Data Requirement

EPA 79.61 and OPPTS 870.3700 and OECD 414

STUDY NO. 05-4287
SPONSOR STUDY NO. 211-DIPE-DEV

GASOLINE DIPE VAPOR CONDENSATE:

EMBRYO-FETAL TOXICITY STUDY IN RATS BY

INHALATION EXPOSURE

Final Report

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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: 8 November 2012

STATEMENT OF COMPLIANCE

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

It was the Sponsor's responsibility to maintain the method of synthesis, fabrication, or derivation of the test fuel, and this was not completed at the time of the study conduct but has been completed since and is on file with the Sponsor. In addition, the Sponsor conducted a non-GLP purity analysis.

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

Nov 2012
Date

Thomas M. Gray, M.S., D.A.B.T.
Sponsor Representative

11/2/12
Date

SIGNATURE PAGE

SCIENTISTS

The following Scientist was responsible for the overall conduct of this study. Departmental supervisory personnel are listed on the personnel page of this report (Appendix Q).

8 NOV 2012

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

SCIENTIFIC REVIEW

The following Scientist has reviewed and approved this report:

8 NOV 2012

Robert M. Parker, Ph.D., D.A.B.T. Date
Director, Developmental and Reproductive Toxicology

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.

Type of Inspection	Date(s) of Inspection	Reported to Study Director and Management
GLP Protocol Review	21-25 Apr 05	25 Apr 05
Exposure Monitoring and Equipment Records	8 Aug 05	11 Aug 05
Formulation Chemistry: Inhalation Dose Analysis	16 Aug 05	16 Aug 05
Terminal Necropsy	22 Aug 05	23 Aug 05
Fetal Soft Tissue Evaluations and Training Records	17 Oct 05	21 Oct 05
Exposure Data and Report	19-20 Dec 05	20 Dec 05
Final Report and Study Data	14 Dec 05- 04-Jan-06	06-Jan-06
Formulation Chemistry Exposure Data and Report	19-20 Dec 05	28 Feb 06
Protocol Amendments 1 and 2	12 Jan 06	12 Jan 06
Pathology Associates Facility Inspection	13 Jul 06	18 Oct 06
Protocol Amendment 3	10 Nov 08	10 Nov 08
Protocol and Draft Protocol Amendment 4[a]	03 May 11	05 May 11
Final Protocol Amendment 4[a]	25 May 11	25 May 11
Protocol Amendment 4	01 Dec 11	01 Dec 11
Final Report Review	22-23 Oct 12	23 Oct 12
Protocol Amendment 5	07 Nov 12	07 Nov 12
Protocol Amendment 5[a]	08 Nov 12	08 Nov 12

Fran Jannone 8 Nov 12

Fran Jannone, B.A., RQAP-GLP Date
Quality Assurance Group Leader

[a]Audits performed by Huntingdon Life Sciences-UK Quality Assurance and reported to Principal Investigator and Management on the date of inspection and Study Director and Management on the dates indicated above.

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY IN RATS VIA INHALATION EXPOSURES

SUMMARY

This study was designed to assess the potential maternal and/or developmental toxicity of Gasoline DIPE Vapor Condensate in the pregnant rat when administered by whole-body inhalation exposure. The study design permitted evaluation of maternal toxicity as revealed by clinical, body weight and food consumption changes and by gross necropsy findings, and evaluation of the developing conceptus, including death, structural abnormalities, or altered growth. This particular study is a near replicate of a previous study conducted by ExxonMobil Biomedical Sciences, Inc. (EMBSI Study Number 171734). The previous study was incomplete due to technical errors made in evaluating the fetuses. The Sponsor needed to repeat the previous study to satisfy the Environmental Protection Agency's Alternative Tier 2 Testing Requirements under Sections 211(b) (2) of the Clean Air Act.

The test substance was administered once daily as a vapor to pregnant Sprague Dawley CD® female rats (24/group) at target concentrations of 2000, 10000 and 20000 mg/m^3 for 6 hours/day, 7 days per week for Gestation Days (GD) 5-20. In addition, a control group (24/sex) received nitrogen enriched air only while in chamber. Exposure levels were determined 4 times per chamber per day. Particle size distribution measurements were also made once per chamber per week. The following parameters were evaluated: viability, clinical observations, body weights and feed consumption. A cesarean section was performed on GD 21. The animals were subjected to a macroscopic postmortem evaluation and corpora lutea/implantation data were recorded and the gravid uterus was weighed. The fetuses were examined for externally visible abnormalities and weighed. Approximately one-half of the fetuses (alternating fetuses) in each litter were examined for soft-tissue abnormalities using a microdissection procedure. The other half of the fetuses were then stained with Alizarin Red S and examined for skeletal abnormalities and ossification variations.

The overall mean (± standard deviation) analytical exposure concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 1982 ± 93, 10072 ± 546, 19776 ± 961 mg/m^3. The exposure regimen resulted in only transient reductions in maternal weight gain (approximately 12-25% reduced from Control at GD 11-14 interval, partly resolving by GD 21) and feed consumption (up to approximately 10%) during the exposure period. These changes were negligible at 2000 mg/m^3 and most noticeable at 20000 mg/m^3. It was considered that a no-observed-adverse-effect level (NOAEL) for maternal effects was evident at 2000 mg/m^3.There was no effect of the exposures at any level on pregnancy outcome, in terms of pre- or post-implantation loss, fetal weight or the incidence of fetal abnormalities and variants. A NOAEL for embryo-fetal effects was therefore established at 20000 mg/m^3.

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1. INTRODUCTION

The study was designed to assess the potential maternal and/or developmental toxicity of Gasoline DIPE (di-isopropyl ether) Vapor Condensate in the pregnant rat when administered by whole-body inhalation exposure. The study design permitted evaluation of maternal toxicity as revealed by clinical, body weight and food consumption changes and by gross necropsy findings, and evaluation of the developing conceptus, including death, structural abnormalities, or altered growth.

This particular study is a near replicate of a previous study conducted by ExxonMobil Biomedical Sciences, Inc. (EMBSI Study Number 171734). The previous study was incomplete due to technical errors made in evaluating the male reproductive system of the fetuses. The Sponsor needed to repeat the previous study to satisfy the Environmental Protection Agency's Alternative Tier 2 Testing Requirements under Sections 211(b) (2) of the Clean Air Act. The potential impact of the test substance on the fetal male reproductive system must still be evaluated within the context of the treatment requirements of those proscribed under the 211(b) Alternative Tier 2 Testing Requirements.

2. MATERIALS AND METHODS

2.1. STUDY MANAGEMENT

2.1.1. SPONSOR

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

2.1.2. SPONSOR REPRESENTATIVE

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

2.1.3. TESTING FACILITY

Huntingdon Life Sciences (HLS)
100 Mettlers Road
East Millstone, New Jersey 08875-2360

2.1.4. STUDY DIRECTOR[1]

Keith P. Hazelden, BSc, CBiol, MIBiol, Eurotox Registered Toxicologist

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

2.1.5. PRINCIPAL INVESTIGATOR – SKELETAL ASSESSMENTS

Michael Mercieca, M.S.
Pathology Associates International (PAI)

2.1.6. PRINCIPAL INVESTIGATOR – STATISTICS

Gareth D. Thomas, B.Sc. (Hons).
Huntingdon Life Sciences, UK

2.2. STUDY DATES

2.2.1. STUDY INITIATION

3 August 2005 (Date Study Director signed the Protocol)

2.2.2. DATE OF ANIMAL RECEIPT

4 August 2005 (GD 0, 1, 2 or 3)

2.2.3. EXPOSURE INITIATION

6 August 2005 (Experimental Start Date - First GD 5)

2.2.4. EXPOSURE TERMINATIONS

24 August 2005 (Last GD 20)

2.2.5. TERMINAL SACRIFICE

22-25 August 2005 (GD 21)

[1]Keith Hazeldon was responsible for the conduct of the study and submission of the draft report; however he is no longer employed with Huntingdon Life Sciences. Gary Hoffman assumes responsibility for finalization of the study report.

2.2.6. EXPERIMENTAL TERMINATION

01 December 2005 (Date of last data collection)

2.2.7. STUDY COMPLETION

8 November 2012 (Date Study Director signed the Final Report)

2.3. EXPERIMENTAL OUTLINE

Group	Group Designation	Exposure Levels (mg/m3)[a]	Treatment Gestation Days	Number of Animals				
				Time-Mated Females	Euthanized Gestation Day 21	Fetal Evaluations (Fetuses/Litter)		
						External	Soft Tissue	Skeletal
1	Control	0 (air only)	5-20	24	All	All	~1/2	~1/2
2	Low	2000	5-20	24	All	All	~1/2	~1/2
3	Mid	10000	5-20	24	All	All	~1/2	~1/2
4	High	20000	5-20	24	All	All	~1/2	~1/2

[a]Exposures were 6 hours per day for 7 consecutive days per week, for gestation days (GD) 5-20. Exposure levels are expressed as mg/m^3 of test substance. The exposures were conducted by whole-body exposure. Control animals were exposed to air enriched with nitrogen only, using the same treatment regimen as the treated groups.

The first day of gestation (GD 0 = day of detection of positive sign of mating) was defined as Day 0 of the study for each animal.

2.4. JUSTIFICATIONS

2.4.1. ROUTE, DURATION AND FREQUENCY OF ADMINISTRATION

The inhalation route was used for the EMBSI study and is one of the potential routes of human exposure to this test substance. The duration and frequency of the exposures were the same as for the EMBSI study, and as recommended in the relevant EPA and OECD test guidelines, ensuring adequate exposure of post-implantation embryo-fetal development.

2.4.2. EXPOSURE LEVEL SELECTION

The exposure levels were the same as those used in the EMBSI study, and as prescribed by the Sponsor based on the lower explosive limit (LEL) of the test substance (the low, intermediate

and high exposure levels were approximately 5, 25, and 50% of the LEL).

2.4.3. TEST ANIMAL SELECTION

The rat was used for the EMBSI study and is an accepted surrogate for humans in the detection of embryo-fetal toxicity. It is the rodent species that is recommended by the relevant EPA and OECD test guidelines. Historical control data are also available with this strain of rat for comparative evaluation, if necessary.

2.4.4. NUMBER OF ANIMALS

The number of animals in this study was considered the minimum necessary to allow for meaningful interpretation of the data as required by the OECD and EPA test guidelines. Some of the endpoints of principal interest (*eg* embryo-fetal death, fetal malformations) are low-frequency events and a group size of ~20 litters tends to provide the necessary degree of consistency between studies. In the expectation of pregnancy rates of 80-100%, the group size of 24 mated female rats was considered appropriate for this study, to provide a sufficient number of litters for evaluation in each group. Also, the number of animals in each group was comparable to the number in the EMBSI study (25/group).

2.5. TEST SUBSTANCE

Gasoline DIPE (di-isopropyl ether) Vapor Condensate

2.5.1. TEST SUBSTANCE CATEGORY

Gasoline product

2.5.2. SUPPLIER

ChevronTexaco Research and Technology Company
100 Chevron Way
Richmond, CA 94802

2.5.3. LOT NUMBER

API 01-06

2.5.4. PURITY

100% Gasoline DIPE Vapor Condensate

2.5.5. DESCRIPTION

Colorless liquid

2.5.6. DATES RECEIVED

7 December 2001
05 August 2002

2.5.7. EXPIRATION DATE

Not available, stable per MSDS.

2.5.8. ANALYSIS

Prior to use in this study, the identity, strength, composition, stability and method of synthesis, fabrication and/or derivation of each batch of the test substance was documented and is maintained by the Sponsor. In addition, the Sponsor conducted a non-GLP purity analysis and the Testing Facility conducted a GLP stability analysis of the test substance by GC prior to the start of this study.

2.5.9. STORAGE

Room temperature (ambient) in an outside solvent shed, except when being used in the inhalation laboratory.

2.5.10. DISPENSING

The test substance was received from ChevronTexaco Research and Technology Company in 100-gallon cylinders. Since only 5-gallon cylinders were practical to be used for exposure operations, the test substance was dispensed, as needed, at the Testing Facility from the 100 gallon cylinders into 5-gallon cylinders using nitrogen pressurization.

2.5.11. ARCHIVAL SAMPLE

An archival sample of the test substance was taken and is stored in the Archives of the Testing Facility under conditions specified for test article storage. Since multiple studies were conducted with the same test substance, a common archival sample was taken and appropriately labeled.

2.5.12. DISPOSITION

The unused portion of the test substance was returned to the Sponsor's designee following completion of the study. Empty test substance containers were returned to the Sponsor's designee on an as-needed basis. The Sponsor was responsible for tracking their disposition.

2.6. TEST ANIMALS

2.6.1. SPECIES

Albino Rats (Outbred) VAF/Plus®
Sprague-Dawley Derived (CD®)
Crl:CD® (SD) IGS BR

2.6.2. SUPPLIER

Charles River Laboratories
Raleigh, NC27610

2.6.3. NUMBER OF ANIMALS

Ordered and Received:
100 time-mated females

Placed on test:
96 females

2.6.4. AGE AT RECEIPT

Approximately 10-12 weeks

2.6.5. AGE AT INITIATION OF EXPOSURES

Approximately 10-12 weeks

2.6.6. WEIGHT AT INITIATION OF EXPOSURES (GRAMS)

	Mean	Range
Females:	244	216-280

Individual weights of animals placed on test were within ±20% of the mean weight.

2.6.7. ACCLIMATION PERIOD

Animals were acclimated for 2-5 days from the day of receipt until initiation of exposures on GD 5.

2.7. ANIMAL ASSIGNMENT

Animals were placed into study groups using a computerized randomization program. This ranked Gestation Day 4 body weights into blocks and randomly assigned each animal within each block into one of the study groups. Disposition of all animals not utilized in the study is maintained in the study file.

2.8. ANIMAL IDENTIFICATION

Each animal was assigned a temporary identification number upon receipt. After selection for study, each animal was ear-tagged by the Testing Facility with a number assigned by the Facility. The number assigned plus the study number comprised the unique animal number for each animal. Each cage was provided with a cage card that was color-coded for exposure level identification and contained the study number and animal number.

2.9. VETERINARY CARE

Animals were monitored by the technical staff for any conditions requiring possible veterinary care, and the staff veterinarian approved the animals for use in the study.

2.10. ANIMAL HUSBANDRY DURING NON-EXPOSURE PERIODS

2.10.1. FACILITIES MANAGEMENT/ANIMAL HUSBANDRY

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

2.10.2. HOUSING

All animals were housed individually in stainless steel suspended cages with wire mesh floors and fronts, except for GD 20-21, when solid-bottom cages with corn-cob bedding were employed (in case any animal littered prematurely). Cage rack positions in the room were rotated each week, so that possible effects due to placement were minimized. Each cage was fitted to secure a glass feeder jar with a stainless steel lid. Clean feed jars and fresh feed were provided at least every 7 days.

2.10.3. FEED

Certified Rodent Diet, No. 5002; (Meal) (PMI Feeds, Inc., St. Louis, MO) was available without restriction, except during actual exposures.

2.10.4. FEED ANALYSIS

Analytical certificates for relevant batches of feed, provided by the manufacturer, are maintained on file at the Testing Facility and are included in this report (Appendix N). There were no known contaminants in the feed that were expected to interfere with the objectives of this study.

2.10.5. WATER

Facility water was supplied by Elizabethtown Water Company, (Westfield, NJ) and was provided *ad libitum* to individual animal cages through an automated watering system, except during actual exposures.

2.10.6. WATER ANALYSIS

Water analyses are conducted monthly by the Elizabethtown Water Company, Westfield, NJ (Raritan-Millstone plant) to ensure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR part 141). In addition, water samples are collected biannually from representative rooms in the Testing Facility. Chemical and microbiological water analyses were conducted on these samples by a subcontract laboratory (Benchmark Analytics, Center Valley, PA). Water analyses (provided by the supplier) and chemical and microbiological water analyses (provided by the subcontractor) results are maintained on file at the Testing Facility and are included in this report (Appendix N). There were no known contaminants in the water that were expected to interfere with this study.

2.10.7. BEDDING

Corn-cob bedding (supplier) was provided in the solid-bottomed plastic cages used from GD 20 (lot number DC295). There were no known contaminants in the bedding that were expected to interfere with this study. An analysis of a representative batch used is included in Appendix N.

2.10.8. ENVIRONMENTAL CONDITIONS

Light/Dark Cycle

A twelve-hour light/dark cycle was provided, controlled by an automatic timer.

Temperature

Temperature was monitored in accordance with Testing Facility SOPs and was maintained within the specified range:

Desired Range:	18 to 26°C
Actual Range:	23 to 24°C
Daily Average Range:	23 to 24°C

Relative Humidity

Relative humidity was monitored in accordance with Testing Facility SOPs and was maintained within the specified range:

Desired Range:	30 to 70%
Actual Range:	49 to 67%
Daily Average Range:	55 to 60%

Air Changes

The animal room was set up to provide 10-15 air changes per hour. The actual number of air changes per hour in each animal room is recorded at least twice each year, and the Testing Facility retains these records.

2.11. TEST SUBSTANCE ADMINISTRATION AND CHAMBER OPERATION

See Appendix B, Inhalation Report.

2.12. EXPERIMENTAL EVALUATIONS

2.12.1. VIABILITY EXAMINATION (CAGE-SIDE)

Observations for mortality, morbidity, and signs of severe toxicity were made at least twice daily.

2.12.2. DETAILED PHYSICAL EXAMINATIONS

In-Chamber: All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Any pertinent behavioral changes and all signs of toxicity (including time of onset, degree and duration) were recorded.

Out-of-Chamber: Animals on study were examined daily from the day after receipt through to terminal euthanasia on GD 21. Examinations included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well

as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded, as well as evaluations of respiration. During the treatment period, these evaluations were performed after exposure.

2.12.3. BODY WEIGHTS

Body weights were recorded on GD 4 (for randomization into test groups), 5, 8, 11, 14, 17, 20 and 21 (day of scheduled sacrifice). Weight gain was calculated for these intervals, and also GD 5-14, 14-21 and 5-21.

2.12.4. FEED CONSUMPTION

Feed consumption was recorded and reported for the intervals: GD 4-5, 5-8, 8-11, 11-14, 14-17, 17-20, 20-21 and 5-21.

2.13. POSTMORTEM EVALUATIONS

2.13.1. METHOD OF EUTHANASIA

All dams were euthanized by carbon dioxide inhalation, by approximately noon on each day. All live fetuses were euthanized with intraperitoneal sodium pentobarbital.

2.13.2. MACROSCOPIC EXAMINATION

A macroscopic postmortem examination was performed on all the dams. Macroscopic lesions or tissues with significant findings were saved in 10% Neutral Buffered Formalin (NBF).

Apparently non-pregnant uteri were stained with ammonium sulphide (modified Salewski test) to confirm the non-pregnant status or detect implantation sites not otherwise visible.

Each dam had the following examinations of the reproductive tract on GD 21:

The intact uterus (ovaries attached) was removed from the abdominal cavity. The gravid uterus, including the cervix, was weighed. Corpora lutea were counted and the number per ovary

recorded. The number and location of the following was recorded for each uterine horn:

- live fetuses
- dead fetuses (no significant degeneration)
- late embryo-fetal deaths (recognizable dead fetus undergoing degeneration, regardless of size)
- early embryonic deaths (evidence of implantation but no recognizable fetus)

Each placenta was examined macroscopically. The maternal carcass, uterus, ovaries and placentas were then discarded.

2.14. FETAL EVALUATIONS

2.14.1. EXTERNAL EVALUATIONS

All live fetuses were weighed and individually identified within cassettes. Each live and dead retained fetus was given a macroscopic external examination for defects, including observation of the palate.

2.14.2. FETAL SOFT TISSUE (VISCERAL) EVALUATION

Approximately one-half of the fetuses in each litter (alternating fetuses within the litter, nominally) were placed in Modified Davidson's fixative for preservation and decalcification. These fetuses were subjected to soft tissue examination by gross dissection of the torso, and a razor blade sectioning technique for the head (Wilson and Warkany, 1965). All malformations and variations were recorded. Particular attention was paid to the reproductive tract for altered signs of development. During the dissection process, the sex of each fetus was confirmed by internal inspection of the gonads. Following complete dissection of the fetuses, all carcasses and sections were preserved in 10% neutral buffered formalin.

2.14.3. FETAL SKELETAL EVALUATIONS

Approximately one-half of the fetuses in each litter (alternating fetuses within the litter, nominally) were eviscerated, during which process the sex of each fetus was determined by internal inspection and recorded. The eviscerated fetuses were placed in 70%

isopropyl alcohol for preservation and processed for staining of the skeleton using Alizarin Red S. Subsequently, these fetuses were evaluated for skeletal malformations and ossification variations by an approved subcontractor, Pathology Associates International (PAI), 15 Worman's Mill Court, Suite 1, Fredrick, MD 21701, USA. These skeletal specimens were then stored in 100% glycerin with a mold inhibitor.

2.14.4. DEAD FETUSES

No conceptuses in this category (recently dead fetuses) occurred in this study. All late embryonic/fetal and early embryonic deaths were discarded.

2.15. STATISTICAL ANALYSIS

Analysis was performed by the Statistics Department of Huntingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. The Testing Facility was responsible for GLP compliance and the archiving of the raw data and original final statistical report.

A copy of the statistical report is provided as Appendix M. This provides details of the methods and statistical results.

2.15.1. CONTINUOUS DATA

The following mean measures were analyzed:

Maternal body weight values and body weight changes during gestation
Maternal feed consumption values (presented as grams of feed/kg of body weight/day)
Gravid uterine weight
Corpora lutea
Implantation sites
Pre-implantation loss as %
Early embryonic deaths ('early resorptions')
Late embryo-fetal deaths ('late resorptions')
Total embryo-fetal deaths, and as % of implantation sites
Live fetuses
Number of males and females per litter

2.15.2. INCIDENCE DATA

The following measures were analyzed:

Maternal necropsy findings
Maternal pregnancy data (including corpora lutea, implantation sites, resorptions, etc.)
Soft tissue (visceral) and skeletal fetal findings, of which only 5 visceral and 7 skeletal findings required analysis

2.15.3. FETAL BODY WEIGHT

Fetal body weight was analyzed by sex and as a composite for both sexes.

2.16. DATA STORAGE

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

At the completion of the study (submission of the signed final report), all raw data, preserved specimens and retained samples, as well as the original study protocol (and any amendments) and the original final report will be maintained in the Archives of the Testing Facility for one year following issue of the Final report. The Sponsor will determine the final disposition of these materials.

2.17. REGULATORY REFERENCES

2.17.1. TEST GUIDELINES

This study was designed to meet or exceed the pertinent requirements of:

US EPA Vehicle Emissions Inhalation Exposure Guideline 79.61, CFR Vol. 59, No. 122, 27 June 1994.

Organization for Economic Cooperation and Development (22 January 2001). OECD Guidelines for Testing of Chemicals; OECD Guideline 414: Prenatal Developmental Toxicity Study

US EPA OPPTS Health Effects Test Guidelines 870.3700, Prenatal Developmental Toxicity Study EPA 712-C-98-207, August 1998.

2.17.2. GOOD LABORATORY PRACTICES

This study was conducted in accordance with US EPA 79.60, CFR Vol. 59, No. 122, 27 June 1994. This study was performed according to protocol and Huntingdon Life Sciences' Standard Operating Procedures. The Testing Facility was responsible for the GLP compliance and archiving of raw data produced by subcontractors (PAI, and HLS Dept. of Statistics).

2.17.3. ANIMAL WELFARE ACT COMPLIANCE

This study complied 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.

2.18. PROTOCOL DEVIATIONS

The following protocol deviations occurred during the study, but did not affect the integrity of the study:

1. Due to technician oversight, the Set B animals (1907-1912, 2907-2912, 3907-3912 and 4907-4912) were not given clean feeders and fresh feed after seven days of use. They were fed on Gestation Day 2 but were not given new feeders until Gestation Day 10.

2. Due to technician oversight, gravid uterine weights for Animal Nos. 1912 and 4910 were not taken. The uteri were opened and all the fetuses were removed prior to recording necessary weights.

3. Fetus number 13 from dam number 2921 did not have an external examination as per the protocol.

3. RESULTS AND CONCLUSIONS

3.1. TEST SUBSTANCE ANALYSIS

(Appendix A)

GC analysis of the test substance showed no significant changes in composition, comparing pre-study results to those obtained during prior studies at this Testing Facility.

3.2. CHAMBER MONITORING

(Appendix B)

Pre-study chamber distribution analyses showed that the test substance was evenly distributed within each chamber. Chamber monitoring showed that the chamber oxygen levels were at least 19%. Chamber room monitoring showed that no test substance was present in the room and that the sound and light levels were acceptable.

The target and mean (± standard deviation) analytical (IR) and nominal concentrations (see Appendix B) are summarized as follows:

Group		Target Concentration (mg/m^3)	Analytical Concentration (mg/m^3)	Nominal Concentration (mg/m^3)
1	Air Control	0	0.00 ± 0.00	0 ± 0
2	Gasoline DIPE Vapor Condensate	2000	1982 ± 93	1995 ± 40
3	Gasoline DIPE Vapor Condensate	10000	10072 ± 546	11474 ± 513
4	Gasoline DIPE Vapor Condensate	20000	19776 ± 961	19842 ± 602

The analytically measured (IR) exposure levels of the airborne test substance were acceptably close, in the opinion of the study director, to the targeted exposure levels and to the nominal concentrations. Chamber environmental conditions averaged 24°C temperature and 41% relative humidity.

Mean particle size distribution measurements for the exposures are summarized as follows:

Group		Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation	Total Mass Concentration (mg/m^3)
1	Air Control	3.263 ± 4.3	2.033 ± 0.2	3.56E-03
2	Gasoline DIPE Vapor Condensate	4.061 ± 3.4	2.435 ± 0.5	5.15E-03
3	Gasoline DIPE Vapor Condensate	3.762 ± 2.6	2.677 ± 0.6	6.12E-03
4	Gasoline DIPE Vapor Condensate	3.118 ± 3.3	2.223 ± 0.1	6.29E-03

These results indicated that the atmospheres were essentially gas/vapor only, as expected, since there was no substantial difference between the test substance chambers and the air control chamber.

Analysis of the major components in the neat test substance and the test atmospheres (see Appendix A) showed an acceptably close comparison, in the opinion of the study director, between the neat test substance and the vaporized test substance. These data demonstrated that the test animals were exposed, as expected, to all of the major components of the test substance in their acceptably proper proportions. The data were consistent pretest and during the study indicating stability of the test substance and the atmosphere generation techniques.

3.3. MATERNAL DATA

3.3.1. MORTALITY AND PREGNANCY

(Table 1; Appendices C and M)

All animals survived until the scheduled termination of the study and pregnancy rates were 92-100% across all the groups.

3.3.2. CLINICAL OBSERVATIONS

(Table 2; Appendices B and D)

During the exposure and non-exposure periods, there was little overt reaction to treatment. There was only an increased incidence of red nasal discharge among the 10000 and 20000 mg/m^3 exposed animals, as compared with the other groups. Chromodacryorrhea was also observed at a low incidence at 20000 mg/m^3, in animals that did not show the nasal discharge. These two findings (interpreted as secretion of porphyrin) are common reactions to mild stress in rats. An animal (#4908) exposed at 20000 mg/m^3 was noted with an abdominal mass on GD 17 -21 and confirmed as a mammary gland mass at necropsy (see 3.3.5.).

3.3.3. BODY WEIGHTS

(Figure 1; Tables 3, 4 and 8; Appendices E, F, J and M)

The exposure regimen resulted in only minor, non-statistically significant reductions in maternal body weight (97.6%, 96.9% and 96.2% of the Control group value on GD 21 for the 2000, 10000 and 20000 mg/m^3 groups, respectively).

The exposure regimen resulted in only transient reductions in maternal weight gain (up to approximately 25% reduced from Control at GD 11-14 interval, partly resolving by GD 21) during the exposure period. These changes were negligible at 2000 mg/m^3 and most noticeable at 20000 mg/m^3.

3.3.4. FEED CONSUMPTION

(Figure 2; Table 5; Appendices G and M)

The exposure regimen resulted in only transient reductions in feed consumption (up to approximately 10% reduced) during the exposure period. These changes were negligible at 2000 mg/m^3 and most noticeable at 20000 mg/m^3.

3.3.5. MACROSCOPIC POSTMORTEM EVALUATIONS

(Table 6; Appendices H and M)

The only maternal necropsy finding was of a mass in the mammary tissue of one animal (#4908) at 20000 mg/m^3. This finding was considered incidental, and not related to treatment.

3.3.6. UTERINE IMPLANTATION DATA

(Table 7; Appendices I and M)

There was no effect of the exposures on the pregnancies. Pre- and post-implantation losses and litter size were similar in all the groups.

3.3.7. GRAVID UTERINE WEIGHT DATA

(Table 8; Appendices J and M)

The maternal net body weight change from Day 5 minus the gravid uterine weight ("net weight change from Day 5" in Table 8 and Appendix J) was significantly reduced (95% of the control group value in the 20000 mg/m^3 group); however, this reduction was not considered adverse because the reduction was minimal and within normal animal variation.

Uterine weight was reduced (96% of the control group value) but this reduction was not considered adverse because the differences were slight, within normal animal variation and there were no statistically significant differences between the treated group and control group.

The percent reduction in uterine weight and in maternal net body weight change from Day 5 minus the gravid uterine weight in the exposed groups was comparable with the minor reduction in maternal body weights and body weight changes observed in these groups.

