Study Title

Inhalation Approximate Lethal Concentration and Pulmonary Pathology of [redacted] Exposed Rats

Author

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Study Completed On

November 22, 1989

Performing Laboratory

E. I. du Pont de Nemours and Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
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[redacted]

Laboratory Project ID

Haskell Laboratory Report No. 651-89

GENERAL INFORMATION

Material Tested:	[redacted]
Medical Research No.:	[redacted]
Haskell No.:	17,699
Physical Form:	White powder
Composition:	[redacted]
Contaminants:	[redacted]
Synonym:	[redacted]
Other Codes:	[redacted]
CAS Registry No.:	[redacted]
Stability:	The test material was assumed to be stable throughout the exposure phase of the study.
Sponsor:	Polymer Products Department E. I. du Pont de Nemours and Company, Inc. Wilmington, Delaware

GENERAL INFORMATION (Cont'd)

Material Submitted By: [redacted]

Polymer Products Department
E. I. du Pont de Nemours and Company, Inc.
Washington Laboratory
Parkersburg, West Virginia

Study Initiation Date: 4/26/89

In-Life Phase Initiated - Completed: 5/3/89 - 6/8/89

Notebook: [redacted]

There are 19 pages in this report.

Distribution: [redacted]

Inhalation Approximate Lethal Concentration and Pulmonary Pathology of [redacted] Exposed Rats

SUMMARY

The acute inhalation toxicity and pulmonary pathology of [redacted] was assessed in groups of male Crl:CD®BR rats. Test atmospheres were generated by suspension of the particulate test material in a high-pressure air stream. The atmospheric concentration of aerosol was measured by gravimetric analysis. To determine an Approximate Lethal Concentration (ALC), groups of 6 rats were exposed nose-only for a single, 4-hour period to [redacted] at concentrations of 1100 or 2400 mg/m^3. After exposure, rats were observed for clinical signs of toxicity during a 14-day recovery period. To assess pulmonary pathology, a separate group of 9 rats was exposed nose-only to [redacted] at a concentration of 980 mg/m^3 for 4 hours. Three subgroups of 3 rats were killed at 2, 7 and 30 days post exposure; the lungs from these rats were weighed at necropsy, perfused with formalin, stained with hematoxylin and eosin, and examined by light microscopy. A procedural control group of 6 male Crl:CD®BR rats, was used for histomorphologic comparison; subgroups of 3 rats from this group were killed at 2 and 30 days.

Three of 6 rats died within 24 hours of exposure to 2400 mg/m^3 [redacted] the highest concentration tested. Clinical signs of toxicity observed immediately after exposure included compound-stained fur, brown oral discharge and red ocular discharge. Other than slight to moderate body weight losses (up to 6% of initial body weight within 24 hours of exposure), no clinical signs of toxicity were observed during the postexposure period. Rats began to regain body weight by day 2 post exposure.

To evaluate pulmonary pathology, rats were exposed to a sublethal concentration (approximately 1000 mg/m^3) of [redacted]. Although no gross changes were found in any animal at necropsy, several compound-related microscopic changes were noted. These changes included the accumulation of test material within the alveoli and alveolar macrophages, and minute, focal areas of inflammation within the alveoli and alveolar ducts. Although test material was still discernible within alveolar macrophages throughout the study, other microscopic pulmonary changes were transient, of minimal severity and had resolved within 7 days of exposure.

Under the conditions of this study, the ALC for [redacted] is 2400 mg/m^3. This material is considered to have very low toxicity on an acute inhalation basis. Acute exposures were associated with minimal, transient inflammatory pulmonary changes; the presence of test material within alveolar macrophages throughout the study indicates ongoing clearance of deposited material.

