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HASKELL LABORATORY REPORT NO. 401-85



Subchronic Feeding Study of Ammonium

Perfluorononanoate (C-9) in Mice

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
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Subchronic Feeding Study of AmmoniumPerfluorononanoate (C-9) in MiceSUMMARY

Groups of 5 male and 5 female mice were fed diets that contained 0, 1, 3, 10, 30, 100, 300, 1,000, 3,000, or 10,000 ppm ammonium perfluorononanoate (greater than 99% pure). Food consumption was not monitored. Clinical signs of toxicity were observed at lethal and nonlethal concentrations. Deaths were observed at concentrations of 300 ppm and higher. All mice fed C-9 at dietary concentrations of 1,000 ppm or higher were killed in extremis on test day 6 and the liver weights were recorded. Mice from the 100 and 300 ppm groups were similarly killed on test day 8. Mice from the remaining test groups and the control group were killed after 14 consecutive days of dietary administration of the test material.

Administration of C-9 at concentration of 100 ppm or higher in the diet resulted in slight to severe weight loss; administration of dietary concentrations of 300 ppm or higher caused death.

Male and female mice fed diets of 3 ppm or higher for 14 consecutive days had significantly increased mean absolute and mean relative liver weights when compared to the controls. The mean relative liver weights of male mice fed the 1 ppm diet were also significantly heavier than the controls. Mean body weights of male and female mice from the 1, 3, 10, and 30 ppm groups were similar to those of control mice throughout the test. Comparison of mean body and liver weights of mice fed diets containing C-9 at concentrations greater than 30 ppm were not possible because there were no concurrent control groups.

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JWS/cgs(5)

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[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]

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INTRODUCTION

The purpose of this study was to evaluate the effects of ammonium perfluorononanoate (C-9) on the liver weights of male and female mice after dietary administration of the test material for 14 consecutive days.

MATERIALS AND METHODS

A. Animal Husbandry

Male and female 5 to 6-week old, Crl:CD®-1(ICR)BR mice were received from Charles River Breeding Laboratories, Kingston, New York. The mice were housed 3 per cage (sexes separate) in suspended, stainless steel, wire-mesh cages with dimensions of 4.5" x 7" x 5" (w x d x h). Mice were quarantined, weighed, and observed for general health for approximately one week prior to testing. Prior to test start, mice were transferred to individual cages and each mouse was assigned a unique identification number which was recorded on tape affixed above the cage. Purina Certified Rodent Chow® #5002 and water were available ad libitum during the pretest period. 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 $22 \pm 3^{\circ}\text{C}$ and relative humidity of $50 \pm 20\%$. Excursions outside these ranges were of brief duration and did not adversely affect the outcome of the study.

B. Protocol

After the pretest period, mice were randomly assigned to study groups on the basis of body weight and freedom from any grossly apparent clinical signs of disease or injury. Mice of each sex were randomly divided into 10 groups of 5 male and 10 groups of 5 female mice. The mean body weights for each group of mice within a sex were approximately equal. The test mice were fed diets that consisted of ammonium perfluorononanoate blended with ground rodent chow (#5002) and supplied ad libitum during the test phase. Verification of the concentration of the test material in the prepared diets was performed by personnel of the Polymer Products Department.

The day that mice were first administered the test diet was test day 1. The mice were weighed and observed daily (weekends included when warranted by signs of toxicity). All mice killed in extremis or by design were euthanized by chloroform asphyxiation and exsanguinated. Liver weights were recorded for all mice killed in extremis as well as those found dead or killed by design. Body weights and liver weights were analyzed using a one-way analysis of variance. Pairwise comparisons between control and treatment groups were made using least significant difference and/or Dunnett's test.

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C. Test Material

Physical Form:

White solid

Purity:

Synonyms:

- Ammonium perfluorononanoate C-9

- C-9

Other Codes:

Stability:

Verification of the concentration of the test material in the prepared diets was performed by personnel of the Polymer Products Department. The test material was assumed to be stable throughout the exposure phase of the study.

D. Records Retention

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

RESULTSA. Diet Information

Sufficient diet was prepared for each test concentration to last throughout the 2-week feeding period. One sample each was taken from the top, middle and bottom of the mixed diet during preparation of the 100 and 10,000 ppm diets. These samples plus a control diet sample were analyzed for C-9 only by S. R. Laas, Polymer Products Department. The results are presented in Appendix I. The diets were refrigerated until used.

B. Clinical Signs

Clinical signs were similar for male and female mice.

