were likely related to test substance exposure. No other test substance-related clinical abnormalities were observed. Body weights and feed consumption were measured at least weekly. All mean feed consumption, feed utilization, body weights, and body weight gains for male and female rats from all exposure levels were comparable with those of the control groups throughout the study.

At study termination, animals were anesthetized with isoflurane and blood was obtained from the posterior vena cava for clinical chemistry and hematology analyses. Fasted body weight and selected organ weights were measured at necropsy. Selected tissues were collected from all animals. Mean red blood cell counts, hemoglobin concentrations, and hematocrit values were higher ($p \leq 0.05$) for the 1.00 mg/L male group, mean white blood cell counts and lymphocyte counts were lower ($p \leq 0.05$) for the 1.0 and 0.3 mg/L female groups, and mean atypical lymphocyte counts were higher ($p \leq 0.05$) for the 0.3 mg/L female groups when compared with the control group. Macrocytosis was observed for male rats from the 1.00 and 0.30 mg/L groups. Evaluation of blood cell morphology did not suggest any other test substance-related effects. All other hematologic parameters for rats from all exposure levels were comparable to control groups. Mean urea nitrogen levels were higher ($p \leq 0.05$) for the 1.00 mg/L male and female groups and the 0.30 mg/L female groups when compared with the control groups. Mean total bilirubin levels were higher ($p \leq 0.05$) for the 1.00 mg/L male group and mean triglyceride levels were higher for the 1.00 mg/L female group when compared with the control group. All other

clinical chemistry parameters for rats from all exposure levels were comparable to the control groups.

The mean relative liver weight was higher ($p \leq 0.05$) for the 1.00 mg/L male group when compared with the control group. A lower ($p \leq 0.05$) mean brain weight for the 1.00 mg/L female group appeared to be related to a low brain weight observed for a single animal from this group. When the brain weights were analyzed statistically with this animal excluded, the mean weights were comparable among the groups. All other terminal body weight and organ weight for rats from all exposure levels were comparable to the control groups.

Test substance-related changes observed at necropsy were limited to pale livers for the 1.00 mg/L male and the 1.00 and 0.30 mg/L female groups, and of pale hearts for the 1.00 mg/L male and female groups. No other test substance-related gross lesions were observed on necropsy examinations. Histopathologic examination of tissues indicated test substance-related effects in the heart and liver. Heart effects consisted of myocyte vacuolation for the 1.00 mg/L male group and the 1.00 and 0.30 mg/L female groups, myocardial necrosis for the 1.00 and 0.30 mg/L male and female groups, and myocarditis for all exposed male and female groups. Liver effects consisted of hepatocellular cytoplasmic vacuolation for all male and female test substance exposed groups. No other test substance-related changes were observed during the histopathology examinations.

Based on the heart and liver lesions which were observed at 0.05 mg/L, the lowest concentration tested, a no-observed-effect concentration (NOEC) was not determined. In addition, the effect on the heart was considered to be adverse. Thus, a no-observed-adverse-effect concentration (NOAEC) was not identified. Effects on the liver were considered adaptive and not considered to constitute an adverse effect.

STUDY AND TEST SUBSTANCE INFORMATION**Testing Facility**

Toxicological Sciences Laboratory
Health and Environment Laboratories
Eastman Kodak Company
Rochester, New York 14652-6272
USA

Project Participants

Study Director:	Raymond M. David, Ph.D.
Toxicologist:	Lisa G. Bernard, M.S.
Study Technicians:	Reade A. Moulton James F. Murphy, B.S.
Hematologist/Clinical Chemist:	Robert E. Emmons, B.S.
Histopathologist:	Robert H. Garman, D.V.M., DACVP, Consultants in Veterinary Pathology
Analytical Chemist:	Thomas J. Walton, B.S.

Sponsor

Eastman Chemical Company
P.O. Box 431
Kingsport, TN 37662-5280

Sponsor's Representative:
Karen R. Miller, Ph.D.