SUMMARY (Cont'd)

Work by: R Valentine for RT Turner
R. T. Turner
Technician

Study Director: R Valentine
Rudolph Valentine, Ph.D.
Research Toxicologist
Acute and Developmental Toxicology Division

Reviewed and Approved for Issue: R Valentine 11/22/89
Rudolph Valentine
Study Director

RV:alr:125.4

QUALITY ASSURANCE DOCUMENTATION

STUDY: [redacted]
H# 17,699

Inhalation Approximate Lethal Concentration and Pulmonary Pathology of [redacted] Exposed Rats

AUDITS:

Items Audited	Audit Dates	Audit Number	Findings Reported to Study Director and Management
Pathology Report #70-89 and Necropsy Records	9/13/89	----	9/13/89
Protocol, records and Final Report	11/9,10,13/89	[redacted]	11/14/89

Reported by: Joseph C. Hamill 11/16/89
Joseph C. Hamill
Quality Assurance Auditor
Date

INTRODUCTION

The purpose of this study was to determine an Approximate Lethal Concentration (ALC) for [redacted]. The ALC was defined as the lowest concentration tested that caused the death of 1 or more rats either during the exposure or during a 14-day postexposure observation period. To assess the pulmonary pathology associated with exposure to the test material, an additional group of 9 rats was exposed to a sublethal concentration of [redacted] Subgroups of these rats were killed at 2, 7 and 30 days post exposure; the lung tissues from these rats were weighed, processed and examined by light microscopy. Except as documented in the study records, this study was conducted according to the applicable Good Laboratory Practice Regulations.

MATERIALS AND METHODS

A. Animal Husbandry

Young adult male Crl:CD®BR rats were received from Charles River Breeding Laboratories, Raleigh, North Carolina. Each rat was assigned a unique 6-digit identification number which corresponded to a numbered card affixed to the cage. Rats were quarantined for approximately one week prior to testing, and were weighed and observed three times during the quarantine period. During the test, rats were housed in pairs in 8" x 14" x 8" suspended, stainless steel, wire-mesh cages. The rat assigned the lower number in each cage was identified by a slash in the right ear. Prior to exposure, rats' tails and cage cards were color-coded with water-insoluble markers so that individual rats could be identified after exposure. Except during exposure, Purina Certified Rodent Chow® #5002 and water were available ad libitum.

Animal rooms were maintained on a timer-controlled, 12 hour/12 hour light/dark cycle. Environmental conditions of the rooms were targeted for a temperature of 23 ± 2°C and relative humidity of 50 ± 10%. Excursions outside these ranges were judged to have been of insufficient magnitude and/or duration to have adversely affected the validity of the study.

B. Exposure Protocol

Approximate Lethal Concentration Determination

Groups of 6 male Crl:CD®BR rats (approximately 7-8 weeks old and weighing 256-295 g) were restrained in perforated, stainless steel cylinders with conical nose pieces. The restrainers were inserted into a

face plate on the exposure chamber such that only the nose of each rat protruded into the chamber. The rats were exposed nose-only to aerosol atmospheres of [redacted] for a single, 4-hour period. Rats were weighed prior to exposure and were observed for clinical signs during and immediately after exposure. Surviving rats were observed daily; rats were also weighed daily for 14 days post exposure, weekends and holidays excluded except when warranted by the rats' condition. All surviving rats were killed 14 days after exposure.

Assessment of Pulmonary Pathology

To assess pulmonary pathology, 1 group of 9 male Crl:CD®BR rats (approximately 7 weeks old and weighing 250-276 g at study initiation) was exposed to [redacted] Based on the results of the ALC determination, a sublethal design concentration of 1000 mg/m^3 was selected to assess lung pathology. Subgroups of 3 rats were killed at 2, 7 and 30 days post exposure by sodium pentobarbital anesthesia and exsanguination. The lungs were weighed and examined for gross lesions at necropsy, perfused with buffered formalin, embedded, sectioned, stained with hematoxylin and eosin and examined by light microscopy. For comparative purposes, an unexposed control group of 6 male Crl:CD®BR rats (approximately 7 weeks old and weighing 232-273 g at study initiation) was used. Subgroups of 3 rats rats from this control group were killed at 2 and 30 days; the lung tissues from these rats were evaluated in the same manner as the treated group. Rats on the extended recovery period were checked daily for morbidity/mortality; rats were weighed and observed for clinical signs daily, weekends excluded, for approximately 3 weeks and then once a week for the remainder of the study.

