STUDY NO. 00-4207
SPONSOR STUDY NO. 211-B-2G

BASELINE GASOLINE VAPOR CONDENSATE

A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTIVE TOXICITY STUDY IN RATS

Final Report

Volume IV of IV

Submitted to: American Petroleum Institute (API)
1220 L Street, Northwest
Washington, D.C. 20005-4070

Attn: Thomas M. Gray, M.S., D.A.B.T.

Date: 18 December 2008

TABLE OF CONTENTS

Volume IV

COVER PAGE 1432
TABLE OF CONTENTS 1433

APPENDICES

CC. Reproductive Assessment Report 1434
DD. GFAP Report 1456
EE. Certificates of Analysis – (Feed, Bedding, and Water) 1516
FF. Protocol and Protocol Amendments 1594
GG. Testing Facility Personnel 1654
HH. Report Amendments 1655

REPRODUCTIVE ASSESSMENT REPORT

FOR

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY
STUDY IN RATS

HLS STUDY NUMBER: 00-4207

PREPARED FOR

HUNTINGDON LIFE SCIENCES (HLS)
METTLERS ROAD
P.O. Box 2360
EAST MILLSTONE, NJ 08875-2360

PREPARED BY

CHARLES RIVER LABORATORIES
PATHOLOGY ASSOCIATES (PAI)- MARYLAND

HLS Study No.: 00-4207

TABLE OF CONTENTS

Section		Page No.
I.	RESERVED FOR QUALITY ASSURANCE STATEMENT	4
II.	MATERIALS AND METHODS	5
	A. Male Fertility Sample Collection	5
	B. Sperm Motility Evaluation	5
	C. Caudal Epididymis Weight and Total Sperm Count Determination	6
	D. Sperm Morphology Evaluation	6
	E. Ovary Sample Collection and Processing	6
	F. Ovarian Follicle Evaluation	7
	G. Statistical Analyses	7
	H. GLP Compliance Statement	7
	I. Sample and Data Archiving	7
III.	RESULTS	8
	A. Sperm Motility	8
	B. Total Sperm Count	8
	C. Sperm Morphology	8
	D. Caudal Epididymis Weight	9
	E. Ovarian Follicle Evaluation	9
IV.	DISCUSSION AND CONCLUSIONS	9
V.	REFERENCES	10

HLS Study No.: 00-4207

INDEX OF TABLES AND APPENDICES

Table		Page No.
1.	Summary of P_0 Generation Male Reproductive Parameters	11
2.	Summary of F_1 Generation Male Reproductive Parameters	12
3.	Summary of F_1 Generation Female Ovarian Follicle Evaluation	13

Appendix		Page No.
A.	Individual P_0 Generation Caudal Epididymis Weight, Sperm Motility and Sperm Count Data	14
B.	Individual F_1 Generation Caudal Epididymis Weight, Sperm Motility and Sperm Count Data	16
C.	Individual P_0 Generation Sperm Morphology Data	18
D.	Individual F_1 Generation Sperm Morphology Data	20
E.	Individual F_1 Generation Female Ovarian Follicle Count Data	22

Baseline Gasoline Vapor Condensate
A Two-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

Reproductive Assessment Report

Study Number: 00-4207

QUALITY ASSURANCE STATEMENT

This reproductive assessment project has been inspected and audited by the PAI Quality Assurance Unit (QAU) as required by the Good Laboratory Practice (GLP) regulations promulgated by the U.S. Environmental Protection Agency (EPA). The reproductive assessment report is an accurate reflection of the recorded data. The following table is a record of the inspections/audits performed and reported by the QAU.

Date of Inspection	Phase Inspected	Date Findings Reported to Study Pathologist/ PAI Management	Date Findings Reported to Study Director/Study Director Management
09/11/01	Total Sperm Count	09/13/01	04/12/02
04/11/02	Total Sperm Count	04/12/02	04/12/02
04/11/02	Ovarian Follicle Quantification	04/12/02	04/12/02
04/15/02	Individual Animal Data	04/16/02	04/16/02
04/15/02	Draft Reproductive Assessment Report	04/16/02	04/16/02
07/31/02	2nd Draft Reproductive Assessment Report	07/31/02	07/31/02
05/22/03	3rd Draft Reproductive Assessment Report	05/22/03	05/22/03
05/30/03	4th Draft Reproductive Assessment Report	05/30/03	05/30/03
03/08/04	5th Draft Reproductive Assessment Report	03/08/04	03/08/04
05/02/07	Final Reproductive Assessment Report	05/02/07	05/02/07

Joseph Stashick
Senior Quality Assurance Auditor

May 02, 2007
Date

Pathology Associates • 15 Worman's Mill Court, Suite I, Frederick, MD 21701 • 301.663.1644 • FAX: 301.663.8994 • www.criver.com

Baseline Gasoline Vapor Condensate
A Two-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

II. MATERIALS AND METHODS

Male fertility assessments, including sperm motility, homogenization-resistant testicular spermatid count, epididymal sperm count, sperm morphology and caudal epididymis weight were conducted by PAI on all surviving male rats from control and high-exposure groups of each generation (P_0 and F_1). The ovarian follicle evaluation was conducted on each ovary from 10 female rats from the control and high-exposure group of the F_1 generation.

A. Male Fertility Sample Collection

All surviving male rats from each generation (P_0 and F_1) were euthanized by exsanguination following anesthesia with inhaled carbon dioxide before the last F_1 and F_2 litters, respectively, had been weaned. For all animals, the abdominal cavity was opened and the reproductive organs exposed. For motility assessment, the left vas deferens was dissected away from the testis and immediately placed in a petri dish containing 10 ml of a solution consisting of 1% Bovine Serum Albumin dissolved in Phosphate Buffered Saline. The solution was prewarmed to a temperature of approximately 38°C. A minimum 3 minute period was allowed for the sperm to swim out.

For total sperm count assessment, the left epididymis and left testis were then removed and placed on dry ice. The frozen epididymides and testes were then transferred to Charles River Laboratories, Pathology Associates (formerly known as Pathology Associates International until January 8, 2001), Frederick, MD. and stored frozen at -70°C until evaluation for caudal epididymal sperm count and homogenization-resistant testicular spermatid count.

Only the control and high exposure animals were examined for percent motility, total epididymal sperm count, testicular spermatid count and sperm morphology.

B. Sperm Motility Evaluation

Following the swim out period, a sperm sample was obtained using a 100 µm deep cannula. The cannula was immediately loaded into the prewarmed stage of the Hamilton Thorne IVOS (Ver. 10.9i)

automated sperm analyzer. The analyzer automatically selected five fields and each motion image was digitally saved and permanently stored on optical media. The images were subsequently analyzed and the percent motility determined for each animal.

C. Caudal Epididymis Weight and Total Sperm Count Determination

Each frozen epididymis was removed from the freezer, thawed and the caudal section was trimmed and weighed. Each frozen testis was removed from the freezer, thawed, the tunica removed and the testicular parenchyma weighed. The cauda epididymis and testicular parenchyma were homogenized in deionized water and the suspensions were transferred to plastic test tubes and vortexed. A 100 µl sample was transferred to a violet reaction vial containing a Hoechst dye (H33342) which uniquely stains the head of the sperm. A sample of the stained sperm was placed into a 20 µm deep Cell-Vu glass slide which was loaded into the Hamilton Thorne IVOS analyzer (Ver. 10.9i and 12.1c for P_0 and F_1 generations, respectively). Twenty fields were automatically selected by the analyzer for each animal and the number of cauda epididymal sperm or homogenization-resistant testicular spermatids determined. The counts reported were adjusted for caudal epididymal or testicular weight.

D. Sperm Morphology Evaluation

Two Eosin stained slides were prepared for each animal from the caudal epididymis total count preparation. The slides were evaluated and a minimum of 200 sperm cells/animal was examined for morphological development.

E. Ovary Sample Collection and Processing

The ovaries from 10 control and high-exposure F_1 female rats were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and evaluated microscopically for the numbers of primordial and growing follicles and the presence of *corpora lutea*.

The ovaries were embedded together without distinguishing left or right. Five ovarian sections were taken at least 100 µm apart from the inner third of each ovary. For analysis, the ovarian sections were considered left or right based on their position on the slide. The ovarian sections were prepared by HLS personnel and evaluated by PAI personnel.

F. Ovarian Follicle Evaluation

Each ovarian section was methodically evaluated and the number of primordial and growing follicles counted and recorded. The counts are reported per ovary and total for primordial and growing follicles. The sections were also examined to confirm the presence or absence of *corpora lutea*. The *corpora lutea* data was not summarized.

G. Statistical Analyses

The means and standard deviations for the sperm motility, caudal epididymal sperm count, testicular homogenization-resistant spermatid count, sperm morphology and numbers of primordial and growing follicles by ovary and total were calculated and compared across groups using the Kruskal-Wallis nonparametric ANOVA test. If a significant effect occurred ($p<0.05$), the Mann-Whitney U test was used for pair-wise comparisons of each treated group to the control group. Animals with fewer than 25 sperm cells present in the motility analysis were excluded from the group mean calculation and subsequent statistical analysis. Animals with at least 25 sperm cells were included in the group mean calculation subsequent and statistical analysis; however, animals with fewer than 50 cells were identified to have a low count. The mean caudal epididymis weight for each group was calculated and compared using one-way analysis of variance (ANOVA) technique. If ANOVA is significant, Dunnett's test was used for pair-wise comparisons of each treated group to the control group at the 5% and 1% risk levels. Statistical analyses were performed using an IBM™ compatible computer with SAS computer programs (SAS/STAT User's Guide, 1989).

H. GLP Compliance Statement

The portion of this study performed by PAI was conducted in compliance with the 79.60, CFR Vol. 59, No. 122, 27 June 1994.

I. Sample and Data Archiving

Frozen epididymis and testis samples not processed for sperm count determination were transferred to and stored frozen at -70°C in PAI's long-term archive facility. The optical media used for permanent storage of the sperm motility images, raw data printouts from the sperm motility, epididymal and testicular sperm count determination, sperm morphology analysis, ovarian follicle count enumeration, and any supporting documentation, as well as the sperm morphology and ovarian histology slides will be maintained

at PAI until the study has been completed. Upon finalizing the report, all non-frozen study material will be returned to HLS for archiving. Disposition of the frozen tissue samples will be directed by correspondence from HLS.

III. RESULTS

A. Sperm Motility

Table 1-2 (Summary Data)
Appendix A-B (Individual Data)

No apparent treatment-related effects were observed in the P_0 or F_1 male sperm motility data. Group mean values were 92%, or higher, in the control and high exposure P_0 and F_1 generation males. No evidence of reduced motility due to treatment with Baseline Gasoline Vapor Condensate (BGVC) was observed.

B. Total Sperm Count

Table 1-2 (Summary Data)
Appendix A-B (Individual Data)

No apparent treatment-related effects were observed in the homogenization-resistant testicular spermatid count or caudal epididymal sperm count in either generation. The mean numbers of testicular spermatids and epididymal sperm were comparable between control and 20,000 mg/m^3 exposure groups.

C. Sperm Morphology

Table 1-2 (Summary Data)
Appendix C-D (Individual Data)

No apparent treatment-related effects were observed in the sperm morphology data of either generation. A statistically significant reduction in the percent of abnormal sperm was observed in the 20,000 mg/m^3 exposure group of the P_0 generation. A decrease in this parameter is not considered biologically meaningful.

D. Caudal Epididymis Weight

Table 1-2 (Summary Data)
Appendix A-B (Individual Data)

No apparent treatment-related effects were observed in the caudal epididymis weight data for either generation. Group mean values were 0.29 grams for the P_0 generation and ranged from 0.25-0.26 grams for the F_1 generation. No biologically meaningful differences were observed.

E. Ovarian Follicle Evaluation

Table 3 (Summary Data)
Appendix E (Individual Data)

No apparent treatment-related effects were observed in the ovarian follicle count data for the F_1 animals evaluated. Group mean values were comparable between the control and high exposure group for the left, right and total primordial and growing follicle counts. *Corpora lutea* were present for all animals.

IV. DISCUSSION AND CONCLUSIONS

The percent sperm motility, caudal epididymal sperm count, homogenization-resistant testicular spermatid count, sperm morphology, caudal epididymis weight and primordial and growing follicle counts, as individual ovaries and total per animal, were not affected by treatment with Baseline Vapor Gasoline Condensate at a dosage level of 20,000 mg/m^3. No biologically meaningful differences were observed between the study groups.

Michael D. Mercieca, B.S.
Reproductive Toxicologist
Charles River Laboratories
Pathology Associates (PAI)

5/2/07
Date

V. REFERENCES

Dunlap, W.P., Marx, M.S. and Agamy, G.G. 1981. Fortran IV functions for calculating probabilities associated with Dunnett's test. Behav. Res. Methods and Instrumentation 13: 363-366.

Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. Journal of the American Statistical Association 50:1096-1121.

Dunnett, C. W. 1964. New tables for multiple comparisons with a control. Biometrics 20:482-491.

Kruskal, W.H. and Wallis, W.A. 1952. Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association 47: 583-621.

Kruskal, W.H. and Wallis, W.A. 1953. Errata for Kruskal-Wallis (1952). Journal of the American Statistical Association 48: 907-911.

Kruskal, W.H. 1957. Historical notes on the Wilcoxon unpaired two-sample test. Journal of the American Statistical Association 52: 356-360.

SAS/STAT User's Guide, 1989a. SAS Institute Inc., Version 6, Fourth Edition, Cary, NC:SAS Institute Inc., 1989. Vol. 1, p. 209-244. Vol. 2 p. 1195-1210.

HLS STUDY NO.: 00-4207

TABLE 1

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

SUMMARY OF P_0 GENERATION MALE REPRODUCTIVE PARAMETERS

Group:	I	IV
Exposure Level: BGVC[a] (mg/m^3)	0	20,000
MOTILITY (%)		
Mean	96	94
SD	3	7
N	26	24
EPIDIDYMAL COUNT (MILLION SPERM/GRAM)		
Mean	935.5	955.3
SD	234.5	305.3
N	26	26
TESTICULAR COUNT (MILLION SPERMATIDS/GRAM)		
Mean	124.1	115.4
SD	24.1	26.4
N	26	26
SPERM MORPHOLOGY[b] (% ABNORMAL)		
Mean	1.7	1.2*
SD	0.6	0.5
N	26	26
CAUDAL EPIDIDYMIS WEIGHTS (GRAM)		
Mean	0.29	0.29
SD	0.03	0.03
N	26	26

[a]BASELINE GASOLINE VAPOR CONDENSATE.
[b]MEAN AND STANDARD DEVIATIONS WERE CALCULATED USING THE TOTAL NUMBER OF ABNORMAL SPERM AS A PERCENTAGE OF THE NUMBER OF SPERM EXAMINED.

*SIGNIFICANTLY DIFFERENT FROM CONTROL ($p < 0.05$).

HLS STUDY NO.: 00-4207

TABLE 2

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

SUMMARY OF F_1 GENERATION MALE REPRODUCTIVE PARAMETERS

Group:	I	IV
Exposure Level: BGVC[a] (mg/m^3)	0	20,000
MOTILITY (%)		
Mean	92	95
SD	6	4
N	25	25
EPIDIDYMAL COUNT (MILLION SPERM/GRAM)		
Mean	882.8	933.5
SD	181.6	199.4
N	25	25
TESTICULAR COUNT (MILLION SPERMATIDS/GRAM)		
Mean	98.4	100.6
SD	14.8	14.7
N	25	25
SPERM MORPHOLOGY[b] (% ABNORMAL)		
Mean	0.7	0.5
SD	1.0	0.5
N	25	25
CAUDAL EPIDIDYMIS WEIGHTS (GRAM)		
Mean	0.26	0.25
SD	0.03	0.03
N	25	25

[a]BASELINE GASOLINE VAPOR CONDENSATE.
[b]MEAN AND STANDARD DEVIATIONS WERE CALCULATED USING THE TOTAL NUMBER OF ABNORMAL SPERM AS A PERCENTAGE OF THE NUMBER OF SPERM EXAMINED.

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL.

HLS STUDY NO.: 00-4207

TABLE 3

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

SUMMARY OF F_1 GENERATION FEMALE OVARIAN FOLLICLE EVALUATION

PRIMORDIAL FOLLICLE COUNT

Group:		I	IV
Exposure Level:	BGVC[a] (mg/m^3)	0	20,000
LEFT	Mean	54.5	63.2
	SD	29.9	32.1
	N	10	10
RIGHT	Mean	51.1	52.1
	SD	17.8	23.2
	N	10	10
TOTAL	Mean	105.6	115.3
	SD	32.8	41.2
	N	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL.

GROWING FOLLICLE COUNT

Group:		I	IV
Level:	BGVC[a] (mg/m^3)	0	20,000
LEFT	Mean	3.3	3.5
	SD	2.7	2.3
	N	10	10
RIGHT	Mean	3.3	3.8
	SD	2.1	1.5
	N	10	10
TOTAL	Mean	6.6	7.3
	SD	3.5	2.9
	N	10	10

NONE SIGNIFICANTLY DIFFERENT FROM CONTROL.

[a]BASELINE GASOLINE VAPOR CONDENSATE.

HLS STUDY NO.: 00-4207

APPENDIX A

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL P_0 GENERATION CAUDAL EPIDIDYMIS WEIGHT,
SPERM MOTILITY AND SPERM COUNT DATA

Group I: 0 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Caudal Epididymis Weight (g)	Motility (%)	- - - - C O U N T - - - - Caudal Epididymal Sperm (million sperm/gram)	Homogenization Resistant Testicular Spermatid
1001	0.35	95	1325.7	100.8
1002	0.32	96	777.4	125.7
1003	0.29	97	1005.5	130.0
1004	0.31	98	862.2	117.2
1005	0.35	90	1176.9	100.7
1006	0.29	94	1117.2	136.2
1007	0.30	96	1106.9	110.4
1008	0.26	96	774.4	106.5
1009	0.26	90	1143.7	135.2
1010	0.30	96	705.8	104.7
1011	0.27	94	724.2	126.7
1012	0.27	99	711.4	197.9
1013	0.30	98	1064.5	108.8
1014	0.30	100	1284.4	139.0
1015	0.29	97	1129.2	164.6
1016	0.26	93	1188.2	148.1
1017	0.28	98	843.0	101.3
1018	0.28	97	1301.7	108.8
1019	0.30	97	1079.9	98.6
1020	0.28	99	657.1	120.9
1021	0.31	96	877.1	161.8
1022	0.26	99	605.2	105.3
1023	0.27	96	732.8	113.8
1024	0.25	98	509.1	102.8
1025	0.28	99	781.0	118.4
1026	0.29	96	837.9	142.5

HLS STUDY NO.: 00-4207

APPENDIX A

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL P_0 GENERATION CAUDAL EPIDIDYMIS WEIGHT,
SPERM MOTILITY AND SPERM COUNT DATA

Group IV: 20,000 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Caudal Epididymis Weight (g)	Motility (%)	- - - - C O U N T - - - - Caudal Epididymal Sperm (million sperm/gram)	Homogenization Resistant Testicular Spermatid (million sperm/gram)
4001	0.29	98	710.2	134.1
4002	0.30	95	867.8	100.7
4003	0.35	99	902.5	102.9
4004	0.28	96	590.9	141.3
4005	0.28	97	863.7	92.9
4006	0.24	80	1099.2	139.0
4007	0.30	[a]	1592.9	91.9
4008	0.27	79	758.5	123.9
4009	0.29	100	1388.5	103.9
4010	0.28	89	611.6	179.7
4011	0.29	96	678.3	109.5
4012	0.29	96	965.6	83.7
4013	0.26	97	1019.1	120.1
4014	0.30	99	1550.5	106.1
4015	0.32	89	672.6	96.7
4016	0.20	94	324.0	79.3
4017	0.29	96	1009.5	134.2
4018	0.27	90	1242.8	99.5
4019	0.31	[b]	694.3	112.0
4020	0.27	95	998.5	134.7
4021	0.26	97	894.5	122.0
4022	0.31	98	955.5	121.7
4023	0.25	98	944.2	97.4
4024	0.32	76	1439.1	182.6
4025	0.31	97	914.5	111.8
4026	0.28	94	1148.8	79.0

[a]NO SPERM PRESENT FOR ANALYSIS.
[b]FEWER THAN 25 SPERM CELLS COUNTED IN 5 FIELDS ANALYZED; EXCLUDED FROM STATISTICAL ANALYSIS.

HLS STUDY NO.: 00-4207

APPENDIX B

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL F_1 GENERATION CAUDAL EPIDIDYMIS WEIGHT,
SPERM MOTILITY AND SPERM COUNT DATA

Group I: 0 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Caudal Epididymis Weight (g)	Motility (%)	- - - - C O U N T - - - - Caudal Epididymal Sperm (million sperm/gram)	Homogenization Resistant Testicular Spermatid
1027	0.17	86	928.8	88.5
1028	0.27	91	720.3	102.5
1029	0.23	89	668.3	84.4
1030	0.31	98	1062.5	83.7
1031	0.26	97	908.9	102.0
1032	0.29	93	728.3	82.7
1034	0.29	97	1016.8	115.9
1035	0.26	98	808.4	106.9
1036	0.24	99	836.5	107.6
1037	0.25	98	941.1	118.8
1038	0.27	97	782.3	118.5
1039	0.27	85	724.2	106.5
1040	0.24	94	897.5	70.2
1041	0.23	93	1359.3	92.2
1042	0.28	96	914.9	70.1
1043	0.31	85	1072.6	81.4
1044	0.26	79	957.2	119.0
1045	0.25	97	786.3	101.8
1046	0.27	90	879.1	95.7
1047	0.25	86	924.3	90.2
1048	0.29	93	919.4	97.3
1049	0.24	83	479.2	103.9
1050	0.26	98	740.0	97.7
1051	0.32	96	784.2	124.2
1052	0.30	90	1230.4	98.4

HLS STUDY NO.: 00-4207

APPENDIX B

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL F_1 GENERATION CAUDAL EPIDIDYMIS WEIGHT,
SPERM MOTILITY AND SPERM COUNT DATA

Group IV: 20,000 mg/m³ (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Caudal Epididymis Weight (g)	Motility (%)	- - - - C O U N T - - - - Caudal Epididymal Sperm (million sperm/gram)	Homogenization Resistant Testicular Spermatid
4027	0.26	95	1009.4	81.9
4028	0.21	92	1035.7	111.4
4029	0.23	96	1191.1	122.8
4030	0.27	98	1068.9	104.6
4031	0.21	91	926.1	107.3
4032	0.29	91	1063.7	96.8
4033	0.18	97	488.0	105.3
4034	0.25	94	941.1	106.7
4035	0.26	96	764.1	85.0
4036	0.26	91	1150.2	102.8
4037	0.26	94	627.4	114.8
4038	0.21	97	891.3	113.2
4039	0.28	94	970.9	108.0
4040	0.27	97	1010.8	103.2
4042	0.25	99	1062.4	105.9
4043	0.25	96	798.9	92.1
4044	0.23	99	627.4	83.8
4045	0.23	99	1204.8	86.3
4046	0.32	91	1182.9	87.7
4047	0.32	97	849.6	70.7
4048	0.23	83	1000.2	80.4
4049	0.26	92	892.8	124.9
4050	0.25	93	644.1	97.5
4051	0.28	94	1187.5	126.9
4052	0.24	97	749.4	94.0

HLS STUDY NO.: 00-4207

APPENDIX C

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL P_0 GENERATION SPERM MORPHOLOGY DATA

Group I: 0 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Normal	Head: Amorphous	Head: Small	Head: Enlarged	Head: Double	Tail: Coiled	Tail: Bent	Tail: Double	Other
1001	197	3	0	0	0	0	0	0	0
1002	196	4	0	0	0	0	0	0	0
1003	197	3	0	0	0	0	0	0	0
1004	198	2	0	0	0	0	0	0	0
1005	196	4	0	0	0	0	0	0	0
1006	200	0	0	0	0	0	0	0	0
1007	198	2	0	0	0	0	0	0	0
1008	195	4	1	0	0	0	0	0	0
1009	197	3	0	0	0	0	0	0	0
1010	196	4	0	0	0	0	0	0	0
1011	196	3	1	0	0	0	0	0	0
1012	197	3	0	0	0	0	0	0	0
1013	197	3	0	0	0	0	0	0	0
1014	196	4	0	0	0	0	0	0	0
1015	195	5	0	0	0	0	0	0	0
1016	195	5	0	0	0	0	0	0	0
1017	196	3	1	0	0	0	0	0	0
1018	196	2	2	0	0	0	0	0	0
1019	198	2	0	0	0	0	0	0	0
1020	196	4	0	0	0	0	0	0	0
1021	197	3	0	0	0	0	0	0	0
1022	197	3	0	0	0	0	0	0	0
1023	195	5	0	0	0	0	0	0	0
1024	196	4	0	0	0	0	0	0	0
1025	197	3	0	0	0	0	0	0	0
1026	198	2	0	0	0	0	0	0	0

HLS STUDY NO.: 00-4207

APPENDIX C

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL P_0 GENERATION SPERM MORPHOLOGY DATA

Group IV: 20,000 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Normal	Head: Amorphous	Head: Small	Head: Enlarged	Head: Double	Tail: Coiled	Tail: Bent	Tail: Double	Other
4001	197	3	0	0	0	0	0	0	0
4002	198	2	0	0	0	0	0	0	0
4003	199	1	0	0	0	0	0	0	0
4004	197	3	0	0	0	0	0	0	0
4005	199	1	0	0	0	0	0	0	0
4006	196	4	0	0	0	0	0	0	0
4007	197	3	0	0	0	0	0	0	0
4008	199	1	0	0	0	0	0	0	0
4009	198	2	0	0	0	0	0	0	0
4010	196	3	1	0	0	0	0	0	0
4011	198	2	0	0	0	0	0	0	0
4012	199	1	0	0	0	0	0	0	0
4013	196	3	1	0	0	0	0	0	0
4014	198	2	0	0	0	0	0	0	0
4015	197	2	1	0	0	0	0	0	0
4016	198	2	0	0	0	0	0	0	0
4017	198	2	0	0	0	0	0	0	0
4018	196	4	0	0	0	0	0	0	0
4019	198	2	0	0	0	0	0	0	0
4020	199	1	0	0	0	0	0	0	0
4021	197	2	1	0	0	0	0	0	0
4022	197	3	0	0	0	0	0	0	0
4023	196	4	0	0	0	0	0	0	0
4024	199	1	0	0	0	0	0	0	0
4025	198	1	1	0	0	0	0	0	0
4026	197	3	0	0	0	0	0	0	0

HLS STUDY NO.: 00-4207

APPENDIX D

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL F_1 GENERATION SPERM MORPHOLOGY DATA

Group I: 0 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Normal	Head: Amorphous	Head: Small	Head: Enlarged	Head: Double	Tail: Coiled	Tail: Bent	Tail: Double	Other
1027	200	0	0	0	0	0	0	0	0
1028	198	2	0	0	0	0	0	0	0
1029	199	1	0	0	0	0	0	0	0
1030	198	1	0	1	0	0	0	0	0
1031	199	1	0	0	0	0	0	0	0
1032	198	2	0	0	0	0	0	0	0
1034	200	0	0	0	0	0	0	0	0
1035	200	0	0	0	0	0	0	0	0
1036	197	3	0	0	0	0	0	0	0
1037	200	0	0	0	0	0	0	0	0
1038	190	5	0	5	0	0	0	0	0
1039	199	1	0	0	0	0	0	0	0
1040	199	1	0	0	0	0	0	0	0
1041	199	1	0	0	0	0	0	0	0
1042	200	0	0	0	0	0	0	0	0
1043	199	1	0	0	0	0	0	0	0
1044	200	0	0	0	0	0	0	0	0
1045	198	0	0	2	0	0	0	0	0
1046	199	1	0	0	0	0	0	0	0
1047	199	1	0	0	0	0	0	0	0
1048	199	0	0	1	0	0	0	0	0
1049	200	0	0	0	0	0	0	0	0
1050	197	3	0	0	0	0	0	0	0
1051	200	0	0	0	0	0	0	0	0
1052	198	1	0	1	0	0	0	0	0

HLS STUDY NO.: 00-4207

APPENDIX D

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL F_1 GENERATION SPERM MORPHOLOGY DATA

Group IV: 20,000 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

Animal No.	Normal	Head: Amorphous	Head: Small	Head: Enlarged	Head: Double	Tail: Coiled	Tail: Bent	Tail: Double	Other
4027	200	0	0	0	0	0	0	0	0
4028	199	1	0	0	0	0	0	0	0
4029	200	0	0	0	0	0	0	0	0
4030	200	0	0	0	0	0	0	0	0
4031	198	1	0	1	0	0	0	0	0
4032	197	3	0	0	0	0	0	0	0
4033	200	0	0	0	0	0	0	0	0
4034	198	2	0	0	0	0	0	0	0
4035	200	0	0	0	0	0	0	0	0
4036	199	1	0	0	0	0	0	0	0
4037	198	2	0	0	0	0	0	0	0
4038	197	3	0	0	0	0	0	0	0
4039	200	0	0	0	0	0	0	0	0
4040	200	0	0	0	0	0	0	0	0
4042	200	0	0	0	0	0	0	0	0
4043	199	1	0	0	0	0	0	0	0
4044	198	1	0	1	0	0	0	0	0
4045	198	2	0	0	0	0	0	0	0
4046	199	1	0	0	0	0	0	0	0
4047	200	0	0	0	0	0	0	0	0
4048	197	2	1	0	0	0	0	0	0
4049	199	1	0	0	0	0	0	0	0
4050	200	0	0	0	0	0	0	0	0
4051	200	0	0	0	0	0	0	0	0
4052	198	2	0	0	0	0	0	0	0

HLS STUDY NO.: 00-4207

APPENDIX E

BASELINE GASOLINE VAPOR CONDENSATE
A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

INDIVIDUAL F_1 GENERATION FEMALE OVARIAN FOLLICLE COUNT DATA

Group I: 0 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

	PRIMORDIAL COUNT			GROWING COUNT			C.L.'s
Animal No.	LEFT	RIGHT	TOTAL	LEFT	RIGHT	TOTAL	PRESENT
1532	30	27	57	2	4	6	Y
1534	66	74	140	7	3	10	Y
1535	37	33	70	7	2	9	Y
1536	87	68	155	7	6	13	Y
1542	35	51	86	3	3	6	Y
1543	42	54	96	1	3	4	Y
1545	88	31	119	3	1	4	Y
1546	11	73	84	1	7	8	Y
1549	46	61	107	1	4	5	Y
1552	103	39	142	1	0	1	Y

C.L.=CORPORA LUTEA

Group IV: 20,000 mg/m^3 (BASELINE GASOLINE VAPOR CONDENSATE)

	PRIMORDIAL COUNT			GROWING COUNT			C.L.'s
Animal No.	LEFT	RIGHT	TOTAL	LEFT	RIGHT	TOTAL	PRESENT
4527	40	10	50	1	1	2	Y
4528	28	70	98	4	4	8	Y
4529	40	56	96	2	5	7	Y
4532	36	35	71	3	3	6	Y
4537	89	82	171	5	6	11	Y
4540	87	34	121	3	3	6	Y
4544	72	83	155	2	6	8	Y
4546	42	63	105	4	4	8	Y
4547	68	43	111	2	3	5	Y
4548	130	45	175	9	3	12	Y

C.L.=CORPORA LUTEA

GFAP Levels in Specific Rat Brain Areas Following A Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in A Two-Generation Reproduction Toxicity Study

HLS Study No.: **00-4207**
Sponsor Study No.: 211-B-2G
Date: 18 May 2007

Summary Report

Baseline Gasoline Vapor Condensate: A Two-Generation Whole-Body Inhalation Reproduction Toxicity Study in the Rat

Huntingdon Life Sciences, Inc. Study No. 00-4207
Sponsor Study No 211-B-2G
Measurement of Glial Fibrillary Acidic Protein

Principal Investigator:	Study Director:
James P. O'Callaghan, Ph.D. Molecular Neurotoxicology Laboratory Health Effects Laboratory Division Centers for Disease Control and Prevention National Institute for Occupational Safety and Health 1095 Willowdale Road Morgantown, WV 26505 304-285-6079 304-285-6220 (FAX) jdo5@cdc.gov	Gary M. Hoffman, B.A., DABT Huntingdon Life Sciences Mettlers Road East Millstone, NJ 00875-2360 732-873-2550 732-873-3992 (FAX) hoffmang@princeton.huntingdon.com

STATEMENT OF COMPLIANCE

This study was conducted in the spirit of compliance with 79.60, CFR Vol. 59, No. 122, 27 June 1994. This study was performed according to protocol and Standard Operating Procedures.

James P. O'Callaghan, Ph.D.
Principal Investigator

5/21/07
Date

Gary M. Hoffman, B.A., D.A.B.T.
Study Director

Date

SIGNATURE PAGE

SCIENTIST

The following Scientist was responsible for the overall conduct of this study.

James P. O'Callaghan, Ph.D.
Principal Investigator

5/21/07
Date

SCIENTIFIC REVIEW

The following Scientist has reviewed and approved this report.

Gary M. Hoffman, B.A., D.A.B.T.
Study Director

18 Dec 08
Date

Third-Party QA Statement

Study Number HLS 00-4207

GFAP Levels in Specific Rat Brain Areas Following a Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in a Two-Generation Reproduction Toxicity Study

The Sponsor's third-party QA contractor inspected/audited the following aspects of this study for compliance with SOPs and the study protocol:

Area Inspected	Date of Inspection	Date Reported to Sponsor
Protocol	9/1-6/00, 3/23/01	9/6/00, 3/25/01
Facility Inspection	4/3//01	4/10/01
Draft Final Report Audit	3/20/03	4/9/03

Christine Sexsmith

5/30/07
Date

Christine Sexsmith
Sexsmith Consulting Services, LLC
107 Lisa Court
McMurray, PA 15317

csexsmith@verizon.net

QUALITY ASSURANCE STATEMENT

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Huntingdon Life Sciences, East Millstone, New Jersey, and the dates that findings were reported to the Study Director and Management. This report reflects the raw data as far as can be reasonably established.

Type of Inspection	Date(s) of Inspection	Reported to Study Director and Management
GLP Protocol Review	9 & 14 Mar 01	14 Mar 01
Exposure, Monitoring & Equipment Records	30 Apr 01	2 May 01
Physical Observations	11 Jun 01	11 Jun 01
Necropsy & GFAP Evaluations	28-29 Aug 01	29 Aug 01
GFAP Report	10 & 11 Jun 02	11 Jun 02

Fran Jannone

Fran Jannone, B.A., R.Q.A.P.
Quality Assurance Group Leader

18 Dec 08
Date

TABLE OF CONTENTS

Page
Summary 7
Introduction 7
Materials, Methods/Procedures 8
I. Basic Protocol 1: GFAP Sandwich ELISA
A. Introduction
B. Materials
C. Protocol Steps
1. Standard Curve Preparation
2. Sample Preparation
3. GFAP Assay
II. Support Protocol 1: Brain Tissue Preparation
A. Introduction
B. Materials
C. Protocol Steps
III. Support Protocol 2: Assay for Total Protein
A. Introduction
B. Materials
C. Protocol Steps
IV. Reagents and Solutions
V. General Commentary on GFAP Assays; Specific Commentary on the GFAP ELISA
A. Background Information
B. Critical Parameters
C. Trouble Shooting
D. Typical Results
E. Time Considerations
Statistics 17
Results and Conclusions 17
References 18
Figure Legends 21
Figure 1: Sample Standard Curve
Figure 2: µg GFAP/mg Total Protein per Brain Region
Tables 24
Table 1: GFAP Standard Curve Preparation
Table 2: Microtiter Plate Template
Table 3: Sample Values for GFAP Standard Curve
Table 4: Troubleshooting Guide
Table 5: Mean GFAP Levels in Specific Regions of First Generation Male Rat Brains Following a Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in a Two-Generation Reproduction Toxicity Study
Table 6: Mean GFAP Levels in Specific Regions of First Generation Female Rat Brains Following a Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in a Two-Generation Reproduction Toxicity
Table 7: Individual GFAP Levels in Specific Regions of First Generation Rat Brains Following a Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in a Two-Generation Reproduction Toxicity Study

Appendix A = Testing Facility Exposure and In-Life Data

Summary

Toxicant-induced injury of the adult or developing central nervous system of the rat results in hypertrophy of astrocytes at the site of injury. The hallmark of this response is the enhanced expression of the major intermediate filament protein of astrocytes, GFAP. Exposure to Baseline Gasoline Vapor Condensate, with the exception of a minor increase in Group III male hippocampus, did not consistently elevate GFAP levels in any of the nine brain regions examined in either males or females of 1st generation rat pups in a two-generation reproduction toxicity study. The data suggest that exposure to Baseline Gasoline Vapor Condensate under the regimen employed does not result in gliosis in any of the nine brain regions examined.