Nonlethal Concentrations

Mice fed C-9 at concentrations of 100 ppm and less exhibited slight to severe sporadic weight loss (i.e., 3 - 16% of body weight).

Lethal Concentrations

Mice fed C-9 at concentrations of 300 ppm and higher exhibited severe weight loss (i.e., 32 - 45% of body weight), ruffled fur, lethargy, low posture and limpness. Deaths began to occur in the 10,000 ppm group on test day 4.

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C. Body Weight and Liver Weight Analysis

Body weights of the male and female mice from the 1, 3, 10, and 30 ppm concentration groups were similar to those of the control mice throughout the feeding study. The mean body weight growth curves for the male and female mice are presented in Figures 1 and 2.

The mean absolute and mean relative liver weights of male and female mice fed diets containing C-9 at concentrations of 3, 10, and 30 ppm were significantly heavier when compared to the controls. In addition, male mice from the 1 ppm concentration group had significantly heavier mean relative liver weights when compared to the control group (Tables 1 and 2).

Comparison of mean body and liver weights of mice fed diets above 30 ppm were not possible because there were no concurrent control groups. The body and liver weights of these animals are presented in Appendix II.

CONCLUSIONS

Dietary administration of C-9 to male and female mice at concentrations of 3, 10, or 30 ppm for 14 consecutive days caused significant increases in mean absolute and relative liver weights. Dietary administration of C-9 at a concentration of 1 ppm caused a significant increase in the mean relative, but not absolute, liver weight of male mice. The mean liver weights of female mice were unaffected by C-9 at a dietary concentration of 1 ppm. C-9 at dietary concentrations of 1, 3, 10 or 30 ppm had no effect on the mean body weights of either male or female mice. Administration of C-9 at concentrations of 100 ppm or higher in the diet resulted in slight to severe weight loss; administration at concentrations of 300 ppm or higher caused death.

