

TRADE SECRET

Study Title

[REDACTED] INHALATION APPROXIMATE LETHAL
CONCENTRATION (ALC) IN RATS

LABORATORY PROJECT ID: HL-1997-00598

AUTHOR: David P. Kelly, B.S.

STUDY COMPLETED ON: August 29, 1997

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

MEDICAL RESEARCH NUMBER: [REDACTED]

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[REDACTED]

GENERAL INFORMATION

Substance Tested:

[REDACTED]

Synonyms/Codes:

[REDACTED]

- H-22387
- H-22632

Haskell Number(s): 22387, 22632

CAS Registry Number(s):

[REDACTED]

Components:

[REDACTED]

Purity:

[REDACTED]

Composition:

[REDACTED]

Contaminants:

[REDACTED]

Physical Characteristics: water clear liquid

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

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GENERAL INFORMATION (Continued)

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

Study Initiated - Completed: February 13, 1997 - (see report cover page)

In-Life Initiated - Completed: February 13, 1997 - July 9, 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.

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SUMMARY

Groups of six male Crl:CD®(SD)BR rats each were exposed nose-only for a single, four-hour period to a water mixture of [REDACTED] (H-22387 and H-22632) in air. Test atmospheres were generated by aerosolization, and concentrations of [REDACTED] were measured by gravimetric filter analysis. Aerosol concentrations were reported as wet and dry aerosol. Rats were weighed and observed for clinical signs of toxicity during a 14- to 15-day recovery period.

Rats were exposed to atmospheres of [REDACTED] at wet aerosol concentrations of 320, 330, or 570 mg/m³, corresponding to dry aerosol concentrations of 210, 270, or 420 mg/m³. The mass median aerodynamic diameters of the aerosols tested were 5.0, 2.3, and 3.0 µm, respectively. Rats died following exposure to [REDACTED] at concentrations of 320 and 570 mg/m³ (wet), corresponding to 210 and 420 mg/m³ (dry). Deaths occurred immediately after exposure or within two days. No deaths occurred after exposure at concentrations of 320 mg/m³ (wet), 270 mg/m³ (dry).

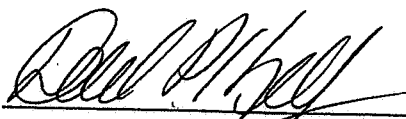
Under the conditions of this study, the approximate lethal concentration (ALC) for aerosolized [REDACTED] 320 mg/m³ (wet aerosol), 210 mg/m³ (dry aerosol). On an acute inhalation basis and based on the dry aerosol concentration [REDACTED] is considered to be moderately toxic (ALC 200-800 mg/m³).

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SIGNATURES

Date

Reviewed and
Approved for Issue:



David P. Kelly, B.S.
Research Toxicologist
Study Director

8/29/97

STUDY PERSONNEL

The following individuals were responsible for conduct of the study:

Management: Scott E. Loveless, Ph.D.

Study Director: David P. Kelly, B.S.

Primary Technicians: M. Ahmad Pulliam, B.S.
Dwayne A. Lavoie, B.S.

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.

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INTRODUCTION

The objective of this study was to determine a four-hour inhalation approximate lethal concentration (ALC) of [REDACTED] 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. Data for this study was obtained using two protocols: an inhalation screen protocol (SOP AI005-T) and an approximate lethal concentration protocol (SOP AI001-T).

MATERIALS AND METHODS

A. Test Substance

The test substance [REDACTED] (H-22387 and H-22632) was supplied by the sponsor as a mixture of [REDACTED] in water. This was diluted to [REDACTED] at Haskell Laboratory to facilitate spraying.

B. Animals

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

Rats have historically been used in safety evaluation studies for acute inhalation toxicity testing. The CrI: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.

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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 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 or numbered 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

Three groups of six male rats each were exposed to atmospheres of [REDACTED] in air. Rats were exposed nose-only for a single, four-hour period.

Rats were approximately eight to nine weeks old and weighed between 255 to 289 grams at the time of exposure.

