TRADE SECRET

Study Title

Inhalation Approximate Lethal Concentration (ALC) of H-22359 in Rats

Laboratory Project ID

HL-1997-00273

Author

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Study Completed

June 17, 1997

Performing Laboratory

E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
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Medical Research Project Number [redacted]

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR 792) Good Laboratory Practice Standards except for the deviation documented and explained below.

1. Characterization of the test substance was conducted at a non-GLP-compliant laboratory.

This deviation did not affect the integrity of the study.

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

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Date

Study Director: John R. Bamberger 13 June 1997
John R. Bamberger, M.A.
Toxicologist

GENERAL INFORMATION

Test Substance: [redacted]

Synonyms/Codes: [redacted]

Haskell Number(s): 22359

Composition: [redacted]

Known Impurities: [redacted]

Physical Form: White to yellow solution or slurry

Stability: In the absence of evidence to the con rary, the test substance was assumed to be stable under the conditions of administration.

Sponsor: E.I. du Pont de Nemours and Company
Wilmington, Delaware, 19898
U.S.A.

Study Initiated - Completed: February 17, 1997 - (see report cover page)

In-Life Study Initiated - Completed: February 18, 1997 - March 7, 1997

All original data and the original of this final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain, 200 Todds Lane, Wilmington, Delaware 19802.

SUMMARY

Two groups of six male Crl:CD®(SD)BR rats each were exposed nose-only for single, four-hour periods to aerosols of 2.3 or 3.9 mg/L H-22359 in air. Test atmospheres were generated by aerosolizing the test substance with a nebulizer and measured by gravimetric analysis. After exposure, rats were weighed and observed for clinical signs of toxicity during a 14-day recovery period.

All rats exposed at 2.3 mg/L survived the exposure and subsequent 14-day recovery period. Under the conditions of this study, the approximate lethal concentration (ALC) for H-22359 is 3.9 mg/L. At this concentration, one rat died during the exposure. Clinical signs of toxicity observed during this study at both concentrations tested included irregular respiration, gasping, oral, ocular, and/or nasal discharges, and moderate to severe weight losses. On an acute inhalation basis, H-22359 is considered to have very low toxicity (ALC greater than 2.0 mg/L).

Approved by: Judith C. Stadler 6/17/97
Judith C. Stadler, Ph.D., D.A.B.T.
Staff Toxicologist

Date

Authored,
Reviewed and
Approved for Issue
by Study Director: John R. Bamberger 17 June 1997
John R. Bamberger, M.A.
Toxicologist

QUALITY ASSURANCE DOCUMENTATION

(H-22359)

Dates of Inspection:

Conduct - 2/18/97

Records, Report(s) - 5/22,27/97

Date(s) Findings Reported to:

Study Director - 5/28/97

Management - 6/13/97

Reported by: James Mackay II 6/13/97

James Mackay II
Quality Assurance Auditor

Date

STUDY PERSONNEL

The following individuals were responsible for conduct of the study:

Management:	Scott E. Loveless, Ph.D.
Study Director:	John R. Bamberger, M.A.
Inhalation Consultant:	Judith C. Stadler, Ph.D., D.A.B.T.
Technician(s):	Kenneth L. Reed
Toxicology Report Preparation:	Maryanne M. Wilford, B.A.

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

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

INTRODUCTION

The objective of this study was to determine a four-hour inhalation approximate lethal concentration (ALC) of H-22359 in male rats. The ALC is defined as the lowest atmospheric concentration tested which caused the death of one or more exposed rats either on the day of exposure or within at least 14 days following exposure. The inhalation route of exposure was chosen based on the expected route of potential human exposure and was requested by the sponsor.

MATERIALS AND METHODS

A. Test Substance

The test substance, H-22359, was supplied by the sponsor as a white to yellow slurry. Prior to exposures, the slurry was heated in a water bath to an approximate temperature of 40°C to bring the slurry back to a solution suitable for spraying.

B. Animals

Young adult, male, Crl:CD®(SD)BR rats were obtained from Charles River Breeding Laboratories. The rats were approximately seven weeks old upon arrival.