Introduction

A characteristic feature of chemical-induced damage of the nervous system is selectivity; exposure to different nervous system toxicants results in damage to different brain regions and cell types (Switzer, 1991; Balaban, 1992; O'Callaghan et al., 1995). The differential susceptibility of nervous system cell types to injury often is referred to as "selective vulnerability"(Spencer and Schuamburg, 1980; Baumgarten and Zimmerman, 1992). An implicit assumption underlying this concept is that intrinsic properties of individual neural cell types render them susceptible to damage by specific chemical exposures (Baumgarten and Zimmerman, 1992). Unfortunately, our knowledge of the mechanisms that confer such vulnerability to specific toxic insults is limited. Thus, often there is no *a priori* basis for predicting the cell types affected by toxic exposures of the nervous system. Given the extreme cellular and molecular heterogeneity of the nervous system (McKay and Hockfield, 1982; Sutcliffe, 1988), the fact that targets of chemical-induced neurotoxicity are diverse and unpredictable should not be surprising. This biologically-based situation does, however, make assessment of neurotoxicity difficult because one must face the problem of deciding where to look for damage. Overcoming this obstacle requires a "marker" of neural injury that can be used to localize (i.e. "mark") sites of damage anywhere in the nervous system.

A universal cellular reaction to damage of the central nervous system is hypertrophy of astrocytes. The hallmark of this response, often termed "reactive gliosis," is the enhanced expression of the major intermediate filament protein of astrocytes, glial fibrillary acidic protein (GFAP). Thus, an increase in the brain concentration of GFAP serves as a biochemical indicator of neurotoxicity. To validate the use of GFAP as a biomarker of neurotoxicity, prototype neurotoxicants have been administered to experimental animals and the effects of these agents on the tissue content of GFAP have been determined by immunoassay (O'Callaghan, 1991; Norton et al., 1992). Assays of GFAP were found to reveal dose-, time- and region-dependent patterns of neurotoxicity at toxicant dosages below those that cause light microscopic evidence of cell loss or damage (O'Callaghan, 1988; Norton et al., 1992). Moreover, the temporal and regional increments in GFAP correspond to the temporal and regional patterns of neuronal damage, as revealed by sensitive silver stains (Balaban, 1988). These findings indicate that assays of GFAP represent a sensitive, simple and quantitative approach for evaluation of nervous system damage (O'Callaghan, 1991; Norton et al., 1992).

As part of the U.S. Environmental Protection Agency's testing requirements under the Clean Air Act, identification and characterization of the potential adverse effects of gasoline and various oxygenate-gasoline blends is to be determined. Neurotoxicity

assessment constitutes a portion of these testing activities. Subchronic (13-week) inhalation exposures to gasoline and gasoline plus each of 6 fuel additives have been performed along with a two-generation reproduction toxicity study that includes a neurotoxicity component for gasoline and gasoline plus MTBE vapor condensates only. The purpose of the present study was to use the GFAP assay for assessing the potential neurotoxic effects of Baseline Gasoline Vapor Condensate on 1st generation rat pups at weaning (28 days after parturition) in a two-generation reproduction toxicity study. A control (air) and three exposure levels to the test condensate (2,000, 10,000 and 20,000 mg/m^3) were used. Although the EPA Guidelines (CFR 59, No. 122, 79.67, 1994) specify six regions to be analyzed, we expanded our analysis to include an additional three areas of the brain to maximize the potential for detecting enhanced expression of GFAP due to exposure to the test substance.

Materials, Methods/Procedures

STUDY DATES

STUDY INITIATION
24 April 2001 (Date Study Director signed the Protocol)

DATE OF ANIMAL RECEIPT
17 April 2001

EXPOSURE INITIATION
30 April 2001 (Experimental Start Date)

EXPOSURE TERMINATION
28 August 2001

TERMINAL SACRIFICE
29 August 2001

EXPERIMENTAL TERMINATION
Day Month Year (Date Final Report is signed by the Principal Investigator)

STUDY COMPLETION
Day Month Year (Date Final Report is signed by the Study Director)

I. Basic Protocol 1: GFAP Sandwich ELISA

A. **Introduction**: The GFAP sandwich ELISA is suitable for assaying the concentration of GFAP present in homogenates of brain tissue. This method has successfully been applied to analysis of at least the following species: mouse, rat, guinea pig, dog, monkey, man, chicken, pigeon, trout and cod. Because GFAP is evolutionarily conserved, it is likely that this assay can be very broadly applied across many species. The assay does not require preparation of any special materials or reagents; all components are available from commercial sources at modest cost. The 96-well microplate format lends itself to processing large numbers of samples and it makes the assay suitable for automation with a variety of liquid handling systems. It takes a minimum of 5 hours to process a single 96-well plate after preparation of the tissue homogenates (Support Protocol 1).

B. **Materials**:

Vortex Mixer or Ultrasonic Cell Disruptor (e.g. PGC cat. # 81-6721-

02, 2 mm probe)
Pipettes
Hot/Stir Plate
Microplate Reader
96-well Microtiter Plates (Immulon 2, Dynatech)
Pipette tips
Rabbit anti-Glial Fibrillary Acidic Protein Antibody (DAKO, Cat. #Z0334, Lot #096(401))
Non-Fat Dry Milk (Carnation)
Mouse anti-Glial Fibrillary Acidic Protein Antibody (Oncogene Research Products Cat. #IFO3L, Lot #D15158-3)
Phosphate Buffered Saline (e.g. Pierce Cat. #28374)
Alkaline Phosphatase conjugated anti-mouse IgG (Jackson Immuno Research Cat #315-055-003)
Alkaline Phosphatase Substrate Kit (e.g. Bio-Rad Cat. #172-1063)
Triton X-100 (e.g. Bio-Rad Cat. #161-0407)
Sodium Hydroxide (e.g., 0.4N NaOH, Fisher LC 243204)

C. Protocol Steps:

1. Standard Curve Preparation

1. Prepare a GFAP standard: The preferred standard consists of an aliquot of a brain homogenate prepared as described in Support Protocol 1. This standard should be prepared from the same species that was used to prepare the samples to be assayed for GFAP because GFAP immunoreactivity with a given set of immunodetection reagents will differ among different species. A large number of standards can be prepared in advance from a single "pool" of a 1% SDS homogenate prepared as described in Support Protocol 1. This homogenate can be aliquoted and stored frozen at -70°C prior to use. *Thus, the GFAP standard essentially consists of a control sample. This is preferable over using a pure GFAP standard because using control tissue as a standard obviates any influence of the tissue "matrix" on the assay performance.* To express the data in units of GFAP per unit of total protein, aliquots of a 1% SDS homogenate are still to be used as a GFAP standard. This is accomplished by "standardizing the standard" with addition of a known amount of pure GFAP to the 1% SDS homogenate (i.e. an internal standard). Immunoreactivity values generated from standard curves of the GFAP "spiked" homogenate and the homogenate alone then are used to determine the concentration of GFAP in homogenate. For analysis of GFAP in regions of rat brain, we routinely use aliquots of a hippocampal homogenate as a standard. It contains approximately 2.5 μg GFAP per mg of total protein. Other species (e.g. mouse) contain different levels of GFAP in hippocampus.
2. Prepare dilutions of the GFAP standard: Remove a tube of the GFAP standard from the freezer, thaw at room temperature and vortex or sonify prior to dilution. Using a rat hippocampal homogenate as a typical standard (~2.5 μg GFAP/mg total protein), use the total protein value for this homogenate (~ 10 mg/ml) to prepare a standard curve in PBS plus 0.5% Triton X-100. For rat hippocampal homogenate, the protein values for the standard curve should be between approximately 0.25 to 10 μg/100μl/microplate well (i.e. 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10μg total protein/100μl). Table 1 shows an example of dilutions

needed to prepare a standard curve from a homogenate of rat hippocampus (hippocampus std.). *Typically, standards are run in duplicate. Detergents act as wetting agents, therefore, more than a single use of a pipette tip with SDS- or Triton X-100-containing samples can lead to carry-over errors. Thus, it is recommended to use only a single pipette tip per sample and to withdraw the sample only a single time per tip.*

2. Sample Preparation

1. Thaw and mix samples: Remove samples from the freezer, thaw at room temperature and vortex or sonify prior to dilution.
2. Prepare dilutions of the samples: Dilute the samples in PBS + 0.5% Triton X-100 to a concentration of approximately 10μg total protein/ 100μl. Samples high in GFAP (e. g. cerebellum) may need to be diluted to a concentration of 5μg total protein/100μl of PBS + 0.5% Triton X-100. Samples low in GFAP (e.g. striatum) may need to be diluted to 20μg total protein/100μl of PBS + 0.5% Triton X-100. These dilution factors are determined empirically. *The best practice is to prepare multiple dilutions of each sample to insure that optical density readings for a given sample fall on the linear portion of the standard curve. Typically, samples (like standards) are run in duplicate. Detergents act as wetting agents, therefore, more than a single use of a pipette tip with SDS- or Triton-X-100-containing samples can lead to carry-over errors. Thus, it is recommended to use only a single pipette tip per sample and to withdraw the sample only a single time per tip.*

3. GFAP Assay

1. Coat Immulon-2 flat bottom plates with rabbit anti-GFAP. Add 1.0μg total immunoglobulin protein /100 μl PBS /well. (~25μl of anti-GFAP [Dako] in 10 ml of PBS is the quantity needed per plate).
2. Incubate the coated plate at 37°C for 1 hour. This step may be done at the beginning of the assay or it may be done the night before with storage overnight at 4°C. Perform all other incubation and reagent addition steps at room temperature.
3. Empty the plate into a sink and tap on absorbent paper to remove excess liquid. *This latter procedure is important to eliminate the possibility of any reagent carry-over between steps.*
4. Wash plates 4X with PBS (200 μl per well), tapping and blotting between each wash.
5. Block 1 hour with 5% non-fat powdered milk in PBS at 100 μl per well.
6. Empty plate, tap on absorbent paper (upside down) to remove excess liquid, load diluted standard curve and samples in a volume of 100 μl per well. Incubate for 1 hour. The template, Table 2, is an example of a typical 96-well microplate layout for GFAP standards and unknowns.
7. Wash 4X with PBS + 0.5% Triton X-100, 200 μl/well.
8. Incubate for 1 hour in monoclonal anti-GFAP (1:500)(Chemicon) + alkaline phosphatase conjugated anti-mouse IgG (1:3000)(Jackson ImmunoResearch) made up in 5% non-fat dry milk + 0.5% Triton X-100, 100 μl /well.
9. Wash 4X with PBS + 0.5% Triton X-100, 200 μl/well.
10. Add P-nitrophenylphosphate substrate (Bio-Rad) in a volume of 100 μl/well and incubate for 20 minutes.

11. Stop reaction with 0.4N NaOH, 100 µl/well.
12. "Pop" any bubbles in the plate wells with a needle or pipette tip to insure uniform and accurate readings of standard and sample ODs. Read plate at 405 nm.
13. Calculate the GFAP concentration in the samples by comparing their optical density (OD) values to those obtained for the linear portion of the GFAP standard curve. Software programs linked to specific plate readers should be programmed to plot OD vs. GFAP values in linear vs. log linear fashion. Typically, we utilize the 4-parameter curve fit equation and generate curves as shown in Fig. 1 and Table 3. Most programs allow for automatic subtraction of blanks and incorporation of dilution factors. Data are expressed as µg GFAP/per mg total protein or, if the absolute amount of GFAP in the standard is not available, data are expressed as GFAP-like immunoreactivity/per mg total protein. Total protein concentration in the samples is estimated from the total protein assay described in Support Protocol 2. Data also can be expressed on the basis of tissue wet weight (µg GFAP/gram wet weight or GFAP immunoreactivity/gram wet weight). Although this approach permits elimination of the total protein assay (Basic Support Protocol 2), we find that the GFAP values obtained are slightly more variable.

II. Support Protocol 1: Brain tissue preparation

A. Introduction: This support protocol describes the procedure for preparing brain tissue for subsequent analysis of GFAP by Sandwich ELISA (Basic Protocol 1). This procedure does not describe or recommend a specific approach for dissecting brain tissue. *It is noted, however, that reliable dissections are essential for obtaining reproducible results with the GFAP ELISA (see commentary).*

B. Materials:

Dissecting Instruments
Balance
Ultrasonic Cell Disruptor (e.g. PGC cat. # 81-6721-02, 2 mm probe)
Pipettes
Hot/Stir Plate
Pipette tips
Microfuge tubes

C. Protocol Steps:

1. Sacrifice animals and remove the brain as rapidly as possible.
2. Dissect brain regions. If a number of regions are to be dissected, this process can be aided by keeping the brain firm on a cold plate (e.g. Thermoelectrics cold plate, Aldrich Chemical Co. or simply an inverted petri dish placed on ice) maintained at approximately 4°C. Rat or mouse brains can be dissected into 10-15 regions, free hand, within approximately 10 minutes. For this study, nine regions were dissected: Striatum, Hippocampus, Cortex, Olfactory Bulb, Thalamus, Hypothalamus, Cerebellum, Pituitary, and Rest of Brain. *This number of regions can be prepared on a cold plate or at room temperature without degradation of GFAP as assessed by immunoblot analysis. All brain*

regions can be stored frozen indefinitely in capped microfuge tubes at this step in the protocol, or you can proceed to the next step.

3. Weigh and homogenize the dissected brain parts. Tare an appropriately labeled microfuge or other suitable storage tube, or weigh boat for the large brain areas that don't fit into microfuge tubes, ie; Cerebellum, Cortex, Rest of Brain and Thalamus). Place individual brain regions in the tube or weigh boat, obtain the weight, transfer large brain areas to large glass homogenization tube, and immerse the tissue in 10 volumes of hot (85-95°C) 1% (w/v) SDS. For example, 0.1 grams of tissue would be immersed in 1.0 ml of SDS. While the SDS is still hot, homogenize the tissue by sonification with an ultrasonification microprobe. Large brain areas are sonified in the glass homogenization vessel with the ultrasonification microprobe, then homogenized using a motor-driven Teflon pestle. Samples should be stored frozen (-70°C) at time of sacrifice prior to assay. *Samples prepared and stored in this manner retain their GFAP content for at least 5 years.*

III. Support Protocol 2: Assay for Total Protein

A. **Introduction**: This support protocol describes the procedure for assaying the concentration of total protein in the SDS-homogenates. The procedure described essentially is the bicinchoninic acid (BCA) method described by Smith et al. (1985) which is available in kit form (see materials). To assay total protein concentration of the SDS-homogenates the assay must be compatible with 1% SDS. Use of the BCA assay is not an absolute requirement as other detergent compatible methods are available (e.g. Bio-Rad DC protein assay). Bovine serum albumin is used as the protein standard in the described procedure. Other protein standards can be substituted.

B. Materials:

Microplate Reader
Pipettes
Pipette tips
96-well Microtiter Plates
Microfuge tubes
Incubator
Vortex Mixer
Miscellaneous Laboratory Glassware
BCA Protein Kit (Pierce #23223)
Bovine Serum Albumin (BSA) (Sigma A7888)
Sodium Dodecyl Sulfate (Bio-Rad #161-0302)

C. Protocol Steps:

1. Prepare total protein standards. Prepare a 1 mg/ml solution of BSA in 1% (w/v) SDS. *Aliquots of this standard can be stored frozen at -70°C for future use. Thaw as needed, but do not re-freeze.*
2. Prepare a total protein standard curve. Prepare dilutions of the BSA standard in 1% SDS as follows: 1.0, 2.5, 5.0, 7.5, and 10 μg/10μl of 1% SDS (no dilution is required for last standard). Vortex each tube and add 10 μl of each standard to

a well of the microtiter plate; add 10 µl of 1% SDS to serves as a blank. *Typically, standards are run in duplicate. Detergents act as wetting agents, therefore, more than a single use of a pipette tip with SDS-containing samples can lead to carry-over errors. Thus, it is recommended to use only a single pipette tip per sample and to withdraw the sample only a single time per tip.*

3. Prepare dilutions of the samples. Thaw the samples, vortex and dilute a 10 µl aliquot with 190 µl of 1% SDS. Vortex the dilution tube and add a 10 µl aliquot into a well of a microtiter plate.
4. Add the protein assay reagent. Add 200 µl of the BCA reagent (composed of 50:1 ratio of solution A: solution B of the Pierce BCA reagent) to each standard and sample.
5. Incubate the plate at 37°C for 30 minutes. *Other incubation temperatures are permissible; follow direction provided with the kit.*
6. "Pop" any bubbles in the microtiter plate wells with a needle or pipette tip to insure uniform and accurate readings and read the plate at 562 nm.
7. Calculate the concentration of total protein in the samples from the standard curve. Software programs linked to specific plate readers should be programmed to plot OD vs. total protein in a linear fashion. Most programs allow for automatic subtraction of blanks and incorporation of dilution factors. Because the samples were prepared in 10 volumes of diluent, typically, total protein values are approximately 10 mg/ml.

IV. Reagents and Solutions

1. **Phosphate Buffered Saline (PBS)**- One packet of PBS is mixed thoroughly with 500 ml of deionized water to give a final concentration of: 137 mM NaCl/1.0 mM KCl/2 mM KH_2PO_4/8.0 mM $Na_2HPO4{\cdot}7H_2O$/pH 7.4 (can be stored at 4°C for at least a month). For this and all subsequent reagents and solutions, determine the total volume that needs to be prepared based on the use of 100 µl/well and 96 wells per plate (washes take 200 µl/well).
2. **PBS+0.5% Triton X-100**- 2.5ml of Triton X-100 is added to 500 ml of PBS (can be stored at 4°C for at least a month).
3. **Blocking agent(BLOTTO)- (**PBS + 5% powdered milk or PBS + 0.5% Triton X-100 + 5% powdered milk). Five grams of non-fat powdered milk is added per 100 ml of PBS or per 100 ml of PBS + Triton X-100. Prepare at least 100 ml of each to facilitate dissolving the powdered milk; PBS may be warmed slightly to facilitate this process. Make these solutions up fresh the day of assay and do not save. Also, do not retain the powdered milk for greater than a month or two (room temperature). The dry milk tends to discolor and will not go into solution at shelf times longer than 2 months.
4. **Polyclonal anti-GFAP** (Dako)- Add 25µl of antibody solution /10ml of PBS. The assay is based on the use of this antibody as a "capture" reagent. Substitution of an antibody from another vendor may not yield suitable results. Make this solution fresh on the day of use and do not save.
5. **Monoclonal anti-GFAP** (Chemicon; formerly Boehringer Mannheim) **combined with Alkaline Phosphatase-conjugated anti-mouse IgG** (Jackson ImmunoResearch)- Make up a stock solution of monoclonal anti-GFAP and alkaline phosphatase-conjugated anti-mouse IgG as per the vendors' instructions. Store both stocks at 4°C as per the vendor's instructions. Add 20µl of the monoclonal antibody solution stock and 3.3 µl of the alkaline

phosphatase conjugate stock/10ml powdered milk +0.5% Triton X-100. The assay is based on the use of the monoclonal antibody as a "detection" reagent and the alkaline phosphatase conjugate to bind to the detection antibody and generate a colored reaction product proportional to the amount of antigen (GFAP) present in the samples. Substitution of antibodies from other vendors may not yield suitable results. Make these solutions fresh on the day of use and do not save.

6. **P-nitrophenylphosphate substrate** (BioRad)- Mix 2ml of diethanolamine buffer on a stirrer with 2 p-nitrophenylphosphate tablets and 8 ml of deionized water. Make this solution fresh on the day of use and do not save.

V: General Commentary on GFAP Assays; Specific Commentary on the GFAP ELISA

A. Background Information

It has long been known that damage to the central nervous system results in astrogliosis (gliosis, reactive gliosis, glial activation), a response to brain injury characterized by hypertrophy and, less often, hyperplasia of astrocytes, a sub-type of CNS glia (Eng, 1988; Norenberg, 1994). At the electron microscopic level, astrogliosis is characterized by the accumulation of glial filaments. GFAP was found to be the major protein component of these filaments (Eng, 1988). As such, GFAP serves as a biomarker for filament accumulation and, therefore, of gliosis (Eng, 1988; Norton et al., 1992; O'Callaghan, 1993). With the development of antibodies to GFAP, immunohistochemical analysis of this protein soon documented that gliosis occurs in response to diverse insults of the CNS, including trauma, disease, and toxic exposures (Eng, 1988; Norenberg, 1994; Norton et al., 1992; O'Callaghan, 1993; O'Callaghan et al., 1995). Thus, a large body of evidence now has been accumulated demonstrating the ubiquity of the glial response to all types of CNS damage based on immunohistochemistry of GFAP. Only recently, however, have methods been introduced to assay levels of GFAP as a means of quantifying gliosis.

While GFAP immunohistochemistry has proven useful for revealing patterns of gliosis after brain injury, this approach does not lend itself to quantification or the analysis of large numbers of samples. Small (25-50%), but toxicologically significant increases, also may be difficult to detect by immunohistochemistry. These drawbacks, combined with the need to develop quantitative biomarkers of neurotoxicity (O'Callaghan et al., 1995), and to define quantitative aspects of toxicant- and disease-induced gliosis, has prompted the development and implementation of a number of GFAP assays. These assays have been applied to examine gliosis in specific brain areas already known to be affected by disease or other insult. In addition, they also can be broadly applied in a risk assessment context (U.S. EPA) to screen for potential sites of neural damage resulting from toxic exposures of the CNS. Recently, analysis of GFAP has been used to demonstrate that the degree of cortical gliosis in postmortem brain tissue from victims of Alzheimer's disease correlates with the severity of dementia scores in these individuals prior to death (G. Webster Ross, submitted). Analysis of GFAP in cerebrospinal fluid (CSF) also has been applied to the human condition as an indicator of the severity of traumatic injury to the brain (Rosengren et al., 1994). Finally, analysis of GFAP can be used as an indicator of the presence of brain or spinal cord contamination of meat (Schmidt et al., 1999).

Of the number of GFAP assays that have appeared in the literature over the last 15 years, all essentially fall into two categories: 1) solid phase immunoassays where GFAP is

immobilized on a solid support matrix and detected by mono- or polyclonal antibodies or 2) liquid-phase assays where GFAP from brain extracts or solubilized brain tissue (or CSF) is "captured" by one antibody and then detected by another antibody raised in a different host species (Butler et al., 1986). The assay described in this study is of the second type and it has a number of advantages over the solid phase assays. Specifically, solid-phase detection and "quantification" of GFAP most commonly involves the time-consuming resolution of a protein mixture by SDS-PAGE, followed by electrophoretic transfer to a solid support membrane. Anti-GFAP antibodies coupled to a variety of detection reagents then can be used for quantification of GFAP bound to the membranes. Unfortunately, this approach has been found to severely underestimate the concentration of GFAP in the resolved mixture of proteins and the effects of treatments known to increase GFAP (O'Callaghan et al., 1999). Other solid phase assays for GFAP have been developed that do not rely on prior resolution of protein mixtures by SDS-PAGE (Wang et al., 1990; O'Callaghan, 1991b). These assays incorporate manual spotting of brain homogenates on solid supports, with or without the aid of a template. The membranes are then incubated with anti-GFAP polyclonal or monoclonal antibodies, which, in turn, are bound by ^{125}I Protein A. Quantification is achieved by gamma spectrometry or by densitometry of the autoradiographs. These assays give a linear signal over a fairly large range of spotted protein. However, they require large amounts of reagents, including radiolabeled reagents, and they do not have impressive throughput.

The sandwich ELISA for GFAP described in this study or similar ELISAs described previously (Eng et al., 1986; Kretzschmar et al., 1985; O'Callaghan, 1991b; Rosengren et al., 1994), have several advantages in comparison to the other methods for assaying GFAP described above. They are easier to perform because they have fewer steps. They are more sensitive. Although they may require a greater number of reagents, ELISAs adapted to the microplate format permit the use of very small volumes, which results in a significant overall cost reduction. The 96-well microplate format also has the advantage of speed and high throughput. From sample application to data collection, all steps can be performed in the plate. Moreover, the microplate-based format permits the entire assay to be automated through the use of robotic liquid handling processors. Finally, radioactivity is not involved, making the assay safer to perform and allowing the user to avoid costly and time-consuming radioactivity disposal procedures. While most of the GFAP sandwich ELISAs described to date are similar and share the advantages afforded by this technique, the assay described in this study may have a few additional advantages. Because it is based on detergent-solubilized homogenates of a given brain area, any treatment effects can be directly related to effects in that brain area, rather than an arbitrarily defined extract or sub-fraction that may contain only a portion of the total GFAP in that area. Using a solubilized homogenate rather than a subfraction of a given brain area also facilitates comparisons of quantitative data on GFAP to immunohistochemical staining of GFAP in that area. Moreover, it also helps rule out inter-laboratory differences associated with assaying GFAP content in one type of extract in one lab, and another type of extract/fraction in another lab. Finally, the same SDS-denatured homogenate used to assay GFAP can be subjected to multiple assays for additional glial or neuronal proteins, thereby permitting comparisons to be made among multiple markers of neurotoxicity in a single sample. For example, the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, causes a large increase in GFAP that results from damage to dopaminergic nerve terminals, damage that can be quantified by immunoassay of tyrosine hydroxylase (TH), a marker of dopamine containing nerve terminals in the target region (O'Callaghan et al., 1990). Both markers can be assayed from aliquots of the same tissue

sample and, on this basis, we find that larger decreases in TH predict greater increases in GFAP.

B. Critical Parameters

The most critical aspect of the GFAP assay is the absolute requirement for preparation of consistently dissected regions of the brain (see Support Protocol 1). Consistent dissections yield consistent GFAP values with the use of only a few animals per dose or time point (see Anticipated Results). The particular regions to be dissected depend on the questions being addressed. If a target region is known or suspected, dissections can be limited to the region of interest. If the GFAP assay is being applied in a screening context, multiple (10-15) brain regions must be dissected in order to avoid the possibility of diluting localized increases in GFAP. The possibility exists that extremely localized increases in GFAP may fail to be detected with the assay. While GFAP immunohistochemistry is relatively insensitive in comparison to the GFAP assay, and it may not detect small increases in GFAP, it can reveal small "hot spots" of gliosis (e.g. see effects of MK-801) (Fix et al., 1995). Such discrete astrocytic responses could escape quantification with the GFAP assay due to dilution of signal by surrounding tissue. No one approach can be broadly applied to detect all toxicant-induced damage of the CNS. Therefore, it is prudent to use the GFAP ELISA in conjunction with GFAP immunohistochemistry and other sensitive morphological approaches for detection of neural damage, such as silver degeneration stains (Switzer, 2000), Fluoro-Jade (Schmued et al., 2000), and stains that detect activated microglia (Streit et al., 1999).

In terms of the GFAP ELISA itself, the key requirements for optimal performance of the assay include: 1) use of the specified antibodies, 2) addition of the reagents at room temperature, 3) fresh (daily) preparation of all reagents containing antibodies and/or non-fat dry milk and 4) mixing of the standards and samples prior to their dilution or addition to the microplate wells. A troubleshooting guide is provided in Table 4 that covers most problems encountered with the assay.

C. Troubleshooting (see Table 4)

D. Typical Results

Typical GFAP assay values obtained for different regions of the rat brain are presented in Fig. 2 (i.e. historical data). Absolute values for GFAP (μg/mg total protein) may vary depending on the GFAP standard used and the species subjected to evaluation. Region-to-region differences in GFAP values from untreated animals of a given species, however, should remain stable, if consistent dissections are performed.

E. Time Considerations

The GFAP assay requires approximately 5 hours for one person to process a 96-well microplate. The time required to prepare brain samples depends on the number of brain areas to be dissected and on whether the areas are stored frozen prior to homogenization With practice, 10 brain areas can be prepared (and homogenized) from 50 rats in a day. Two people are required: one to dissect the brains and one (or more) to weigh and homogenize the tissue. The total protein assay requires approximately 1 hour for one person to process a 96-well microplate.

Statistics:

The effect of treatment on GFAP concentration was determined by separate one-way ANOVAs for each of the nine brain areas from males and females utilizing the JMP® statistical package (SAS, 1995). The significance level was set a $P<0.05$ and, to ensure detection of between group treatment effects, The Least Significant-Difference test (Keppel, 1973) was used in *post-hoc* analyses.

Results and Conclusions

The results of the GFAP analysis are presented in Tables 5 and 6. Results are reported according to gender, because sex-dependent responses to toxic substances are not uncommon, including responses reflected in levels of GFAP. Control levels for GFAP varied markedly according to brain region, consistent with known historical levels observed for GFAP across different brain regions (see Fig. 2). Exposure to Baseline Gasoline Vapor Condensate only caused a slight elevation in levels of GFAP in Group III male hippocampus of 1st generation rat pups in a two-generation reproduction toxicity study. This one minor increase was not dose-related and likely is a result of variability in the dissection procedure. These data suggest that under the exposure conditions employed, damage-induced gliosis did not occur to a significant extent in the brain regions examined. Toxicant-induced gliosis is highly dose-, region-, and time-dependent. It is possible, therefore, that exposure to higher concentrations, examination of more brain regions, examination of more discrete dissections of a given brain region, or the inclusion of more time points, might have resulted in positive findings. Nevertheless, it is remarkable that a brain-wide survey of GFAP revealed almost no increases from baseline in any of the examined regions. A significant decrease in GFAP levels was observed in the **thalamus** of the Group IV males. This isolated effect likely is due to the difficulty of obtaining reliable dissections of this region. Regardless of its statistical significance no adverse biological significance can be assigned to a GFAP *decrease* at the present time. In conclusion, exposure to Baseline Gasoline Vapor Condensate did not result in gliosis in nine representative brain regions in males or females of 1st generation rat pups in a two-generation reproduction toxicity study.

References

Balaban, C.D. 1992. The use of selective silver degeneration stains in neurotoxicology: Lessons from studies of selective neurotoxicants. In: The Vulnerable Brain and Environmental Risks, Vol 1, Malnutrition and Hazard Assessment. R.L. Issacson and K.F. Jensen. Eds.: 223-238, Plenum. New York, NY.

Baumgarten, H.G.& B. Zimmermann. 1992. Cellular and subcellular targets of neurotoxins: the concept of selective vulnerability. In: Handbook of Experimental Pharmacology, Vol. 102 Selective Neurotoxicity. H. Herken and F. Hucho. Eds., pp. 1-28. Springer-Verlag. Heidelberg.

Butler, J.E., Spradling, J.E., Suter, M., Dierks, S.E., Heyermann, H., and Peterman, J.H. 1986. The immunochemistry of sandwich ELISAs-I. The binding characteristic of immunoglobulins to monoclonal and polyclonal capture antibodies adsorbed on plastic and their detection by symmetrical and asymmetrical antibody-enzyme conjugates. *Mol. Immunol.* 23: 971982.

Eng, L.F., Stöcklin, E., Lee, Y.-L. Shiurba, R.A., Coria, F., Halks-Miller, M. Mozsgai, C., Fukayama, G. and Gibbs, M. 1986. Astrocyte culture on nitrocellulose membranes and plastic: Detection of cytoskeletal proteins and mRNAs by immunocytochemistry and in situ hybridization. *J. Neurosci. Res.* 16: 239-250.

Eng, L.F. 1988. Regulation of glial intermediate filaments in astrogliosis. *In* Biochemical Pathology of Astrocytes. (M.D. Norenberg, L. Hertz & A. Schousboe, eds.) pp. 79-90. A.R. Liss, New York.

Fix, A.S., Wightman, K.A. and O'Callaghan, J.P. 1995. Reactive gliosis induced by MK-801 in the rat posterior cingulate/retrosplenial cortex: GFAP evaluation by sandwich ELISA and immunocytochemistry. *Neurotoxicology* 16: 229-237.

Keppel, G. Design and Analysis: A Researcher's Handbook, Prentice-Hall, Inc., Englewood Cliffs, N.J., 1973.

Kretzschmar, H.A., DeArmond, S.J., Forno, L.S. 1985. Measurement of GFAP in hepatic encephalopathy by ELISA and transblots. *J. Neuropathol. Exp. Neurol* 44: 459-471.

McKay, R.D.G. and S.J. Hockfield. 1982. Monoclonal antibodies distinguish antigenically discrete neuronal types in the vertebrate central nervous system. *Proc. Natl. Acad. Sci. USA* 79: 6747-6751.

Martin, P.M. and O'Callaghan, J.P. (1995). Biochemical immunohistology. In: Central Nervous System Trauma: Research Techniques, S.T. Ohnishi and T. Ohnishi, eds., pp. 509-516, CRC Press, Boca Raton.

Norenberg, M.D. 1994. Astrocyte responses to CNS injury. *J. Neuropathol. Exp. Neurol* 53: 213-220.

Norton, W.T., Aquino, D.A., Hozumi, I., Chiu, F.-C., and Brosnan, C.F. 1992. Quantitative aspects of reactive gliosis: A review. *Neurochem. Res.* 17: 877-885.

O'Callaghan, J.P. 1988. Neurotypic and gliotypic proteins as biochemical markers of neurotoxicity. *Neurotoxicol. Teratol.* 10: 445-452.

O'Callaghan, J.P. 1991. Quantification of glial fibrillary acidic protein: Comparison of slot-immunobinding assays with a novel sandwich ELISA. *Neurotoxicol. Teratol.* 13: 275-281.

O'Callaghan, J.P. 1993. Quantitative features of reactive gliosis following toxicant-induced damage of the CNS. *Ann. NY Acad. Sci.* 679: 195-210.

O'Callaghan, J.P. 1999. Quantitative immunoblots of proteins resolved from brain homogenates: Underestimation of specific protein concentration and of treatment effects. *Anal. Biochem.* 274: 18-26.

O'Callaghan, J.P., Miller, D.B. and Reinhard, J.F., Jr. 1990. Characterization of the origins of astrocyte response to injury using the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine. *Brain Res.* 521: 73-80.

O'Callaghan, J.P., Jensen, K.F. and Miller, D.B. 1995. Quantitative aspects of drug- and toxicant-induced astrogliosis. *Neurochem. Int.* 26: 115-124.

Rosengren, L.E., Wikkelsø, C. and Hagberg, L. 1994. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of adults. *J. Neurosci. Methods* 51: 197-204.

SAS Institute, Inc., JMP, Statistics and Graphics Guide, Version 3, 1995.

Schmidt, G.R., Hossner, K.L., Yemm, R.S., Gould, D.H. and O'Callaghan, J.P. 1999. An enzyme-linked immunosorbent assay for glial fibrillary acidic protein as an indicator of the presence of brain or spinal cored in meat. *J. Food Protect.* 62: 394-397.

Schmued, L.C. and Hopkins, K.J. 2000. Fluoro-Jade: novel fluorochromes for detecting toxicant-induced neuronal degeneration. *Toxicol. Pathol.* 28: 91-99.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. Klenk, D.C. 1985. Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150: 76-85.

Spencer, P.S. and H.H. Schaumburg. 1999. Experimental and Clinical Neurotoxicology, 2nd Edition, Oxford Unviversity Press, Oxford, Cary, NC

Streit, W.J. , Walter, S.A., and Pennell, N.A. 1999. Reactive microgliosis. *Prog. Neurobiol,* 57: 563-581.

Sutcliffe, J.G. 1988. mRNA in the mammalian central nervous system. *Ann. Rev. Neurosci.* 11: 157-198.

Switzer, R.C. 1991. Strategies for Assessing Neurotoxicity. *Neurosci. Biobehav. Rev.* 15: 89-93.

Switzer, R.C. 2000. Application of silver degeneration stains for neurotoxicity testing. *Toxicol. Pathol.* 28: 70-83.

U.S. Environmental Protection Agency, Pesticide Assessment Guidelines, Subdivision F. Hazard Evaluation: Human and Domestic Animals. Addendum 10, Series 81, 82, 83, Neurotoxicity. EPA-540/09-91-123, Springfield, VA: National Technical Information Service.

Wang, S., Rosengren, L.E., Karlsson, J.-E., Stigbrand, T. and Haglid, K.G. 1990. A simple quantitative dot-immunobinding assay for glial and neuronal marker proteins in SDS-solubilized brain tissue extracts. *J. Neurosci. Methods* 33: 219-227.

Figure Legends

Figure 1. Sample GFAP standard curve. GFAP values in nanograms correspond to levels found in .25 –10 μg total hippocampal homogenate protein. Dilutions of this homogenate were used to construct the GFAP standard curve shown.

Figure 2. Levels of GFAP found in different regions of untreated rat brain OB, olfactory bulbs; Str, striatum; Hip, hippocampus; Hypo, hypothalamus; Ctx, cortex; Cbm, cerebellum; BS, brain stem. Values are mean ± SEM. Adapted from Martin and O'Callaghan, 1995.