3.4. FETAL DATA

3.4.1. Fetal Body Weights

(Table 7; Appendices I, K and M)

There was no effect of the exposures on fetal weight at GD 21. The very marginal (3-4%) difference from Control that occurred in the 20000 mg/m3 group, while achieving statistical significance in total fetal and female weights, was considered not to represent an effect of the exposures, particularly in context with the lack of other signs of effect on pregnancy outcome in the study and the values are comparable to the historical control range for published data from Charles River Labs (see Appendix P).

3.4.2. Fetal Observations

(Table 9; Appendices L and M)

A very small number of major abnormalities occurred sporadically in the study, with no relationship to the exposures.

Similarly, there were sporadic incidences across the groups, of structural variants, both soft-tissue and skeletal. No association with the exposures was inferred.

Although the incidence of 14^{th} (supernumerary, rudimentary) ribs was higher at 20000 mg/m^3 than in the other groups, the difference from Control did not achieve statistical significance, and remained consistent with the range of incidences that are seen in this strain of animal. The mean group incidence for this strain in this laboratory is approximated by the 2000 and 10000 mg/m^3 group mean incidences (7 and 5 fetuses in 5 litters, respectively), while the 20000 mg/m^3 exposure group incidence (12 fetuses in 9 litters) is nearer the upper limit of the range seen previously (14 fetuses in 10 litters) (see Appendix P).

There was no evidence of any effect of the exposures on the general state of skeletal ossification.

4. CONCLUSIONS

Exposure of pregnant rats to atmospheres of 0 (Air Control), 2000, 10000 or 20000 mg/m^3 of Gasoline DIPE Vapor Condensate, 6 hours/day on gestation days 5-20, resulted in only transient reductions in maternal weight gain and feed consumption during the exposure period. These changes were negligible at 2000 mg/m^3 and most noticeable at 20000 mg/m^3. It was considered that a no-observed-adverse-effect level (NOAEL) for maternal effects was evident at 2000 mg/m^3. There was no effect of the exposures at any level on pregnancy outcome, in terms of pre- or post-implantation loss, fetal weight or the incidence of fetal abnormalities and variants. A NOAEL for embryo-fetal effects was therefore established at 20000 mg/m^3.

REFERENCES

Salewski, E. 1964. Farbemethode zum makroskopischen machweis von implantationsstellen am uterus der ratte. *Archiv. Path. Exp. Pharmakol*. 247: 367.

Wilson, J.S. and Warkany, J.1965, Teratology: Principles and Techniques, Chicago: The University of Chicago Press, Page 271.

Note: Appendix M provides details of the methods and statistical results.

CALCULATIONS

Feed Consumption (g/kg/day):

[(grams of feed presented – grams of feed remaining)/average of the previous and current body weights in kilograms]/no. days

Preimplantation Loss:

no. of corpora lutea – no. of implantation sites

Percent Preimplantation Loss:

[(no. of corpora lutea – no. of implantation sites)/no. of corpora lutea] X 100

Postimplantation Loss:

total no. of early and late resorptions and dead fetuses

Percent Postimplantation Loss:

(no. of early and late resorptions and dead fetuses/no. of implantation sites) X 100

Mean % Live Fetuses (number per animal):

(no. of male or female live fetuses/ total number of live fetuses) X 100

Mean % Dead Fetuses (number per animal):

(no. of dead fetuses/ total number of implantation sites) X 100

Mean Percent Resorptions (implants per animal):

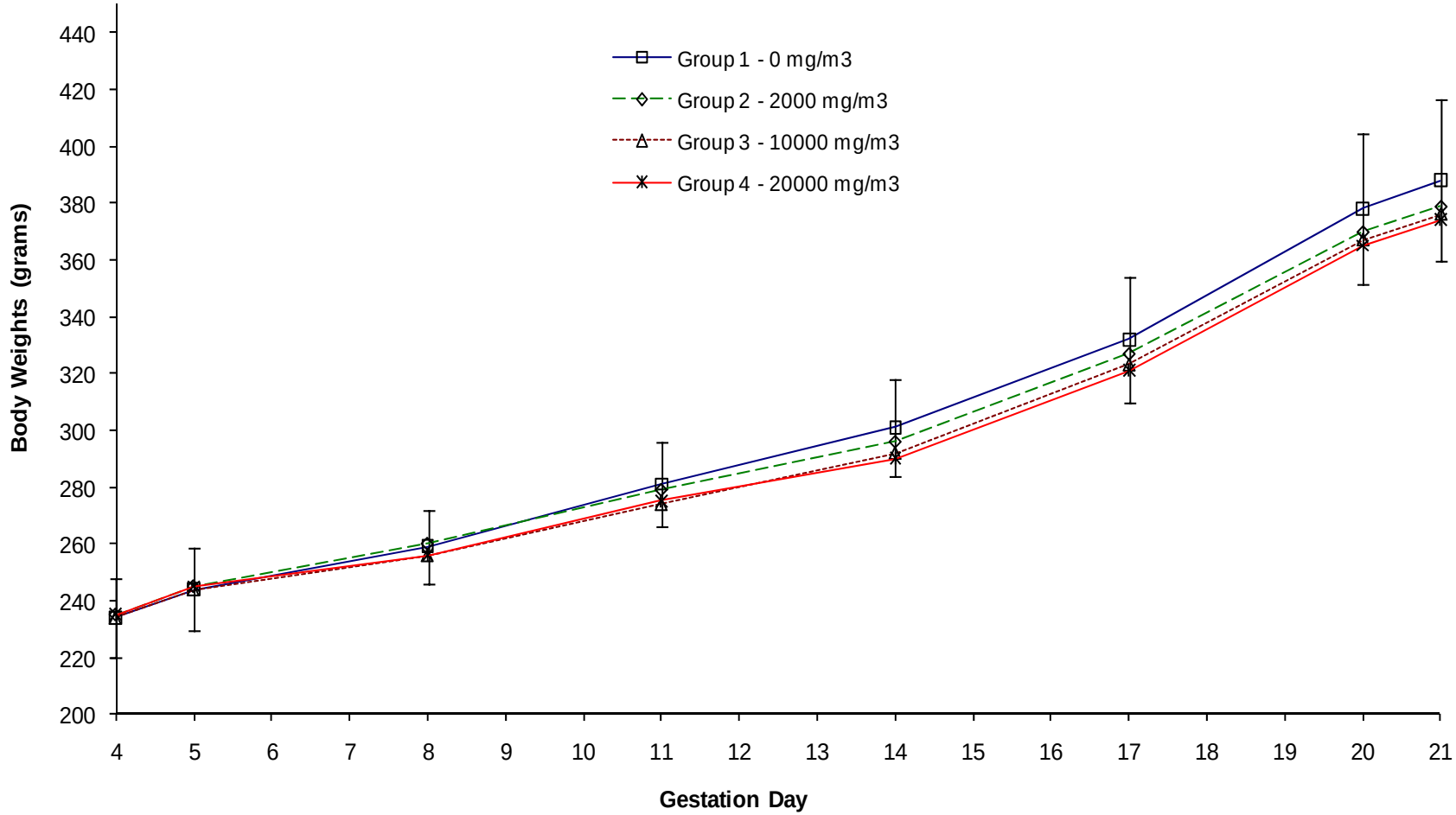
total no. of early or late resorptions/ total number of implantation sites) X 100

	General Preface	

General Notes

Individual animal data values presented in this report may be rounded. Unrounded individual animal data values are used to calculate the reported mean and standard deviation values. Therefore, use of the reported individual values to reproduce means, standard deviations and/or to perform any subsequent calculations may produce minor discrepancies between the calculated values and those presented in this report.

	Mean Gestation Body Weights (grams)	Figure 1



	Mean Gestation Feed Consumption (g/kg/day)	Figure 2

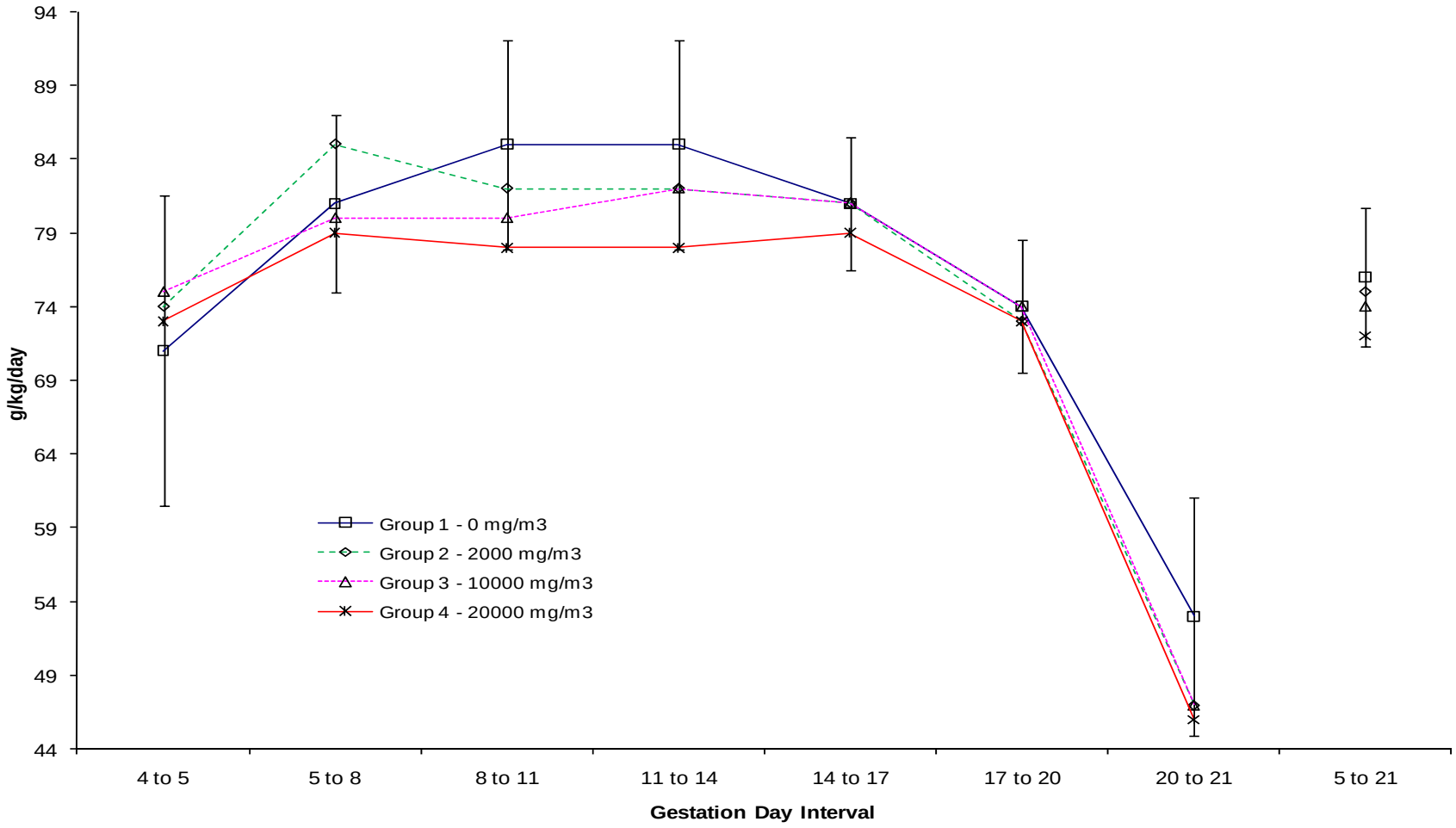


TABLE 1

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF SURVIVAL AND PREGNANCY

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
No. of females mated	N	24	24	24	24
Pregnant	N	23	23	24	22
- Died/sacrificed moribund	N	0	0	0	0
- Elective sacrifice	N	0	0	0	0
Nonpregnant	N	1	1	0	2
- Died/sacrificed moribund	N	0	0	0	0
- Elective sacrifice	N	0	0	0	0
Total no. of females died/	N	0	0	0	0
sacrificed moribund	%	0.0	0.0	0.0	0.0
Examined at scheduled c-section	N	24	24	24	24
- Nonpregnant	N	1	1	0	2
- With total implant loss	N	0	0	0	0
	%	0.0	0.0	0.0	0.0
- With viable fetuses	N	23	23	24	22
	%	95.8	95.8	100.0	91.7

TABLE 2

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF CLINICAL OBSERVATIONS DURING GESTATION - (frequency/animals)

DOSE GROUP:	1	2	3	4
DOSE LEVEL (MG/M3):	0	2000	10000	20000
DAY 4 to 21				
Normal				
WITHIN NORMAL LIMITS	431/24	421/24	382/24	349/23
TERMINAL SACRIFICE	24/24	24/24	24/24	24/24
Dermal-General				
ALOPECIA - GENERAL	0/ 0	0/ 0	0/ 0	8/ 1
Ocular				
CHROMODACRYORRHEA - UNILATERAL	0/ 0	0/ 0	0/ 0	14/ 3
Oral/Buccal				
INCISORS MALOCCLUDED	0/ 0	0/ 0	0/ 0	18/ 1
NASAL DISCHARGE - RED	1/ 1	11/ 3	50/15	43/ 9
Palpable masses				
MASS	0/ 0	0/ 0	0/ 0	5/ 1

TABLE 3

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF GESTATION BODY WEIGHTS (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
DAY 4	MEAN	234	235	234	235
	S.D.	14.0	16.6	15.7	14.6
	N	23	23	24	22
DAY 5	MEAN	244	245	244	245
	S.D.	14.6	16.5	13.9	15.2
	N	23	23	24	22
DAY 8	MEAN	259	260	256	256
	S.D.	12.8	18.0	14.4	16.3
	N	23	23	24	22
DAY 11	MEAN	281	279	274	275
	S.D.	14.9	19.6	15.6	19.5
	N	23	23	24	22
DAY 14	MEAN	301	296	292	290
	S.D.	17.0	20.3	14.8	19.5
	N	23	23	24	22
DAY 17	MEAN	332	327	323	321
	S.D.	22.2	24.3	18.7	24.9
	N	23	23	24	22
DAY 20	MEAN	378	370	367	365
	S.D.	26.3	29.9	24.2	27.1
	N	23	23	24	22
DAY 21	MEAN	388	379	376	374
	S.D.	28.6	32.7	25.9	27.4
	N	23	23	24	22

TABLE 4

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF GESTATION BODY WEIGHT GAIN (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
DAYS 4 TO 5	MEAN	10	11	10	10
	S.D.	6.1	4.7	5.2	3.2
	N	23	23	24	22
DAYS 5 TO 8	MEAN	15	15	13	11
	S.D.	4.5	4.7	5.5	4.7
	N	23	23	24	22
DAYS 8 TO 11	MEAN	22	19	18	20
	S.D.	4.7	3.6	3.9	5.5
	N	23	23	24	22
DAYS 11 TO 14	MEAN	20	17	17	15
	S.D.	4.2	5.0	3.7	4.0
	N	23	23	24	22
DAYS 14 TO 17	MEAN	31	31	31	31
	S.D.	7.3	6.9	7.6	6.8
	N	23	23	24	22
DAYS 17 TO 20	MEAN	46	44	44	44
	S.D.	7.6	8.9	9.9	7.7
	N	23	23	24	22
DAYS 20 TO 21	MEAN	10	9	9	8
	S.D.	5.2	5.0	4.9	5.1
	N	23	23	24	22
DAYS 5 TO 14	MEAN	57	50	48	45
	S.D.	8.4	7.8	7.7	9.5
	N	23	23	24	22
DAYS 14 TO 21	MEAN	86	84	84	83
	S.D.	15.5	16.3	17.0	10.0
	N	23	23	24	22
DAYS 5 TO 21	MEAN	144	134	132	129
	S.D.	20.6	21.5	21.0	16.0
	N	23	23	24	22

TABLE 5

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF GESTATION FEED CONSUMPTION (GRAMS/KG/DAY)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
DAYS 4 TO 5	MEAN	71	74	75	73
	S.D.	10.5	11.5	11.4	10.2
	N	22	23	24	22
DAYS 5 TO 8	MEAN	81	85	80	79
	S.D.	6.0	14.1	6.0	6.0
	N	22	23	24	21
DAYS 8 TO 11	MEAN	85	82	80	78
	S.D.	7.1	4.6	5.5	5.7
	N	22	23	24	21
DAYS 11 TO 14	MEAN	85	82	82	78
	S.D.	7.1	4.7	3.9	4.0
	N	22	23	22	21
DAYS 14 TO 17	MEAN	81	81	81	79
	S.D.	4.5	4.5	6.0	4.4
	N	22	23	22	21
DAYS 17 TO 20	MEAN	74	73	74	73
	S.D.	4.5	3.3	3.7	4.3
	N	23	23	22	22
DAYS 20 TO 21	MEAN	53	47	47	46
	S.D.	8.1	7.5	5.8	7.8
	N	23	23	24	22
DAYS 5 TO 21	MEAN	76	75	74	72
	S.D.	4.7	4.0	3.6	3.8
	N	23	23	24	22

TABLE 6

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF MATERNAL NECROPSY OBSERVATIONS

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
FEMALES	N	24	24	24	24
GROSS EXAM	N	0	0	0	1
MASS	N	0	0	0	1
	%	0.0	0.0	0.0	4.2

TABLE 7

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF CESAREAN SECTION DATA

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
Pregnant (at scheduled sacrifice)	N	23	23	24	22
Dams with no Viable Fetuses	N	0	0	0	0
Dams with Viable Fetuses	N	23	23	24	22
Corpora Lutea	TOTAL	343	334	354	334
No. per animal	MEAN	14.9	14.5	14.8	15.2
	S.D.	2.78	2.06	2.52	2.63
Implantation Sites	TOTAL	311	310	315	307
No. per animal	MEAN	13.5	13.5	13.1	14.0
	S.D.	2.71	1.50	2.85	2.38
Preimplantation Loss	TOTAL	32	24	39	27
No. per animal	MEAN	1.4	1.0	1.6	1.2
	S.D.	1.88	1.46	2.96	1.77
% per animal	MEAN%	9.3	6.6	10.4	7.6
	S.D.	13.46	7.99	18.58	9.14
Live Fetuses	TOTAL	300	286	302	292
No. per animal	MEAN	13.0	12.4	12.6	13.3
	S.D.	2.64	2.64	3.06	2.60
Males	TOTAL	146	133	154	135
	MEAN%	48.5	46.1	51.2	46.8
	S.D.	10.94	14.75	12.50	13.92
Females	TOTAL	154	153	148	157
	MEAN%	51.5	53.9	48.8	53.2
	S.D.	10.94	14.75	12.50	13.92

TABLE 7

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF CESAREAN SECTION DATA

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
Postimplantation Loss	TOTAL	11	24	13	15
No. per animal	MEAN	0.5	1.0	0.5	0.7
	S.D.	0.67	2.08	0.83	0.65
% implants per animal	MEAN%	3.4	7.9	5.1	5.2
	S.D.	4.66	14.89	8.64	5.15
Dead Fetuses	TOTAL	0	0	0	0
No. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00
% of implants per animal	MEAN%	0.0	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00
Resorptions: Early	TOTAL	10	23	13	15
No. per animal	MEAN	0.4	1.0	0.5	0.7
	S.D.	0.66	2.07	0.83	0.65
% of implants per animal	MEAN%	3.1	7.6	5.1	5.2
	S.D.	4.66	14.80	8.64	5.15
Resorptions: Late	TOTAL	1	1	0	0
No. per animal	MEAN	0.0	0.0	0.0	0.0
	S.D.	0.21	0.21	0.00	0.00
% of implants per animal	MEAN%	0.3	0.3	0.0	0.0
	S.D.	1.39	1.60	0.00	0.00

TABLE 7

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF CESAREAN SECTION DATA

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
Fetal Body Weight (g)	MEAN	5.7	5.8	5.7	5.5
	S.D.	0.29	0.30	0.28	0.32
	N	23	23	24	22
Male Fetuses	MEAN	5.9	5.9	5.8	5.7
	S.D.	0.32	0.33	0.32	0.33
Female Fetuses	MEAN	5.6	5.6	5.5	5.4
	S.D.	0.28	0.33	0.25	0.31

TABLE 8

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

SUMMARY OF GRAVID UTERINE WEIGHT AND NET BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
UTERUS WEIGHT	MEAN	101	97	96	96
	S.D.	19.4	19.8	22.5	15.7
	N	22	23	24	20
ADJUSTED WEIGHT	MEAN	286	283	280	273
	S.D.	18.1	19.0	17.1	15.9
	N	22	23	24	20
NET WEIGHT CHANGE FROM DAY 5	MEAN	43	37	36	29
	S.D.	11.1	9.1	11.7	10.6
	N	22	23	24	20
WEIGHT CHANGE FROM DAY 5	MEAN	144	134	132	129
	S.D.	20.6	21.5	21.0	16.0
	N	23	23	24	22

UTERUS WEIGHT = ABSOLUTE GRAVID UTERINE WEIGHT
ADJUSTED WEIGHT = TERMINAL BODY WEIGHT - UTERUS WEIGHT
NET WEIGHT CHANGE FROM DAY 5 = ADJUSTED WEIGHT – DAY 5 BODY WEIGHT
WEIGHT CHANGE FROM DAY 5 = TERMINAL BODY WEIGHT – DAY 5 BODY WEIGHT

Females	Summary of Fetal Observations	Table 9

		Fetuses				Litters			
Observations	Group	1	2	3	4	1	2	3	4
(Major abnormalities in CAPITALS)	Number examined	300	286	302	292	23	23	24	22
HYDROCEPHALY[a], ANOPHTHALMIA; HYPOPLASTIC APICAL LUNG LOBE, THYMUS AND SPLEEN				1				1	
MICROPHTHALMIA/ANOPHTHALMIA, UNILATERAL				1				1	
EXOCCIPITAL FUSED TO 1ST CERVICAL ARCH					1				1
MANDIBLE SHORTENED, MODERATE			1				1		
THORACIC VERTEBRA 13, LUMBAR VERTEBRA 1: ACENTRIC, MALFORMED					1				1
FORELIMB SHORTENED, RIGHT			1				1		
ANAL ATRESIA, ABSENT SACRAL AND CAUDAL VERTEBRAE		1				1			
Additional subclavian artery		1				1			
Innominate artery absent		1		2	1	1		2	1
Undescended thymic tissue		10	11	5	11	6	8	3	8

[a]Noted as DOMED CRANIUM in external observations.

Females	Summary of Fetal Observations	Table 9

		Fetuses				Litters			
Observations	Group	1	2	3	4	1	2	3	4
(Major abnormalities in CAPITALS)	Number examined	300	286	302	292	23	23	24	22
Renal papilla (e) not fully developed		1		2	1	1		2	1
Ureter(s) distended		1		1	1	1		1	1
Left umbilical artery		1	4	1	2	1	3	1	2
Testis displaced, medially, left		1				1			
7th cervical rib(s) present			1		3		1		3
Sternebra(e) malaligned		11	11	13	3	9	9	10	3
Rib(s) rudimentary, 13th		1			3	1			1
Rib(s) rudimentary, 14th		2	7	5	12	1	5	5	9
25 presacral vertebrae		1			3	1			1
27 presacral vertebrae					1				1

Females	Summary of Fetal Observations	Table 9

		Fetuses				Litters			
Observations	Group	1	2	3	4	1	2	3	4
(Major abnormalities in CAPITALS)	Number examined	300	286	302	292	23	23	24	22
Hyoid body/arch(es) unossified		1		1	1	1		1	1
Sternebra 5 and/or 6 unossified		1		3	3	1		3	2
Metatarsals: 1 unossified, bilateral					2				1

	Analytical Report	Appendix A

STUDY TITLE

Analytical Report For:

Gasoline DIPE Vapor Condensate: Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure

AUTHOR

Yonggang Wang

REPORT DATE

19 October 2012

STUDY NUMBER

05-4287

SIGNATURES

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Date

Table of Contents

1. Summary

A neat sample of the Gasoline DIPE (Diisopropyl Ether) Vapor Condensate (G/DIPE VC) test substance was assayed before initiation of animal exposures to demonstrate stability by comparison to prior data at this Testing Facility.

Inhalation chamber samples of the Gasoline DIPE Vapor Condensate were analyzed during each week of the study to demonstrate the stability of the test substance and the comparability of the test atmospheres to the neat test substance.

The analytical method (HLS-001-02R2) was validated at Huntingdon Life Sciences (HLS). The analytical method involves, when appropriate, the collection of chamber air samples onto charcoal tubes, extraction with carbon disulfide, and quantification using Gas Chromatography with Flame Ionization Detection (FID).

2. Experimental Procedures

The analytical method (HLS-001-02R2) was validated by Formulation Chemistry at HLS. Details of the analytical method are maintained in this study file.

The neat test substance and the charcoal tube samples containing the test substance were received from the Inhalation Department at HLS. Samples analyzed to determine the relative concentration of the major components of Gasoline DIPE Vapor Condensate were extracted from the charcoal tubes with Carbon Disulfide (CS_2). The extracted solutions were analyzed by Gas Chromatography equipped with a Supelco Petrocol™ DH 150 (150m x 0.25mm, 1.0 μm) column and Flame Ionization Detector (FID). PE Nelson TotalChrom 6.2.1 installed on a personal computer was used for data collection and processing.

Date of sample analysis is listed as follows:

Interval	Date of Exposures	Date Received	Date Analyzed
Pretest/Stability	N/AV	29 Mar 05	30-31 Mar 05
Pretest /Trials	02 & 05 Aug 05	02 & 05 Aug 05	03-05 Aug 05
Exposure-4	09 & 11 Aug 05	09 & 11 Aug 05	09 -11 Aug 05
Exposure-11	16 Aug 05	16 Aug 05	16 -18 Aug 05
Exposure-18	23 Aug 05	23 Aug 05	23 - 24 Aug 05

3. Results and Discussion

Before the study, a Gasoline DIPE Vapor Condensate sample was analyzed to determine the stability of the area percent of the test substance's major components. During the trials and exposures, Gasoline DIPE Vapor Condensate air samples were analyzed to determine the area percent of the test substance's major components in the chamber. The results of the stability analysis, pretest, summary of animal exposures and individual animal exposures are presented in Tables I, II, III and IVA-IVC. Typical chromatograms of Groups 1, 2, 3, and 4 are presented in Figures I to IV.

Table I. Chamber Components Confirmation
Area Percent of Gasoline DIPE Vapor Condensate Test Substance Stability
Pretest

Area %

Compound	Mean TM Std-1 for 00-4205[a]	Mean TM Std-2 00-4205[a]	TM Std-1 05-4287[b]	TM Std-2 05-4287[b]
Isobutane	1.61	1.60	1.34	1.42
N-Butane	9.57	9.59	9.05	9.25
3-Methyl-1-butene	0.35	0.34	0.34	0.34
Isopentane	32.26	32.35	32.58	32.50
N-Pentane	7.38	7.46	9.99	9.93
Trans-2-pentene	1.11	1.14	2.16	2.16
2,3-Dimethylbutane	1.76	1.78	1.10	1.08
2-Methylpentane	5.31	5.29	5.00	5.45
3-Methylpentane	3.21	3.20	3.23	3.16
N-hexane & **DIPE**	22.24	22.16	22.17	21.78
Methylcyclopentane	1.44	1.43	1.44	1.39
2,4-Dimethylpentane	1.16	1.17	0.93	1.15
Benzene	2.29	2.28	2.20	2.18
2-Methylhexane	1.33	1.32	1.33	1.26
2,3-Dimethylpentane	1.38	1.37	1.38	1.35
3-Methylhexane	1.54	1.53	1.49	1.48
Isooctane	1.71	1.69	1.65	1.58
Toluene	3.08	3.09	2.43	2.34
Total	98.73	98.79	99.81	99.80

ND = none detected. <LOQ = Less than the limit of quantification = less than 25% of the area count of the component in the test substance standard.

[a] These values are obtained from the Summary report for Study 00-4205.
[b] These values are obtained from standard injections made on 31 March 2005 for DIPE test substance stability (see Sequence # 001 in 05-4287).

Note: The discrepancy in the N-Pentane and Trans-2-pentene values are due to the analytical columns used for each study analyses. The analytical column used for Study 00-4205 analyses produced a split peak for N-Pentane and Trans-2-pentene but the column used for this study separated the two peaks entirely.