Rats were observed for mortality and response to alerting stimuli during the exposure and observed for mortality and clinical signs of toxicity immediately after they were removed from

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the restrainers following exposure. During a 14- to 15-day postexposure period, all surviving rats were observed each day for mortality, and were weighed and observed for clinical signs of toxicity at least twice 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 [REDACTED] in air with a Spraying Systems nebulizer. The test substance was metered into the nebulizer with a Harvard Apparatus model 22 Syringe Infusion Pump. Filtered houseline air, introduced at the nebulizer, atomized the test substance and carried the aerosol into the exposure chamber. Chamber concentrations of [REDACTED] were controlled by varying the test substance feed rate to the atmosphere generator.

Test atmospheres were exhausted through a bubbler, a dry-ice cold trap, 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 (cylindrical) with a nominal internal volume of 34 L. A dispersion plate at the chamber inlet 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 the polymethylmethacrylate 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 [REDACTED] was determined by gravimetric analysis at approximately 30-minute intervals during 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. After samples were collected, the filters were immediately weighed to determine the wet aerosol concentration. After overnight drying in a dessicator, the filter samples were weighed to determine the dry aerosol weight. The filters were weighed on a Cahn model C-30 Microbalance®. The atmospheric concentration of [REDACTED] was calculated from the difference in the pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled.

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Samples to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 3 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. The airflow was monitored continually with a calibrated Brooks model 1355 Sho-Rate Rotometer and recorded one to four times during 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 two to five times during exposure. Chamber relative humidity was targeted at $50 \pm 10\%$. The relative humidity was measured with an Omega Digital Psychrometer and recorded two to three times during exposure. Chamber oxygen concentration was targeted to at least 19%. The oxygen concentration was measured with a Biosystems model 3100R Oxygen Analyzer and recorded two to three times during exposure.

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RESULTS

A. Exposure Conditions (Table 1)

Animals were exposed to [REDACTED] concentrations of 210, 270, or 420 mg/m³. The aerosols generated during the 270 mg/m³ exposure were considered to be marginally respirable in rats as the mass median aerodynamic diameters (MMAD) was 5.0 μ m.

TABLE 1

CHARACTERIZATION OF TEST ATMOSPHERES AND ASSOCIATED ANIMAL MORTALITY

AEROSOL CONCENTRATION (mg/m ³) ^a							AEROSOL SIZE			MORTALITY (# deaths/ # exposed)
Wet Filters			Dry Filters			n	MMAD (μm) ^b	GSD ^c	Percent < 3 μm ^d	
Mean	S.D.	Range	Mean	S.D.	Range					
320	98	210 - 480	270	87	160 - 400	8	5.0	3.1	33	0 / 6
330	35	290 - 390	210	25	190 - 260	8	2.3	2.9	66	5 / 6
570	500	210 - 1800	420	250	150 - 940	12	3.0	2.3	53	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 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 3 μ m.

Chamber temperature ranged from 20 to 23°C, chamber relative humidity ranged from 76 to 86%, chamber airflow ranged from 15 to 25 L/min, and the oxygen concentration was 21%. The chamber relative humidity was high because of the aerosolized water content of the test mixture.

B. Mortality, Clinical Signs, and Body Weights

Deaths occurred at [REDACTED] concentrations of 210 mg/m³ or greater. Rats died within two days of exposure.

A diminished alerting response was observed near the end of the 270 mg/m³ exposure. Following exposures, when the rats were removed from the chambers, the clinical signs of toxicity observed included irregular respiration, lung noise, lethargy, and wet perineum.

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Surviving rats showed slight to severe weight loss on the day after exposure. Normal weight gain rates were resumed within one week after exposure.

CONCLUSION

In these studies, the difference in aerosol concentrations between a lethal level, where 5 of 6 rats died, and a non-lethal level was minimal. It is speculated that the reason rats died at 330 mg/m³ (wet aerosol), 210 mg/m³ (dry), and none died at 320 mg/m³ (wet), 270 mg/m³ (dry), was that the aerosol particle was larger (5 µm) in the 270 mg/m³ experiment and smaller in the 210 mg/m³ experiment. Although both aerosol sizes are inhalable, the smaller aerosol would reach deeper in the lung, and possibly cause more acute damage. From an industrial health standpoint, it is recommended that production of small aerosols of this material be minimized.

Under the conditions of this study, the approximate lethal concentration (ALC) for aerosolized [REDACTED] is 320 mg/m³ (wet aerosol), 210 mg/m³ (dry aerosol). On an acute inhalation basis and based on the dry aerosol concentration [REDACTED] is considered to be moderately toxic (ALC 200-800 mg/m³).

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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