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Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, S. W.
Washington, DC 20460
Attn: 8(e) Coordinator

Reference: 8EHQ-94-13095

8EHQ-1195-13095

Subject: Final report on a Four-Week Inhalation Toxicity Study in the Rat for 2,5-Dihydrofuran (CAS number 1708-29-8).

Dear Madam or Sir:

The following information is being submitted as a follow-up to the letter dated June 24, 1994, advising the Administrator of a finding of substantial risk. The final report has just now been received from the contract laboratory and is being submitted in support of the previous data.

In summary, exposure concentrations were selected based on the results of a one-week probe study during which two male and two female rats per group were exposed to nominal concentrations of 1250, 2500, 5000, or 7500 ppm (actual measured concentrations were 1142, 1686, 3692, or 6889 ppm) of the test substance for 6 hours/day for 5 days. Based on mortality at 5000 and 7500 ppm and convulsions at 2500 ppm, test substance concentrations of 0, 125, 400, and 1250 ppm were selected for the four week study. All animals (5 male and 5 female for each exposure group) were observed daily before, during, and after exposure. Body weights and feed consumption were measured weekly.

Rats exposed to 1250 ppm showed reduced activity and partially-closed eyes during exposure. All female 1250 ppm rats also exhibited head shaking movements and three of the five animals had chewing motions during exposure. Additional signs of toxicity for the 1250 ppm group were observed 18 hours after most exposures with animals exhibiting tremors and gait disturbances. An abbreviated functional observation batter (FOB) was performed prior to the first exposure and on Days 8, 15, 22, and 29. The FOB indicated that animals in the 1250 ppm group had increased incidence and severity of tremors. Additional findings included a higher incidence ($p \leq 0.05$) of reduced muscle tone (soft, flabby muscles), an abnormal gait (splayed



Eastman Chemical Company

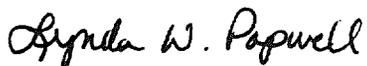
walking), and an elevated ($p \leq 0.05$) hindlimb hypotonic gait score. A significant reduction in terminal body weight in the 1250 ppm group influenced the following absolute and relative organ weights: brain, kidney (males only), thymus, spleen, and testes. There were no exposure-related gross or microscopic observations in the nervous system of animals on this study. Exposure-related changes observed in the nasal passages consisted of squamous metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium, and serocellular exudate in the middle or dorsal meatus. One 1250 ppm rat was found dead prior to exposure on Day 6 and two 1250 ppm female rats were found dead prior to Day 19.

Rats exposed to 400 ppm also had some reduced activity and partially-closed eyes during exposure, but the frequency and severity were less than that for the 1250 ppm group. No other clinical signs of toxicity were seen after exposure. A significant reduction in terminal body weight in the 400 ppm group influenced the following absolute and relative organ weights: brain, kidney (males only), thymus, spleen, and testes. There were no exposure-related gross or microscopic observations in the nervous system of animals on this study. Exposure-related changes observed in the nasal passages consisted of squamous metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium, and serocellular exudate in the middle or dorsal meatus.

Rats exposed to 125 ppm exhibited minimal reduced activity during the first three days of exposure, and partially-closed eyes only on the first day of exposure. No clinical signs were noted except for a single incidence of porphyrin nasal discharge in 2 of the female rats. A slight decrease in body weight was observed in the 125 ppm group. 125 ppm was considered to be the no-observed-adverse-effect level (NOAEL).

If additional information concerning this study is required, please contact Dr. W. Mills Dyer, Jr., M.D., of my staff. His telephone number is (423) 229-3538.

Sincerely yours,



Lynda W. Popwell
Vice President
Health, Safety and Environment and Quality

fdp95194.doc

Enclosure

STUDY TITLE

**2,5-DIHYDROFURAN
SYNONYMS: DHF**

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

**HAEL No. 94-0214 KAN/EAN 908701
CAS No. 001708-29-8 PM No. 14504-00**

FINAL REPORT

AUTHORS

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PERFORMING LABORATORY

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

LABORATORY PROJECT ID

940200I2

STUDY SPONSOR

Eastman Chemical Company
Kingsport, TN 37662-5394

STUDY COMPLETION DATE

October 16, 1995

QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 94-0200-1 STUDY DIRECTOR: DAVID, R.M.
ACCESSION NUMBER: 908701

PAGE 1
10/05/95

STUDY TYPE: BASIC REPEATED INHALATION

M. James
(AUDITOR, QUALITY ASSURANCE UNIT)

10/5/95
DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY
ASSURANCE UNIT OF HAEL, EASTMAN KODAK COMPANY ROCHESTER, N.Y.
AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
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06/03/94	PROTOCOL SUBMISSION 1 WEEK PROBE STUDY	
06/06/94	CHAMBER CONCENTRATION ANALYSIS CLINICAL SIGNS DURING DOSE CHAMBER PARAMETERS TAKEN DAY 0 OF THE 1 WEEK PROBE	
08/30/95	FINAL REPORT REVIEW 1-WEEK PROBE STUDY	08/30/95
09/13/95	FINAL REPORT REVIEW 1-WEEK PROBE STUDY	09/13/95

QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 94-0214-1 STUDY DIRECTOR: DAVID, R.M.
ACCESSION NUMBER: 908701

PAGE 1
10/05/95

STUDY TYPE: BASIC REPEATED INHALATION (28-DAY)

M. James
(AUDITOR, QUALITY ASSURANCE UNIT)

10/5/95
DATE

THIS STUDY WAS INSPECTED BY 1 OR MORE PERSONS OF THE QUALITY
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AND WRITTEN STATUS REPORTS WERE SUBMITTED ON THE FOLLOWING DATES:

INSPECTION DATES	PHASE(S) INSPECTED	STATUS REPORT DATES
06/21/94	DATA UNDER ANALYSIS PROTOCOL SUBMISSION	06/21/94
06/22/94	PRE-EXPOSURE FOB DATA TABLES ANALYSIS CHAMBER CONCENTRATION ANALYSIS PRE-EXPOSURE FOB SUMMARY TABLES	06/22/94
06/28/94	TEST SYSTEM IDENTIFICATION FUNCTIONAL OBSERVATIONAL BATTERY TESTING QUANTITATIVE MEASUREMENT OF GRIP STRENGTH TESTING FOOT SPLAY TEST	06/29/94
07/14/94	RECORDS REVIEW FOBS FOR DAY 8	
07/15/94	RECORDS REVIEW FOBS FOR DAY 8	
07/19/94	RECORDS REVIEW FOBS FOR DAY 8	
07/20/94	RECORDS REVIEW FOBS FOR DAY 8	
07/20/94	BLEEDING-NECROPSY-HEMATOLOGY-CLINICAL CHEMISTRY PERFUSION SPECIMEN COLLECTION	07/20/94