Test Substance Characterization

Test Substance Name:	Cyclopropyl methyl ketone
Synonym:	1-cyclopropylethanone, MCPK, CPMK
HAEL No.:	1999-0215
EAN No.:	905571
CAS No.:	000765-43-5
PM No.:	20644-00
SRID No.:	8-99Blend
Physical State and Appearance:	Clear, colorless liquid
Source of Test Substance:	Eastman Chemical Company, Kingsport, TN
Laboratory Project ID:	99021511

Study Dates

Study Initiation Date: September 30, 1999
Experimental Start Date: October 4, 1999
Experimental Completion Date: February 26, 2000

Purity, Structure Confirmation, and Stability Determination

The purity of the test substance was determined by gas chromatography with flame ionization detection (GC/FID) to be 99.4% prior to use on the study and 99.6% at study termination. Based on these data, the test substance was considered to be stable during the test period. The structure of the test substance was confirmed using gas chromatography with mass spectroscopy (GS/MS). The mass spectrum of the test substance was consistent with published spectra for this substance. The analytical reports are provided in Appendix C beginning on page 2.

PURPOSE

The purpose of this study was to evaluate the subacute effects of the test substance in rats following repeated inhalation exposures for four weeks.

MATERIALS AND METHODS**Test System**

Five male and five female Sprague-Dawley rats (CD(SD)BR/VAF Plus) obtained from Charles River Laboratories, Stone Ridge (Kingston), NY were randomly assigned to each exposure group. The male and female rats were 48 or 50 days of age and weighed 244.3 ± 10.7 or 179.4 ± 7.8 grams (mean \pm SD), respectively, at the start of the study. Rats were chosen for this study because they are a common representative species for inhalation toxicity studies. Also, the rat is the rodent species recommended for use in the Organisation for Economic Cooperation and Development (OECD) and European Economic Community (EEC) Test Guidelines.

HusbandryHousing

Animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited vivarium in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). During nonexposure

Husbandry, continued

Housing, continued

periods, rats were singly housed in stainless-steel, wire-mesh cages in a room separate from the exposure room. No other study was housed in the same room as this study. Exposure tubes were washed daily. Housing cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed daily.

Environmental Conditions

The study room was maintained at 21.1 - 24.1°C and 40.2 - 65.7% relative humidity. A photoperiod of 12 hours light from ~6 a.m. to ~6 p.m. was maintained.

Acclimation Period

The animals were isolated upon arrival and allowed to acclimate for a period of at least five days prior to assignment to this study. Animals were judged to be healthy prior to testing.

Feed

Certified Rodent Diet [Purina Rodent Chow #5002, meal (PMI Feed, Inc. Richmond, IN)] was available *ad libitum* except during exposure. Feed containers were cleaned and refilled at least once a week. No known contaminants which would interfere with the outcome of this study were present in the feed. Analyses of feed are maintained on file within the testing laboratory.

Water

Water was available *ad libitum*, except during exposure, through an automatic watering system. The source of the water was the local public water system. There have been no contaminants identified in periodic water analyses that would be expected to interfere with the conduct of the study. Semiannual analyses of water are maintained on file within the testing laboratory.

Identification

Upon arrival, all rats were identified by uniquely-numbered metal ear tags. Ear tags which were lost during the study were replaced. During randomization, study-specific animal numbers were assigned to each animal. Cage cards, color-coded for each group, contained the study-specific animal number and the ear tag number.

Experimental Design

Randomization

The test animals were culled from the stock population based on body weight and randomly assigned to groups using a stratified randomization program. Variation among the body weights of individual animals in the culled population did not vary more than 20% from the mean for each sex.

Test Guideline

This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals: Guideline 412, Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (Adopted May 12, 1981) and European Economic Community (EEC), Annex V., Test B.8. Repeated Dose (28 Days) Toxicity (Inhalation), as required by Council Directive 92/69/EEC, July 31, 1992.

Selection of Exposure Concentrations

Exposure concentrations were selected by the Sponsor based on test results of a one-week probe study during which 5 male and 5 female rats per group were exposed to 2.0, 1.0, 0.5, or 0.0 mg/L of the test substance 6 hours per day for 6 consecutive week days (Wednesday to Wednesday). All exposure conditions were as described below. Based upon myocardial vacuolation and cardiomyopathy observed for the 0.5 mg/L male and female rats, reduced feed consumption and body weight gain observed for the 0.5 mg/L male rats, and elevated urea nitrogen levels and decreased bone marrow cellularity observed for the 0.5 mg/L female rats, test substance concentrations of 1.00, 0.30, 0.05, and 0.00 mg/L were selected for the four-week study. The results of the one-week inhalation probe study were reported in a separate report (TX-99-131).