Figure 1

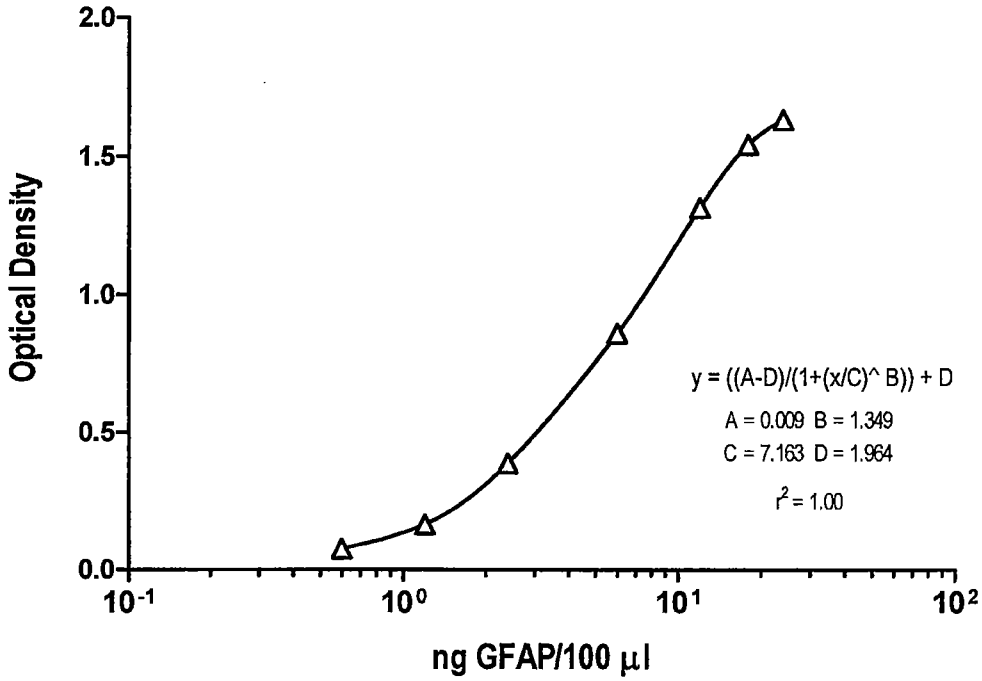
2.0
1.5
1.0
0.5
0.0
Optical Density
y = ((A-D)/(1+(x/C)^ B)) + D
A = 0.009 B = 1.349
C = 7.163 D = 1.964
r2 = 1.00
10-1
100
101
102
ng GFAP/100 µl

Figure 2

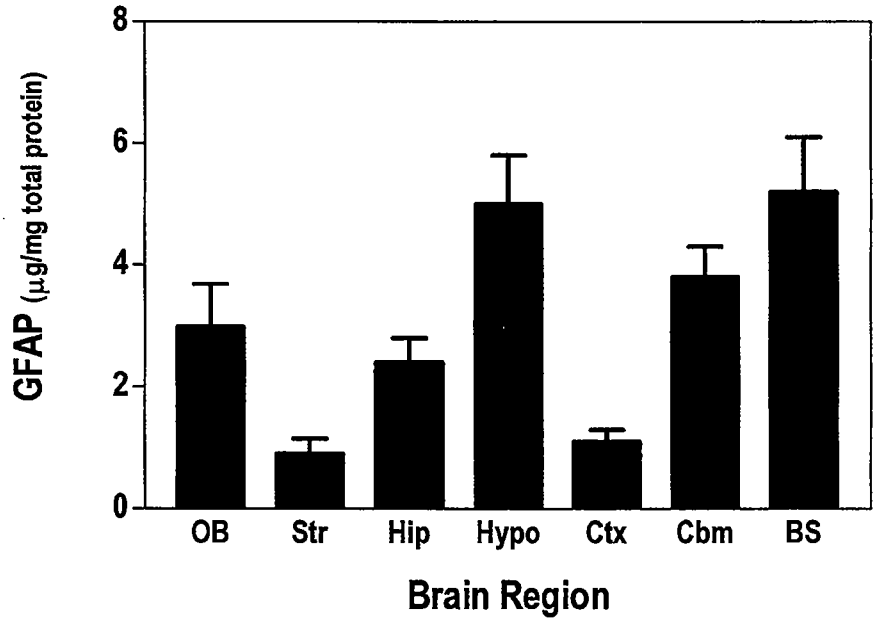
GFAP (μg/mg total protein)
8
6
4
2
0
OB
Str
Hip
Hypo
Ctx
Cbm
BS
Brain Region

TABLE 1: GFAP Standard Curve Preparation

Tube #	µg of total protein/100µl/well (ng of GFAP)	µl of Hippo-campus Std (10.34 mg/ml)	Serial Dilution	µl of PBS+ 0.5% Triton X-100
1	10 µg (25.00ng)	29 µl		2971 µl
2	7.5 µg (18.75ng)		2063 µl from tube #1	687 µl
3	5.0 µg (12.50ng		1833 µl from tube #2	917 µl
4	2.5 µg (6.25ng)		1000 µl from tube #3	1000 µl
5	1.0 µg (2.50ng)		800 µl from tube #4	1200 µl
6	0.5 µg (1.25ng)		1000 µl from tube #5	1000 µl
7	0.25 µg (0.625ng)		700µl from tube #6	700 µl

Table 2: Microtiter Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	Blk	? 1	? 1	? 9	? 9	? 17	? 17	? 25	? 25	? 33	? 33
B	Std 1	Std 1	? 2	? 2	? 10	? 10	? 18	? 18	? 26	? 26	? 34	? 34
C	Std 2	Std 2	? 3	? 3	? 11	? 11	? 19	? 19	? 27	? 27	? 35	? 35
D	Std 3	Std 3	? 4	? 4	? 12	? 12	? 20	? 20	? 28	? 28	? 36	? 36
E	Std 4	Std 4	? 5	? 5	? 13	? 13	? 21	? 21	? 29	? 29	? 37	? 37
F	Std 5	Std 5	? 6	? 6	? 14	? 14	? 22	? 22	? 30	? 30	? 38	? 38
G	Std 6	Std 6	? 7	? 7	? 15	? 15	? 23	? 23	? 31	? 31	? 39	? 39
H	Std 7	Std 7	? 8	? 8	? 16	? 16	? 24	? 24	? 32	? 32	? 40	? 40

Blk= Blank; Std= Standard; ? = Unknowns

Table 3: Sample Values for GFAP Standard Curve

PLATE BLANK	Well	OD	Mean	Std Dev	CV
BL	A1	0.001	0.0	0.001	0.0
	A2	-0.001			

STANDARDS µg total protein (ng GFAP)	Value	Well	OD	Mean	Std Dev	CV
STD01	10µg (25.00ng)	B1	1.694	1.632	0.087	5.4
		B2	1.570			
STD02	7.5 µg (18.75ng)	C1	1.537	1.543	0.008	0.5
		C2	1.549			
STD03	5.0µg (12.50ng)	D1	1.295	1.314	0.027	2.1
		D2	1.334			
STD04	2.5µg (6.25ng)	E1	0.857	0.859	0.002	0.3
		E2	0.861			
STD05	1.0 µg (2.50ng)	F1	0.386	0.386	0.000	0.1
		F2	0.386			
STD06	0.5 µg (1.25ng)	G1	0.175	0.164	0.016	10.0
		G2	0.152			
STD07	0.25 µg (.625ng)	H1	0.072	0.076	0.006	7.6
		H2	0.080			

BL=Blank; STD=Standard; OD=Optical Density; Std Dev=Standard Deviation; CV=Coefficient of Variation

Table 4: Troubleshooting Guide

Problem	Possible Cause	Solution
No color reaction	Incorrect preparation of color reagent	If color reaction has not been terminated, remove reagent, add new color reagent and continue assay
	Antibody was not as specified in the protocol	Obtain correct antibody and repeat assay
	One or more antibodies were omitted or used at the wrong dilution	Repeat assay with proper reagents used at the correct dilutions
Color reaction abnormally low	Incubator was set at less that 37°C	Repeat assay with incubator temperature set at 37°C
	Antibody solution too dilute; incorrect preparation of color reagent	Repeat assay with correct reagent dilutions
Color reaction abnormally high	P-nitrophenylphosphate substrate kit is too old	Repeat assay with fresh kit
	Color reaction was not terminated	Repeat assay and terminate reaction with 0.4 N NaOH
Standard curve not sigmoid	Incorrect plate template set in the plate reader	Use correct plate template and re-read plate
	Incorrect standard dilution	Repeat assay with correct standard dilution
Samples not on linear portion of curve	Incorrect standard dilution	Repeat assay with correct dilution of standard
	Incorrect sample dilution	Run multiple dilutions of samples to obtain OD values from the linear portion of the curve
Duplicates are not similar	Carry over from using same tip	Change tips after each use
	Poor pipetting technique	Check precision by weighing
	Plate washer malfunction	Check plate washer for even dispensing and aspiration
	Bubbles throughout the plate	Pop bubbles and re-read plate
Color reaction obtained for standards and samples, but OD values not as expected	Plate read at incorrect wavelength	Read plate at 405 nm

Table 5: Mean GFAP Levels in Specific Regions of First Generation Male Rat Brains Following a Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in a Two-Generation Reproduction Toxicity Study

Brain Area	Group I Air Control 0 mg/m^3	Group II Test Substance 2,000 mg/m^3	Group III Test Substance 10,000 mg/m^3	Group IV Test Substance 20,000 mg/m^3
Striatum	0.33 ± 0.01*	0.38 ± 0.02	0.40 ± 0.04	0.33 ± 0.05
Hippocampus	2.01 ± 0.14	2.36 ± 0.22	2.48 ± 0.20	1.75 ± 0.20
Cortex	0.61 ± 0.05	0.72 ± 0.08	0.76 ± 0.07	0.54 ± 0.07
Olfactory Bulb	1.34 ± 0.08	1.19 ± 0.07	1.28 ± 0.08	1.13 ± 0.09
Thalamus	0.86 ± 0.07	0.88 ± 0.07	0.94 ± 0.07	0.65 ± 0.05+
Hypothalamus	1.81 ± 0.18	1.99 ± 0.20	1.73 ± 0.14	1.36 ± 0.16
Cerebellum	3.04 ± 0.07	3.34 ± 0.26	3.13 ± 0.18	2.47 ± 0.27
Rest of Brain	2.56 ± 0.25	3.00 ± 0.22	3.07 ± 0.27	2.41 ± 0.39

*Each value represents the mean ± SEM (Standard Error of Mean) for the concentration of GFAP (μg/mg Total Protein) n= 5; see Results and Conclusion and Table 7
+Statistically different from Air Control, P<0.05

Table 6: Mean GFAP Levels in Specific Regions of First Generation Female Rat Brains Following a Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in a Two-Generation Reproduction Toxicity Study

Brain Area	Group I Air Control 0 mg/m^3	Group II Test Substance 2,000 mg/m^3	Group III Test Substance 10,000 mg/m^3	Group IV Test Substance 20,000 mg/m^3
Striatum	0.34 ± 0.04*	0.42 ± 0.02	0.37 ± 0.04	0.35 ± 0..03
Hippocampus	2.04 ± 0.09	2.18 ± 0.09	2.26 ± 0.14	2.06 ± 0.09
Cortex	0.60 ± 0.02	0.67 ± 0.04	0.69 ± 0.04	0.63 ± 0.07
Olfactory Bulb	1.29 ± 0.11	1.37 ± 0.11	1.22 ± 0.09	1.12 ± 0.07
Thalamus	0.77 ± 0.03	0.86 ± 0.06	0.93 ± 0.06	0.73 ± 0.07
Hypothalamus	1.89 ± 0.12	1.68 ± 0.09	1.75 ± 0.18	1.68 ± 0.20
Cerebellum	2.84 ± 0.18	3.42 ± 0.30	2.94 ± 0.23	2.44 ± 0.27
Rest of Brain	2.96 ± 0.52	2.94 ± 0.20	3.32 ± 0.42	2.64 ± 0.22

*Each value represents the mean ± SEM (Standard Error of Mean) for the concentration of GFAP (μg/mg Total Protein) n= 5; see Results and Conclusion and Table 7

Table 7: Individual GFAP Levels in Specific Regions of First Generation Rat Brains Following a Whole-Body Inhalation Exposure to Baseline Gasoline Vapor Condensate in a Two-Generation Reproduction Toxicity Study

Group	Animal no.	Sex	Striatum	Hippocampus	Cortex	Olfactory Bulb	Thalamus	Hypothalamus	Cerebellum	Pituitary	Rest of Brain
Group I Air Control 0 mg/m3	1508-3	Male	0.326•	1.566	0.483	1.493	0.687	1.360	3.042	0.054	2.602
	1523-5		0.338	2.429	0.756	1.306	0.870	2.020	3.158	*	2.752
	1514-3		0.293	1.935	0.566	1.244	0.767	1.492	2.846	*	2.170
	1508-4		0.366	2.135	0.652	1.553	1.119	2.345	2.964	*	3.376
	1525-8		0.318	1.967	0.565	1.117	0.837	1.826	3.207	0.087	1.906
	1525-16	Female	0.325	1.877	0.602	1.316	0.827	1.874	3.141	*	2.824
	1514-11		0.307	1.993	0.547	1.684	0.710	1.903	2.539	*	2.133
	1508-10		0.351	2.189	0.623	1.220	0.735	2.190	2.398	0.121	2.918
	1508-6		0.254	1.847	0.568	1.114	0.749	1.451	2.802	*	2.002
	1523-9		0.473	2.313	0.634	1.106	0.835	2.042	3.334	0.060	4.917
Group II Test Substance 2,000 mg/m3	2501-3	Male	0.424	3.167	0.998	1.277	1.079	2.619	3.318	*	3.306
	2519-2		0.363	2.374	0.670	1.271	0.865	1.889	2.747	0.044	3.122
	2513-2		0.415	2.341	0.782	1.165	0.926	2.226	3.946	*	3.043
	2502-7		0.377	1.996	0.679	1.302	0.893	1.695	3.914	0.075	3.376
	2508-5		0.328	1.918	0.484	0.925	0.633	1.507	2.776	0.198	2.164
	2513-14	Female	0.434	2.281	0.711	1.399	0.968	1.595	3.253	*	3.046
	2508-7		0.469	2.326	0.630	1.675	0.901	1.930	4.520	*	3.645
	2501-7		0.430	2.229	0.821	1.497	0.871	1.525	3.330	*	2.781
	2519-12		0.330	1.836	0.598	1.049	0.924	1.468	3.285	*	2.787
	2502-8		0.413	2.251	0.612	1.206	0.621	1.863	2.687	*	2.415
Group III Test Substance 10,000 mg/m3	3526-3	Male	0.412	2.651	0.856	1.400	1.118	1.909	2.872	0.117	3.772
	3512-1		0.292	1.768	0.537	1.116	0.722	1.718	2.628	*	2.101
	3506-8		0.396	2.565	0.679	1.500	0.860	2.146	3.609	*	3.215
	3513-2		0.377	2.449	0.946	1.111	1.045	1.433	3.431	*	3.143
	3525-1		0.522	2.960	0.773	1.275	0.947	1.459	3.120	*	3.127
	3506-13	Female	0.479	1.994	0.627	1.130	0.920	1.492	2.631	0.048	2.702
	3526-13		0.402	2.739	0.847	1.570	1.135	1.978	3.746	*	4.845
	3513-11		0.341	2.357	0.715	1.157	0.859	2.223	2.702	0.055	3.028
	3525-10		0.406	2.277	0.670	1.229	0.900	1.207	3.174	*	3.533
	3512-9		0.242	1.948	0.600	1.024	0.819	1.844	2.451	*	2.478
Group IV Test Substance 20,000 mg/m3	4520-4	Male	0.339	1.625	0.431	1.080	0.640	1.252	2.226	*	1.951
	4506-1		0.376	2.333	0.666	1.279	0.759	1.441	3.304	0.065	3.938
	4524-3		0.146	1.164	0.362	0.801	0.493	0.968	1.812	*	1.723
	4505-7		0.366	1.580	0.517	1.272	0.637	1.204	2.182	*	2.205
	4513-2		0.414	2.024	0.703	1.198	0.733	1.944	2.836	*	2.233
	4506-14	Female	0.460	2.364	0.882	1.151	0.843	1.377	3.106	0.052	2.664
	4520-11		0.320	2.004	0.549	1.210	0.632	1.340	1.520	*	2.466
	4524-8		0.331	2.152	0.590	0.925	0.642	1.975	2.378	*	2.035
	4513-13		0.390	1.940	0.647	1.302	0.936	2.304	2.842	*	3.386
	4505-12		0.258	1.844	0.485	1.004	0.617	1.403	2.372	*	2.642

• Each value represents the concentration of GFAP (ug/mg Total Protein)

* Pituitary samples too dilute to detect GFAP; therefore, not of value and not further summarized or discussed.

	Animal Exposure and Animal Data Preface	Appendix A

INTRODUCTION: The following is data generated at Huntingdon Life Sciences, East Millstone, NJ. The separately issued main study report should be referenced for details of the procedures used for test atmosphere generation/characterization and animal evaluations.

STUDY DATES:	Date of Parental Animal Receipt:	17 April 2001
	Experimental Initiation Date:	30 April 2001 (in-life)
	Experimental Completion Date:	29 August 2001 (in-life)
	Draft Report Date:	28 June 2002

EXPOSURES AND IN-LIFE SUMMARY: The actual measured results during the exposures of the parental animals (before, during and after gestation, until the day before pups were sacrificed) were comparable to the targeted exposure levels. There were no exposure-related effects seen in the pups with regards to clinical observations or body weights.

TABLE OF CONTENTS

TABLES

A. Chamber Monitoring Results 1486
B. Individual Pup Clinical Observations 1511
C. Individual Pup Body Weights (grams) 1513
D. Individual Pup Termination History 1515

	GFAP Report	Table A

Chamber Monitoring Records
Preface

Key to Abbreviations

MMAD	=	Mass Median Aerodynamic Diameter
GSD	=	Geometric Standard Deviation
TMC	=	Total Mass Concentration

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group I - 0 mg/m^3 (Air Control)

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
0	30-Apr-01	1	0	0	0	0	0	0				25	33
1	1-May-01	2	0	0	0	0	0	0	1.8340	1.698	2.06E-03	25	36
2	2-May-01	3	0	0	0	0	0	0				25	43
3	3-May-01	4	0	0	0	0	0	0				24	44
4	4-May-01	5	0	0	0	0	0	0				24	51
5	5-May-01	6	0	0	0	0	0	0				24	55
6	6-May-01	7	0	0	0	0	0	0				22	36
7	7-May-01	8	0	0	0	0	0	0				25	40
8	8-May-01	9	0	0	0	0	0	0	12.4800	2.969	7.74E-03	25	42
9	9-May-01	10	0	0	0	0	0	0				25	44
10	10-May-01	11	0	0	0	0	0	0				25	44
11	11-May-01	12	0	0	0	0	0	0				25	45
12	12-May-01	13	0	0	0	0	0	0				24	49
13	13-May-01	14	0	0	0	0	0	0				25	38
14	14-May-01	15	0	0	0	0	0	0				26	75
15	15-May-01	16	0	0	0	0	0	0	3.4180	1.773	3.27E-03	25	37
16	16-May-01	17	0	0	0	0	0	0				25	38
17	17-May-01	18	0	0	0	0	0	0				25	41
18	18-May-01	19	0	0	0	0	0	0				25	46
19	19-May-01	20	0	0	0	0	0	0				25	48
20	20-May-01	21	0	0	0	0	0	0				25	47
21	21-May-01	22	0	0	0	0	0	0				25	47

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group I - 0 mg/m^3 (Air Control)

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment Mean: Temperature (°C)	Humidity (%)
22	22-May-01	23	0	0	0	0	0	0	1.9910	2.026	1.29E-03	29	49
23	23-May-01	24	0	0	0	0	0	0				26	53
24	24-May-01	25	0	0	0	0	0	0				25	50
25	25-May-01	26	0	0	0	0	0	0				25	48
26	26-May-01	27	0	0	0	0	0	0				25	50
27	27-May-01	28	0	0	0	0	0	0				25	54
28	28-May-01	29	0	0	0	0	0	0				25	50
29	29-May-01	30	0	0	0	0	0	0	8.7220	3.265	2.60E-03	25	46
30	30-May-01	31	0	0	0	0	0	0				26	35
31	31-May-01	32	0	0	0	0	0	0				25	34
32	1-Jun-01	33	0	0	0	0	0	0				26	42
33	2-Jun-01	34	0	0	0	0	0	0				26	50
34	3-Jun-01	35	0	0	0	0	0	0				26	52
35	4-Jun-01	36	0	0	0	0	0	0				26	45
36	5-Jun-01	37	0	0	0	0	0	0	1.1450	1.803	1.96E-03	25	48
37	6-Jun-01	38	0	0	0	0	0	0				25	50
38	7-Jun-01	39	0	0	0	0	0	0				25	44
39	8-Jun-01	40	0	0	0	0	0	0				25	42
40	9-Jun-01	41	0	0	0	0	0	0				25	40
41	10-Jun-01	42	0	0	0	0	0	0				25	41

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group I - 0 mg/m^3 (Air Control)

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
42	11-Jun-01	43	0	0	0	0	0	0				26	48
43	12-Jun-01	44	0	0	0	0	0	0	0.9046	1.896	1.70E-03	26	52
44	13-Jun-01	45	0	0	0	0	0	0				26	50
45	14-Jun-01	46	0	0	0	0	0	0				26	52
46	15-Jun-01	47	0	0	0	0	0	0				26	54
47	16-Jun-01	48	0	0	0	0	0	0				26	53
48	17-Jun-01	49	0	0	0	0	0	0				26	55
49	18-Jun-01	50	0	0	0	0	0	0				24	61
50	19-Jun-01	51	0	0	0	0	0	0	0.9532	1.924	2.24E-03	26	49
51	20-Jun-01	52	0	0	0	0	0	0				26	48
52	21-Jun-01	53	0	0	0	0	0	0				25	53
53	22-Jun-01	54	0	0	0	0	0	0				24	51
54	23-Jun-01	55	0	0	0	0	0	0				24	51
55	24-Jun-01	56	0	0	0	0	0	0				24	56
56	25-Jun-01	57	0	0	0	0	0	0				24	54
57	26-Jun-01	58	0	0	0	0	0	0	4.4210	2.920	4.24E-03	24	54
58	27-Jun-01	59	0	0	0	0	0	0				24	44
59	28-Jun-01	60	0	0	0	0	0	0				24	43
60	29-Jun-01	61	0	0	0	0	0	0				24	41
61	30-Jun-01	62	0	0	0	0	0	0				24	48
62	1-Jul-01	63	0	0	0	0	0	0				24	46

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group I - 0 mg/m^3 (Air Control)

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
63	2-Jul-01	64	0	0	0	0	0	0				24	44
64	3-Jul-01	65	0	0	0	0	0	0	3.8110	1.944	4.83E-03	24	44
65	4-Jul-01	66	0	0	0	0	0	0				24	48
66	5-Jul-01	67	0	0	0	0	0	0				24	42
67	6-Jul-01	68	0	0	0	0	0	0				24	44
68	7-Jul-01	69	0	0	0	0	0	0				24	44
69	8-Jul-01	70	0	0	0	0	0	0				24	47
70	9-Jul-01	71	0	0	0	0	0	0				24	41
71	10-Jul-01	72	0	0	0	0	0	0	1.1830	2.162	2.83E-03	24	41
72	11-Jul-01	73	0	0	0	0	0	0				24	45
73	12-Jul-01	74	0	0	0	0	0	0				24	45
74	13-Jul-01	75	0	0	0	0	0	0				24	45
75	14-Jul-01	76	0	0	0	0	0	0				24	47
76	15-Jul-01	77	0	0	0	0	0	0				24	46
77	16-Jul-01	78	0	0	0	0	0	0				24	43
78	17-Jul-01	79	0	0	0	0	0	0	0.8646	1.760	2.50E-03	24	44
79	18-Jul-01	80	0	0	0	0	0	0				24	45
80	19-Jul-01	81	0	0	0	0	0	0				24	50
81	20-Jul-01	82	0	0	0	0	0	0				24	50
82	21-Jul-01	83	0	0	0	0	0	0				24	45
83	22-Jul-01	84	0	0	0	0	0	0				23	49

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group I - 0 mg/m^3 (Air Control)

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
84	23-Jul-01	85	0	0	0	0	0	0				24	47
85	24-Jul-01	86	0	0	0	0	0	0	0.8876	1.677	4.87E-03	24	51
86	25-Jul-01	87	0	0	0	0	0	0				24	59
87	26-Jul-01	88	0	0	0	0	0	0				24	48
88	27-Jul-01	89	0	0	0	0	0	0				24	44
89	28-Jul-01	90	0	0	0	0	0	0				24	46
90	29-Jul-01	91	0	0	0	0	0	0				24	48
91	30-Jul-01	92	0	0	0	0	0	0				23	51
92	31-Jul-01	93	0	0	0	0	0	0	1.7190	1.748	8.21E-04	23	45
93	1-Aug-01	94	0	0	0	0	0	0				23	53
94	2-Aug-01	95	0	0	0	0	0	0				22	53
95	3-Aug-01	96	0	0	0	0	0	0				22	61
96	4-Aug-01	97	0	0	0	0	0	0				23	60
97	5-Aug-01	98	0	0	0	0	0	0				23	41
98	6-Aug-01	99	0	0	0	0	0	0				22	48
99	7-Aug-01	100	0	0	0	0	0	0	0.8421	1.321	7.59E-03	21	72
100	8-Aug-01	101	0	0	0	0	0	0				23	50
101	9-Aug-01	102	0	0	0	0	0	0				23	51
102	10-Aug-01	103	0	0	0	0	0	0				24	53
103	11-Aug-01	104	0	0	0	0	0	0				23	52
104	12-Aug-01	105	0	0	0	0	0	0				23	43

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group I - 0 mg/m^3 (Air Control)

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
105	13-Aug-01	106	0	0	0	0	0	0				24	45
106	14-Aug-01	107	0	0	0	0	0	0	4.5440	2.637	8.57E-03	24	42
107	15-Aug-01	108	0	0	0	0	0	0				24	43
108	16-Aug-01	109	0	0	0	0	0	0				24	44
109	17-Aug-01	110	0	0	0	0	0	0				24	44
110	18-Aug-01	111	0	0	0	0	0	0				24	44
111	19-Aug-01	112	0	0	0	0	0	0				24	44
112	20-Aug-01	113	0	0	0	0	0	0				24	45
113	21-Aug-01	114	0	0	0	0	0	0	0.8419	1.482	1.07E-03	22	43
114	22-Aug-01	115	0	0	0	0	0	0				21	48
115	23-Aug-01	116	0	0	0	0	0	0				21	52
116	24-Aug-01	117	0	0	0	0	0	0				21	51
117	25-Aug-01	118	0	0	0	0	0	0				21	52
118	26-Aug-01	119	0	0	0	0	0	0				21	51
119	27-Aug-01	120	0	0	0	0	0	0				21	47
120	28-Aug-01	121	0	0	0	0	0	0	0.8008	1.687	3.14E-03	21	45
		Mean	**0**		**0**				**2.853**	**2.038**	**3.52E-03**	**24.2**	**47.4**
		S.D.	**0**		**0**				**3.162**	**0.545**	**2.35E-03**	**1.3**	**6.3**

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group II - 2000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
0	30-Apr-01	1	2440	2018	2030	1980	1940	2120				26	32
1	1-May-01	2	2430	1965	1970	1950	1970	1970	2.5140	1.849	2.98E-03	25	35
2	2-May-01	3	2460	2098	2080	2100	2110	2100				25	42
3	3-May-01	4	2470	1973	2000	1920	1890	2080				24	43
4	4-May-01	5	2450	1978	2040	2050	1780	2040				24	50
5	5-May-01	6	2450	1978	2120	1700	2050	2040				24	54
6	6-May-01	7	2800	2010	1850	2110	2070	2010				22	35
7	7-May-01	8	2520	2068	2050	2020	2070	2130				25	38
8	8-May-01	9	2290	2048	2160	2000	2170	1860	3.4440	2.265	6.28E-03	25	41
9	9-May-01	10	2410	2003	2030	2020	1990	1970				25	42
10	10-May-01	11	2410	1963	1950	1890	1990	2020				25	42
11	11-May-01	12	2410	1970	1940	1920	2010	2010				25	43
12	12-May-01	13	2260	2003	2010	2030	1990	1980				24	48
13	13-May-01	14	2650	2198	2400	2170	2120	2100				25	37
14	14-May-01	15	2360	2000	1990	1990	2010	2010				25	36
15	15-May-01	16	2470	2058	2280	1880	2000	2070	2.6370	2.097	1.29E-03	25	35
16	16-May-01	17	2420	2130	2160	2130	2090	2140				25	36
17	17-May-01	18	2370	2158	2170	2170	2130	2160				25	39
18	18-May-01	19	2320	2048	2080	2090	2050	1970				25	44
19	19-May-01	20	2340	2085	2010	2090	2120	2120				25	47
20	20-May-01	21	2270	2028	1960	2100	2050	2000				25	44
21	21-May-01	22	2310	1943	1790	1940	1900	2140				25	45

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group II - 2000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
22	22-May-01	23	2310	2003	2230	1970	1970	1840	1.4230	1.825	9.59E-04	28	48
23	23-May-01	24	2340	2028	2100	1980	2030	2000				26	50
24	24-May-01	25	2380	2000	2000	1960	1960	2080				25	48
25	25-May-01	26	2470	2063	2230	1970	2020	2030				25	47
26	26-May-01	27	2600	2035	1840	2090	2050	2160				26	48
27	27-May-01	28	2530	2020	2060	2040	2000	1980				26	51
28	28-May-01	29	2610	2038	2160	1990	2000	2000				26	49
29	29-May-01	30	2500	2013	2000	2000	2010	2040	0.9373	1.927	1.23E-03	25	45
30	30-May-01	31	2500	2023	2070	2000	2020	2000				26	35
31	31-May-01	32	2720	2000	2000	2000	2000	2000				26	33
32	1-Jun-01	33	2590	1993	1970	2000	2000	2000				26	35
33	2-Jun-01	34	2470	2175	2400	2200	2070	2030				26	48
34	3-Jun-01	35	2770	2048	2030	2030	2030	2100				26	49
35	4-Jun-01	36	2590	2003	2030	2010	2020	1950				26	44
36	5-Jun-01	37	2840	2125	2080	2000	2040	2380	0.8336	1.646	1.43E-03	26	45
37	6-Jun-01	38	2570	2070	2130	2010	2010	2130				26	47
38	7-Jun-01	39	2450	2025	2040	2010	2030	2020				26	43
39	8-Jun-01	40	2460	2043	2210	1960	1990	2010				26	42
40	9-Jun-01	41	2400	2008	2060	1960	2020	1990				26	39
41	10-Jun-01	42	2350	2000	2010	2000	1990	2000				26	39
42	11-Jun-01	43	2550	2100	2090	2280	2010	2020				26	45

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group II - 2000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
43	12-Jun-01	44	2490	2013	2050	2000	2020	1980	0.9419	1.766	1.60E-03	26	51
44	13-Jun-01	45	2490	2048	2070	2020	2000	2100				26	48
45	14-Jun-01	46	2390	2005	2000	2000	2000	2020				26	50
46	15-Jun-01	47	2500	2023	2080	2010	2000	2000				26	52
47	16-Jun-01	48	2540	2043	2110	2000	2070	1990				26	51
48	17-Jun-01	49	2540	2005	2000	2000	2000	2020				26	52
49	18-Jun-01	50	2530	2003	2000	2000	2010	2000				26	43
50	19-Jun-01	51	2440	1998	2010	2030	1980	1970	0.8474	1.484	1.59E-03	26	48
51	20-Jun-01	52	2390	2005	2110	1970	2000	1940				26	46
52	21-Jun-01	53	2550	1998	2000	2000	1990	2000				26	51
53	22-Jun-01	54	2400	1998	2010	1980	2000	2000				25	49
54	23-Jun-01	55	2400	1978	2000	1950	1980	1980				24	49
55	24-Jun-01	56	2460	1978	2000	1940	1940	2030				24	52
56	25-Jun-01	57	2350	1988	2000	1930	2000	2020				24	51
57	26-Jun-01	58	2370	2020	2000	1960	2090	2030	2.1620	2.336	3.92E-03	24	52
58	27-Jun-01	59	2580	2005	2000	1980	2000	2040				25	42
59	28-Jun-01	60	2230	2003	2010	2000	2000	2000				24	41
60	29-Jun-01	61	2440	2112	2260	2140	2070	1980				25	38
61	30-Jun-01	62	2440	2135	2300	2140	2060	2040				24	45
62	1-Jul-01	63	2410	2060	2190	1890	2030	2130				25	43
63	2-Jul-01	64	2540	2043	2210	1990	1970	2000				24	41

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group II - 2000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
64	3-Jul-01	65	2410	1985	2010	1980	1990	1960	4.6170	2.199	2.81E-03	24	42
65	4-Jul-01	66	2150	2008	2000	1950	2050	2050				24	45
66	5-Jul-01	67	2460	1998	2010	1940	1970	2070				24	41
67	6-Jul-01	68	2470	2015	2000	2090	1980	1990				24	43
68	7-Jul-01	69	2430	2000	2020	2010	2000	1970				24	41
69	8-Jul-01	70	2400	1995	1960	2000	1990	2030				24	46
70	9-Jul-01	71	2470	1993	2000	1990	2000	1980				24	39
71	10-Jul-01	72	2500	1990	2010	2000	2000	1950	0.8530	1.773	2.18E-03	24	38
72	11-Jul-01	73	2480	2008	2000	2000	1990	2040				25	43
73	12-Jul-01	74	2460	2013	2050	2000	2000	2000				24	43
74	13-Jul-01	75	2490	2000	2000	1980	1950	2070				24	43
75	14-Jul-01	76	2450	2013	2150	2000	1940	1960				24	45
76	15-Jul-01	77	2260	1850	1860	1680	1940	1920				24	44
77	16-Jul-01	78	2430	2008	2000	2030	2000	2000				25	41
78	17-Jul-01	79	2440	2083	2160	2040	2040	2090	0.8424	2.182	3.33E-03	25	42
79	18-Jul-01	80	2330	1943	2000	1940	1950	1880				25	45
80	19-Jul-01	81	2430	2000	2040	1960	2000	2000				25	47
81	20-Jul-01	82	2370	1988	1960	2030	1960	2000				25	50
82	21-Jul-01	83	2430	2033	2000	2010	1930	2190				24	49
83	22-Jul-01	84	2520	2383	3560	1960	2000	2010				24	48
84	23-Jul-01	85	2430	2000	2000	1950	2070	1980				24	44

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group II - 2000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
85	24-Jul-01	86	2400	1978	1990	1900	2000	2020	1.1250	2.596	7.41E-03	25	47
86	25-Jul-01	87	2470	2035	2250	1960	1960	1970				24	54
87	26-Jul-01	88	2270	1995	2050	2010	1970	1950				25	44
88	27-Jul-01	89	2330	2003	1980	1990	2040	2000				25	43
89	28-Jul-01	90	2350	2000	2010	2000	2010	1980				25	46
90	29-Jul-01	91	2290	1995	2000	1980	2000	2000				25	46
91	30-Jul-01	92	2420	2000	2030	1950	1980	2040				24	47
92	31-Jul-01	93	2340	1965	2000	1810	2000	2050	2.5960	1.901	1.33E-03	23	45
93	1-Aug-01	94	2400	2010	2090	1960	2000	1990				23	54
94	2-Aug-01	95	2440	2003	2000	2000	2010	2000				22	54
95	3-Aug-01	96	2450	2010	2030	2000	1980	2030				23	58
96	4-Aug-01	97	2410	1985	2030	1970	2020	1920				23	56
97	5-Aug-01	98	2450	2015	2030	2020	2030	1980				23	39
98	6-Aug-01	99	2260	1970	1940	1950	2040	1950				23	46
99	7-Aug-01	100	2310	2003	2010	1980	2040	1980	0.8364	1.542	7.65E-03	23	58
100	8-Aug-01	101	2510	2000	2050	1950	2000	2000				24	48
101	9-Aug-01	102	2430	2008	1950	2050	2000	2030				24	51
102	10-Aug-01	103	2480	2013	1980	2060	2000	2010				24	54
103	11-Aug-01	104	2430	2013	2040	2000	2030	1980				23	55
104	12-Aug-01	105	2420	2003	2000	2030	1980	2000				24	43
105	13-Aug-01	106	2400	1995	1950	1900	2090	2040				24	43