QUALITY ASSURANCE STATEMENT

QUALITY ASSURANCE INSPECTION STATEMENT
(21 CFR 58.35(B)(7), 40 CFR 792.35(B)(7), AND 40 CFR 160.35(B)(7))

STUDY: 94-0214-1 STUDY DIRECTOR: DAVID, R.M. PAGE 2
ACCESSION NUMBER: 908701 10/05/95

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	FOBS FOR DAY 8	
08/04/94	RECORDS REVIEW	
	FOBS FOR DAY 15	
08/08/94	RECORDS REVIEW	
	FOBS FOR DAY 15	
08/16/94	RECORDS REVIEW	08/16/94
	FOBS FOR DAY 15	
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	FOBS FOR DAY 22	
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	FOBS FOR DAY 22	
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08/30/95	FINAL REPORT REVIEW	08/30/95
09/13/95	FINAL REPORT REVIEW	09/13/95
10/05/95	FINAL REPORT REVIEW	10/05/95

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

To the best of the signer's knowledge and belief, the study described by this report was conducted in compliance with the following Good Laboratory Practice Standards:

- United States Food and Drug Administration, 21 CFR Part 58,
- United States Environmental Protection Agency, 40 CFR Part 792 (TSCA),
- United States Environmental Protection Agency, 40 CFR 160 (FIFRA), and
- Annex 2 of the Organization for Economic Cooperation and Development Guidelines for Testing of Chemicals (C(81)30(Final)).



Raymond M. David, Ph.D., DABT
Study Director

10/16/95

Month/Day/Year

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STUDY TITLE

**2,5-DIHYDROFURAN
SYNONYMS: DHF**

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

**HAEL No. 94-0214 KAN/EAN 908701
CAS No. 001708-29-8 PM No. 14504-00**

ABSTRACT

Male and female Sprague-Dawley rats were exposed for 6 hr/day, 5 days/wk for four weeks to target concentrations of 0, 125, 400, or 1250 ppm of the test substance. The mean time-weighted average (TWA) exposure concentrations were within 10% of the target concentrations. Animals were observed daily before, during, and after exposure. Body weights and feed consumption were measured weekly. Three 1250 ppm rats died during the study. During exposure, the 1250 ppm group had reduced activity and partially-closed eyes. All female 1250 ppm rats also exhibited head shaking movements and three of five animals had chewing motions during exposure. Other clinical signs consisted of unkempt haircoats, porphyrin nasal discharges, and piloerection. Additional signs of toxicity for the 1250 ppm group were observed 18 hours after most exposures with animals exhibiting tremors and gait disturbances. Two 1250 ppm females had brief (10-15 sec. duration) convulsions induced by handling on Days 7 or 20. One of these animals exhibited abnormal behavior shortly before and after the convulsion; this animal also exhibited tremors with pawing motions and head shaking. Other clinical signs for this group were considered spurious. Animals in the 400 ppm group also had reduced activity and partially-closed eyes during exposure, but the frequency and severity were less than for the 1250 ppm group. No other clinical signs of toxicity during exposure were noted for the 400 ppm group, and no clinical signs of toxicity were seen after exposure. The 125 ppm group exhibited minimal reduced activity during exposure only on Days 0, 1, and 2, and partially-closed eyes were observed during the first exposure for the 125 ppm group. No clinical signs of toxicity were noted for the 125 ppm group except for single incidences of porphyrin nasal discharge which were observed in two 125 ppm female rats. Mean feed consumption values for all test substance-exposed groups were significantly lower ($p \leq 0.05$) than for the control group on Day 4, and was reduced for all but the 125 ppm group thereafter. Mean body weights reflect the decrease in feed consumption with significantly lower body weights for all treated groups on Day 4, and lower mean body weights for all but the 125 ppm group thereafter.

An abbreviated functional observational battery (FOB) was performed prior to the first exposure (Day -3) and on Days 8, 15, 22, and 29. The FOB indicated that animals in the 1250 ppm group had increased incidence and severity of tremors. Additional findings included a higher incidence ($p \leq 0.05$) of reduced muscle tone (soft, flabby muscles), an abnormal gait (splayed walking), and an elevated ($p \leq 0.05$) hindlimb hypotonic gait score. One 400 ppm male rat had tremors on Day 29. A reduced ($p \leq 0.05$) response to touch was noted on Day 15 for the 400 and 1250 ppm

male rats when the data were normalized to the baseline response, but this was not considered to be toxicologically significant. Other findings in the FOB were considered to be spurious.

After four weeks of exposure, the animals were euthanatized and blood was collected for analysis. The carcasses were perfused and tissues collected for histopathology. Selected organs were weighed. Tissue samples of the central nervous system, peripheral nerves, and all other tissues were collected for histopathology. No toxicologically significant changes were observed in hematology or serum chemistry endpoints. Abnormal red blood cell morphology was observed in one 400 ppm rat and one 1250 ppm rat. Other findings were considered to be not significant. Mean terminal body weights were reduced in a concentration-dependent manner for all test substance-exposed groups when compared to the control group; this reduction was significant ($p \leq 0.05$) for the 400 and 1250 ppm groups. The lower body weight for these groups influenced the weights (absolute and relative to body weight) of the brain, kidneys (males only), thymus, spleen, and testes. There were no exposure-related gross or microscopic observations in the nervous system of animals on this study. Exposure-related changes observed in the nasal passages consisted of squamous metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium, and serocellular exudate in the middle or dorsal meatus for the 400 and 1250 ppm groups. The lesions in the nasal passages may reflect irritation of mucosa of the nasal passages. All other histopathologic changes were considered to reflect reduced feed consumption and lower body weight.

Based on the decrease in body weight in the 125 ppm group, a no-observed-effect level (NOEL) was not determined. However, based on the absence of histopathologic changes in the nasal passages and absence of any clinical signs of neurotoxicity, 125 ppm was considered to be the no-observed-adverse-effect level (NOAEL).