Exposure

The inhalation exposures were conducted in flow-past nose-only units (CH Technologies, Westwood, NJ) at target vapor concentrations of 1.00, 0.30, 0.05, or 0.00 mg/L. Animals were singly housed in restraint tubes during the 6-hour exposures. The animals were acclimated to the restraint tubes for three hours each day on the two weekdays immediately prior to the initiation of exposure. A picture of the exposure unit and placement of restraint tubes within the unit is provided in Appendix A. The exposure units were maintained under positive pressure relative to room air. The air flow, temperature, and humidity of the supply air were recorded approximately once every 30 minutes. Chamber vapor concentrations were recorded at least once each hour.

Experimental Design, continuedExposure Atmosphere Generation

The test atmosphere was generated by passing compressed air over the surface of the test substance to evaporate it. The resultant vapor was directed via Teflon tubing to a tee just upstream of the inhalation chamber where it was mixed with additional filtered, conditioned outside air to produce a total airflow of 9.7 to 15.0 Lpm (96.7 to 150.0 air changes per hour). The air flow rates were adjusted to produce the desired chamber target vapor concentration. The resultant vapor was directed via Teflon tubing to the nose-only unit. A diagram of the generation system is provided in Appendix A. A Micro Laser Particle Counter (model μ LPC-301, Particle Measuring Systems, Inc., Boulder, CO) was used to measure the number and size of particulates in the chamber. The results indicated that an aerosol of the test substance was not present.

Weekly GC/FID Vapor Concentration Determination

Once each week (Days 1, 8, 15, 22, and 29), samples of chamber test atmosphere were collected into Tedlar bags. These samples were analyzed using a GC/FID. The results of these analyses are reported in Appendix C beginning on page 26.

Daily MIRAN Vapor Concentration Determination

Chamber vapor concentrations were monitored with a multipositional air sampling and analysis system. The system consisted of a single MIRAN IA infrared gas analyzer (Wilks Foxboro Analytical, South Norwalk, CT) and a computer-operated four-port sampling valve (Valco Instruments, Houston, TX).

Chamber vapor samples were continuously collected from each chamber through TEFLON tubing (0.48 mm i.d.). The valve position was periodically changed to sample from each chamber at least once each hour. The voltage output of the MIRAN and chamber concentrations were printed in real-time and captured on electronic media. Voltage data were converted to concentration by linear interpolation between the calibration data points immediately on each side of the sampled data. A time-weighted average exposure concentration was calculated using the following formula:

$$TWA = \frac{\sum(T_2 - T_1)(C_1 + C_2)/2}{\sum(T_2 - T_1)}$$

where: TWA = time-weighted average exposure concentration (mg/L)
 T₁ = the earlier time from each consecutive concentration determination (increment from 1 to n-1)
 T₂ = the later time from each consecutive concentration determination (increment from 2 to n)
 C₁ = the concentration at time T₁
 C₂ = the concentration at time T₂

Experimental Design, continuedMIRAN IA Infrared Analyzer Operating Parameters And Calibration

The infrared analyzer operating parameters were as follows:

MIRAN No.	3
Pathlength (m)	14.25
Wavelength (μm)	8.6
Slit width (mm)	1
Response Time (sec)	4
Range (Absorption)	1A
Gain	x10
Cell Temperature ($^{\circ}\text{C}$)	25
Cell Pressure (atm)	0.833
Cell Volume (L)	5.64

The wavelength used for monitoring concentration was selected based on a comparison of infrared spectra of the test substance to that of air.

The infrared analyzer was calibrated by making serial injections [Hamilton microliter syringe (Hamilton Company, Reno, NV)] of the test substance into a closed-loop cell. The concentration was determined using the following formula:

$$C = \frac{(V_1)(\rho)}{(V_2)(P)}$$

where: C	=	concentration (mg/L)
V ₁	=	Injection volume (μL)
ρ	=	test substance density (g/mL)
V ₂	=	MIRAN cell volume (5.64 L/1 atm)
P	=	Cell pressure (atm)

Three sets of serial injections were made to produce a mean calibration curve of test substance concentration versus infrared analyzer output voltage.