FIGURE 1
GROWTH CURVES OF MALE MICE FED C-9

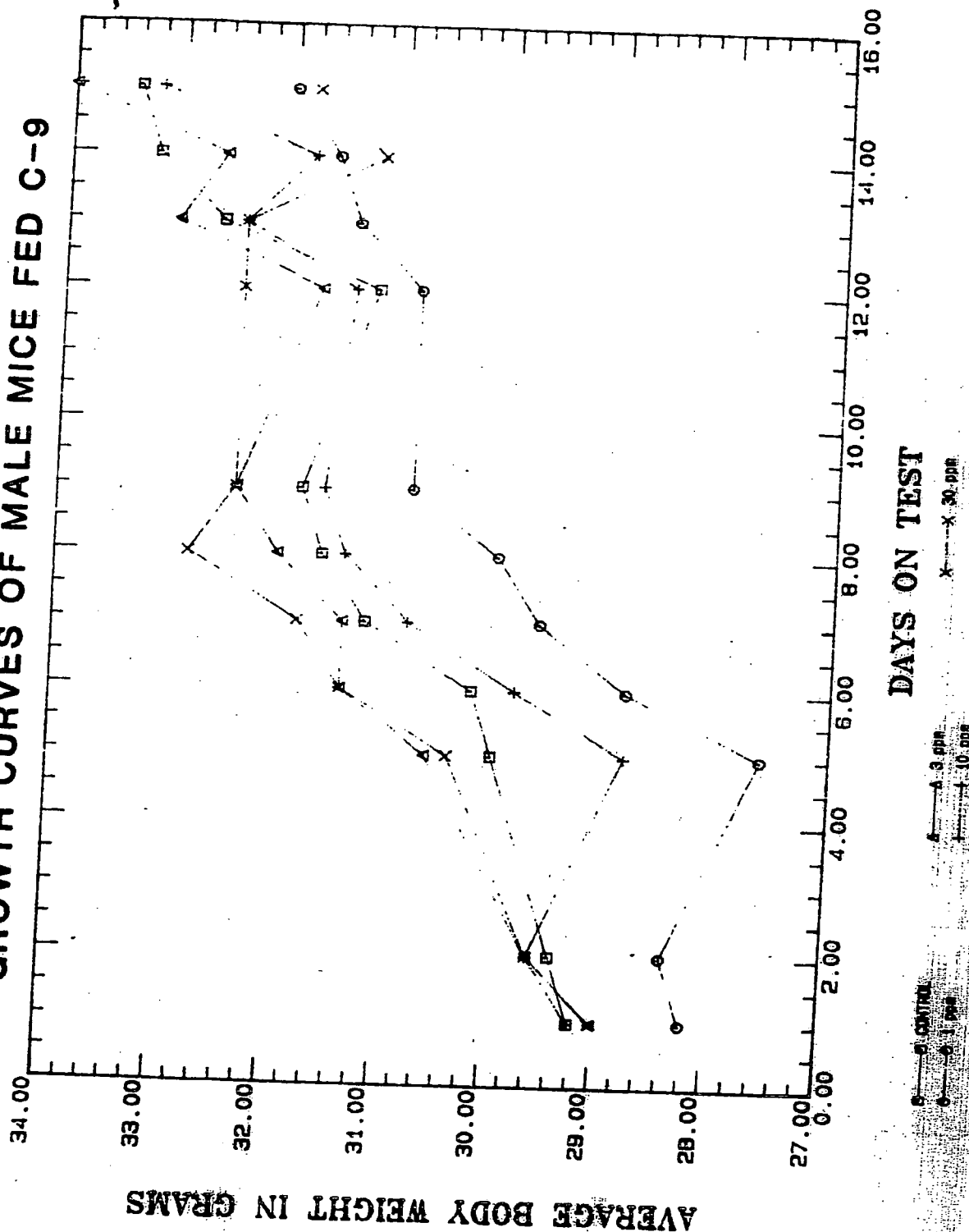
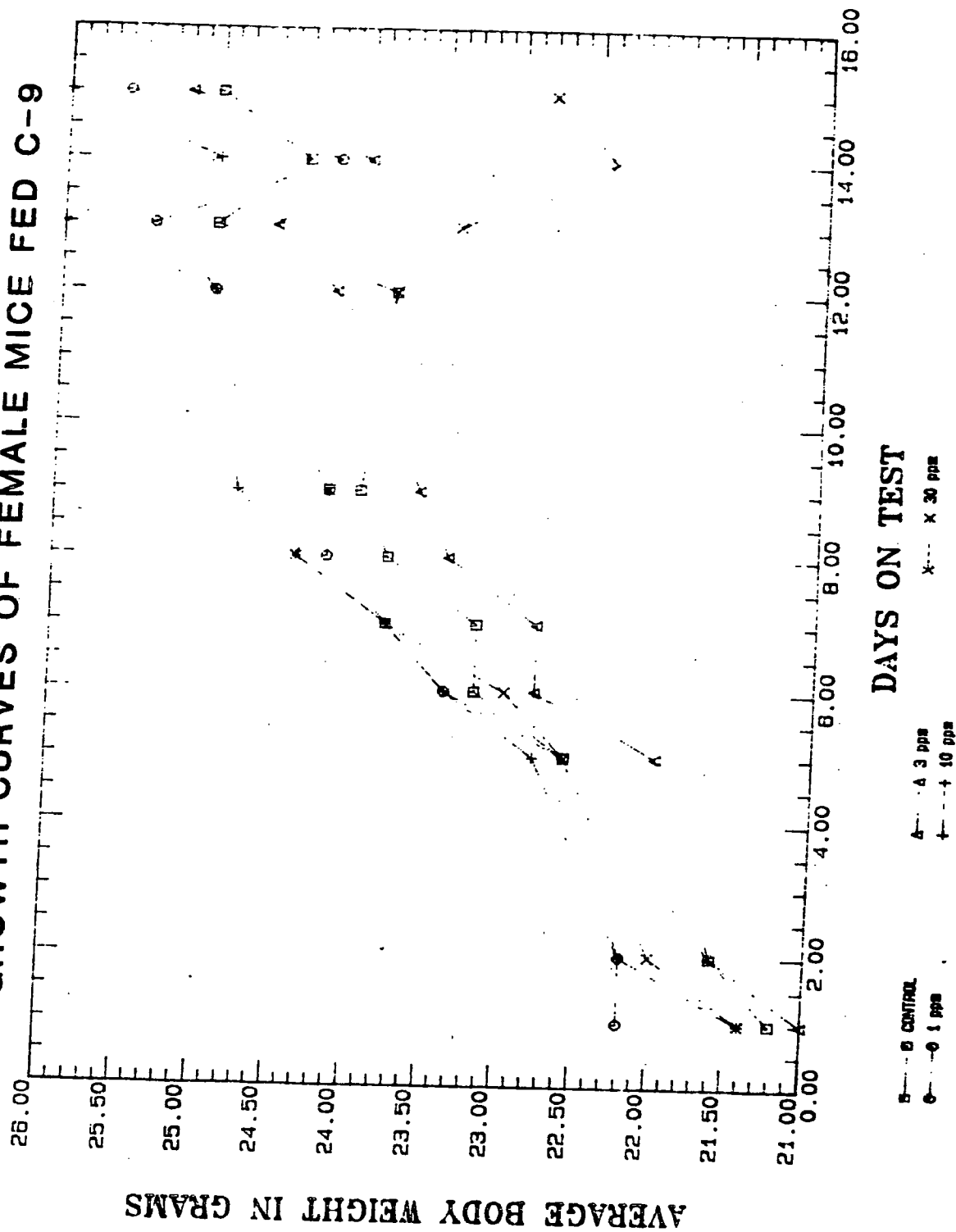