Purpose

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

Performing Laboratory

Toxicological Sciences Laboratory
Health and Environment Laboratories (HAEL)
Eastman Kodak Company
Rochester, NY 14652-6272

Sponsor

Eastman Chemical Company
First American Center
P.O. Box 1994
Kingsport, TN 37662-5394

Authorized Representative: W. Mills Dyer, Jr., M.D.

Test Procedures

This study was conducted according to the Organization for Economic Cooperation and Development (OECD) Guidelines for Testing of Chemicals Guideline: TG-412, Repeated Dose Inhalation Toxicity; and European Economic Community: Annex V., Test B.8, Sub-Acute Inhalation Toxicity.

MATERIALS AND METHODS

Test Substance Characterization

Chemical Name:	2,5-Dihydrofuran
Synonym:	DHF
Source of Test Substance	Eastman Chemical Company Kingsport, TN 37662-5394
HAEL No.:	94-0214
KAN/EAN:	908701
Lot No.:	X23849-84
CAS No.:	001708-29-8
PM No.:	14504-00
Experiment No.:	940200I2
Empirical formula:	C ₄ H ₆ O
Vapor Pressure:	126 mm/Hg at 20 °C (68 °F)
Molecular weight:	70.09
Boiling point:	66 °C (151°F)
Specific gravity (H ₂ O=1):	0.927 at 25 °C (77 °F)
Physical State and Appearance:	Clear, colorless liquid
Received at Performing Laboratory:	June 17, 1994

The test substance was tested as received.

Test Substance Purity and Identity

The purity of the test chemical was determined, using gas chromatography with a flame ionization detector, to be >99.9% prior to the study and 97.5% at study termination. Based on these data, the test substance was considered to be stable during the test period. The test substance was stabilized with ~250 ppm of butylated hydroxytoluene (BHT) to prevent peroxide formation during storage. The structure of the test substance was confirmed using mass spectrometry. The mass spectrum of the test substance was consistent with the structure of 2,5-dihydrofuran based on comparison with reference library spectra. Chemical analyses were performed by Environmental Analytical Services, Chemicals Quality Services Division, Eastman Kodak Company. The analytical report is provided in the Appendix.

Selection of Exposure Concentrations

Exposure concentrations were selected based on the results of a one-week probe study during which two male and two female rats per group were exposed to 1250, 2500, 5000, or 7500 ppm of the test substance 6 hours/day for 5 days. Based on mortality at 5000 and 7500 ppm and convulsions at 2500 ppm, test substance concentrations of 0, 125, 400, and 1250 ppm were selected for the four-week study.

Exposure

The inhalation exposures were conducted in 420 L stainless-steel and glass inhalation chambers at target vapor concentrations of 0, 125, 400, and 1250 ppm. Animals were singly housed during the 6-hour exposures. A diagram of the chamber and placement of cages within the chamber is provided in the Appendix. The animals were moved sequentially each day to new cage positions within the chamber. Cage positions 10 through 19 were used for this study. The exposure chambers were maintained under negative pressure relative to room air. The air flow, temperature, and humidity were recorded every 30 minutes. Chamber vapor concentrations were recorded at least once each hour.

Exposure Atmosphere Generation

The test atmosphere was generated by metering the test substance into glass distillation columns packed with glass beads. Nitrogen gas was passed through the glass bead-packed column at 5 Lpm because the test substance quickly forms peroxides when exposed to oxygen. The resultant vapor was directed via glass tubing to a tee just upstream of the 420 L inhalation chamber where it was mixed with filtered, conditioned dilution air (outside air) to produce a total airflow of 94 to 102 Lpm (13 to 15 air changes per hour). A continuous, dynamic test atmosphere was generated using this system. A diagram of the generation system is provided in the Appendix.

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.

The effluent from the chamber was passed through a water containing sodium bisulfite to neutralize the peroxides, coarse prefilters, HEPA filters, and charcoal filters.

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 (3/16" 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 concentration 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 (ppm)
 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

MIRAN® IA Infrared Analyzer Operating Parameters and Calibration

The infrared analyzer operating parameters were as follows:

MIRAN® No.	3
Pathlength (m)	2.25
Wavelength (mm)	3.48
Slit width (mm)	1
Response Time (sec)	1
Range (Absorption)	1A
Gain	x10
Cell Temperature (°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) of the test substance into a closed-loop cell. The concentration was determined using the following formula:

$$C = \frac{(\rho)(V_1)(R)(T + 273)(1000)}{(MW)(P)(V_2)}$$

where: C = concentration (ppm)
 ρ = test substance density (g/mL)
 V₁ = serial injection volume
 R = gas constant (0.08205 atmL/moleK)
 T = MIRAN® cell temperature (25°C)
 1000 = Conversion from mL to mL
 MW = test substance molecular weight (g/mole)
 P = MIRAN® cell pressure (0.833 atm)
 V₂ = MIRAN® cell volume (5.64 L)

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 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 concentration was within 10% of that expected, the calibration was accepted.

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:

$$NC = \frac{(G)(MV)(10^6)}{(V)(MW)}$$

where: NC = nominal concentration (ppm)
G = amount of test substance vaporized (grams)
MV = molar volume at 1 atm and 25°C (24.45 L/mole)
V = total daily chamber air flow (L)
MW = test substance molecular weight (g/mole)

Chamber Vapor Homogeneity

A test to determine variations in concentration at different positions within the exposure chambers was conducted prior to study initiation. The air from the breathing zones of cage positions 10, 12, 14, 16, 18, and 19 was sampled as described under Vapor Concentration Determination and compared to the concentration at a fixed reference position (cage 15). Based on deviations from the reference position of less than 4%, the chamber atmosphere was considered to be homogeneous.

Air Flow Measurement

Total chamber air flow was a combination of nitrogen, which was used to vaporize the test substance and to carry the vapor from the generation system to the inhalation chamber, and dilution air. The nitrogen flow rate was continuously monitored using a rotameter. The dilution air was adjusted and monitored throughout the exposure using a Kurz (Model 441) Air Velocity Meter (Kurz Instruments, Inc., Monterey, CA). The dilution air flow rate was calculated using the following formula:

$$Q = \frac{(A)(V)}{1000}$$

where: Q = Supply air flow rate (Lpm)
A = Cross sectional area of the dilution air duct (cm²) [πr^2 , r = 1 in.]
V = Supply air linear velocity (cm/min.) [air velocity meter readings are in ft./min.]
1000 = Conversion Factor

Oxygen Level

The oxygen content of the chamber exposure atmosphere was measured during exposure from the reference position using an Model K Oxygen Indicator (Johnson-Williams Products, Bacharach Instrument Co., Mountain View, CA). The oxygen content of the chamber exposure atmosphere was $\geq 20\%$.