Rats have historically been used in safety evaluation studies for acute inhalation toxicity testing. The Crl:CD®(SD)BR rat has been chosen based on consistently acceptable health status and the extensive experience with the strain at this laboratory.

C. Animal Husbandry

1. Quarantine and Animal Selection

Rats were quarantined after arrival for approximately six days prior to testing. During the quarantine period, rats were weighed and observed for clinical signs of disease three times. Rats were obtained from the general population of stock rats released from quarantine and were selected for use on this study from those rats exhibiting a normal pattern of weight gain and no overt signs of disease.

2. Housing

Rats were housed either singly or in pairs in suspended, stainless steel, wire-mesh cages.

3. Animal Room Environment

Animal rooms were maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the rooms were targeted to be within a temperature range of 23 ± 1°C and a relative humidity range of 50 ± 10%. Excursions outside these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

4. Identification

Each rat was assigned a unique six-digit identification number which corresponded to a numbered card affixed to the cage. Prior to exposure, the tail of each rat was color-coded with water-insoluble markers so that individual rats could be identified after exposure.

5. Feed and Water

Except during exposure, Purina® Certified Rodent Chow® #5002 and tap water from United Water Delaware were available *ad libitum*.

6. Health Monitoring Program

Haskell Laboratory has an animal health monitoring program. The following procedures are performed periodically:

- Water samples are analyzed for total bacterial counts, and the presence of coliforms, lead, and other contaminants.
- Feed samples are analyzed for the presence of bacteria and fungi.
- Samples from freshly washed cages and cage racks are analyzed to ensure adequate sanitation by the cage washers.

Haskell Laboratory uses certified animal feed. The feed is guaranteed by the manufacturer to meet specified nutritional requirements and to be free of a list of specified contaminants.

The animal health monitoring program is administered by the laboratory animal veterinarian. Data are maintained separately from study records and are not included in the final report. Evaluation of these data did not indicate any conditions that affected the validity of the study.

D. Study Design

Two groups of six male rats each were exposed to aerosols of H-22359 in air. Rats were exposed nose-only for a single four-hour period. Because of the dense atmosphere resulting from the high aerosol concentration of the test substance, observations for clinical signs could not be conducted during exposures. The rats were observed for mortality and clinical signs of toxicity immediately after they were removed from the

restrainers following exposure. During a 14-day postexposure period, all surviving rats were observed each day for mortality. Rats in the 2.3 mg/L group were weighed and observed for clinical signs of toxicity on test days 2, 3, 8, and 15. Rats in the 3.9 mg/L group were weighed and observed for clinical signs of toxicity on test days 2, 3, 4, 9, and 15. At the end of the recovery period, all surviving rats were sacrificed by carbon dioxide asphyxiation and discarded.

E. Inhalation Exposure System

1. Atmosphere Generation

Aerosol atmospheres of H-22359 were generated by metering the liquid test substance into a heated (40-50°C) Spraying Systems nebulizer with a Harvard Apparatus model 22 Syringe Infusion Pump equipped with heated syringes (40-50°C). Filtered, houseline air introduced into the nebulizer atomized the liquid test substance and carried the resulting aerosol into the exposure chamber. The concentration of H-22359 was controlled by varying the amount of test substance delivered to the nebulizer.

Test atmospheres were exhausted through a dry-ice cold trap, an aerosol scrubber, and an MSA charcoal/HEPA filter cartridge prior to discharge into the fume hood.

2. Chamber Construction and Design

The exposure chamber was constructed of glass with a nominal internal volume of approximately 20 liters. A baffle located immediately inside the chamber was used to promote uniform distribution of aerosol.

3. Animal Exposures

Rats were exposed nose-only to the test substance to avoid concurrent exposure by the dermal or oral routes. During exposure, rats were individually restrained in perforated stainless steel cylinders with conical nose pieces. The restrainers were inserted into the faceplate of the exposure chamber so that only the nose of each rat extended into the chamber.

F. Characterization of Chamber Atmosphere

1. Test Substance Sampling and Analysis

The atmospheric concentration of H-22359 was determined by gravimetric analysis at approximately 30-minute intervals during each exposure. Known volumes of chamber atmospheres were drawn from the reference sampling port through a 25 mm filter cassette that contained a pre-weighed Gelman glass fiber (Type A/E) filter. The filters were weighed on a Cahn model C-31 Microbalance. The atmospheric concentration of H-22359 was calculated from the difference in the pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled.