C. Atmosphere Generation

Particulate atmospheres of [redacted] were generated by suspension of the test material in a high-pressure air stream. The test material was metered into a Fluid Energy Processing and Equipment Company Model 00 Jet-O-Mizer with a K-Tron Model T-20 twin screw volumetric feeder equipped with an electronic speed control. Test material entering the jet mill was entrained in an air stream (approximately 52 L/min) and was pulverized by impaction with other particles within the mill's reduction chamber. The test material exited the jet mill and was swept through a short, Tygon® transfer tube directly into a cylindrical, borosilicate glass, 38-L exposure chamber. Particles entering the chamber were dispersed with a 4 cm circular baffle/impaction plate located at the outlet of the transfer tube; this baffle acted like an impaction plate to remove larger particles and promote uniform distribution. Chamber atmospheres were exhausted through a high-capacity fiberpac dust filter, dry-ice cold trap and a MSA cartridge filter prior to discharge into a fume hood.

D. Analytical

The atmospheric concentration of [redacted] was determined at approximately 30-minute intervals during each exposure by gravimetric analysis. Known volumes of chamber atmospheres were drawn through preweighed, Gelman glass fiber (Type A/E) filters. Filters were weighed on a Cahn Model 26 or 28 Automatic Electrobalance®. The atmospheric concentration of [redacted] was calculated from the difference in the pre- and post-sampling filter weights.

Particle size (mass median aerodynamic diameter and percent less than 3 and 10 um) was determined with a Sierra Series 210 cascade impactor during each exposure.[1] During each exposure, chamber temperature was measured with a mercury thermometer, relative humidity was measured with a Bendix Model 566 psychrometer, and chamber oxygen concentration was measured with a Biosystems® Model 3100R oxygen analyzer.

E. Statistical Analysis

At termination, lung and final body weights were obtained. Data were analyzed by one-way analysis of variance. Statistical differences were declared at the $p = 0.05$ probability level.

F. Records Retention

All raw data and the final report will be stored in the archives of Haskell Laboratory for Toxicology and Industrial Medicine, Newark, Delaware, or in the Du Pont Records Management Center, E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware.

RESULTS

A. Exposure Conditions and Associated Mortality

Aerosols of [redacted] were readily observed during the exposures. Chamber temperatures ranged from 25-26°C, relative humidity varied from 48-54%, and chamber oxygen concentration was 21.0%. Atmospheric concentrations of [redacted] and rat mortality data for each exposure are summarized in the following table.

Characterization of [redacted] Atmospheres and Rat Mortality

Aerosol Concentration (mg/m^3)[a] Mean	S.D.	Range	n	MMAD[b]	GSD[c]	Percent < 3 um[d]	Percent < 10 um[e]	Mortality[f]
980	210	630 - 1200	9	1.4	2.4	80	98	0/9[g]
1100	330	580 - 1500	8	1.5	2.3	79	98	0/6
2400	300	2000 - 2800	8	1.8	2.5	73	98	3/6

[a] Values shown represent the mean, standard deviation (S.D.), range and number of observations (n) for each exposure. Aerosol concentrations were based on filter weights obtained immediately after sampling.
[b] Mass median aerodynamic diameter in um.
[c] Geometric standard deviation.
[d] Percent by weight of particles with aerodynamic diameter (AD) less than 3 um.
[e] Percent by weight of particles with aerodynamic diameter (AD) less than 10 um.
[f] Mortality is expressed as the number dead/number exposed.
[g] Exposure used for assessment of pulmonary pathology.

B. Clinical Observations

Clinical signs of toxicity could not be taken during the exposures since the dense aerosol prevented visual observation of the rats. Upon release from the restrainers immediately after exposure, rats exhibited compound-stained fur, red ocular discharge, and brown oral discharge. No deaths occurred either during or immediately after exposure.

Deaths occurred within 24 hours of exposure to [redacted] at an aerosol concentration of 2400 mg/m^3. With the exception of slight to moderate weight losses (up to 6% of initial body weight) in

some rats within 1 day of exposure, no clinical signs were noted in rats during the 14-day postexposure recovery period. All surviving rats began to regain weight by day 2 post exposure.