Table II. Chamber Components Confirmation
Area Percent of Gasoline DIPE Vapor Condensate
Pretest /Trials

Area %

	DIPE STD (Tank #4)	DIPE STD (Tank #5)	Spiked Control 1	Spiked Control 2	Sample 101	Sample 201	Sample 301	Sample 401
Compound	**002_008**	**002_009**	**002_005**	**002_006**	**002_007**	**002_002**	**002_003**	**002_004**
Isobutane	1.40	1.59	1.44	1.45	ND	1.52	1.73	1.64
N-Butane	9.30	9.70	9.41	9.52	ND	9.62	10.55	10.19
3-Methyl-1-butene	0.36	0.35	0.36	0.35	ND	0.36	0.35	0.36
Isopentane	32.61	32.29	32.74	33.00	ND	32.08	32.85	32.85
N-Pentane	10.15	9.81	10.19	10.27	ND	9.99	10.08	10.13
Trans-2-pentene	2.35	2.18	2.43	2.41	ND	2.30	2.25	2.28
2,3-Dimethylbutane	1.70	1.63	1.42	1.40	ND	1.66	1.34	1.37
2-Methylpentane	5.23	5.28	5.24	5.14	ND	5.48	5.17	5.03
3-Methylpentane	3.24	3.19	3.34	3.34	ND	3.24	3.14	3.23
N-hexane & **DIPE**	19.12	20.13	19.87	19.25	ND	19.64	18.65	19.66
Methylcyclopentane	1.46	1.44	1.15	1.23	ND	1.47	1.41	1.30
2,4-Dimethylpentane	1.22	1.19	1.27	1.20	ND	1.20	1.17	1.22
Benzene	2.32	2.23	2.38	2.32	ND	2.42	2.24	2.31
2-Methylhexane	1.28	1.27	1.34	1.28	ND	1.32	1.21	1.29
2,3-Dimethylpentane	1.28	1.24	1.37	1.32	ND	1.29	1.24	1.26
3-Methylhexane	1.52	1.48	1.59	1.55	ND	1.54	1.49	1.55
Isooctane	1.65	1.50	0.50	1.09	ND	1.58	1.24	0.65
Toluene	3.11	2.93	3.09	3.02	ND	3.22	2.91	2.98
Total	99.30	99.43	99.13	99.14	0.00	99.93	99.02	99.30

ND = none detected. <LOQ = Less than the limit of quantification = less than 25% of the area count of the component in the test substance standard.

Table III. Summary of Chamber Components Confirmation
Mean Area Percent of Gasoline DIPE Vapor Condensate (Exposures 4, 11, and 18)
Area %

Compound	Spiked Control-1	TM Standard-1 (Tank # 5)	Samples Group 1	Samples Group 2	Samples Group 3	Samples Group 4	All Samples [b]	Spiked Control-2	TM Standard-2 (Tank # 5)
Isobutane	0.95	1.21	ND	1.59	1.71	1.44	1.58	1.30	1.48
N-Butane	6.92	7.70	ND	10.43	10.56	9.32	10.10	8.67	9.40
3-Methyl-1-butene	0.37	0.33	ND	0.36	0.36	0.34	0.35	0.37	0.37
Isopentane	30.72	31.26	ND	32.88	32.67	32.50	32.68	32.94	32.07
N-Pentane[a]	9.74[a]	8.92	ND	9.35	9.29	9.67[a]	9.44[a]	9.05[a]	9.26[a]
Trans-2-pentene	a	1.89	ND	2.11	2.08	a	a	a	a
2,3-Dimethylbutane	1.54	1.46	ND	1.47	1.46	1.34	1.42	1.52	1.67
2-Methylpentane	5.72	5.38	ND	5.26	5.06	5.00	5.11	5.56	5.37
3-Methylpentane	3.63	3.55	ND	3.21	3.17	3.20	3.19	3.42	3.26
N-hexane & **DIPE**	22.50	21.71	ND	19.27	19.62	20.39	19.76	20.90	20.64
Methylcyclopentane	1.67	1.68	ND	1.46	1.43	1.47	1.45	1.59	1.50
2,4-Dimethylpentane	1.44	1.41	ND	1.21	1.18	1.24	1.21	1.34	1.25
Benzene	2.87	2.50	ND	2.31	2.25	2.31	2.29	2.42	2.35
2-Methylhexane	1.66	1.58	ND	1.46	1.27	1.34	1.36	1.48	1.38
2,3-Dimethylpentane	1.58	1.52	ND	1.30	1.27	1.36	1.31	1.51	1.38
3-Methylhexane	1.79	1.73	ND	1.56	1.48	1.54	1.53	1.70	1.56
Isooctane	1.51	1.65	ND	1.31	1.48	1.58	1.46	1.82	1.61
Toluene	3.74	3.70	ND	3.16	2.94	2.91	3.00	3.57	3.23
Total	98.35	99.18	ND	99.70	99.28	96.95	97.24	99.16	97.78

ND = none detected.
[a] N-Pentane co-eluted with Trans-2-pentene during the analysis for exposure 11.
[b] average of Groups II, III and IV only

Table IV-A: Gasoline DIPE Vapor Condensate Characterization ("Exposure 4")

Area %

	DIPE STD 1 (Tank #5)	DIPE STD 2 (Tank #5)	Spiked Control 1	Spiked Control 2	Sample 1001	Sample 2001	Sample 3001	Sample 4001
Compound	**003_002**	**003_009**	**003_006**	**003_007**	**003_008**	**003_003**	**003_010[a]**	**003_005**
Isobutane	1.50	1.45	1.18	1.03	ND	1.98	1.66	1.67
N-Butane	9.07	8.68	7.55	6.26	ND	12.46	10.22	10.32
3-Methyl-1-butene	0.37	0.34	0.32	0.30	ND	0.38	0.37	0.36
Isopentane	32.26	32.02	30.69	30.24	ND	32.72	32.80	32.45
N-Pentane	9.62	9.56	9.36	8.99	ND	9.51	10.14	9.99
Trans-2-pentene	2.17	2.13	2.14	2.07	ND	2.23	2.32	2.28
2,3-Dimethylbutane	1.65	1.54	1.53	1.57	ND	1.36	1.39	1.50
2-Methylpentane	5.28	5.42	5.52	5.85	ND	4.36	5.09	5.11
3-Methylpentane	3.29	3.40	3.43	3.71	ND	2.80	3.21	3.11
N-hexane & **DIPE**	20.26	20.25	21.94	20.90	ND	18.42	19.25	19.67
Methylcyclopentane	1.50	1.58	1.62	1.85	ND	1.28	1.47	1.41
2,4-Dimethylpentane	1.28	1.28	1.32	1.50	ND	1.16	1.20	1.17
Benzene	2.34	2.41	2.54	2.88	ND	2.18	2.32	2.24
2-Methylhexane	1.36	1.40	1.48	1.65	ND	1.33	1.25	1.22
2,3-Dimethylpentane	1.36	1.43	1.46	1.75	ND	1.30	1.25	1.26
3-Methylhexane	1.60	1.64	1.73	2.03	ND	1.57	1.49	1.45
Isooctane	1.43	1.66	1.82	2.23	ND	1.37	1.50	1.41
Toluene	3.19	3.36	3.73	4.70	ND	3.39	3.05	2.87
Total	99.53	99.55	99.36	99.51	0.00	99.80	99.98	99.49

ND = none detected.
[a] Resample results

Table IV-B: Gasoline DIPE Vapor Condensate Characterization ("Exposure 11")

Area %

	DIPE STD 1 (Tank #5)	DIPE STD 2 (Tank #5)	Spiked Control 1	Spiked Control 2	Sample 1002	Sample 2002	Sample 3002	Sample 4002
Compound	**004_001**	**004_008**	**004_005**	**004_006**	**004_007**	**004_002**	**004_003**	**004_004**
Isobutane	1.18	1.50	0.90	1.36	ND	0.99	1.86	1.21
N-Butane	6.84	9.32	7.17	9.65	ND	7.21	10.76	8.24
3-Methyl-1-butene	0.32	0.45	0.37	0.44	ND	0.33	0.36	0.31
Isopentane	30.09	31.63	30.61	32.93	ND	32.12	32.39	31.99
N-Pentane	8.81	10.11[a]	11.54[a]	10.23[a]	ND	10.57	9.81	10.78[a]
Trans-2-pentene	1.96	[a]	[a]	[a]	ND	2.37	2.19	[a]
2,3-Dimethylbutane	1.57	1.73	1.80	1.48	ND	1.61	1.34	1.15
2-Methylpentane	5.67	5.33	5.76	5.16	ND	6.03	5.04	4.96
3-Methylpentane	3.59	3.21	3.78	3.39	ND	3.77	3.09	3.34
N-hexane & **DIPE**	22.48	21.18	21.85	20.75	ND	19.63	19.52	20.55
Methylcyclopentane	1.75	1.48	1.76	1.56	ND	1.72	1.42	1.61
2,4-Dimethylpentane	1.46	1.25	1.50	1.32	ND	1.34	1.15	1.34
Benzene	2.69	2.39	2.85	2.38	ND	2.63	2.25	2.54
2-Methylhexane	1.62	1.37	1.63	1.47	ND	1.53	1.23	1.51
2,3-Dimethylpentane	1.68	1.39	1.62	1.46	ND	1.40	1.27	1.50
3-Methylhexane	1.91	1.55	1.91	1.64	ND	1.75	1.46	1.68
Isooctane	1.88	1.60	1.16	1.65	ND	1.18	1.42	1.75
Toluene	4.17	3.33	3.75	3.01	ND	3.67	3.03	2.96
Total	99.67	98.82	99.96	99.88	0.00	99.85	99.59	97.42

[a] N-pentane and Trans-2-pentene are coeluted..
ND = None Detected

Table IV-C: Gasoline DIPE Vapor Condensate Characterization ("Exposure 18")

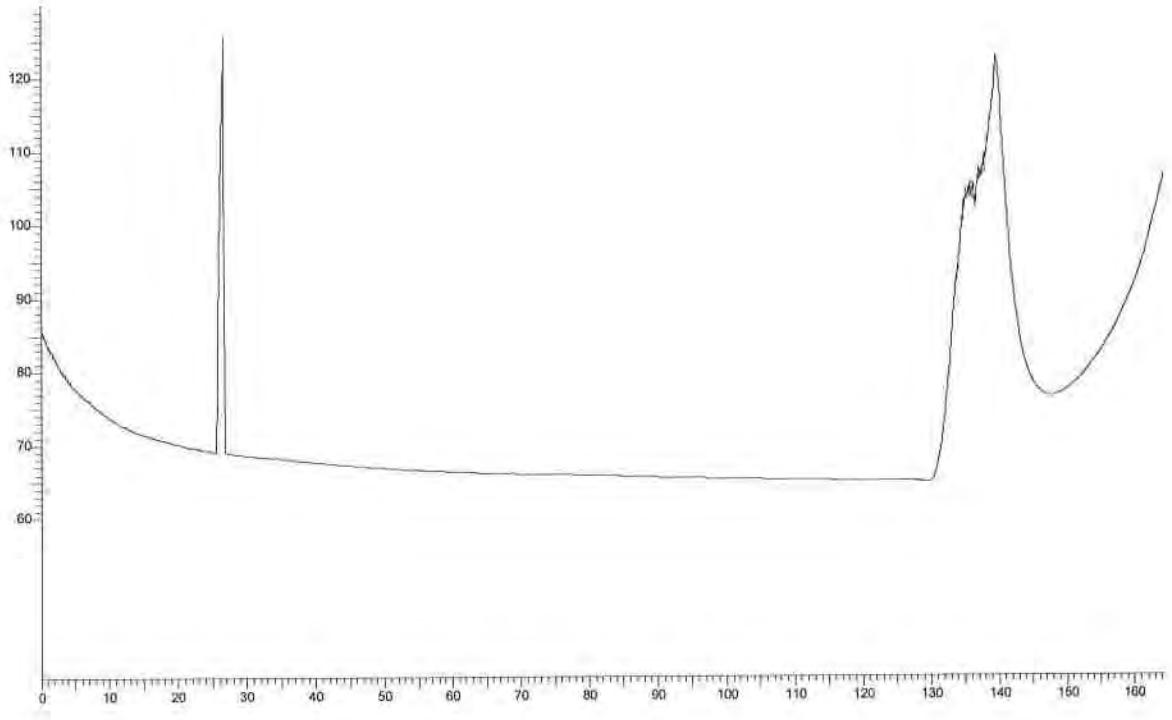
Area %

	DIPE STD 1 (Tank #5)	DIPE STD 2 (Tank #5)	Spiked Control 1	Spiked Control 2	Sample 1003	Sample 2003	Sample 3003	Sample 4003
Compound	**005_002**	**005_009**	**005_001**	**005_007**	**005_008**	**005_004**	**005_005**	**005_006**
Isobutane	0.95	1.50	0.77	1.52	ND	1.79	1.60	1.44
N-Butane	7.20	10.19	6.03	10.11	ND	11.62	10.69	9.41
3-Methyl-1-butene	0.31	0.32	0.41	0.36	ND	0.36	0.34	0.35
Isopentane	31.42	32.57	30.87	32.65	ND	33.79	32.83	33.07
N-Pentane	8.32	8.10	8.31	7.92	ND	7.97	7.92	8.24
Trans-2-pentene	1.55	2.15	1.95	1.66	ND	1.73	1.72	1.91
2,3-Dimethylbutane	1.17	1.75	1.28	1.51	ND	1.45	1.66	1.37
2-Methylpentane	5.19	5.35	5.87	5.68	ND	5.38	5.05	4.94
3-Methylpentane	3.76	3.17	3.68	3.17	ND	3.07	3.20	3.16
N-hexane & **DIPE**	22.38	20.49	23.71	21.04	ND	19.75	20.09	20.94
Methylcyclopentane	1.80	1.43	1.64	1.36	ND	1.37	1.41	1.38
2,4-Dimethylpentane	1.50	1.21	1.49	1.21	ND	1.12	1.19	1.20
Benzene	2.48	2.26	3.23	2.01	ND	2.11	2.19	2.15
2-Methylhexane	1.73	1.36	1.88	1.31	ND	1.22	1.32	1.30
2,3-Dimethylpentane	1.52	1.33	1.66	1.32	ND	1.21	1.30	1.32
3-Methylhexane	1.68	1.48	1.74	1.44	ND	1.35	1.48	1.49
Isooctane	1.65	1.58	1.54	1.59	ND	1.37	1.53	1.59
Toluene	3.73	2.99	3.73	3.00	ND	2.41	2.74	2.90
Total	98.34	99.23	99.79	99.86	0.00	99.07	98.26	98.16

[a] N-pentane and Trans-2-pentene are co-eluted.
ND = None Detected

Figure I. A Typical Gas Chromatogram of Group 1 (Air Control) Chamber Sample

(File # 003_008.rst)



Response (mV) vs Time (minutes)

Figure II. A Typical Gas Chromatogram of Group 2 (G/DIPE VC) Chamber Sample

(File # 003_003.rst)

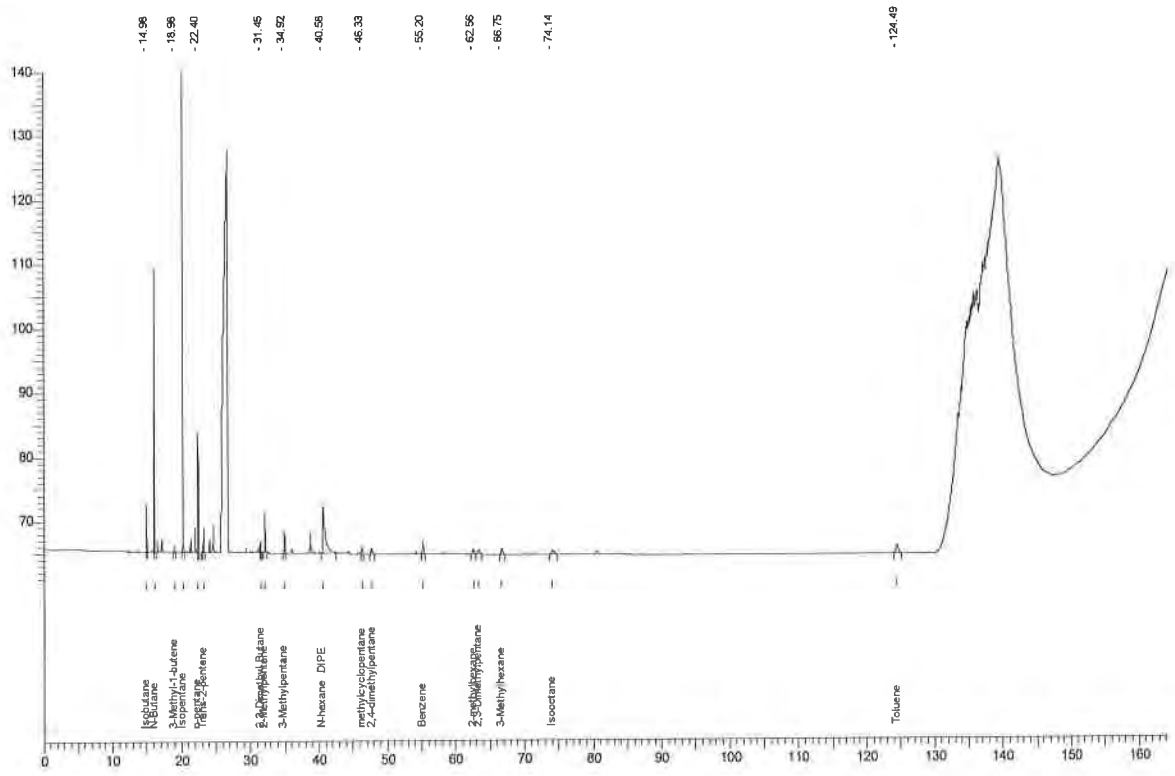
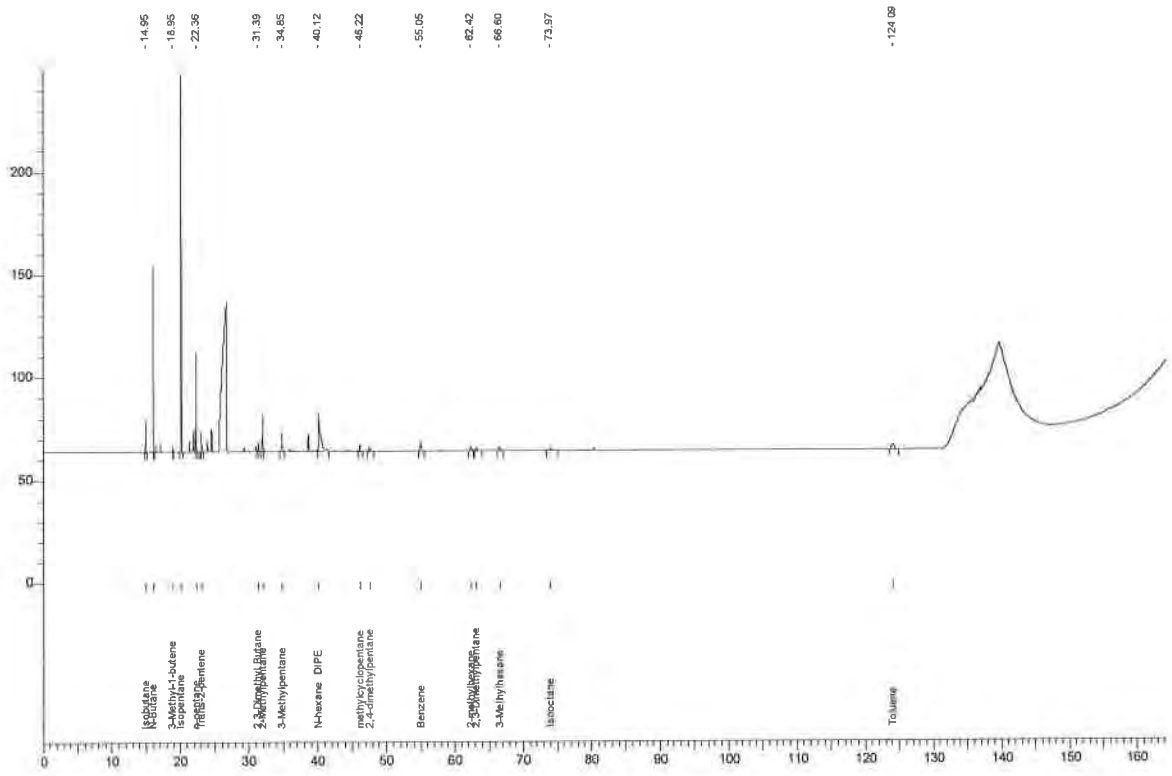


Figure III. A Typical Gas Chromatogram of Group 3 (G/DIPE VC) Chamber Sample

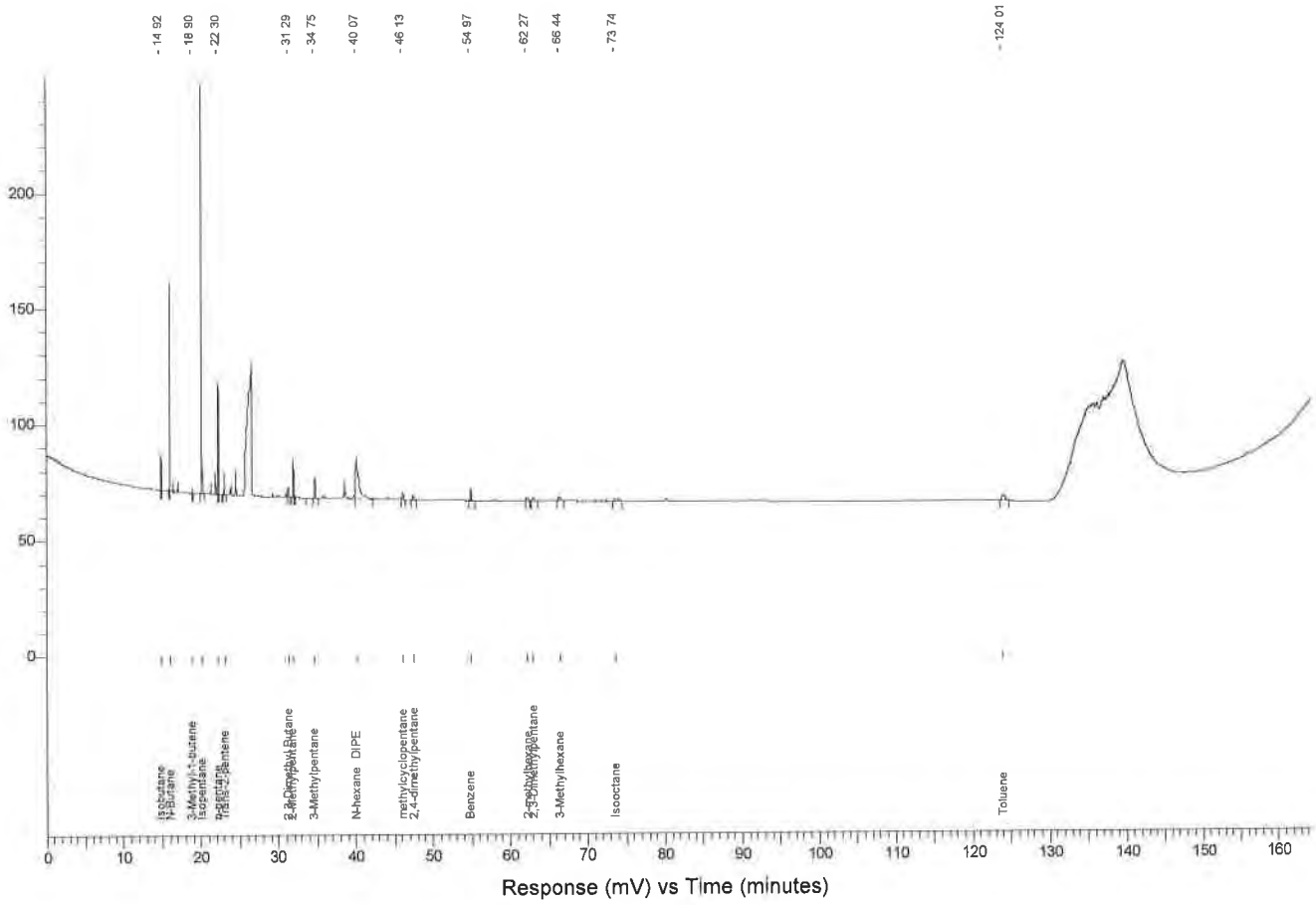
(File # 003_010.rst)



Response (mV) vs Time (minutes)

Figure IV. A Typical Gas Chromatogram of Group 4 (G/DIPE VC) Chamber Sample

(File # 003_005)



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1. INTRODUCTION

This appendix presents the methodology for exposure generation, monitoring and results.

2. MATERIALS AND METHODS

2.1. HUSBANDRY DURING EXPOSURE PERIODS

2.1.1. HOUSING

Animals were individually housed in stainless steel wire mesh cages within a 1000 Liter glass and stainless steel whole-body exposure chamber. The placement of the animals in the whole-body exposure chamber was rotated daily to ensure uniform exposure of the animals. A description of the animal rotation is included in the raw data.

2.1.2. FEED

None was provided during exposure.

2.1.3. WATER

None was provided during exposure.

2.1.4. ENVIRONMENTAL CONDITIONS

Chamber temperature and relative humidity were recorded every half-hour during exposure and maintained, to the maximum extent possible, within the ranges presented below. Excursions outside the specified range did not affect the integrity of the study.

Temperature

Desired: 20 to 24°C
Actual: 20 to 25°C

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The daily average temperature within the chamber was outside the desired range on 7/19 days and 12/19 days for Groups I and III, respectively.

Relative Humidity

Desired: 40 to 60%
Actual: 36 to 54%

The daily average relative humidity within the chamber was outside the desired range on 2/19 days and 1/19 days for Groups I and III, respectively.

2.2. TEST SUBSTANCE ADMINISTRATION AND CHAMBER OPERATIONS

2.2.1. ROUTE OF ADMINISTRATION

Inhalation via whole-body exposures.

2.2.2. TEST SUBSTANCE ADMINISTRATION

The test substance was administered once daily as a vapor in the breathing air of the animals. The test atmosphere was generated by an appropriate procedure determined during pre-study trials. The trials were performed to evaluate the optimal set of conditions and equipment to generate a stable and uniform atmosphere at the target exposure levels. The method was described in the raw data of the study and is outlined in this report.

2.2.3. TARGET EXPOSURE LEVELS

Group 1 - 0 mg/m^3
Group 2 - 2000 mg/m^3
Group 3 - 10000 mg/m^3
Group 4 - 20000 mg/m^3

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2.2.4. DURATION AND FREQUENCY OF ADMINISTRATION

The test substance was administered once daily for 6 hours/day, 7 days per week for Gestation Days (GD) 5-20.

2.2.5. CHAMBER OPERATIONS

The whole-body exposure chambers each had a volume of approximately 1000 Liters. The chambers were operated at a minimum flow rate of 200 Liters per minute. The final airflow was set to provide at least one air change (calculated by dividing the chamber volume by the airflow rate) in 5.0 minutes (12 air changes/hour) and a T_{99} equilibrium time (calculated by multiplying the air change by the exponential factor 4.6) of at most 21-22 minutes. Initial settings for each group were as follows:

Group	Airflow Rate (Lpm)	Air Change (min)	T_{99} (min)
1	210	4.7	22
2	208	4.8	22
3	216	4.6	21
4	210	4.7	22

The chamber atmospheres were exhausted through the in-house filtering system, which consisted of a coarse filter, a HEPA filter, and an activated charcoal bed.

Chamber static pressure was recorded every half-hour during exposure. The chambers were operated for a minumum of 23 minutes (approximate T_{99}) following the exposure with clean air only to allow the atmosphere to clear.

Refer to Figures 1 and 2, and Table III for equipment details.

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2.2.6. EXPOSURE PROCEDURE

Group 1

Houseline nitrogen was delivered from a regulator with a backpressure gauge via ¼" tubing to a flowmeter regulated by a metering valve. This nitrogen flow was then directed into the turret of the 1000 Liter glass and stainless steel exposure chamber where it was mixed with room air as it was drawn into the chamber. This nitrogen flow simulated the generation nitrogen flow for Groups 2, 3 and 4.

Groups 2-4

Houseline nitrogen was delivered from a regulator with a backpressure gauge through a stainless steel cross to create three flow systems: the test substance pressurization flow, the purge flow and the volatilization flow.

The test substance pressurization flow was directed via ¼" tubing through a metering valve, attached to a backpressure gauge, into the vapor inlet valve of the test substance cylinder. The metering valve was used to adjust and maintain the pressure within the cylinder. From the pressurized cylinder, the test substance flowed from the liquid outlet valve through a quick-disconnect fitting and through a filter to prevent equipment contamination. From the filter tube, the test substance flowed to a liquid flowmeter via $^{1}/_{8}$" tubing. The outlet of the flowmeter was regulated by a built-in metering valve. From this flowmeter and metering valve, the test substance flowed via $^{1}/_{8}$" tubing onto the glass helix of a counter current volatilization chamber. The glass helix was heated by a nichrome wire, which was controlled by an autotransformer and was inserted in the center of the glass tube that supported the helix.

The nitrogen for the purge flow system was directed, via ¼" tubing to a flowmeter regulated by a metering valve. The purge nitrogen was delivered via $^{1}/_{8}$" tubing to the bottom of the tube containing the nichrome wire. This nitrogen flow continuously purged the

area surrounding the nichrome wire within the tube, thereby protecting the wire from oxidation.

The nitrogen for the volatilization system was directed via ¼" tubing to a flowmeter regulated by a metering valve. From the flowmeter, the volatilization nitrogen flowed via ¼" tubing to a ball and socket joint at the bottom of the volatilization chamber. This nitrogen flowed up through the volatilization chamber passing over the coil and volatilizing the test substance. The pressure within the counter-current volatilization chamber was maintained slightly negative to the room and was monitored with a pressure gauge.

This test substance laden nitrogen exited the top of the volatilization chamber through a glass elbow, which directed the flow, via ½" tubing, to the turret of 1000 Liter glass and stainless steel exposure chamber. As the test substance laden nitrogen was drawn into the chamber, it was mixed with room air.

Refer to Figures 1 and 2, and Table III for equipment details.

2.3. EXPOSURE CONCENTRATION DETERMINATION

2.3.1. NOMINAL CONCENTRATION

A nominal exposure concentration was calculated daily. The flow of air through the chambers was monitored using appropriate calibrated equipment. The nominal concentrations in mg/m^3 were determined by weighing the generation apparatus containing the test substance before and after the exposure and dividing the difference in these weights by the total volume of air used during the exposure (flowrate multiplied by total exposure time).