An infrared analyzer calibration check was performed just prior to each exposure by injecting a measured amount of the test substance into the MIRAN closed loop. The infrared analyzer output voltage was converted to the test substance concentration and compared to the calculated expected concentration. If the variation of the calibration concentrations were within 10% of that expected, the calibration was accepted.

Experimental Design, continued

Nominal Concentration Determination

The nominal concentration was calculated by dividing the amount of test substance consumed from the reservoir (determined gravimetrically) by the total chamber air flow using the formula:

$$NC = \frac{(G)(C)}{(V)(T)}$$

where: NC = Nominal concentration (mg/L)
G = Amount of test substance vaporized (grams)
C = Conversion from g to mg
V = Mean chamber air flow (Lpm)
T = Length of exposure (minutes)

Chamber Vapor Homogeneity

A test to determine variations in concentration at different positions within the exposure chambers was conducted. The concentration of the test substance in the air from the breathing zones of restraint tubes 1, 4, 7, and 10 was sampled as described on Page 15 under Daily Miran Vapor Concentration Determination and compared to the concentration at a fixed reference position. Based on deviations from the reference position of less than 10%, the chamber atmosphere was considered to be homogeneous.

Air Flow Measurement

Total chamber air flow was a combination of filtered, compressed air, which was used to vaporize the test substance and to carry the vapor from the generation system to the inhalation chamber, and additional filtered, compressed air dilution air. The air flow rate was continuously monitored using calibrated flowmeters (Gilmont Instruments, Barrington, IL).

Oxygen Level

During pre-study tests, the oxygen content of the chamber exposure atmosphere was measured at an animal port using an MAS MiniOX Monitor (MAS Instrument Division, Pittsburgh, PA). The oxygen content of the chamber exposure atmosphere was $\geq 20\%$.

Chamber Temperature And Humidity

The temperature and humidity of the air supplied to the chambers was measured using an Omega Digital Thermo-Hygrometer (Omega Engineering, Inc., Stamford, CT) and was recorded approximately every 30 minutes during exposure.

Experimental Design, continuedDisposition of Groups

Animals were distributed into groups as follows:

Group	Exposure Concentrations	Number of Animals	Animal Numbers	
			Males	Females
1	Control / 0.00 mg/L	5 Males & 5 Females	301 - 305	321 - 325
2	Low / 0.05 mg/L	5 Males & 5 Females	306 - 310	326 - 330
3	Mid / 0.30 mg/L	5 Males & 5 Females	311 - 315	331 - 335
4	High / 1.00 mg/L	5 Males & 5 Females	316 - 320	336 - 340

Animals were exposed 6 hours per day, five days per week (Monday to Friday), for four consecutive weeks, and for an additional two days (Monday and Tuesday) of the fifth week. All surviving animals were euthanized and necropsied on the day following the last exposure.

Clinical Observations

Rats were observed hourly during exposure for clinical signs of toxicity. However, due to the restraint of the animals in tubes, the during-exposure observations were limited to changes in respiration, eyes, and mucous membranes.

Clinical examinations (hands-on) were conducted after each exposure, each weekend morning, and in the morning prior to necropsy. Moribundity and mortality observations were conducted prior to each exposure. On weekends, afternoon observations were not performed. Observations included, but were not limited to, examination of the hair, skin, eyes, mucous membranes, motor activity, feces, urine, respiratory system, circulatory system, autonomic nervous system, central nervous system, and behavior patterns.

Body Weight and Feed Consumption Determinations

Body weights were measured, prior to exposure, on Days 0, 7, 14, 21, and 28. Feed consumption was measured, prior to exposure, on Days 7, 14, 21, and 28. Animals were fasted the day prior to necropsy. Terminal body weights were measured after exsanguination, but prior to necropsy.

Experimental Design, continued

Blood Collection and Euthanasia

Animals were fasted overnight beginning after the last exposure. The following day, animals were anesthetized with Isoflurane (Henry Schein, Melville, NY) and blood was collected from the posterior vena cava. The blood was placed into vacutainer tubes and allowed to clot for analyses of serum. Other tubes containing an anticoagulant were used for analyses of whole blood samples. Blood smears were also prepared for blood cell counts. Following blood collection, the animals were euthanatized by exsanguination. Animals were bled and euthanatized in a random order based on a computer-generated list.