Chamber Temperature and Humidity

Chamber temperature and humidity were measured using wet/dry bulb hygrometers and were recorded twice each hour during exposure.

Animals

Five male and five female Sprague-Dawley rats (CD[®](SD)BR/VAF Plus[™]) obtained from Charles River Kingston (Stone Ridge, NY) were randomly assigned to each exposure group. The animals were isolated upon arrival and were judged to be healthy prior to testing. The male and female rats were 52 days of age and weighed 264.3 ± 7 or 197.8 ± 12 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 toxicity studies.

Housing

Animals were housed in an American Association for Accreditation of Laboratory Animal Care-accredited vivarium (Building 320) in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication 86-23). During nonexposure periods, rats were singly housed in stainless-steel, wire-mesh cages in a room separate from the exposure room. The study room was maintained at 67 - 73°F and 55 - 66% relative humidity. A photoperiod of 12 hours light from 6 a.m. to 6 p.m. was maintained. No other animals were housed in this room. Exposure cages were washed daily. Housing cages and racks were washed once a week. Absorbent paper, used to collect excreta, was changed daily.

Feed and Water

Certified Rodent Diet (Agway[®] Prolab[®] RMH 3200, ground chow) and water were available *ad libitum* except during exposures. Water was available through an automatic watering system. The source of the water was the Monroe County (NY) Water Authority. Feed containers were cleaned weekly for all animals and were refilled at least once a week. Analyses of feed and quarterly analyses of water are maintained on file within the testing laboratory. Previous analyses of the feed and water have not identified contaminants in concentrations which would interfere with the outcome of this study.

Identification

Upon arrival, all rats were identified by uniquely-numbered metal ear tags. Ear tags that were lost during the study were replaced. During randomization, study-specific animal numbers were assigned to each animal and placed on the color-coded cage card along with the ear tag number. During FOB procedures, cage cards were removed to eliminate information of dose level or study-specific animal numbers. Only ear tag numbers were used to identify animals during this procedure.

Randomization

The test animals were culled from the population based on body weight and randomly assigned to groups using computer-generated lists from the Automated Animal Toxicology System. The body weights of individual animals in the culled population did not exceed 20% of the mean for each sex. Following randomization, the body weights of all groups were compared by analysis of variance to insure that there were no statistically significant differences prior to initiation of dosing.

Disposition of Groups

Animals were distributed into groups as follows:

Group	Exposure Concentration	Number of Animals	Animal Number	
			Male	Female
1	Control / 0 ppm	5 Males & 5 Females	751 - 755	771 - 775
2	Low / 125 ppm	5 Males & 5 Females	756 - 760	776 - 780
3	Mid / 400 ppm	5 Males & 5 Females	761 - 765	781 - 785
4	High / 1250 ppm	5 Males & 5 Females	766 - 770	786 - 790

Animals were exposed 6 hours/day for five days per week [Monday to Friday] for four consecutive weeks, and for an additional two (males) or three days (females) of the fifth week. All surviving animals were euthanatized and necropsied on the day following the last exposure [Days 30 (males) and 31 (females)].

Clinical Observations

While performing clinical observations, the observers were not blind to treatment. Rats visible through chamber windows were observed for clinical signs during exposure. Tapping sounds were made on the outside of the chamber with a key or other metal object to assess the animals' activity level. Before and after exposure, each rat was removed from its cage and examined. Cageside observations were conducted once a day on weekends. Observations included, but were not limited to, examination of the hair, skin, eyes and 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, 4, 7, 14, 21, and 28. Feed consumption was measured on Days 4, 7, 14, 21, and 28. Animals were fasted the day prior to necropsy. Fasted body weights were collected at termination after perfusion.

Abbreviated Functional Observational Battery (FOB)

Animals were observed the Friday prior to the first exposure (Day -3) and prior to exposure on Days 8, 15, 22, and 29 of the study. Animals were evaluated in random order using the ear tag as the identification. Two people, one functioning as an observer and the other as a recorder, performed the FOB. The observer was blind to treatment. The same observer was used for all the FOB evaluations except for one day on which that individual was unavailable. On that day, another person who also was blind to the treatment observed the animals. The recorder noted the level of vocalization during the examination period. A copy of the scoring system and categorical entries is listed in the Appendix.

The animals were observed for the following endpoints with a score of 1 being normal and scores of 2 to 4 being given for different levels of behavior.

- Severity of convulsions and tremors
- Ranking of reactivity
- Alertness
- Coordination of movement
- Sensory function (vision and pain perception)
- Pinna reflex
- Righting reflex
- Approach response
- Touch response

Descriptive categories of differing behavior were noted for the following endpoints.

- Pupillary size (constriction or dilation)
- Description and induction of convulsions and tremors
- Alertness
- Auditory orientation (ranking of reactivity)
- Vocalization
- Description of body position and gait abnormalities
- Stereotypy and bizarre behavior
- Unusual respiration
- Muscle tone

A quantitative assessment of forelimb and hindlimb strength grip strength was performed using an apparatus equipped with a digital push-pull gauge (Model DFIS, John Chatillon & Sons, Inc., Kew Gardens, NY). The rat was placed on a rectangular screen and then lifted back and upwardly until it released its grip. The grip strength was repeated for a total of three readings. The mean of the three readings was reported as the grip strength measurement.

A quantitative assessment of foot splay was performed by releasing a rat 32 cm above a bench, in a horizontal position and allowing it to drop on a sheet of paper (Edwards and Parker, 1977). The foot splay was determined by measuring the distance between marks caused by ink placed on the outside digit of each hindfoot with a water soluble marker.

Historical positive control data demonstrating the sensitivity of the FOB procedure are available.

Blood Collection

Animals were fasted beginning after the last exposure. The following day, blood was collected via cardiac puncture for hematology and clinical chemistry while the animals were anesthetized with sodium pentobarbital.