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group II - 2000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
106	14-Aug-01	107	2600	2013	1980	2070	2000	2000	0.9904	2.343	3.35E-03	24	43
107	15-Aug-01	108	2520	2003	2000	2010	2000	2000				24	43
108	16-Aug-01	109	2500	2028	1900	2080	2050	2080				25	43
109	17-Aug-01	110	2420	1990	2000	2000	2000	1960				25	43
110	18-Aug-01	111	2470	1995	1940	2040	2010	1990				25	41
111	19-Aug-01	112	2510	1995	2040	1900	2000	2040				25	41
112	20-Aug-01	113	2530	2040	2290	1980	2070	1820				25	44
113	21-Aug-01	114	2410	2008	2000	2030	2000	2000	3.1920	2.870	2.09E-03	23	41
114	22-Aug-01	115	2420	2000	1980	2030	1990	2000				22	47
115	23-Aug-01	116	2470	2043	2280	1930	1980	1980				21	61
116	24-Aug-01	117	2470	1985	2000	1980	1960	2000				21	58
117	25-Aug-01	118	2430	1995	1960	1960	2060	2000				22	49
118	26-Aug-01	119	2430	2043	2080	2070	2020	2000				22	48
119	27-Aug-01	120	2380	2033	2060	2000	2000	2070				22	45
120	28-Aug-01	121	2410	1998	2030	2030	1980	1950	0.7675	2.080	2.91E-03	22	43
		Mean	**2445**		**2020**				**1.753**	**2.038**	**3.02E-03**	**24.6**	**45.2**
		S.D.	**106**		**105**				**1.161**	**0.364**	**2.08E-03**	**1.2**	**5.6**

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group III - 10000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
0	30-Apr-01	1	10400	10190	11100	10100	8760	10800				26	32
1	1-May-01	2	10100	9633	9680	9920	9920	9010	1.6510	1.701	1.65E-03	25	35
2	2-May-01	3	10600	10450	10400	10500	10700	10200				25	41
3	3-May-01	4	9820	9203	10100	7340	9070	10300				24	42
4	4-May-01	5	10400	10230	10100	10400	10000	10400				24	49
5	5-May-01	6	10100	9835	10000	9770	9890	9680				24	53
6	6-May-01	7	10100	9553	8310	10000	10100	9800				22	35
7	7-May-01	8	10500	10400	10200	10100	10300	10800				25	38
8	8-May-01	9	10200	9805	10000	9860	9680	9680	4.5220	2.383	7.00E-03	25	40
9	9-May-01	10	10500	10070	10300	9980	10400	9610				26	42
10	10-May-01	11	10300	10050	10500	10000	10000	9710				25	42
11	11-May-01	12	10400	9973	9370	9920	10300	10300				25	42
12	12-May-01	13	10800	10220	9920	10000	9770	11200				25	46
13	13-May-01	14	10400	10120	10100	10700	9740	9920				25	36
14	14-May-01	15	10500	10130	10000	10100	10300	10100				26	35
15	15-May-01	16	10500	10150	10200	9980	9920	10500	1.4010	1.622	5.49E-04	26	35
16	16-May-01	17	10600	10200	10100	9680	10600	10400				26	36
17	17-May-01	18	10200	10460	10700	10400	10900	9830				26	38
18	18-May-01	19	10000	10030	9830	10100	10200	10000				26	53
19	19-May-01	20	10200	10070	9920	10200	10200	9950				26	45
20	20-May-01	21	10000	10280	10000	10300	10200	10600				26	43
21	21-May-01	22	10800	10320	9680	10600	10300	10700				26	44

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group III - 10000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
22	22-May-01	23	10100	10150	10000	10600	10000	9980	1.6880	1.973	1.11E-03	29	47
23	23-May-01	24	10100	9980	9280	10400	9740	10500				26	49
24	24-May-01	25	10100	10200	10500	10000	10300	9980				26	46
25	25-May-01	26	10000	10300	10000	10500	10600	10100				26	47
26	26-May-01	27	9790	10080	9800	10300	10200	10000				26	47
27	27-May-01	28	9870	10030	10000	10100	10000	10000				26	50
28	28-May-01	29	10400	10300	10300	10000	10300	10600				26	47
29	29-May-01	30	9820	10070	10500	9460	10300	10000	1.3810	2.172	1.92E-03	26	44
30	30-May-01	31	9800	10180	10000	10300	10300	10100				26	35
31	31-May-01	32	9220	9820	10200	9830	10100	9160				26	33
32	1-Jun-01	33	9920	10200	10700	9950	10300	9860				26	35
33	2-Jun-01	34	10200	10590	9640	11900	10600	10200				26	47
34	3-Jun-01	35	10300	10300	10100	10100	10100	10900				26	49
35	4-Jun-01	36	10300	10020	9980	10000	10100	10000				26	44
36	5-Jun-01	37	10200	10100	10000	10100	9980	10300	0.8545	1.690	1.41E-03	26	45
37	6-Jun-01	38	9990	10450	11600	9680	10500	10000				26	46
38	7-Jun-01	39	10000	9993	9950	10100	10000	9920				26	41
39	8-Jun-01	40	9610	10028	10700	9950	10000	9460				26	40
40	9-Jun-01	41	9650	10090	10400	10000	9980	9980				26	39
41	10-Jun-01	42	9310	10290	10100	10200	10900	9950				26	39
42	11-Jun-01	43	9960	10170	10300	10800	9890	9680				26	46

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group III - 10000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
43	12-Jun-01	44	10600	9858	7830	10500	10600	10500	1.1770	1.654	2.02E-03	26	49
44	13-Jun-01	45	10000	10490	10900	9950	10400	10700				26	46
45	14-Jun-01	46	9520	10010	10100	10100	9980	9860				26	48
46	15-Jun-01	47	10700	10550	10700	10600	10300	10600				26	51
47	16-Jun-01	48	10200	10180	10500	10000	10200	10000				26	50
48	17-Jun-01	49	10100	10120	10000	10500	10000	9980				26	50
49	18-Jun-01	50	10300	10060	10100	10200	10000	9950				26	41
50	19-Jun-01	51	9960	10030	9830	10100	10000	10200	0.9191	2.123	3.68E-03	26	46
51	20-Jun-01	52	9940	10090	9640	10300	10400	10000				27	46
52	21-Jun-01	53	10000	9995	10000	9980	10000	10000				26	50
53	22-Jun-01	54	10100	10050	10100	10100	10000	9980				25	48
54	23-Jun-01	55	9880	10050	10000	9950	9830	10400				24	51
55	24-Jun-01	56	9880	9998	10000	10000	10100	9890				25	52
56	25-Jun-01	57	10000	10640	10000	9770	10300	12500				24	51
57	26-Jun-01	58	10400	10090	9860	10000	10200	10300	1.7990	2.072	3.40E-03	25	52
58	27-Jun-01	59	10200	10100	9980	10100	10000	10300				25	42
59	28-Jun-01	60	10400	10070	10100	10200	10000	9980				25	42
60	29-Jun-01	61	10100	10630	11000	10600	10700	10200				25	38
61	30-Jun-01	62	10000	10590	11000	11000	10400	9950				25	44
62	1-Jul-01	63	9940	10400	10700	9890	10600	10400				25	43
63	2-Jul-01	64	10100	10120	10300	10000	9860	10300				25	41

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group III - 10000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
64	3-Jul-01	65	9940	10250	10400	10100	10200	10300	4.9280	2.112	4.51E-03	25	42
66	5-Jul-01	67	10100	10300	10400	10300	10400	10100				24	41
67	6-Jul-01	68	9660	10058	10300	10400	9730	9800				24	43
68	7-Jul-01	69	9360	10060	10700	9950	10100	9490				25	41
69	8-Jul-01	70	9700	10020	9770	10000	10200	10100				25	46
70	9-Jul-01	71	9700	9998	9890	10100	10000	10000				25	40
71	10-Jul-01	72	9660	9980	10000	9520	10100	10300	1.3170	2.547	2.41E-03	25	38
72	11-Jul-01	73	9740	9928	10000	10000	9710	10000				25	43
73	12-Jul-01	74	9780	10160	10600	10000	10300	9740				25	43
74	13-Jul-01	75	9600	10008	10000	10000	9830	10200				25	43
75	14-Jul-01	76	10200	9745	9430	9920	10200	9430				25	44
76	15-Jul-01	77	10100	10600	10300	11600	10000	10500				25	44
77	16-Jul-01	78	10000	10120	10700	10100	9860	9800				24	42
78	17-Jul-01	79	10500	10600	10300	10700	11200	10200	1.6940	2.790	4.35E-03	25	43
79	18-Jul-01	80	10300	10080	9920	10300	10100	9980				24	45
80	19-Jul-01	81	9740	10200	10700	9890	10100	10100				25	49
81	20-Jul-01	82	9650	9995	10000	9980	10000	10000				24	51
82	21-Jul-01	83	9710	10010	10400	9830	9800	10000				24	47
83	22-Jul-01	84	9750	10070	9890	10100	10300	10000				24	46
84	23-Jul-01	85	9360	10480	10400	10700	10700	10100				25	46

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group III - 10000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
85	24-Jul-01	86	9460	10100	10000	9890	10100	10400	15.6200	3.639	1.29E-02	25	50
86	25-Jul-01	87	10400	10750	10900	10500	10700	10900				25	54
87	26-Jul-01	88	9950	10750	11100	10700	10300	10900				25	45
88	27-Jul-01	89	9360	10050	10100	10100	10100	9890				25	44
89	28-Jul-01	90	9820	10200	10900	9920	9860	10100				25	45
90	29-Jul-01	91	9820	10030	10100	10200	9890	9920				25	45
91	30-Jul-01	92	9910	10020	9680	10200	10100	10100				24	49
92	31-Jul-01	93	9970	10020	9890	9980	10200	10000	1.6550	1.467	7.51E-04	23	44
93	1-Aug-01	94	9870	9915	9680	9850	9950	10200				23	54
94	2-Aug-01	95	9860	10030	10000	10000	10100	10000				23	48
95	3-Aug-01	96	10000	10030	9830	10100	10200	9980				23	54
96	4-Aug-01	97	10400	10400	10400	10600	10500	10100				24	58
97	5-Aug-01	98	10200	10490	11600	10700	9830	9830				24	40
98	6-Aug-01	99	9590	10010	10100	9980	10000	9950				24	46
99	7-Aug-01	100	9690	9998	10000	9890	10100	10000	0.8296	1.303	7.11E-03	24	52
100	8-Aug-01	101	9680	9965	10000	9860	10000	10000				24	51
101	9-Aug-01	102	9850	9753	8310	10300	10400	10000				24	49
102	10-Aug-01	103	9920	10020	10000	10000	10100	9980				24	54
103	11-Aug-01	104	9950	10010	9950	10100	10100	9890				23	50
104	12-Aug-01	105	9880	10050	10000	10100	10100	10000				24	41
105	13-Aug-01	106	9520	10080	10000	9830	10400	10100				25	44

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group III - 10000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment Mean: Temperature (°C)	Humidity (%)
106	14-Aug-01	107	9890	10050	10000	10100	10000	10100	1.0930	2.171	3.06E-03	24	41
107	15-Aug-01	108	9720	10100	9980	10000	10000	10400				24	41
108	16-Aug-01	109	9700	10110	10400	9830	10100	10100				24	42
109	17-Aug-01	110	9550	9990	10000	9680	10300	9980				24	40
110	18-Aug-01	111	9960	10030	10000	10100	10000	10000				25	39
111	19-Aug-01	112	9930	9983	9830	10100	10000	10000				25	40
112	20-Aug-01	113	9790	10120	10000	10000	10100	9980				24	43
113	21-Aug-01	114	9740	9980	9830	10100	9890	10100	0.8375	1.418	1.06E-03	23	42
114	22-Aug-01	115	10000	10130	10000	10000	10100	10400				22	48
115	23-Aug-01	116	9700	10020	10000	10000	10100	9980				22	50
116	24-Aug-01	117	9890	9885	10000	9830	9710	10000				22	53
117	25-Aug-01	118	9810	10150	10300	9980	9830	10500				22	48
118	26-Aug-01	119	10100	10550	10700	10600	10500	10400				22	48
119	27-Aug-01	120	9720	10080	10100	10000	10100	10100				22	43
120	28-Aug-01	121	9490	10020	10300	10000	9830	9950	0.7432	1.432	2.48E-03	22	41
		Mean	**9992**		**10130**				**2.451**	**2.015**	**3.41E-03**	**24.9**	**44.6**
		S.D.	**325**		**417**				**3.487**	**0.580**	**3.05E-03**	**1.2**	**5.2**

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group IV - 20000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
0	30-Apr-01	1	20200	20480	19100	22000	20400	20400				25	31
1	1-May-01	2	19700	19550	19700	18000	20400	20100	1.5110	1.625	1.29E-03	25	33
2	2-May-01	3	19700	20330	20000	20800	20700	19800				25	39
3	3-May-01	4	19300	19580	18600	19200	21400	19100				24	42
4	4-May-01	5	19800	20130	19300	19800	20600	20800				24	45
5	5-May-01	6	19500	19700	19700	19500	19700	19900				24	50
6	6-May-01	7	19300	19600	17700	20000	20600	20100				23	33
7	7-May-01	8	19400	19000	20600	14800	20100	20600				25	36
8	8-May-01	9	18600	18800	19200	19500	19700	16800	1.2770	1.818	2.46E-03	25	39
9	9-May-01	10	20200	20000	18500	19900	21400	20200				25	41
10	10-May-01	11	19900	20200	18700	21000	21100	20000				25	39
11	11-May-01	12	19600	19980	19800	20100	20000	20000				25	39
12	12-May-01	13	20400	20400	20300	20200	20700	20400				25	44
13	13-May-01	14	19600	19230	19200	20200	19400	18100				25	35
14	14-May-01	15	20400	19980	18500	20800	20600	20000				26	33
15	15-May-01	16	20700	20580	20700	20000	20700	20900	1.5800	1.620	9.19E-04	26	33
16	16-May-01	17	20100	19800	20000	19600	18700	20900				26	34
17	17-May-01	18	20000	20200	20200	20500	20600	19500				25	35
18	18-May-01	19	19600	19850	19500	20300	20400	19200				26	41
19	19-May-01	20	19400	20200	20900	20200	20000	19700				26	44
20	20-May-01	21	19500	20450	19600	20600	20800	20800				26	41
21	21-May-01	22	20000	20450	20200	21000	20500	20100				26	42

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group IV - 20000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
22	22-May-01	23	19300	19980	20000	19900	20000	20000	1.8820	1.985	1.59E-03	29	45
23	23-May-01	24	19200	19980	19800	19900	20200	20000				26	47
24	24-May-01	25	19400	19980	20000	19500	20400	20000				26	43
25	25-May-01	26	18100	19900	20000	19800	19500	20300				26	43
26	26-May-01	27	18300	20180	20000	20000	20800	19900				26	43
27	27-May-01	28	18400	20100	20000	20100	20800	19500				26	47
28	28-May-01	29	18500	19880	20100	20000	19800	19600				26	44
29	29-May-01	30	18400	19750	20400	18600	20200	19800	1.2850	1.880	1.57E-03	26	43
30	30-May-01	31	19700	20150	20000	20000	19700	20900				26	33
31	31-May-01	32	20100	21930	23600	21000	21600	21500				26	32
32	1-Jun-01	33	20500	20000	19800	19700	20500	20000				26	33
33	2-Jun-01	34	19900	20450	21100	20700	19500	20500				26	46
34	3-Jun-01	35	20300	20250	20200	20000	20100	20700				26	46
35	4-Jun-01	36	19100	19380	19200	18500	19800	20000				26	42
36	5-Jun-01	37	19700	20380	20000	20700	20800	20000	1.0490	2.006	1.86E-03	26	44
37	6-Jun-01	38	19700	20400	20400	20000	20600	20600				26	44
38	7-Jun-01	39	19500	20330	21500	19800	20000	20000				26	40
39	8-Jun-01	40	19200	19680	18900	19800	20000	20000				26	39
40	9-Jun-01	41	18100	20230	21400	19800	20000	19700				26	36
41	10-Jun-01	42	17400	19980	20000	20000	19900	20000				26	36
42	11-Jun-01	43	19200	19980	20000	19900	20100	19900				26	43

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group IV - 20000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
43	12-Jun-01	44	19400	19200	18000	19800	20100	18900	0.8612	1.815	1.50E-03	26	46
44	13-Jun-01	45	19700	20100	19900	19200	20400	20700				26	45
45	14-Jun-01	46	18800	19950	19200	20000	20200	20400				26	47
46	15-Jun-01	47	19300	20000	20000	20000	20000	20000				26	49
47	16-Jun-01	48	19500	19900	20000	20000	20000	19600				26	47
48	17-Jun-01	49	19400	20000	19600	20400	20000	20000				26	48
49	18-Jun-01	50	19700	19900	19000	19800	20400	20400				26	41
50	19-Jun-01	51	19200	19950	20400	19800	19500	20100	0.8340	1.745	4.23E-03	26	43
51	20-Jun-01	52	19200	20000	19900	19200	20400	20500				26	43
52	21-Jun-01	53	19500	20030	20000	20000	20000	20100				26	47
53	22-Jun-01	54	19500	20000	20000	19900	20000	20100				25	44
54	23-Jun-01	55	19100	20450	20300	19900	20400	21200				24	46
55	24-Jun-01	56	18400	20000	20000	20000	20100	19900				25	49
56	25-Jun-01	57	18700	20280	19900	19800	20400	21000				24	47
57	26-Jun-01	58	19600	20030	18900	19800	20000	21400	0.9786	2.192	2.95E-03	25	48
58	27-Jun-01	59	19600	19650	18300	20100	19800	20400				25	38
59	28-Jun-01	60	19600	19980	19800	20000	20000	20100				25	38
60	29-Jun-01	61	19700	20080	20400	18700	20800	20400				25	35
61	30-Jun-01	62	19500	19680	17700	20300	20400	20300				25	43
62	1-Jul-01	63	19800	20230	20900	19100	20100	20800				25	40
63	2-Jul-01	64	19400	19930	19900	20000	19900	19900				25	38

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group IV - 20000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
64	3-Jul-01	65	19600	20000	20100	20000	19900	20000	10.9200	2.534	4.26E-03	25	39
65	4-Jul-01	66	19700	20000	19800	19600	20200	20400				25	42
66	5-Jul-01	67	19600	20130	20200	19900	20300	20100				25	37
67	6-Jul-01	68	19600	20000	20700	18900	19900	20500				25	40
68	7-Jul-01	69	19900	20050	19800	19800	20300	20300				25	38
69	8-Jul-01	70	19700	20000	19500	20000	20400	20100				25	43
70	9-Jul-01	71	19600	20050	20000	20100	20100	20000				25	37
71	10-Jul-01	72	19900	20330	20000	20200	20400	20700	17.0200	2.925	1.24E-02	25	36
72	11-Jul-01	73	19400	19880	20000	19500	19600	20400				25	40
73	12-Jul-01	74	19100	20030	20700	19900	19500	20000				24	40
74	13-Jul-01	75	18800	19880	20100	20000	19700	19700				25	40
75	14-Jul-01	76	19600	19550	19000	19900	19800	19500				25	40
76	15-Jul-01	77	19600	20080	20700	21200	18100	20300				25	42
77	16-Jul-01	78	19300	20000	19900	19900	19900	20300				24	39
78	17-Jul-01	79	19500	19930	19000	20300	20000	20400	0.8420	1.902	3.67E-03	25	40
79	18-Jul-01	80	19100	19950	19300	20400	20100	20000				25	42
80	19-Jul-01	81	19300	20080	18900	20500	20400	20500				25	44
81	20-Jul-01	82	19100	20030	20300	19900	19900	20000				25	44
82	21-Jul-01	83	19700	19800	19200	19800	19500	20700				25	41
83	22-Jul-01	84	18800	20300	20400	20600	21000	19200				25	41
84	23-Jul-01	85	19000	20000	19200	20400	20300	20100				24	41

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group IV - 20000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
85	24-Jul-01	86	19400	20000	18600	20400	20600	20400	0.9485	2.448	5.62E-03	25	43
86	25-Jul-01	87	19700	20030	20300	19900	20200	19700				25	47
87	26-Jul-01	88	19300	19980	19700	20200	19800	20200				25	40
88	27-Jul-01	89	19200	20000	20000	20000	20000	20000				25	39
89	28-Jul-01	90	17900	19930	20700	19400	18900	20700				25	41
90	29-Jul-01	91	19200	20030	19900	20400	19900	19900				25	42
91	30-Jul-01	92	18600	19980	18900	20700	20200	20100				25	40
92	31-Jul-01	93	19500	20000	19800	20400	20000	19800	1.5940	1.579	7.60E-04	24	39
93	1-Aug-01	94	19400	19930	20400	19500	19500	20300				23	46
94	2-Aug-01	95	19400	20050	19900	20100	20400	19800				23	44
95	3-Aug-01	96	19000	19980	20000	19800	20100	20000				23	50
96	4-Aug-01	97	18700	18430	19500	17000	17500	19700				24	51
97	5-Aug-01	98	19900	20250	20000	20700	20900	19400				23	36
98	6-Aug-01	99	19000	20130	19900	20400	20400	19800				24	41
99	7-Aug-01	100	18500	20050	20400	20400	19900	19500	0.8417	1.654	7.32E-03	24	44
100	8-Aug-01	101	18800	19600	18900	20100	20200	19200				25	43
101	9-Aug-01	102	19100	19830	18900	20600	20000	19800				24	47
102	10-Aug-01	103	19600	19930	20400	19700	19500	20100				25	46
103	11-Aug-01	104	19300	19680	18600	20200	19900	20000				23	45
104	12-Aug-01	105	19400	19780	20000	19500	19500	20100				25	40
105	13-Aug-01	106	19400	19700	18600	20200	19900	20100				25	39

BASELINE GASOLINE VAPOR CONDENSATE:A TWO-GENERATION REPRODUCTION
STUDY IN RATS VIA WHOLE-BODY INHALATION EXPOSURES

Chamber Monitoring Results
Cumulative Exposure Record
Group IV - 20000 mg/m^3

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
106	14-Aug-01	107	19400	19900	19800	19600	19800	20400	0.9033	2.182	2.84E-03	25	37
107	15-Aug-01	108	19400	20030	20000	19600	20100	20400				25	37
108	16-Aug-01	109	19100	20030	20400	19800	19900	20000				25	37
109	17-Aug-01	110	19700	20050	20000	20000	20400	19800				25	37
110	18-Aug-01	111	19300	20030	20000	20000	20000	20100				25	36
111	19-Aug-01	112	19200	19930	20100	20100	19900	19600				25	37
112	20-Aug-01	113	19400	20030	19500	20100	20300	20200				25	39
113	21-Aug-01	114	19300	20050	20000	20100	20000	20100	0.9855	2.031	1.38E-03	24	38
114	22-Aug-01	115	19600	20000	19900	20000	20100	20000				22	41
115	23-Aug-01	116	19600	20000	19600	20200	20200	20000				22	46
116	24-Aug-01	117	19400	19950	19500	20100	20000	20200				22	46
117	25-Aug-01	118	19800	20180	19000	20100	20800	20800				22	46
118	26-Aug-01	119	19700	20130	20600	20100	19900	19900				22	45
119	27-Aug-01	120	18900	20000	19900	20200	20100	19800				22	40
120	28-Aug-01	121	19400	20100	20400	19900	20100	20000	0.8617	1.928	3.74E-03	22	39
		Mean	**19379**		**19990**				**2.565**	**1.993**	**3.35E-03**	**25.0**	**41.2**
		S.D.	**526**		**676**				**4.291**	**0.356**	**2.85E-03**	**1.1**	**4.4**

MALE PUPS	GFAP Report	Table B

Individual Pup Clinical Observations

Exposure Day	0	4	7	14	21	28	Exposure Day	0	4	7	14	21	28
Group I – 0 mg/m^3							Group III – 10,000 mg/m^3						
1566-4	WNL	WNL	WNL	WNL	WNL	WNL	3551-3	WNL	WNL	WNL	WNL	WNL	WNL
1567-3	WNL	WNL	WNL	WNL	WNL	WNL	3554-5	WNL	WNL	WNL	WNL	WNL	WNL
1555-3	WNL	WNL	WNL	WNL	WNL	WNL	3568-1	WNL	WNL	WNL	WNL	WNL	WNL
1568-6	WNL	WNL	WNL	WNL	WNL	WNL	3563-7	WNL	WNL	WNL	WNL	WNL	WNL
1564-3	WNL	WNL	WNL	WNL	WNL	WNL	3560-4	WNL	WNL	WNL	WNL	WNL	WNL
Group II – 2,000 mg/m^3							Group IV – 20,000 mg/m^3						
2574-6	WNL	WNL	WNL	WNL	WNL	WNL	4573-2	WNL	WNL	WNL	WNL	WNL	WNL
2561-7	WNL	WNL	WNL	WNL	WNL	WNL	4565-2	WNL	WNL	WNL	WNL	WNL	WNL
2558-6	WNL	WNL	WNL	WNL	WNL	WNL	4564-2	WNL	WNL	WNL	WNL	WNL	WNL
2565-2	WNL	WNL	WNL	WNL	WNL	WNL	4552-4	WNL	WNL	WNL	WNL	WNL	WNL
2557-3	WNL	WNL	WNL	WNL	WNL	WNL	4553-7	WNL	WNL	WNL	WNL	WNL	WNL

WNL= Within Normal Limits

FEMALE PUPS	GFAP Report	Table B

Individual Pup Clinical Observations

Exposure Day	0	4	7	14	21	28	Exposure Day	0	4	7	14	21	28
Group I – 0 mg/m^3							Group III – 10,000 mg/m^3						
1566-12	WNL	WNL	WNL	WNL	WNL	WNL	3551-12	WNL	WNL	WNL	WNL	WNL	WNL
1567-11	WNL	WNL	WNL	WNL	WNL	WNL	3554-12	WNL	WNL	WNL	WNL	WNL	WNL
1555-8	WNL	WNL	WNL	WNL	WNL	WNL	3568-12	WNL	WNL	WNL	WNL	WNL	WNL
1568-15	WNL	WNL	WNL	WNL	WNL	WNL	3563-8	WNL	WNL	WNL	WNL	WNL	WNL
1564-10	WNL	WNL	WNL	WNL	WNL	WNL	3560-10	WNL	WNL	WNL	WNL	WNL	WNL
Group II – 2,000 mg/m^3							Group IV – 20,000 mg/m^3						
2574-11	WNL	WNL	WNL	WNL	WNL	WNL	4573-12	WNL	WNL	WNL	WNL	WNL	WNL
2561-8	WNL	WNL	WNL	WNL	WNL	WNL	4565-14	WNL	WNL	WNL	WNL	WNL	WNL
2558-13	WNL	WNL	WNL	WNL	WNL	WNL	4564-12	WNL	WNL	WNL	WNL	WNL	WNL
2565-13	WNL	WNL	WNL	WNL	WNL	WNL	4552-8	WNL	WNL	WNL	WNL	WNL	WNL
2557-9	WNL	WNL	WNL	WNL	WNL	WNL	4553-14	WNL	WNL	WNL	WNL	WNL	WNL

WNL= Within Normal Limits

Table C

Baseline Gasoline Vapor Condensate: A Two-Generation Reproduction Study in Rats via Whole-Body Inhalation Exposures

MALE PUPS	**Lactation Day**					
Animal Number	**1**	**4**	**7**	**14**	**21**	**28**
1508-3	8.7	12.9	18.1	28.8	43.5	86.9
1508-4	8.3	11.9	16.1	26.7	45.4	87.4
1514-3	7.6	12.4	17.1	26.2	44.1	74.7
1523-5	7.2	9.5	12.6	22.7	37.7	70.5
1525-8	7.2	9.9	16.2	25.9	42.7	70.0
Mean	**7.8**	**11.3**	**16.0**	**26.1**	**42.7**	**77.9**
S.D.	**0.7**	**1.5**	**2.1**	**2.2**	**3.0**	**8.6**
N	**5**	**5**	**5**	**5**	**5**	**5**
2501-3	7.1	10.6	14.9	25.3	39.7	69.1
2502-7	6.4	9.4	13.5	24.6	38.1	71.4
2508-5	6.4	9.4	15.0	25.5	40.4	67.0
2513-2	8.2	10.7	16.3	25.2	42.7	76.3
2519-2	7.1	9.8	14.5	25.6	44.2	73.4
Mean	**7.0**	**10.0**	**14.8**	**25.2**	**41.0**	**71.4**
S.D.	**0.7**	**0.6**	**1.0**	**0.4**	**2.4**	**3.6**
N	**5**	**5**	**5**	**5**	**5**	**5**
3506-8	8.5	11.7	17.7	30.5	49.0	77.7
3512-1	8.4	11.4	16.9	27.1	48.1	77.3
3513-2	8.1	11.8	16.6	25.3	40.4	67.8
3525-1	6.6	8.9	13.0	20.8	31.3	57.2
3526-3	8.4	11.7	16.1	26.6	47.5	73.6
Mean	**8.0**	**11.1**	**16.1**	**26.1**	**43.3**	**70.7**
S.D.	**0.8**	**1.2**	**1.8**	**3.5**	**7.5**	**8.5**
N	**5**	**5**	**5**	**5**	**5**	**5**
4505-7	7.6	12.0	15.8	23.3	40.0	66.9
4506-1	6.1	8.7	13.5	24.5	37.4	65.6
4513-2	7.3	10.8	15.5	24.4	44.3	75.5
4520-4	7.5	10.1	13.0	21.7	35.2	66.1
4524-3	5.9	8.4	12.0	21.3	29.9	49.7
Mean	**6.9**	**10.0**	**14.0**	**23.0**	**37.4**	**64.8**
S.D.	**0.8**	**1.5**	**1.6**	**1.5**	**5.4**	**9.3**
N	**5**	**5**	**5**	**5**	**5**	**5**

Table C

Baseline Gasoline Vapor Condensate: A Two-Generation Reproduction Study in Rats via Whole-Body Inhalation Exposures

FEMALE PUPS	**Lactation Day**					
Animal Number	**1**	**4**	**7**	**14**	**21**	**28**
1508-6	7.4	10.9	15.0	24.0	42.0	75.8
1508-10	8.0	11.6	15.8	25.2	44.0	81.3
1514-11	7.4	11.3	15.3	22.9	39.7	64.6
1523-9	6.3	7.6	11.1	19.3	33.7	59.1
1525-16	6.8	10.3	15.3	26.3	43.9	65.0
Mean	**7.2**	**10.3**	**14.5**	**23.5**	**40.7**	**69.2**
S.D.	**0.6**	**1.6**	**1.9**	**2.7**	**4.3**	**9.1**
N	**5**	**5**	**5**	**5**	**5**	**5**
2501-7	6.7	9.8	13.5	22.5	34.9	57.2
2502-8	6.7	8.1	12.7	23.2	34.6	68.0
2508-7	6.2	9.0	14.2	25.0	39.0	63.3
2513-14	6.9	9.7	13.2	21.6	35.2	64.1
2519-12	6.2	9.1	13.5	24.1	39.6	62.5
Mean	**6.5**	**9.1**	**13.4**	**23.3**	**36.7**	**63.0**
S.D.	**0.3**	**0.7**	**0.5**	**1.3**	**2.4**	**3.9**
N	**5**	**5**	**5**	**5**	**5**	**5**
3506-13	7.7	11.6	16.2	28.1	49.5	77.0
3512-9	7.5	10.9	15.5	26.2	45.0	70.1
3513-11	7.8	11.2	15.6	24.9	38.8	64.8
3525-10	6.4	8.6	12.3	22.6	33.4	59.7
3526-13	8.0	12.2	16.5	26.4	46.5	69.4
Mean	**7.5**	**10.9**	**15.2**	**25.6**	**42.6**	**68.2**
S.D.	**0.6**	**1.4**	**1.7**	**2.0**	**6.5**	**6.4**
N	**5**	**5**	**5**	**5**	**5**	**5**
4505-12	7.1	10.8	14.5	21.9	36.4	63.5
4506-14	5.5	7.5	11.3	21.5	31.9	57.2
4513-13	6.9	10.7	15.2	23.4	41.5	66.4
4520-11	7.9	10.7	13.6	23.1	36.8	69.3
4524-8	5.8	8.7	12.1	20.6	32.0	58.0
Mean	**6.6**	**9.7**	**13.3**	**22.1**	**35.7**	**62.9**
S.D.	**1.0**	**1.5**	**1.6**	**1.2**	**4.0**	**5.2**
N	**5**	**5**	**5**	**5**	**5**	**5**

	Individual Pup Termination History	Table D

Animal Number	Date of Death	Type of Death[a]	Study Interval[b]	Animal Number	Date of Death	Type of Death[a]	Study Interval[b]
Group I – 0 mg/m^3 (air only)							
1508-3	8/29/01	T	LD 28	1508-6	8/29/01	T	LD 28
1508-4	8/29/01	T	LD 28	1508-10	8/29/01	T	LD 28
1514-3	8/29/01	T	LD 28	1514-11	8/29/01	T	LD 28
1523-5	8/29/01	T	LD 28	1523-9	8/29/01	T	LD 28
1525-8	8/29/01	T	LD 28	1525-16	8/29/01	T	LD 28
Group II – 2,000 mg/m^3							
2501-3	8/29/01	T	LD 28	2501-7	8/29/01	T	LD 28
2502-7	8/29/01	T	LD 28	2502-8	8/29/01	T	LD 28
2508-5	8/29/01	T	LD 28	2508-7	8/29/01	T	LD 28
2513-2	8/29/01	T	LD 28	2513-14	8/29/01	T	LD 28
2519-2	8/29/01	T	LD 28	2519-12	8/29/01	T	LD 28
Group III – 10,000 mg/m^3							
3506-8	8/29/01	T	LD 28	3506-13	8/29/01	T	LD 28
3512-1	8/29/01	T	LD 28	3512-9	8/29/01	T	LD 28
3513-2	8/29/01	T	LD 28	3513-11	8/29/01	T	LD 28
3525-1	8/29/01	T	LD 28	3525-10	8/29/01	T	LD 28
3526-3	8/29/01	T	LD 28	3526-13	8/29/01	T	LD 28
Group IV – 20,000 mg/m^3							
4505-7	8/29/01	T	LD 28	4505-12	8/29/01	T	LD 28
4506-1	8/29/01	T	LD 28	4506-14	8/29/01	T	LD 28
4513-2	8/29/01	T	LD 28	4513-13	8/29/01	T	LD 28
4520-4	8/29/01	T	LD 28	4520-11	8/29/01	T	LD 28
4524-3	8/29/01	T	LD 28	4524-8	8/29/01	T	LD 28

[a]T = Terminal Sacrifice.
[b]LD = Lactation Day.

