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Approximate Lethal Concentration by Inhalation (ALC) of Ammonium Perfluorononanoate (C-9)

Haskell Laboratory Report No. 293-85

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

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Approximate Lethal Concentration by Inhalation (ALC) of Ammonium Perfluorononanoate (C-9)

Summary

To determine an ALC, groups of 6 male Crl:CD®(SD)BR rats were exposed to dust atmospheres of C-9 for a single, 4-hour period, followed by a 14-day recovery period. After determining an ALC, 2 groups of 10 male rats were exposed for 4 hours to a marginally lethal and approximately 1/10th of a lethal concentration of C-9. For each test group, a control group of 10 male rats was exposed simultaneously to air only. Five rats per group were killed on the 5th day of recovery, and 5 rats per group were killed on the 12th day of recovery for a gross examination of the liver.

Under the conditions of this test, the ALC for C-9 was 590 mg/m^3. This material is considered moderately toxic by inhalation.

Rats exposed to 67 mg/m^3 had significantly elevated mean liver weights and liver-to-body weight ratios on the 5th and 12th days after exposure. Rats exposed to 590 mg/m^3 had significantly elevated liver-to-body weight ratios on the 12th day after exposure. Liver weights for these rats were not significantly different than controls on the 5th day of recovery, and mean liver weights were significantly depressed on the 12th day of recovery. However, these seemingly inconsistent changes were due to severe body weight loss in rats exposed to 590 mg/m^3.

Gross pathologic examination of rats exposed to 590 mg/m^3 revealed discolored livers with prominent lobular patterns in 4/5 rats killed on the 5th day of recovery, and similar gross liver lesions in 2/5 rats killed on the 12th day of recovery.

Work by: Robert T. Turner 6/3/85
Robert T. Turner
Technician

Study Director: Laura A. Kinney 6/3/85
Laura A. Kinney
Chemist

Approved by: Nancy C. Chromey 6/3/85
Nancy C. Chromey, Ph.D.
Section Supervisor
Acute Investigations

LAK:smk:HLR9.14

Haskell Laboratory Report No. 293-85

[redacted] Haskell No. 15,437

Material Tested: Nonanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluoro-, ammonium salt

Sponsor: Polymer Products Department
E. I. du Pont de Nemours and Company
Wilmington, Delaware

Material Submitted by: [redacted]
Polymer Products Department
E. I. du Pont de Nemours and Company
Parkersburg, West Virginia

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

Study Initiated/Completed: 1/3/85 - 2/3/85

[Notebook E-38738, pp. 55-110.]

There are 15 pages in this report.

Distribution: [redacted]

INTRODUCTION

The purpose of this study was to determine a 4-hour inhalation ALC for C-9 in male rats. The ALC was defined as the lowest atmospheric concentration tested that caused the death of 1 or more rats either on the day of exposure or within 14 days post exposure. In addition, following the determination of the ALC, additional exposures were conducted to monitor the effects of C-9 inhalation on the liver. 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®(SD)BR rats were received from Charles River Breeding Laboratories, Kingston, New York. Each rat was assigned a unique 6-digit identification number which corresponded to a numbered card affixed to the cage. Rats were quarantined for one week prior to testing, and were weighed and observed twice 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.

B. Exposure Protocol

To determine an ALC, groups of 6 rats, 8 weeks old and weighing between 234 and 298 grams, were restrained in perforated, stainless steel cylinders with conical nose pieces. Each group was exposed nose-only for a single, 4-hour period to a dust atmosphere of C-9 in air. Rats were weighed prior to exposure, and were observed for clinical signs of toxicity during exposure. Surviving rats were weighed and observed daily for 14 days post exposure, weekends excluded except when deemed necessary by the rats' condition.

To monitor the effects of C-9 inhalation on the liver, 2 groups of 10 rats, 8 weeks old and weighing between 237 and 277 grams, were restrained and exposed to a marginally lethal and approximately 1/10th of a lethal exposure concentration of C-9, respectively. Two groups of 10 rats, 8 weeks old and weighing between 231 and 267 grams, were restrained and exposed to air only. Each control group was exposed concurrently with

one of the test groups. Five rats per group were killed 5 days after exposure, and 5 rats per group were killed 12 days after exposure for pathologic examination of the liver.

C. Test Material

Physical Form: White solid
Purity: [redacted]
Synonyms: • Ammonium perfluorononanoate
• C-9
Other Codes: [redacted]
Stability: The test material was assumed to be stable throughout the test.