Hematology and Clinical Chemistry Examinations

All clinical pathology assays were conducted by the Clinical Biochemistry Team, using a Roche Analytical Instruments Cobas Bio serum chemistry analyzer, Ortho Diagnostics Systems ELT 8/ds hematology analyzer, Helena Laboratories Titan Gel Electrophoresis System for A/G ratio and albumin, Corning Flame 480 for sodium and potassium, and Corning chloride Analyzer 925 for chloride. Hematology tests included: hemoglobin concentration, hematocrit, red blood cell count, white blood cell count, red blood cell indices, and platelet count. Slides containing blood smears were examined for cellular morphology and differential white blood cell count. Clinical chemistry tests included: aspartate aminotransferase, alanine aminotransferase, sorbitol dehydrogenase, alkaline phosphatase, creatinine, urea nitrogen, glucose, total bilirubin, total protein, albumin, albumin/globulin ratio, γ -glutamyltransferase, calcium, phosphorus, sodium, potassium, and chloride.

Necropsy

Animals were anesthetized with sodium pentobarbital containing heparin (10% by volume) and perfused through the ascending aorta after collection of blood for analysis. Perfusates were 4% paraformaldehyde followed by 5% glutaraldehyde, both in 0.1 M phosphate buffer (pH 7.4 at 4°C). Animals were necropsied in a random pattern using a computer-generated list.

The following tissues were fixed in 10% neutral buffered formalin (NBF): nasal passages, trachea, larynx, lungs, heart, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, pancreas, liver, salivary glands, kidneys, urinary bladder, pituitary gland, adrenal glands, thyroid glands, parathyroid glands, thymus, spleen, mesenteric lymph nodes, bone marrow (femoral), brain (including the forebrain, cerebrum, midbrain, cerebellum, pons, medulla oblongata), spinal cord, testes, right sciatic nerve, right tibial nerve, epididymides, male accessory sex glands, ovaries, vagina, uterus, Fallopian tubes, and gross lesions. The left sciatic and tibial nerves were fixed for an additional period of at least 2 hours in 5% glutaraldehyde and then stored in a sodium phosphate buffer. After processing was completed, the remaining tissues were stored in NBF.

Organ Weights

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

Histopathology

All tissues, except for the peripheral nervous system, were embedded in paraffin, sectioned at 5 μm , and stained with hematoxylin and eosin (H&E). The nasal passages were decalcified prior to being embedded and sectioned. The lungs were sectioned along planes that allowed examination of the major bronchi and bronchioles. Tissue samples of the peripheral nervous system, including the left sciatic and tibial nerves, dorsal and ventral roots, and the dorsal root ganglia were embedded in plastic (EPON), sectioned at 1 μm , and stained with 1% toluidine blue.

All tissues from the control and high-concentration groups; the nasal passages, thymus, spleen, bone marrow, testes, epididymides, and accessory sex glands from the mid-concentration group; and the nasal passages and thymus (one male only) from the low-concentration group were examined microscopically.

Statistical Procedures

Mean values were calculated for analytical concentration, chamber temperature, chamber relative humidity, chamber air flow, body weight, and body weight change. Feed consumption, body weight, and body weight change data were evaluated using the following statistical tests: Bartlett's test ($p \leq 0.01$), one-way analysis of variance (ANOVA) ($p \leq 0.05$), and Duncan's multiple range test (feed consumption and body weight) ($p \leq 0.05$) or Dunnett's t-test (body weight change) ($p \leq 0.05$) to indicate statistical significance.

Continuous FOB data and behavior scores were analyzed using a repeated measures analysis of variance/multivariate analysis. Baseline (pretest) values were subtracted from test-day values to normalize the variance. Data from individual test days were also analyzed using a one-way analysis of variance and pairwise comparisons made to control group values using a Dunnett's t-test. Categorical data were analyzed using a two-way and multiway frequency table/log-linear model. Time points indicating significant changes were further analyzed using Fisher's Exact test. A probability of $p \leq 0.05$ (two-tailed) was used to determine significance.

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 and Mann-Whitney U-test.

Data Storage

The final report, data sheets, and all nonperishable raw data have been stored in the HAEL archives. An aliquot of the test substance is stored in the chemical repository of the Health and Environment Laboratories.

Project Participants

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FOB Observers

Hematologist/Clinical Chemist
Pathologist/Laboratory Animal Medicine
Analytical Chemist
Statistician

Study Dates

Study Initiation Date	June 20, 1994
Experimental Start Date	June 20, 1994
Experimental Termination Date	October 2, 1995

Protocol and Standard Operating Procedure Deviations

Due to the administration of heparin to the animals prior to blood collection, prothrombin time could not be determined.

Due to the time required for the necropsy of perfused animals, half of the animals were necropsied on Day 30 while the other half were necropsied on Day 31.

These protocol deviations had no effect on the outcome of the study.

There were no other protocol or standard operating procedure deviations during the study.

RESULTS

Selection of Exposure Concentrations

A one-week probe study was conducted in which two male and two female rats per group were exposed to 1250, 2500, 5000, or 7500 ppm of the test substance 6 hours/day for 5 days. All animals in the 5000 and 7500 ppm groups died within the first two days of the study. One male and one female in the 2500 ppm group had convulsions prior to exposure on Day 2. The nature of this effect was considered to be adverse enough that the highest concentration for the four-week study would be set lower than 2500 ppm. Therefore, all the animals in this group were euthanatized on Day 2. All animals in the 1250 ppm group survived exposure and gained weight; reduced activity of minimal to moderate severity was observed during exposure. After exposure on Day 1, one male had depressed activity and on Day 2, both females had abnormal gait. Decreased fecal volumes were observed in an occasional animal prior to the next exposure. Based on the convulsions at 2500 ppm and the absence of convulsions at 1250 ppm, test substance concentrations of 0, 125, 400, and 1250 ppm were selected for the four-week study.

Exposure Conditions

The time-weighted average analytical concentrations and the nominal exposure concentrations are presented in the summary tables. Animals were exposed to target concentrations of 0, 125, 400, or 1250 ppm for 22 (males) or 23 exposures (females). The mean time-weighted average (TWA) exposure concentrations (\pm standard deviation) were 129 ± 10 , 403 ± 24 , and 1249 ± 51 ppm for male rats and were 128 ± 11 , 403 ± 24 , and 1247 ± 51 ppm for female rats. No test substance was detected in the control chamber. Daily TWA exposure concentrations were within 11% of the target concentrations for the mid- and high-concentrations and within 19% for the low-concentration. Nominal concentrations were 151 ± 12 , 396 ± 26 , and 2098 ± 213 ppm for male rats and were 151 ± 12 , 397 ± 26 , and 2113 ± 220 ppm for female rats the same groups. The daily mean chamber temperatures and chamber relative humidity were 21.9 - 23.5 °C and 58.1 - 61.2%, respectively. Daily mean values are provided in the Appendix.