Hematology and Clinical Chemistry Examinations

Clinical pathology assays were conducted using the following instruments: Roche Analytical Instruments Cobas Fara II serum chemistry analyzer (Roche Diagnostic Systems, Nutley, NJ), Technicon H-1 System hematology analyzer (Bayer Corporation, Diagnostics Division, Tarrytown, NY), Helena Laboratories Titan Gel Electrophoresis System [Helena Laboratories, Beaumont, TX (A/G ratio and albumin)], BBL Fibrosystems analyzer [BBL division of Becton, Dickinson and Company, Cockeysville, MD (prothrombin times)], and NOVA CRT5 [NOVA Biomedical Corporation, Waltham, MA (sodium and potassium)].

Hematology tests included: hemoglobin concentration, hematocrit, red blood cell count, white blood cell count, red blood cell indices, prothrombin time, and platelet count. Slides containing blood smears were examined for cellular morphology and differential white blood cell count. Clinical chemistry tests included: alanine aminotransferase, sorbitol dehydrogenase, creatinine, urea nitrogen, glucose, total bilirubin, total protein, albumin, albumin/globulin ratio, total cholesterol, triglycerides, calcium, phosphorus, sodium, and potassium.

Necropsy

Following exsanguination, the animals were weighed and necropsied. The following tissues were fixed in 10% buffered formalin: nasal passages, trachea, larynx, lungs, heart, stomach, duodenum, jejunum, ileum, cecum, colon, liver, salivary glands, kidneys, urinary bladder, adrenal glands, thymus, thyroid gland, spleen, sternum (with bone marrow), mesenteric lymph nodes, cervical lymph nodes, brain (including sections of medulla/pons, cerebellar cortex, and cerebral cortex), sciatic nerve, cervical spinal cord, ovaries, vagina, uterus, Fallopian tubes, testes, epididymides, male accessory sex glands, and gross lesions.

Experimental Design, continuedOrgan Weights

The lungs, liver, kidneys, adrenal glands, spleen, heart, thymus, brain, testes, and epididymides were weighed. Paired organs were weighed together.

Histopathology

For the 1.00 and 0.00 mg/L groups, all tissues were embedded in paraffin and sectioned at 4 μ m. The nasal passages and sternum were decalcified prior to being embedded and sectioned. The lungs were sectioned along a plane to allow visual examination of the major bronchi and bronchioles. The resulting tissue sections were stained with hematoxylin and eosin (H&E) stains and examined for histopathology. The liver and heart from the 0.30 and 0.05 mg/L male and female groups and the bone marrow and sternum from the 0.30 and 0.05 mg/L female groups were also examined microscopically.

Data Storage

The final report, tissues, paraffin blocks, slides, data sheets, all nonperishable raw data, and an aliquot of the test substance have been stored in the testing facility archive managed under GLP-mandated conditions.

Calculations and Statistical Procedures

Mean values were calculated for time-weighted average atmospheric concentration, chamber temperature, chamber relative humidity, chamber airflow, chamber nominal concentration, body weight, body weight gain, feed consumption, feed utilization, serum chemistries, hematology values, organ weights, and organ-to-body weight ratios. Body weight, body weight gain, feed consumption, feed utilization, clinical pathology, and organ weight data were evaluated using Bartlett's test ($p \leq 0.01$), one-way analysis of variance (ANOVA) ($p \leq 0.05$), and Dunnett's t-test ($p \leq 0.05$) to indicate statistical significance (MINITAB Statistical Software, State College, PA). When the variances of the means were not considered equal by the Bartlett's test ($p \leq 0.01$), the data were evaluated using a Kruskal-Wallis H-test ($p \leq 0.05$) followed by a Mann-Whitney U-test ($p \leq 0.05$) (MINITAB Statistical Software, State College, PA).

Protocol and Standard Operating Procedure Deviations

There were no SOP or protocol deviations during the study.

