AR226_3299

TRADE SECRET

Study Title

H-25914: Inhalation Approximate Lethal Concentration (ALC) in Rats

AUTHOR: Michael P. DeLorme, Ph.D.

STUDY COMPLETED ON: September 9, 2003

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
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LABORATORY PROJECT ID: DuPont-13308

[redacted]

Company Sanitized. Does not contain TSCA CBI

CERTIFICATION

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

Issued by Study Director: Michael P. DeLorme 09 SEP 03

Michael P. DeLorme, Ph.D.
Research Toxicologist

Date

Company Sanitized. Does not contain TSCA CBI

STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: • H-25914
[redacted]

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

Haskell Number: 25914

CAS Registry Number: [redacted]

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Amber 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: June 26, 2003 / (see report cover page)

STUDY PERSONNEL

Study Director: Michael P. DeLorme, Ph.D.
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SUMMARY

Two groups of 6 male Crl:CD®(SD)IGS BR rats each were exposed nose-only for a single, 4-hour period to H-25914 in air. H-25914 is a suspension [redacted] and test atmospheres were generated by aerosolization. Airborne concentrations of H-25914 were determined by gravimetric analysis. Since H-25914 was a dilute suspension, sample filters were weighed immediately following air sample collection for determination of "wet" aerosol concentration (suspension), and then subsequently desiccated for determination of the "dry" aerosol concentration (polymer). Animals were weighed and observed for clinical signs of toxicity during a 14-day recovery period.

Rats were exposed to mean wet aerosol concentrations of 1200 or 4900 mg/m³, which corresponded to dry aerosol concentrations of 830 or 1600 mg/m³, respectively. The mass median aerodynamic diameters measured for the wet aerosol tested ranged from 2.2 to 3.5 µm. No rats died during exposure to H-25914 or during the recovery period.

The only clinical sign of toxicity observed during this study was alopecia of the front paws in animals exposed to 4900 mg/m³ wet aerosol (1600 mg/m³ dry). The observation of alopecia was transient in nature and was observed up to 14 days postexposure. Slight body weight losses were observed in 2 of 6 animals exposed to1200 mg/m³ wet aerosols (830 mg/m³ dry), while all animals exposed to 4900 mg/m³ wet aerosol (1600 mg/m³ dry) demonstrated an average 4.5% decrease in body weight 1 day postexposure. However, by the second day following exposure all animals demonstrated normal weight gain.

Under the conditions of this study the approximate lethal concentration (ALC) for aerosolized polymer in H-25914 is greater than 1600 mg/m³ (dry). Based on the dry aerosol concentration and on an acute inhalation basis, H-25914 is considered to be, at worst, slightly toxic (ALC 800 – 2000 mg/m³) according to Haskell Laboratory toxicity classifications.

INTRODUCTION

The objective of this study was to determine a 4-hour inhalation approximate lethal concentration (ALC) of H-25914 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.

STUDY DESIGN

Two groups of 6 male rats each were exposed to aerosol atmospheres of H-25914 in air. Rats were exposed nose-only for a single, 4-hour period. Following exposure, rats were retained for a 14-day recovery period.

Rats were approximately 8 or 9 weeks old and weighed between 241 and 290 grams at the time of exposure.

Rats were observed for mortality and clinical signs of toxicity immediately after exposure as they were removed from the restrainers. 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 the recovery period, all rats were sacrificed by carbon dioxide asphyxiation and discarded.

MATERIALS AND METHODS

A. Test Substance

The test substance, H-25914, was supplied by the sponsor as an amber liquid that was a [redacted] The purity of the organic polymer 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 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 1 time.

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, had 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 that was recorded on a card affixed to the cage. Prior to exposure, the tail of each animal and cage card were coded 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. The animal room was 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 nebulization 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 5851E mass flow controller, carried the resulting atmosphere into the exposure chamber. Chamber concentrations of test substance were controlled by varying the syringe infusion pump rate to the nebulizer.