Calculation

$$\text{Conc } (mg/m^3) = \frac{\text{amount consumed (g) x 1000 mg/g x 1000 L/m}^3}{\text{exposure duration (min) x airflow (Lpm)}}$$

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2.3.2. CHAMBER SAMPLING

Determinations of the exposure levels were made using a MIRAN® Ambient Air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the normal sampling portal through the MIRAN® and measurements were recorded at least 4 times during each exposure. The exposure levels were determined by comparison of the measured absorbance to a calibrated response curve constructed using the same instrument settings.

Also, one charcoal tube sample per chamber per week was analyzed by gas chromatography (GC) to characterized 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.

See Table III for equipment list.

2.3.3. PARTICLE SIZE DISTRIBUTION

Particle size distribution measurements were performed once during each week of exposure using a TSI Aerodynamic Particle Sizer. The samples were drawn for 20 seconds at a rate of 5.0 Lpm. A computer was used to program the system to the appropriate settings prior to sampling. The particle size distributions were calculated by the computer and printed out. The mass median aerodynamic diameter, geometric standard deviation and total mass concentration were calculated. For comparison, room air samples were similarly collected at least once weekly.

2.3.4. CHAMBER AND EXPOSURE ROOM ENVIRONMENT

Chamber oxygen levels (maintained at least 19%) were measured pretest and at the beginning (Day 3), middle (Day 12) and end of the study (Day 19).

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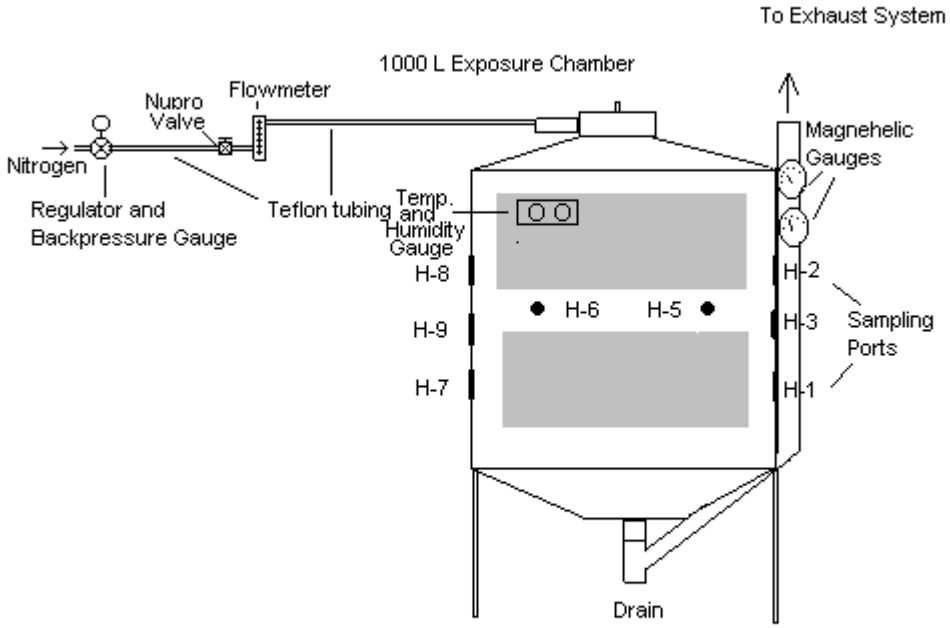
Air samples were taken in the vapor generation area pretest and at the beginning (Day 3), middle (Day 12) and end of the study (Day 19). Light (maintained approximately 30 to 40 foot-candles at 1.0 meters above the floor) and noise levels (maintained below 85 decibels) in the exposure room were measured pretest and at the beginning (Day 3), middle (Day 12) and end of the study (Day 19).

Group 1	Inhalation Report	Appendix B

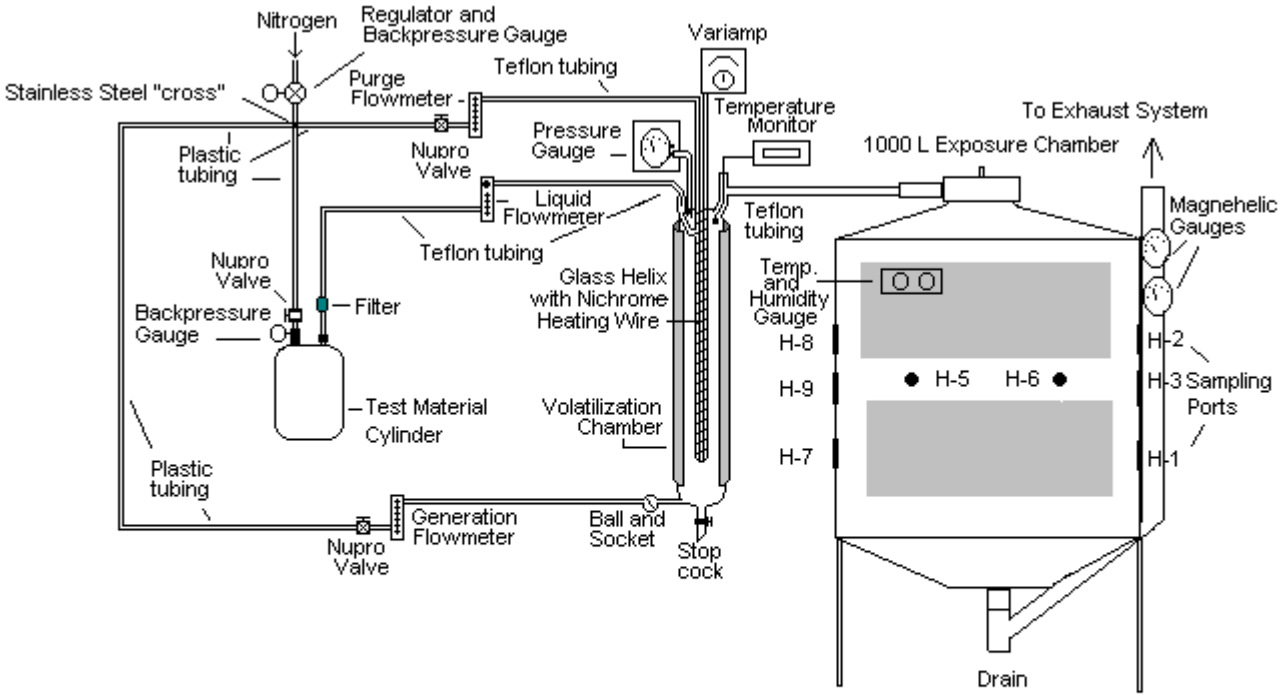
Figure I
Chamber Generation System
And Whole Body Exposure Chamber



Note:
1. Animals were individually housed on two levels within the exposure chamber.

Groups 2 - 4	Inhalation Report	Appendix B

Figure II
Chamber Generation System
And Whole Body Exposure Chamber



Notes:
1. Animals were individually housed on two levels within the exposure chamber.

	Inhalation Report	Appendix B

Table I
Summary of In-Chamber Observations

Exposure Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Group 1 – 0 mg/m^3																			
Within Normal Limits	6	12	18	24	24	24	24	24	24	24	24	24	24	24	24	24	18	12	6
Group 2 – 2000 mg/m^3																			
Within Normal Limits	6	12	18	24	24	24	24	24	24	24	24	24	24	24	24	24	18	12	6
Group 3 – 10000 mg/m^3																			
Within Normal Limits	6	12	18	24	24	24	24	24	24	24	24	24	24	24	24	24	18	12	6
Group 4 – 20000 mg/m^3																			
Within Normal Limits	6	12	18	24	24	24	24	24	24	24	24	24	24	24	24	24	18	12	6

Appendix B - Table II

Chamber Monitoring Results
Cumulative Exposure Record
Group 1 - 0 mg/m^3 (Air only)

Day	Date	Exposure Number	Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal (mg/m^3)	Mean (mg/m^3)	Individual (mg/m^3)				MMAD (µm)	GSD	TMC (mg/m^3)	Mean Temperature (°C)	Humidity (%)
0	6-Aug-05	1	0	0.00	0.00	0.00	0.00	0.00				23	42
1	7-Aug-05	2	0	0.00	0.00	0.00	0.00	0.00				23	41
2	8-Aug-05	3	0	0.00	0.00	0.00	0.00	0.00				24	39
3	9-Aug-05	4	0	0.00	0.00	0.00	0.00	0.00	0.8011	2.031	4.25E-03	24	40
4	10-Aug-05	5	0	0.00	0.00	0.00	0.00	0.00				24	41
5	11-Aug-05	6	0	0.00	0.00	0.00	0.00	0.00				24	40
6	12-Aug-05	7	0	0.00	0.00	0.00	0.00	0.00				25	40
7	13-Aug-05	8	0	0.00	0.00	0.00	0.00	0.00				25	42
8	14-Aug-05	9	0	0.00	0.00	0.00	0.00	0.00				24	43
9	15-Aug-05	10	0	0.00	0.00	0.00	0.00	0.00				25	39
10	16-Aug-05	11	0	0.00	0.00	0.00	0.00	0.00	0.7675	1.792	4.78E-03	24	40
11	17-Aug-05	12	0	0.00	0.00	0.00	0.00	0.00				25	40
12	18-Aug-05	13	0	0.00	0.00	0.00	0.00	0.00				25	40
13	19-Aug-05	14	0	0.00	0.00	0.00	0.00	0.00				25	40
14	20-Aug-05	15	0	0.00	0.00	0.00	0.00	0.00				24	40
15	21-Aug-05	16	0	0.00	0.00	0.00	0.00	0.00				25	40
16	22-Aug-05	17	0	0.00	0.00	0.00	0.00	0.00				24	40
17	23-Aug-05	18	0	0.00	0.00	0.00	0.00	0.00	8.2210	2.276	1.64E-03	24	41
18	24-Aug-05	19	0	0.00	0.00	0.00	0.00	0.00				23	41
		Mean	**0**		**0.00**				**3.263**	**2.033**	**3.56E-03**	**24**	**40**
		S.D.	**0**		**0.00**				**4.3**	**0.2**	**0.0**	**0.7**	**1.0**

Appendix B - Table II

Chamber Monitoring Results
Cumulative Exposure Record
Group 2 - 2000 mg/m^3

Day	Date	Exposure Number	Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal (mg/m^3)	Mean (mg/m^3)	Individual (mg/m^3)				MMAD (µm)	GSD	TMC (mg/m^3)	Temperature (°C)	Humidity (%)
0	6-Aug-05	1	2000	1900	1800	1800	1900	1900				23	43
1	7-Aug-05	2	2000	1900	2000	1900	1800	1900				23	42
2	8-Aug-05	3	2000	2000	2200	1800	2000	2000				23	40
3	9-Aug-05	4	2100	2100	2200	2000	2000	2000	3.882	2.954	7.23E-03	24	41
4	10-Aug-05	5	2000	2000	2100	2000	1900	2100				24	43
5	11-Aug-05	6	2000	2000	2000	2200	2100	1800				24	42
6	12-Aug-05	7	2000	2000	2100	2000	2000	2000				24	41
7	13-Aug-05	8	2000	2000	2000	1900	2000	2000				24	43
8	14-Aug-05	9	2000	2000	1900	2000	2000	2000				24	45
9	15-Aug-05	10	2000	2000	2100	2000	2000	2000				24	41
10	16-Aug-05	11	1900	2000	2000	2000	2000	1900	0.7935	2.294	6.14E-03	24	42
11	17-Aug-05	12	2000	2000	2000	1900	2100	2100				24	41
12	18-Aug-05	13	2000	2000	1900	1900	2000	2000				24	42
13	19-Aug-05	14	2000	2000	1900	2100	2000	2000				24	42
14	20-Aug-05	15	1900	2000	2000	1800	2000	2000				24	42
15	21-Aug-05	16	2000	2000	2000	1900	2100	2000				24	42
16	22-Aug-05	17	2000	1900	1900	1900	1900	2000				24	42
17	23-Aug-05	18	2000	2000	2000	1900	2000	2000	7.508	2.057	2.08E-03	23	43
18	24-Aug-05	19	2000	2000	2100	1800	2000	2100				23	42
		Mean	**1995**		**1982**				**4.061**	**2.435**	**5.15E-03**	**24**	**42**
		S.D.	**40**		**93**				**3.4**	**0.5**	**0.0**	**0.5**	**1.1**

Appendix B - Table II

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

Day	Date	Exposure Number	Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal (mg/m^3)	Mean (mg/m^3)	Individual (mg/m^3)				MMAD (µm)	GSD	TMC (mg/m^3)	Mean Temperature (°C)	Mean Humidity (%)
0	6-Aug-05	1	11000	10000	10000	9600	10000	11000				23	42
1	7-Aug-05	2	11000	9800	9400	9700	10000	9900				24	41
2	8-Aug-05	3	11000	9900	10000	10000	9800	9900				24	41
3	9-Aug-05	4	11000	9600	9300	9500	9700	9900	0.8112	2.450	5.75E-03	24	41
4	10-Aug-05	5	12000	10000	11000	10000	10000	10000				25	41
5	11-Aug-05	6	12000	10000	9700	9900	9400	11000				25	41
6	12-Aug-05	7	11000	9700	9900	9600	9400	9700				25	40
7	13-Aug-05	8	12000	10000	10000	10000	10000	10000				25	42
8	14-Aug-05	9	12000	9800	9700	10000	10000	9400				25	44
9	15-Aug-05	10	12000	9800	9600	9900	9700	10000				25	40
10	16-Aug-05	11	12000	10000	9900	9800	11000	11000	4.952	3.334	1.07E-02	25	41
11	17-Aug-05	12	12000	10000	10000	10000	10000	10000				25	40
12	18-Aug-05	13	11000	10000	10000	10000	10000	10000				25	39
13	19-Aug-05	14	11000	9800	9600	9700	9900	9900				25	42
14	20-Aug-05	15	12000	10000	11000	9700	9700	10000				25	42
15	21-Aug-05	16	12000	11000	11000	11000	11000	10000				25	42
16	22-Aug-05	17	11000	10000	11000	10000	10000	10000				24	40
17	23-Aug-05	18	11000	10000	11000	9100	9600	11000	5.523	2.246	1.91E-03	24	41
18	24-Aug-05	19	11000	11000	11000	12000	10000	11000				23	41
		Mean	**11474**		**10072**				**3.762**	**2.677**	**6.12E-03**	**25**	**41**
		S.D.	**513**		**546**				**2.6**	**0.6**	**0.0**	**0.7**	**1.1**

Appendix B - Table II

Chamber Monitoring Results
Cumulative Exposure Record
Group 4 - 20000 mg/m^3

Day	Date	Exposure Number	Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal (mg/m^3)	Mean (mg/m^3)	Individual (mg/m^3)				MMAD (µm)	GSD	TMC (mg/m^3)	Mean Temperature (°C)	Mean Humidity (%)
0	6-Aug-05	1	20000	19000	18000	20000	20000	19000				23	42
1	7-Aug-05	2	21000	21000	19000	20000	22000	21000				23	41
2	8-Aug-05	3	19000	20000	21000	20000	19000	19000				24	40
3	9-Aug-05	4	21000	20000	21000	20000	20000	20000	0.7811	2.137	4.84E-03	24	40
4	10-Aug-05	5	20000	20000	18000	18000	22000	20000				24	41
5	11-Aug-05	6	20000	20000	18000	20000	21000	20000				24	41
6	12-Aug-05	7	20000	20000	19000	20000	20000	20000				24	41
7	13-Aug-05	8	20000	20000	19000	19000	19000	21000				24	44
8	14-Aug-05	9	19000	19000	21000	20000	18000	18000				24	45
9	15-Aug-05	10	20000	20000	20000	20000	19000	19000				24	41
10	16-Aug-05	11	19000	20000	20000	20000	19000	19000	0.7640	2.260	9.80E-03	24	41
11	17-Aug-05	12	20000	21000	21000	21000	21000	20000				24	41
12	18-Aug-05	13	20000	20000	19000	20000	20000	20000				24	41
13	19-Aug-05	14	20000	20000	20000	21000	18000	21000				24	42
14	20-Aug-05	15	19000	20000	19000	20000	20000	20000				24	42
15	21-Aug-05	16	19000	20000	20000	19000	20000	19000				24	42
16	22-Aug-05	17	20000	20000	21000	20000	20000	20000				24	40
17	23-Aug-05	18	20000	20000	20000	20000	20000	20000	7.810	2.272	4.24E-03	23	41
18	24-Aug-05	19	20000	19000	17000	20000	20000	20000				23	40
		Mean	**19842**		**19776**				**3.118**	**2.223**	**6.29E-03**	**24**	**41**
		S.D.	**602**		**961**				**3.3**	**0.1**	**0.0**	**0.4**	**1.3**

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Table III
Equipment List

Exposure Chamber
1000 Liter glass and stainless steel chamber (Wahmann).

Compound Reservoir
Sponsor cylinder (100 gallons) and smaller cylinders (5 gallons) filled from Sponsor cylinder and used on study.

Compound Generator
Counter-Current Volatilization Unit, coiled glass rod insert, (Crown Glass Co., Inc. heated by a nichrome wire).

Flowmeters
Flowmeter, size 0-5, 0-20, 0-40 Lpm (Dwyer® Instruments Inc.).
Top Trak™ Mass Flow Meter size 0-1 Lpm, Model 821-1 (Sierra Instruments), calibrated prestudy with a Gilibrator®Bubble Generator, P/N D800286, S/N 569-S, flow cell assembly P/N D80026B, BD #1860).
Flowmeter, size 0 – 65 mm, P/N G6-02-R3, G6-03-R3, G6-04-R3 (Key Instruments).

Pressure/Vacuum Gauges
Union Carbide backpressure gauge, P/N SG 6383.
Norgreen backpressure gauge, P/N 9892K23.
U.S. Gauge backpressure gauge, P/N 12672-1.
Ashcroft backpressure gauge, P/N 733-47.
Matheson® backpressure gauge, P/N 63-3161.
Dwyer® Magnehelic® gauge.

Variable Auto Transformer
Variable Autotransformer, Type 3PN 1010 (Staco Energy Products Company).

Chamber Air Flow
Dwyer® Magnehelic® gauge (Dwyer® Instruments Inc.), calibrated prestudy with a Dry Gas Meter, Model 8586799, (Singer).

Chamber Static Pressure
Dwyer® Magnehelic® gauge (Dwyer® Instruments Inc.), calibrated prestudy with Dwyer® Mark II Manometer, Model 25 (Dwyer® Instruments, Inc.).

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Table III
Equipment List

Regulators
Norgreen, P/N 9892K23
Union Carbide, P/N SG 3800 30.

Metering Valve
Metering Valve, Model SS-4L (Nupro® Co.).

Timers
Gralab Universal Timer, Model 171.

Absorbent Tube Sampling
Charcoal Tubes, ORBO-32, Lot #2000 (Supelco).

Vacuum Pumps
Thomas Industries Inc., Model 707CM50.
Neptune Dyna-pump®, Model 4K.

Tubing
Clear plastic, sizes 3/16", ¼", ½" (Norton, Baxter).
½" stainless steel.
Stainless steel cross(Swage).
Teflon®, size 1/8", ¼", ½".
Plastic "T".

Air Analyzer
MIRAN® 1A-CVF Ambient Air Analyzer (Wilks) with a Cole Parmer Strip Chart Recorder, Model 201, and a Micronta® LCD Benchtop Digital Multimeter, Model No. 22-195
Balston® Microfibre™ Disposable Filter Unit was attached in-line.
Syringe, size 0 - 25 mL, Gas-tight No. 1025 (Hamilton).

Particle Sizer
TSI Aerodynamic Particle Sizer, Model 331001, and a DELL computer, Model 486P/25, equipped with an Epson LQ-5707 printer, Model P630B.

	Inhalation Report	Appendix B

Table III
Equipment List

Environmental Monitoring
VWR Temperature and Humidity Gauge, tested prestudy with a Big Digit Traceable Hygrometer/Thermometer.
Digital Sound Meter 840029 (SPER Scientific)
Quantum Instruments Photo Meter 1.
Oxygen/Gas Analyzer, Model 1214S (Gastech)

Thermometer
T° Sentry Digital Alarm Module, Model 110.

Balance
Pelouze, No. 4040
Mettler PM30000K (Mettler Instrument Corporation).

Miscellaneous
Quick-disconnect fitting with toggle valve (REGO®).
DFU® filter, Grade DQ (Balston).

	Inhalation Report	Appendix B

Table IV
Chamber Distribution Records

Group (target)	Date	Port	Analytical (IR) Concentration (mg/m3)	Ratio to H-1
2 (2000 mg/m^3)	02 August 2005	H-1	1900	1.00
		H-2	2000	1.05
		H-1	2100	1.00
		H-7	2100	1.00
		H-8	2000	0.95
3 (10000 mg/m^3)	02 August 2005	H-1	11000	1.00
		H-2	9900	0.90
		H-1	10000	1.00
		H-7	10000	1.00
		H-8	9900	0.99
4 (20000 mg/m^3)	02 August 2005	H-1	19000	1.00
		H-2	19000	1.00
		H-1	19000	1.00
		H-7	19000	1.00
		H-1	20000	1.00
		H-8	20000	1.00

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Table V
Miran Calibration

Methodology for Gasoline DIPE Vapor Condensate

Settings: The instrument settings for the Miran 2225 Unit are summarized below:

wavelength, microns	10.2
pathlength, dial setting	5.30
slit width, mm	1
range, absorbance	1A
response, seconds	1
gain	High
chart speed, cm/min	1
chart volts	1

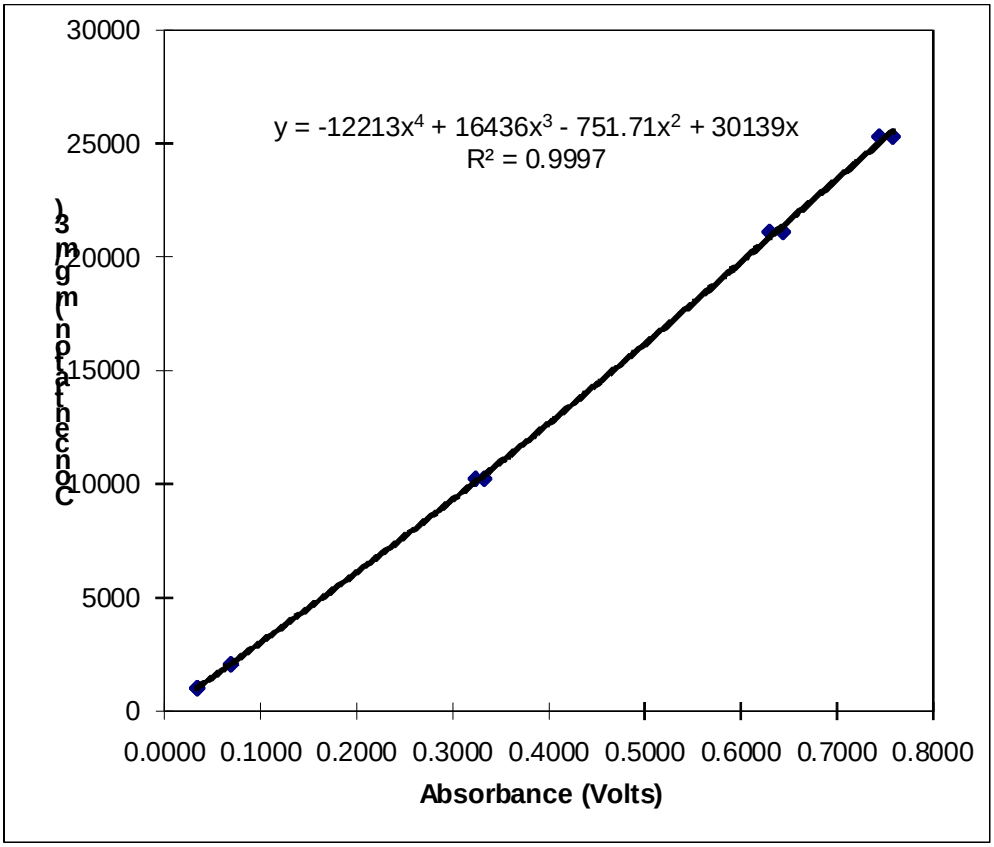
Calibrations: The Miran® was turned on and allowed to warm up for approximately 10 minutes. The cell was flushed with room air for approximately one minute. The loop was closed, the unit was zeroed and the calibration series was performed as shown below. The resultant data were plotted to obtain a calibration curve. Each observer used a separate syringe for calibration.

Injection Volume (µL)	Calculated Concentration[1] (mg/m^3)	Absorbance		
		Operator 1 (volts)	Operator 2 (volts)	Average (volts)
8.5	1025	0.0338	0.0331	0.0335
17	2050	0.0681	0.0691	0.0686
85	10248	0.323	0.333	0.328
175	21099	0.630	0.644	0.637
210	25319	0.744	0.758	0.751

[1]Calculated Conc. (mg/m^3) = $\frac{\text{Injection volume (µL) x Density (0.68 mg/µL) x 1000 L/m}^3}{\text{5.64 L (Volume of Miran cell)}}$

	Inhalation Report	Appendix B

Table V
Miran Calibration



	Inhalation Report	Appendix B

Table V
Miran Calibration

Calibration Checks: A three-point calibration check of the Miran® was performed for each exposure prior to sampling the chambers. The parameters are shown below:

Injection Volume (µL)	Calculated Concentration (mg/m3)	Expected Absorbance Reading (volts)	Acceptable Absorbance Range (volts)
17	2050	0.0686	0.0583 – 0.0789
85	10248	0.328	0.2788 – 0.377
175	21099	0.637	0.541 – 0.733

The absorbance was recorded after each injection. The absorbance was considered satisfactory if it was within 15% of the original calibration series. If any of the absorbance values fell outside the 15% range, the injection was rechecked as follows. The volume for the value that was out of range was reinjected twice. The closer pair of the three injections were averaged and the results were compared to the original curve. If the average of the pair was within the 15% range, the original was accepted. If the value of the average was outside the 15% range, the Study Director decided if a new graph was to be prepared.

	Inhalation Report	Appendix B

Table VI
Testing Room and Chambers Environmental Monitoring

Interval	**Location**	**Test Substance (mg/m^3)**	**Light (Ft Candles)**	**Noise (dB)**	**Oxygen (%)**	**Particle Sizing (mg/m^3)[a]**
Pretest	Room 813 - Front	0	32.3	62.8	-	
	Room 813 - Back	0	38.6	59.5	-	
	Group 1 Chamber	-	-	-	20	2.23×10^{-2}
	Group 2 Chamber	-	-	-	20	1.62×10^{-2}
	Group 3 Chamber	-	-	-	20	3.29×10^{-2}
	Group 4 Chamber	-	-	-	20	3.30×10^{-2}
Day 3	Room 813 – Front	0	30.0	64.1	-	
	Room 813 – Back	0	38.0	61.2	-	
	Group 1 Chamber	-	-	-	20	
	Group 2 Chamber	-	-	-	20	
	Group 3 Chamber	-	-	-	20	
	Group 4 Chamber	-	-	-	20	
Day 12	Room 811 – Front	0	30.4	63.8	-	
	Room 811 – Back	0	38.7	59.3	-	
	Group 1 Chamber	-	-	-	20	
	Group 2 Chamber	-	-	-	20	
	Group 3 Chamber	-	-	-	20	
	Group 4 Chamber	-	-	-	20	
Day 19	Room 811 – Front	0	31.1	63.9	-	
	Room 811 – Back	0	38.0	60.1	-	
	Group 1 Chamber	-	-	-	20	
	Group 2 Chamber	-	-	-	20	
	Group 3 Chamber	-	-	-	20	
	Group 4 Chamber	-	-	-	20	

[a]Pretest results presented above. For Weeks 1, 2 and 3 results, see CMR (Table II of Appendix B).