	Certificates of Analysis	Appendix EE

1. Feed Certificates of Analysis 1517
2. Water Certificates of Analysis 1567
3. Bedding Certificates of Analysis 1585

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0111270-1
Lot Code: JAN 21 01 3A
Entered: 1/23/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.36	%
FIBER (CRUDE)	4.38	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.064	PPM
CALCIUM	0.817	%
LEAD	0.172	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.546	%
SELENIUM	0.394	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.04
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0112839-2
Lot Code: FEB 19 01 3B
Entered: 2/21/2001

Assay	Analysis	Units
PROTEIN	21.4	%
FAT ACID (HYDRO.)	5.9	%
FIBER (CRUDE)	4.52	%
ARSENIC	0.341	PPM
CADMIUM	LESS THAN 0.05	PPM
CALCIUM	0.858	%
LEAD	0.169	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.655	%
SELENIUM	0.344	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0114619-1
Lot Code: APR 01 01 1A
Entered: 3/26/2001

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	5.85	%
FIBER (CRUDE)	4.2	%
ARSENIC	0.335	PPM
CADMIUM	0.071	PPM
CALCIUM	0.911	%
LEAD	0.159	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.637	%
SELENIUM	0.281	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.12
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0114619-3
Lot Code: APR 01 01 1C
Entered: 3/26/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.91	%
FIBER (CRUDE)	4.27	%
ARSENIC	0.255	PPM
CADMIUM	0.068	PPM
CALCIUM	0.951	%
LEAD	0.158	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.627	%
SELENIUM	0.306	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.11
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0114261-2
Lot Code: MAR 15 01 2B
Entered: 3/19/2001

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	5.79	%
FIBER (CRUDE)	4.98	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.055	PPM
CALCIUM	0.954	%
LEAD	0.174	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.617	%
SELENIUM	0.348	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0116204-2
Lot Code: APR 18 01 1B
Entered: 4/20/2001

Assay	Analysis	Units
PROTEIN	21.4	%
FAT ACID (HYDRO.)	5.59	%
FIBER (CRUDE)	3.82	%
ARSENIC	0.275	PPM
CADMIUM	0.056	PPM
CALCIUM	0.943	%
LEAD	0.109	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.722	%
SELENIUM	0.283	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.12
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0116765-3
Lot Code: MAY 01 01 2C
Entered: 5/2/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	6.04	%
FIBER (CRUDE)	4.26	%
ARSENIC	0.282	PPM
CADMIUM	0.057	PPM
CALCIUM	0.807	%
LEAD	0.152	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.669	%
SELENIUM	0.287	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.05
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0116765-1
Lot Code: MAY 01 01 2A
Entered: 5/2/2001

Assay	Analysis	Units
PROTEIN	21.5	%
FAT ACID (HYDRO.)	5.84	%
FIBER (CRUDE)	4.43	%
ARSENIC	0.327	PPM
CADMIUM	0.057	PPM
CALCIUM	0.872	%
LEAD	0.148	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.667	%
SELENIUM	0.274	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.05
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0117638-3
Lot Code: MAY 11 01 2A
Entered: 5/17/2001

Assay	Analysis	Units
PROTEIN	21.5	%
FAT ACID (HYDRO.)	5.58	%
FIBER (CRUDE)	4.29	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	LESS THAN 0.05	PPM
CALCIUM	0.783	%
LEAD	0.137	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.717	%
SELENIUM	0.300	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.06
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0118944-3
Lot Code: JUN 11 01 2C
Entered: 6/13/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.69	%
FIBER (CRUDE)	4.35	%
ARSENIC	0.225	PPM
CADMIUM	0.051	PPM
CALCIUM	0.956	%
LEAD	0.171	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.613	%
SELENIUM	0.313	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.06
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0119450-3
Lot Code: JUN 21 01 1C
Entered: 6/22/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.83	%
FIBER (CRUDE)	4.11	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.071	PPM
CALCIUM	0.945	%
LEAD	0.186	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.706	%
SELENIUM	0.312	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.04
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0119990-1
Lot Code: JUL 01 01 3A
Entered: 7/3/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.66	%
FIBER (CRUDE)	4.13	%
ARSENIC	0.364	PPM
CADMIUM	0.07	PPM
CALCIUM	1.01	%
LEAD	0.185	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.756	%
SELENIUM	0.310	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.04
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0119450-1
Lot Code: JUN 21 01 1A
Entered: 6/22/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.51	%
FIBER (CRUDE)	4.5	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.07	PPM
CALCIUM	0.881	%
LEAD	0.184	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.689	%
SELENIUM	0.291	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0120470-2
Lot Code: JUL 09 01 3B
Entered: 7/11/2001

Assay	Analysis	Units
PROTEIN	21.3	%
FAT ACID (HYDRO.)	5.67	%
FIBER (CRUDE)	4.57	%
ARSENIC	0.435	PPM
CADMIUM	0.059	PPM
CALCIUM	0.930	%
LEAD	0.162	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.742	%
SELENIUM	0.297	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0121626-4
Lot Code: AUG 01 01 3B
Entered: 8/1/2001

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	5.84	%
FIBER (CRUDE)	4.33	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.066	PPM
CALCIUM	0.996	%
LEAD	0.156	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.718	%
SELENIUM	0.321	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.03
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0122383-3
Lot Code: AUG 13 01 3C
Entered: 8/15/2001

Assay	Analysis	Units
PROTEIN	20.9	%
FAT ACID (HYDRO.)	5.67	%
FIBER (CRUDE)	4.62	%
ARSENIC	0.223	PPM
CADMIUM	0.052	PPM
CALCIUM	0.918	%
LEAD	0.147	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.629	%
SELENIUM	0.263	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0122383-2
Lot Code: AUG 13 01 3B
Entered: 8/15/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.33	%
FIBER (CRUDE)	4.68	%
ARSENIC	0.229	PPM
CADMIUM	0.055	PPM
CALCIUM	0.901	%
LEAD	0.163	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.666	%
SELENIUM	0.250	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.03
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0123118-2
Lot Code: AUG 28 01 2B
Entered: 8/30/2001

Assay	Analysis	Units
PROTEIN	22.6	%
FAT ACID (HYDRO.)	5.59	%
FIBER (CRUDE)	4.8	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.055	PPM
CALCIUM	0.869	%
LEAD	0.14	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.780	%
SELENIUM	0.129	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.07
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0123118-3
Lot Code: AUG 28 01 2C
Entered: 8/30/2001

Assay	Analysis	Units
PROTEIN	22.6	%
FAT ACID (HYDRO.)	5.46	%
FIBER (CRUDE)	4.97	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.057	PPM
CALCIUM	0.784	%
LEAD	0.144	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.763	%
SELENIUM	0.242	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.05
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0123327-1
Lot Code: SEP 01 01 3A
Entered: 9/4/2001

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	5.72	%
FIBER (CRUDE)	4.35	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.054	PPM
CALCIUM	0.866	%
LEAD	0.143	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.693	%
SELENIUM	0.329	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0124569-2
Lot Code: OCT 01 01 1B
Entered: 9/28/2001

Assay	Analysis	Units
PROTEIN	21.7	%
FAT ACID (HYDRO.)	5.79	%
FIBER (CRUDE)	4.31	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.053	PPM
CALCIUM	0.953	%
LEAD	0.176	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.681	%
SELENIUM	0.263	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0125416-2
Lot Code: OCT 10 01 1B
Entered: 10/12/2001

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	5.58	%
FIBER (CRUDE)	4.45	%
ARSENIC	<0.200	PPM
CADMIUM	0.059	PPM
CALCIUM	0.913	%
LEAD	0.174	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.602	%
SELENIUM	0.295	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0125416-3
Lot Code: OCT 10 01 1C
Entered: 10/12/2001

Assay	Analysis	Units
PROTEIN	21.3	%
FAT ACID (HYDRO.)	5.75	%
FIBER (CRUDE)	4.2	%
ARSENIC	<0.200	PPM
CADMIUM	0.057	PPM
CALCIUM	1.01	%
LEAD	0.174	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.643	%
SELENIUM	0.309	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	LESS THAN 0.02
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0126324-3
Lot Code: NOV 01 01 3C
Entered: 11/1/2001

Assay	Analysis	Units
PROTEIN	21.5	%
FAT ACID (HYDRO.)	5.5	%
FIBER (CRUDE)	4.69	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.053	PPM
CALCIUM	1.04	%
LEAD	0.185	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.709	%
SELENIUM	0.401	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.15
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0127004-3
Lot Code: NOV 13 01 1C
Entered: 11/14/2001

Assay	Analysis	Units
PROTEIN	21.2	%
FAT ACID (HYDRO.)	5.65	%
FIBER (CRUDE)	4.18	%
ARSENIC	0.212	PPM
CADMIUM	LESS THAN 0.05	PPM
CALCIUM	1.03	%
LEAD	0.177	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.712	%
SELENIUM	0.288	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.17
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.

For additional information, please contact:
1) Customer Service at (314) 962-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed.
The use of the term "Less Than" does not imply that traces of analyte were present.

Elizabethtown Water Company
Physical & Chemical Analyses

Date April 4, 2001

:eral Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 7:45 a.m.4-4-01, MT

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	49							
Turbidity (NTU)	0.5 NTU	0.04							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	3.5/2Cc (1)							
Threshold Taste	3 TTN *	3.4							
Conductivity (micromhos / cm)	-	320							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	82							
Alkalinity	-	33							
pH	6.5-8.5 *	5.[illegible]							
[illegible]rine, Free / Total (mg / l Cl)	-	<0.05/1.20							
Calcium (as mg / l CaCO3)	-	50							
Magnesium (as mg / l CaCO3)	-	32							
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05							
Sulfates (mg / l SO4)	250 mg/ l *	40.3							
Chlorides (mg / l Cl)	250 mg/ l *	48.0							
Fluoride (mg / l F)	1.2 mg / l	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	196							
Total Suspended Solids (mg / l)	-	<0.1							
Ammonia Nitrogen (mg / l N)	-	0.06							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.90							
Dissolved Oxygen (mg / l O2)	-	11.3							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	<0.05							
[illegible]nganese (mg / l MN)	-	<0.05							
T.O.C. (mg / l)	-	1.6							

EXACT COPY
gm 18 Apr 02

REVIEWED
TM 30 Apr 01

* Secondary or Recommended MCL

Laboratory Analyst: KK

(1) taste and odor are non-regulated parameters which are not required to monitor by Elizabethtown TM 30 Apr 01

Revised 05/05/96

Elizabethtown Water Company
Physical & Chemical Analyses

Date May 2, 2001

General Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 7:35 a.m.5-2-01, KK

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	69							
Turbidity (NTU)	0.5 NTU	0.10							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	4.8/4Cc							
Threshold Taste	3 TTN *	4.7 (1)							
Conductivity (micromhos / cm)	-	354							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	106							
Alkalinity	-	49							
pH	6.5-8.5 *	6.6							
lorine, Free / Total (mg / l Cl)	-	2.1/2.2							
C m (as mg / l CaCO3)	-	70							
Magnesium (as mg / l CaCO3)	-	36							
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05							
Sulfates (mg / l SO4)	250 mg/ l *	61.7							
Chlorides (mg / l Cl)	250 mg/ l *	59.4							
Fluoride (mg / l F)	1.2 mg / l	<0.10							
Total Dissolved Solids (mg / l)	500 mg / l *	234							
Total Suspended Solids (mg / l)	-	0.7							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	0.73							
Dissolved Oxygen (mg / l O2)	-	8.4							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	<0.05							
nganese (mg / l MN)	-	<0.05							
(mg / l)	-	1.5							

EXACT COPY

REVIEWED 14 May 01

* Secondary or Recommended MCL

(1) Taste + odor are non-regulated parameters which are not required to monitor by Elizabethtown

Laboratory Analyst: Ed

14 May 01

Revised 08/05/98

Elizabethtown Water Company
Physical & Chemical Analyses

Date June 5, 2001

eral Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 7:50 a.m.6-5-01, MJ

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	66							
Turbidity (NTU)	0.5 NTU	0.11							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	3.1/2Cc (1)							
Threshold Taste	3 TTN *	2.8							
Conductivity (micromhos / cm)	-	293							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	80							
Alkalinity	-	38							
pH	6.5-8.5 *	7.2							
rine, Free / Total (mg / l Cl)	-	<0.05/1.05							
Calcium (as mg / l CaCO3)	-	60							
Magnesium (as mg / l CaCO3)	-	20							
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05							
Sulfates (mg / l SO4)	250 mg/ l *	39.7							
Chlorides (mg / l Cl)	250 mg/ l *	32.1							
Fluoride (mg / l F)	1.2 mg / l	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	230							
Total Suspended Solids (mg / l)	-	0.2							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.5							
Dissolved Oxygen (mg / l O2)	-	9.1							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	<0.05							
ganese (mg / l MN)	-	<0.05							
T.O.C. (mg / l)	-	1.7							

EXACT COPY
18 Apr 02

REVIEWED
26 June 01

* Operational or Recommended MCL

(1) Taste and odor are non-regulated parameters for which monitoring is not required by Elizabethtown

Elizabethtown Water Company
Physical & Chemical Analyses

Date July 10, 2001

C ·al Source Raritan-Millstone Plant

Sa...ple No. 1 Plant Delivered Water - 8:00 a.m.7-10-01, Ed

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	77							
Turbidity (NTU)	0.5 NTU	0.20							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	3.2/2Cc							
Threshold Taste	3 TTN *	3.1 (1)							
Conductivity (micromhos / cm)	-	352							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	108							
Alkalinity	-	52							
pH	6.5-8.5 *	7.1							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/1.10							
C n (as mg / l CaCO3)	-	82							
Magnesium (as mg / l CaCO3)	-	26							
Iron, Total (mg / l Fe)	0.3 mg/ l *	0.05							
Sulfates (mg / l SO4)	250 mg/ l *	49.9							
Chlorides (mg / l Cl)	250 mg/ l *	33.1							
Fluoride (mg / l F)	1.2 mg / l	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	248							
Total Suspended Solids (mg / l)	-	0.1							
Ammonia Nitrogen (mg / l N)	-	0.06							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.25							
Dissolved Oxygen (mg / l O2)	-	8.4							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	<0.05							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	0.36							
Manganese (mg / l MN)	-	<0.05							
...(mg / l)	-	2.1							

EXACT COPY
@ 18 Apr 02

REVIEWED
TM 26 July 01

* Secondary or Recommended MCL

(1) Taste + odor are non-regulated parameters for which monitoring is not required by Elizabethtown
TM 7/26/01

Laboratory Analyst: MT

Revised 08/05/98

Elizabethtown Water Company
Physical & Chemical Analyses

Date August 7, 2001

General Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 7:45 a.m.8-7-01, MT

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	82							
Turbidity (NTU)	0.5 NTU	0.25							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	3.5/2Cc							
Threshold Taste	3 TTN *	3.2 (1)							
Conductivity (micromhos / cm)	-	310							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	106							
Alkalinity	-	53							
pH	6.5-8.5 *	7.1							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/1.30							
Calcium (as mg / l CaCO3)	-	70							
Magnesium (as mg / l CaCO3)	-	36							
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05							
Sulfates (mg / l SO4)	250 mg/ l *	47.7							
Chlorides (mg / l Cl)	250 mg/ l *	33.1							
Fluoride (mg / l F)	1.2 mg / l	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	252							
Total Suspended Solids (mg / l)	-	0.5							
Ammonia Nitrogen (mg / l N)	-	0.06							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.20							
Dissolved Oxygen (mg / l O2)	-	6.9							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	0.83							
Manganese (mg / l MN)	-	<0.05							
T.O.C. (mg / l)	-	1.8							

EXACT COPY 18 Apr 02

REVIEWED TMS 8/9/01

* Secondary or Recommended MCL

(1) Taste/odor are not regulated parameters TMS 8/9/01

Laboratory Analyst: KK

Revised 06/05/96

Elizabethtown Water Company
Physical & Chemical Analyses

Date September 11, 2001

General Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 8:00 a.m.9-11-01, ED

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	76							
Turbidity (NTU)	0.5 NTU	0.11							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	3.1/1Cc	(1)						
Threshold Taste	3 TTN *	3.0							
Conductivity (micromhos / cm)	-	356							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	110							
Alkalinity	-	49							
pH	6.5-8.5 *	7.0							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/1.00							
C[illegible] (as mg / l CaCO3)	-	84							
Magnesium (as mg / l CaCO3)	-	26							
Iron, Total (mg / l Fe)	0.3 mg/ l *	0.05							
Sulfates (mg / l SO4)	250 mg/ l *	55.0							
Chlorides (mg / l Cl)	250 mg/ l *	36.0							
Fluoride (mg / l F)	1.2 mg / l	0.12							
Total Dissolved Solids (mg / l)	500 mg / l *	278							
Total Suspended Solids (mg / l)	-	0.2							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	0.94							
Dissolved Oxygen (mg / l O2)	-	8.0							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	0.22							
Manganese (mg / l MN)	-	<0.05							
[illegible] (mg / l)	-	1.9							

EXACT COPY
18 Apr 02

REVIEWED
10 Oct 01

* Secondary or Recommended MCL

(1) Taste + odor are non-regulated parameters for which monitoring is not required by Elizabethtown
10 Oct 01

Laboratory Analyst: MT

Revised 08/05/98

Elizabethtown Water Company
Physical & Chemical Analyses

Date October 2, 2001

General Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 8:00 a.m. 10-2-01, ED

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	64							
Turbidity (NTU)	0.5 NTU	0.13							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	3.0/1Cc							
Threshold Taste	3 TTN *	3.0							
Conductivity (micromhos / cm)	-	362							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	114							
Alkalinity	-	50							
pH	6.5-8.5 *	6.8							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/0.95							
[illegible] (as mg / l CaCO3)	-	82							
Magnesium (as mg / l CaCO3)	-	32							
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05							
Sulfates (mg / l SO4)	250 mg/ l *	55.5							
Chlorides (mg / l Cl)	250 mg/ l *	35.2							
Fluoride (mg / l F)	1.2 mg / l	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	282							
Total Suspended Solids (mg / l)	-	0.3							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.24							
Dissolved Oxygen (mg / l O2)	-	10.4							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	0.12							
Manganese (mg / l MN)	-	<0.05							
T[illegible] (mg / l)	-	1.9							

REVIEWED TM 29 Oct 01

EXACT COPY
18 Apr 02

* Secondary or Recommended MCL

Laboratory Analyst: MT

Revised 08/05/96

Elizabethtown Water Company
Physical & Chemical Analyses

Date November 6, 2001

eral Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 7:30 a.m. 11-6-01, KK

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	55							
Turbidity (NTU)	0.5 NTU	0.12							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	2.9/2Cc							
Threshold Taste	3 TTN *	3.1 ①							
Conductivity (micromhos / cm)	-	328							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	106							
Alkalinity	-	50							
pH	6.5-8.5 *	6.8							
Chlorine, Free / Total (mg / l Cl)	-	<0.05/0.85							
.um (as mg / l CaCO3)	-	74							
Magnesium (as mg / l CaCO3)	-	32							
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05							
Sulfates (mg / l SO4)	250 mg/ l *	55.5							
Chlorides (mg / l Cl)	250 mg/ l *	38.1							
Fluoride (mg / l F)	1.2 mg / l	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	280							
Total Suspended Solids (mg / l)	-	0.88							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg /l	0.91							
Dissolved Oxygen (mg / l O2)	-	10.9							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	<0.05							
Manganese (mg / l MN)	-	<0.05							
.C. (mg / l)	-	1.36							

EXACT COPY REVIEWED
18Apr02 TM 3Dec01

* Secondary or Recommended MCL

① Taste is a nonregulated parameter for which Elizabethtown is not required to monitor TM 3Dec01

Laboratory Analyst: Ed

Revised 08/05/96

Elizabethtown Water Company
Physical & Chemical Analyses

Date December 12, 2001

ral Source Raritan-Millstone Plant

Sample No. 1 Plant Delivered Water - 7:30 a.m. 12-12-01, Ed

Sample No. 2

Sample No. 3

Sample No. 4

Sample No. 5

Sample No. 6

Sample No. 7

Sample No. 8

Parameter	MCL (mg/l)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	49							
Turbidity (NTU)	0.5 NTU	0.09							
Color	10 *	1							
Threshold Odor 40 ° C	3 TON *	3.3/1Cc							
Threshold Taste	3 TTN *	3.3 (1)							
Conductivity (micromhos / cm)	-	365							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	116							
Alkalinity	-	53							
pH	6.5-8.5 *	6.9							
C[illegible]rine, Free / Total (mg / l Cl)	-	<0.05/1.16							
C[illegible]lcium (as mg / l CaCO3)	-	86							
Magnesium (as mg / l CaCO3)	-	30							
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05							
Sulfates (mg / l SO4)	250 mg/ l *	59.4							
Chlorides (mg / l Cl)	250 mg/ l *	34.5							
Fluoride (mg / l F)	1.2 mg / l	<0.05							
Total Dissolved Solids (mg / l)	500 mg / l *	258							
Total Suspended Solids (mg / l)	-	0.40							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	1.30							
Dissolved Oxygen (mg / l O2)	-	11.5							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-							
Surfactants (mg / l LAS)	0.5 mg / l *	-							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	-	<0.010							
Phosphate (mg / l PO3)	-	0.81							
[illegible]anese (mg / l MN)	-	<0.05							
T.O.C. (mg / l)	-	2.10							

EXACT COPY REVIEWED

13 Apr 02 31 Jan 02

* Secondary or Recommended MCL

Laboratory Analyst: Ed

(1) Taste and odor are not regulated parameters not required to be monitored by Elizabethtown

Revised 08/05/98

Elizabethtown Water Company
Physical & Chemical Analyses

Date January 22, 2002

General Source	Raritan-Millstone Plant
Sample No. 1	Plant Delivered Water - 8:00 a.m. 1-22-02, KK
Sample No. 2	Plant Delivered Water - 8:00 a.m. 2-20-02, MT
Sample No. 3	Plant Delivered Water - 7:30 a.m. 3-12-02, KK/AS
Sample No. 4	
Sample No. 5	
Sample No. 6	
Sample No. 7	
Sample No. 8	

Parameter	MCL (mg/l)	Jan. 1.0	Feb. 2.0	Mar. 3.0	4.0	5.0	6.0	7.0	8.0
Temperature ° F	-	38	41	45					
Turbidity (NTU)	0.5 NTU	0.17	0.13	0.25					
Color	10 *	2	1	2					
Threshold Odor 40 ° C	3 TON *	2.2/1Cc	3.0/2cc	2.8/1cc					
Threshold Taste	3 TTN *	2.6	2.9	2.8					
Conductivity (micromhos / cm)	-	492	399	399					
Hardness, Total (as mg / l CaCO3)	250 mg / l *	118	120	118					
Alkalinity	-	45	64	39					
pH	6.5-8.5 *	6.9	7.1	7.0					
Chlorine, Free / Total (mg / l Cl)	-	<0.05/0.53	<0.05/1.80	<0.05/0.75					
ium (as mg / l CaCO3)	-	76	80	80					
Magnesium (as mg / l CaCO3)	-	42	40	38					
Iron, Total (mg / l Fe)	0.3 mg/ l *	<0.05	0.06	<0.05					
Sulfates (mg / l SO4)	250 mg/ l *	25.8	54.8	50.4					
Chlorides (mg / l Cl)	250 mg/ l *	89.9	63.3	45.1					
Fluoride (mg / l F)	1.2 mg / l	<0.01	<0.10	<0.1					
Total Dissolved Solids (mg / l)	500 mg / l *	320	298	262					
Total Suspended Solids (mg / l)	-	0.1	0.1	0.1					
Ammonia Nitrogen (mg / l N)	-	0.08	0.07	<0.05					
Nitrate Nitrogen (mg / l N)	10 mg / l	1.7	1.79	1.8					
Dissolved Oxygen (mg / l O2)	-	12.6	11.6	11.0					
BOD 5 (mg / l O2)	-	-	-	-					
Langelier Index	+/- 1.0	-	-	-					
Surfactants (mg / l LAS)	0.5 mg / l *	-	-	-					
Hydrogen Sulfide (mg / l H2S as S)	-	0	0	0					
Nitrite Nitrogen (mg / l N)	-	<0.010	<0.010	<0.010					
Phosphate (mg / l PO3)	-	<0.05	<0.05	<0.05					
Manganese (mg / l MN)	-	<0.05	<0.05	<0.05					
J.C. (mg / l)	-	1.9	1.6	1.6					

EXACT COPY
18 Apr 02

REVIEWED
9 Apr 02

* Secondary or Recommended MCL

Laboratory Analyst: KK/MT

Revised 08/05/98

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3681567

Collected:09/06/2001 10:45 by SA

Account Number: 02698

Submitted: 09/06/2001 18:50
Reported: 09/21/2001 at 15:54
Discard: 10/06/2001
#1 Rm. 803 INH Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water.

803IN SDG#: ANI27-03

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Method Detection Limit	Units	Dilution Factor
01055	Lead (furnace method)	7439-92-1	0.0062	0.00077	mg/l	1
	The action level for lead in the lead and copper rule is 0.015 mg/l. Because health effects are possible, especially in young children, EPA guidance recommends that corrective action be taken when the action level is met or exceeded.					
01753	Copper	7440-50-8	0.0580	0.0024	mg/l	1
	The action level for copper in the lead and copper rule is 1.3 mg/l. Copper at these levels is not considered a direct health hazard, but can affect the taste of the water. Excessive copper levels may indicate a corrosive water if the system has copper plumbing.					

State of New Jersey Lab Certification No. 77011

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
01055	Lead (furnace method)	EPA 200.9	1	09/14/2001 23:35	Jessica L. Boyd	1
01753	Copper	EPA 200.7	1	09/14/2001 19:57	Brian L. Boyd	1
05281	Undigested Sample Prep	EPA 200.9	1	09/13/2001 08:45	Deborah A. Krady	1

EXACT COPY
gp 18 Apr 02

REVIEWED
M 27 Sep 01

MEMBER ACIL

Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3681569

Collected:09/06/2001 11:10 by SA

Account Number: 02698

Submitted: 09/06/2001 18:50
Reported: 09/21/2001 at 15:54
Discard: 10/06/2001
#1 Rm.705 V-1 Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water.

705V1 SDG#: ANI27-05

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Method Detection Limit	Units	Dilution Factor
01055	Lead (furnace method)	7439-92-1	N.D.	0.00077	mg/l	1
	The action level for lead in the lead and copper rule is 0.015 mg/l. Because health effects are possible, especially in young children, EPA guidance recommends that corrective action be taken when the action level is met or exceeded.					
01753	Copper	7440-50-8	0.0156 J	0.0024	mg/l	1
	The action level for copper in the lead and copper rule is 1.3 mg/l. Copper at these levels is not considered a direct health hazard, but can affect the taste of the water. Excessive copper levels may indicate a corrosive water if the system has copper plumbing.					

State of New Jersey Lab Certification No. 77011

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
01055	Lead (furnace method)	EPA 200.9	1	09/14/2001 23:56	Jessica L. Boyd	1
01753	Copper	EPA 200.7	1	09/14/2001 20:06	Brian L. Boyd	1
05281	Undigested Sample Prep	EPA 200.9	1	09/13/2001 08:45	Deborah A. Krady	1

EXACT COPY
18 Apr 02

REVIEWED
27 Sep 01

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3725257

Collected:11/12/2001 10:05 by GA

Account Number: 02698

Submitted: 11/12/2001 17:30
Reported: 11/29/2001 at 21:33
Discard: 12/14/2001
#2 INH 803 Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
00307	Heterotrophic Plate Count	n.a.	< 1.	1.	cfu/ml	n.a.
	This result is an estimated count. All plates used to calculate the result are outside the established counting range of 30 to 300 colony forming units (cfu) per dilution.					
00564	Pseudomonas aeruginosa	n.a.	Absent	1.	/gram	n.a.
	A 100 gram sample was analyzed for Pseudomonas aeruginosa. The result was negative.					
06477	Total Coliform	n.a.	See Below	1.	/100ml	n.a.
	Total Coliform	Negative	/100ml			
	E. coli	Negative	/100ml			

The water this test result represents is considered BACTERIOLOGICALLY SAFE for drinking according to standards established by the Environmental Protection Agency (EPA). If the source of your water supply is a well, we recommend that you retest your well water every 6 to 12 months to verify that it continues to be bacteriologically safe.

State of New Jersey Lab Certification No. 77011

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
00307	Heterotrophic Plate Count	Standard Methods, 19th ed 1995	1	11/12/2001 20:15	Connie D. Rhodes	n.a.
00564	Pseudomonas aeruginosa	Reference: USP, 23 rd Ed 1995	1	11/13/2001 08:20	Earl R. Custer	n.a.
06477	Total Coliform	Standard Methods 19th ed, 1995	1	11/12/2001 20:50	Connie D. Rhodes	n.a.

EXACT COPY

REVIEWED
TM 4Dec01
BApr02

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3725259

Collected:11/12/2001 10:35 by GA

Account Number: 02698

Submitted: 11/12/2001 17:30
Reported: 11/29/2001 at 21:34
Discard: 12/14/2001
#4 V-1 705 Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Limit of Quantitation	Units	Dilution Factor
00307	Heterotrophic Plate Count	n.a.	1.	1.	cfu/ml	n.a.
	This result is an estimated count. All plates used to calculate the result are outside the established counting range of 30 to 300 colony forming units (cfu) per dilution.					
00564	Pseudomonas aeruginosa	n.a.	Absent	1.	/gram	n.a.
	A 100 gram sample was analyzed for Pseudomonas aeruginosa. The result was negative.					
06477	Total Coliform	n.a.	See Below	1.	/100ml	n.a.
	Total Coliform	Negative	/100ml			
	E. coli	Negative	/100ml			
	The water this test result represents is considered BACTERIOLOGICALLY SAFE for drinking according to standards established by the Environmental Protection Agency (EPA). If the source of your water supply is a well, we recommend that you retest your well water every 6 to 12 months to verify that it continues to be bacteriologically safe.					

State of New Jersey Lab Certification No. 77011

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
00307	Heterotrophic Plate Count	Standard Methods, 19th ed 1995	1	11/12/2001 20:15	Connie D. Rhodes	n.a.
00564	Pseudomonas aeruginosa	Reference: USP, 23 rd Ed 1995	1	11/13/2001 08:20	Earl R. Custer	n.a.
06477	Total Coliform	Standard Methods 19th ed, 1995	1	11/12/2001 20:50	Connie D. Rhodes	n.a.

EXACT COPY REVIEWED
18 Apr 02 4 Dec 01

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3566610

Collected:03/08/2001 09:30 by GA

Account Number: 02698

Submitted: 03/08/2001 16:30
Reported: 03/23/01 at 07:08 AM
Discard: 3/31/01
#4 Rm. 805(Inh) Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water

805IN

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Method Detection Limit	Units	Dilution Factor
01055	Lead (furnace method)	7439-92-1	N.D.	0.00093	mg/l	1
	The action level for lead in the lead and copper rule is 0.015 mg/l. Because health effects are possible, especially in young children, EPA guidance recommends that corrective action be taken when the action level is met or exceeded.					
01753	Copper	7440-50-8	0.0243 J	0.0027	mg/l	1
	The action level for copper in the lead and copper rule is 1.3 mg/l. Copper at these levels is not considered a direct health hazard, but can affect the taste of the water. Excessive copper levels may indicate a corrosive water if the system has copper plumbing.					
00307	Heterotrophic Plate Count	n.a.	N.D.	1.	cfu/ml	n.a.
	This result is an estimated count. All plates used to calculate the result are outside the established counting range of 30 to 300 colony forming units (cfu) per dilution.					
00564	Pseudomonas aeruginosa	n.a.	See Below		See Below	n.a.
	A 100 gram sample was analyzed for Pseudomonas. The result was negative.					
06477	Total Coliform	n.a.	See Below	1.	/100ml	n.a.
	Total Coliform	Negative	/100ml			
	E. coli	Negative	/100ml			
	The water this test result represents is considered BACTERIOLOGICALLY SAFE for drinking according to standards established by the Environmental Protection Agency (EPA). If the source of your water supply is a well, we recommend that you retest your well water every 6 to 12 months to verify that it continues to be bacteriologically safe.					

State of New Jersey Lab Certification No. 77011

REVIEWED
TM 29 Mar 01

Respectfully submitted,

Microbiologist: Earl R. Custer
Earl R. Custer
Sr. Microbiologist/Coordinator

EXACT COPY
GP 13 Apr 02

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3566610

Collected:03/08/2001 09:30 by GA

Account Number: 02698

Submitted: 03/08/2001 16:30
Reported: 03/23/01 at 07:08 AM
Discard: 3/31/01
#4 Rm. 805(Inh) Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water

805IN

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
01055	Lead (furnace method)	EPA 200.9	1	03/15/2001 12:51	Jessica L. Boyd	1
01753	Copper	EPA 200.7	1	03/14/2001 08:23	Joanne M. Gates	1
00307	Heterotrophic Plate Count	Standard Methods, 18th ed 1992	1	03/09/2001 07:30	Karen J. Ocamb	n.a.
00564	Pseudomonas aeruginosa	Reference: USP, 23 rd Ed 1995	1	03/09/2001 11:15	Melissa L. Billings	n.a.
06477	Total Coliform	Standard Methods 19th ed, 1995	1	03/09/2001 07:30	Karen J. Ocamb	n.a.
05281	Undigested Sample Prep	EPA 200.9	1	03/13/2001 23:45	Annamaria Stipkovits	1

REVIEWED
TM 29 Mar 01

EXACT COPY
13 Apr 02

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3566609

Collected:03/08/2001 09:10 by GA

Account Number: 02698

Submitted: 03/08/2001 16:30
Reported: 03/23/01 at 07:08 AM
Discard: 3/31/01
#3 Rm. 706(V-1) Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water

RM706

CAT No.	Analysis Name	CAS Number	As Received Result	As Received Method Detection Limit	Units	Dilution Factor
01055	Lead (furnace method)	7439-92-1	N.D.	0.00093	mg/l	1
	The action level for lead in the lead and copper rule is 0.015 mg/l. Because health effects are possible, especially in young children, EPA guidance recommends that corrective action be taken when the action level is met or exceeded.					
01753	Copper	7440-50-8	0.0093 J	0.0027	mg/l	1
	The action level for copper in the lead and copper rule is 1.3 mg/l. Copper at these levels is not considered a direct health hazard, but can affect the taste of the water. Excessive copper levels may indicate a corrosive water if the system has copper plumbing.					
00307	Heterotrophic Plate Count	n.a.	N.D.	1.	cfu/ml	n.a.
	This result is an estimated count. All plates used to calculate the result are outside the established counting range of 30 to 300 colony forming units (cfu) per dilution.					
00564	Pseudomonas aeruginosa	n.a.	See Below		See Below	n.a.
	A 100 gram sample was analyzed for Pseudomonas. The result was negative.					
06477	Total Coliform	n.a.	See Below	1.	/100ml	n.a.
	Total Coliform	Negative	/100ml			
	E. coli	Negative	/100ml			
	The water this test result represents is considered BACTERIOLOGICALLY SAFE for drinking according to standards established by the Environmental Protection Agency (EPA). If the source of your water supply is a well, we recommend that you retest your well water every 6 to 12 months to verify that it continues to be bacteriologically safe.					

State of New Jersey Lab Certification No. 77011

REVIEWED
TM 29 Mar 01

Respectfully submitted,

Microbiologist: Earl R. Custer
Earl R. Custer
Sr. Microbiologist/Coordinator

EXACT COPY
18 Apr 02

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

Lancaster Laboratories
Where quality is a science.

Lancaster Laboratories Sample No. PW 3566609

Collected:03/08/2001 09:10 by GA

Account Number: 02698

Submitted: 03/08/2001 16:30
Reported: 03/23/01 at 07:08 AM
Discard: 3/31/01
#3 Rm. 706(V-1) Grab Water Sample

Huntingdon Life Sciences
PO Box 2360
Mettlers Road
East Millstone NJ 08875-2360

Animal Drinking Water

RM706

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
01055	Lead (furnace method)	EPA 200.9	1	03/15/2001 12:46	Jessica L. Boyd	1
01753	Copper	EPA 200.7	1	03/14/2001 08:18	Joanne M. Gates	1
00307	Heterotrophic Plate Count	Standard Methods, 18th ed 1992	1	03/09/2001 07:30	Karen J. Ocamb	n.a.
00564	Pseudomonas aeruginosa	Reference: USP, 23 rd Ed 1995	1	03/09/2001 11:15	Melissa L. Billings	n.a.
06477	Total Coliform	Standard Methods 19th ed, 1995	1	03/09/2001 07:30	Karen J. Ocamb	n.a.
05281	Undigested Sample Prep	EPA 200.9	1	03/13/2001 23:45	Annamaria Stipkovits	1

REVIEWED
TM 29 Mar 01

EXACT COPY
18 Apr 02

MEMBER ACIL
Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425
Lancaster, PA 17605-2425
717-656-2300 Fax: 717-656-2681

SteriGenics™

Certificate of Irradiation

Irradiation Facility: Westerville

Call: Production A

Customer Name: The Anderson Inc.
Customer PO Number:
Irradiation Run Date: 11/02/99
Irradiation Run Number: ANDW AND 6900013

MATERIALS PROCESSED:

Qty	Product	Description	Lot Number	LOT #
1,440	BED'O COB 1/4" BAG	10/TOTE 23.75 X 11.75 X 8"	1/4 BOC 425URB	DK219
2	SAMPLE-506		1/4 BOC 425URB	DK219
320	BED'O COB 1/8" BAG	10/TOTE	1/8 BOC 825URB	DK139
2	SAMPLE-504	53910 LARGE BAG	1/8 BOC 825URB	DK139
1,764 Bags	Total			

Minimum Dose: 25.2 kGy
Maximum Dose: 47.3 kGy

SteriGenics certifies that the materials listed above (as described by its Manufacturer) received the indicated doses within the precision and accuracy of the dosimetry system employed:

Certified By:
Date: 11/04/99

EXACT COPY
@18Apr02

ISO 9002 REGISTERED

SteriGenics International, Inc. • 303 Enterprise Drive • Westerville, Ohio 43081-8827 • 614-888-4077 • FAX 614-888-4223

NAMSA
Ensuring Medical Device Safety and Compliance™

Corp. Hdqtrs: 2261 Tracy Road, Northwood, OH 43619-1397 / 419.666.9455 / Fax 419.666.2954
3400 Cobb International Blvd., Kennesaw, GA 30152-7601 / 770.427.3101 / Fax 770.426.5692
9 Morgan, Irvine, CA 92618-2078 / 949.951.3110 / Fax 949.951.3280
Affiliates: France • Germany • Israel • Taiwan • United Kingdom

Confidential
MS005-FEE

Lab No. 99T 13976 00
P.O. No. DK089

TED WEAVER
THE ANDERSONS INC.
PO BOX 119

ID No. DK089 & DK219

MAUMEE, OH 43537-1639

STERILITY TEST
Testing Follows USP Methods or the Client's Procedures

Test Article:	Bed-O'Cobs Product Code 825RCTB (DK089) & Product Code 425RCTB (DK219)
Procedure/Test Method:	S-00165-01-00/Immersion
Test Article Received:	11-12-99
Test Start Date:	11-15-99
Test Termination Date:	11-29-99
Number of Products Tested:	2 bags

STERILITY TEST RESULTS
Test Article Identity Maintained as Submitted by Client

PRODUCT

Articles Tested	Number of Articles Tested	Type of Media	Incubation Temperature (Degrees C)	Number of Days Incubated	Number of Positive Articles
Whole Product - DK089	1	SCDB-400 ml	20-25	14	0
Whole Product - DK089	1	FTM-400 ml	30-35	14	0
Whole Product - DK219	1	SCDB-400 ml	20-25	14	0
Whole Product - DK219	1	FTM-400 ml	30-35	14	0

SCDB = Soybean Casein Digest Broth FTM = Fluid Thioglycollate Medium

Results and conclusions apply only to the test article tested. No further evaluation of these results is made by NAMSA. Any extrapolation of these data to other samples is the responsibility of the sponsor. All procedures were conducted in conformance with good laboratory practice and EN45001 Quality Standards (TÜV Product Services 1/96).