Test atmospheres were exhausted through a water-filled scrubber 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 34 L. A polycarbonate baffle inside the chamber promoted uniform chamber 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 that 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-25914 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 containing a pre-weighed Gelman glass fiber (Type A/E) filter. The filters were desiccated for at least 1 day prior to air sampling. Immediately following the collection of the air sample, the filters were removed from the cassette and weighed on either a Cahn model C-30 or C-31 Microbalance®. The postsampling filter weight was recorded and used to determine the total aerosol concentration (wet aerosol) in the exposure chamber. The filters were then placed in a desiccator for at least 1 day, and reweighed. The desiccated filter mass was then used to determine the dry aerosol concentration. The content of the sample on the desiccated filter was not analyzed, but was assumed to be the fluorinated organic polymer.

2. Particle Size Determination

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]

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 NIST digital traceable thermometer and recorded 3 times during each exposure. Chamber relative humidity was targeted at 50 ± 20%. 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.

RESULTS AND DISCUSSION

A. Exposure Conditions
(Table 1)

Animals were exposed to H-25914 aerosols at concentrations of 1200 or 4900 mg/m³ (wet), which corresponded to mean dry aerosol concentrations of 830 or 1600 mg/m³, respectively. The atmospheres generated in this study were considered to be respirable in rats, with mass median aerodynamic diameters (MMAD) ranging from 2.2 to 3.5 µm.

TABLE 1

CHARACTERIZATION OF TEST ATMOSPHERES
AND ASSOCIATED ANIMAL MORTALITY

AEROSOL CONCENTRATION (mg/m³)[a]							AEROSOL SIZE			MORTALITY
Wet Aerosol			Dry Aerosol				MMAD		Percent	(# deaths/
Mean	S.D.	Range	Mean	S.D.	Range	n	(µm)[b]	GSD[c]	<10 µm[d]	# exposed)
1200	130	1000 - 1400	830	60	740 - 910	8	2.2	2.4	96	0 / 6
4900	1500	3100 - 7000	1600	360	1100 - 2100	9	3.5	3.5	82	0 / 6

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 having aerodynamic equivalent diameters of less than 10 µm.

Chamber temperature ranged from 20 to 22°C, chamber relative humidity ranged from 70 to 72%, chamber airflow was 18 L/min, and the oxygen concentration was 20.8%. Although chamber relative humidity was outside the targeted parameters, this variation was considered not to have adversely affected the validity of this study.

B. Mortality, Clinical Signs, and Body Weights

No deaths occurred during the study.

No clinical signs of toxicity were observed in the animals immediately after exposure. However, 2 days following exposure to 4900 mg/m³ wet H-25914 aerosol (1600 mg/m³ dry), 1 of 6 animals demonstrated alopecia of the front paws that went unresolved at 14 days postexposure. At 7 and 14 days following exposure to 4900 mg/m³ wet aerosol, 4 animals demonstrated alopecia of the front paws. No alopecia was observed in animals exposed to 1200 mg/m³ wet H-25914 aerosol (830 mg/m³ dry). Weight losses were observed in all animals exposed to 4900 mg/m³ wet

aerosol (1600 mg/m³ dry) 1 day postexposure. The weight loss ranged from 1.8 – 7.6% initial body mass and by 2 days postexposure, all animals demonstrated normal weight gains. Following the 1212 mg/m³ wet H-25914 aerosol (830 mg/m³ dry) exposure 2 rats lost <1 and 4.3% of their initial body weight at 1 day postexposure. By 2 days following the 1212 mg/m³ wet H-25914 aerosol (830 mg/m³ dry) exposure all animals demonstrated normal weight gains.

CONCLUSIONS

Under the conditions of this study, the approximate lethal concentration (ALC) for the organic polymer in H-25914 is greater than 1600 mg/m³ dry aerosol. Based on the dry aerosol concentration and on an acute inhalation basis, H-25914 is considered to be, at worst, slightly toxic (ALC 800 – 2000 mg/m³) according to Haskell Laboratory toxicity classifications.

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.