APPENDIX C

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL ANIMAL TERMINATION HISTORY

FEMALES GROUP 1 0 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	GESTATION DAY	PREGNANCY STATUS
1901	TERMINAL SACRIFICE	22-AUG-05	21	P
1902	TERMINAL SACRIFICE	22-AUG-05	21	P
1903	TERMINAL SACRIFICE	22-AUG-05	21	P
1904	TERMINAL SACRIFICE	22-AUG-05	21	P
1905	TERMINAL SACRIFICE	22-AUG-05	21	P
1906	TERMINAL SACRIFICE	22-AUG-05	21	P
1907	TERMINAL SACRIFICE	23-AUG-05	21	P
1908	TERMINAL SACRIFICE	23-AUG-05	21	P
1909	TERMINAL SACRIFICE	23-AUG-05	21	P
1910	TERMINAL SACRIFICE	23-AUG-05	21	P
1911	TERMINAL SACRIFICE	23-AUG-05	21	P
1912	TERMINAL SACRIFICE	23-AUG-05	21	P
1913	TERMINAL SACRIFICE	24-AUG-05	21	P
1914	TERMINAL SACRIFICE	24-AUG-05	21	P
1915	TERMINAL SACRIFICE	24-AUG-05	21	NP
1916	TERMINAL SACRIFICE	24-AUG-05	21	P
1917	TERMINAL SACRIFICE	24-AUG-05	21	P
1918	TERMINAL SACRIFICE	24-AUG-05	21	P
1919	TERMINAL SACRIFICE	25-AUG-05	21	P
1920	TERMINAL SACRIFICE	25-AUG-05	21	P
1921	TERMINAL SACRIFICE	25-AUG-05	21	P
1922	TERMINAL SACRIFICE	25-AUG-05	21	P
1923	TERMINAL SACRIFICE	25-AUG-05	21	P
1924	TERMINAL SACRIFICE	25-AUG-05	21	P

NP-NOT PREGNANT, P-PREGNANT

APPENDIX C

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL ANIMAL TERMINATION HISTORY

FEMALES GROUP 2 2000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	GESTATION DAY	PREGNANCY STATUS
2901	TERMINAL SACRIFICE	22-AUG-05	21	P
2902	TERMINAL SACRIFICE	22-AUG-05	21	P
2903	TERMINAL SACRIFICE	22-AUG-05	21	P
2904	TERMINAL SACRIFICE	22-AUG-05	21	P
2905	TERMINAL SACRIFICE	22-AUG-05	21	P
2906	TERMINAL SACRIFICE	22-AUG-05	21	P
2907	TERMINAL SACRIFICE	23-AUG-05	21	P
2908	TERMINAL SACRIFICE	23-AUG-05	21	P
2909	TERMINAL SACRIFICE	23-AUG-05	21	P
2910	TERMINAL SACRIFICE	23-AUG-05	21	P
2911	TERMINAL SACRIFICE	23-AUG-05	21	P
2912	TERMINAL SACRIFICE	23-AUG-05	21	P
2913	TERMINAL SACRIFICE	24-AUG-05	21	P
2914	TERMINAL SACRIFICE	24-AUG-05	21	P
2915	TERMINAL SACRIFICE	24-AUG-05	21	P
2916	TERMINAL SACRIFICE	24-AUG-05	21	P
2917	TERMINAL SACRIFICE	24-AUG-05	21	P
2918	TERMINAL SACRIFICE	24-AUG-05	21	P
2919	TERMINAL SACRIFICE	25-AUG-05	21	NP
2920	TERMINAL SACRIFICE	25-AUG-05	21	P
2921	TERMINAL SACRIFICE	25-AUG-05	21	P
2922	TERMINAL SACRIFICE	25-AUG-05	21	P
2923	TERMINAL SACRIFICE	25-AUG-05	21	P
2924	TERMINAL SACRIFICE	25-AUG-05	21	P

NP-NOT PREGNANT, P-PREGNANT

APPENDIX C

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL ANIMAL TERMINATION HISTORY

FEMALES GROUP 3 10000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	GESTATION DAY	PREGNANCY STATUS
3901	TERMINAL SACRIFICE	22-AUG-05	21	P
3902	TERMINAL SACRIFICE	22-AUG-05	21	P
3903	TERMINAL SACRIFICE	22-AUG-05	21	P
3904	TERMINAL SACRIFICE	22-AUG-05	21	P
3905	TERMINAL SACRIFICE	22-AUG-05	21	P
3906	TERMINAL SACRIFICE	22-AUG-05	21	P
3907	TERMINAL SACRIFICE	23-AUG-05	21	P
3908	TERMINAL SACRIFICE	23-AUG-05	21	P
3909	TERMINAL SACRIFICE	23-AUG-05	21	P
3910	TERMINAL SACRIFICE	23-AUG-05	21	P
3911	TERMINAL SACRIFICE	23-AUG-05	21	P
3912	TERMINAL SACRIFICE	23-AUG-05	21	P
3913	TERMINAL SACRIFICE	24-AUG-05	21	P
3914	TERMINAL SACRIFICE	24-AUG-05	21	P
3915	TERMINAL SACRIFICE	24-AUG-05	21	P
3916	TERMINAL SACRIFICE	24-AUG-05	21	P
3917	TERMINAL SACRIFICE	24-AUG-05	21	P
3918	TERMINAL SACRIFICE	24-AUG-05	21	P
3919	TERMINAL SACRIFICE	25-AUG-05	21	P
3920	TERMINAL SACRIFICE	25-AUG-05	21	P
3921	TERMINAL SACRIFICE	25-AUG-05	21	P
3922	TERMINAL SACRIFICE	25-AUG-05	21	P
3923	TERMINAL SACRIFICE	25-AUG-05	21	P
3924	TERMINAL SACRIFICE	25-AUG-05	21	P

NP-NOT PREGNANT, P-PREGNANT

APPENDIX C

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL ANIMAL TERMINATION HISTORY

FEMALES GROUP 4 20000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	GESTATION DAY	PREGNANCY STATUS
4901	TERMINAL SACRIFICE	22-AUG-05	21	P
4902	TERMINAL SACRIFICE	22-AUG-05	21	P
4903	TERMINAL SACRIFICE	22-AUG-05	21	P
4904	TERMINAL SACRIFICE	22-AUG-05	21	P
4905	TERMINAL SACRIFICE	22-AUG-05	21	P
4906	TERMINAL SACRIFICE	22-AUG-05	21	P
4907	TERMINAL SACRIFICE	23-AUG-05	21	P
4908	TERMINAL SACRIFICE	23-AUG-05	21	NP
4909	TERMINAL SACRIFICE	23-AUG-05	21	P
4910	TERMINAL SACRIFICE	23-AUG-05	21	P
4911	TERMINAL SACRIFICE	23-AUG-05	21	P
4912	TERMINAL SACRIFICE	23-AUG-05	21	P
4913	TERMINAL SACRIFICE	24-AUG-05	21	P
4914	TERMINAL SACRIFICE	24-AUG-05	21	P
4915	TERMINAL SACRIFICE	24-AUG-05	21	P
4916	TERMINAL SACRIFICE	24-AUG-05	21	P
4917	TERMINAL SACRIFICE	24-AUG-05	21	P
4918	TERMINAL SACRIFICE	24-AUG-05	21	P
4919	TERMINAL SACRIFICE	25-AUG-05	21	NP
4920	TERMINAL SACRIFICE	25-AUG-05	21	P
4921	TERMINAL SACRIFICE	25-AUG-05	21	P
4922	TERMINAL SACRIFICE	25-AUG-05	21	P
4923	TERMINAL SACRIFICE	25-AUG-05	21	P
4924	TERMINAL SACRIFICE	25-AUG-05	21	P

NP-NOT PREGNANT, P-PREGNANT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 1 0 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	1 0	1	1 2	3	4	5	6	7	8	9	2 0	1
1901	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1902	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1903	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1904	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1905	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1906	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1907	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1908	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1909	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																					P		
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P		P	P
1910	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1911	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 1 0 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1912	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1913	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1914	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1915	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1916	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1917	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1918	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1919	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1920	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1921	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1922	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 1 0 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1923	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
1924	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 2 2000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	1 0	1	1 2	3	1 4	5	1 6	7	1 8	9	2 0	1
2901	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																P	P						
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P			P	P	P	P	P	
2902	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
2903	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
2904	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
2905	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
2906	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
2907	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
2908	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED														P	P	P							
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P								P	P	P	P	P	
2909	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
2910	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 2 2000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	1 0	1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0	2 1
2911	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2912	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2913	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2914	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2915	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2916	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2917	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2918	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED								P	P	P	P							P	P				
	WITHIN NORMAL LIMITS		P	P	P	P			P	P	P	P	P	P							P	P	P	P
2919	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2920	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2921	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 2 2000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
2922	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2923	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2924	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 3 10000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
3901	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3902	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																				P			
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P		P	P	
3903	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																			P	P			
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P			P	P	
3904	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3905	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3906	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																	P						
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P		P	P	P	P	P	P	P	P	P	
3907	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED										P	P				P								
	WITHIN NORMAL LIMITS			P	P	P	P	P		P	P	P	P	P		P	P	P	P	P	P	P	P	P
3908	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																		P	P	P	P		
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P							P
3909	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED												P											
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P		P	P	P	P	P	P	P	P	P	P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 3 10000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
3910	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED														P									
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P		P	P	P	P	P	P	P	P	P	P	
3911	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED					P		P	P	P	P	P	P	P	P	P							P	
	WITHIN NORMAL LIMITS			P	P		P	P									P	P	P		P			
3912	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
3913	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
3914	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	
3915	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED											P	P	P	P	P	P							
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P									P	P	P	P	P	P	
3916	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED															P	P	P						
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P					P	P	P	P	P	P	
3917	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																P	P						
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P					P	P	P	P	P	
3918	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 3 10000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	1 0	1 1	1 2	1 3	1 4	1 5	1 6	1 7	1 8	1 9	2 0	2 1
3919	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3920	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED													P				P						
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P		P	P	P		P	P	P	P	P	P
3921	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED								P					P	P									
	WITHIN NORMAL LIMITS		P	P	P	P	P		P	P	P	P	P			P	P	P	P	P	P	P	P	P
3922	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																P	P						
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P			P	P	P	P	P
3923	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3924	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED									P	P	P	P	P				P	P	P				
	WITHIN NORMAL LIMITS		P	P	P	P	P								P	P	P	P				P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 4 20000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4901	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4902	TERMINAL SACRIFICE																							P
	ALOPECIA - GENERAL																	2	2	2	2	2	2	2
	CHROMODACRYORRHEA - UNILATERAL											P												
	WITHIN NORMAL LIMITS				P	P	P	P	P		P	P	P	P										
4903	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED											P	P	P								P		
	WITHIN NORMAL LIMITS				P	P	P	P	P				P	P	P	P	P	P	P	P		P	P	
4904	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4905	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4906	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4907	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4908	TERMINAL SACRIFICE																							P
	INCISORS MALOCCLUDED			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	MASS																			2	2	2	2	2
4909	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4910	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED																		P		P	P		
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P		P			P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 4 20000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4911	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4912	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS				P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4913	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4914	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED							P	P	P		P												
	WITHIN NORMAL LIMITS			P	P	P	P				P		P	P	P	P	P	P	P	P	P	P	P	P
4915	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4916	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED													P	P			P	P	P				
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P			P	P				P	P	P	P
4917	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED								P	P		P			P	P	P			P				
	WITHIN NORMAL LIMITS			P	P	P	P	P			P		P	P					P	P		P	P	P
4918	TERMINAL SACRIFICE																							P
	CHROMODACRYORRHEA - UNILATERAL														P									
	WITHIN NORMAL LIMITS			P	P	P	P	P	P	P	P	P	P	P		P	P	P	P	P	P	P	P	P
4919	TERMINAL SACRIFICE																							P
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
4920	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED								P	P		P	P											
	WITHIN NORMAL LIMITS		P	P	P	P	P				P			P	P	P	P	P	P	P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX D

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL CLINICAL OBSERVATIONS DURING GESTATION

GROUP 4 20000 MG/M3

FEMALE#	OBSERVATIONS	DAY OF GESTATION	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
4921	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED														P									
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P	P	P	P	P		P	P	P	P	P	P	P	P	P	
4922	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED											P				P	P	P						
	WITHIN NORMAL LIMITS		P	P	P	P	P	P	P		P	P	P						P	P	P	P	P	P
4923	TERMINAL SACRIFICE																							P
	NASAL DISCHARGE - RED									P	P		P	P	P	P	P	P	P	P	P		P	
	WITHIN NORMAL LIMITS		P	P	P	P	P		P	P											P		P	P
4924	TERMINAL SACRIFICE																							P
	CHROMODACRYORRHEA - BILATERAL		P																					
	CHROMODACRYORRHEA - UNILATERAL			P	P		P	P	P	P	P	P	P	P	P	P	P	P	P					
	WITHIN NORMAL LIMITS					P														P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

APPENDIX E

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHTS (GRAMS)

GROUP 1 0 MG/M3

	DAY OF GESTATION							
ANIMAL#	4	5	8	11	14	17	20	21
1901	212	230	259	275	303	332	380	389
1902	213	228	241	261	280	308	354	360
1903	229	223	245	264	286	322	368	384
1904	230	248	260	275	299	328	387	397
1905	233	254	271	295	316	343	387	393
1906	249	264	278	296	318	342	393	406
1907	209	218	235	256	266	291	328	334
1908	220	239	256	288	311	342	388	400
1909	232	239	255	278	299	335	392	400
1910	233	240	255	269	284	312	361	372
1911	247	258	272	294	314	351	398	411
1912	253	264	274	295	313	345	396	397
1913	232	239	250	270	286	314	351	353
1914	241	245	253	278	296	324	374	384
1915x NP	245	243	257	258	253	263	263	264
1916	246	261	270	298	318	357	410	414
1917	249	254	269	297	316	357	405	415
1918	259	269	283	310	338	378	420	434
1919	214	223	245	268	288	306	351	368
1920	222	232	246	261	280	292	313	313
1921	231	236	253	275	295	324	373	382
1922	235	242	257	282	295	324	368	382
1923	243	247	264	292	317	359	405	426
1924	245	259	274	297	316	353	395	405
MEAN	234	244	259	281	301	332	378	388
S.D.	14.0	14.6	12.8	14.9	17.0	22.2	26.3	28.6
N	23	23	23	23	23	23	23	23

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX E

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHTS (GRAMS)

GROUP 2 2000 MG/M3

	DAY OF GESTATION							
ANIMAL#	4	5	8	11	14	17	20	21
2901	209	221	233	245	263	286	323	327
2902	216	230	234	245	262	287	330	333
2903	224	236	246	267	280	302	350	354
2904	232	244	271	288	313	342	385	397
2905	235	252	268	285	306	339	393	404
2906	241	257	275	290	313	337	381	385
2907	202	221	233	255	273	307	353	365
2908	214	224	240	256	274	299	342	355
2909	228	235	252	270	286	312	353	364
2910	234	248	263	279	302	342	389	395
2911	246	258	274	297	310	342	393	401
2912	270	280	294	318	335	376	435	454
2913	229	234	251	273	291	322	369	372
2914	239	244	255	279	281	302	327	328
2915	244	257	271	290	307	343	389	399
2916	248	257	276	293	312	357	405	418
2917	254	261	280	295	306	334	366	381
2918	266	277	290	315	326	355	398	405
2919x NP	211	218	217	228	235	237	232	240
2920	222	229	237	254	270	298	335	347
2921	228	239	256	278	295	326	366	370
2922	233	229	250	269	278	318	339	350
2923	242	257	274	292	312	353	408	426
2924	243	253	267	285	301	335	387	396
MEAN	235	245	260	279	296	327	370	379
S.D.	16.6	16.5	18.0	19.6	20.3	24.3	29.9	32.7
N	23	23	23	23	23	23	23	23

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX E

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHTS (GRAMS)

GROUP 3 10000 MG/M3

	DAY OF GESTATION							
ANIMAL#	4	5	8	11	14	17	20	21
3901	210	230	232	245	262	284	320	326
3902	219	242	253	273	295	313	356	363
3903	219	231	249	267	291	310	362	375
3904	233	245	263	285	299	327	379	379
3905	237	248	256	268	286	322	379	388
3906	246	245	267	279	295	338	386	392
3907	206	218	229	245	267	302	337	344
3908	212	226	236	255	273	294	333	338
3909	231	245	255	278	296	325	370	370
3910	241	246	257	274	287	318	367	377
3911	243	254	263	285	302	336	393	401
3912	264	275	280	299	315	345	390	399
3913	232	235	260	276	293	328	354	370
3914	241	250	267	290	305	333	375	390
3915	246	258	268	283	292	309	324	324
3916	248	257	270	292	309	348	399	408
3917	252	256	275	297	319	357	401	408
3918	258	263	280	294	304	337	377	394
3919	216	227	237	254	273	309	354	368
3920	218	227	242	262	281	313	365	374
3921	223	230	243	255	272	295	329	334
3922	233	239	253	271	294	338	393	408
3923	241	249	263	275	293	323	373	389
3924	245	258	260	283	305	344	388	398
MEAN	234	244	256	274	292	323	367	376
S.D.	15.7	13.9	14.4	15.6	14.8	18.7	24.2	25.9
N	24	24	24	24	24	24	24	24

APPENDIX E

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHTS (GRAMS)

GROUP 4 20000 MG/M3

	DAY OF GESTATION							
ANIMAL#	4	5	8	11	14	17	20	21
4901	209	220	226	243	260	281	330	328
4902	213	225	239	255	274	297	342	352
4903	228	244	248	267	278	306	369	376
4904	231	236	249	270	285	319	370	380
4905	237	252	260	275	293	327	379	383
4906	239	250	262	279	302	329	378	389
4907	211	216	229	248	267	291	335	340
4908x NP	214	218	222	230	221	228	237	233
4909	222	231	233	243	255	279	313	324
4910	235	242	264	286	301	328	373	386
4911	241	254	264	282	294	326	378	387
4912	247	257	271	291	306	332	379	386
4913	235	246	257	272	289	322	365	372
4914	244	254	262	285	297	327	368	380
4915	245	253	261	279	291	317	357	371
4916	246	254	261	278	295	326	374	389
4917	258	269	282	303	318	362	408	417
4918	259	265	275	300	308	350	381	394
4919x NP	212	217	215	217	225	223	221	218
4920	220	224	237	258	275	306	338	348
4921	229	234	247	268	279	315	348	358
4922	233	246	261	276	294	327	373	366
4923	236	247	250	275	280	308	345	351
4924	261	271	286	325	343	389	439	442
MEAN	235	245	256	275	290	321	365	374
S.D.	14.6	15.2	16.3	19.5	19.5	24.9	27.1	27.4
N	22	22	22	22	22	22	22	22

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX F

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHT GAIN (GRAMS)

GROUP 1 0 MG/M3

	DAY OF GESTATION									
ANIMAL#	4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 14	14 - 21	5 - 21
1901	18	29	16	28	29	48	9	73	86	159
1902	15	14	19	20	28	47	5	53	80	132
1903	-6	22	19	22	35	46	17	63	98	161
1904	19	12	15	24	29	59	10	51	98	149
1905	21	17	24	22	27	44	6	63	77	139
1906	14	15	18	22	24	51	14	55	88	143
1907	8	18	21	10	25	37	6	49	68	116
1908	19	17	32	23	31	46	12	72	89	161
1909	8	16	22	21	36	57	8	60	101	161
1910	7	15	15	14	28	50	11	44	88	132
1911	12	14	22	20	37	47	13	56	97	153
1912	11	10	21	17	33	51	1	49	85	133
1913	8	11	20	16	29	37	2	46	68	114
1914	4	9	25	18	28	50	10	51	88	139
1915x NP	-2	14	1	-6	11	-1	1	10	11	21
1916	15	9	28	20	39	53	4	57	96	153
1917	5	15	29	19	41	48	10	62	99	161
1918	10	15	27	27	40	43	14	69	96	165
1919	9	21	24	20	18	45	17	65	80	145
1920	9	14	16	19	12	21	0	48	33	81
1921	6	17	22	20	29	49	9	59	87	146
1922	7	15	25	14	28	45	14	54	87	141
1923	5	17	28	25	42	46	21	69	109	179
1924	14	14	23	19	37	43	10	56	89	146
MEAN	10	15	22	20	31	46	10	57	86	144
S.D.	6.1	4.5	4.7	4.2	7.3	7.6	5.2	8.4	15.5	20.6
N	23	23	23	23	23	23	23	23	23	23

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX F

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHT GAIN (GRAMS)

GROUP 2 2000 MG/M3

	DAY OF GESTATION									
ANIMAL#	4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 14	14 - 21	5 - 21
2901	12	11	13	17	23	37	4	41	64	105
2902	14	3	11	17	25	43	3	32	70	102
2903	12	10	22	13	22	48	5	44	74	119
2904	12	27	17	26	28	43	12	70	84	153
2905	17	16	17	22	33	54	11	55	98	153
2906	16	18	15	24	24	44	4	57	72	128
2907	19	13	21	18	34	46	13	52	93	145
2908	11	16	16	18	25	43	13	50	81	130
2909	7	17	18	17	26	41	11	51	78	129
2910	13	16	16	23	41	46	7	54	94	148
2911	12	16	23	14	32	51	8	53	90	143
2912	10	14	24	17	41	59	19	55	119	175
2913	5	17	22	19	31	46	3	57	81	138
2914	5	11	24	3	21	25	0	37	47	84
2915	12	14	18	18	36	46	10	50	92	143
2916	9	19	17	20	45	48	13	55	105	161
2917	8	19	15	11	28	32	15	45	76	120
2918	11	13	25	11	29	42	8	49	79	128
2919x NP	6	-1	11	7	2	-5	9	17	6	23
2920	8	8	17	16	27	38	12	41	77	118
2921	11	17	22	17	31	40	4	56	75	132
2922	-4	21	18	9	40	21	11	48	73	121
2923	15	16	19	20	41	55	18	55	114	168
2924	10	14	18	16	34	52	9	48	95	143
MEAN	11	15	19	17	31	44	9	50	84	134
S.D.	4.7	4.7	3.6	5.0	6.9	8.9	5.0	7.8	16.3	21.5
N	23	23	23	23	23	23	23	23	23	23

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX F

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHT GAIN (GRAMS)

GROUP 3 10000 MG/M3

	DAY OF GESTATION									
ANIMAL#	4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 14	14 - 21	5 - 21
3901	19	3	13	16	22	36	6	32	64	96
3902	24	11	20	21	19	43	7	52	69	121
3903	12	17	18	24	20	52	13	59	84	144
3904	13	17	22	14	28	52	-1	53	80	133
3905	11	8	12	18	37	57	8	38	102	140
3906	-1	21	13	16	43	48	6	49	97	146
3907	13	10	16	22	34	35	8	49	77	126
3908	14	10	19	18	21	39	6	47	66	113
3909	14	10	23	18	30	45	0	51	75	126
3910	5	11	17	13	31	49	10	41	90	131
3911	12	9	22	17	33	57	8	48	98	146
3912	10	6	18	16	30	45	8	40	84	124
3913	3	25	15	17	36	26	16	57	77	135
3914	10	16	24	14	28	42	15	54	85	139
3915	12	10	15	9	17	15	1	34	32	67
3916	10	13	22	17	39	51	10	51	100	151
3917	4	18	22	22	39	44	6	62	89	151
3918	5	18	14	10	32	41	16	42	89	131
3919	12	10	17	19	36	45	14	46	95	141
3920	9	15	21	18	32	52	9	54	93	147
3921	6	13	12	17	24	34	5	42	63	105
3922	6	14	19	22	44	55	15	54	114	169
3923	8	14	12	18	31	50	16	44	96	140
3924	14	2	23	23	38	45	10	47	93	140
MEAN	10	13	18	17	31	44	9	48	84	132
S.D.	5.2	5.5	3.9	3.7	7.6	9.9	4.9	7.7	17.0	21.0
N	24	24	24	24	24	24	24	24	24	24

APPENDIX F

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION BODY WEIGHT GAIN (GRAMS)

GROUP 4 20000 MG/M3

	DAY OF GESTATION									
ANIMAL#	4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 14	14 - 21	5 - 21
4901	12	5	18	17	21	48	-2	40	68	108
4902	12	14	16	20	23	45	11	50	78	127
4903	16	4	20	11	28	63	7	35	98	132
4904	5	13	21	15	34	52	10	49	95	144
4905	15	8	15	18	34	51	4	42	90	131
4906	11	12	16	23	27	49	12	52	87	139
4907	5	12	19	19	25	44	4	50	73	123
4908x NP	5	4	8	-9	6	10	-5	3	11	14
4909	9	2	9	13	24	33	11	24	69	93
4910	7	22	22	15	27	45	13	59	85	144
4911	13	10	18	13	32	52	9	40	93	133
4912	10	14	20	15	26	47	7	49	80	129
4913	10	11	15	17	32	44	7	44	83	127
4914	10	8	23	13	30	42	12	43	83	126
4915	8	8	18	12	26	40	14	38	80	118
4916	8	7	18	17	31	48	16	41	94	135
4917	11	14	20	15	44	46	8	49	99	148
4918	6	11	24	8	42	32	13	43	86	129
4919x NP	6	-3	2	8	-2	-2	-3	7	-7	1
4920	5	13	21	16	31	32	10	50	73	124
4921	5	13	21	12	36	33	10	45	79	124
4922	12	15	15	18	34	46	-6	48	73	120
4923	11	3	25	5	28	37	6	33	71	104
4924	11	15	39	18	47	49	4	71	100	171
MEAN	10	11	20	15	31	44	8	45	83	129
S.D.	3.2	4.7	5.5	4.0	6.8	7.7	5.1	9.5	10.0	16.0
N	22	22	22	22	22	22	22	22	22	22

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX G

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION FEED CONSUMPTION -- (GRAMS/KG/DAY)

GROUP 1 0 MG/M3

	DAY OF GESTATION							
ANIMAL#	4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 21
1901	66	89	79	91	79	75	48	77
1902	83	82	83	88	81	77	44	76
1903	43	66	93	90	84	72	58	77
1904	76	82	79	83	78	71	46	73
1905	72	79	87	84	81	72	43	74
1906	63	71	72	78	77	76	58	72
1907	63	80	82	77	82	73	44	73
1908	95	89	96	94	90	79	58	84
1909	77	86	85	87	79	72	58	78
1910	75	79	76	82	82	72	55	74
1911	76	79	81	79	78	73	43	72
1912	70	78	76	72	72	72	36	67
1913	81	80	81	75	81	72	51	73
1914	66	77	80	82	82	78	58	76
1915x NP	56	75	67	67	69	63	72	69
1916	76	78	84	84	83	74	46	75
1917	73	86	91	89	83	75	54	80
1918	73	74	86	84	83	73	66	78
1919	54	85	92	92	75	69	58	78
1920	78	92	100	104	93	91	65	91
1921	70	83	92	89	81	74	54	79
1922	71	81	91	82	77	73	63	78
1923	CF	CF	CF	CF	CF	79	62	71
1924	65	81	87	83	78	71	57	76
MEAN	71	81	85	85	81	74	53	76
S.D.	10.5	6.0	7.1	7.1	4.5	4.5	8.1	4.7
N	22	22	22	22	22	23	23	23

NP=NOT PREGNANT x=EXCLUDED FROM MEAN
CF=Contaminated Feeder

APPENDIX G

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION FEED CONSUMPTION -- (GRAMS/KG/DAY)

GROUP 2 2000 MG/M3

ANIMAL#		DAY OF GESTATION 4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 21
2901		84	89	83	82	79	76	39	75
2902		83	81	79	76	79	79	52	74
2903		70	73	79	76	74	72	50	71
2904		44	80	78	87	78	75	52	75
2905		73	82	78	82	82	75	35	72
2906		79	80	75	80	73	65	41	69
2907		78	89	90	81	83	72	43	76
2908		86	145	86	91	87	76	47	89
2909		94	80	90	86	83	77	55	79
2910		85	88	82	84	87	76	35	76
2911		74	80	83	76	75	71	38	71
2912		76	84	84	82	82	75	44	75
2913		76	86	88	83	82	75	48	77
2914		72	74	82	72	78	73	45	71
2915		73	75	76	79	89	72	52	74
2916		72	83	82	82	86	72	47	75
2917		75	90	83	82	78	71	50	76
2918		77	80	81	83	76	70	40	72
2919x	NP	76	78	81	79	72	74	65	75
2920		59	79	74	78	79	76	68	76
2921		76	87	84	84	82	73	53	77
2922		45	78	76	76	86	67	47	71
2923		78	87	80	86	76	74	54	76
2924		77	94	88	89	84	77	41	79
MEAN		74	85	82	82	81	73	47	75
S.D.		11.5	14.1	4.6	4.7	4.5	3.3	7.5	4.0
N		23	23	23	23	23	23	23	23

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX G

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION FEED CONSUMPTION -- (GRAMS/KG/DAY)

GROUP 3 10000 MG/M3

	DAY OF GESTATION							
ANIMAL#	4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 21
3901	76	70	67	75	73	70	52	68
3902	74	72	74	83	75	70	40	69
3903	72	84	85	86	74	72	58	77
3904	81	88	89	88	82	74	42	77
3905	71	77	77	81	80	74	47	73
3906	47	71	79	80	86	75	39	72
3907	92	87	85	90	94	78	47	80
3908	88	88	90	86	85	78	50	79
3909	69	75	77	80	76	69	39	69
3910	74	77	80	78	73	65	43	69
3911	84	90	85	78	80	74	48	76
3912	73	75	76	82	84	74	54	74
3913	55	85	79	87	86	77	50	77
3914	66	78	81	79	73	67	49	71
3915	84	81	84	84	86	76	42	75
3916	87	78	75	CF	88	CF	38	70
3917	66	78	83	86	87	72	47	75
3918	81	82	79	85	80	76	56	76
3919	83	82	83	84	84	79	55	78
3920	69	80	83	80	78	75	42	73
3921	97	78	71	77	73	72	42	69
3922	64	85	87	CF	CF	CF	51	74
3923	71	83	79	82	77	73	48	74
3924	75	69	78	82	CF	79	50	72
MEAN	75	80	80	82	81	74	47	74
S.D.	11.4	6.0	5.5	3.9	6.0	3.7	5.8	3.6
N	24	24	24	22	22	22	24	24

CF=Contaminated Feeder

APPENDIX G

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GESTATION FEED CONSUMPTION -- (GRAMS/KG/DAY)