Mortality

One 1250 ppm male rat (# 769) was found dead prior to exposure on Day 6 and two 1250 ppm female rats (# 787 and # 789) were found dead prior to exposure on Day 19. No other spontaneous mortality occurred during the study.

Clinical Observations

The animals were observed hourly during exposure. All the clinical signs observed are listed on the summaries (pages 33 - 40) regardless of their duration.

During exposure, male and female rats exhibited a concentration-dependent reduced level of general activity. For the 1250 ppm group, reduced activity was minimal to minor during exposure and was observed during most exposures. Partially-closed eyes were observed during the first

and second exposures for the 1250 ppm group. Most female 1250 ppm rats exhibited head shaking movements on Days 17 and 18, and rat # 788 had head shaking movements on Days 21-30. Rat # 790 also had head shaking motions on Day 25. Rat # 788 also had chewing motions on Days 7, 17, 21, 23, 25, and 28. Rat # 790 had chewing motions on Days 17 and 18, and rat # 789 also had chewing motions on Day 18. Chewing motions and head shaking movements were generally minimal to minor severity. Incidental clinical signs consisted of unkempt haircoats, porphyrin nasal discharges, and piloerection. For the 400 ppm group, the reduced activity level was minimal during exposure and was observed only on Days 0, 1, 2, 7, 10, and 17. Partially-closed eyes were observed during the first and second exposures for the 400 ppm group, and no other clinical signs of toxicity were noted at any time during exposure. The 125 ppm group exhibited minimal reduced activity levels only on Days 0, 1, and 2. Partially-closed eyes were observed during the first exposure for the 125 ppm group, but no other clinical signs of toxicity were noted at any time during exposure. No abnormal clinical signs were observed for the control group.

Clinical signs of toxicity before or after exposure were limited to the 1250 ppm group. In general, there was no difference between the observations noted before exposure and those seen after exposure. Tremors of minimal to minor severity were observed for three of the 1250 ppm male rats (# 766, # 767, and # 770) and all five of the 1250 ppm female rats; the tremors were observed in at least one animal on 9 or 16 days during the study for the male and female rats, respectively. Tremors were not initiated by activity or stimuli. Convulsions were observed in two 1250 ppm female rats prior to exposure on Days 7 (# 790) and 20 (# 786). In both cases, convulsions consisted of extended limbs lasting approximately 10-20 seconds, and were induced by handling. Gait disturbances were observed in the 1250 ppm male (4/5 animals) and female rats (5/5 animals) during most of the study. The gait disturbances were described as walking up on the toes. Other abnormalities in gait consisted of limping with the left hind leg, hopping with the hindlimbs, walking with small steps (with the sacral region held low to the ground), a duck-walk type gait, a waddling gait, walking with splayed hindlimbs, circling, a hunched posture, and walking with the tail held straight up with the distal portion bent at a slight angle ($< 90^\circ$).

Several treatment-related observations were noted in only an occasional animal. One 1250 ppm female (# 786) exhibited abnormal behavior shortly before and after having a convulsion on Day 20; the behavior consisted of the animal being easily startled and subsequent jumping ~15 cm high. On the same day, this animal (# 786) also exhibited tremors which was marked by pawing motion and head shaking. Minimal to minor reduced activity levels were observed for one 1250 ppm male rats (# 770) on Days 9-11 and 16-30 and for one 1250 ppm female rats (# 788) on Day 11. Minimal dehydration was observed for one male (# 767) and one female (# 788) 1250 ppm rat. Minimal to moderate reductions in feces volume were observed for the 1250 ppm male and female rats on 17 separate days during the study. Unkempt haircoats were observed for the 1250 ppm male and female rats on 23 and 9 days, respectively, during the study. Diarrhea and urine-soaked inguinal hair occurred in an occasional 1250 ppm female rat, and single incidences of porphyrin nasal discharges were observed for two 1250 ppm female rats.

A decreased amount of feces was observed for all 400 ppm animals on Day 4, and one animal (# 765) had an unkempt haircoat on Days 18, 19, and 20. No other clinical signs of toxicity were

noted for 400 ppm rats. Single incidences of porphyrin nasal discharge were observed for two 125 ppm female rats.

Body Weight and Feed Consumption Determinations

Mean feed consumption values for all test substance-exposed groups were significantly lower ($p \leq 0.05$) than for the control group on Day 4. Mean feed consumption levels for the 1250 ppm male and female groups continued to be significantly lower ($p \leq 0.05$) for the remainder of the study. Mean feed consumption values for the 400 ppm groups were significantly lower ($p \leq 0.05$) than for the control group from Day 14 until the end of the study. The mean feed consumption values for the 125 ppm male and female groups were comparable to that of the control group after Day 4 for the remainder of the study except for Day 14 when feed consumption for the 125 ppm female group was significantly ($p \leq 0.05$) lower.

Mean body weights for the 125 ppm male group, and the 400 and 1250 ppm male and female groups, were significantly lower ($p \leq 0.05$) than the control groups on Day 4 with the 0, 125, 400, and 1250 ppm groups exhibiting mean percent body weight changes of 12, 6, -1, and -22% for the male rats and 6, 1, -3, and -18% for female rats. Mean body weights for the male and female 1250 ppm and the male 400 ppm groups continued to be significantly lower ($p \leq 0.05$) than those of the control groups for the remainder of the study. The mean body weights for the female 400 ppm groups were also lower ($p \leq 0.05$) than those of the control group on Days 14 and 21, but by study termination, the mean body weight was not different from that of the control group. The mean body weight for the female 125 ppm group was significantly lower ($p \leq 0.05$) than those of the control only on Day 14 of the study. At study termination, the mean body weights for the 0, 125, 400, and 1250 ppm groups were 156, 147, 128, and 91% of their initial mean body weight for the male rats and 128, 120, 119, and 99% of their initial mean body weight for female rats.