D. Atmosphere Generation

For most exposures, dust atmospheres of C-9 were generated with a K-Tron® Bin Feeder equipped with twin feed screws. The feed rate was regulated with a K-Tron® Volumetric Feed Controller. The bin feeder metered test material into a glass transfer tube. Air introduced at the tube swept the test material through a size-reducing cyclone and into the exposure chamber. The cyclone removed large particles by inertial impaction, while aerodynamic particles passed through the cyclone and into the exposure chamber. The atmospheric concentration of C-9 was controlled by varying the feed rate.

For the lowest exposure concentration, the atmosphere was generated by passing pressurized air through a 2-stage glass generator. A round flask at the bottom of the generator served as a dust reservoir. A cyclone elutriator was inserted above the reservior. A motorized stirring rod with plastic paddles agitated dust in the generator. Air introduced at the bottom of the reservoir and at the cyclone elutriator swept dust particles into the exposure chamber. The atmospheric concentration of C-9 was controlled by varying the 2 airflows.

E. Analytical

The atmospheric concentration of C-9 was determined at approximately 30-minute intervals by drawing calibrated volumes of chamber atmosphere through preweighed, Gelman glass fiber filters. Filters were weighed on a Cahn model 26 Automatic Electrobalance®. The atmospheric concentration of particulate was determined from the filter weight differential before and after sampling.

Particle size (mass median aerodynamic diameter and percent respirable) was determined with a Sierra model 210 cascade impactor during each exposure.[1] During most exposures, chamber temperature was

measured with a mercury thermometer, relative humidity was measured with a Bendix model 566 psychrometer, and chamber oxygen content was measured with a BioMarine model 225 oxygen analyzer.

F. Pathology

Two groups of 10 rats were exposed for 4 hours to 67 or 590 mg/m^3 of C-9 in air, and 2 groups of 10 rats were exposed to air only. Five rats per group were arbitrarily selected and killed by chloroform anesthesia and exsanguination 5 days after exposure. The remaining 5 rats/group were killed 12 days after exposure. Rats were necropsied and the livers were weighed. Mean liver weights and liver-to-body weight ratios for test rats were compared to their respective controls by Least Significant Difference and Dunnett's tests. Significance was judged at the 0.05 probability level.

G. Records Retention

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

RESULTS

A. Exposure Conditions and Associated Mortality

During most exposures, the chamber walls were coated with the test material. Chamber temperatures ranged between 23-27°C, relative humidities ranged from 19-45%, and chamber oxygen contents were 21%. Atmospheric characterization and mortality data are summarized in the following table.

Characterization of C-9 Atmospheres and Associated Rat Mortality

Concentration (mg/m^3) Mean	S.D.	Range	% Respirable[a]	MMD(um)[b]	Mortality (# deaths/# exposed)
620	180	490 - 980	91	3.4	0/6
910	760	10 - 1700	94	3.2	4/6
1600	420	920 - 1900	93	3.4	6/6
4600	600	4100 - 5900	90	4.2	6/6
67[c]	35	12 - 120	95	3.5	-
590[c]	290	300 - 1200	75	3.7	1/5[d]

a Percent by weight of particles with aerodynamic diameter less than 10 um.
b Mass median aerodynamic diameter.
c Exposures for subsequent pathologic examination.
d Although this exposure was conducted for subsequent pathologic examination of the rats, 1/5 rats designated to be killed 12 days after exposure died on the 12th day.

B. Clinical Observations

During or immediately following exposure, some rats exposed to the 5 lowest exposure concentrations had red or brown facial discharges. At 620 mg/m^3 and 1600 mg/m^3, rats had test material on their heads. Rats exposed to 4600 mg/m^3 had labored breathing, profuse clear nasal and oral discharges, a diminished startle response and test material on their heads. All rats died during exposure to 4600 mg/m^3.

During the postexposure period, 1/5 remaining rats exposed to 590 mg/m^3 died 12 days post exposure, 4/6 rats exposed to 910 mg/m^3 died 9-11 days post exposure, and 6/6 rats exposed to 1600 mg/m^3 died 4-8 days post exposure. Rats exposed to 67 mg/m^3 lost 1-9% of initial body weight 1 day post exposure, followed by normal weight gain. At concentrations greater than 67 mg/m^3, rats lost approximately 6-15% of initial body weight 1 day post exposure, and continued to lose weight either throughout the recovery period or until they died. Most surviving rats exposed to 590, 620 or 910 mg/m^3 weighed only 54-71% of initial body weight when they were sacrificed 12 days post exposure or at the end of the recovery period, respectively.

No adverse clinical signs were observed in rats exposed to 67 mg/m^3 throughout the recovery period. Common clinical signs at higher concentrations included hunched posture, ruffled or discolored fur, red

or brown facial discharges, wet or stained perineum, pallor, lung noise or labored breathing, lethargy, limpness and hair loss. Clinical signs were observed throughout the recovery period.