GROUP 4 20000 MG/M3

ANIMAL#		DAY OF GESTATION 4 - 5	5 - 8	8 - 11	11 - 14	14 - 17	17 - 20	20 - 21	5 - 21
4901		71	CF	CF	CF	CF	77	47	62
4902		60	70	73	77	73	76	52	70
4903		83	78	75	72	71	71	43	68
4904		58	71	76	76	76	67	42	68
4905		83	80	82	81	79	72	39	72
4906		81	76	77	81	75	65	42	69
4907		84	81	84	84	83	79	50	77
4908x	NP	63	67	66	60	66	69	46	62
4909		73	67	67	75	77	77	40	67
4910		69	92	82	79	78	73	57	77
4911		68	74	75	75	80	72	47	70
4912		71	81	77	76	74	72	45	71
4913		85	83	78	78	77	73	49	73
4914		68	76	75	79	84	77	45	73
4915		88	90	80	77	75	69	42	72
4916		74	83	81	80	83	80	46	76
4917		76	79	74	76	81	72	47	71
4918		68	75	72	71	83	66	59	71
4919x	NP	74	68	70	72	66	66	54	66
4920		73	82	84	82	88	78	55	78
4921		72	82	72	72	81	70	54	72
4922		59	77	70	79	78	79	21	67
4923		92	77	80	77	81	71	45	72
4924		53	80	92	87	86	73	42	77
MEAN		73	79	78	78	79	73	46	72
S.D.		10.2	6.0	5.7	4.0	4.4	4.3	7.8	3.8
N		22	21	21	21	21	22	22	22

NP=NOT PREGNANT x=EXCLUDED FROM MEAN
CF=Contaminated Feeder

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 1 0 MG/M3

ANIMAL#	ORGAN	OBSERVATION
1901		NO REMARKABLE OBSERVATIONS
1902		NO REMARKABLE OBSERVATIONS
1903		NO REMARKABLE OBSERVATIONS
1904		NO REMARKABLE OBSERVATIONS
1905		NO REMARKABLE OBSERVATIONS
1906		NO REMARKABLE OBSERVATIONS
1907		NO REMARKABLE OBSERVATIONS
1908		NO REMARKABLE OBSERVATIONS
1909		NO REMARKABLE OBSERVATIONS
1910		NO REMARKABLE OBSERVATIONS
1911		NO REMARKABLE OBSERVATIONS
1912		NO REMARKABLE OBSERVATIONS
1913		NO REMARKABLE OBSERVATIONS
1914		NO REMARKABLE OBSERVATIONS
1915 NP	SALEWSKI'S TEST	NO FOCI PRESENT
1916		NO REMARKABLE OBSERVATIONS

NP=NOT PREGNANT

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 1 0 MG/M3

ANIMAL#	ORGAN	OBSERVATION
1917		NO REMARKABLE OBSERVATIONS
1918		NO REMARKABLE OBSERVATIONS
1919		NO REMARKABLE OBSERVATIONS
1920		NO REMARKABLE OBSERVATIONS
1921		NO REMARKABLE OBSERVATIONS
1922		NO REMARKABLE OBSERVATIONS
1923		NO REMARKABLE OBSERVATIONS
1924		NO REMARKABLE OBSERVATIONS

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 2 2000 MG/M3

ANIMAL#	ORGAN	OBSERVATION
2901		NO REMARKABLE OBSERVATIONS
2902		NO REMARKABLE OBSERVATIONS
2903		NO REMARKABLE OBSERVATIONS
2904		NO REMARKABLE OBSERVATIONS
2905		NO REMARKABLE OBSERVATIONS
2906		NO REMARKABLE OBSERVATIONS
2907		NO REMARKABLE OBSERVATIONS
2908		NO REMARKABLE OBSERVATIONS
2909		NO REMARKABLE OBSERVATIONS
2910		NO REMARKABLE OBSERVATIONS
2911		NO REMARKABLE OBSERVATIONS
2912		NO REMARKABLE OBSERVATIONS
2913		NO REMARKABLE OBSERVATIONS
2914		NO REMARKABLE OBSERVATIONS
2915		NO REMARKABLE OBSERVATIONS
2916		NO REMARKABLE OBSERVATIONS

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 2 2000 MG/M3

ANIMAL#		ORGAN	OBSERVATION
2917			NO REMARKABLE OBSERVATIONS
2918			NO REMARKABLE OBSERVATIONS
2919	NP	SALEWSKI'S TEST	NO FOCI PRESENT
2920			NO REMARKABLE OBSERVATIONS
2921			NO REMARKABLE OBSERVATIONS
2922			NO REMARKABLE OBSERVATIONS
2923			NO REMARKABLE OBSERVATIONS
2924			NO REMARKABLE OBSERVATIONS

NP=NOT PREGNANT

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 3 10000 MG/M3

ANIMAL#	ORGAN	OBSERVATION
3901		NO REMARKABLE OBSERVATIONS
3902		NO REMARKABLE OBSERVATIONS
3903		NO REMARKABLE OBSERVATIONS
3904		NO REMARKABLE OBSERVATIONS
3905		NO REMARKABLE OBSERVATIONS
3906		NO REMARKABLE OBSERVATIONS
3907		NO REMARKABLE OBSERVATIONS
3908		NO REMARKABLE OBSERVATIONS
3909		NO REMARKABLE OBSERVATIONS
3910		NO REMARKABLE OBSERVATIONS
3911		NO REMARKABLE OBSERVATIONS
3912		NO REMARKABLE OBSERVATIONS
3913		NO REMARKABLE OBSERVATIONS
3914		NO REMARKABLE OBSERVATIONS
3915		NO REMARKABLE OBSERVATIONS
3916		NO REMARKABLE OBSERVATIONS

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 3 10000 MG/M3

ANIMAL#	ORGAN	OBSERVATION
3917		NO REMARKABLE OBSERVATIONS
3918		NO REMARKABLE OBSERVATIONS
3919		NO REMARKABLE OBSERVATIONS
3920		NO REMARKABLE OBSERVATIONS
3921		NO REMARKABLE OBSERVATIONS
3922		NO REMARKABLE OBSERVATIONS
3923		NO REMARKABLE OBSERVATIONS
3924		NO REMARKABLE OBSERVATIONS

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 4 20000 MG/M3

ANIMAL#		ORGAN	OBSERVATION
4901			NO REMARKABLE OBSERVATIONS
4902		EXTERNAL EXAM	ALOPECIA- GENERAL EXTERNAL FINDING CONFIRMED
4903			NO REMARKABLE OBSERVATIONS
4904			NO REMARKABLE OBSERVATIONS
4905			NO REMARKABLE OBSERVATIONS
4906			NO REMARKABLE OBSERVATIONS
4907			NO REMARKABLE OBSERVATIONS
4908	NP	GROSS EXAM	MASS; LEFT MAMMARY GLAND - LOWER LATERAL, 2.0 X 2.5 CM.,TAN, FIRM EXTERNAL FINDING CONFIRMED TISSUE SAVED
		EXTERNAL EXAM	INCISOR MALOCLUDED EXTERNAL FINDING CONFIRMED
		SALEWSKI'S TEST	NO FOCI PRESENT
4909			NO REMARKABLE OBSERVATIONS
4910			NO REMARKABLE OBSERVATIONS
4911			NO REMARKABLE OBSERVATIONS
4912			NO REMARKABLE OBSERVATIONS
4913			NO REMARKABLE OBSERVATIONS

NP=NOT PREGNANT

APPENDIX H

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL MATERNAL NECROPSY OBSERVATIONS

GROUP 4 20000 MG/M3

ANIMAL#		ORGAN	OBSERVATION
4914			NO REMARKABLE OBSERVATIONS
4915			NO REMARKABLE OBSERVATIONS
4916			NO REMARKABLE OBSERVATIONS
4917			NO REMARKABLE OBSERVATIONS
4918			NO REMARKABLE OBSERVATIONS
4919	NP	SALEWSKI'S TEST	NO FOCI PRESENT
4920			NO REMARKABLE OBSERVATIONS
4921			NO REMARKABLE OBSERVATIONS
4922			NO REMARKABLE OBSERVATIONS
4923			NO REMARKABLE OBSERVATIONS
4924			NO REMARKABLE OBSERVATIONS

NP=NOT PREGNANT

APPENDIX I

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FEMALE REPRODUCTION DATA AND MEAN FETAL WEIGHTS (GRAMS)

GROUP 1 0 MG/M3

ANIMAL#	CORPORA LUTEA	%PREIMPL. LOSS	IMPLANT SITES	FETUSES LIVE	FETUSES DEAD (n)	FETUSES DEAD %	RESORPTIONS EARLY	RESORPTIONS LATE	RESORPTIONS TOTAL (n)	RESORPTIONS TOTAL %	SEX MALE	SEX FEMALE	AVERAGE FETAL BODY WEIGHT MALES	AVERAGE FETAL BODY WEIGHT FEMALES	AVERAGE FETAL BODY WEIGHT LITTER
1901	16	25.0	12	11	0	0.0	1	0	1	8.3	6	5	6.1	5.7	5.9
1902	16	25.0	12	12	0	0.0	0	0	0	0.0	8	4	6.1	5.7	6.0
1903	15	6.7	14	14	0	0.0	0	0	0	0.0	7	7	6.3	5.6	5.9
1904	22	31.8	15	15	0	0.0	0	0	0	0.0	8	7	5.7	5.4	5.5
1905	14	0.0	14	13	0	0.0	1	0	1	7.1	6	7	5.4	5.6	5.5
1906	15	6.7	14	13	0	0.0	1	0	1	7.1	6	7	5.6	5.4	5.5
1907	9	0.0	9	9	0	0.0	0	0	0	0.0	6	3	6.4	6.1	6.3
1908	14	7.1	13	12	0	0.0	1	0	1	7.7	7	5	5.9	5.5	5.7
1909	15	0.0	15	15	0	0.0	0	0	0	0.0	7	8	6.4	6.0	6.2
1910	14	7.1	13	13	0	0.0	0	0	0	0.0	6	7	6.0	6.0	6.0
1911	19	10.5	17	15	0	0.0	2	0	2	11.8	6	9	5.7	5.5	5.6
1912	15	0.0	15	15	0	0.0	0	0	0	0.0	5	10	5.4	5.1	5.2
1913	13	0.0	13	11	0	0.0	2	0	2	15.4	5	6	5.8	5.3	5.5
1914	12	0.0	12	12	0	0.0	0	0	0	0.0	5	7	6.1	5.9	6.0
1915	NP														
1916	17	11.8	15	14	0	0.0	1	0	1	6.7	6	8	5.6	5.4	5.5
1917	18	5.6	17	17	0	0.0	0	0	0	0.0	7	10	5.7	5.4	5.5
1918	15	0.0	15	14	0	0.0	0	1	1	6.7	10	4	5.6	5.3	5.5
1919	15	6.7	14	14	0	0.0	0	0	0	0.0	5	9	6.1	5.7	5.8
1920	9	55.6	4	4	0	0.0	0	0	0	0.0	1	3	6.3	6.1	6.1
1921	15	0.0	15	15	0	0.0	0	0	0	0.0	8	7	5.4	5.3	5.4
1922	14	7.1	13	13	0	0.0	0	0	0	0.0	7	6	6.1	5.8	6.0
1923	16	6.3	15	15	0	0.0	0	0	0	0.0	8	7	6.1	5.6	5.9
1924	15	0.0	15	14	0	0.0	1	0	1	6.7	6	8	5.7	5.4	5.6
MEAN	14.9	9.3	13.5	13.0	0.0	0.0	0.4	0.0	0.5	3.4	6.3	6.7	5.9	5.6	5.7
S.D.	2.78	13.46	2.71	2.64	0.00	0.00	0.66	0.21	0.67	4.66	1.70	1.99	0.32	0.28	0.29
N	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23

NP=NOT PREGNANT

APPENDIX I

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FEMALE REPRODUCTION DATA AND MEAN FETAL WEIGHTS (GRAMS)

GROUP 2 2000 MG/M3

ANIMAL#	CORPORA LUTEA	%PREIMPL. LOSS	IMPLANT SITES	FETUSES LIVE	FETUSES DEAD (n)	FETUSES DEAD %	RESORPTIONS EARLY	RESORPTIONS LATE	RESORPTIONS TOTAL (n)	RESORPTIONS TOTAL %	SEX MALE	SEX FEMALE	AVERAGE FETAL BODY WEIGHT MALES	AVERAGE FETAL BODY WEIGHT FEMALES	AVERAGE FETAL BODY WEIGHT LITTER
2901	13	15.4	11	10	0	0.0	1	0	1	9.1	7	3	5.6	5.3	5.5
2902	13	7.7	12	11	0	0.0	1	0	1	8.3	6	5	5.9	5.5	5.7
2903	13	7.7	12	11	0	0.0	1	0	1	8.3	7	4	5.8	5.5	5.7
2904	14	0.0	14	12	0	0.0	2	0	2	14.3	5	7	6.4	6.0	6.1
2905	18	11.1	16	16	0	0.0	0	0	0	0.0	6	10	6.1	5.7	5.8
2906	15	13.3	13	12	0	0.0	1	0	1	7.7	3	9	6.0	5.9	5.9
2907	13	0.0	13	13	0	0.0	0	0	0	0.0	8	5	5.5	5.2	5.4
2908	21	28.6	15	14	0	0.0	1	0	1	6.7	6	8	5.4	5.1	5.2
2909	11	0.0	11	10	0	0.0	1	0	1	9.1	5	5	6.3	5.8	6.0
2910	14	0.0	14	14	0	0.0	0	0	0	0.0	4	10	6.3	5.7	5.9
2911	15	6.7	14	14	0	0.0	0	0	0	0.0	5	9	6.1	5.6	5.8
2912	17	0.0	17	17	0	0.0	0	0	0	0.0	12	5	6.0	5.9	6.0
2913	14	0.0	14	14	0	0.0	0	0	0	0.0	6	8	5.7	5.6	5.6
2914	14	0.0	14	4	0	0.0	10	0	10	71.4	1	3	6.6	6.2	6.3
2915	13	7.7	12	11	0	0.0	1	0	1	8.3	3	8	6.0	5.9	5.9
2916	16	6.3	15	15	0	0.0	0	0	0	0.0	8	7	5.5	5.3	5.4
2917	14	7.1	13	12	0	0.0	1	0	1	7.7	5	7	5.5	5.2	5.3
2918	14	7.1	13	13	0	0.0	0	0	0	0.0	9	4	5.9	5.8	5.9
2919	NP														
2920	13	0.0	13	11	0	0.0	1	1	2	15.4	7	4	5.4	5.0	5.3
2921	14	7.1	13	11	0	0.0	2	0	2	15.4	5	6	5.7	5.8	5.7
2922	16	25.0	12	12	0	0.0	0	0	0	0.0	5	7	5.9	5.7	5.8
2923	15	0.0	15	15	0	0.0	0	0	0	0.0	5	10	6.2	5.9	6.0
2924	14	0.0	14	14	0	0.0	0	0	0	0.0	5	9	6.3	5.9	6.1
MEAN	14.5	6.6	13.5	12.4	0.0	0.0	1.0	0.0	1.0	7.9	5.8	6.7	5.9	5.6	5.8
S.D.	2.06	7.99	1.50	2.64	0.00	0.00	2.07	0.21	2.08	14.89	2.24	2.29	0.33	0.33	0.30
N	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23

NP=NOT PREGNANT

APPENDIX I

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FEMALE REPRODUCTION DATA AND MEAN FETAL WEIGHTS (GRAMS)

GROUP 3 10000 MG/M3

	CORPORA	%PREIMPL.	IMPLANT	FETUSES			RESORPTIONS				SEX		AVERAGE FETAL BODY WEIGHT		
ANIMAL#	LUTEA	LOSS	SITES	LIVE	DEAD (n)	DEAD %	EARLY	LATE	TOTAL (n)	TOTAL %	MALE	FEMALE	MALES	FEMALES	LITTER
3901	11	9.1	10	10	0	0.0	0	0	0	0.0	6	4	5.9	5.6	5.8
3902	13	0.0	13	12	0	0.0	1	0	1	7.7	6	6	5.6	5.5	5.6
3903	14	7.1	13	13	0	0.0	0	0	0	0.0	5	8	5.2	5.1	5.1
3904	13	0.0	13	13	0	0.0	0	0	0	0.0	8	5	6.1	5.5	5.8
3905	15	0.0	15	15	0	0.0	0	0	0	0.0	8	7	5.3	4.9	5.1
3906	14	0.0	14	14	0	0.0	0	0	0	0.0	7	7	5.5	5.6	5.6
3907	13	0.0	13	11	0	0.0	2	0	2	15.4	6	5	5.8	5.5	5.6
3908	13	0.0	13	10	0	0.0	3	0	3	23.1	4	6	5.7	5.4	5.5
3909	14	7.1	13	12	0	0.0	1	0	1	7.7	6	6	5.9	5.6	5.8
3910	16	12.5	14	14	0	0.0	0	0	0	0.0	7	7	6.1	5.7	5.9
3911	16	0.0	16	16	0	0.0	0	0	0	0.0	5	11	5.9	5.3	5.5
3912	14	7.1	13	12	0	0.0	1	0	1	7.7	5	7	6.1	5.9	6.0
3913	20	60.0	8	8	0	0.0	0	0	0	0.0	6	2	6.5	5.9	6.4
3914	18	16.7	15	14	0	0.0	1	0	1	6.7	6	8	5.5	5.2	5.3
3915	10	70.0	3	2	0	0.0	1	0	1	33.3	1	1	6.4	5.3	5.8
3916	16	12.5	14	14	0	0.0	0	0	0	0.0	9	5	5.7	5.6	5.7
3917	21	33.3	14	14	0	0.0	0	0	0	0.0	4	10	5.5	5.2	5.3
3918	15	0.0	15	13	0	0.0	2	0	2	13.3	5	8	5.8	5.4	5.5
3919	13	0.0	13	13	0	0.0	0	0	0	0.0	8	5	5.6	5.3	5.5
3920	14	0.0	14	14	0	0.0	0	0	0	0.0	8	6	6.1	5.8	6.0
3921	13	7.7	12	11	0	0.0	1	0	1	8.3	5	6	5.8	5.8	5.8
3922	17	0.0	17	17	0	0.0	0	0	0	0.0	8	9	6.0	5.6	5.8
3923	16	0.0	16	16	0	0.0	0	0	0	0.0	13	3	5.6	5.5	5.6
3924	15	6.7	14	14	0	0.0	0	0	0	0.0	8	6	5.9	5.6	5.8
MEAN	14.8	10.4	13.1	12.6	0.0	0.0	0.5	0.0	0.5	5.1	6.4	6.2	5.8	5.5	5.7
S.D.	2.52	18.58	2.85	3.06	0.00	0.00	0.83	0.00	0.83	8.64	2.26	2.32	0.32	0.25	0.28
N	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24

APPENDIX I

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FEMALE REPRODUCTION DATA AND MEAN FETAL WEIGHTS (GRAMS)

GROUP 4 20000 MG/M3

	CORPORA	%PREIMPL.	IMPLANT	FETUSES			RESORPTIONS				SEX		AVERAGE FETAL BODY WEIGHT		
ANIMAL#	LUTEA	LOSS	SITES	LIVE	DEAD (n)	DEAD %	EARLY	LATE	TOTAL (n)	TOTAL %	MALE	FEMALE	MALES	FEMALES	LITTER
4901	15	6.7	14	13	0	0.0	1	0	1	7.1	6	7	5.6	5.3	5.5
4902	11	0.0	11	10	0	0.0	1	0	1	9.1	3	7	5.8	5.3	5.5
4903	17	11.8	15	14	0	0.0	1	0	1	6.7	9	5	5.8	5.6	5.7
4904	18	0.0	18	18	0	0.0	0	0	0	0.0	10	8	5.4	5.0	5.2
4905	16	0.0	16	16	0	0.0	0	0	0	0.0	9	7	5.4	5.0	5.2
4906	19	15.8	16	16	0	0.0	0	0	0	0.0	7	9	5.5	5.3	5.4
4907	13	15.4	11	10	0	0.0	1	0	1	9.1	4	6	6.0	5.6	5.7
4908	NP														
4909	11	9.1	10	9	0	0.0	1	0	1	10.0	6	3	6.0	5.7	5.9
4910	14	0.0	14	13	0	0.0	1	0	1	7.1	8	5	5.7	5.5	5.6
4911	17	0.0	17	16	0	0.0	1	0	1	5.9	6	10	5.6	5.3	5.4
4912	11	0.0	11	11	0	0.0	0	0	0	0.0	6	5	6.5	5.7	6.2
4913	15	6.7	14	14	0	0.0	0	0	0	0.0	4	10	6.0	5.7	5.8
4914	14	14.3	12	10	0	0.0	2	0	2	16.7	6	4	6.3	5.9	6.1
4915	16	6.3	15	14	0	0.0	1	0	1	6.7	3	11	5.6	5.1	5.2
4916	16	0.0	16	16	0	0.0	0	0	0	0.0	10	6	5.0	4.7	4.9
4917	16	6.3	15	14	0	0.0	1	0	1	6.7	6	8	6.0	5.8	5.9
4918	21	38.1	13	13	0	0.0	0	0	0	0.0	4	9	5.9	5.3	5.5
4919	NP														
4920	14	14.3	12	11	0	0.0	1	0	1	8.3	5	6	5.4	5.1	5.2
4921	13	15.4	11	11	0	0.0	0	0	0	0.0	6	5	5.7	5.4	5.6
4922	16	6.3	15	15	0	0.0	0	0	0	0.0	5	10	5.4	5.2	5.2
4923	13	0.0	13	11	0	0.0	2	0	2	15.4	7	4	5.7	5.2	5.5
4924	18	0.0	18	17	0	0.0	1	0	1	5.6	5	12	5.6	5.4	5.4
MEAN	15.2	7.6	14.0	13.3	0.0	0.0	0.7	0.0	0.7	5.2	6.1	7.1	5.7	5.4	5.5
S.D.	2.63	9.14	2.38	2.60	0.00	0.00	0.65	0.00	0.65	5.15	2.05	2.49	0.33	0.31	0.32
N	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22

NP=NOT PREGNANT

APPENDIX J

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GRAVID UTERINE WEIGHT AND NET BODY WEIGHT CHANGE (GRAMS)

GROUP 1 0 MG/M3

ANIMAL#	UTERUS WEIGHT	ADJUSTED WEIGHT	NET WEIGHT CHANGE FROM DAY 5	WEIGHT CHANGE FROM DAY 5
1901	91	298	68	159
1902	94	265	38	132
1903	112	272	49	161
1904	111	286	38	149
1905	98	295	41	139
1906	98	308	44	143
1907	72	262	44	116
1908	92	308	69	161
1909	119	281	42	161
1910	104	268	29	132
1911	117	294	35	153
1912	NT			133
1913	84	269	30	114
1914	103	281	36	139
1915x	NP			21
1916	108	306	45	153
1917	126	289	35	161
1918	108	326	57	165
1919	113	255	32	145
1920	36	277	45	81
1921	108	274	38	146
1922	106	276	34	141
1923	121	304	57	179
1924	107	298	39	146
MEAN	101.	286.	43.	144.
S.D.	19.4	18.1	11.1	20.6
N	22	22	22	23

UTERUS WEIGHT = ABSOLUTE GRAVID UTERINE WEIGHT
ADJUSTED WEIGHT = TERMINAL BODY WEIGHT - UTERUS WEIGHT
NET WEIGHT CHANGE FROM DAY 5 = ADJUSTED WEIGHT – DAY 5 BODY WEIGHT
WEIGHT CHANGE FROM DAY 5 = TERMINAL BODY WEIGHT – DAY 5 BODY WEIGHT

NP=NOT PREGNANT NT=NOT TAKEN x=EXCLUDED FROM MEAN

APPENDIX J

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GRAVID UTERINE WEIGHT AND NET BODY WEIGHT CHANGE (GRAMS)

GROUP 2 2000 MG/M3

ANIMAL#	UTERUS WEIGHT	ADJUSTED WEIGHT	NET WEIGHT CHANGE FROM DAY 5	WEIGHT CHANGE FROM DAY 5
2901	73	253	32	105
2902	85	247	17	102
2903	86	268	32	119
2904	98	299	55	153
2905	120	284	33	153
2906	95	290	33	128
2907	95	270	50	145
2908	95	259	35	130
2909	86	278	43	129
2910	109	286	38	148
2911	109	292	35	143
2912	134	320	40	175
2913	101	271	37	138
2914	37	290	46	84
2915	91	308	52	143
2916	113	304	47	161
2917	86	295	34	120
2918	105	300	23	128
2919x NP				23
2920	81	266	36	118
2921	88	282	43	132
2922	93	257	27	121
2923	126	300	42	168
2924	113	283	30	143
MEAN	97.	283.	37.	134.
S.D.	19.8	19.0	9.1	21.5
N	23	23	23	23

UTERUS WEIGHT = ABSOLUTE GRAVID UTERINE WEIGHT
ADJUSTED WEIGHT = TERMINAL BODY WEIGHT - UTERUS WEIGHT
NET WEIGHT CHANGE FROM DAY 5 = ADJUSTED WEIGHT – DAY 5 BODY WEIGHT
WEIGHT CHANGE FROM DAY 5 = TERMINAL BODY WEIGHT – DAY 5 BODY WEIGHT

NP=NOT PREGNANT x=EXCLUDED FROM MEAN

APPENDIX J

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GRAVID UTERINE WEIGHT AND NET BODY WEIGHT CHANGE (GRAMS)

GROUP 3 10000 MG/M3

ANIMAL#	UTERUS WEIGHT	ADJUSTED WEIGHT	NET WEIGHT CHANGE FROM DAY 5	WEIGHT CHANGE FROM DAY 5
3901	80	246	16	96
3902	88	275	33	121
3903	95	280	49	144
3904	103	276	31	133
3905	107	281	33	140
3906	104	288	43	146
3907	83	261	43	126
3908	75	263	38	113
3909	90	280	36	126
3910	108	269	23	131
3911	117	283	29	146
3912	96	303	28	124
3913	68	302	67	135
3914	100	289	39	139
3915	19	305	47	67
3916	110	298	41	151
3917	100	308	51	151
3918	101	293	30	131
3919	93	275	48	141
3920	114	259	33	147
3921	86	248	19	105
3922	136	272	33	169
3923	121	268	19	140
3924	112	286	28	140
MEAN	96.	280.	36.	132.
S.D.	22.5	17.1	11.7	21.0
N	24	24	24	24

UTERUS WEIGHT = ABSOLUTE GRAVID UTERINE WEIGHT
ADJUSTED WEIGHT = TERMINAL BODY WEIGHT - UTERUS WEIGHT
NET WEIGHT CHANGE FROM DAY 5 = ADJUSTED WEIGHT – DAY 5 BODY WEIGHT
WEIGHT CHANGE FROM DAY 5 = TERMINAL BODY WEIGHT – DAY 5 BODY WEIGHT

APPENDIX J

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL GRAVID UTERINE WEIGHT AND NET BODY WEIGHT CHANGE (GRAMS)

GROUP 4 20000 MG/M3

ANIMAL#		UTERUS WEIGHT	ADJUSTED WEIGHT	NET WEIGHT CHANGE FROM DAY 5	WEIGHT CHANGE FROM DAY 5
4901		91	237	17	108
4902		78	274	49	127
4903		103	272	29	132
4904		123	257	21	144
4905		110	273	21	131
4906		119	270	20	139
4907		79	261	45	123
4908x	NP				14
4909		70	254	23	93
4910		NT			144
4911		113	274	20	133
4912		94	292	35	129
4913		105	267	21	127
4914		84	296	42	126
4915		97	275	22	118
4916		108	281	27	135
4917		114	303	34	148
4918		94	300	35	129
4919x	NP				1
4920		76	272	47	124
4921		84	274	40	124
4922		104	262	16	120
4923		81	270	23	104
4924	PART	124x	318x	47x	171
MEAN		96.	273.	29.	129.
S.D.		15.7	15.9	10.6	16.0
N		20	20	20	22

UTERUS WEIGHT = ABSOLUTE GRAVID UTERINE WEIGHT
ADJUSTED WEIGHT = TERMINAL BODY WEIGHT - UTERUS WEIGHT
NET WEIGHT CHANGE FROM DAY 5 = ADJUSTED WEIGHT – DAY 5 BODY WEIGHT
WEIGHT CHANGE FROM DAY 5 = TERMINAL BODY WEIGHT – DAY 5 BODY WEIGHT

NP=NOT PREGNANT NT=NOT TAKEN PART=PARTIAL DELIVERY; 5 LIVE PUPS DELIVERED x=EXCLUDED FROM MEAN

APPENDIX K

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FETAL BODY WEIGHTS (GRAMS)