Samples to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 1, 3, and 10 µm diameter) were taken with a Sierra® Series 210 Cyclone Preseparator/Cascade Impactor and Sierra® Series 110 Constant Flow Air Sampler.[1]

2. Environmental Monitoring

Chamber airflow was set at the beginning of each exposure to achieve at least 12 air changes per hour. Total chamber airflow was monitored continually with a calibrated Brooks model 1355 Rotometer, and was recorded two or three times during each exposure. Chamber temperature was targeted at 23 ± 2°C. Chamber temperature was monitored continually with an Omega model 650 Thermocouple Thermometer, and was recorded three or four times during each exposure. Chamber relative humidity was targeted at 50 ± 10%. Chamber relative humidity was measured with an Omega model RH-5100-c Digital Psychrometer, and was recorded two or three times during each exposure. Chamber oxygen concentration was targeted to at least 19%. Chamber oxygen concentration was measured with a Biosystems model 3100R Oxygen Analyzer, and was recorded two or three times during each exposure.

RESULTS

A. Exposure Conditions

Two groups of six male rats each were exposed to aerosol atmospheres of 2.3 or 3.9 mg/L of H-22359 in air. The test atmospheres were considered respirable in rats with mass median aerodynamic diameter (MMAD) for aerosol particles ranging from 3.9 to 4.0 µm. Pertinent test atmosphere characteristics and associated animal mortality are in Table 1.

Chamber temperature ranged from 21 to 23°C, chamber relative humidity ranged from 85 to 98%, chamber airflow was maintained at 15 L/min, and the oxygen concentration was 21%. The measured relative humidity was above the targeted range of 40-60%. The elevated relative humidity was expected based on the physical characteristics of the test substance and the method of generation. The results of the study indicate the deviation of relative humidity from the targeted range did not adversely affect the welfare of the rats.

TABLE 1

CHARACTERIZATION OF TEST ATMOSPHERES
AND ASSOCIATED ANIMAL MORTALITY

AEROSOL CONCENTRATION (mg/L)[a]			AEROSOL SIZE			MORTALITY
Mean	S.D.	Range	MMAD (µm)[b]	GSD[c]	Percent <10 µm[d]	(# deaths/ # exposed)
2.3	0.63	1.3 - 3.3	4.0	2.7	83	0 / 6
3.9	0.47	3.2 - 4.4	3.9	2.6	84	1 / 6

a Represents the mean, standard deviation (S.D.), and range for each exposure. Based on eight samples per exposure. Values are reported to two significant figures.
b The MMAD (Mass Median Aerodynamic Diameter) is based on one particle size sample taken during each exposure.
c Geometric Standard Deviation.
d Percent aerosol mass having aerodynamic equivalent diameters of less than 10 µm.

B. Mortality, Clinical Signs, and Body Weights

No deaths occurred in rats exposed at 2.3 mg/L. One rat exposed at 3.9 mg/L died during the exposure.

Following each exposure, when the rats were removed from the chamber, the clinical signs of toxicity frequently observed included irregular respiration, gasping, and ocular, nasal, and/or oral discharges. Clinical signs of toxicity in rats from the 2.3 or 3.9 mg/L groups resolved by one day and three days, respectively, following exposure. No clinical signs of toxicity attributable to H-22359 were observed during the 14-day recovery period.

All surviving rats from each group experienced moderate to severe weight losses within one day of exposure. Weight losses ranged from 5.4 to 14% of initial body weight. Body weights of rats began to increase by study day three and increased over the remainder of the 14-day recovery period.

CONCLUSION

The purpose of this study was to determine an ALC for H-22359. Under the conditions of this study, the ALC for H-22359 is 3.9 mg/L. On an acute inhalation basis, aerosols of H-22359 are considered to have very low toxicity (ALC greater than 2.0 mg/L).

REFERENCES

1. Calculation described in Sierra Instruments, Inc., Bulletin 7-79-219IM, Instruction Manual: Series 210 Ambient Cascade Impactors and Cyclone Preseparators.