C. Pathology

Gross pathologic examination of rats exposed to 67 mg/m^3 revealed large livers in 5/5 rats killed on the 5th day of recovery. After 12 days of recovery, none of the rats' livers were noted as large. At 590 mg/m^3, 4/5 rats killed on the 5th day of recovery had discolored livers with prominent lobular patterns or markings. These patterns were probably attributable to diffuse fatty change with a zonal distribution. On the 12th day of recovery, gross liver lesions were noted in 2/5 rats. The Pathology report is attached as Appendix I.

Statistical analyses showed significantly elevated mean liver weights and liver-to-body weight ratios in rats exposed to 67 mg/m^3 on both the 5th and the 12th days of recovery. In the 590 mg/m^3 exposure group, mean liver weights and liver-to-body weight ratios were not significantly different from controls on the 5th day of recovery. On the 12th day of recovery, mean liver weights were significantly lower than controls; however, liver-to-body weight ratios were significantly elevated. The lack of significant liver weight changes on the 5th day of recovery, and the depressed mean liver weights on the 12th day of recovery were due to a large loss of body weight in rats exposed to 590 mg/m^3. Mean body weights for rats exposed to 590 mg/m^3 were only 70% and 48% of controls on the 5th and 12th days of recovery, respectively. However, mean liver weights were 83% and 72% of controls, respectively. Statistical analyses of mean liver weights and liver-to-body weight ratios are presented in Appendix II.

CONCLUSION

Under the conditions of this study, the ALC for C-9 was 590 mg/m^3. This material is considered moderately toxic by inhalation (ALC between 200 and 800 mg/m^3). However, C-9 caused elevated liver weights in rats exposed to 67 mg/m^3 on the 5th and 12th days after exposure, and gross liver lesions in rats exposed to 590 mg/m^3.

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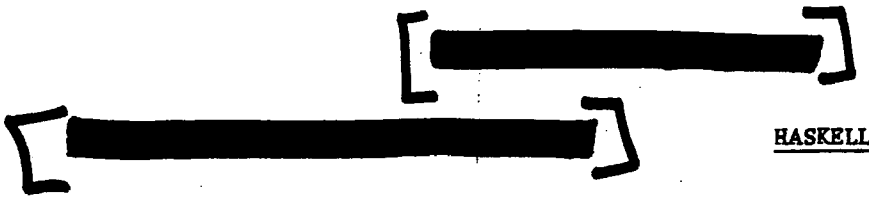
[1] Calculation described in Sierra Instruments, Inc., Bulletin 7-79-219IM, Instruction Manual: Series 210 Ambient Cascade Impactors and Cyclone Preseparators.

Appendix I

E. I. DU PONT DE NEMOURS & COMPANY
INCORPORATED
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
P.O. BOX 50, ELKTON ROAD
NEWARK, DELAWARE 19711

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT



HASKELL LABORATORY NO. 15437

NONANOIC ACID, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9 -HEXADECAFLUORO-, AMMONIUM SALT

C-9

APPROXIMATE LETHAL CONCENTRATION (ALC) TOXICITY STUDY IN MALE Crl:CD®(SD)BR RATS

GROSS PATHOLOGY

POLYMER PRODUCTS DEPARTMENT

DATE ISSUED: MAY 16, 1985

ALC TOXICITY STUDY IN MALE RATS WITH C-9

Introduction and Results

Male Crl:CD®(SD)BR rats exposed to C-9 for 4 hours via inhalation were necropsied and livers were weighed and examined at 5 and 12 days post-exposure. Method of euthanasia was chloroform anesthesia and exsanguination.

Table I identifies the exposure groups and contains the gross observations made at necropsy. The liver of only a few exposed animals was noted as large at necropsy, however, liver weights (weights and statistics will not be presented in this report) indicate that the liver of all exposed animals was large. The prominent lobular pattern noted in 4 of 5 high dose rats examined on day 5 post-exposure is most likely attributable to diffuse fatty change with a zonal distribution (centrilobular or periportal). A portion of the increase in liver size may be due to fatty change, however, it is more likely that the liver functions as the primary site of metabolism of the compound and that the increase in size is due to a proliferation of smooth endoplasmic reticulum.

Acknowledgement

Joan A. Wolfe was pathology supervisor for this study.

