AR226_3285

TRADE SECRET

Study Title

H-25777: Inhalation Approximate Lethal Concentration (ALC) in Rats

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STUDY COMPLETED ON: July 8, 2003

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell Laboratory for Health and Environmental Sciences
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LABORATORY PROJECT ID: DuPont-12966

WORK REQUEST NUMBER: [redacted]

SERVICE CODE NUMBER: [redacted]

CERTIFICATION

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

Anatomic Pathology Evaluation by: _____ 8-July-2003
Peter C. Mann, D.V.M. Date
Diplomate A.C.V.P.
Veterinary Pathologist

Issued by Study Director: _____ 8-JULY-2003
David P. Kelly, B.S. Date
Research Toxicologist

Company Sanitized. Does not contain TSCA CBI

STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: [redacted]

- H-25777

[redacted]

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

Haskell Number: 25777

Composition: [redacted]

Physical Characteristics: Pale yellow 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: May 8, 2003 / (see report cover page)

Company Sanitized. Does not contain TSCA CBI

STUDY PERSONNEL

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

Two groups of 6 male Crl:CD®(SD)IGS BR rats were exposed nose-only for a single, 4-hour period to H-25777 in air. Test atmospheres were generated by heating the test substance to form a condensation aerosol in the test chamber. The chamber concentrations of H-25777 were measured gravimetrically. Rats were weighed and observed for clinical signs of toxicity during a 1- or 14-day recovery period.

Rats were exposed to atmospheres of H-25777 at mean concentrations of 0.10 or 1.8 mg/L. The mass median aerodynamic diameters for the aerosol tested were 5.8 and 6.8 µm, respectively. Following exposure, 3 rats from the 0.10 mg/L exposure were retained for a 1-day recovery period; the remaining 3 rats were retained for a 14-day recovery period. Rats from the 1.8 mg/L exposure were retained for a 14-day recovery period. At the end of their respective recovery period, all rats from the 0.10 mg/L group were sacrificed for anatomic pathology evaluation. All rats from the 1.8 mg/L exposure were sacrificed and discarded. No rats died following exposure to H-25777 or during the recovery period.

Other than slight body weight losses measured in 3 of 6 rats one day after exposure to 1.8 mg/L of H-25777, there were no significant clinical signs observed in the exposed rats at either test concentration. Histological examination of rats exposed to 0.10 mg/L showed no treatment-related effects.

Under the conditions of this study, the approximate lethal concentration (ALC) for H-25777 is greater than 1.8 mg/L. According to Haskell Laboratory toxicity classifications, H-25777 is considered to be at most slightly toxic (aerosol ALC 0.8-2.0 mg/L) in male rats. However, the mass median aerodynamic diameter (MMAD) of the tested aerosol at this concentration was 6.8 µm. Targeted MMADs in inhalation studies are from 1 to 4 µm to allow maximum lung exposure. Had the generated aerosol been smaller at this concentration, it is possible that the toxicity could have been somewhat greater.

INTRODUCTION

The objective of this study was to determine a 4-hour inhalation approximate lethal concentration (ALC) of H-25777 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.

STUDY DESIGN

Two groups of 6 male rats each were exposed to aerosol atmospheres of H-25777 in air. Rats were exposed nose-only for a single, 4-hour period. Following exposure, 3 rats from the 0.10 mg/L exposure were retained for a 1-day recovery period; the remaining 3 rats were retained for a 14-day recovery period. Rats from the 1.8 mg/L exposure were retained for a 14-day recovery period.

Rats were approximately 8 weeks old and weighed between 258 and 279 grams at the time of exposure.

Animals were observed for mortality 4 times during each exposure and observed for mortality and clinical signs of toxicity immediately after exposure. During the recovery period, all rats were observed each day for mortality. Rats were weighed and observed for clinical signs of toxicity on the day following exposure and at least twice more during the recovery period. At the end of both recovery periods, all rats from the 0.10 mg/L group were sacrificed for anatomic pathology evaluation. All rats from the 1.8 mg/L exposure were sacrificed by carbon dioxide asphyxiation and discarded.

MATERIALS AND METHODS

A. Test Substance

The test substance, H-25777, was supplied by the sponsor as a pale yellow solid. Purity was [redacted] The test substance was assumed to be stable throughout the exposure phase of the study; no evidence of instability was observed.