FIGURE 2
GROWTH CURVES OF FEMALE MICE FED C-9



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APPENDIX I

Diet Analysis

Polymer Products Department
Research and Development Division
Experimental Station

cc [REDACTED]

ANALYTICAL REPORT

August 10, 1984

Rayanne Ferenz
CR&DD
Haskell

ANALYSIS OF PERFLUORONONANOATE (C9) IN RODENT CHOW
(Job No. 841-0896; PRAL Nos 84-5799 to 5805)

Six samples of rodent chow, 3 nominally 100 ppm and 3 nominally 10,000 ppm have been analyzed by the derivatization/gas chromatographic method using electron capture detector originally developed for C8 in blood (ES-567). No problems were encountered in applying the method to rodent chow. The samples gave very clean chromatograms with no interfering peaks in the C8 to C10 region. Samples were run in duplicate with C10 as an internal standard added to samples and standards. Unfortunately the C9 we had available as a standard has some C8 and possibly C10 contaminants.

The C9 you used to spike the rodent chow appears much purer. We have reported results based on peak height only because of interfering peaks in the integration of the standards. The C10 in the standards does affect the absolute values, but the relative values between the 6 samples should be valid. The results all summarized in the attached table.

The precision for the high level samples is not as good as for the 100 ppm samples as very large dilutions were necessary to bring the samples within the calibration range. In addition, a smaller sample of rat chow was used.

If you need a more accurate analysis, you could send us a sample of your C9 for a standard and we could rerun the samples. If you have any questions, don't hesitate to call me.

S. R. Leas
S. R. Leas

M. J. Vilone

mag
Attachment

Keywords:
GC
Perfluorononanoate
C9

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TABLE I

PERFLUORONONANOATE IN RODENT CHOW

<u>Pral No.</u>	<u>Designation</u>	<u>ug Perfluorononanoate/g sample</u> <u>Duplicate Analyses (a)</u>
84-5799	0 ppm	None detected (b)
84-5800	100 ppm - Top	86; 85
84-5801	100 ppm - Middle	97; 90
84-5802	100 ppm - Bottom	83; 78
84-5803	10,000 ppm - Top	11,000; 10,500
84-5804	10,000 ppm - Middle	11,200; 11,400
84-5805	10,000 ppm - Bottom	11,700; 11,100

(a) Analysis of duplicate aliquots of rat chow; approx. 0.2 g used in each analysis of 100 ppm samples; approx. 0.1 g used in each analysis of 10,000 ppm samples.

(b) Detection limit not determined; no peak observed under conditions used for analyzing 100 ppm sample.

TABLE 1

MALE MICE LIVER & BODY WEIGHT SUMMARY

MEAN ABSOLUTE AND RELATIVE DATA
[REDACTED] H 15437

GROUP	CONC.	FINAL WT.	ABSOLUTE WT.	RELATIVE WT.
0 ppm		33.4(0.000)	1.811(0.000)	5.422(0.000)
1 ppm		32.0(0.204)	2.162(0.081)	6.752(0.017)+
3 ppm		34.0(0.580)	2.365(0.009)#	6.952(0.007)#
10 ppm		33.2(0.853)	3.171(0.000)#	9.528(0.000)#
30 ppm		31.8(0.149)	3.793(0.000)#	11.953(0.000)#
TEST - HOMOGENEITY		0.221		
TEST - TREND		0.411	0.000	0.000
BARTLETT'S TEST		0.082	0.000	0.000
			0.015	0.082

=====

Values in parentheses - P VALUE OF STUDENT 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

HOMOGENEITY - P VALUE OF F TEST OF WHETHER GROUP MEANS ARE EQUAL.

TREND - P VALUE OF F TEST OF WHETHER THERE IS DOSE-RELATED
CHANGE IN GROUP MEANS.