Conclusion: Test results met test acceptance criteria.

Record Storage: All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSA archive files.

Test Facility: NAMSA, 2261 Tracy Road, Northwood, OH 43619-1397.

EXACT COPY
18 Apr 02

ms Date Completed 11-29-99 Approved By Michelle R. Pierce

Michelle R. Pierce

10080

Authorization for duplication of this report, except in whole, is reserved pending NAMSA's written approval.

ILLIKER
Ensuring Quality Worldwide
ILLIKER LABORATORIES OF OHIO
1224 KINNEAR ROAD, SUITE 114 • COLUMBUS, OH 43212
TEL: 614/ 486 0150 • FAX: 614/ 486 0151

LABORATORY REPORT

REPORT NO.	DATE	PAGE
196389	12/01/99	1

PURCHASE ORDER

LOT# DK139 & DK219

MR. TED A. WEAVER
SALES MANAGER
THE ANDERSON'S, INC.
480 W. DUSSEL DR.
P.O. BOX 119
MAUMEE OH 43537

SAMPLES RECEIVED: 11/10/99
RECEIVED FROM: MAUMEE OH

REVISED DATE: 12/06/99
REISSUED DATE: 1/05/.

ANALYTICAL RESULTS

SAMPLE DESCRIPTION

BED O' COBBS
1/8" & 1/4"
COMPOSITE SAMPLE
DK 139 AND DK219

ORGANOCHLORINE PESTICIDE SCREEN

PESTICIDE	AMOUNT (PPM)	MDL (PPM)	TOLERANCE (PPM)
A,B,D-BHC	ND	0.05	
ACETOCLOR	ND	0.02	
ALACHLOR (LASSO)	ND	0.02	
ALERT (PIRATE)	ND	0.04	
ALDRIN	ND	0.01	
BANFLURALIN (BALAN, BENEFIN)	ND	0.03	
BHC (BENZAHEX)	ND	0.02	
BIFENOX (MODOWN)	ND	0.05	
BIFENTHRIN	ND	0.04	
BROMACIL	ND	0.1	
CAPTAFOL	ND	0.04	
CAPTAN	ND	0.05	
CHLORDANE	ND	0.25	
CHLOROBENZILATE (ACRABEN)	ND	0.4	
CHLORTHALONIL (BRAVO)	ND	0.01	
CYANAZINE	ND	0.1	
CYFLUTHRIN	ND	0.15	
CYPERMETHRIN	ND	0.05	
DACTHAL (CHLORTHAL)	ND	0.03	
DDD	ND	0.03	
DDE	ND	0.05	
DDT	ND	0.05	
DICHLOBENIL (CASORON)	ND	0.03	
DICHLONE	ND	0.05	
DICLORAN (BOTRAN)	ND	0.02	
DICOFOL (KELTHANE)	ND	0.05	
DIELDRIN	ND	0.01	
DYRENE (ANILAZINE)	ND	0.1	
ENDOSULFAN ALPHA	ND	0.01	
ENDOSULFAN BETA	ND	0.01	
ENDOSULFAN SULFATE	ND	0.01	
ENDRIN	ND	0.01	

EXACT COPY
18 Apr 02

The results of this report relate only to the samples tested. This report shall not be reproduced unless written approval has been obtained from the laboratory.

* CONTINUED *

SILLIKER
Securing Quality Worldwide
SILLIKER LABORATORIES OF OHIO
1224 KINNEAR ROAD, SUITE 114 • COLUMBUS, OH 43212
TEL: 614/ 486 0150 • FAX: 614/ 486 0151

LABORATORY REPORT

REPORT NO.	DATE	PAGE
196389	12/01/99	2

PURCHASE ORDER

LOT# DK139 & DK219

CONTINUED:

ANALYTICAL RESULTS

SAMPLE DESCRIPTION			
BED O' COBBS	ESFENVALERATE (ASANA)	ND	0.02
1/8" & 1/4"	ETHALFLURALIN (SONALAN)	ND	0.03
COMPOSITE SAMPLE	FLUVALINATE	ND	0.4
DK 139 AND DK219	FOLPET	ND	0.05
	HEPTACHLOR	ND	0.01
	HEPTACHLOR EPOXIDE	ND	0.02
	IPRODIONE (ROVRAL)	ND	0.05
	LINDANE (GAMMA-BHC)	ND	0.02
	LINURON	ND	0.05
	METHOXYCHLOR	ND	0.05
	METRIBUZIN	ND	0.02
	MIREX	ND	0.02
	MYCLOBUTANIL (RALLY)	ND	0.05
	NITROFEN (TOK)	ND	0.05
	OXADIAZON (RONSTAR)	ND	0.05
	OXYFLUORFEN (GOAL)	ND	0.04
	PENDAMETHALIN	ND	0.02
	PENTACHLORONITROBENZENE (PCNB)	ND	0.02
	PERMETHRIN	ND	0.05
	PERTHANE (ETHYLAN)	ND	0.05
	POLYCHLORINATED BIPHENYLS	ND	0.25
	PROCYMIDONE	ND	0.05
	PROFLURALIN (TOLBAN)	ND	0.03
	PRONAMIDE (KERB, PROPYZAMIDE)	ND	0.2
	PYRETHRINS (TOTAL)	ND	0.1
	TETRADIFON (TEDION)	ND	0.05
	TOXAPHENE (ATTAC) STROBANE	ND	0.25
	TRIDIMEFON (BAYLETON)	ND	0.05
	TRIFLURALIN (TREFLAN)	ND	0.02
	VEGADEX (DIETHYLDITHIOCARB. ACID)	ND	0.05
	VINCLOZOLIN (RONILAN)	ND	0.02

MICHELE SMOOT, PH.D.
LABORATORY DIRECTOR

The results of this report relate only to the samples tested. This report shall not be reproduced unless written approval has been obtained from the laboratory.

EXACT COPY
18 Apr 02

ILLIKER
Ensuring Quality Worldwide
ILLIKER LABORATORIES OF OHIO
1224 KINNEAR ROAD, SUITE 114 • COLUMBUS, OH 43212
TEL: 614/ 486 0150 • FAX: 614/ 486 0151

LABORATORY REPORT

REPORT NO.	DATE	PAGE
196389	12/01/99	1

PURCHASE ORDER
LOT# DK139 & DK219

SAMPLES RECEIVED: 11/10/99
RECEIVED FROM: MAUMEE OH

REVISED DATE: 12/06/99
REISSUED DATE: 1/05/00

MR. TED A. WEAVER
SALES MANAGER
THE ANDERSON'S, INC.
480 W. DUSSEL DR.
P.O. BOX 119
MAUMEE OH 43537

ANALYTICAL RESULTS

SAMPLE DESCRIPTION

BED O' COBBS
1/8" & 1/4"
COMPOSITE SAMPLE
DK 139 AND DK219

ORGANOPHOSPHATE/ORGANONITROGEN SCREEN

PESTICIDE	AMOUNT (PPM)	MDL (PPM)	TOLERANCE (PPM)
ACEPHATE	ND	0.05	
AMETRYN	ND	0.05	
ATRAZINE	ND	0.05	
AZINPHOS-METHYL (GUTHION)	ND	0.05	
BENTHIOCARB	ND	0.1	
BOLSTAR (SULPROFOS)	ND	0.05	
CARBOPHENOTHION (TRITHION)	ND	0.05	
CHLORFENVINPHOS (SUPONA)	ND	0.05	
CHLOROPROPHAM	ND	0.05	
CHLORPYRIFOS (DURSBAN)	ND	0.03	
CHLORPYRIFOS-METHYL	ND	0.05	
CIODRIN (CROTOPHOS)	ND	0.05	
COUMAPHOS (CO-RAL)	ND	0.04	
CYANAZINE (BLADEX)	ND	0.05	
DEF	ND	0.05	
DEMETON (SYSTOX) O/S ANALOGUES	ND	0.05	
DIAZINON	ND	0.03	
DIBROM (NALED)	ND	0.05	
DICROTOPHOS (DIDRIN)	ND	0.05	
DIMETHOATE (CYGON)	ND	0.03	
DIOXATHION (DELNAV)	ND	0.05	
DIPHENYL AMINE	ND	0.1	
DISULFOTON (DISYSTON)	ND	0.05	
EPN	ND	0.05	
ETHION	ND	0.05	
ETHOPROP (MOCAP)	ND	0.05	
FENAMIPHOS (NEMACUR)	ND	0.05	
FENITROTHION (SUMITHION)	ND	0.05	
FENITHION (BAYTEX)	ND	0.05	
FONOFOS (DYFONATE)	ND	0.05	
HEXAZINONE (VELPAR)	ND	0.05	

The results of this report relate only to the samples tested. This report shall not be reproduced unless written approval has been obtained from the laboratory.

* CONTINUED *

EXACT COPY
18 Apr 02

ILLIKER
...uring Quality Worldwide
ILLIKER LABORATORIES OF OHIO
1224 KINNEAR ROAD, SUITE 114 • COLUMBUS, OH 43212
TEL: 614/ 486 0150 • FAX: 614/ 486 0151

LABORATORY REPORT

REPORT NO.	DATE	PAGE
196389	12/01/99	2

PURCHASE ORDER:
LOT# DK139 & DK219

ANALYTICAL RESULTS

SAMPLE DESCRIPTION			
BED O' COBBS 1/8" & 1/4" COMPOSITE SAMPLE DK 139 AND DK219	HOSTATHION (TRIAZOPHOS)	ND	0.05
	IMAZALIL	ND	0.05
	IMIDAN (PHOSMET)	ND	0.05
	ISOFENPHOS (OFTANOL)	ND	0.05
	MALATHION	ND	0.05
	METALAXYL	ND	0.05
	METAHAMIDOPHOS	ND	0.05
	METHIDATHION (SUPRACIDE)	ND	0.05
	METHYL PARATHION	ND	0.05
	METOLACHLOR (DUAL)	ND	0.05
	METRIBUZIN (SENCOR)	ND	0.05
	MEVINPHOS (PHOSDRIN)	ND	0.05
	MOLINATE (ORDRAM)	ND	0.05
	MYCLOBUTANIL	ND	0.05
	PARATHION	ND	0.04
	PHORATE (THIMET)	ND	0.05
	PHOSALONE (ZOLONE)	ND	0.05
	PHOSPHAMIDON (DIMECRON)	ND	0.05
	PRIMIPHOS-METHYL	ND	0.05
	PROFENOPHOS (CRUACRON)	ND	0.05
	PROMETRYNE	ND	0.05
	PROPETAMPHOS (SAFROTIN)	ND	0.05
	RONNEL (FENCHLORFOS)	ND	0.05
	SIMAZINE	ND	0.05
	TERBACIL	ND	0.5
	TETRACHLORVINPHOS (GARDONA)	ND	0.05
	THIABENDAZOLE	ND	0.05
	THIONAZIN (ZINOPHOS)	ND	0.05

ND=NONE DETECTED

EXACT COPY
18 Apr 02

ILLIKER
Delivering Quality Worldwide
ILLIKER LABORATORIES OF OHIO
1224 KINNEAR ROAD, SUITE 114 • COLUMBUS, OH 43212
TEL: 614/486 0150 • FAX: 614/486 0151

LABORATORY REPORT

REPORT NO.	DATE	PAGE
197827	12/10/99	1
PURCHASE ORDER		
LOT#		

SAMPLES RECEIVED: 12/08/99
RECEIVED FROM: MAUMEE OH

REISSUED DATE: 1/05/00

MR. TED A. WEAVER
SALES MANAGER
THE ANDERSON'S, INC.
480 W. DUSSEL DR.
P.O. BOX 119
MAUMEE OH 43537

ANALYTICAL RESULTS

SAMPLE DESCRIPTION	AEROBIC PLATE COUNT/G
BED O COBS DK 139	<10
BED O COBS DK 219	<10

MICHELE SMOOT, PH.D.
LABORATORY DIRECTOR

The results of this report relate only to the samples tested. This report shall not be reproduced unless written approval has been obtained from the laboratory.

EXACT COPY
18 Apr 02

SILLIKER
Assuring Quality Worldwide
SILLIKER LABORATORIES OF OHIO
1224 KINNEAR ROAD, SUITE 114 • COLUMBUS, OH 43212
TEL: 614/ 486 0150 • FAX: 614/ 486 0151

LABORATORY REPORT

REPORT NO.	DATE	PAGE
196389	12/01/99	1

PURCHASE ORDER
LOT# DK139 & DK219

SAMPLES RECEIVED: 11/10/99
RECEIVED FROM: MAUMEE OH

REVISED DATE: 12/06/99
REISSUED DATE: 1/05/00

MR. TED A. WEAVER
SALES MANAGER
THE ANDERSON'S, INC.
480 W. DUSSEL DR.
P.O. BOX 119
MAUMEE OH 43537

ANALYTICAL RESULTS

SAMPLE DESCRIPTION	SALMONELLA EIA METHOD SAMPLE WEIGHT	RESULT	COLIFORMS MPN/G	YEASTS/G	MOLDS/G	SHIGELLA SAMPLE WEIGHT	RESULT
BED O' COBBS 1/8" & 1/4" COMPOSITE SAMPLE DK 139 AND DK219	25G	NEGATIVE	<3	<10	<10	25G	NEGATIVE

SAMPLE DESCRIPTION	PREP METHOD	CADMIUM	LEAD	ARSENIC
BED O' COBBS 1/8" & 1/4" COMPOSITE SAMPLE DK 139 AND DK219	MICROWAVE WET ASH	<0.05 MG/100G	<0.1 MG/100G	<0.1MG/100G

SAMPLE DESCRIPTION	FUMONISIN (PPB)	VOMITOXIN	MERCURY (HYDRIDE GENERATOR)
BED O' COBBS 1/8" & 1/4" COMPOSITE SAMPLE DK 139 AND DK219	LIMITS 0.1PPM B1 0.1 B2,B3 ND*	LIMITS 0.1 PPM 0.2 DETECTED	<0.01 PPM

The results of this report relate only to the samples tested. This report shall not be reproduced unless written approval has been obtained from the laboratory.

* CONTINUED *

EXACT COPY
18 Apr 02

ELLIKER
Quality Worldwide
ELLIKER LABORATORIES OF OHIO
2224 KINNEAR ROAD, SUITE 114 • COLUMBUS, OH 43212
TEL: 614/486 0150 • FAX: 614/486 0151

LABORATORY REPORT

REPORT NO.	DATE	PAGE
196389	12/01/99	1

PURCHASE ORDER:
LOT# DK139 & DK219

SAMPLES RECEIVED: 11/10/99
RECEIVED FROM: MAUMEE OH

REVISED DATE: 12/06/99
REISSUED DATE: 1/05/00

MR. TED A. WEAVER
SALES MANAGER
THE ANDERSON'S, INC.
480 W. DUSSEL DR.
P.O. BOX 119
MAUMEE OH 43537

ANALYTICAL RESULTS

SAMPLE DESCRIPTION

BED O' COBBS
1/8" & 1/4"
COMPOSITE SAMPLE
DK 139 AND DK219

AFLATOXIN (PPB)

	TEST LIMIT	RESULT
B1	1 PPB	ND
B2	1 PPB	ND
G1	1 PPB	ND
G2	1 PPB	ND

ND=NONE DETECTED

MICHELE SMOOT, PH.D.
LABORATORY DIRECTOR

The results of this report relate only to the samples tested. This report shall not be reproduced unless written approval has been obtained from the laboratory.

EXACT COPY
18 Apr 02

PROTOCOL

BASELINE GASOLINE VAPOR CONDENSATE

A TWO-GENERATION WHOLE-BODY INHALATION REPRODUCTION TOXICITY STUDY IN RATS

CONFIDENTIAL

HLS Study No.: 00-4207
Sponsor Study No.: 211-B-2G
Final Protocol
Date: 18 April 2001

PROTOCOL SIGNATURES / PREFACE

(Confidential Information – to be distributed on a need-to-know basis)

Study Title: Baseline Gasoline Vapor Condensate: A Two-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

HLS Study No.: 00-4207

Sponsor Study No.: 211-B-2G

This is the Final Protocol. It has been reviewed and approved by:

24 April 2001

Gary M. Hoffman, B.A., D.A.B.T. Date
Study Director
Huntingdon Life Sciences (HLS)

4/23/01

Thomas M. Gray, M.S., D.A.B.T. Date
Sponsor Representative
Sponsor: American Petroleum Institute (API)
Address: 1220 L Street, Northwest
Washington, D.C. 20005-4070
Phone No.: 202-682-8480
Fax No.: 202-682-8270
Email: grayt@api.org

CONFIDENTIAL STUDY SPECIFIC INFORMATION

Ship Unused Test Substance and Empty Test Substance Containers to:

Name: Michael C. Henley
Sponsor: Chevron Research and Technology Company
Address: 100 Chevron Way
Richmond, CA 94802-0627
Phone No.: 510-242-3062
Fax No.: 510-242-5542
Email: mche@chevron.com

TABLE OF CONTENTS

1. INTRODUCTION **5**

2. STUDY PERSONNEL **5**

3. REGULATORY REFERENCES **6**

3.1. TEST GUIDELINE 6
3.2. GOOD LABORATORY PRACTICES 6
3.3. FACILITIES MANAGEMENT/ANIMAL HUSBANDRY 6
3.4. ANIMAL WELFARE ACT COMPLIANCE 6
3.5. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE: 7

4. QUALITY ASSURANCE MONITORING **7**

5. ALTERATION OF DESIGN **8**

6. PROPOSED STUDY DATES **8**

7. EXPERIMENTAL DESIGN **9**

8. TEST SUBSTANCE **10**

8.1. TEST SUBSTANCE: 10
8.2. IDENTIFICATION OF TEST SUBSTANCE: 10
8.3. ARCHIVAL SAMPLES: 10
8.4. UNUSED TEST SUBSTANCE: 10

9. TEST ANIMALS (P_0) **10**

9.1. SPECIES 10
9.2. SUPPLIER 11
9.3. JUSTIFICATION FOR TEST SYSTEM SELECTION 11
9.4. ANIMAL REQUIREMENTS/SPECIFICATIONS 11
9.5. ACCLIMATION PERIOD 11
9.6. ANIMAL HUSBANDRY 11
9.7. ANIMAL HUSBANDRY DURING EXPOSURE 13
9.8. SELECTION FOR STUDY 14
9.9. ANIMAL IDENTIFICATION 14

10. MATING, GESTATION AND LACTATION PROCEDURES **14**

10.1. ESTROUS CYCLING 14
10.2. MATING PROCEDURE 14
10.3. PARTURITION AND LACTATION 15
10.4. SELECTION OF F_1 PARENTAL GENERATION 15

11. TEST SUBSTANCE ADMINISTRATION **15**

11.1. ROUTE OF ADMINISTRATION 15
11.2. JUSTIFICATION FOR ROUTE OF ADMINISTRATION 15

11.3. JUSTIFICATION FOR EXPOSURE LEVEL SELECTION 15
11.4. FREQUENCY AND DURATION OF INHALATION ADMINISTRATION 16
11.5. TEST SUBSTANCE ADMINISTRATION 16
11.6. EXPOSURE CONCENTRATION DETERMINATION 17
11.7. PARTICLE SIZE DISTRIBUTION ANALYSIS 17
11.8. CHAMBER ENVIRONMENT 17
11.9. SUMMARY OF CHAMBER ACTIVITY 18

12. EXPERIMENTAL EVALUATIONS 18

12.1. OBSERVATIONS 18
12.2. BODY WEIGHTS 19
12.3. FEED CONSUMPTION 19
12.4. LITTER EVALUATIONS (F_1, F_2) 20

13. POSTMORTEM 21

13.1. MACROSCOPIC POSTMORTEM EXAMINATION 21

14. PRESERVATION OF RECORDS AND SPECIMENS 28

15. STATISTICAL EVALUATIONS 29

15.1. CONTINUOUS DATA 29
15.2. INCIDENCE DATA 30

16. REPORT 30

16.1. STATUS REPORT 30
16.2. FINAL REPORT 31

17. REFERENCES 32

18. APPENDIX A 33

1. INTRODUCTION

HLS Study No.	00-4207
Sponsor Study No.	211-B-2G
Study Title	Baseline Gasoline Vapor Condensate: A Two-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats
Testing Facility	Huntingdon Life Sciences Mettlers Road PO Box 2360 East Millstone, NJ 08875
Purpose	The objective of this reproduction study is to provide general information concerning the effects of a test substance on the integrity and performance of the male and female reproductive systems, including gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation, and weaning, and on the growth and development of the offspring. The study will also provide information about the effects of the test substance on neurotoxicity, neonatal morbidity and mortality, and data on prenatal and postnatal developmental toxicity.

2. STUDY PERSONNEL

Study Director:	Gary M. Hoffman, B.A., D.A.B.T. Tel.: 732-873-2550 x 2920 Fax: 732-873-3992 Email: hoffmang@Princeton.Huntingdon.com
Alternate:	Carol S. Auletta, B.A., D.A.B.T. x2960 Senior Director of Toxicology

Additional personnel will be documented in the project file and presented in the final report.

3. REGULATORY REFERENCES

3.1. TEST GUIDELINE

This study is designed to meet or exceed the guideline requirements of the EPA (Environmental Protection Agency) Health Effects Test Guidelines, OPPTS 870.3800, Reproduction and Fertility Effects (August 1998) and US EPA GFAP Assay 79.67, CFR Vol. 59, No. 122, 27 June 1994.

3.2. GOOD LABORATORY PRACTICES

This study will be conducted in accordance with 79.60, CFR Vol. 59, No. 122, 27 June 1994. This study will be performed according to protocol and Huntingdon Life Sciences' Standard Operating Procedures.

3.3. FACILITIES MANAGEMENT/ANIMAL HUSBANDRY

Currently acceptable practices of good animal husbandry will be followed, e.g., *Guide for the Care and Use of Laboratory Animals*; National Academy Press, 1996. Huntingdon Life Sciences Inc., is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

3.4. ANIMAL WELFARE ACT COMPLIANCE

This study will comply with all appropriate parts of the Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, Federal Register, Volume 54, No. 168, August 31, 1989, pp. 36112-36163 effective October 30, 1989 and 9 CFR Part 3 Animal Welfare Standards; Final Rule, Federal Register, Volume 56, No. 32, February 15, 1991, pp. 6426-6505 effective March 18, 1991. The Sponsor should make particular note of the following:

1. The Sponsor's signature on all protocol documents for the study described herein signifies that there are no generally accepted non-animal alternatives and the study does not unnecessarily duplicate previous experiments.

2. All procedures used in this study have been designed to avoid discomfort, distress and pain to the animals. All methods are described in this study protocol or in written laboratory standard operating procedures.

3. Any procedures outlined in this study protocol which are expected to cause more than momentary or slight pain or distress to the animals will be performed with appropriate sedatives, analgesics or anesthetics unless the withholding of these agents is justified for scientific reasons, in writing, by the Sponsor and the Study Director and approved by the IACUC; in which case the procedure will continue

for the minimum time necessary. Documentation of the justification for withholding treatment for pain or distress and IACUC approval of the procedures will be made prior to study initiation on the IACUC Protocol Review form.

4. Animals experiencing more than momentary or slight pain or distress due to emergency situations such as injury or illness will be treated by the Testing Facility's veterinarian staff with approved analgesics or agents to relieve pain. If possible, the Study Director will be consulted prior to treatment; however, the veterinary staff is authorized to administer emergency treatment as necessary. Any subsequent treatment or euthanasia will be administered after consultation with the Study Director. The Sponsor will be advised by the Study Director of all emergency situations in as timely a manner as possible.

5. Methods of euthanasia used during this study are in conformance with the above referenced regulations.

6. The numbers of animals used in this study was selected to ensure that an adequate number of pregnant females are available for valid statistical interpretation of the data as required by the referenced guidelines. OPPTS 870.3800 Guidelines, Section 2, number (B), item (v) states: Each control group should contain a sufficient number of mating pairs to yield at least 20 pregnant females. Each test group should contain a similar number of mating pairs. The 26 females/group will assure us of having at least 20 pregnant females/group based on 80%- 100% pregnancy rate at HLS.

3.5. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE:

The IACUC Protocol Review Subcommittee has reviewed this protocol and found it to be in compliance with all appropriate regulations.

4. QUALITY ASSURANCE MONITORING

The Huntingdon Life Sciences Quality Assurance Unit, East Millstone, NJ will monitor the facilities, equipment, personnel, methods, practices, records and controls used in this study to assure that they are in conformance with this protocol, company standard operating procedures, and the appropriate Good Laboratory Practice regulations.

5. ALTERATION OF DESIGN

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such changes will be honored by the Testing Facility and will be followed by a written verification. All protocol modifications will be signed by the Study Director and a Sponsor representative. Any modifications potentially

affecting animal welfare will also be signed by two members of the Institutional Animal Care and Use Committee prior to the modification's implementation.

6. PROPOSED STUDY DATES

Study initiation date:	Date Study Director signs protocol
Receipt of test animals:	17 April 2001
Initiation of exposures (P): (Experimental start date)	30 April 2001
Experimental termination:	19 January 2002 (date of last data collection)
Submission of audited draft report:	17 May 2002
Study completion date:	Date final report is signed by Study Director.

7. EXPERIMENTAL DESIGN

			Number of Animals									
			Mated Adults				Microscopic Pathology Adult Generations[b]				Macroscopic Postmortem Examinations-Pups[c]	
			P_0		F_1		P_0		F_1			
Group	Group Designation	Exposure Levels (mg/m^3)[a]	M	F	M	F	M	F	M	F	F_1	F_2
I	Control	0 (air only)	26	26	26	26	10	10	10	10	3/sex/litter	3/sex/litter
II	Low	2,000	26	26	26	26	TBD	TBD	TBD	TBD	3/sex/litter	3/sex/litter
III	Middle	10,000	26	26	26	26	TBD	TBD	TBD	TBD	3/sex/litter	3/sex/litter
IV	High	20,000	26	26	26	26	10	10	10	10	3/sex/litter	3/sex/litter

[a] Exposures daily (7 days/week) for 6 hours per day.
[b] Histologic examinations will be performed on reproductive tissues for the control and high-exposure animals.
[c] F_1 pups (5/sex/group/assessment) not selected for F_1 mating will be evaluated for standard Tier 2 Neuropathology (as per 40 CFR 79.66) and GFAP assessments (as per 40 CFR 79.67). The GFAP and Neuropathology assessments will be conducted in F_1 pups on Postpartum Day 28.

M = Male; F = Female; TBD = to be determined based on Group IV evaluations; The first day of exposure will be Day 0.

8. TEST SUBSTANCE

8.1. TEST SUBSTANCE: Baseline Gasoline Vapor Condensate

Description, lot number, storage, expiration date (if available) and handling procedures, as well as other pertinent information will be documented in the study data.

8.2. IDENTIFICATION OF TEST SUBSTANCE:

Unless otherwise noted, the identity, strength, composition, stability and method of synthesis, fabrication and/or derivation of each batch of the test substance will be documented by the Sponsor before its use in the study. This documentation will be maintained by the Sponsor at the address indicated on page 2 of this protocol. The Sponsor will conduct a purity analysis of the test substance by GC prior to the start of this study.

8.3. ARCHIVAL SAMPLES:

An archival sample from each lot of test substance will be taken and stored in the Archives of the Testing Facility. If multiple studies are conducted with the same substance, a common archival sample may be taken and appropriately labeled.

8.4. UNUSED TEST SUBSTANCE:

The unused portion of the test substance as well as any empty test substance containers will be returned to the Sponsor's designee (see page 2) following completion of the study. Empty test substance containers will be returned to the Sponsor's designee on an as needed basis. The Sponsor will be responsible for tracking their disposition.

In the event the Sponsor wishes the Testing Facility to arrange for disposal, a cost for this service will be provided.

9. TEST ANIMALS (P_0)

Albino Rats (Outbred) VAF/Plus®

9.1. SPECIES

Sprague Dawley–derived CD®
[Crl: CD® IGS BR]

9.2. SUPPLIER

Charles River Laboratories
Kingston, New York

9.3. JUSTIFICATION FOR TEST SYSTEM SELECTION

The rat is used as a surrogate to humans in the detection of reproductive effects and is a species in which known reproductive toxicants have been detected. The rat is a rodent animal model commonly utilized in reproduction studies and is recommended in the referenced guidelines. In addition, a historical control database with this strain of animal and supplier facility is available for comparative evaluation.

9.4. ANIMAL REQUIREMENTS/SPECIFICATIONS

9.4.1. NUMBER

	Total	Males	Females
Placed on test	208	104	104

9.4.2. AGE AND WEIGHT

Males and Females: Approximately four weeks at receipt; and five to six weeks at initiation of treatment. Males will weigh approximately 150-200 grams and females 125-175 grams at initiation of exposures. Animals outside this weight range will be used at the discretion of the Study Director.

Females: Nulliparous and non-pregnant

9.5. ACCLIMATION PERIOD

Approximately two weeks; all animals will be checked for viability twice daily. Prior to assignment to study all animals will be examined to ascertain suitability for study.

9.6. ANIMAL HUSBANDRY

9.6.1. HOUSING

Animals will be housed in suspended, stainless steel cages with wire mesh fronts and floors. Animals at receipt will be housed 2/cage during the initial week of acclimation. Thereafter, animals will be housed individually except as follows:

Mating: One male and one female co-housed continuously (except during treatment) until mated or 14 days have elapsed, whichever occurs first. Mating pairs will be assigned randomly.

Lactation: Dam with litter (solid plastic "shoebox" cage)

Postweaning: Single weanling/cage

9.6.2. FEED & FEED ANALYSIS

Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, MO) ad libitum.

Analytical certifications of batches of feed used during the study are provided by the manufacturer and will be maintained on file at the Testing Facility. There are no known contaminants in the feed that are expected to interfere with the results of this study.

9.6.3. WATER & WATER ANALYSIS

Facility water supply (Elizabethtown Water Company, Westfield, NJ); without restriction, via an automated water delivery system to individual animal cages.

Water analyses are conducted by Elizabethtown Water company to ensure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR part 141). Water analyses, provided by the supplier, will be maintained on file at the Testing Facility. In addition, water samples are collected biannually from representative rooms in the Testing Facility; chemical and microbiological water analyses are conducted on these samples by a subcontract laboratory. Results are maintained on file at the Testing Facility. There are no known contaminants in the water that are expected to interfere with the results of this study.

9.6.4. BEDDING SUBSTANCE

Ground corncob bedding (Bed-O'-Cobs® 1/4 inch irradiated, The Andersons, Maunee, OH) will be provided for each mated female on Day 18 of gestation. Fresh bedding will be provided at least weekly and as needed during the lactation period (litters will be weaned on Day 28 of lactation).

9.6.5. BEDDING ANALYSIS

Analyses for each batch of bedding used on study provided by the supplier will be maintained at the Testing Facility. There are no known contaminants in the bedding that are expected to interfere with the results of this study.

9.6.6. VETERINARY CARE

Animals are monitored by the technical staff for any conditions requiring possible veterinary care. If any such conditions are identified, a staff veterinarian will be notified for an examination and evaluation. Animals will be treated as outlined in the Animal Welfare Act Compliance section of this protocol.

9.6.7. ENVIRONMENTAL CONDITIONS

Light/Dark Cycle

Twelve-hour light/dark cycle daily via automatic timer.

Temperature

Temperature will be monitored in accordance with Testing Facility SOPs to ensure that the desired range of 18-26°C is maintained to the maximum extent possible.

Humidity

Humidity will be monitored in accordance with Testing Facility SOPs to ensure that the desired range of 30-70% is maintained to the maximum extent possible.

Air Changes

Air changes will be maintained at a rate of 12-15 per hour and will be recorded once prior to exposures.

9.7. ANIMAL HUSBANDRY DURING EXPOSURE

9.7.1. HOUSING Individually in cages.

9.7.2. FEED None.

9.7.3. WATER None.

9.8. SELECTION FOR STUDY

More animals than required for the study will be purchased and acclimated. Animals considered suitable for study on the basis of pretest physical examinations, body weight data and any other pretest evaluations will be randomly assigned, by sex, to control or treated groups in an attempt to equalize mean group body weights. Individual weights of animals placed on test shall not exceed 20% of the mean weight for each sex. Disposition of all animals not used in the study will be maintained in the study file.

9.9. ANIMAL IDENTIFICATION

Each animal will be assigned a temporary identification number upon receipt. After selection for study (P_0 generation), each animal will be ear-tagged with a number assigned by the Testing Facility. This number plus the study number will comprise the unique identification for each study animal. The selected F_1 parental animals will be ear-tagged with a unique number at the time of selection. If the tag is lost, it will be replaced or the animal will be tail tattooed for identification. Each animal's cage will be provided with a card that will be color-coded for exposure level identification and will contain the study number and animal number.

10. MATING, GESTATION AND LACTATION PROCEDURES

10.1. ESTROUS CYCLING

Daily vaginal smears will be taken at approximately the same time each day, and the stage of estrous will be determined for each female for three weeks prior to cohabitation for the P_0 and F_1 females. These evaluations will continue until there is evidence of mating or until the 14-day mating period ends. Care will be taken to ensure that pseudo-pregnancy is not induced.

10.2. MATING PROCEDURE

After both sexes have been exposed to the test substance for ten weeks (premating treatment period), one male and one female from the same exposure group will be caged together until a sign of mating (microscopic observation of sperm in the vaginal smear and/or a copulation plug in the vagina) is observed or for 14 consecutive days. Males and females will not be paired during the mating period while being exposed. The day evidence of mating is observed will be defined as Day 0 of gestation. If mating has not occurred after this interval, the animals will be separated without further opportunity for mating. During mating of the F_1 generation, cohabitation of male and female littermates will be prohibited.

10.3. PARTURITION AND LACTATION

On Day 18 of gestation, several days prior to expected parturition, each female will be transferred to a solid plastic "shoebox" cage. Bedding substance will be provided and changed at least weekly and as needed. Examination for signs of parturition will be made twice daily (morning and afternoon). Evidence of difficult or prolonged parturition (dystocia), if observed, will be recorded. The day on which parturition is first observed will be defined as Day 0 of lactation. Females which were caged with males but exhibited no evidence of mating, will be transferred to plastic shoe-box cages when the first animals mated reach their day 18 of gestation.

10.4. SELECTION OF F_1 PARENTAL GENERATION

At weaning of each F_1 litter, one pup/sex/litter will be chosen at random to continue on study as the F_1 parental generation. If less than 26 litters are available at weaning, additional pups will be randomly selected to provide the 26 pups/sex. In the selection procedure, macroscopically malformed or obviously-diseased animals within the litters will be excluded from the pool of animals eligible for selection if, in the judgment of the Study Director, their condition may adversely affect long-term survival. Runts, if otherwise normal, will not be excluded from the selection procedure. Pups as chosen will be housed one weanling/cage. In this selection procedure, each litter will contribute at least one pup/sex, when possible, so as to maximize the representation of animals from different litters in the parental generation in each group.

11. TEST SUBSTANCE ADMINISTRATION

11.1. ROUTE OF ADMINISTRATION

Inhalation via whole-body exposures.

11.2. JUSTIFICATION FOR ROUTE OF ADMINISTRATION

The inhalation route is one of the potential routes of human exposure to this test substance and is the route specified in the referenced guidelines.