B. Test Species

Young adult, male Crl:CD®(SD)IGS BR rats were received from Charles River Laboratories, Inc., Raleigh, North Carolina. The rats were approximately 7 or 8 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

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.

2. Animal Selection

Prior to each exposure, 6 male rats were selected for use on the study from the rats that were released from quarantine. The selected animals had been gaining weight at a normal rate and demonstrated no overt signs of disease, and were the appropriate age and body weight. No attempt was made to randomly group animals.

3. Identification

Each rat was assigned an animal number which was recorded on a card affixed to the cage. Prior to exposure, the tail of each animal and cage card were marked with water-insoluble pens so that each animal could be identified after exposure and during the recovery period.

4. Housing

Except during exposure, rats were housed singly in stainless steel, wire-mesh cages suspended above cage boards.

5. Animal Room Environment

Rats were housed in proximity to the inhalation chambers. Animal rooms were maintained at a temperature of 18-26°C (targeted to 22-24°C) and a relative humidity of 30-70% (targeted to 40-60%). Animal rooms were artificially illuminated (fluorescent light) on an approximate 12 hour light/dark cycle. Excursions outside of these ranges were of insufficient magnitude and/or duration to have adversely affected the validity of the study.

6. Feed and Water

Except during exposure, PMI® Nutrition International, LLC Certified Rodent LabDiet® 5002 and tap water were available *ad libitum*.

7. Animal Health and Environmental 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. Inhalation Exposure System

1. Atmosphere Generation

Chamber atmospheres were generated by aerosolization of the solid test substance in air with a glass, round-bottom flask placed in a heating mantle. The flask was heated to approximately 100°C to melt the test substance. Filtered, high-pressure air, metered into the flask by a Brooks model 5851E mass flow controller, carried the evolved vapor through heated glass tubing. For the 0.10 mg/L exposure, 2 chambers were used. The vapor passed from the heated tubing into the first chamber where a condensation aerosol was formed. The aerosol then passed into the second chamber for animal exposure. For the 1.8 mg/L exposure, the vapor passed from the heated tubing in the exposure chamber where an aerosol was formed.

Test atmospheres were exhausted through an MSA charcoal/HEPA filter cartridge prior to discharge into the fume hood.

2. Chamber Construction and Design

The exposure chambers were constructed of glass (cylindrical) with a nominal internal volume of 34 L. A baffle inside each chamber promoted uniform distribution of the test atmosphere.

3. Exposure Mode

During exposure, animals 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 the nose of each animal extended into the exposure chamber.

E. Characterization of Chamber Atmosphere

1. Test Substance Sampling and Analysis

The atmospheric concentration of H-25777 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-25777 was calculated from the difference in the pre- and post-sampling filter weights divided by the volume of chamber atmosphere sampled.

2. Particle Size Determination

A sample to determine particle size distribution (mass median aerodynamic diameter and percent particles less than 1, 3, or 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)

3. Environmental Monitoring

Chamber airflow was set at the beginning of each exposure to achieve at least 10 air changes per hour. The airflow was monitored continually with a calibrated Brooks model 5851E mass flow controller and recorded initially and whenever changes were made during each exposure. Chamber temperature was targeted at 22 ± 2°C. The temperature was monitored continually with a digital traceable thermometer and recorded 3 times during each exposure. Chamber relative humidity was targeted at 50 ± 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.

F. Anatomic Pathology Evaluation

All animals from the 0.10 mg/L exposure underwent a gross pathology examination at sacrifice. In addition, the lungs, trachea, nose (4 cross sections), larynx, and pharynx were processed for histopathology and microscopically examined. Lung weights were obtained for the 3 animals sacrificed 1-day after exposure to 0.10 mg/L.

RESULTS AND DISCUSSION

A. Exposure Conditions
(Table 1)

Animals were exposed to H-25777 at mean concentrations of 0.10 or 1.8 mg/L. The atmospheres generated in this study were considered to be marginally respirable in rats, as the mass median aerodynamic diameters (MMAD) were 5.8 and 6.8 µm. Several attempts were made to produce a smaller, more respirable aerosol size; however, it appeared that this could not be accomplished at these high concentrations due to the condensation aerosol forming large particle agglomerates.