RESULTS

Exposure Conditions

A summary of exposure conditions is presented in the summary tables. The mean weekly analytical (GC/FID) concentrations (\pm standard deviation), of the test substance in air test atmospheres were 1.02 ± 0.060 , 0.33 ± 0.014 , and 0.05 ± 0.003 mg/L compared with target concentrations of 1.00, 0.30, and 0.05 mg/L, respectively. The analytical report for concentration verification can be found in Appendix C beginning on page 26. The means of daily time-weighted average (MIRAN) concentrations (\pm standard deviation) for each exposure were 1.02 ± 0.021 , 0.31 ± 0.013 , and 0.05 ± 0.003 mg/L for the same groups. Both the mean weekly analytical (GC/FID) and mean daily time-weighted average (MIRAN) concentrations were within 10% of the target concentrations. Nominal concentrations were 1.19 ± 0.066 , 0.38 ± 0.038 , and 0.07 ± 0.016 mg/L for the same groups. No test substance was detected in the control chamber. Mean temperature and relative humidity of the incoming air were 19.3 ± 0.4 and $70.0 \pm 1.5\%$, respectively. Daily mean values for each exposure are provided in Appendix A.

Mortality

No mortality occurred during the study.

Clinical Observations

Clinical signs are summarized on pages 29 - 33 (male) and 34 - 38 (female). Individual animal data are presented in Appendix A.

Porphyrin discharges around the eyes and/or nose were observed for all groups, including the control groups. These discharges were considered to be related to the nose-only method of exposure and not the test substance.

For the 1.00 mg/L group, reduced amounts of feces were observed for one male rat on two days and for all female rats on two to thirteen days during the study, and soft feces were observed for one male and one female rat on one day each during the study. Alopecia observed for one male 1.00 mg/L rat was not considered test substance-related.

Body Weight and Feed Consumption

Feed consumption was measured weekly and is presented graphically and as means and standard deviations on pages 39 - 40 (male) and 42 - 43 (female). Feed utilization data are presented as means and standard deviations on pages 41 (male) and 44 (female). Body weights were measured weekly and are presented graphically and as means and standard deviations on pages 45 - 46 (male) and 48 - 49 (female). Body weight gains are presented as means and standard deviations on pages 47 (male) and 50 (female). Individual animal data are presented in Appendix A.

Mean feed consumption, feed utilization, body weight, and body weight gains for rats from all exposure levels were comparable to the those of the respective control group throughout the study.

Hematology

Hematology data are presented as means and standard deviations on pages 51 - 52 (male) and 53 - 54 (female). Cell morphology data are presented as means and standard deviations on pages 55 (male) and 56 (female). Individual animal data are presented in Appendix A.

For male rats, mean red blood cell counts, hemoglobin concentrations, and hematocrit values were higher ($p \leq 0.05$) for the 1.00 mg/L group when compared with the control group. For female rats, mean white blood cell counts and lymphocyte counts were lower ($p \leq 0.05$) for the 1.0 and 0.3 mg/L groups, and mean atypical lymphocyte counts were higher ($p \leq 0.05$) for the 0.3 mg/L groups when compared with the control group.

Macrocytosis was observed for two male rats from the 1.00 mg/L group (#317 and 318) and for one male rat from the 0.30 mg/L group (#312). Anisocytosis and poikilocytosis were seen in blood smears of rats from all groups and Howell-Jolly bodies were observed in the blood for one female rat each from the 0.30 (#332) and 0.00 mg/L groups (#325); since these changes were not observed in a concentration-dependent manner, they were not considered test substance-related. In addition, the severity of all red blood cell abnormalities was minimal.

All other hematologic parameters for rats from all exposure levels were comparable to the those of the respective control group.

Clinical Chemistry

Serum clinical chemistry data are presented as means and standard deviations on pages 57 - 58 (male) and 59 - 60 (female). Individual animal data are presented in Appendix A.

For male rats, mean urea nitrogen and total bilirubin levels were higher ($p \leq 0.05$) for the 1.00 mg/L group when compared with the control group. For female rats, mean urea nitrogen levels were higher for the 1.00 and 0.30 mg/L groups and mean triglyceride levels were higher for the 1.00 mg/L group when compared with the control group. In addition, mean total cholesterol levels were higher for the 0.05 mg/L female group when compared with the control group; this difference was not considered test substance-related. All other clinical chemistry parameters for rats from all exposure levels were comparable to the those of the respective control group.

