

AR 226-3239

TRADE SECRET*Study Title*

H-24678: Inhalation Approximate Lethal Concentration (ALC) in
Rats

Laboratory Project ID: DuPont-8684

AUTHOR: John R. Bamberger, M.A.

STUDY COMPLETED ON: April 5, 2002

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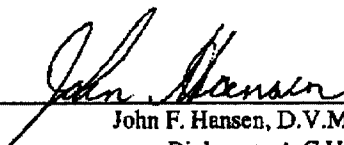
[REDACTED]
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CERTIFICATION

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

**Anatomic Pathology
Evaluations Reported by:**

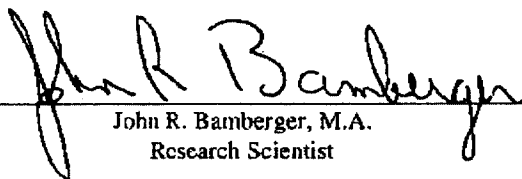


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05 April 2002

Date

Issued by Study Director:



John R. Bamberger, M.A.
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05 April 2002

Date

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STUDY INFORMATION

Substance Tested: [REDACTED]

Synonyms/Codes: [REDACTED]

• H-24678

Haskell Number: 24678

Composition: [REDACTED]

Known Impurities: [REDACTED]

Physical Characteristics: Amber brown liquid

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: November 28, 2001 / (see report cover page)

In-Life Initiated/Completed: November 29, 2001 / December 27, 2001

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STUDY PERSONNEL

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SUMMARY

Three groups of 6 male Crl:CD[®](SD)IGS BR rats each were exposed nose-only for a single, 4-hour period to aerosols of H-24678 in air at concentrations of 23, 57, or 120 mg/m³ (dry weight). Test atmospheres were generated by atomization, and concentrations of H-24678 were measured gravimetrically. Rats were weighed and observed for clinical signs of toxicity during a 14-day recovery period. Surviving rats from the 57 mg/m³ group underwent pathological evaluation at the end of the recovery period.

The mass median aerodynamic diameters for the aerosol tested ranged from 1.3 to 1.8 μ m. Rats died following exposure to H-24678 at concentrations of 57 mg/m³ or greater; deaths occurred within 1 day of exposure. Clinical signs of toxicity noted during the study included a diminished response to an auditory stimulus in rats during exposure to 57 or 120 mg/m³. In addition, rats exposed at 120 mg/m³ exhibited lethargy, lung noise, and labored breathing immediately following the exposure.

One rat exposed at 23 mg/m³ exhibited slight body-weight loss within 1 day of exposure (2.1% of pre-exposure body weight) but began to regain weight by test day 2. Rats exposed at 57 mg/m³ exhibited slight to moderate body-weight losses within 2 days of exposure (3.1 to 14% of pre-exposure body weight) but began to regain weight by test day 3.

Under the conditions of this study, the approximate lethal concentration (ALC) for H-24678 is 57 mg/m³. According to Haskell Laboratory toxicity classifications, H-24678 is considered to be extremely toxic (ALC less than 0.08 mg/L) 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-24678 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-24678, was supplied by the sponsor as an amber brown liquid. 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 approximately 1 week 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-24678 in air. Rats were exposed nose-only for a single, 4-hour period.

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Rats were approximately 8 or 9 weeks old and weighed between 211 and 316 grams at the time of exposure.

Rats were observed for mortality and response to alerting stimuli 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 weighed and observed for clinical signs of toxicity daily until weight gains were observed then once weekly thereafter. At the end of the recovery period, all surviving rats were sacrificed by carbon dioxide asphyxiation. Three rats from the 57 mg/m³ group underwent a gross pathology examination. Tissues were taken from these 3 rats and further processed for microscopic pathology examination.

E. Inhalation Exposure System

1. Atmosphere Generation

Chamber atmospheres were generated by atomization of the test substance 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, high-pressure air, metered into the nebulizer by a Brooks model 1355 Sho-Rate Rotometer, carried the resulting atmosphere into the exposure chamber. Chamber concentrations of test substance were controlled by varying the test substance feed rate to the atmosphere generator.

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 polycarbonate 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.

F. Characterization of Chamber Atmosphere

1. Test Substance Sampling and Analysis

The atmospheric concentration of H-24678 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-30 Microbalance®, placed in a dessicator for 1 day, and reweighed on the same balance. The atmospheric concentration of H-24678 was calculated from the difference in the pre- and post-sampling dry 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 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 a calibrated Brooks model 1355 Sho-Rate Rotometer and recorded initially and whenever changes were made during the exposure. Chamber airflow was inadvertently not recorded during the 23 or 120 mg/m^3 exposures. Chamber temperature was targeted at $22 \pm 2^\circ\text{C}$. The temperature was monitored continually with a glass alcohol thermometer and recorded 4 times during each exposure. Chamber relative humidity was targeted at $50 \pm 10\%$. The relative humidity was measured with an Omega model RH5100C 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.