11.3. JUSTIFICATION FOR EXPOSURE LEVEL SELECTION

Exposure levels were selected by the Sponsor, based on results from a 13-week inhalation study conducted at the Testing Facility, that utilized this test article in rats (00-6125). The exposure levels were also selected based on the lower flammable limits of the test substance.

11.4. FREQUENCY AND DURATION OF INHALATION ADMINISTRATION

Frequency: P_0 males and females will receive 70 consecutive days (ten weeks) of exposure prior to mating for six hours/day. P_0 males and females will continue to be exposed daily throughout a 14 day cohabitation period. The mated females will continue to be exposed daily from Day 0 through 19 of gestation. Beginning on day 5 of lactation, nursing P_0 females will be exposed daily until weaning of F_1 offspring. P_0 females with no confirmed day of mating would continue to be exposed for 25 days following completion of the mating period. P_0 females with a confirmed day of mating that do not deliver will be euthanized on presumed day 25 of gestation.

P_0 males will be exposed daily until euthanasia. P_0 males will be euthanized after delivery of the last F_1 litter.

Selected F_1 males and females will begin treatment at 28 days of age (day of weaning) and will continue treatment for at least 70 days (ten weeks) prior to being paired to produce the F_2 generation. Some F_1 offspring may receive additional treatments (approximately 1 to 14 treatments) depending on the date that weaning occurs. F_1 males and females will continue to be exposed throughout a 14 day cohabitation period. The mated F_1 females will continue to be exposed daily from Day 0 through 19 of gestation. F_1 females with no confirmed day of mating would continue to be exposed for 25 days following completion of the mating period. F_1 females with a confirmed day of mating that do not deliver will be euthanized on presumed day 25 of gestation.

F_1 males will be exposed daily until euthanasia. F_1 males will be euthanized after delivery of the last F_2 litter.

Duration: The test substance will be administered for 6 hours/day during all exposure periods.

11.5. TEST SUBSTANCE ADMINISTRATION

The test substance will be administered as a vapor in the breathing air of the animals. The test atmosphere will be generated by an appropriate procedure determined during pre-study trials. The trials will be performed to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the target exposure levels and maintain uniform conditions throughout the exposure chambers. The method will be described in the raw data of the study and in the report.

The whole-body exposure chambers will each have a volume of approximately 1500 liters. The chambers will be operated at a minimum flow rate of 300 liters per minute. The final airflow will be set to provide at least one air change in 5.0 minutes (12 air changes/hour) and a T_{99} equilibrium time of at most 23 minutes. This

chamber size and air flowrate is considered adequate to maintain the oxygen level at least 19% and the animal loading factor below 5%. At the end of the 6-hour exposure, all animals will remain in the chamber for a minimum of 30 minutes. During this time the chamber will be operated at approximately the same flow rate using clean air only.

11.6. EXPOSURE CONCENTRATION DETERMINATION

A nominal exposure concentration will be calculated. The flow of air through the chamber will be monitored using appropriate calibrated equipment. The test substance consumed during the exposure will be divided by the total volume of air passing through the chamber (volumetric flow rate times total exposure time) to give the nominal concentration.

During each exposure, measurements of airborne concentrations will be performed in the animals' breathing zone at least 4 times using an appropriate sampling procedure and IR analytical procedure. Also, one sample per chamber per week will be analyzed by gas chromatography (GC) to characterize at least 10 major components (comprising at least 80% by weight of the test substance) to show test substance stability and comparison between the neat liquid test substance and the vaporized test atmospheres.

If more than the normal amount of trials is required because of test substance generation or monitoring problems (80 technician hours), the Sponsor will be consulted prior to additional trials (additional cost).

11.7. PARTICLE SIZE DISTRIBUTION ANALYSIS

During each week of exposure, particle size determinations will be performed using a TSI Aerodynamic Particle Sizer to characterize the aerodynamic particle size distribution of any aerosol present.

11.8. CHAMBER ENVIRONMENT

Chamber temperature, humidity, airflow rate and static pressure will be monitored continuously and recorded every 30 minutes during exposure. Chamber temperature and relative humidity will be maintained, to the maximum extent possible, between 20 to 24°C and 40 to 60%, respectively. Chamber oxygen levels (maintained at least 19%) will be measured pretest and at the beginning, middle and end of the study.

Air samples will be taken in the vapor generation area pretest and at the beginning, middle and end of the study. Light (maintained approximately 30 foot-candles at 1.0 meter above the floor) and noise levels (maintained below 85 decibels) in the exposure room will be measured pretest and at the beginning, middle and end of the study.

11.9. SUMMARY OF CHAMBER ACTIVITY

The minimum frequency of chamber activity is summarized below:

Activity	Frequency/chamber
Measured Test Substance Concentration	4X/day
Measured Test Substance Characterization	1X/week
Particle Size	1X/week
Temperature	13X/day
Relative Humidity	13X/day
Airflow Rate	13X/day
Static Pressure	13X/day
Nominal Test Substance Concentration (excluding the air control chamber)	1X/day
Rotation Pattern of Exposure Cages	1X/week
Loading/Unloading Verification	1X/day

12. EXPERIMENTAL EVALUATIONS

12.1. OBSERVATIONS

12.1.1. VIABILITY EXAMINATIONS (CAGESIDE)

P_0 and F_1 Male and Female Parents: Observations for mortality, and signs of toxicity or pharmacological effects will be made at least twice daily (morning and afternoon). Animals in extremely poor health or in a possible moribund condition will be identified for further monitoring and possible euthanasia. All animals found dead will be submitted for a macroscopic examination. Parental animals found dead after normal working hours will be refrigerated until a necropsy can be performed.

12.1.2. DETAILED PHYSICAL EXAMINATIONS

The animals will be observed as a group at least once during each exposure.

Each P_0 and F_1 parental generation animals will be removed from its cage and examined at least once pretest (P_0), at the study start (first day of treatment for the P_0) and weekly thereafter during the study period (P_0 and F_1). For the F_1 parental generation, these weekly evaluations will start with the formal initiation of the premating treatment period on Postpartum Day 28. F_1 females will continue to be observed weekly until there is evidence of mating. Once mated, F_1 females will be observed on day 0, 7, 14 and 20 of gestation and on day 1, 4, 7, 14, 21 and 28 of lactation. F_1 females without evidence of mating will continue to be observed weekly until euthanized. Examinations will include, but not be limited to, observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia as well as evaluations of respiration, and palpation for tissue masses. During the exposure period, physical examinations will be performed post-exposure.

12.2. BODY WEIGHTS

Males:

P_0 males: At least once during pretest, at the time of randomizing into study groups, on the day treatment initiates, weekly throughout the study (including the mating period) and at termination.

F_1 males: On the day treatment initiates and weekly throughout the study (including the mating period) and at termination.

Females:

P_0 females: At least once during pretest and at the time of randomizing into study groups.

P_0 and F_1 females: On the day treatment initiates and weekly during the premating growth and mating periods; day 0, 4, 7, 14 and 20 of gestation; day 1, 4, 7, 14, 21 and 28 of lactation; and weekly thereafter until euthanasia. Females that do not deliver pups will be weighed weekly until euthanasia.

12.3. FEED CONSUMPTION

Males:

P_0: One week pretest and during the exposure period of the P_0 and F_1: weekly during the premating treatment period (food consumption will not be measured during the mating period when animals will be co-housed) and through the postmating period until euthanasia.

Females:

P_0: One week pretest and during the exposure period of the P_0 and F_1: weekly during the premating growth periods (food consumption will not be measured during the mating period when animals are co-housed) and on day 0, 4, 7, 14 and 20 of gestation and on day 1, 4, 7, 14, 21 and 28 of lactation, and weekly thereafter until euthanasia. Females without evidence of mating and that do not deliver pups will have food consumption measured weekly until euthanasia.

12.4. LITTER EVALUATIONS (F_1, F_2)

12.4.1. OBSERVATIONS

F_1 and F_2 litters will be observed as soon as possible after delivery for the number of live and dead pups, sex of the pups and pup abnormalities. The anogenital distance will be qualitatively determined on day 0 for all pups of the F_1 generation (to determine the sex). If treatment-related effect is observed in either the F_1 sex ratios or age of vaginal opening or preputial separation, the anogenital distance will be recorded for the F_2 pups (additional cost). All pups in the litter will be uniquely identified by toe tattoo on the day of delivery completion. Thereafter, litters will be observed twice daily (morning, afternoon) for the presence of dead pups. These dead pups and pups euthanized in a moribund condition will be examined to the extent possible for defects and/or cause of death and preserved in neutral, phosphate-buffered 10% formalin. Litter size will be recorded daily from Lactation Day 1 to 28.

12.4.2. CULLING PUPS

On Day 4 of lactation, each F_1 and F_2 litter with more than 10 pups will be culled to that number with sex distribution equalized (five/sex) when possible. Pups will be culled randomly. Preferential culling of runts will not be performed.

12.4.3. PHYSICAL EXAMINATIONS

Each F_1 and F_2 pup will be given a physical examination on the day of delivery completion and on day 4, 7, 14, 21 and 28 of lactation.

12.4.4. PUP BODY WEIGHT DATA

F_1 and F_2 individual pup weights will be recorded on day 1, 4 (precull intervals) and 7, 14, 21 and 28 of lactation (postcull intervals).

12.4.5. PUP SEXING DATA

F_1 and F_2 individual sexing data will be recorded on the day of delivery completion and reconfirmed, and recorded day 4, 7, 14, 21 and 28 of lactation.

12.4.6. PHYSICAL MATURATION LANDMARKS - SELECTED F_1 ANIMALS

All F_1 pups selected to become the F_1 parental animals (26 sex/group) will be observed daily for either vaginal opening (examinations will begin on Postnatal Day 28) or preputial separation (examinations will begin on Postnatal Day 35) and the body weight of each pup will be recorded when the criteria is reached.

13. POSTMORTEM

13.1. MACROSCOPIC POSTMORTEM EXAMINATION

13.1.1. PARENTAL ANIMALS (P_0, F_1)

13.1.1.1. Method of Euthanasia

Exsanguination following anesthesia with inhaled carbon dioxide.

13.1.1.2. Moribund Animals

Animals showing signs of severe debility, particularly if death appears imminent will be euthanized to prevent loss of tissues through autolysis.

13.1.1.3. Terminal Necropsy Males (P_0, F_1)

P_0 and F_1 parental males will be euthanized within 3 days after the last F_1 and F_2 litters, respectively, have been delivered. This will permit some evaluation of fertility (i.e., number of litters delivered) prior to euthanasia.

13.1.1.4. Terminal Necropsy Females (P_0, F_1)

All P_0 and F_1 parental females with litters will be euthanized after day 28 of lactation.

13.1.1.5. Macroscopic Examination

Complete macroscopic postmortem examinations will be performed on all adult animals, including animals euthanized in a moribund condition or found dead and all abnormal observations will be recorded. The necropsy of the parental animals will include examination of the external surface and all orifices; the external surfaces of the brain and spinal cord; the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. Examination of all parental P_0 females, which were co-housed with P_0 males, will include a vaginal smear examined to determine the stage of the estrous cycle and a count of uterine implantation scars, if present.

13.1.1.6. Organ Weights

The following organs will be weighed at terminal euthanasia of all the P_0 and F_1 animals in each group:

Males	Females
Epididymides (total and caudal)	Ovaries
Prostate	Uterus (with oviducts and cervix)
Seminal Vesicles with coagulating glands and their fluids	
Testes	
Adrenal Glands	
Brain	
Kidneys	
Liver	
Lungs	
Pituitary	
Spleen	

Prior to weighing, all organs will be carefully dissected and properly trimmed to remove fat and other contiguous tissue in a uniform manner. Organs will be weighed as soon as possible after dissection to avoid drying. Paired organs will be weighed together. Organ weight data will be presented as absolute values and relative to terminal body weight and brain weight. Organ weights will not be recorded for an animal dying spontaneously or euthanized moribund.

13.1.1.7. Tissues Preserved

Tissues listed following will be obtained at necropsy and preserved for all P_0 and F_1 parents:

Males	Females
Epididymis (right)	Ovaries
Prostate	Uterus (with oviducts and cervix)
Seminal Vesicles (with coagulating glands and their fluids)	Vagina
Testis (right)	
Adrenal Glands	
Lungs	
Pituitary	
Macroscopic Lesions	
Target Organs (as determined from this study and previous studies, e.g.13-week inhalation study 00-6125)	

All tissues listed above and brain, kidneys, liver and spleen will be fixed in 10% Neutral Buffered Formalin (NBF) except the testes and epididymides will be fixed in modified Davidson's solution for at least 48 hrs prior to permanent storage in NBF.

13.1.2. WEANLINGS (F_1, F_2)

13.1.2.1. Method of Euthanasia

F_1 and F_2 weanling pups will be euthanized by carbon dioxide asphyxiation.

13.1.2.2. Moribund Animals

F_1 and F_2 animals showing signs of severe debility, particularly if death appears imminent, will be euthanized to prevent loss of tissues through autolysis.

13.1.2.3. Dead and Culled Pups (F_1 and F_2)

F_1 and F_2 pups found dead at birth or during the lactation period will be examined to the extent possible for defects and/or the cause of death, and for the presence or absence of milk in the stomach. Dead pups will not be eviscerated. Viscera will remain intact and the pup will be preserved in 10% NBF. Cannibalized pups will be examined to the

extent possible and discarded. Culled F_1 and F_2 pups will be examined for external abnormalities. If unremarkable, these pups will then be euthanized via carbon dioxide asphyxiation and discarded. Culled pups with external abnormalities will be preserved intact in 10% NBF at the discretion of the Study Director.

13.1.2.4. Terminal Necropsy Pups (F_1 and F_2)

Macroscopic Examination and Tissues Preserved

F_1 and F_2 pups (3/sex/litter) will be given a macroscopic examination at weaning for any structural abnormalities or pathological changes. Special attention will be given to the organs of the reproductive system; brain, liver, kidneys, pituitary, adrenal gland, spleen, heart and thymus gland. Abnormal tissues and/or target organs, if identified, will be preserved in 10% neutral buffered formalin (NBF).

Organ Weights

The F_1 and F_2 pups (1 pup/sex/litter) for which organ weight data will be collected will be randomly selected. The following organs will be weighed from one pup that was selected for complete macroscopic examination at weaning:

brain
spleen
thymus gland

Organ weight data will be presented as absolute values and relative to terminal body weight and brain weight.

13.1.3. SPERM COUNT, MOTILITY AND MORPHOLOGY ASSESSMENTS

All P_0 and F_1 parental males euthanized at termination in each group will have the following sperm evaluations available for analysis: 1) motility; 2) a count of homogenization-resistant testicular sperm; 3) a count of caudal epididymal sperm; and 4) sperm morphology (cauda epididymis). Only samples for the high-exposure and control group will be analyzed if there is no effect in the high-exposure group. Evaluations will be performed by Pathology Associates International, Frederick, MD as follows:

- The right testis and right epididymis from each animal will be removed intact, weighed (testes weighed together) and preserved in modified Davidson's solution for at least 48 hrs prior to permanent storage in NBF for histopathological evaluation.

- The left epididymis will be removed intact, weighed, and frozen on dry ice for transport to Pathology Associates International, Frederick, MD. The epididymides will be stored frozen at -70° C until evaluation for caudal sperm count. Each epididymis will be thawed and the caudal portion removed and weighed. A homogenized sample of the caudal epididymis will be stained and examined using the Hamilton Thorne IVOS sperm analyzer. For each stained preparation, 20 fields will be counted. The total number of sperm in the caudal epididymis will be calculated and adjusted for the caudal epididymal weight. Additionally, for each male, two sperm morphology slides will be prepared, stained with Eosin and evaluated for morphological development.

- The left vas deferens will be excised and placed in a prewarmed solution of phosphate buffered saline and 1% Bovine Serum Albumin. After a minimum three minutes "swimout" period, a sample will be placed in a Hamilton Thorne IVOS sperm analyzer and five fields will be stored on an optical disk. These fields will subsequently be analyzed for percent motility.

- The left testis will be removed and frozen on dry ice for transport to PAI (Frederick, MD). The testis will be stored frozen at -20°C until processed for counting of homogenization-resistant sperm.

  Tissues retained from these evaluations will be discarded following issuance of the final report following consultation with the Sponsor. The Testing Facility will be responsible for the GLP compliance of this subcontractor. All raw data, the protocol and all reports generated at PAI will be maintained. After submission of the final report, all of the above will be shipped to Huntingdon Life Sciences to be archived.

13.1.4. MICROSCOPIC PATHOLOGY EVALUATIONS

Slides of tissues listed in the table in Section 13.1.1.7. will be prepared and stained with hematoxylin and eosin (H&E) and examined microscopically for 10 animals/sex/group from P_0 and F_1 parental animals in the control and high-exposure groups. All macroscopic lesions will be evaluated (additional cost). If microscopic findings indicative of an effect of test substance administration are seen in the initial 10 animals/sex of high-exposure animals, then examinations should be made of those tissues/organs for the remaining control and high-exposure animals and/or

all low- and middle-exposure animals (Sponsor consulted, additional cost). [Note: any abnormalities not noted during macroscopic postmortem examinations that are seen during histological processing will be recorded]. Additionally reproductive organs of the low- and middle-exposure animals suspected of reduced fertility (e.g. those that failed to mate, conceive, sire or deliver healthy offspring, or for which estrous cyclicity or sperm number motility or morphology were affected) will also be subjected to microscopic examination for additional cost at the discretion of the Sponsor.

Histopathological examinations of the testes will be conducted to identify potential treatment-related effects such as retained spermatids, missing germ cell layers or types, multinucleated giant cells, or sloughing of spermatogenic cells into the lumen. The examination of the intact epididymis will be of a longitudinal section that will permit examination of the caput, corpus and cauda regions. These examinations will identify such lesions as sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, or the absence of clear cells in the cauda epididymal epithelium.

Histopathological examination of the ovary will include evaluation of five sections taken at least 100 μm apart from the inner third of each ovary. These examinations can detect depletion of the primordial follicle population and enumerate the total number of primordial follicles for comparison with the ovaries from control animals. These examinations can also confirm the presence or absence of growing follicles and corpora lutea in comparison to control ovaries. Qualitative evaluations of the P_0 animals will be performed by HLS. Quantitative evaluations of the P_0 animals (Sponsor consulted, additional cost) and F_1 animals (required) will be performed by Pathology Associates International, Frederick, MD.

13.1.5. NEUROPATHOLOGY EVALUATIONS

F_1 pups (5/sex/group), not selected for mating, will be randomly selected by computer for Neuropathology evaluation. Selected pups will be anesthetized with an intraperitoneal injection (~1 mL/kg) of ketamine/xylazine/acepromazine and transcardially perfused with phosphate buffered saline followed by 1% glutaraldehyde and 4% paraformaldehyde in the same buffer. After perfusion, the required tissues will be dissected out. Measurement of the size (length and width) and weight of the whole brain (cerebrum, cerebellum and pons-medulla) shall be made. All tissues will then be placed into a fresh solution of the same fixative prior to processing. Tissues listed following will be preserved for all designated F_1 pups for neuropathology evaluation:

No.[a]	Tissue	Preserved (Groups)	Microscopic Examination (Groups)	
		All	I and IV	II and III
6	Brain (forebrain, central cerebrum, hippocampus, basal ganglia, midbrain, cerebellum and pons, medulla)	X	X	
2	Eye with optic nerve	X	X	
6	Spinal cord (cervical, thoracic, lumbar, cross and longitudinal sections)	X	X	
2	Sciatic nerve (cross and longitudinal sections)	X	X	
2	Tibial nerve (cross and longitudinal sections)	X	X	
2	Sural nerve (cross and longitudinal sections)	X	X	
2	Trigeminal ganglia	X	X	
4	Dorsal root ganglia (from C_3-C_6 and L_4-L_6)	X	X	
4	Dorsal root fibers (from C_3-C_6 and L_4-L_6)	X	X	
4	Ventral root fibers (from C_3-C_6 and L_4-L_6)	X	X	
6	Lungs and trachea	X	X	X
	Tissues with macroscopic findings	X	X	X

[a] Number of organs/sections that will be preserved/examined per animal.

Peripheral nerves will be post-fixed in 1% osmium tetroxide, processed and embedded in epoxy resin and microtoned at approximately 2 microns. All other tissues, including the brain, spinal cord, trigeminal ganglia, dorsal root ganglia, dorsal and ventral root fibers, lungs and trachea will be processed by standard techniques, embedded in paraffin and microtomed at approximately 6 microns.

The tissues listed above will be examined microscopically for all animals as indicated. Tissues with macroscopic lesions will be examined in all animals (additional cost). Any abnormalities not noted during macroscopic postmortem examinations that are seen during histological processing will be recorded. If microscopic findings indicative of an effect of test substance administration are seen in high-exposure animals, then

examinations will be made of these tissues/organs for low- and mid-exposure animals (additional cost). Additional examinations will be made only after consultation with the Sponsor and receipt of authorization from the Sponsor.

If any evidence of neuropathology is found, then a subjective diagnosis will be performed to evaluate exposure-response relationships (additional cost). All regions of the nervous system exhibiting any evidence of neuropathological changes will be included in the analysis. Sections from all exposure groups from each region will be coded and examined in a randomized order without knowledge of the code. The frequency and severity of each lesion will be recorded. After all samples have been rated, the code will be broken and statistical analysis performed to evaluate exposure-response relationships.

13.1.6. GFAP EVALUATION

F_1 pups (5/sex/group), not selected for mating, will be randomly selected by computer for GFAP evaluation. Selected pups will be euthanized (by carbon dioxide asphyxiation) by US Center for Disease Control (CDC) Health Effects Laboratory personnel and the brains will be removed, weighed and processed for GFAP evaluation. HLS personnel will ship the samples on dry ice to the US Center for Disease Control Health Effects Laboratory, Morgantown, WV (attn: James P. O'Callaghan, Ph.D.). Upon receipt, samples will be further processed for determination of GFAP response as detailed in the referenced guidelines and procedures (see Appendix A). The Sponsor will be responsible for the spirit of GLP compliance of this subcontractor.

14. PRESERVATION OF RECORDS AND SPECIMENS

All data documenting experimental details and study procedures and observations will be recorded and maintained as raw data.

At the completion of the study, all reports, raw data, preserved specimens and retained samples will be maintained in the Testing Facility's Archives for a period of one year after submission of the signed final report.

The Sponsor will be contacted in order to determine the final disposition of these materials. The Sponsor is responsible for all costs associated with the storage of these materials beyond one year from the issuance of the final report and for any costs associated with the shipment of these materials to the Sponsor or to any other facility designated by the Sponsor.

15. STATISTICAL EVALUATIONS

The following items will be analyzed statistically in the final report:

15.1. CONTINUOUS DATA

- Body weights (all recorded intervals - premating, gestation, lactation and postmating)[a]
- Body weight change[a]
  -entire premating period (males and females)
  -over each weighing interval during the gestation and lactation periods to include Days 0-20 of gestation and Days 0-28 of lactation
  -males during the postmating period (weekly and over the entire period)
- Feed consumption values (all recorded intervals)[a]
  -premating growth period (weekly)
  -postmating period (weekly for males)
  -gestation (Days 0-4, 4-7, 7-14 and 14-20)
  -lactation (all recorded intervals)
- Organ weight data (absolute and relative to the terminal body weight and brain weights)[a]
- Gestation length (F_1 and F_2 litters)[a]
- Pup body weights (all recorded intervals during lactation)[a]
- Number of pups (live, dead, total) at birth (F_1 and F_2 litters) and during lactation (days 1, 4, 7, 14, 21 and 28)[a]
- Mean age-to-criteria for vaginal opening and preputial separation (selected F_1 animals)[a]
- Mean sperm count (homogenization-resistant testicular sperm and caudal epididymal sperm) and motility data[b]

[a] Statistical evaluation of equality of means will be made by the appropriate one way analysis of variance (ANOVA) technique, followed by a multiple comparison procedure, if needed. If ANOVA shows no difference, no additional comparisons will be made. If ANOVA is significant, Dunnett's test will be used to determine which data, if any, differ from the control.
[b] Sperm motility, total count and morphology data will be compared between groups using the Kruskal-Wallis nonparametric ANOVA test.

15.1.1. STATISTICAL ANALYSES CONTINUOUS DATA - MULTIPLE GROUP ANALYSES

Data will be compared between the control and treated groups. All statistical tests will be conducted at the 5% and 1%, two-sided risk levels.

15.1.2. STATISTICAL ANALYSES CONTINUOUS DATA - MULTIPLE GROUP ANALYSES FOR SPERM AND MOTILITY ANALYSIS

If a significant difference occurs ($p<0.05$), the Wilcoxon (Mann-Whitney U) test will be used for pair-wise comparisons of each treated group to the vehicle control group. Statistical analyses will be performed using an IBM compatible computer with SAS computer programs (SAS/STAT User's Guide, 1989).

15.2. INCIDENCE DATA

- Mortality rates
- Mating indices (male and female)
- Pregnancy rates
- Male fertility indices
- Live birth indices
- Pup viability indices (Days 0-4) and lactation indices (Days 4-21)

15.2.1. INCIDENCE DATA ANALYSIS

Analyzed using the Chi-square test (2 x n). If Chi-square analysis is not significant, no additional analyses will be performed. If Chi-square is significant, a Fisher Exact Test with Bonferonni correction will be performed to identify differences between the groups.

16. REPORT

16.1. STATUS REPORT

Periodic verbal and written updates on study progress will be provided by the Study Director. In general, written status reports will be submitted weekly and at termination of the study. These reports will include:

- Exposure data
- Mortality rates
- Mean weekly body weight and body weight gain data
- Mean weekly feed consumption premating period
- Summary of detailed physical examinations
- Mating indices (males and females)
- Male fertility indices
- Pregnancy rates
- Gestation length
- Number of pups at birth (live, dead and total) and number of live pups surviving during lactation

- Mean pup body weights (lactation)
- Individual female litter data
- Maternal gestation and lactation body weights and weight gains
- Maternal feed consumption - gestation/lactation
- Summary of macroscopic postmortem evaluations (adults, weanlings)

16.2. FINAL REPORT

One unbound hard copy and one electronic copy of a draft report will be submitted following termination of the study. The Main Study and the GFAP satellite study will be separately reported. After receipt and review of the Sponsor's comments, appropriate changes will be made and two hard copies and one electronic copy of a signed, final report will be issued. (Additional copies will be provided at additional cost). The Main Study report will minimally include:

16.2.1. GENERAL

- Compliance Statement
- Quality Assurance Statement
- Abstract
- Introduction
- Experimental Design
- Materials and Methods
- Protocol Deviations
- Discussion of study results
- Conclusion and No Observed Effect Level (NOEL) or No-Observed-Adverse-Effect Level (NOAEL) statement, if applicable
- References for experimental methodology
- Senior personnel participating in the study
- Protocol and any amendments

16.2.2. DATA TABULATIONS FOR PARENTAL GENERATIONS (P, F_1)

- Mortality - termination history
- Physical in-life observations (summarized and individual data presented monthly throughout the study)
- Mating indices
- Pregnancy rates
- Male fertility indices
- Mean body weight data (all interval)
- Mean feed consumption data (all intervals)
- Mean weight gain data (premating, postmating [males], gestation and lactation intervals)
- Estrous cycle data

- Macroscopic postmortem observations (adults, weanlings)
- Microscopic pathology examinations
- Organ weight data (parental and pups)
- Sperm assessment data

16.2.3. DATA TABULATIONS FOR LITTERS AND PUPS (F_1 AND F_2)

- Mean gestation length
- Mean number of pups (live, dead and total) at birth and live pups at Days 4, 7, 14, 21 and weaning (Day 28)
- Litter survival indices
- Pup live birth index
- Pup viability and lactation indices
- Mean pup body weights (all recorded intervals during lactation)
- Pup sex ratio at birth and weaning
- Pup day of sexual maturation and body weight at sexual maturation
- Pup macroscopic postmortem observations
- Individual female litter data

16.2.4. APPENDICES

All exposure data, analytical methodology and individual animal data (adults, pups) including but not limited to the following will be presented in the appendices: body weight and body weight gain, feed consumption, physical observation data, litter data, organ weight data, sperm assessment data, estrous cycle data, macroscopic postmortem findings, microscopic examination data and feed/water/bedding data.

17. REFERENCES

Dunlap, W.P. and J.A. Duffy (1975) "Fortran IV functions for calculating exact probabilities associated with Z, Chi-square, T and F values." Behav. Res. Methods and Instrumentations, 7:59-60

Dunlap, W.P., M.S. Marx and G.J. Agamy (1981) "Fortran IV functions for calculating probabilities associated with Dunnett's Test", Behav. Res. Methods and Instrumentations, 13:363-366.

Hollander, M. and D.A. Wolf (1973) Nonparametric Statistical Methods, Wiley, New York, NY, pp. 120-123.

Siegel, S. (1956) Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, pp. 98-99 and 104-106.

18. APPENDIX A
GFAP Evaluations

GFAP SATELLITE PROCEDURE

1.0 PURPOSE

The purpose of this study is to evaluate the potential of the test substance to induce GFAP following inhalation exposure of rats.

2.0 FACILITIES and STAFF

2.1 Subcontractor Sponsor
Huntingdon Life Sciences
Princeton Research Center (PRC)
Mettlers Road
East Millstone, New Jersey 08875-2360

2.2 Study Director:
Gary M. Hoffman, B.A., DABT
Huntingdon Life Sciences
Phone: (732) 873-2550 Ext. 2920
Fax: (732) 873-3992
E-mail: hoffmang@princeton.huntingdon.com

2.3 Principal Investigator:
James P. O'Callaghan, Ph.D.
US Centers for Disease Control (CDC) and Prevention
Health Effects Laboratory Division – NIOSH
1095 Willowdale Road
Morgantown, WV 26505-2888
Phone No.: 304-285-6079 or 6233
Fax No.: 304-285-6220
Email: jdo5@cdc.gov

2.4 Testing Facility Project: 00-4207

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1 Test Substance: Baseline Gasoline Vapor Condensate
3.2 Control: Negative: Air control
3.3 Test Substance Retention Sample
The retention of a reserve sample of the test substance will be the responsibility of Huntingdon Life Sciences.

4.0 SUBCONTRACTOR AND KEY PERSONNEL

4.1 Name: US Centers for Disease Control and Prevention Health Effects Laboratory Division – NIOSH

4.2 Address: 1095 Willowdale Road
Morgantown, WV 26505-2888

4.3 Principal Investigator: James P. O'Callaghan, Ph.D.

5.0 TEST SCHEDULE

5.1 Proposed Experimental Initiation Date: 30 April 2001

5.2 Proposed Experimental Completion Date: 27 August 2001

5.3 Proposed Draft Report Date: 17 May 2002

6.0 TEST SYSTEM

Closed-colony, random-bred rodents are acceptable models for GFAP studies. Sprague-Dawley rats were selected because of the availability of historical control data. Information on the test system is provided by Huntingdon Life Sciences and is contained in their files.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

Information on the experimental design and methodology has been provided by Huntingdon Life Sciences and is contained in their files. All *in vivo* portions of the study, including inhalation exposures of the P_0 parental generation, will be performed by Huntingdon Life Sciences. F_1 pups not selected for breeding will be anesthetized and brain samples will be collected by CDC personnel on site at Huntingdon Life Sciences. The brain samples will be shipped to CDC and processed and evaluated as indicated in the following procedures. The potential of the test substance to increase GFAP in a dose-responsive manner will be measured relative to a control group.

The study design will be as follows:

Group	Number per Sex
Negative Air Control	5
Test Substance (mg/m^3)	
2,000	5
10,000	5
20,000	5

7.1 Exposure Levels Selection

The exposure levels for the GFAP assay will be as shown in the above table.

7.2 Controls: The negative control group of animals will be exposed to air only.

7.3 Brain Collection and Processing and Evaluations

See the following procedures.

8.0 Report

A report of the results of this portion of the study conducted at CDC will be prepared and will accurately describe all methods used for generation, results and analysis of the data.

9.0 Records and Archives

All raw data, the protocol and all reports generated at CDC will be maintained. After submission of the final report, all of the above will be shipped to Huntingdon Life Sciences to be archived.

10.0 Specimens

All specimens will be held in storage as long as the quality of the preparation affords evaluation or in accordance with the relevant Good Laboratory Practice Regulations.

11.0 Regulatory Requirements/Good Laboratory Practice

This study will be conducted in accordance with the spirit of:
US EPA GFAP Assay 79.67, CFR Vol. 59, No. 122, 27 June 1994.
US EPA GLP 79.60, CFR Vol. 59, No. 122, 27 June 1994.

GFAP ELISA ASSAY Standard Operating Procedure

1 SCOPE OF APPLICATION

The following is an SOP to be used in the quantification of the levels of glial fibrillary acidic protein (GFAP) in rat or mouse brain tissue. This procedure will be conducted by:

Principal Investigator: Dr. James P. O'Callaghan
US Centers for Disease Control and Prevention Health Effects
Laboratory Division – NIOSH

2 PREREQUISITES

2.1 EQUIPMENT and SUPPLIES

2.1.1 EQUIPMENT

Ultrasonic Cell Disruptor
Pipettes
Hot/Stir Plate
Plate Reader

2.1.2 CHEMICAL REAGENTS

αGlial Fibrillary Acidic Protein (DAKO Z0334) - Lot # 096 (302)
Carnation Non-Fat Dry Milk
αGlial Fibrillary Acidic Protein (Chemicon MAB 3402-Lot 14119825-07)
Phosphate Buffered Saline (Pierce #28374)
Alkaline Phosphatase conjugated αmouse IgG(Jackson ImmunoResearch #315-055-003)
Alkaline Phosphatase Substrate Kit (Bio-Rad #172-1063)
Triton X-100 (Bio-Rad #161-0407)
Sodium Hydroxide (Fisher #S320)

2.1.3 SUPPLIES

Microtiter Plates (Immulon 2, Dynatech)
Pipet tips

2.2 TRAINING REQUIREMENTS

Able to use a plate reader and to accurately dilute tissue samples.

3 CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

1. Powdered milk should be purchased from a grocery store and used within six months. Old powdered milk gives a yellow color in all samples and masks any GFAP results.

2. When preparing the alkaline phosphatase substrate (p-nitrophenylphosphate), observe the color. The color should be a faint yellow, if darker a new kit should be used.

4 PROCEDURE

4.1 STANDARD CURVE PREPARATION

1. Remove a tube of the GFAP standard (see O'Callaghan et al., 1990 for method of preparation) from the freezer, thaw at room temperature and vortex prior to aliquoting.

2. Preparation of the standard curve requires dilution of the GFAP standard. The standard has a known total protein concentration (11.5 mg/ml) and a known concentration of GFAP (2.4µg GFAP/ mg total protein). The total protein value is used to prepare a standard curve in PBS with 0.5% Triton X-100. The protein values for the standard curve should be between 0.25 to 10 µg/100µl (i.e. 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10µg/100µl).

GFAP Standard Curve Preparation

Tube #	µg of Protein/100µl/well (µg of GFAP)	µl of Hippo Standard	Serial Dilution	µl of PBS+ 0.5% Triton X-100
1	10µg (.024µg)	17.8 µl		1982µl
2	7.5µg (.018µg)	13.0µl		1987µl
3	5.0µg(.012µg)		1.0ml from tube #1(10µg)	1ml
4	2.5µg(.006µg)		500µl from tube #3(5µg)	500µl
5	1.0µg(.0024µg)		500µl from tube #3(5µg)	2 ml
6	0.5µg(.0012µg)		1ml from tube #5(1µg)	1ml
7	0.25µg(.0006µg)		500µl from tube #6(0.5µg)	500µl

4.2 SAMPLE PREPARATION

1. Control and experimental animals are euthanized, brain regions are dissected, weighed, and placed in the appropriately labeled tube.

2. At this point, the samples are sonified in 10 volumes (i.e. a volume that is 10X the weight of the tissue e. g. tissue weight = .045 grams 10X volume= 450µl 1%SDS)of hot 1% SDS (85-90°C) is added and then sonified. In both cases, the samples are stored at -70°C.

3. Before the frozen tissues can be used in the GFAP ELISA, they are removed from the freezer, placed on dry ice, then sonified in hot 1% SDS (85-90°C) 10X volume (step2). Continue with step 4.

4. Experimental samples already sonified in 1%SDS are removed from the freezer, allowed to thaw at room temperature, and diluted in PBS with 0.5% Triton X-100 to a concentration of 10µg total protein/ 100µl of PBS with 0.5% Triton X-100. Samples high in GFAP (e. g. cerebellum) may need to be diluted to a concentration of 5µg total protein/ 100µl of PBS with 0.5% Triton X-100. Samples low in GFAP (e.g. striatum) may need to be diluted to 20µg total protein/ 100µl of PBS with 0.5% Triton X-100. The dilution of the samples is critical because the optical density readings for each samples needs to fall on the linear portion of the standard curve.