C. Body and Organ Weight Analysis

Two rats (#459627 and 459579) were omitted from statistical comparisons since their identification numbers could not be accurately established. No statistically significant differences in final lung weight or lung to body weight ratios were found when compared to controls for any group. Statistical analyses are shown in Appendix I.

D. Pathology

No gross abnormalities were detected in any animal at the time of necropsy. Pathology data from 2 rats (#459627 and 459579) from the 30-day post-treatment groups were not included in this report since the identification numbers from these rats could not be accurately established. Microscopically, a granular, yellow-brown material was present in the alveoli and alveolar macrophages of all compound-exposed rats 2 days after exposure; this material was observed in alveolar macrophages throughout the 30-day study. Subacute inflammation, consisting of small focal interstitial and alveolar infiltrates of macrophages and neutrophils, associated with alveoli and alveolar ducts was also observed. These focal inflammatory changes were limited in number, of minimal severity and had resolved by 7 days post exposure. Pathology Report No. 70-89 is attached as Appendix II.

DISCUSSION AND CONCLUSION

Under the conditions of this study, the ALC for [redacted] is 2400 mg/m^3. Based on the atmospheric concentration of aerosol, [redacted] is considered have very low toxicity on an acute inhalation basis (ALC greater than 2000 mg/m^3). Acute exposures were associated with minimal, transient inflammatory pulmonary changes; the presence of test material within alveolar macrophages throughout the study indicates ongoing clearance of deposited material.

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

Inhalation Approximate Lethal Concentration and
Pulmonary Pathology of [redacted] Exposed Rats

Appendix I

Final Lung and Body Weights

Inhalation Approximate Lethal Concentration and
Pulmonary Pathology of [redacted] Exposed Rats

Final Lung and Body Weights

Group: Unexposed Controls
Days Post Exposure: 2

Rat Number	Final Lung Weight (g)	Final Body Weight (g)	Lung/Body Weight Ratio
459574	1.475	289	0.00510
459575	1.738	291	0.00597
459576	1.580	294	0.00537
Mean ± (SD)	1.598 (0.132)	291 (3)	0.00548 (0.00045)

Group: 980 mg/m^3
Days Post Exposure: 2

Rat Number	Final Lung Weight (g)	Final Body Weight (g)	Lung/Body Weight Ratio
459618	1.518	275	0.00552
459619	*	267	*
459620	1.385	258	0.00537
Mean ± (SD)	1.452 (0.0940)	267 (9)	0.00544 (0.00011)

Group: 980 mg/m^3
Days Post Exposure: 7

Rat Number	Final Lung Weight (g)	Final Body Weight (g)	Lung/Body Weight Ratio
459621	1.635	331	0.00494
459622	2.006	296	0.00678
459623	2.296	332	0.00692
Mean ± (SD)	1.979 (0.331)	320 (21)	0.00621 (0.00110)

ONE-WAY ANALYSIS OF VARIANCE

P value for lung weight data = 0.104; not significant
P value for lung/body weight data = 0.459; not significant

* Invalid lung weight, data not presented.

Inhalation Approximate Lethal Concentration and
Pulmonary Pathology of [redacted] Exposed Rats

Final Lung and Body Weights (Cont'd)

Group: Control
Days Post Exposure: 30

Rat Number	Final Lung Weight (g)	Final Body Weight (g)	Lung/Body Weight Ratio
459577	1.581	452	0.00350
459578	1.497	336	0.00446
459579	**	**	**
Mean ± (SD)	1.539 (0.059)	394 (82)	0.00398 (0.00068)

Group: 980 mg/m^3
Days Post Exposure: 30

Rat Number	Final Lung Weight (g)	Final Body Weight (g)	Lung/Body Weight Ratio
459624	1.861	465	0.00400
459626	2.033	502	0.00405
459627	**	**	**
Mean ± (SD)	1.947 (0.122)	484 (26)	0.00403 (0.00003)

ONE-WAY ANALYSIS OF VARIANCE

P value for lung weight data = 0.051; not significant
P value for lung/body weight data = 0.927; not significant

** Unable to establish animal identification numbers; data not used for statistical analyses.

Inhalation Approximate Lethal Concentration and
Pulmonary Pathology of [redacted] Exposed Rats