G. Anatomic Pathology Evaluation

The 3 surviving animals in the 57 mg/m^3 group had the lungs, trachea, pharynx/larynx, and nose grossly examined at sacrifice. In addition, the tissues from these rats were processed for histology and microscopically examined.

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RESULTS AND DISCUSSION

A. Exposure Conditions (Table 1)

Animals were exposed to H-24678 at concentrations of 23, 57, or 120 mg/m³. The concentrations reported are based on the solids component of the test substance. The solvent component of the test substance was measured by gas chromatography during each exposure. The mean concentration of solvent measured during the study was less than 53 ppm. This concentration is negligible compared to the reported acute inhalation toxicity of the solvent. Therefore, the concentrations reported do not include the solvent component of the test substance. The atmospheres generated in this study were considered to be respirable in rats, as the mass median aerodynamic diameters (MMAD) ranged from 1.3 to 1.8 µm.

Chamber temperature ranged from 22 to 23°C, chamber relative humidity ranged from 40 to 45%, chamber airflow was 20 L/min, and the oxygen concentration was 21%. Chamber airflow was inadvertently not recorded during the 23 or 120 mg/m³ exposures.

B. Mortality, Clinical Signs, and Body Weights

Deaths occurred at concentrations of 57 mg/m³ H-24678 or greater. Three of 6 rats died at a concentration of 57 mg/m³ and all rats died at 120 mg/m³. Rats died within 1 day of exposure.

Clinical signs of toxicity noted during the study included a diminished response to an auditory stimulus in rats during exposure to 57 or 120 mg/m³. In addition, rats exposed at 120 mg/m³ exhibited lethargy, lung noise, and labored breathing immediately following the exposure.

One rat exposed at 23 mg/m³ exhibited slight body-weight loss within 1 day of exposure (2.1% of pre-exposure body weight) but began to regain weight by test day 2. Rats exposed at 57 mg/m³ exhibited slight to moderate body-weight losses within 2 days of exposure (3.1 to 14% of pre-exposure body weight) but began to regain weight by test day 3.

C. Anatomic Pathology Evaluation (Appendix A)

1. Gross Pathology

There were no gross pathologic observations in any of the tissues examined.

2. Microscopic Pathology

There were no microscopic pathologic observations in any of the tissues examined.

CONCLUSIONS

Under the conditions of this study, the approximate lethal concentration (ALC) for H-24678 is 57 mg/m³. According to Haskell Laboratory toxicity classifications, H-24678 is considered to be extremely toxic (ALC less than 0.08 mg/L) 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

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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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 (μ m) ^b	GSD ^c	Percent <10 μ m ^d	
23	3.7	14 - 27	8	1.4	1.9	~100	0 / 6
57	13	42 - 77	8	1.8	2.0	~100	3 / 6
120	11	110 - 140	8	1.3	1.9	~100	6 / 6

a Represents the mean, standard deviation (S.D.), and range for each exposure, based on n samples per exposure. Values represent dry weight of the test substance and are reported to 2 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 μ m.

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APPENDIX A

Individual Animal Pathology Data

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Individual Animal Pathology Data

Concentration : 57 mg/m³ Sex: Males

Animal Ref Microscopic & Macroscopic Findings

654083 Terminal Sacrifice
 Killed on Day : 14
 Animal is signed off from necropsy

Gross Pathology :

No Macroscopic Abnormality Observed :
LUNGS, TRACHEA, PHARYNX/LARYNX, NOSE

Histopathology :

No Microscopic Abnormality Observed :
LUNGS, TRACHEA, PHARYNX/LARYNX, NOSE

654084 Terminal Sacrifice
 Killed on Day : 14
 Animal is signed off from necropsy

Gross Pathology :

No Macroscopic Abnormality Observed :
LUNGS, TRACHEA, PHARYNX/LARYNX, NOSE

Histopathology :

No Microscopic Abnormality Observed :
LUNGS, TRACHEA, PHARYNX/LARYNX, NOSE

654086 Terminal Sacrifice
 Killed on Day : 14
 Animal is signed off from necropsy

Gross Pathology :

No Macroscopic Abnormality Observed :
LUNGS, TRACHEA, PHARYNX/LARYNX, NOSE

Histopathology :

No Microscopic Abnormality Observed :
LUNGS, TRACHEA, PHARYNX/LARYNX, NOSE

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