Organ Weights

Means and standard deviations of selected organ weights and organ-to-body weight ratios are presented on pages 61 - 62 (male) and 63 - 64 (female). Individual animal data are listed in Appendix A.

The mean relative, but not absolute, liver weight was higher ($p \leq 0.05$) for the 1.00 mg/L male rats when compared to the male control group.

The mean absolute, but not relative, brain weight was lower ($p \leq 0.05$) for the 1.00 mg/L female rats when compared to the female control group. The brain weight for one animal in this group (Rat 337) was much lower than for the other animals in the group (1.2 versus 1.7-1.8). When the mean absolute brain weights were analyzed statistically with this animal excluded, the mean weights were comparable among the groups. Since no histopathology was noted, the significance of the lower brain weight in a single animal could not be determined.

The mean terminal body weights and all other organ weight measurements for rats from all exposure levels were comparable to the those of the respective control group.

Gross Pathology

See the pathologist's report beginning on page 65 for details of the gross pathology examinations. Test substance-related changes observed at the time of necropsy consisted of pale livers for the three of five 1.00 mg/L male rats and all 1.00 and 0.30 mg/L female rats, and of pale hearts for the two of five male and three of five female 1.00 mg/L rats. No other test substance-related gross lesions were observed on necropsy examinations.

Histopathology

See the pathologist's report beginning on page 65 for details of the histopathology examinations.

Histopathologic examination of tissues indicated test substance-related effects in the heart and liver. Lesions in the heart consisted of muscle cell microvesicular sarcoplasmic vacuolation for the all male and four female 1.00 mg/L rats and for four female 0.30 mg/L rats, myocardial necrosis for four male and four female 1.00 mg/L rats and for two male and three female 0.30 mg/L rats, and myocarditis for four male and four female 1.00 mg/L rats, 2 male and all female 0.30 mg/L rats, and 1 male and 1 female 0.05 mg/L rats. One female 0.0 mg/L rat had a focus of mild myocarditis, however the lesion was of a different pattern than that present in the rats exposed to the test substance.

Lesions in the liver consisted of hepatocellular cytoplasmic vacuolation which was observed for all male and female 1.00 and 0.30 mg/L rats (mild to moderate in severity), and all male and female 0.05 mg/L rats (minimal to moderate in severity). Hepatocellular cytoplasmic vacuolation was also present in the control animals but at a lower severity (minimal to mild) and with a more diffuse distribution.

No other test substance-related changes were observed during the histopathology examinations.

DISCUSSION

Exposure of rats to vapors of the test substance produced no mortality and only minimal signs of overt toxicity. However, changes in hematology, clinical chemistry, and organ weight values and microscopic lesions were indicative of test substance-related toxicity in the heart and liver.

Cardiac effects consisted of pale hearts for the 1.00 mg/L group, myocardial vacuolation for the 1.00 and 0.30 mg/L groups, myocardial necrosis for the 1.00 and 0.30 mg/L group, and myocarditis for all test substance-exposed groups. It is possible that exposure to the test substance resulted in changes to the heart that are consistent with the hypersensitivity associated with exposure to cyclopropane, an inhalation anesthetic (Cavender, F., 1994; Van Vleet, et. al., 1991). Exposure to high concentrations of cyclopropane can result in hypersensitivity to catecholamines which may lead to lesions similar to those observed in this study. Similar effects have been observed following exposure to other structurally similar substances (methyl cyclopropanecarboxylate, cyclopropanemethanol, and cyclopropanecarboxaldehyde) (TX-97-240, 1998; TX-98-114, 1998; TX-98-56, 1998). Since the test substance and cyclopropane have structural similarities, it seems likely that the test substance may also affect the heart in a similar fashion as cyclopropane.