GROUP 1 0 MG/M3

FEMALE#	MEAN	FETUS# 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1901	5.9	5.6	5.9	5.9	5.8	6.0/	6.3	5.3	5.4	E	6.0	6.2	6.5							
1902	6.0	5.6	6.6	6.0	5.7	6.3	5.9/	6.3	5.9	5.6	5.9	6.0	6.0							
1903	5.9	5.7	6.3	5.3	5.6	6.2	6.4	5.6	5.5	6.2/	6.2	6.0	5.4	6.2	6.5					
1904	5.5	5.3	5.1	5.6	5.4	5.7/	5.4	5.9	6.0	5.7	5.4	5.3	5.6	5.5	5.7	5.3				
1905	5.5	3.2	5.7	6.4	E	5.6	5.6/	6.1	5.9	5.8	5.8	5.3	5.7	5.2	5.3					
1906	5.5	5.6	5.3	5.2	5.6	5.4	5.4	E /	6.1	5.4	5.8	5.2	5.3	5.8	5.4					
1907	6.3	6.4	6.3	6.0	6.2/	6.2	6.1	6.6	6.5	6.3										
1908	5.7	6.0	5.8	6.4	5.6	5.4	5.7/	5.5	E	6.0	6.1	5.3	5.8	5.3						
1909	6.2	6.3	6.6	6.1	6.4	6.3/	6.2	6.0	5.7	6.7	6.2	6.4	6.4	5.7	5.9	6.1				
1910	6.0	5.9	6.0	5.2	6.0	6.3	5.7	5.9	6.1	5.8	6.2	6.2/	6.4	6.3						
1911	5.6	5.8	5.8	5.5	5.5	E	5.6	5.4/	5.6	5.4	5.7	5.4	5.7	5.6	E	6.2	5.6	5.2		
1912	5.2	5.6	5.0	5.3	5.0	4.7	5.0	5.2	5.2	5.4/	5.7	5.1	5.2	5.2	5.3	5.6				
1913	5.5	5.0	5.9	E	5.2	5.5	5.9	E /	5.6	5.6	5.5	5.4	6.1	5.3						
1914	6.0	6.0	6.3	6.4	6.1/	6.1	5.4	5.8	5.8	6.2	5.9	6.1	5.9							
1915	NP																			
1916	5.5	5.3	5.4	5.5	5.2	5.6	5.7	5.4	6.0/	E	5.6	5.6	5.0	5.6	5.3	5.5				
1917	5.5	5.5	5.1	5.7	5.4	5.1	5.7	5.2	5.2	5.5	5.7	6.1/	5.3	5.7	5.4	5.9	5.5	5.7		
1918	5.5	5.4	5.3	5.6	5.6	5.7	5.4/	6.0	5.7	5.1	5.5	L	5.5	5.4	5.9	5.2				
1919	5.8	5.6	6.2	6.0	6.2	5.3	5.0	5.8	5.7/	5.9	6.6	5.6	5.7	6.0	6.1					
1920	6.1 /	6.2	6.0	6.1	6.3															
1921	5.4	5.1	5.3	5.1	5.6	5.2	5.5	5.3/	5.5	5.9	5.9	4.8	5.3	5.2	5.2	5.4				
1922	6.0	5.8	6.5	5.6	5.9	5.8/	6.5	6.2	6.0	6.0	6.1	5.7	5.4	6.0						
1923	5.9	6.1	5.9	5.5	5.6	6.0	5.5	5.2	5.7	6.1/	6.2	5.8	6.1	5.8	6.4	6.1				
1924	5.6	5.7	E	5.9	5.6	5.3	5.8	5.3	5.6/	5.3	5.4	5.7	5.6	5.5	5.6	5.3				

MEAN 5.7
S.D. 0.29
N 23

E-Early resorption L-Late resorption
/-Denotes position of cervix

NP=NOT PREGNANT

APPENDIX K

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FETAL BODY WEIGHTS (GRAMS)

GROUP 2 2000 MG/M3

FEMALE#	MEAN	FETUS# 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
2901	5.5	5.4/	5.6	5.3	5.3	5.7	5.1	5.6	5.3	E	6.1	5.9								
2902	5.7	5.2	5.7	6.0	5.7	5.5	6.1	5.9/	5.3	6.2	E	5.5	5.9							
2903	5.7	5.1	5.8	5.3	5.5	5.5	E	5.9/	5.9	5.9	5.9	5.9	6.1							
2904	6.1	5.9	5.9	6.4	6.0	5.8	5.7	6.4	E	E /	6.8	5.9	6.4	6.3	6.2					
2905	5.8	6.1	6.2	5.9	5.6	5.6	5.9	5.9	5.7	6.3	5.6/	6.2	5.8	5.5	5.8	6.1	5.2			
2906	5.9	6.0	5.8	6.1/	6.0	5.5	E	5.5	5.9	6.2	6.1	6.2	6.1	5.8						
2907	5.4	4.9	5.8	5.1	6.0	5.4	5.1	5.5/	5.7	5.3	5.4	5.2	5.2	5.3						
2908	5.2	5.3	4.9	4.9	5.7	5.6	5.1/	5.1	5.6	4.8	E	5.3	5.4	4.9	5.1	5.2				
2909	6.0	5.7	E	6.5	6.4	5.8/	6.4	6.2	5.8	5.7	5.9	5.8								
2910	5.9	5.7	5.5	6.0	5.7	5.3	5.8/	6.1	6.4	6.0	6.2	5.9	6.5	5.2	6.4					
2911	5.8	5.5	6.0	5.4	5.7	5.7/	5.4	5.6	5.4	5.7	6.3	6.1	5.7	6.2	6.1					
2912	6.0	6.4	5.9	5.9	6.4	6.0	5.8	6.0	5.8	6.1	6.1/	6.1	5.9	6.1	6.3	5.5	5.9	5.8		
2913	5.6	5.6	5.6	5.5	5.7	5.6	5.7	5.5	5.7	5.1/	5.6	5.6	6.0	5.6	5.8					
2914	6.3	E	E	6.1	E	E /	E	E	E	6.6	6.4	E	E	E	6.2					
2915	5.9	5.4	E	6.2	6.0	6.2	5.7	6.2	5.3/	5.9	6.2	6.1	5.6							
2916	5.4	5.5	5.3	5.3	5.1	5.6	5.3	4.9	5.8/	5.4	5.7	5.4	5.6	5.7	5.2	5.5				
2917	5.3	5.2	E	5.0	5.5	5.0/	5.6	5.6	5.4	5.6	5.4	4.9	5.3	5.3						
2918	5.9	5.7	6.0	5.9	6.0	5.3	5.4	6.1	5.4	5.9	6.0/	6.1	6.5	6.3						
2919	NP																			
2920	5.3	5.5	5.7	L	5.4/	5.1	5.3	5.2	4.9	E	5.1	4.5	5.6	5.3						
2921	5.7	E	E	5.7	6.0	5.4	5.8	5.6	5.7	6.0/	5.8	5.6	6.0	5.6						
2922	5.8	5.4	6.0	5.9	5.4	5.8	5.6/	6.0	5.9	5.8	6.0	5.8	5.8							
2923	6.0	5.4	5.9	5.8	6.1	6.3	6.3/	6.5	6.4	5.7	6.0	6.2	6.3	6.0	5.4	6.1				
2924	6.1	5.9	6.5	5.9	6.5	6.1	6.0	5.3/	5.9	5.7	6.1	6.5	6.1	6.3	6.2					
MEAN	5.8																			
S.D.	0.30																			
N	23																			

E-Early resorption L-Late resorption
/-Denotes position of cervix

NP=NOT PREGNANT

APPENDIX K

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FETAL BODY WEIGHTS (GRAMS)

GROUP 3 10000 MG/M3

FEMALE#	MEAN	FETUS# 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
3901	5.8	5.5	6.0	5.9	5.7	5.7	6.2	5.7/	6.0	5.1	5.9									
3902	5.6	5.6	5.9	5.3	E	5.2	5.5	5.2	5.4	5.8/	5.6	5.8	5.8	5.6						
3903	5.1	5.4	5.2	5.6	5.2	5.3/	5.0	5.1	4.9	5.0	4.8	5.1	4.8	5.3						
3904	5.8	5.4	5.9	6.1	4.9	6.0	6.2	5.8/	6.1	5.8	6.3	5.6	5.8	5.9						
3905	5.1	5.1	5.4	4.7	4.9	4.8	4.9	4.8	5.2	4.7	5.4/	5.3	5.9	5.0	5.5	5.2				
3906	5.6	5.4	5.7	4.6	5.2	5.5	5.6	5.5	5.5	5.6	5.9/	5.9	5.9	6.1	5.8					
3907	5.6	5.7	E	6.0	5.6	5.6	5.3/	6.0	5.5	E	5.6	5.4	5.7	5.8						
3908	5.5	5.6	5.7	E	6.1	5.1	5.2	5.4/	5.6	5.3	5.1	E	E	5.8						
3909	5.8	6.0	5.8	5.6	E	5.8	5.9	5.6	5.3	5.4	5.7/	6.1	6.1	5.7						
3910	5.9	5.9	6.0	6.2	5.8	6.0	6.0/	5.7	6.0	6.2	4.3	6.0	6.4	5.9	6.4					
3911	5.5	5.7	6.3	5.9	6.0	6.2	5.5	5.6	3.7	5.7	5.4/	3.3	6.2	5.5	5.7	5.6	6.0			
3912	6.0	6.1	E	5.9	6.4	6.3/	6.3	5.8	5.8	5.9	6.0	5.9	6.0	5.7						
3913	6.4 /	7.0	6.3	5.8	6.7	6.2	6.4	6.4	5.9											
3914	5.3	5.2	5.3	5.3	5.8	5.0	E /	5.6	5.4	5.2	5.4	4.9	5.3	5.0	5.3	5.9				
3915	5.8 /	E	6.4	5.3																
3916	5.7	5.6	5.5	5.7	5.5	5.8	5.4	5.9/	5.7	5.7	5.6	5.6	5.7	5.8	5.8					
3917	5.3	4.6	5.3	5.2	5.6	5.5/	5.4	5.0	5.4	5.7	4.9	4.8	5.5	5.7	5.3					
3918	5.5	5.7	E	5.6	5.8	5.8	5.9/	E	5.5	5.3	5.7	5.5	5.4	5.1	4.8	5.7				
3919	5.5	5.6	5.8	5.5	5.8	5.1	5.5/	5.9	5.7	4.9	5.3	5.6	5.7	5.3						
3920	6.0	6.1	5.7	6.7	6.4	6.0	5.5	6.2/	6.2	5.8	5.8	5.8	6.1	5.5	5.8					
3921	5.8	5.6	6.3	6.0	E	5.6	6.2	5.5/	6.0	6.1	5.9	5.8	4.9							
3922	5.8	6.2	6.0	5.8	5.5	5.8	5.3	5.7	5.6	5.8/	5.8	5.7	5.2	5.5	6.2	5.7	6.3	6.1		
3923	5.6	5.5	5.5	5.1	5.5	5.5	5.8	5.8	5.7/	5.6	5.7	5.5	5.4	6.1	5.3	5.7	5.5			
3924	5.8	5.6	5.9	5.8	6.0	6.0	5.2	5.6	5.8/	6.3	5.8	5.8	5.8	6.0	5.6					
MEAN	5.7																			
S.D.	0.28																			
N	24																			

E-Early resorption
/-Denotes position of cervix

APPENDIX K

GASOLINE DIPE VAPOR CONDENSATE: EMBRYO-FETAL TOXICITY STUDY
IN RATS BY INHALATION EXPOSURE

INDIVIDUAL FETAL BODY WEIGHTS (GRAMS)

GROUP 4 20000 MG/M3

FEMALE#	MEAN	FETUS# 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
4901	5.5	5.7	5.9	5.6	5.3/	5.5	5.4	5.2	5.3	5.4	5.6	5.4	E	5.2	5.4					
4902	5.5	6.0	5.5	E	5.6	5.4/	5.4	5.3	5.3	6.0	5.1	5.1								
4903	5.7	5.6	5.7	5.7	5.7	E	5.9/	5.4	5.9	5.2	5.8	5.5	6.1	6.0	5.8	5.7				
4904	5.2	4.8	5.1	5.8	5.1	4.9	4.8	5.1/	5.5	5.1	5.1	5.8	5.2	5.3	4.9	5.3	4.9	5.1	5.6	
4905	5.2	5.2	5.3	5.1	5.5	5.2	5.6	5.1	5.0	4.5	4.9/	5.2	5.2	5.7	5.5	5.1	5.4			
4906	5.4	5.5	5.4	5.3	5.4	5.1	5.9	5.5	5.4/	5.5	5.0	5.0	5.3	5.4	5.4	5.6	5.3			
4907	5.7	6.0	5.6	5.6	6.1	5.6	5.6/	E	5.7	6.0	5.4	5.9								
4908	NP																			
4909	5.9	6.1	6.0	E	5.8	6.3	5.4	6.2	6.1/	6.0	5.4									
4910	5.6	5.5	5.7	5.2	6.0	5.8	5.8	5.7	5.7/	E	5.5	5.0	5.8	5.8	5.8					
4911	5.4	5.1	5.6	5.1	5.6	5.8	4.4	5.3	5.4	5.4	5.5	5.6	5.4/	E	5.1	5.6	5.6	5.9		
4912	6.2	6.7	6.7	5.3	6.0	6.3	6.6/	5.6	5.8	5.7	6.0	6.9								
4913	5.8	6.0	5.8	5.6	5.7	5.8/	6.1	5.7	5.4	5.3	6.1	6.0	5.9	5.8	5.6					
4914	6.1	5.9	E	6.5	5.9	6.3	E	5.9	6.2/	5.7	6.4	6.1	6.6							
4915	5.2	5.5	4.7	5.3	5.2	5.4	E	5.3	5.1	4.3	5.2/	4.8	6.1	5.4	5.5	5.0				
4916	4.9	5.0	5.4	4.8	4.9	4.7	4.5	5.3/	5.1	5.0	5.1	4.9	4.8	4.2	5.2	4.7	4.8			
4917	5.9	6.0	5.9	5.8	5.6	6.0	5.7	5.7	E	6.2/	6.0	5.8	5.8	6.1	6.0	6.1				
4918	5.5	5.2	5.3	5.3	5.4	5.2/	5.5	5.4	6.2	5.3	5.3	5.9	5.8	5.7						
4919	NP																			
4920	5.2	5.2	5.0	E	5.2	5.1	5.1/	5.3	5.4	5.8	5.0	5.2	5.3							
4921	5.6	5.4	5.8	5.2	5.6	6.4	5.6/	5.3	5.3	5.8	5.6	5.0								
4922	5.2	5.0	4.9	5.8	5.0	5.1	5.6	5.3	5.3	5.3/	5.3	5.2	5.3	4.9	5.2	5.5				
4923	5.5	5.3	5.2	5.6	5.9	4.8	5.5	5.6	5.8/	5.2	5.9	E	5.8	E						
4924	5.4	4.7	5.7	5.3	5.9	5.2	5.6	5.6	5.4	5.3	5.8	E	5.5/	5.6	5.1	5.6	5.2	5.8	5.4	
MEAN	5.5																			
S.D.	0.32																			
N	22																			

E-Early resorption
/-Denotes position of cervix

NP=NOT PREGNANT

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 1 - 0 MG/M^3						
1901	6s, 5v	12	M	6.5	s	Sternebra(e) malaligned, #4 and #5, slight
1903	7s, 7v	3	F	5.3	s	Rib(s) 14th rudimentary, left
		10	M	6.2	v	Additional subclavian artery, left
		11	M	6.0	s	Rib(s) 14th rudimentary, bilateral
1904	7s, 8v	6	F	5.4	s	Hyoid body/arch(es), unossified body
		11	F	5.3	v	Renal papilla (e) not fully developed (grade 1), left
1905	7s, 6v	1	M	3.2	s	ANAL ATRESIA AND FILAMENTOUS TAIL VERTEBRAL AGENESIS: SACRAL VERTEBRAE #1 AND #2 SMALL IN SIZE; SACRAL VERTEBRAE #3 AND #4 AND ALL CAUDAL VERTEBRAE ABSENT; ILIA AND ISCHIA CLOSELY APPROXIMATED Sternebra(e) 5 and/or 6 unossified, #5
		2	F	5.7	v	Undescended thymic tissue, right
1908	6s, 6v	6	F	5.7	s	25 presacral vertebra(e) Rib(s) 13th rudimentary, right
1909	8s, 7v	8	F	5.7	v	Undescended thymic tissue, left
		9	M	6.7	s	Sternebra(e) malaligned, #2 through #4, slight

s = skeletal examination, v = visceral examination , M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 1 - 0 MG/M^3						
1910	6s, 7v	2	F	6.0	s	Sternebra(e) malaligned, #4, slight
		9	F	5.8	v	Left umbilical artery
1911	8s, 7v	4	M	5.5	v	Undescended thymic tissue, left
		9	M	5.4	v	Undescended thymic tissue, right
1912	7s, 8v	3	F	5.3	v	Undescended thymic tissue, left
		9	F	5.4	v	Undescended thymic tissue, right Ureter(s) distended, bilateral
		12	F	5.2	s	Sternebra(e) malaligned, #4 and #5, slight
1913	6s, 5v	6	M	5.9	s	Sternebra(e) malaligned, #3 through #5, slight to moderate
1914	6s, 6v	8	F	5.8	s	Sternebra(e) malaligned, #4 and #5, slight
1916	7s, 7v	1	F	5.3	v	Undescended thymic tissue, right
		3	M	5.5	v	Undescended thymic tissue, left

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 1 - 0 MG/M^3						
1918	7s, 7v	11	L			Crown-rump distance 2.4 cm
1919	7s, 7v	9	F	5.9	s	Sternebra(e) malaligned, #4 and #5, slight
		11	F	5.6	s	Sternebra(e) malaligned, #4 and #5, slight
		13	F	6.0	s	Sternebra(e) malaligned, #4 and #5, slight
1921	8s, 7v	2	F	5.3	v	Innominate artery absent
		6	M	5.5	v	Undescended thymic tissue, left
		12	M	5.3	v	Undescended thymic tissue, right Displaced testis medially, left
1923	8s, 7v	7	F	5.2	s	Sternebra(e) malaligned,#3 and #4, slight to moderate
1924	7s, 7v	9	F	5.3	s	Sternebra(e) malaligned, #3 through #5, slight

s = skeletal examination, v = visceral examination, M=Male, F=Female, L=Late Resorption, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 2 - 2000 MG/M^3						
2901	5s, 5v	2	M	5.6	v	Undescended thymic tissue, left
		3	F	5.3	s	Sternebra(e) malaligned, #3 through #4, slight
		8	M	5.3	v	Undescended thymic tissue, bilateral
2902	5s, 6v	2	F	5.7	s	Sternebra(e) malaligned, #3 and #4, slight
2903	6s, 5v	2	M	5.8	v	Undescended thymic tissue, left
		3	M	5.3	s	Sternebra(e) malaligned, #4 and #5 Rib(s) 14th rudimentary, right
2904	6s, 6v	14	F	6.2	s	RIGHT FORELIMB SHORTENED
2905	8s, 8v	1	M	6.1	s	Sternebra(e) malaligned, #3 through #5, slight
		7	F	5.9	s	Sternebra(e) malaligned, #4 and #5, slight
		10	F	5.6	v	Undescended thymic tissue, right
2908	7s, 7v	14	M	5.1	v	Left umbilical artery
2909	5s, 5v	6	M	6.4	s	Rib(s) 14th rudimentary, right
		11	M	5.8	v	Left umbilical artery

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 2 - 2000 MG/M^3						
2910	7s, 7v	3	F	6.0	v	Undescended thymic tissue, bilateral
		4	F	5.7	s	Rib(s) 14th rudimentary, bilateral
		5	F	5.3	v	Undescended thymic tissue, left
		6	F	5.8	s	Rib(s) 14th rudimentary, bilateral
		8	F	6.4	s	Rib(s) 14th rudimentary, left
		13	F	5.2	v	Undescended thymic tissue, right
2912	8s, 9v	4	M	6.4	s	7th cervical rib(s) present, left
		10	F	6.1	s	SHORT MANDIBLE, moderate
		15	M	5.5	v	Undescended thymic tissue, left
						Left umbilical artery
		17	F	5.8	v	Left umbilical artery
2913	7s, 7v	1	M	5.6	s	Sternebra(e) malaligned, #4 and #5, slight
						Rib(s) 14th rudimentary, bilateral
2917	6s, 6v	4	M	5.5	s	Rib(s) 14th rudimentary, bilateral
		13	F	5.3	v	Undescended thymic tissue, bilateral
2918	6s, 7v	2	M	6.0	s	Sternebra(e) malaligned, #4 and #5, slight

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 2 - 2000 MG/M^3						
2920	5s, 6v	3	L			Crown-rump distance 2.9 cm
		12	M	5.6	s	Sternebra(e) malaligned, #4 and #5, slight
2921	5s, 5v	3	M	5.7	s	Sternebra(e) malaligned, #3, slight
2923	8s, 7v	1	F	5.4	s	Sternebra(e) malaligned, #3 through #5, slight
		7	F	6.5	s	Sternebra(e) malaligned, #3 and #4, slight
		14	F	5.4	v	Undescended thymic tissue, left
2924	7s, 7v	11	F	6.5	v	Undescended thymic tissue, left

s = skeletal examination, v = visceral examination, M=Male, F=Female, L=Late Resorption, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 3 - 10000 MG/M^3						
3901	5s, 5v	1	M	5.5	s	Sternebra(e) malaligned, #4, slight
3903	7s, 6v	9	F	5.0	s	Sternebra(e) malaligned, #3 and #4, slight
3905	8s, 7v	1	F	5.1	s	Sternebra(e) malaligned, #5, slight Rib(s) 14th rudimentary, right
		5	F	4.8	s	Sternebra(e) malaligned, #5, slight
3906	7s, 7v	1	M	5.4	v	Innominate artery absent
		9	F	5.6	v	Renal papilla (e) not fully developed (grade 1), left Ureter(s) distended left
3907	6s, 5v	6	F	5.3	s	Sternebra(e) malaligned, #5, slight
		8	F	5.5	s	Hyoid body/arch(es), unossified body
3910	7s, 7v	6	M	6.0	s	Rib(s) 14th rudimentary, right
		7	F	5.7	v	Innominate artery absent

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 3 - 10000 MG/M^3						
3911	7s, 9v	8	F	3.7	v	MICROPHTHALMIA/ANOPHTHALMIA, left Undescended thymic tissue, right Left umbilical artery
		11	F	3.3	v	DOMED CRANIUM, moderate; HYDROCEPHALY ANOPHTHALMIA, bilateral Hypoplastic lung lobe(s), apical lobe Hypoplastic thymus, bilateral lobes Hypoplastic spleen
		13	F	5.5	s	Rib(s) 14th rudimentary, bilateral
3912	6s, 6v	6	M	6.3	v	Undescended thymic tissue, bilateral
		8	M	5.8	v	Undescended thymic tissue, bilateral
		12	F	6.0	v	Undescended thymic tissue, right
3913	4s, 4v	1	M	7.0	s	Rib(s) 14th rudimentary, right
		5	M	6.2	s	Sternebra(e) malaligned, #4, slight
3914	7s, 7v	5	M	5.0	v	Undescended thymic tissue, left
		15	M	5.9	s	Rib(s) 14th rudimentary, left
3916	7s, 7v	2	M	5.5	s	Sternebra(e) 5 and/or 6 unossified, #5
		8	M	5.7	s	Sternebra(e) malaligned, #3 and #4, slight

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 3 - 10000 MG/M^3						
3917	7s, 7v	11	F	4.8	s	Sternebra(e) 5 and/or 6 unossified, #5
3918	6s, 7v	14	F	4.8	s	Sternebra(e) malaligned, #3 and #4, slight
3921	6s, 5v	6	M	6.2	s	Sternebra(e) malaligned, #3 through #5, slight
		8	F	6.0	s	Sternebra(e) malaligned, #3 through #5, slight
3922	8s, 9v	2	F	6.0	s	Sternebra(e) malaligned, #4 and #5
		10	F	5.8	s	Sternebra(e) malaligned, #3 through #5, slight
3923	8s, 8v	16	M	5.5	v	Renal papilla (e) not fully developed (grade 1), left
3924	7s, 7v	4	M	6.0	s	Sternebra(e) malaligned, #4, slight
		6	F	5.2	s	Sternebra(e) 5 and/or 6 unossified, #5

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 4 - 20000 MG/M^3						
4901	7s, 6v	2	M	5.9	v	Undescended thymic tissue, left
		5	F	5.5	s	Hyoid body/arch(es) unossified body
		10	M	5.6	v	Undescended thymic tissue, bilateral
4902	5s, 5v	5	F	5.4	s	7th cervical rib(s) present, right Rib(s) 14th rudimentary, bilateral
		11	F	5.1	s	Sternebra(e) 5 and/or 6 unossified, #5
4904	9s, 9v	3	M	5.8	v	Undescended thymic tissue, left
4905	8s, 8v	12	F	5.2	v	Undescended thymic tissue, left
		14	M	5.5	v	Undescended thymic tissue, left
		15	M	5.1	s	Sternebra(e) malaligned, #2 through #4, slight to moderate
4906	8s, 8v	5	F	5.1	v	Undescended thymic tissue, right
		11	F	5.0	v	Undescended thymic tissue, left
4907	5s, 5v	1	M	6.0	s	VERTEBRAL ANOMALY: THORACIC CENTRUM #13 AND LUMBAR CENTRUM #1 ABSENT; THORACIC ARCHES #13 AND LUMBAR ARCHES #1 MALFORMED AND CLOSELY APPROXIMATED
		11	M	5.9	v	Undescended thymic tissue, left

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 4 - 20000 MG/M^3						
4909	5s, 4v	4	F	5.8	s	Rib(s) 14th rudimentary, right
		6	M	5.4	s	EXOCCIPITAL AND CERVICAL ARCH #1 FUSED, right Sternebra(e) malaligned, #4 and #5, slight
4910	6s, 7v	13	F	5.8	s	27 presacral vertebra(e) Rib(s) 14th rudimentary, bilateral
4911	8s, 8v	5	M	5.8	s	Rib(s) 14th rudimentary, right
		16	F	5.6	s	Rib(s) 14th rudimentary, left
4912	5s, 6v	2	M	6.7	s	Rib(s) 14th rudimentary, right
		8	F	5.8	s	Sternebra(e) malaligned, #3 through #5, slight to moderate
4915	7s, 7v	3	F	5.3	s	Rib(s) 14th rudimentary, right
		8	F	5.1	s	Rib(s) 14th rudimentary, left
		15	F	5.0	v	Innominate artery absent
4916	8s, 8v	13	F	4.2	v	Left umbilical artery

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females | INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS | APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 4 - 20000 MG/M^3						
4917	7s, 7v	4	F	5.6	v	Undescended thymic tissue, left
		10	F	6.0	s	7th cervical rib(s) present, right
		12	M	5.8	s	Rib(s) 14th rudimentary, right
		14	F	6.0	s	Rib(s) 14th rudimentary, left
4921	6s, 5v	1	M	5.4	s	7th cervical rib(s) present, right 25 presacral vertebra(e) Sternebra(e) 5 and/or 6 unossified, #5 Rib(s) 13th rudimentary, right
		2	M	5.8	v	Undescended thymic tissue, right
		3	M	5.2	s	25 presacral vertebra(e) Sternebra(e) 5 and/or 6 unossified, #5 Rib(s) 13th rudimentary, bilateral Metatarsals, 1 unossified, bilateral
		11	F	5.0	s	25 presacral vertebra(e) Rib(s) 13th rudimentary, bilateral Metatarsals, 1 unossified, bilateral
4923	6s, 5v	4	M	5.9	v	Undescended thymic tissue, left
		12	M	5.8	s	Rib(s) 14th rudimentary, left

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

Females	INDIVIDUAL FETAL EXTERNAL, VISCERAL AND SKELETAL OBSERVATIONS	APPENDIX L

Animal Number	Exam	Fetus Number	Sex	Weight (g)	Fate	Abnormalities (only fetuses with abnormalities are listed) Major abnormalities in CAPITALS
Group 4 - 20000 MG/M^3						
4924	8s, 9v	1	F	4.7	v	Renal papilla (e) not fully developed (grade 1), bilateral
						Ureter(s) distended, left
		14	F	5.1	v	Left umbilical artery
		17	F	5.8	s	Rib(s) 14th rudimentary, left

s = skeletal examination, v = visceral examination, M=Male, F=Female, g=grams

05-4287

GASOLINE DIPE VAPOR CONDENSATE

EMBRYO-FETAL TOXICITY STUDY IN RATS BY INHALATION EXPOSURE

Statistical Analysis

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COMPLIANCE STATEMENT

This phase of the study was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No 3106 as amended by Statutory Instrument 2004 No. 994).

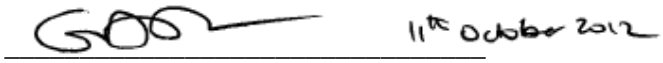
OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44).

US EPA 79.60, CFR Vol. 59, No. 122, 27 June 1994.

These principles of Good Laboratory Practice are accepted by the regulatory authorities of the United States of America and Japan on the basis of intergovernmental agreements.

Principal Investigator



Gareth D. Thomas, B.Sc. (Hons).

INTRODUCTION AND EXPERIMENTAL DESIGN

This rat study had a control and three groups treated at 2000 mg/m^3, 10000 mg/m^3 and 20000 mg/m^3. There were up to 24 dams per group.

The following parameters were subjected to statistical analysis:

Continuous maternal parameters

Maternal body weight (d4, d5, d8, d11, d14, d17, d20, d21)
Maternal body weight change (d4-d5, d5-d8, d8-d11, d11-d14, d14-d17, d17-d20, d5-d14, d14-d21, d5-d21)
Maternal feed consumption (d4-d5, d5-d8, d8-d11, d11-d14, d14-d17, d17-d20, d20-d21, d5-d21)
Maternal uterus weight (absolute and adjusted)
Maternal net bodyweight change from d5 minus gravid uterine weight

Reproductive performance

Corpora lutea
%Preimplantation loss
Number of implant sites
Fetuses alive
Early resorptions
Late resorptions
Total resorptions
%Total resorptions/implant sites
Number of males
Number of females

There were no dead fetuses, therefore the "Fetuses dead" and "%Fetuses dead/total" data were not analyzed.

Fetal body weight

Mean fetal body weight: males
Mean fetal body weight: females
Mean fetal body weight: litter (pooled males and females)

Discrete findings

Maternal pregnancy summary
Maternal necropsy findings
Fetal findings: external, visceral and skeletal (only certain visceral and skeletal findings required statistical analysis).