BARTLETT'S TEST - P VALUE OF TEST OF HOMOGENEITY OF VARIANCE

TABLE 2

FEMALE MICE LIVER & BODY WEIGHT SUMMARY

MEAN ABSOLUTE AND RELATIVE DATA

[REDACTED] H 15437

GROUP	CONC.	FINAL WT.	ABSOLUTE WT.	RELATIVE WT.
0 PPM		25.0(0.000)	1.414(0.000)	5.664(0.000)
1 PPM		25.6(0.580)	1.663(0.107)	6.513(0.139)
3 PPM		25.2(0.853)	1.854(0.007)#	7.343(0.006)#
10 PPM		26.0(0.359)	2.493(0.000)#	9.600(0.000)#
30 PPM		22.8(0.052)	3.167(0.000)#	13.896(0.000)#
TEST - HOMOGENEITY		0.058	0.000	0.000
TEST - TREND		0.109	0.000	0.000
BARTLETT'S TEST		0.626	0.207	0.626

=====

Values in parentheses - P VALUE OF STUDENT 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

HOMOGENEITY - P VALUE OF F TEST OF WHETHER GROUP MEANS ARE EQUAL.

TREND - P VALUE OF F TEST OF WHETHER THERE IS DOSE-RELATED
CHANGE IN GROUP MEANS.

BARTLETT'S TEST - P VALUE OF TEST OF HOMOGENEITY OF VARIANCE

APPENDIX II

Individual Body and Liver Weight Data from
Animals that were Removed from Test Diets

Mice fed diet containing 1,000 ppm or higher were placed on control diet on test day 5 because of moderate to severe body weight loss and death (1/10 mice) in the 10,000 ppm group. On test day 6, the surviving mice from these groups were sacrificed in extremis and liver weights were recorded. Similarly, mice from the 100 and 300 ppm groups were sacrificed on test day 8. The initial and final body weights and the liver weights of these mice are presented here for completeness.

APPENDIX II

Body and Liver Weights for Mice Fed
100, 300, 1,000, 3,000, and 10,000 ppm C-9

<u>Concentration (ppm)</u>	<u>Mouse No.</u>	<u>Initial Body Weight (g)</u>	<u>Final Body Weight (g)</u>	<u>Liver Weight (g)</u>
100 Males	34716	31	27	3.413
	34717	28	22	2.380
	34718	29	24	2.658
	34719	31	29	3.466
	34720	29	27	3.219
100 Females	34779	21	22	3.021
	34780	20	16	2.150
	34781	23	20	2.663
	34782	22	17	1.854
	34786	22	18	2.205
300 Males	34721	29	17	1.932
	34722	30	19	2.415
	34723	29	18	1.885
	34724	30	18	1.910
	34725	30	19	2.327
300 Females	34787	22	F.D.[8]*	1.468
	34788	22	15	2.138
	34789	24	F.D.[8]	1.785
	34790	21	14	1.589
	34791	23	F.D.[8]	1.868
1,000 Males	34727	29	17	1.22
	34728	31	19	1.984
	34729	29	17	1.70
	34730	30	20	1.99
	34732	28	18	1.34
1,000 Females	34792	22	14	1.673
	34793	22	F.D.[6]	1.34
	34794	23	F.D.[5]	1.71
	34795	20	F.D.[6]	1.24
	34796	22	F.D.[6]	1.390

F.D. = Found Dead; number in brackets is equal to days on test.

APPENDIX II (Continued)

Body and Liver Weights for Mice Fed
100, 300, 1,000, 3,000, and 10,000 ppm C-9

Concentration (ppm)	Mouse No.	Initial Body Weight (g)	Final Body Weight (g)	Liver Weight (g)
3,000 Males	34733	28	F.D.[6]	1.834
	34734	29	F.D.[6]	1.694
	34735	31	17	1.79
	34736	29	F.D.[6]	1.634
	34737	30	17	1.684
3,000 Females	34797	20	F.D.[5]	1.24
	34799	22	F.D.[6]	1.243
	34800	21	F.D.[6]	1.262
	34801	20	F.D.[6]	1.352
	34802	21	F.D.[5]	1.27
10,000 Males	34738	31	17	1.39
	34739	30	20	1.650
	34740	30	F.D.[6]	2.080
	34741	29	18	1.602
	34742	29	17	1.523
10,000 Females	34803	22	F.D.[6]	1.530
	34804	21	F.D.[5]	1.32
	34805	22	12	1.691
	34806	22	13	1.20
	34807	22	F.D.[4]	1.119

*F.D. = Found Dead; number in brackets is equal to days on test.