Liver effects consisted of a higher mean relative liver weight for the 1.00 mg/L male group, pale livers for the 1.00 and 0.30 mg/L groups, and an increase in the severity of hepatocellular cytoplasmic vacuolization for all test-substance-exposed groups. In addition, mean total bilirubin levels were higher for the 1.00 mg/L male group and mean triglyceride levels were higher for the 1.00 mg/L female group. Inhibition of fatty acid oxidation to acetyl CoA in the liver (Bahl *et al.*, 1978) may have contributed to the elevated triglyceride levels. In addition, the reduced fatty acid oxidation can result in accumulations of fatty acids in the liver which, in turn, may be related to increased liver weight and hepatocellular cytoplasmic vacuolization. The pathologist considered the hepatocellular cytoplasmic vacuolization observed in this study to be an accumulation of lipid and the cause of the pale livers. However, the changes in the liver are considered to be adaptive changes in metabolism since serum enzymes such as ALT and SDH, which are considered to reflect hepatocellular damage, were not altered. In addition, hepatocellular cytoplasmic vacuolization was reversible following cessation of exposure for a structurally similar substance (cyclopropanecarboxaldehyde) (TX-98-56, 1998).

Mean red blood cell counts, hemoglobin concentrations, and hematocrit values were higher for the 1.00 mg/L male group, mean white blood cell counts and lymphocyte counts were lower for the 1.00 and 0.30 mg/L female groups, and mean atypical lymphocyte counts were higher for the 0.30 mg/L groups. In addition, minimal macrocytosis of the red blood cells was observed for one to two male rats from the 1.00 and 0.30 mg/L groups. Although the hematology changes suggest an effect on the bone marrow, no histopathological changes were observed in the marrow and the marrow was not considered a target organ for toxicity of the test substance. Alternatively, these hematologic changes may have been caused by the animals being slightly dehydrated.

Increases in the concentration of serum urea nitrogen were observed for the 1.00 mg/L male group and for the 1.00 and 0.30 mg/L female groups. While urea nitrogen has been used as an indicator of kidney function (Meeks, 1989), the mean serum creatinine levels and the mean kidney weights were comparable among the groups and the kidneys from all animals were normal when examined by light microscopy. As a result, the significance of the elevated serum urea nitrogen levels is unclear. Similar elevations in serum urea nitrogen levels were observed during the one-week inhalation toxicity study on this test substance (TX-99-131, 2000) and have been observed following exposure to other structurally similar substances (cyclopropanemethanol [one-week study], cyclopropanemethanol [four-week study], cyclopropanecarboxaldehyde [two-week study], cyclopropanecarboxaldehyde [thirteen-week study]) (TX-98-130, 1998; TX-98-114, 1998; TX-97-3, 1997; TX-98-56, 1997).

CONCLUSION

Based on the heart and liver lesions which were observed at 0.05 mg/L, the lowest concentration tested, a no-observed-effect concentration (NOEC) was not determined. In addition, the effect on the heart was considered to be adverse. Thus, a no-observed-adverse-effect concentration (NOAEC) was not identified. Effects on the liver were considered adaptive and were not considered to constitute an adverse effect.

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Summary of Exposure Conditions

Target Concentration (mg/L)		0.0	0.05	0.30	1.0
Number Of Exposures		22	22	22	22
Analytical (GC/FID) Concentration (mg/L) Analyzed on Days 1, 8, 15, 22, and 29	Mean	0.00	0.05	0.33	1.02
	SD	0.00	0.003	0.014	0.060
	n	5	5	5	5
Extremes of Daily Means	Low	0.000	0.046	0.309	0.978
	High	0.000	0.052	0.346	1.120
Time Weighted Average (MIRAN) Concentration (mg/L)	Mean	0.00	0.05	0.31	1.02
	SD	0.000	0.003	0.013	0.021
	n	22	22	22	22
Extremes of Daily Values	Low	0.00	0.05	0.28	0.98
	High	0.00	0.06	0.33	1.07
Nominal Concentration (mg/L)	Mean	0.00	0.07	0.38	1.19
	SD	0.000	0.016	0.038	0.066
	n	22	22	22	22
Extremes of Daily Values	Low	0.00	0.04	0.34	0.99
	High	0.00	0.10	0.52	1.27
Airflow (Lpm)	Mean	10.51	11.34	12.98	10.83
	SD	0.37	0.68	0.42	0.62
	n	286	286	286	286
Extremes of Daily Values	Low	9.67	10.52	12.15	9.98
	High	11.10	12.92	14.55	12.91

		All Groups
Temperature (°C)	Mean	19.3
	SD	0.4
	n	286
Extremes of Daily Values	Low	19
	High	20
Relative Humidity (%)	Mean	70.0
	SD	1.5
	n	286
Extremes of Daily Values	Low	64
	High	73