Abbreviated Functional Observational Battery

An abbreviated functional observational battery (FOB) was performed prior to the first exposure (Day -3) and on Days 8, 15, 22, and 29. For the 1250 ppm group, the incidence of resting tremors was elevated ($p \leq 0.05$) on Days 22 and 29 for male rats, and on Days 8 and 22 for the female rats compared with the control group; the severity of the tremors was also elevated ($p \leq 0.05$) on Days 22 and 29 for the male rats and on Day 29 for the female rats when the data were normalized to the baseline response. Additionally, the 400 ppm male rats exhibited an elevated ($p \leq 0.05$) severity of tremors on Day 29 when the data was normalized to the baseline response. The higher mean severity score for the group appears to be the result of a higher tremor severity score from one animal.

For the 1250 ppm male rats, additional findings included an elevated ($p \leq 0.05$) number of rats with reduced muscle tone (soft, flabby muscles) and with an abnormal gait (splayed walking) on Days 15 and 29 and an elevated ($p \leq 0.05$) hindlimb hypotonic gait score on Day 15. A reduced ($p \leq 0.05$) response to touch was also noted on Day 15 for the 400 and 1250 ppm male rats when the data were normalized to the baseline response.

Incidental findings consisted of an elevated ($p \leq 0.05$) mean landing foot splay value on Day 22 for the 400 ppm female rats when the data were normalized to the baseline response and an elevated ($p \leq 0.05$) number of 125 ppm male rats exhibiting constricted pupils on Days 8 and 29; these findings were not considered to be toxicologically significant because there was no concentration-related response.

Hematology

The mean corpuscular hemoglobin level for the 1250 ppm male group was significantly reduced ($p \leq 0.05$) when compared to the control group. The mean platelet count for the 400 ppm female group was significantly higher when compared to the control group. These changes were not considered to be toxicologically significant.

The level of erythrocyte poikilocytosis was elevated for the 1250 ppm male and female rats when compared to the control group. Anisocytosis and macrocytosis were observed in the blood from 1250 ppm male rat #770, and Howell-Jolly bodies for 1250 ppm male rat #767. Spherocytosis was noted for a single 1250 ppm female rat (#788). Anisocytosis, microcytosis, spherocytosis, macrocytosis, and elliptocytosis were observed in the blood from a single 400 ppm male rat (#765). The different hematology findings were not considered to be toxicologically significant since they occurred in only one animal per group.

All other hematologic parameters for the treated rats were comparable to the control group values.

Clinical Chemistry

Mean glucose levels were elevated ($p \leq 0.05$) for the 1250 ppm male and female groups compared with the control groups. Mean total protein levels were elevated ($p \leq 0.05$) for the 1250 ppm male, and the 400 and 1250 ppm female groups compared with the control groups. Mean albumin levels were elevated ($p \leq 0.05$) for the 125, 400, and 1250 ppm male, and the 1250 ppm female groups, compared with the control groups. Mean calcium levels were elevated ($p \leq 0.05$) and the chloride levels were reduced ($p \leq 0.05$) for the 400 and 1250 ppm female groups compared with the control group. Mean phosphorus levels were elevated ($p \leq 0.05$) for the 1250 ppm female group compared with the control group. None of the changes in serum chemistries were considered to be toxicologically significant.

Mean sodium levels were reduced ($p \leq 0.05$) and the sorbitol dehydrogenase levels were elevated ($p \leq 0.05$) for the 400 ppm male and female groups, respectively. Mean phosphorous concentrations in the 125 ppm male group were significantly lower than in the control group. These changes in serum chemistries were not considered to be toxicologically significant since they did not occur in a concentration-dependent manner. All other clinical chemistry parameters for rats from all exposure levels were comparable to the control groups.

Organ Weights

Mean terminal body weights were reduced in a concentration-dependent manner for all test substance-exposed groups when compared to the control groups; these reductions were significant ($p \leq 0.05$) for the 400 and 1250 ppm groups. The lower body weight for these groups influenced the weights (absolute and relative to body weight) of several organs. The mean absolute weights of the liver (males only), brain, kidneys (males only), thymus, spleen, and testes for the 1250 ppm rats were significantly ($p \leq 0.05$) lower than for the control group. Mean absolute brain weight for male and female 400 ppm groups, mean absolute spleen weights for the 400 ppm female group, and mean absolute thymus weights for the 400 ppm male group were also significantly ($p \leq 0.05$) lower than for the control group. Additionally, the mean absolute brain weight for the 125 ppm female group was significantly lower than for the control group.

Mean relative (to body weight) liver weights were elevated in a concentration-dependent manner for all test substance-exposed groups when compared to the control groups; these differences were significant ($p \leq 0.05$) for all treated groups except for the 125 ppm female group. Mean relative (to body weight) kidney weights for the 400 ppm female rats and the 1250 ppm male and female rats were significantly higher ($p \leq 0.05$) when compared to the control groups. The mean relative (to body weight) thymus weight for the 1250 ppm male rats and mean relative spleen weight for the 1250 ppm female group were significantly lower ($p \leq 0.05$) when compared to the control group. The mean relative (to body weight) brain weight for the 1250 ppm group was significantly lower ($p \leq 0.05$) when compared to the control group. The mean relative (to body weight) adrenal gland weight for the 1250 ppm female rats was significantly higher ($p \leq 0.05$) when compared to the control group.

All other organ weight measurements for all test substance-exposed groups were comparable to the control group values.

Gross Pathology

Exposure-related changes observed for the 1250 ppm male rat (# 769) that died on Day 6 consisted of moderate atrophy of the spleen, moderate atrophy of the subcutaneous and abdominal adipose tissue, minimal yellowish-red discharge on the hair of the face, and minor dry urine stains on the inguinal hair. Exposure-related changes observed for the 1250 ppm female rats (# 787 and # 789) that died on Day 19 consisted of yellow mucous throughout the intestinal tract, abdominal hair wet with saliva, and a minor porphyrin nasal discharge (# 789 only). Agonal changes included gas in the stomach of Rat # 787, and minimal to minor thymus hemorrhage.

No exposure-related changes were observed for the 1250 ppm male and female rats that survived to necropsy or for the 400 and 125 ppm male and female rats.

Histopathology

There were no exposure-related gross or microscopic observations in the nervous system of animals on this study.