Appendix II

[redacted]

ES 3662 REV 12 85

DUPONT

ESTABLISHED 1802

E. I. DU PONT DE NEMOURS & COMPANY

INCORPORATED

HASKELL LABORATORY FOR TOXICOLOGY

AND INDUSTRIAL MEDICINE

P.O. BOX 50, ELKTON ROAD

NEWARK, DELAWARE 19714

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT

[redacted]

[redacted]

HASKELL LABORATORY NO. 17699

PULMONARY PATHOLOGY OF [redacted] IN RATS

POLYMER PRODUCTS

DATE ISSUED: NOVEMBER 21, 1989

PULMONARY PATHOLOGY OF [redacted]

Introduction

Nine male Crl:CD®BR rats were exposed nose-only to [redacted] at a concentration of 0.98 mg/L for 4 hours. Subgroups of 3 exposed rats were serially-sacrificed at 2, 7, and 30 days post-exposure. Six male Crl:CD®BR rats, not exposed to the test material, were used as a control group. Subgroups of 3 control rats were sacrificed at 2 and 30 days to correspond to the first and last post-exposure subgroups. Euthanasia was by intraperitoneal injection of sodium pentobarbital and exsanguination. Lungs from these animals were examined grossly, weighed, perfused, and fixed in formalin. Representative sections of processed lung tissue were embedded in paraffin, sectioned at 5 micrometers, stained with hematoxylin and eosin, and examined microscopically.

Results

All animals survived until their scheduled sacrifice. No gross abnormalities were detected for any animal at the time of necropsy. Two animals were deleted from the study due to an identification problem. One was a 30 day post-exposure rat (#459627) and the second deleted rat (#459579) was a 30 day cage control.

Table 1 contains microscopic observations of all rat lungs. The foreign material present in alveoli and within alveolar macrophages of the compound-exposed lungs was yellow-brown, isotropic, and finely granular. It was assumed to be the test compound. The subacute inflammation present in the 3 compound-exposed 2 day post-exposure lungs consisted of very small focal interstitial and alveolar infiltrates of macrophages, primarily, and neutrophils. The minute inflammatory foci per lung section were few in number and associated with alveolar ducts and alveoli. These foci were most likely a response to the presence of the test compound. This minimal inflammatory response was transient and had resolved in the 7 and 30 day post-exposure animals.

The other microscopic findings consisting of microfocal hemorrhage and inflammation in one control animal and perivascular lymphocytes in a treated

animal were considered to be incidental findings and not test compound related.

Report by: G. Tracy Makovec

G. Tracy Makovec, D.V.M.
Diplomate, A.C.V.P.
Staff Pathologist

Approved by: William C. Krauss

William C. Krauss, D.V.M.
Manager, Pathology Division

GTM/WCK/wfd
GTM1 1.17

HN-17699 PULMONARY PATHOLOGY OF [redacted]

[redacted]

TABLE 1
MICROSCOPIC OBSERVATIONS IN MALE RAT LUNGS

Animal Number	Recovery Days	Microscopic Observations
0.0 mg/L aerosol		
459574	2	No abnormalities detected
459575	2	No abnormalities detected
459576	2	Microfocal hemorrhage and subacute inflammation, minimal
459577	30	No abnormalities detected
459578	30	No abnormalities detected
0.98 mg/L aerosol		
459618	2	Foreign material in alveoli and terminal airways, mild Foreign material within alveolar macrophages, minimal Microfocal subacute inflammation, minimal
459619	2	Foreign material in alveoli and terminal airways, mild Foreign material within alveolar macrophages, minimal Microfocal subacute inflammation, minimal
459620	2	Foreign material in alveoli and terminal airways, mild Foreign material within alveolar macrophages, minimal Microfocal subacute inflammation, minimal
459621	7	Foreign material within alveolar macrophages, mild
459622	7	Foreign material within alveolar macrophages, mild Lymphocytic infiltrate, perivascular, minimal
459623	7	Foreign material within alveolar macrophages, mild
459624	30	Foreign material within alveolar macrophages, minimal
459626	30	Foreign material within alveolar macrophages, minimal