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TRADE SECRET

Study Title

H-25134: Inhalation Approximate Lethal Concentration (ALC) in
Rats

Laboratory Project ID: DuPont-9603

AUTHOR: Thomas A. Kegelman, A.A.

STUDY COMPLETED ON: May 22, 2002

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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[REDACTED]
[REDACTED]

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CERTIFICATION

I, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Issued by Study Director:

Thomas A. Kogelman
Thomas A. Kogelman, A.A.
Staff Scientist

22 May 2002
Date

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STUDY INFORMATION

Substance Tested: [REDACTED]

Synonyms/Codes: [REDACTED]

H-25134

Submitter's Notebook Number(s): [REDACTED]

Haskell Number: 25134

CAS Registry Number: [REDACTED]

Composition: [REDACTED]

Purity: [REDACTED]

Known Impurities: [REDACTED]

Physical Characteristics: Dark brown solid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

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

Study Initiated/Completed: February 12, 2002 / (see report cover page)

In-Life Initiated/Completed: February 12, 2002 / March 19, 2002

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STUDY PERSONNEL

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SUMMARY

Three groups of 6 male Crl:CD[®](SD)IGS BR rats were exposed nose-only for a single, 4-hour period to H-25134 in air. Test atmospheres were generated by heating the test substance to a targeted temperature of 304°C. Concentrations of the condensation aerosol of H-25134 were measured gravimetrically. Rats were weighed and observed for clinical signs of toxicity during a 14-day recovery period.

Rats were exposed to atmospheres of H-25134 at mean concentrations of 259, 336, or 377 mg/m³. No deaths occurred in the 259 and 336 mg/m³ exposure groups, however, all rats in the 377 mg/m³ group were found dead within 4 hours of the exposure. The lethality data for this study indicated a steep dose response in rats exposed to aerosol concentrations slightly higher than 336 mg/m³, therefore, it was not practical to conduct an exposure that resulted in partial mortality. The mass median aerodynamic diameters for the aerosol tested ranged from 3.4 to 4.4 µm.

The only clinical sign of toxicity observed during this study was ocular discharge. This sign was only observed upon unloading the rats from the restrainers after exposure. In addition, rats in the 259 and 336 mg/m³ exposure groups exhibited slight body-weight (less than 5% of initial body weight) losses on the day following the exposure but began to regain weight by the next day.

Under the conditions of this study, the ALC for H-25134 is 377 mg/m³. According to Haskell Laboratory toxicity classifications, H-25134 is considered to be moderately toxic (ALC between 200 and 800 mg/m³) in male rats.

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INTRODUCTION

The objective of this study was to determine a 4-hour inhalation approximate lethal concentration (ALC) of H-25134 in male rats. The ALC is defined as the lowest atmospheric concentration tested which caused the death of 1 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-25134, was supplied by the sponsor as a dark brown solid with a purity of [REDACTED]. The test substance was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed.

B. Animals

Young adult, male Crl:CD[®](SD)IGS BR rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The rats were approximately 7 weeks old on the day of arrival.

Rats have historically been used in safety evaluation studies for inhalation toxicity testing. The Crl:CD[®](SD)IGS BR rat was selected based on consistently acceptable health status and on extensive experience with the strain at Haskell Laboratory.

C. Animal Husbandry

1. Quarantine and Animal Selection

Rats were quarantined after arrival for 6 days prior to testing. During the quarantine period, rats were weighed and observed for clinical signs of disease 3 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 stainless steel, wire-mesh cages suspended above cage boards.

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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 $23 \pm 1^\circ\text{C}$ and a relative humidity range of $50 \pm 10\%$. 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 6-digit identification number which corresponded to a numbered card affixed to the cage. Prior to exposure, the tail of each rat was coded with water-insoluble markers so that individual rats could be identified after exposure.

5. Feed and Water

Except during exposure, PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 and tap water from United Water Delaware were available *ad libitum*.

6. Health Monitoring Program

As specified in the Haskell Laboratory animal health and environmental monitoring program, the following procedures are performed periodically to ensure that contaminant levels are below those that would be expected to impact the scientific integrity of the study:

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

Certified animal feed is used, guaranteed by the manufacturer to meet specified nutritional requirements and not to exceed stated maximum concentrations of key contaminants, including specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The presence of these contaminants below the maximum concentration stated by the manufacturer would not be expected to impact the integrity of the study.

The animal health and environmental monitoring program is administered by the attending laboratory animal veterinarian. Evaluation of these data did not indicate any conditions that affected the validity of the study.

D. Study Design

Three groups of 6 male rats each were exposed to aerosol atmospheres of H-25134 in air. Rats were exposed nose-only for a single, 4-hour period.

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Rats were approximately 8 weeks old and weighed between 233 and 278 grams at the time of exposure.