Exposure-related changes observed in the nasal passages showed minor to moderate squamous metaplasia of the respiratory epithelium (4/4 male and 5/5 female rats), minor to moderate degeneration of the olfactory epithelium (4/4 male and 5/5 female rats), and minimal serocellular exudate in the middle meatus (1/4 male rats) for the 1250 ppm group and minimal to minor squamous metaplasia (4/5 male and 5/5 female rats), minor to moderate degeneration of the olfactory epithelium (5/5 male and 5/5 female rats), and minimal serocellular exudate in the dorsal meatus (1/5 male and 2/5 female rats) for the 400 ppm male rats. Autolysis of the nasal passages prevented meaningful examination for male # 769 (1250 ppm).

Exposure-related changes observed in the thymus for the 1250 ppm group consisted of minimal to moderate atrophy (3/5 male and 4/5 female rats) and moderate hemorrhage (1/5 male and 1/5 female rats). Exposure-related changes observed in the spleen consisted of minor to moderate atrophy of the splenic lymphatic follicles (2/5 male and 2/5 female rats) and splenic red pulp (2/5 male rats) and minor to moderately reduced extramedullary hematopoiesis (4/5 female rats) in the red pulp for the 1250 ppm group. Exposure-related changes observed in the bone marrow consisted of minor to moderate atrophy (5/5 male and 5/5 female rats) and minor hemorrhages (1/5 male and 2/5 female rats) for the 1250 ppm group. Exposure-related changes in the testes consisted of minimal to moderate atrophy of spermatozoa (3/5), spermatids (3/5), and spermatocytes (2/5) with minimal multinucleated giant cell formation (1/5). The epididymides showed moderate to severe reduction in the number of spermatozoa (2/5), and minor to moderate spermatid degeneration (2/5). The accessory sex glands (prostate, seminal vesicles, and coagulating glands) were moderately atrophied for rat # 767.

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

DISCUSSION

Exposure to the test substance resulted in clear signs of neurotoxicity at a concentration of 1250 ppm. Animals had reduced activity during exposure and female rats in this group had chewing motions and head-shaking movements. Chewing motions could be associated with convulsions, but without examination of the animals, this could not be determined. Convulsions were observed on two occasions prior to exposures and were initiated by handling. No convulsions were observed during the FOB, however. Other indications of CNS excitability (tremors) were observed during the functional observational battery. The incidence and severity of tremors were significantly higher in the 1250 ppm group compared with the control group. In addition, neuromuscular activity was abnormal in the 1250 ppm group with reduced muscle tone, splayed walk, and hypotonic gait. The test substance has some structural similarity to 2,5-dimethylfuran, a cyclized material which can be synthesized from 2,5-hexanedione. This structural similarity suggested that effects on the nervous system might be similar to the peripheral neuropathy seen with 2,5-hexanedione. However, the observations of convulsions and tremors suggest a central nervous system effect rather than a peripheral effect; grip strength values were unchanged and hindlimb paralysis was not evident as might be expected with peripheral neuropathy. Thus, the test substance does not appear to fit the profile of γ -diketone neurotoxins in that effects reflect CNS excitability rather than peripheral neuropathy.

The mechanism of action of the test substance on the nervous system is unknown. CNS effects are not associated with other furan compounds. The results of a subchronic study of tetrahydrofuran (THF) conducted by the NTP (Chhabra *et al.*, 1990) reported only CNS depression during the 13-weeks of exposure, although Chhabra and others did reference an earlier study by Katahira *et al.* (1982) in which rats exposed to THF had clonic muscle spasm. The NTP study did not confirm those findings, however. The lack of neurohistopathologic lesions in the central and peripheral nervous system indicated that the test substance does not act by structural alterations of nerve tissue. On the other hand the absence of histologic lesions is not uncommon for shorter-term studies; some inhalation studies of n-hexane, a known neurotoxin that results in axonopathy, did not demonstrate this lesion even after 14 weeks of exposure to concentrations of 1500 ppm (O'Donoghue, 1985).

No CNS effects were observed in animals of the other groups except for a single male 400 ppm rat that exhibited tremors on Day 29. This animal (#765) also had slight blood changes with anisocytosis, microcytosis, macrocytosis, and elliptocytosis. While these hematologic findings and the occurrence of tremors may be related to the test substance, they appear to be an isolated case of a single animal with unusual susceptibility.

The test substance appears to be a nasal irritant based on the presence of squamous metaplasia of the respiratory epithelium, degeneration of the olfactory epithelium, and serocellular exudates in the dorsal meatus of animals exposed to 400 and 1250 ppm concentrations. Chhabra and coworkers (1990) found that THF also was a nasal irritant. Other histologic lesions observed in the 1250 ppm group appear to be unrelated to the irritant properties of the test substance, and may be related to reduced feed consumption and lower body weight gains. Atrophic changes which were observed in the brain, thymus, spleen, bone marrow, testes, epididymides, and male accessory sex glands of 1250 ppm-exposure group rats were considered secondary to reduced feed intake and loss of body weight. The histopathologic changes in the bone marrow, thymus,

and testes are likely related to the decrease in body weight and have been described for feed-restricted animals (Levin *et al.*, 1993).

Mean total protein concentrations and calcium concentrations in female rats increased in a concentration-dependent manner. However, no toxicological significance can be attributed to these changes in serum chemistry.

Based on the decrease in body weight in the 125 ppm group, a no-observed-effect level (NOEL) was not determined. However, based on the absence of histopathologic changes in the nasal passages and absence of any clinical signs of neurotoxicity, 125 ppm was considered to be the no-observed-adverse-effect level (NOAEL).

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STUDY TITLE

**2,5-DIHYDROFURAN
SYNONYMS: DHF**

A FOUR-WEEK INHALATION TOXICITY STUDY IN THE RAT

**HAEL No. 94-0214
CAS No. 001708-29-8**

**KAN/EAN 908701
PM No. 14504-00**

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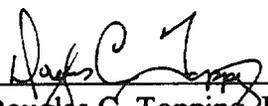
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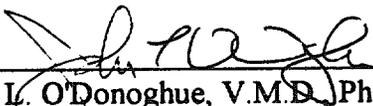
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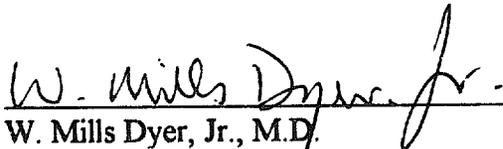
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