TABLE 1

CHARACTERIZATION OF TEST ATMOSPHERES

MEASURED AEROSOL CONCENTRATION (mg/L)[a]				AEROSOL SIZE				
				MMAD		PERCENT[d]		
MEAN	S.D.	RANGE	n	(µm)[b]	GSD[c]	<1 µm	<3 µm	<10 µm
0.10	0.058	0.018 - 0.15	8	5.8	2.2	24	37	77
1.8	0.12	1.1 - 1.5	8	6.8	1.8	8	13	86

a Represents the mean, standard deviation (S.D.), and range for each exposure, based on n samples per exposure. Values are reported to 2 significant figures. Calculations were performed prior to rounding values.
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.

Chamber temperature ranged from 23 to 24°C, chamber relative humidity ranged from 46 to 68%, chamber airflow ranged from 15 to 25 L/min, and the oxygen concentration was 21%.

B. Mortality, Clinical Signs, and Body Weights

No deaths occurred during the study.

Following exposures, when the rats were removed from the restrainers, the clinical signs of toxicity frequently observed included colored discharge of the nose and eyes. These signs are typically observed in inhalation studies with rats under restraint. No clinical signs of toxicity were observed during the remainder of the recovery period.

No body weight losses were observed in rats exposed to 0.10 mg/L immediately after exposure or after the 14-day recovery period.

Three of 6 rats exposed to 1.8 mg/L showed slight weight loss (<10 grams) on the day following exposure, followed by a normal weight gain rate (approximately 5-10 g/day) for the remainder of the recovery period.

C. Anatomic Pathology Evaluation
(Tables 2-3)

Tissues were submitted from 3 rats that were sacrificed one day after a 0.10 mg/L exposure and from 3 rats that were sacrificed 14 days after the exposure. The following tissues were processed and evaluated from each rat: lungs, trachea, nose (4 cross sections), larynx, and pharynx.

TABLE 2

SUMMARY OF MICROSCOPIC FINDINGS IN RATS EXPOSED TO 0.10 mg/L TEST SUBSTANCE

DAYS ATER EXPOSURE (NUMBER EXAMINED)	1 (3)	14 (3)
LUNG		
Not remarkable	1	1
Hemorrhage, alveolar	2	1
Infiltration, mononuclear cell, perivascular	-	1
TRACHEA		
Not remarkable	3	3
NOSE		
Not remarkable	3	3
LARYNX		
Not remarkable	2	3
Infiltration, mononuclear cell	1	-
PHARYNX		
Not remarkable	3	3

There were no appreciable differences between the 2 groups. The alveolar hemorrhages were very minimal, and were likely agonal changes occurring at the time of sacrifice.

Although no concurrent controls were provided, the changes present in the tissues examined were considered to be of the type and severity expected in this strain of rats, and none of them appeared to be the result of treatment.

Lung weights in 3 rats sacrificed 1 day after exposure to H-25777 at 0.10 mg/L did not appear to be different from control rats of similar weights in a recent Haskell Laboratory inhalation study.[2]

TABLE 3

FINAL BODY AND LUNG WEIGHTS ONE DAY POSTEXPOSURE

ANIMAL NUMBER	FINAL BODY WEIGHT (g)	ABSOLUTE LUNG WEIGHT (g)	RELATIVE LUNG WEIGHT (%)[a]
673575	268	1.539	0.57
673576	262	1.540	0.59
673578	271	1.606	0.59

a Relative weight is calculated as follows:
Absolute Lung Weight ÷ Final Body Weight × 100

CONCLUSIONS

Under the conditions of this study, the approximate lethal concebtration (ALC) for H-25777 is greater than 1.8 mg/L. According to Haskell Laboratory toxicity classifications, H-25777 is considered to be at most slightly toxic (aerosol ALC 0.8-2.0 mg/L) in male rats. However, the mass median aerodynamic diameter (MMAD) of the tested aerosol at this concentration was 6.8 µm. Targeted MMADs in inhalation studies are from 1 to 4 µm to allow maximum lung exposure. Had the generated aerosol been smaller at this concentration, it is possible that the toxicity could have been somewhat greater.

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.

2. DuPont Haskell Laboratory (2003). H-24678: Two-Week Inhalation Toxicity Study in Male Rats. Unpublished report [redacted]