5. Samples are diluted according to the total protein value of the sample (e.g. Sample = 11.5mg/ml total protein; the dilution for this sample will be 8.70 µl 991.3 µl of PBS with 0.5% Triton X-100.)

6. If samples have to be diluted to a lower concentration, a 1:1 dilution of the 10µg/100µl dilution results in the 5µg/100µl (200µl of the 10µg/100µl dilution plus 200µl of the PBS with 0.5% Triton X-100).

4.3 REAGENT PREPARATION

1. **Volumes Required-** The volumes of reagents are 100µl/well (10ml per plate and the volume of wash is 200µl/well (20 ml per plate).

2. **Phosphate Buffered Saline (PBS)-** One packet of PBS is mixed thoroughly with 500ml of deionized water.

3. **PBS+0.5% Triton X-100-** 2.5ml of Triton X-100 is added to 500ml of PBS.

4. **Blotto-** PBS + 5% powdered milk. Step 4 uses BLOTTO without Triton X-100, in steps 7 and 9 , BLOTTO contains 0.5% TritonX-100. Before the assay is started, the total volume of BLOTTO needed for the entire assay should be determined (total volume should be made fresh on the day of the assay). The larger volume allows a good mix for the addition of the Triton X-100 detergent. PBS may be warmed slightly; this will facilitate getting the powdered milk into solution.

5. **Polyclonal anti-GFAP** (Dako)- 25µl/10ml of PBS

6. **Monoclonal anti-GFAP** (Chemicon)- 1:500 20µl/10ml Blotto+0.5% Triton X-100

7. **Alkaline Phosphatase**- 1:3000 3.3µl/10ml Blotto+0.5% Triton X-100

8. **P-nitrophenylphosphate substrate**- 2 ml of diethanolamine buffer, 2 p-nitrophenylphosphate tablets in 8ml of deionized water.

4.4 ASSAY PROCEDURE

1. Coat Immulon-2 flat bottom plates with αGFAP, 1.0µg total immunoglobulin protein / 100µl PBS / well. (25µl of αGFAP, Dako Z0334, in 10ml of PBS is the quantity needed per plate).

2. Incubate the coated plate at 37°C for 1 hour. This step may be done at the begining of the assay or it may be done the night before and refrigerated overnight at 4°C.

3. Empty plate, tap on absorbent paper to remove excess (this procedure is important to eliminate the possibility of any reagent carry-over between steps). Wash plates 4X with PBS (200µl per well), tapping and blotting between each wash.

4. Block 1 hour with BLOTTO (5% w/v non-fat powdered milk in PBS) 100µl per well.

5. Empty plate, tap on absorbent paper to remove excess, load diluted standard curve and samples in a volume of 100µl per well. Incubate for 1 hour at room temperature. The template below is an example of a typical plate for this labs GFAP Assay.

Microtiter Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	Blk	1	1	9	9	17	17	25	25	33	33
B	Std 1	Std 1	2	2	10	10	18	18	26	26	34	34
C	Std 2	Std 2	3	3	11	11	19	19	27	27	35	35
D	Std 3	Std 3	4	4	12	12	20	20	28	28	36	36
E	Std 4	Std 4	5	5	13	13	21	21	29	29	37	37
F	Std 5	Std 5	6	6	14	14	22	22	30	30	38	38
G	Std 6	Std 6	7	7	15	15	23	23	31	31	39	39
H	Std 7	Std 7	8	8	16	16	24	24	32	32	40	40

6. Wash 4X with PBS + 0.5% Triton X-100, 200µl/well.

7. Incubate for 1 hour in monoclonal αGFAP (Chemicon) at a dilution of 1:500 plus alkaline phosphatase conjugated anti-mouse IgG (Jackson ImmunoResearch) at a dilution of 1:3000 made up in BLOTTO plus

0.5% Triton X-100, 100µl/well made up in BLOTTO plus 0.5% Triton X-100, 100µl /well.

8. Wash 4X with PBS + 0.5% Triton X-100.

9. Wash 4X with PBS + 0.5% Triton X-100, 200µl/well.

10. Add P-nitrophenylphosphate substrate (Bio-Rad) in a volume of 100µl/well and incubated for 20 minutes.

11. Stop reaction with 0.4N NaOH 100µl/well.

12. Check wells for any bubbles, read plate at 405nm.

4.5 CALCULATIONS

1. Plates are read on a Molecular Devices Spectra Max Plus microplate reader. The plate reader is coupled to a PC running the SoftMax Pro (Molecular Devices) program.

2. A template is set up for each plate in the assay. The template includes the position of the standard curve and value for each point, and the name, number, and dilution factor of the unknown samples. The dilution factor for the samples is determined by the amount of total protein loaded into the wells (e.g. 10µg of total protein=100x dilution factor, 5µg of total protein=200x dilution factor, etc. The dilution factor is the multiplier needed to bring the total protein value up to 1mg of total protein in the final calculations.)

GFAP STANDARD CURVE

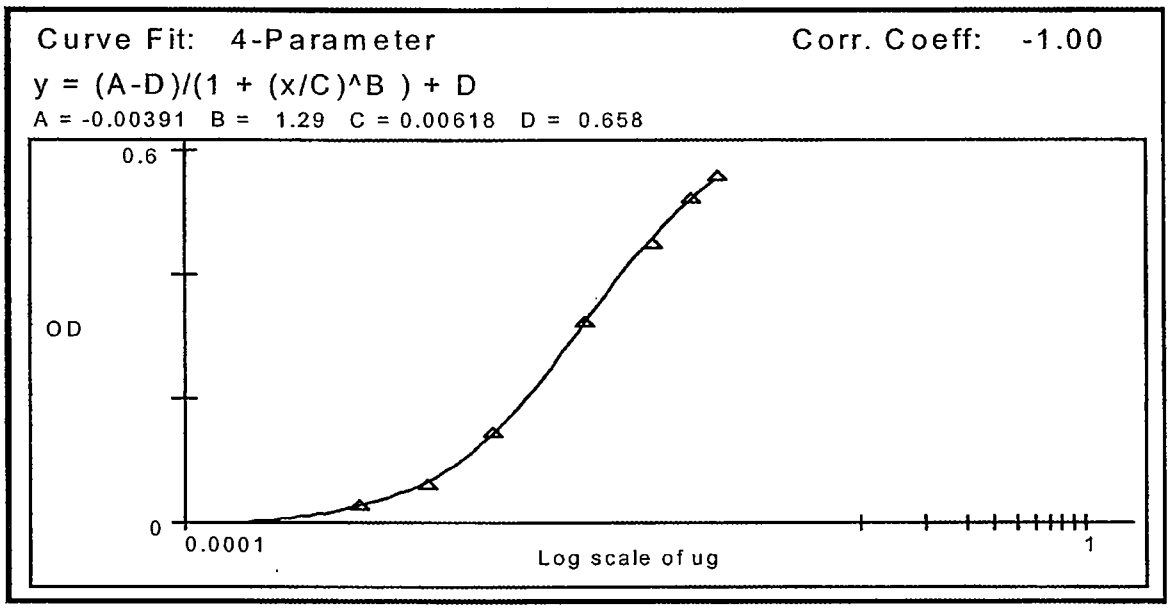

PLATE BLANK	Well	OD	Mean	Std Dev	CV
BL	A1	0.096	0.095	0.001	1.489
	A2	0.094			

STANDARDS	Value	Well	OD	Mean	Std Dev	CV
STD01	6.00e-4ug	B1	0.033	0.03	0.004	14.14
		B2	0.027			
STD02	0.0012ug	C1	0.065	0.065	*****	*****
		C2	0.065			
STD03	**0.0024ug**	D1	**0.146**	**0.145**	**7.07E-04**	**0.486**
		D2	**0.145**			
STD04	**0.006ug**	E1	**0.326**	**0.327**	**0.001**	**0.432**
		E2	**0.328**			
STD05	**0.012ug**	F1	**0.463**	**0.454**	**0.013**	**2.963**
		F2	**0.444**			
STD06	0.018ug	G1	0.53	0.526	0.006	1.075
		G2	0.522			
STD07	0.024ug	H1	0.546	0.561	0.022	3.904
		H2	0.577			

3. The above standard curve is a typical curve generated from the rat hippocampus standard. The OD values for unknown samples need to fall on the straight portion of the curve, between STD 3 and STD 5.

4. The SoftMax Pro program automatically subtracts the blank from each OD reading (plate blank information is given in the table above), means the two readings for each sample, and multiplies the value obtained from the curve for this OD by the dilution factor for the unknown sample.

5. The report generated by the SoftMax Pro program may be imported into an Excel spreadsheet or data may be entered manually. The chart below is an example of the report generated in Softmax Pro for 10 unknown hippocampus samples that has been imported into an Excel spreadsheet. These samples were diluted to 2.5µg of total protein/100µl, therefore the dilution factor for all ten samples is 400(this factor may be included as another column on the spreadsheet).

UNKNOWNS	Mean	Std Dev	CV	Well	OD	Value
HIP01	2.633	0.114	4.323	A3	0.334	2.552
				A4	0.347	2.713
HIP02	2.482	0.099	3.984	B3	0.334	2.552
				B4	0.322	2.412
HIP03	3.099	*****	*****	C3	0.375	3.099
				C4	0.375	3.099
HIP04	2.792	0.093	3.34	D3	0.358	2.858
				D4	0.348	2.726
HIP05	2.206	0.074	3.338	E3	0.298	2.154
				E4	0.308	2.259
HIP06	2.681	0.027	0.999	F3	0.343	2.662
				F4	0.346	2.7
HIP07	3.077	0.094	3.048	G3	0.369	3.011
				G4	0.378	3.144
HIP08	2.631	0.044	1.663	H3	0.338	2.601
				H4	0.343	2.662
HIP09	2.934	0.108	3.697	A5	0.369	3.011
				A6	0.358	2.858
HIP10	2.626	0.087	3.325	B5	0.345	2.688
				B6	0.335	2.564

6. If sample dilutions were prepared by pipetting the exact amount of total protein, the value for GFAP has been calculated and no further calculations need to be done.
7. If sample dilutions were prepared by pipetting 10µl volumes for all samples, the exact amount of total protein needs to be included in the final calculations. This may be done on the Excel spreadsheet using the formula below:

   GFAP value ÷ 1/10 Total protein value=µg GFAP/mg Total Protein

   Unadjusted GFAP Value for Hippo #3 = 3.099

Protein Value for Hippo #3 = 9.883

3.099 ÷ 9.883 x 10 = 3.136 µg GFAP/mg Total Protein

REFERENCES

Characterization of the origins of the astrocyte response to injury using the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. O'Callaghan, J.P. , Miller, D.B., and Reinhard, J.F., Jr. Brain Res. 521: 73-80, 1990.

Quantification of Glial Fibrillary Acidic Protein: Comparison of Slot-Immunobinding Assay with a Novel Sandwich ELISA, O'Callaghan, J.P., Neurotoxicology and Teratology, Vol. 13, pp. 275-281, Pergamon Press plc. 1991

SoftMax User's Manaual, Molecular Devices Corporation, Sunnyvale, CA

Brain Tissue Preparation Standard Operating Procedure

1 SCOPE OF APPLICATION

The following is an SOP to be used for preparing rat or mouse brain tissue to be assayed for total protein concentration and the concentration of glial fibrillary acidic protein (GFAP).

2 PREREQUISITES

2.1 EQUIPMENT and SUPPLIES

2.1.1 EQUIPMENT

Ultrasonic Cell Disruptor
Pipettes
Hot/Stir Plate
Plate Reader

2.1.2 CHEMICAL REAGENTS

Sodium Dodecyl Sulfate, Bio-Rad #161-0302

2.1.3 SUPPLIES

Microfuge tubes
Pipet tips

2.2 TRAINING REQUIREMENTS

Ability to dissect discrete regions of the rat brain.

3 CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

SDS needs to be heated to a temperature of (85-90^0 C).

4 PROCEDURE

4.1 REAGENT PREPARATION

1% SDS

A stock solution of 10% SDS is prepared w/v, 100 gms of SDS in 1 litre of deionized water. The 1% SDS is a dilution of the stock- 10ml of 10X stock in 90ml of deionized water.

4.2 TISSUE PREPARATION

1. Control and Test substance exposed animals are sacrificed, brains then are rapidly removed from the skull case with the aid of pointed scissors (to open the brain case) and curved blunt forceps (to remove the brain).

2. The brain is placed on a cold (approximately 4°C) dissecting platform (Thermoelectrics cold plate, Aldrich Chemical Co., St. Louis, MO) and individual areas are dissected along neuroanatomical landmarks using fine curved forceps and a # 11 scapel blade. The areas dissected include: cerebellum, cortex, hippocampus, hypothalamus, striatum, thalamus, rest of brain. In addition, olfactory bulbs and pituitary are removed from their position in the skull case after brain removal.

3. The dissected brain parts then are weighed in a previously tared and appropriately labelled tube. At this point, each brain region is immersed in 10 volumes of hot 1% SDS (85-90°C) (i.e. 10X volume the weight of the tissue; e.g. tissue weight = .045 grams, 10X volume= 450µl 1%SDS) and then homogenized by sonification with an

ultrasonification microprobe (Heat Systems, Farmingdale, NY). The samples then are stored at -70°C prior to assay.

Sample Storage/Handling and Shipping Standard Operating Procedure

1 SCOPE OF APPLICATION

The following is an SOP to be used for storing/handling and shipping of brain tissue homogenates that serve as standards and unknowns for determining total protein concentration and the concentration of glial fibrillary acidic protein (GFAP) in each homogenate.

2 PREREQUISITES

2.1 EQUIPMENT and SUPPLIES

2.1.1 EQUIPMENT

-70 °C Freezer

2.1.2 CHEMICAL REAGENTS

2.1.3 SUPPLIES

2.2 TRAINING REQUIREMENTS

Ability to handle samples frozen at -70 °C

3 CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

Wear protective gloves when removing samples from -70^0 C freezers.

4 PROCEDURE

4.1 STORAGE/HANDLING

Homogenates will be stored frozen at -70 °C prior to shipping to CDC-NIOSH and shipped frozen on dry ice.

4.2 SHIPPING

Homogenates will be shipped on dry ice in protective Styrofoam containers to CDC-NIOSH.

BCA PROTEIN ASSAY Standard Operating Procedure

1 SCOPE OF APPLICATION

The following is an SOP to be used in the determination of total protein in brain tissue by the Pierce BCA method. BSA (Bovine Serum Albumin) is used as the standard and samples are prepared by sonification in 1% (w/v) SDS.

2 PREREQUISITES

2.1 EQUIPMENT and SUPPLIES

2.1.1 EQUIPMENT

Plate Reader
Pipettes
Incubator
Vortex-Mixer

2.1.2 CHEMICAL REAGENTS

BCA Protein Kit, Pierce #23223
Bovine Serum Albumin (BSA), Sigma A7888
Sodium Dodecyl Sulfate, Bio-Rad #161-0302

2.1.3 SUPPLIES

Microtubes 1.2ml
96 well microtiter plates
Pipette Tips

2.2 TRAINING REQUIREMENTS

Able to use a plate reader and to accurately dilute tissue samples.

3 CAUTIONARY NOTES OR SPECIAL CONSIDERATIONS

BCA reagent is stable for up to one week; standard practice dictates the use of 50 ml quantities, enough to do two full microtiter plates.

SDS serves as a wetting agent and pipetting SDS-containing samples can lead to pipetting errors. Therefore, use only a single pipette tip per sample and withdraw the sample only a single time per tip. This procedure holds for SDS and all SDS-containing samples.

4 PROCEDURE

4.1 REAGENT PREPARATION

1. **1% SDS**
A stock solution of 10%SDS is prepared w/v, 100 gms of SDS in 1 litre of deionized water. The 1%SDS is a dilution of the stock- 10ml of 10X stock in 90 ml deionized water.

2. **BCA Reagent**
This reagent is made according to the directions provided by Pierce, 50ml of solution A and 1ml of solution B. Thoroughly mix while adding solution B.

4.2 STANDARD PREPARATION

Protein Standards: Bovine Serum Albumin (BSA) in 1% (w/v) SDS (sodium dodecyl sulfate).

1. Prepare 1mg/ml BSA in 1% SDS. Weigh out 0.100 gms of dry BSA on an analytical balance. Add the BSA to a 100 ml volumetric flask then gradually add 1% SDS to the flask. Mix gently after each addition of SDS to dissolve the BSA without a lot of bubbling. Continue adding the SDS until the total 100 ml has been added.

2. Aliquot a volume into microfuge tubes (600 µl is enough volume to do one standard curve). Store the tubes at -70°C . Thaw as needed, but do not re-freeze.

3. **Blank:** Remove 200µl of 1%SDS, place it in a dilution tube.

4. **1.0µg/10µl:** Remove 20µl of the prepared BSA standard(1mg/ml), place it in a dilution tube and add 180µl of 1%SDS.

5. **2.5µg/10µl:** Remove 50µl of the prepared BSA standard, place it in a dilution tube and add 150µl of 1% SDS.

6. **5.0µg/10µl:** Remove 100µl of the prepared BSA standard, place it in a dilution tube and add 100µl of 1% SDS.

7. **7.5µg/10µl:** Remove 150µl of the prepared BSA standard, place it in a dilution tube and add 50µl of 1% SDS.

8. **10.0µg/10µl:** Remove 200µl of the prepared BSA standard and place it in a dilution tube.

BSA Standard Curve			
Tube #	µg Protein/ 10µl/well	µl of 1mg/ml BSA Standard	µl of 1%SDS
1	Blank	0	200µl
2	1.0µg	20µl	180µl
3	2.5µg	50µl	150µl
4	5.0µg	100µl	100µl
5	7.5µg	150µl	50µl
6	10.0µg	200µl	0

9. Repeat steps 3 to 8. This generates a second standard curve, independent of the first.

10. Vortex each tube of the diluted BSA standard, remove 10µl of the diluted standard and place it in the designated well on a microtiter plate.

4.3 SAMPLE PREPARATION

The unknown experimental samples are diluted two times, independently.

1. Remove samples from the freezer and allow to thaw at room temperature.

2. Vortex and then remove a 10µl aliquot from the sample and place it into a dilution tube. Add 190µl of 1% SDS to each sample tube.

3. Repeat step 2. This becomes the second dilution for this sample.
4. Vortex the dilution tubes and remove a 10µl aliquot and place it in the appropriate well on the microtiter plate.

5. Repeat steps 1 through 4 for each experimental sample.

6. The following chart is an example of a typical microtiter plate layout, standard curve and 42 unknown samples.

Microtiter Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	Blk	3	3	11	11	19	19	27	27	35	35
B	Std 1	Std 1	4	4	12	12	20	20	28	28	36	36
C	Std 2	Std 2	5	5	13	13	21	21	29	29	37	37
D	Std 3	Std 3	6	6	14	14	22	22	30	30	38	38
E	Std 4	Std 4	7	7	15	15	23	23	31	31	39	39
F	Std 5	Std 5	8	8	16	16	24	24	32	32	40	40
G	1	1	9	9	17	17	25	25	33	33	41	41
H	2	2	10	10	18	18	26	26	34	34	42	42

4.4 PROTEIN ASSAY

1. Add 200µl of reagent to each of the wells containing either the standard curve or sample.

2. Place the plate in the plate reader and mix the plate using the automatic mix button on the plate reader or vortex gently prior to placing the plate into the reader.

3. Incubate the plate at 37°C for 30 minutes.

4. At the completion of the incubation period, the plate is checked for bubbles and then read in the plate reader at a wavelength of 562nm. "Pop" any bubbles with a needle or a pipette tip prior to reading the plate.

4.5 CALCULATIONS

1. Software programs coupled to specific plate readers are used to calculate protein values based on comparing OD readings of the unknowns with those obtained from the standard curve. OD values and standard protein values are plotted in a linear fashion on both axes. The following is a typical standard curve for the BCA Protein Assay when bovine serum albumin is used for the standard. The table gives the actual values for each point on the standard curve and their corresponding OD readings.

BCA Total Protein Standard Curve

Curve Fit: Linear Corr. Coeff: 0.999

$y = A + B * x$

A = 0.0240 B = 0.0395

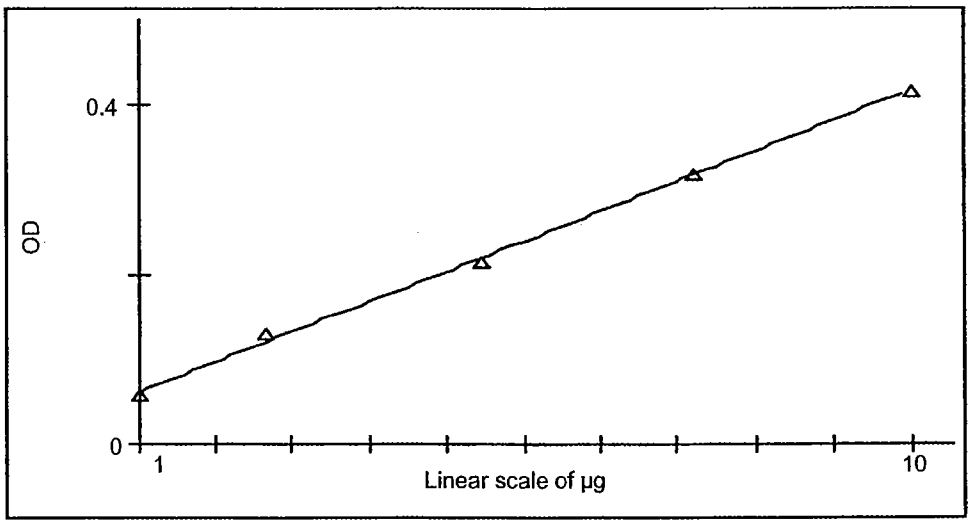

Plate Blank	Well	OD	Mean	Std Dev	CV
BL	A1	0.083	0.082	0.001	1.725
	A2	0.081			

STANDARD	Value		Well	OD	Mean	Std Dev	CV
STD01	1.000	µg	A3	0.06	0.058	0.002	3.626
			A4	0.057			
STD02	2.500	µg	A5	0.131	0.131	7.07E-04	0.542
			A6	0.13			
STD03	5.000	µg	A7	0.22	0.218	0.003	1.297
			A8	0.216			
STD04	7.500	µg	A9	0.311	0.321	0.014	4.406
			A10	0.331			
STD05	10.00	µg	A11	0.419	0.418	0.001	0.338
		A12	0.417				

2. The blank can be automatically subtracted from all other OD readings or it must be done manually if this feature is not available.

3. The dilution factors can be entered into the programs, otherwise they must be considered in the final calculations.

4. The final printout will include a plot of the standard curve and the protein concentration of each unknown expressed as mg/ml or µg/µl. The table below gives the calculated protein values for unknown hippocampus samples in mg/ml or µg/µl. These values include the subtraction of the blank value and a dilution factor of 2.

UNKNOWN	Mean	Std Dev	CV	Well	OD	Value	Dil Factor
UNK01	9.325	0.215	2.306	B1	0.211	9.477	2
				B2	0.205	9.173	
UNK02	10.44	*****	*****	B3	0.23	10.44	2
				B4	0.23	10.44	
UNK03	9.883	0.072	0.725	B5	0.218	9.832	2
				B6	0.22	9.934	
UNK04	11.18	1.254	11.23	B7	0.227	10.29	2
				B8	0.262	12.06	
UNK05	11.45	0.717	6.258	B9	0.24	10.95	2
				B10	0.26	11.96	

5. Example of calculations, if samples are prepared as listed above:

   a. Hippo #3 is prepared as a 1:20 dilution (10µl of standard or unknown sample are placed in a dilution tube and brought up to a final volume of 200µl)

   b. OD reading for this sample is .219

   c. OD of .219 corresponds to ≈ 4.942µg/10µl or .4942µg/µl total protein

   d. This sample has been diluted 1:20 so the value is actually .4942µg/µl x 20= 9.883µg/µl (since the protein sample was loaded in 10 µl volumes, the value can be determined by using a dilution factor of 2, 4.942µg/10µl x2=9.883µg/µl).

5 REFERENCES

BCA Protein Assay Reagent (instruction manual), Pierce, Rockford, IL.

SoftMax Pro User's Manual, Molecular Devices Corporation. Sunnyvale, CA.

Protocol Amendment No. 1

Study Title: Baseline Gasoline Vapor Condensate: A 2-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

Changes

1. Page 12, Feed & Feed Analysis:
Revise: Certified Rodent Diet, No. 5002; (Meal) (PMI Nutrition International, St. Louis, MO) ad libitum **except during exposures**.

2. Page 12, Water & Water Analysis:
Revise: Facility water supply (Elizabethtown Water Company, Westfield, NJ); without restriction **except during exposures**, via an automated water delivery system to individual animal cages.

3. Page 13, Air Changes:
Revise: Air changes will be maintained at a rate of 10 ~~12~~ -15 per hour and will be recorded once prior to exposures.

4. Page 21, Terminal Necropsy Males (P_0, F_1):
Revise: P_0 and F_1 parental males will be euthanized before ~~within 3 days after~~ the last F_1 and F_2 litters, respectively, have been weaned ~~delivered~~.

5. Page 23, Dead and Culled Pups (F_1 and F_2):
Revise: F_1 and F_2 pups found dead at birth **will be identified as stillborn or alive but found dead (lung floatation test). F_1 and F_2 pups found dead** during the lactation period.....

6. Page 24, Dead and Culled Pups (F_1 and F_2):
Revise: Culled F_1 and F_2 pups will be examined for external abnormalities. If unremarkable, these pups will then be euthanized via **IP injection of sodium pentobarbital** ~~carbon dioxide asphyxiation~~ and discarded.

7. Page 24, Macroscopic Examination and Tissues Preserved:
Revise: F_1 and F_2 pups (**randomly selected** 3/sex/litter**, if possible**) will be given.....

8. Page 24, Organ Weights:
Revise: The F_1 and F_2 pups (1 pup/sex/litter**, if possible**) for which organ.....

Protocol Amendment No. 1

9. Page 25, Microscopic Pathology Evaluations:
Revise: Slides of tissues listed in the table in Section 13.1.1.7. will be prepared and stained with hematoxylin and eosin (H&E) and examined microscopically for **randomly selected** 10 animals/sex/group from P_0 and F_1 parental animals in the control and high-exposure groups.

10. Page 26, Neuropathology Evaluations:
Revise: Selected pups will be anesthetized with an **intramuscular** ~~intraperitoneal~~ injection (~1 mL/kg) of ketamine/xylazine/acepromazine.....

Reasons for Changes

1. Clarification of the feed restriction during exposures.

2. Clarification of the water restriction during exposures.

3. Correction of the AAALAC requirements for air changes in a rodent housing room.

4. Clarification of the acceptable necropsy interval for the parental males.

5. Clarification of the evaluation procedures for dead pups.

6. Correction of the sacrifice procedure for the culled pups.

7. Depending on the actual number of pups per sex per litter, these exact requirements may not always be practical but will be randomly selected if possible.

8. Depending on the actual number of pups per sex per litter, these exact requirements may not always be practical but will be randomly selected if possible.

9. Clarification of the selection procedure for the animals to be evaluated.

10. Correction of the actual dosing procedure for the anesthetic.

Protocol Amendment No. 1

Amendment approved by:

IACUC
Huntingdon Life Sciences

31 May 01
Date

IACUC
Huntingdon Life Sciences

31 MAY 01
Date

Gary M. Hoffman, B.A., DABT
Study Director
Huntingdon Life Sciences

31 May 01
Date

Thomas M. Gray, M.S., DABT
Sponsor Representative
American Petroleum Institute

05/27/01
Date

Protocol Amendment No. 2

Study Title: Baseline Gasoline Vapor Condensate: A 2-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

Changes

1. Detailed Physical Examinations, page 19:
Revise: **P_0 and** F_1 females will continue to be observed weekly until there is evidence of mating. Once mated, **P_0 and** F_1 females will be observed on day 0, 7, 14 and 20 of gestation and on day 1, 4, 7, 14, 21 and 28 of lactation. **P_0 and** F_1 females without evidence of mating will continue to be observed weekly until euthanized.

2. Postmortem, Page 21:
Add:
If a dam is found dead or is euthanized in moribund condition prior to weaning her litter, the dam will be macroscopically examined & tissues retained but no organs will be weighed. The litter will be euthanized and externally examined and discarded (if normal) or preserved (if abnormal).

If a dam entirely loses her litter, she shall resume exposures the next day (if prior to lactation day 5) or will otherwise continue exposures. She will be sacrificed on the initial day of weaning for the study (or later, as practical) and macroscopically examined & tissues retained but no organs will be weighed.

3. Organ Weights, page 22:
Revise: Paired organs will be weighed together **(gonads will also be weighed separately)**.

4. Sample Preparation, Page 38:
Revise: 2. At this point, the samples are sonified in 10 volumes (i.e. a volume that is 10X the weight of the tissue e. g. tissue weight = .045 grams 10X volume= 450µl 1% SDS) of hot 1% SDS (85-95°C) is added and then sonified. In both cases, the samples are stored **within 24 hours** at -70°C.

3. Before the frozen tissues can be used in the GFAP ELISA, they are removed from the freezer, placed on dry ice, then sonified in hot 1% SDS (85-95°C) 10X volume (step2). Continue with step 4.

5. Cautionary Notes or Special Considerations, Page 44:
Revise: SDS needs to be heated to a temperature of (85-95°C).

Protocol Amendment No. 2

6. Tissue Preparation, Page 44:
Revise: .....At this point, each brain region is immersed in 10 volumes of hot 1% SDS (85-95°C) (i.e. 10X volume the weight of the tissue; e.g. tissue weight = .045 grams, 10X volume= 450μl 1%SDS) and then homogenized by sonification with an ultrasonification microprobe (Heat Systems, Farmingdale, NY). The samples then are stored **within 24 hours** at -70°C prior to assay.

Reasons for Changes

1. Clarification of procedures for the P_0 females.
2. Clarification of procedures for the dams and their litters.
3. Clarification of procedures for the weighing of the gonads.

4., 5., 6. Clarification of acceptable temperature range for SDS and period within which samples need to be frozen.

Amendment approved by:

24 AUG 01
IACUC
Huntingdon Life Sciences
Date

24 Aug 01
IACUC
Huntingdon Life Sciences
Date

24 Aug 01
Gary M. Hoffman, B.A., DABT
Study Director
Huntingdon Life Sciences
Date

22 August, 2001
Thomas M. Gray, M.S., DABT
Sponsor Representative
American Petroleum Institute
Date

Protocol Amendment No. 3

Study Title: Baseline Gasoline Vapor Condensate: A 2-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

Changes

Sperm Count, Motility and Morphology Assessments, Page 25:

Revise:

The left vas deferens will be excised and placed in a prewarmed solution of phosphate buffered saline and 1% Bovine Serum Albumin. After a minimum three minute "swimout" period, a sample will be placed in a Hamilton Thorne IVOS sperm analyzer. **Five fields will be selected and stored as digital images.** ~~and five fields will be stored on an optical disk.~~ These **images** ~~fields~~ will subsequently be analyzed for percent motility **and transferred to optical media for permanent storage.**

Reasons for Changes

To clarify the sperm motility evaluation procedures.

Amendment approved by:

Gary M. Hoffman, B.A., DABT
Study Director
Huntingdon Life Sciences

28 Sept 01
Date

Thomas M. Gray, M.S., DABT
Sponsor Representative
American Petroleum Institute

28 September, 2001
Date

Protocol Amendment No. 4

Study Title: Baseline Gasoline Vapor Condensate: A 2-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

Changes

1. Test Guideline, page 6:

Add: This study is designed to meet or exceed the guideline requirements of the **US EPA Vehicle Emissions Inhalation Exposure Guideline 79.61, CFR Vol. 59, No. 122, 27 June 1994,...**

2. Frequency and Duration of Inhalation Administration, page 16:

Revise: **P_0 and F_1 females with no confirmed day of mating but with evidence of pregnancy (weight gain) will continue to be exposed until the presumed Day 19 of gestation.** P_0 **and F_1** females with no confirmed day of mating **and with NO evidence of pregnancy (weight gain)** would continue to be exposed for 25 days following completion of the mating period **and then euthanized.**

3. Observations, page 20:

Revise: ....The anogenital distance will be qualitatively determined on day 0 for all pups of the F_1 **and F_2** generation (to determine the sex).....

4. Macroscopic Examination, page 22:

Add: .....Examination of all parental P_0 **and F_1** females, which were co-housed with P_0 **and F_1** males, will include a vaginal smear examined to determine the stage of the estrous cycle and a count of uterine implantation scars, if present.

5. Terminal Necropsy Pups (F_1 and F_2), Organ Weights, page 24:

Add: Organ weight data will be presented as absolute values and relative to terminal body weight and brain weight. **Remaining F_1 and F_2 pups, if any, will be examined for external abnormalities. If unremarkable, these pups will then be euthanized via carbon dioxide asphyxiation and discarded. Remaining F_1 and F_2 pups with external abnormalities will be preserved intact in 10% NBF at the discretion of the Study Director.**

6. Sperm Count, Motility and Morphology Assessments, page 24:

Add: **If a macroscopic abnormality is noted on the left testis or left epididymis, then the right testis and right epididymis will be transferred to PAI and the left testis and left epididymis will be preserved as indicated for possible histopathology.**

Protocol Amendment No. 4

7. Microscopic Pathology Evaluations, page 26:
Add: ... Qualitative evaluations of the P_0 **and** F_1 animals will be performed by HLS.....

8. Statistical Evaluations/Continuous Data, page 29:

Add: **Estrous Cycle data[a] and Ovarian quantitative evaluation (PAI) data**

Revise: Body weight change[a]
-entire premating period (males and females)
-over each weighing interval during the gestation and lactation periods to include Days 0-20 of gestation and Days **1-28** ~~0-28~~ of lactation

Reasons for Changes

1. Omission in original protocol.
2. Clarification of procedures.
3. Omission in original protocol.
4. Omission in original protocol.
5. Omission in original protocol.
6. Clarification of procedures.
7. Omission in original protocol.
8. Omission in original protocol.

Amendment approved by:

14 Jan 03

Gary M. Hoffman, B.A., DABT
Study Director
Huntingdon Life Sciences

Date

1/14/03

Thomas M. Gray, M.S., DABT
Sponsor Representative
American Petroleum Institute

Date

Protocol Amendment No. 5

Study Title: Baseline Gasoline Vapor Condensate: A 2-Generation Whole-Body Inhalation Reproduction Toxicity Study in Rats

Changes

1. Organ Weights, page 22:

Revise the last sentence: Organ weights will not be recorded for an animal dying spontaneously or euthanized moribund **or for a non-pregnant animal.**

2. Statistical Evaluations/Continuous Data, page 29:

Add: **Brain weight, length and width (F1 pups selected for neuropathology)**[a]

Reasons for Changes

1. Omission in original protocol.
2. Omission in original protocol.

Amendment approved by:

15 Dec 08

Gary M. Hoffman, B.A., DABT
Study Director
Huntingdon Life Sciences

Date

12/15/08

Thomas M. Gray, M.S., DABT
Sponsor Representative
American Petroleum Institute

Date

	Testing Facility Personnel	Appendix GG

TITLE/DEPARTMENT	NAME/DEGREE
VICE PRESIDENT, SAFETY ASSESSMENT	Sylvie J. Gosselin, D.V.M., Ph.D., Diplomate A.C.V.P.
VICE PRESIDENT, OPERATIONS	Michael Caulfield
VICE PRESIDENT, ANALYTICAL SERVICES	Dari Dadgar, Ph.D.
SENIOR DIRECTOR, TOXICOLOGY	Carol S. Auletta, M.B.A., D.A.B.T., R.A.C.
DIRECTOR, QUALITY ASSURANCE	Nicki S. Iacono, B.S.
STUDY DIRECTOR	Gary M. Hoffman, B.A., D.A.B.T.
PATHOLOGIST	Katharine M. Whitney, D.V.M., Ph.D., Diplomate A.C.V.P.
STUDY MONITOR	Brian P. Colfer, B.S.
VETERINARIAN	Teresa S. Kusznir, V.M.D.
MANAGER/SUPERVISOR	
Inhalation/Pharmacy	Ellen H. Whiting, AALAS LAT
Reproductive Toxicology	Robert Faust, B.S.
Analytical Services	Kay Saladdin, B.S.
Pathology Services	Barbara A. Litzenberger, B.S., M.T. (ASCP)

	Report Amendments	Appendix HH

There are no amendments for this report at this time.