Report by: Theodore W. Slone
Theodore W. Slone, D.V.M.
Diplomate A.C.V.P.
Staff Pathologist

Approved by: William C. Kruass
William C. Kruass, D.V.M.
Manager, Pathology Division

TWS/WCK/wfd
SLONE 3.13

R-15437 [redacted]

TABLE I

GROSS PATHOLOGY
C-9
MALE RATS - INHALATION

Animal Number	Test Days	Recovery Days	Mode of* Death	Observations
Group IA - Control				
386616	6	5	SD	Liver - discoloration, dark red area, (5 mm), left lobe
386618	6	5	SD	No abnormalities detected
386620	6	5	SD	No abnormalities detected
386622	6	5	SD	No abnormalities detected
386624	6	5	SD	No abnormalities detected
386617	13	12	SD	No abnormalities detected
386619	13	12	SD	No abnormalities detected
386621	13	12	SD	No abnormalities detected
386623	13	12	SD	No abnormalities detected
386625	13	12	SD	No abnormalities detected
Group IB - Control				
386988	6	5	SD	No abnormalities detected
386990	6	5	SD	No abnormalities detected
386992	6	5	SD	No abnormalities detected
386994	6	5	SD	No abnormalities detected
386996	6	5	SD	No abnormalities detected
386989	13	12	SD	No abnormalities detected
386991	13	12	SD	No abnormalities detected
386993	13	12	SD	No abnormalities detected

* SD = Sacrificed by design; FD = Found dead

B-15437 [redacted]

TABLE I Part 2

GROSS PATHOLOGY
C-9
MALE RATS - INHALATION

Animal Number	Test Days	Recovery Days	Mode of Death	Observations
386995	13	12	SD	No abnormalities detected
386997	13	12	SD	No abnormalities detected
Group II - High Dose (0.59 mg/L)				
386545	6	5	SD	Liver - discoloration, lobular pattern prominent
386546	6	5	SD	Liver - discoloration, lobular pattern prominent
386550	6	5	SD	No abnormalities detected
386552	6	5	SD	Liver - discoloration, lobular pattern prominent
386554	6	5	SD	Liver - discoloration, lobular markings prominent, tan streaks scattered
386547	13	12	SD	Liver - foci, white, scattered, (< 2 mm in diameter), all lobes
386549	13	12	FD	No abnormalities detected
386551	13	12	SD	No abnormalities detected
386553	13	12	SD	Liver - discoloration, small, white area, left lobe, (< 2 mm in diameter) Perineum - alopecia, moderate
386555	13	12	SD	Dorsum - alopecia, right, moderate
Group III - Low Dose (0.067 mg/L)				
387033	6	5	SD	Liver - large
387035	6	5	SD	Liver - large

H-15437 [redacted]

TABLE I

Part 3

GROSS PATHOLOGY
C-9
MALE RATS - INHALATION

Animal Number	Test Days	Recovery Days	Mode of Death	Observations
387037	6	5	SD	Liver - large
387039	6	5	SD	Liver - large
387041	6	5	SD	Liver - large
387034	13	12	SD	No abnormalities detected
387036	13	12	SD	No abnormalities detected
387038	13	12	SD	No abnormalities detected
387040	13	12	SD	No abnormalities detected
387042	13	12	SD	No abnormalities detected

APPENDIX II

C9 - MEAN LIVER WEIGHTS AND LIVER-TO-BODY WEIGHT RATIOS
RATS SACRIFICED ON THE 5TH DAY OF RECOVERY

GROUP	FINAL WT.	LIVER	LIVER-TO-BODY WEIGHT RATIO
CONTROL I	275.6 (0.000)	11.306 (0.000)	4.090 (0.000)
67 MG/M^3	276.6 (0.912)	14.501 (0.002)#	5.243 (0.000)#
CONTROL II	293.4 (0.000)	14.511 (0.000)	4.946 (0.000)
590 MG/M^3	204.0 (0.000)#	12.010 (0.164)	5.803 (0.053)

Values in parentheses - P value of Student's t test comparison of treatment mean to control mean.
\+ - Significantly different ($p<0.05$) from control group by LSD
\# - Significantly different ($p<0.05$) from control group by LSD and Dunnett's test

C9 - MEAN LIVER WEIGHTS AND LIVER-TO-BODY WEIGHT RATIOS
RATS SACRIFICED ON THE 12TH DAY OF RECOVERY

GROUP	FINAL WT.	LIVER	LIVER-TO-BODY WEIGHT RATIO
CONTROL I	315.4 (0.000)	14.806 (0.000)	4.678 (0.000)
67 MG/M^3	325.0 (0.374)	20.371 (0.001)#	6.268 (0.000)#
CONTROL II	335.4 (0.000)	15.557 (0.000)	4.649 (0.000)
590 MG/M^3	160.0 (0.000)#	11.198 (0.002)#	7.003 (0.000)#

Values in parentheses - P value of Student's t test comparison of treatment mean to control mean.
\+ - Significantly different ($p<0.05$) from control group by LSD
\# - Significantly different ($p<0.05$) from control group by LSD and Dunnett's test