Rats were observed for mortality during each exposure and 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, and were generally weighed and observed for clinical signs of toxicity daily until weight gains were observed, and then were weighed and observed weekly. 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

Chamber atmospheres were generated by aerosolization of the test substance. The test substance was placed in a 1000-mL round-bottom flask that was heated to 304-309°C via a Unimantle heater. A stirring motor was positioned over the top of the flask with a rod attached to the motor to agitate the melted test material. Filtered, high-pressure air, metered to the flask by a Brooks model 1355 Sho-Rate Rotometer, carried the condensation aerosol into the exposure chamber. Additional filtered, high-pressure air metered downstream from the flask by an identical rotometer, diluted the test atmosphere to the desired concentration. Chamber concentrations of the test substance were controlled by varying the airflow rates through the flask. The temperature of the test substance inside the flask was targeted at 304°C and adjusted by varying the voltage to the Unimantle heater.

Test atmospheres were exhausted through a dry-ice cold trap followed by 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 (cylindrical) with a nominal internal volume of 29 L. A baffle inside the chamber promoted uniform chamber distribution of the test atmosphere.

3. Exposure Mode

During exposure, rats were individually restrained in perforated stainless steel cylinders with conical nose pieces. The restrainers were inserted into a polymethylmethacrylate faceplate which was attached to the exposure chamber so that only the nose of each rat extended into the chamber.

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F. Characterization of Chamber Atmosphere

1. Test Substance Sampling and Analysis

The atmospheric concentration of H-25134 was determined by gravimetric analysis at approximately 30-minute intervals during each exposure. Known volumes of chamber atmosphere were drawn from the 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-33 Microbalance[®]. The atmospheric concentration of H-25134 was calculated from the difference in the pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled.

A sample to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 1, 3, and 10 μm diameter) was taken during each exposure 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. The airflow was monitored continually with calibrated Brooks model 1355 Sho-Rate Rotometers and recorded initially and whenever changes were made during each exposure. Chamber temperature was targeted at $22 \pm 2^\circ\text{C}$. The temperature was monitored continually with an Omega model 650 Thermocouple Thermometer and recorded 3 times during each exposure. Chamber relative humidity was targeted at $50 \pm 10\%$. The relative humidity was measured with an Omega model 5100 Digital Psychrometer and recorded 3 times during each exposure. Chamber oxygen concentration was targeted to be at least 19%. The oxygen concentration was measured with a Biosystems model 3100R Oxygen Analyzer and recorded 3 times during each exposure.

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RESULTS AND DISCUSSION

A. Exposure Conditions (Table 1)

Animals were exposed to H-25134 at concentrations of 259, 336, or 377 mg/m³. The atmospheres generated in this study were considered to be respirable to marginally respirable in rats, as the mass median aerodynamic diameters (MMAD) ranged from 3.4 to 4.4 µm.

TABLE 1

CHARACTERIZATION OF TEST ATMOSPHERES AND ASSOCIATED ANIMAL MORTALITY

AEROSOL CONCENTRATION (mg/m ³) ^a				AEROSOL SIZE			MORTALITY (# deaths/ # exposed)
Mean	S.D.	Range	n	MMAD (µm) ^b	GSD ^c	Percent <10 µm ^d	
259	40.5	173 - 295	8	4.4	1.7	96	0 / 6
336	27.4	286 - 373	8	4.3	1.5	98	0 / 6
377	54.0	290 - 441	8	3.4	1.6	99	6 / 6

a Represents the mean, standard deviation (S.D.), and range for each exposure, based on n samples per exposure. Values are reported to 3 significant figures.

b The MMAD (Mass Median Aerodynamic Diameter) is based on 1 particle size sample taken during each exposure.

c Geometric Standard Deviation.

d Percent aerosol mass having aerodynamic equivalent diameters of less than 10 µm.

Chamber temperature ranged from 19 to 23°C, chamber relative humidity ranged from 30 to 47%, chamber airflow ranged from 19 to 21 L/min, and the oxygen concentration was 21%. Although chamber temperature and relative humidity were slightly outside the targeted parameters, these deviations were considered not to have adversely affected the validity of this study.

An additional exposure was conducted with a mean concentration of 257 mg/m³, however, an excursion occurred during this exposure that exceeded the lethal concentration of the test substance. Because the excursion exceeded the lethal concentration for this test substance, the results of this exposure were not considered when determining the ALC for this study.

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B. Mortality, Clinical Signs, and Body Weights

Deaths occurred at concentrations of 377 mg/m³ H-25134. Rats were found dead within 4 hours of exposure.

Following exposures, when the rats were removed from the restrainers, the only clinical sign of toxicity observed was ocular discharge. During the remainder of the recovery period, no clinical signs of toxicity were observed.

Slight weight losses were observed in rats from the 259 and 336 mg/m³ groups on the day following the exposure, and ranged from 1 to 3 percent of initial body weight. Rats subsequently resumed normal weight gains (approximately 5-10 g/day) and gained weight for the remainder of the recovery period.

CONCLUSIONS

Under the conditions of this study, the approximate lethal concentration (ALC) for H-25134 is 377 mg/m³. According to Haskell Laboratory toxicity classifications, H-25134 is considered to be moderately toxic (ALC 200-800 mg/m³) in male rats.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware (formerly known as the E.I. du Pont de Nemours and Company Records Management Center).

REFERENCES

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

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