METHODOLOGY

Continuous maternal parameters

The analysis was performed at each timepoint separately.

Bartlett's test (Bartlett, 1937) was first applied (Proc GLM, SAS Institute 1999) to determine if the groups had equal variances. If the test was not significant at the 1% level, parametric procedures were used. If the test was significant then non-parametric procedures were used.

For the parametric choice, a one-way analysis of variance (Armitage, Berry and Matthews 2002) was initially applied (Proc GLM, SAS Institute 1999). If this was significant at the 5% level, then each treatment group was compared with the control using Dunnett's test (Dunnett 1955, 1964).

For the non-parametric choice, a Kruskal-Wallis test (Kruskal and Wallis 1952, 1953) was initially applied (Proc Npar1way, SAS Institute 1999). If this was significant at the 5% level, then each treatment group was compared with the control using Steel's test (Steel 1959).

Reproductive performance

All parameters except fetal bodyweight were analyzed using the same methods as for continuous maternal parameters, described above.

Fetal body weight

The fetal body weight was analyzed by separate and combined sexes using mixed models (Proc Mixed, SAS Institute 1999). The separate-sex analysis had dose group as explanatory variable, a random animal effect and litter size as covariate. The combined analysis had dose group and fetal sex and their interaction as explanatory variables, with litter size as covariate. For both sets of analysis, the Satterthwaite method was used for the fixed effects denominator degrees of freedom. If the dose group effect was significant at the 5% level, then each treatment group was compared with the control using Dunnett's test (Dunnett 1955, 1964), with the means being adjusted for litter size.

Discrete findings

Maternal pregnancy summary
Maternal necropsy findings
Fetal findings: external, visceral and skeletal malformations and variations

For maternal parameters:

These parameters were analyzed using non-parametric methods. If the dose group effect in was statistically significant, each dose group was compared with the control group using pairwise Wilcoxon rank sum tests (Wilcoxon 1945).

For fetal findings:

Only findings having an incidence of greater than one were included in the statistical analysis, since for less frequent parameters there was no chance of statistical significance.

These parameters were analyzed by generalized estimating equations (Proc Genmod, SAS Institute 1999) using a log link function and assuming Poisson distributed data. The litter was the basis for analysis where the correlation among littermates was modelled using a compound symmetric structure. Results are presented using the model based standard errors. If the dose group effect in the model was statistically significant, each dose group was compared with the control group using pairwise tests associated with least squares means, in this case being the mean incidence adjusted for litter size. For very low frequency findings this method did not converge. Therefore non-parametric methods, the Kruskal-Wallis test (Kruskal and Wallis 1952, 1953) were applied to the total number of affected foetuses in the litter instead. If this was significant at the 5% level then each treatment group was compared with the control using Wilcoxon rank sum tests (Wilcoxon 1945).

DATA HANDLING

Data were received as text files and Excel spreadsheets and re-formatted for software input. The software used for all the analyses was SAS 8.2 (SAS Institute 1999). For the analysis of uterine weight, adjusted bodyweight and the reanalysis of fetal findings conducted in this updated report, SAS 9.1.3 (SAS Institute 2002) and Quasar 1.1 (Quasar 1.1 2009) were used for the statistical analyses.

RESULTS

The data for animals 1915, 2919, 4908 and 4919 were excluded, where necessary, since the animals did not become pregnant. For maternal net bodyweight change from d5 minus gravid uterine weight, animals 1912 and 4924 were also excluded.

For food consumption, the animal 2908 value for the d5-d8 period was an extreme statistical outlier, being about 11 standard deviations higher than the mean. This value was excluded from the analysis.

Continuous maternal parameters

Summary statistics are presented in Tables 1 to 4 respectively. Bartlett's test was not significant for any parameter at any timepoint. The results can be summarised as follows:

Bodyweight

No significant differences at any timepoint.

Bodyweight change

d5-d8 significant difference at 20000 mg/m^3 (p<0.01).
d8-d11 significant difference at 2000 mg/m^3 (p<0.05) and 10000 mg/m^3 (p<0.01).
d11-d14 significant difference at 2000 mg/m^3 (p<0.05) and 20000 mg/m^3 (p<0.01).
d5-d14 significant difference at 2000 mg/m^3 (p<0.05), 10000 mg/m^3 (p<0.01) and 20000 mg/m^3 (p<0.01).

Maternal net bodyweight change from d5 minus gravid uterine weight

Significant difference at 20000 mg/m^3 (p<0.001).

Maternal feed consumption

d8-d11 significant difference at 10000 mg/m^3 (p<0.05) and 20000 mg/m^3 (p<0.01).
d11-d14 significant difference at 20000 mg/m^3 (p<0.01).
d20-d21 significant difference at 2000 mg/m^3 (p<0.05), 10000 mg/m^3 (p<0.05) and 20000 mg/m^3 (p<0.01).
d5-d21 significant difference at 20000 mg/m^3 (p<0.01).

All changes were reductions from the Control.

Maternal uterus weight and maternal bodyweight minus gravid uterine weight

Maternal uterus weight: no significant differences.
Maternal bodyweight minus gravid uterine weight: no significant differences.

Reproductive performance

Summary statistics are presented in Table 5. Bartlett's test was significant for several parameters. The subsequent analysis was thus either parametric (P) or non-parametric (NP). The methods and results can be summarised as follows:

Corpora lutea	P	No significant differences.
%Preimplantation loss	NP	No significant differences.
Number of implant sites	P	No significant differences.
Fetuses alive	P	No significant differences.
Early resorptions	NP	No significant differences.
Late resorptions	P	No significant differences.
Total resorptions	NP	No significant differences.
%Total resorptions/implant sites	NP	No significant differences.
Number of males	P	No significant differences.
Number of females	P	No significant differences.

Fetal bodyweight

Summary statistics, for means adjusted for litter size, are presented in Table 6.

For the pooled sex (litter) analysis there was a strong sex difference and evidence for a difference between treatment groups (p=0.0487). The subsequent Dunnett's test was significant at the high dose (p=0.016, a reduction from control).

For males, there was a tendency for reduced mean bodyweight at higher doses, but the overall comparison did not attain statistical significance.

For females, there was a significant difference between groups (p=0.016). The subsequent Dunnett's test was significant at the high dose (p=0.007, a reduction from control).

There was a strong effect of litter size in all three analyses.

These results can be summarised as follows:

Male fetal body weight	No significant differences.
Female fetal body weight	Significant decrease at 20000 mg/m^3 (p=0.007).
Litter body weight	Significant decrease at 20000 mg/m^3 (p=0.016).

Discrete findings

For the Maternal pregnancy summary, only one parameter, the number of non-pregnant females, required analysis. This is summarised in Table 7.

The incidence of fetal findings, for those findings with a total incidence of greater than one is summarised in Table 8. Only five Visceral and seven Skeletal findings, all of them variations, required statistical analysis. The analysis was either generalised linear modelling (GL) or non-parametric (NP).

The results can be summarised as follows:

Maternal parameters

Non-pregnant females	NP	No significant differences

Visceral findings

Heart, V Innominate artery absent	NP	No significant differences
Torso, V Left umbilical artery	GL	No significant differences
Torso, V Renal papilla (E) not fully developed	NP	No significant differences
Torso, V Undescended thymus	GL	No significant differences
Torso, V Ureter(s) distended	NP	No significant differences

Skeletal findings

Cervical vertebrae, V 7th cervical rib(s) present	NP	No significant differences
Ribs, V Rib(s) 13th Rudimentary	NP	No significant differences
Ribs, V Rib(s) 14th Rudimentary	GL	No significant differences†
Sacral vertebrae, V 25 Presacral vertebra(e)	NP	No significant differences
Skull, V Hyoid body/arch(es) unossified	NP	No significant differences
Sternebrae, V Sternebra(e) 5 and/or 6 unossified.	NP	No significant differences
Sternebrae, V Sternebra(e) malaligned	GL	Significant decrease from control at 20000 mg/m^3 (p=0.047).

† There was some evidence of a statistically significant increase from control at 20000 mg/m^3 (p=0.067).

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TABLE 1

Maternal body weight (g)

	0 mg/m^3 (n=23)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=22)
Day of gestation	mean (sd)	mean (sd)	mean (sd)	mean (sd)
4	233.8 (14.1)	234.7 (16.6)	233.9 (15.7)	235.4 (14.7)
5	244.0 (14.6)	245.3 (16.6)	243.9 (13.9)	245.0 (15.3)
8	259.3 (12.8)	260.4 (18.1)	256.6 (14.4)	255.6 (16.2)
11	281.5 (14.9)	279.0 (19.7)	274.4 (15.6)	275.4 (19.6)
14	301.5 (17.1)	295.5 (20.2)	292.0 (14.8)	290.2 (19.6)
17	332.1 (22.3)	326.7 (24.2)	322.8 (18.7)	321.1 (24.9)
20	378.1 (26.3)	370.3 (29.9)	366.8 (24.1)	365.5 (27.1)
21	387.8 (28.5)	379.4 (32.7)	375.7 (26.0)	373.6 (27.4)

Analysis performed = Parametric throughout.
No significant differences found for any parameter.
n = number of litters, sd = standard deviation

TABLE 2

Maternal body weight change (g)

Days of gestation	0 mg/m^3 (n=23)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=22)
Period	mean (sd)	mean (sd)	mean (sd)	mean (sd)
4-5	10.3 (6.1)	10.7 (4.7)	10.2 (5.3)	9.6 (3.2)
5-8	15.5 (4.4)	15.0 (4.8)	12.5 (5.4)	10.6 (4.7) **
8-11	22.2 (4.7)	18.6 (3.7) *	17.9 (4.0) **	19.7 (5.6)
11-14	20.0 (4.2)	16.8 (5.1) *	17.5 (3.8)	15.0 (4.0) **
14-17	30.7 (7.3)	31.2 (6.9)	31.0 (7.5)	31.0 (6.7)
17-20	46.2 (7.5)	43.5 (8.9)	44.1 (9.8)	44.5 (7.7)
5-14	57.6 (8.4)	50.2 (7.9) *	47.8 (7.6) **	45.2 (9.4) **
14-21	86.4 (15.3)	84.0 (16.2)	83.8 (17.0)	83.5 (10.1)
5-21	143.9 (20.6)	134.2 (21.5)	131.8 (20.9)	128.6 (16.0)

Analysis performed = Parametric throughout.
* = $p<0.05$, ** = $p<0.01$
n = number of litters, sd = standard deviation

Maternal net bodyweight change from d5 minus gravid uterine weight (g)

	0 mg/m^3 (n=22)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=20)
Parameter	mean (sd)	mean (sd)	mean (sd)	mean (sd)
Absolute	42.95 (11.08)	37.39 (9.16)	35.71 (11.75)	29.35 (10.65) ***

Analysis performed = Parametric throughout.
n = number of litters, sd = standard deviation
*** = $p<0.001$

TABLE 3

Maternal feed consumption (g/kg/day)

Days of gestation	0 mg/m^3 (n=22-23)	2000 mg/m^3 (n=22-23)	10000 mg/m^3 (n=22-24)	20000 mg/m^3 (n=21-22)
Period	mean (sd)	mean (sd)	mean (sd)	mean (sd)
4-5	71.2 (10.5)	74.2 (11.5)	75.0 (11.4)	73.1 (10.3)
5-8	80.8 (5.9)	82.7 (5.5)	79.7 (5.9)	78.8 (5.9)
8-11	85.1 (7.1)	81.8 (4.6)	80.3 (5.5) *	77.4 (5.7) **
11-14	85.0 (7.1)	81.6 (4.6)	82.4 (3.9)	77.8 (3.9) **
14-17	80.9 (4.5)	80.8 (4.5)	80.6 (6.0)	79.2 (4.4)
17-20	74.5 (4.5)	73.4 (3.3)	73.6 (3.8)	73.1 (4.3)
20-21	53.3 (8.1)	46.8 (7.6) *	47.0 (5.8) *	45.9 (7.8) **
5-21	76.2 (4.8)	75.0 (4.1)	73.6 (3.5)	71.5 (3.9) **

Analysis performed = Parametric throughout.
* = $p<0.05$, ** = $p<0.01$
n = number of litters, sd = standard deviation
Some variation in group size due to missing values.

TABLE 4

Maternal uterus weights and maternal bodyweight minus gravid uterine weight (g)

	0 mg/m^3 (n=22)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=20)
Parameter	mean (sd)	mean (sd)	mean (sd)	mean (sd)
Absolute	101.3 (19.3)	96.5 (19.9)	96.1 (22.5)	96.4 (15.6)
Adjusted[A]	286.0 (18.1)	282.7 (19.0)	279.5 (17.2)	275.3 (18.4)

Analysis performed = Parametric throughout.
A = Maternal bodyweight minus gravid uterine weight
No significant differences found for any parameter.
n = number of litters, sd = standard deviation

TABLE 5

Reproductive performance

	0 mg/m^3 (n=23)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=22)
Parameter	mean (sd)	mean (sd)	mean (sd)	mean (sd)
corpora lutea (1)	14.9 (2.8)	14.5 (2.1)	14.8 (2.5)	15.2 (2.6)
%preimplantation loss (2)	9.3 (13.5)	6.6 (8.0)	10.4 (18.6)	7.6 (9.1)
number of implant sites (1)	13.5 (2.7)	13.5 (1.5)	13.1 (2.8)	14.0 (2.4)
fetuses alive (1)	13.0 (2.6)	12.4 (2.6)	12.6 (3.1)	13.3 (2.6)
early resorptions (2)	0.4 (0.7)	1.0 (2.1)	0.5 (0.8)	0.7 (0.6)
late resorptions (1)	0.0 (0.2)	0.0 (0.2)	0.0 (0.0)	0.0 (0.0)
total resorptions (2)	0.5 (0.7)	1.0 (2.1)	0.5 (0.8)	0.7 (0.6)
%total resorp./implant sites (2)	3.4 (4.7)	7.9 (14.9)	5.1 (8.6)	5.2 (5.2)
number of males (1)	6.3 (1.7)	5.8 (2.2)	6.4 (2.3)	6.1 (2.1)
number of females (1)	6.7 (2.0)	6.7 (2.3)	6.2 (2.3)	7.1 (2.5)

Analysis performed = (1) Parametric or (2) Non-parametric
No significant differences found for any parameter.
n = number of litters, sd = standard deviation
Male and female bw n=11

TABLE 6

Mean (adjusted) fetal bodyweight (g)

	0 mg/m^3 (n=23)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=22)
	mean (se)	mean (se)	mean (se)	mean (se)
male	5.88 (0.06)	5.87 (0.06)	5.78 (0.06)	5.71 (0.06)
female	5.58 (0.06)	5.59 (0.06)	5.47 (0.06)	5.36 (0.06) **
litter	5.73 (0.06)	5.73 (0.06)	5.62 (0.06)	5.54 (0.06) *

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$
p values vs control using Dunnett's test following mixed model ANOVA significant at 5%.
n = number of litters, se =standard error of the adjusted mean

TABLE 7

Maternal pregnancy summary and necropsy findings

Parameter	Finding	0 mg/m^3 (n=24)	2000 mg/m^3 (n=24)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=24)
Pregnancy	Non-pregnant	1	1	0	2

Only findings where the total incidence was greater than one are included.
Analysis performed = Generalized linear modelling or Non-parametric tests (see text).
No significant differences found for any parameter.
n = number of litters

TABLE 8

Fetal findings: Visceral

Tissue	Organ	Finding	0 mg/m^3 (n=23)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=22)
Visceral	HEART	V INNOMINATE ARTERY ABSENT	1	0	2	1
	TORSO	V LEFT UMBILICAL ARTERY	1	4	1	2
	TORSO	V RENAL PAPILLA (E) NOT FULLY DEVELOPED	1	0	2	1
	TORSO	V UNDESCENDED THYMUS	10	11	5	11
	TORSO	V URETER(S) DISTENDED	1	0	1	1

Only findings where the total incidence was greater than one are included.
Analysis performed = Generalized linear modelling or Non-parametric tests (see text).
No significant differences found for any parameter.
n = number of litters

TABLE 8 (cont'd)

Fetal findings: Skeletal

Tissue	Organ	Finding	0 mg/m^3 (n=23)	2000 mg/m^3 (n=23)	10000 mg/m^3 (n=24)	20000 mg/m^3 (n=22)
Skeletal	CERVICAL VERT	V 7TH CERVICAL RIB(S) PRESENT	0	1	0	3
	RIBS	V RIB(S) 13TH RUDIMENTARY	1	0	0	3
	RIBS	V RIB(S) 14TH RUDIMENTARY	2	7	5	12
	SACRAL VERT	V 25 PRESACRAL VERTEBRA(E)	1	0	0	3
	SKULL	V HYOID BODY/ARCH(ES) UNOSSIFIED BODY	1	0	1	1
	STERNEBRAE	V STERNEBRA(E) 5 AND/OR 6 UNOSSIFIED	1	0	3	3
	STERNEBRAE	V STERNEBRA(E) MALALIGNED	11	11	13	3*

Only findings where the total incidence was greater than one are included.
Analysis performed = Generalized linear modelling or Non-parametric tests (see text).
* = $p<0.05$
n = number of litters

	Certificates of Analysis	Appendix N

Table of Contents

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0516825-2
Lot Code: MAY 12 05 1B
Entered: 5/31/2005

Assay	Analysis	Units
PROTEIN	20.8	%
FAT ACID (HYDRO.)	6.34	%
FIBER (CRUDE)	3.64	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	LESS THAN 0.05	PPM
CALCIUM	0.943	%
LEAD	0.170	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.698	%
SELENIUM	0.214	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

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0517995-2
Lot Code: JUN 07 05 3B
Entered: 6/24/2005

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	5.62	%
FIBER (CRUDE)	4.01	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.0882	PPM
CALCIUM	1.03	%
LEAD	0.213	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.744	%
SELENIUM	0.316	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

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

LabDiet PMI NUTRITION INTERNATIONAL

Return to Certified Analysis Retrieval

Product Code: 5002M
Product Desc: CERTIFIED RODENT DIET MEAL
Lab Number: L0517995-3
Lot Code: JUN 07 05 3C
Entered: 6/24/2005

Assay	Analysis	Units
PROTEIN	20.9	%
FAT ACID (HYDRO.)	5.87	%
FIBER (CRUDE)	3.75	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.0933	PPM
CALCIUM	1.01	%
LEAD	0.209	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.723	%
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

AFLATOXINS	Aflatoxins	LESS THAN 5 PPB

LAB ID# 39-401

BENCHMARK ANALYTICS
4777 SAUCON CREEK ROAD
CENTER VALLEY, PA 18034-9004

PHONE (610) 974-8100
FAX (610) 974-8104

SEND DATA TO:

NAME: Terry Kusznir
COMPANY: Huntingdon Life Sciences
ADDRESS: PO Box 2360, Mettlers Road
East Millstone, NJ 08875-2360

PHONE: (732) 873-2550 x2100
FAX: (732) 873-3992

TEST REPORT

INV #: 383492-383494
383496-383498

PAGE: 1 OF 6

PO #:

PWS ID#:

PROJECT NAME: ANIMAL DRINKING WATER
SAMPLED BY: CARA KEIPER
LOCATION: SEE BELOW
DATE: 07/13/05
TIME: SEE BELOW

GRAB SAMPLES: 383492-383494
383496-383498
COMPOSITE SAMPLES: NONE
DATE RECEIVED BY LAB: 07/14/05
TIME RECEIVED BY LAB: 0819
RECEIVED FOR LAB BY: R. SMOLENAK

SAMPLE: SITE #1, F-WING ROOM 546 TIME: 1608

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
PSEUDOMONAS * Pseudomonas TArgo	<1	PER 100 ML	SM#19 9215 D	383492	07/14/05	1505	07/15/05	JD
STANDARD PLATE COUNT	<1	CFU/ML	SM#20 9215 B	383494	07/14/05	1105	07/16/05	JD
TOTAL COLIFORM	0	PER 100 ML	EPA 600-R-00-013	383493	07/14/05	1308	07/15/05	MJG
E. COLI	0	PER 100 ML	EPA 600-R-00-013	383493	07/14/05	1308	07/15/05	MJG

SAMPLE: SITE #2, V-1 ROOM 725 TIME: 1600

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
PSEUDOMONAS *	<1	PER 100 ML	SM#19 9215 D	383496	07/14/05	1505	07/15/05	JD
STANDARD PLATE COUNT	<1	CFU/ML	SM#20 9215 B	383498	07/14/05	1105	07/16/05	JD
TOTAL COLIFORM	0	PER 100 ML	EPA 600-R-00-013	383497	07/14/05	1308	07/15/05	MJG
E. COLI	0	PER 100 ML	EPA 600-R-00-013	383497	07/14/05	1308	07/15/05	MJG

REMARKS:

THE ABOVE TEST RESULTS MEET ALL THE REQUIREMENTS OF NELAC.
S PARAMETER IS NOT CURRENTLY UNDER THE NELAC CERTIFICATION PROGRAM.

REVIEWED TArgo

MANAGER: Stephanie O Neil DATE: July 25, 2005

LAB ID# 39-401

BENCHMARK ANALYTICS
4777 SAUCON CREEK ROAD
CENTER VALLEY, PA 18034-9004

PHONE (610) 974-8100
FAX (610) 974-8104

SEND DATA TO:
NAME: Terry Kusznir
COMPANY: Huntingdon Life Sciences
ADDRESS: PO Box 2360, Mettlers Road
East Millstone, NJ 08875-2360

PHONE: (732) 873-2550 x 2100
FAX: (732) 873-3992

INV #: 383495
383499, 503

PAGE: 2 OF 6

PO #:
PWS ID#:

TEST REPORT

PROJECT NAME: ANIMAL DRINKING WATER
SAMPLED BY: CARA KEIPER
LOCATION: SEE BELOW
DATE: 07/13/05
TIME: SEE BELOW

GRAB SAMPLES: 383495
383499, 503
COMPOSITE SAMPLES: NONE
DATE RECEIVED BY LAB: 07/14/05
TIME RECEIVED BY LAB: 0819
RECEIVED FOR LAB BY: R. SMOLENAK

SAMPLE: SITE #1, F-WING ROOM 546 TIME: 1608

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
COPPER	0.0660	MG/L	EPA 200.8	383495	07/15/05	0735	07/15/05	KP
LEAD	0.0017	MG/L	EPA 200.8	383495	07/15/05	0735	07/15/05	KP

SAMPLE: SITE #2, V-1 ROOM 725 TIME: 1600

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
COPPER	0.0177	MG/L	EPA 200.8	383499	07/15/05	0735	07/15/05	KP
LEAD	<0.0005	MG/L	EPA 200.8	383499	07/15/05	0735	07/15/05	KP

SAMPLE: SITE #3, V-2 ROOM 910 TIME: 1625

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
COPPER	0.0548	MG/L	EPA 200.8	383503	07/15/05	0735	07/15/05	KP
LEAD	0.0007	MG/L	EPA 200.8	383503	07/15/05	0735	07/15/05	KP

REMARKS:

REVIEWED

THE ABOVE TEST RESULTS MEET ALL THE REQUIREMENTS OF NELAC ANDRELATE ONLY TO THESE SAMPLES.

MANAGER: Stephanie Arcuri DATE: July 25, 05

LAB ID# 39-401

BENCHMARK ANALYTICS
4777 SAUCON CREEK ROAD
CENTER VALLEY, PA 18034-9004

PHONE (610) 974-8100
FAX (610) 974-8104

SEND DATA TO:		INV #:	383500-383502
NAME:	Terry Kusznir		383504-383506
COMPANY:	Huntingdon Life Sciences		
ADDRESS:	PO Box 2360, Mettlers Road	PAGE:	3 OF 6
	East Millstone, NJ 08875-2360		
		PO #:	
PHONE:	(732) 873-2550 x2100		
FAX:	(732) 873-3992 **TEST REPORT**	PWS ID#:	

PROJECT NAME:	ANIMAL DRINKING WATER	GRAB SAMPLES:	383500-383502
SAMPLED BY:	CARA KEIPER		383504-383506
LOCATION:	SEE BELOW	COMPOSITE SAMPLES:	NONE
DATE:	07/13/05	DATE RECEIVED BY LAB:	07/14/05
TIME:	SEE BELOW	TIME RECEIVED BY LAB:	0819
		RECEIVED FOR LAB BY:	R. SMOLENAK

SAMPLE: SITE #3, V-2 ROOM 910 TIME: 1625

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
PSEUDOMONAS *	<1	PER 100 ML	SM#19 9215 D	383500	07/14/05	1505	07/15/05	JD
STANDARD PLATE COUNT	9	CFU/ML	SM#20 9215 B	383502	07/14/05	1105	07/16/05	JD
TOTAL COLIFORM	0	PER 100 ML	EPA 600-R-00-013	383501	07/14/05	1308	07/15/05	MJG
E. COLI	0	PER 100 ML	EPA 600-R-00-013	383501	07/14/05	1308	07/15/05	MJG

SAMPLE: SITE #4, V-3 ROOM 950 TIME: 1556

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
PSEUDOMONAS *	<1	PER 100 ML	SM#19 9215 D	383504	07/14/05	1505	07/15/05	JD
STANDARD PLATE COUNT	4	CFU/ML	SM#20 9215 B	383506	07/14/05	1105	07/16/05	JD
TOTAL COLIFORM	0	PER 100 ML	EPA 600-R-00-013	383505	07/14/05	1308	07/15/05	MJG
E. COLI	0	PER 100 ML	EPA 600-R-00-013	383505	07/14/05	1308	07/15/05	MJG

REMARKS:

THE ABOVE TEST RESULTS MEET ALL THE REQUIREMENTS OF NELAC.
IS PARAMETER IS NOT CURRENTLY UNDER THE NELAC CERTIFICATION PROGRAM.

REVIEWED

MANAGER: Stephanie Olesa DATE: July 25, 2005

LAB ID# 39-401

BENCHMARK ANALYTICS
4777 SAUCON CREEK ROAD
CENTER VALLEY, PA 18034-9004

PHONE (610) 974-8100
FAX (610) 974-8104

SEND DATA TO:

NAME:	Terry Kusznir	INV #:	383507-383511
COMPANY:	Huntingdon Life Sciences		383515
ADDRESS:	PO Box 2360, Mettlers Road		
	East Millstone, NJ 08875-2360	PAGE:	4 OF 6
PHONE:	(732) 873-2550 x 2100	PO #:	
FAX:	(732) 873-3992 **TEST REPORT**	PWS ID#:	

PROJECT NAME:	ANIMAL DRINKING WATER	GRAB SAMPLES:	383507-383511
SAMPLED BY:	CARA KEIPER		383515
LOCATION:	SEE BELOW	COMPOSITE SAMPLES:	NONE
DATE:	07/13/05	DATE RECEIVED BY LAB:	07/14/05
TIME:	SEE BELOW	TIME RECEIVED BY LAB:	0819
		RECEIVED FOR LAB BY:	C. PASSOW

SAMPLE: SITE #4, V-3 ROOM 950 TIME: 1556

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
COPPER	0.0191	MG/L	EPA 200.7	383507	07/15/05	0735	07/15/05	KP
LEAD	<0.0005	MG/L	EPA 200.9	383507	07/15/05	0735	07/15/05	KP

SAMPLE: SITE #5, INHALATION ROOM 812 TIME: 1618

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
COPPER	0.0203	MG/L	EPA 200.7	383511	07/15/05	0735	07/15/05	KP
LEAD	0.0006	MG/L	EPA 200.9	383511	07/15/05	0735	07/15/05	KP

SAMPLE: L-WING ROOM 458 TIME: 1617

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
COPPER	0.0166	MG/L	EPA 200.7	383515	07/15/05	0735	07/15/05	KP
LEAD	<0.0005	MG/L	EPA 200.9	383515	07/15/05	0735	07/15/05	KP

REMARKS:

THE ABOVE TEST RESULTS MEET ALL THE REQUIREMENTS OF NELAC AND RELATE ONLY TO THESE SAMPLES.

REVIEWED

MANAGER: Stephanie Oliva DATE: July 25, 05

LAB ID# 39-401

BENCHMARK ANALYTICS
4777 SAUCON CREEK ROAD
CENTER VALLEY, PA 18034-9004

PHONE (610) 974-8100
FAX (610) 974-8104

SEND DATA TO:		INV #:	383508-383510
NAME:	Terry Kusznir		383512-383514
COMPANY:	Huntingdon Life Sciences		
ADDRESS:	PO Box 2360, Mettlers Road	PAGE:	5 OF 6
	East Millstone, NJ 08875-2360		
		PO #:	
PHONE:	(732) 873-2550 x2100		
FAX:	(732) 873-3992 **TEST REPORT**	PWS ID#:	

PROJECT NAME:	ANIMAL DRINKING WATER	GRAB SAMPLES:	383508-383510
SAMPLED BY:	CARA KEIPER		383512-383514
LOCATION:	SEE BELOW	COMPOSITE SAMPLES:	NONE
DATE:	07/13/05	DATE RECEIVED BY LAB:	07/14/05
TIME:	SEE BELOW	TIME RECEIVED BY LAB:	0819
		RECEIVED FOR LAB BY:	C. PASSOW

SAMPLE: SITE #5, INHALATION ROOM 812 TIME: 1618

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
PSEUDOMONAS *	<1	PER 100 ML	SM#19 9215 D	383508	07/14/05	1505	07/15/05	JD
STANDARD PLATE COUNT	<1	CFU/ML	SM#20 9215 B	383510	07/14/05	1105	07/16/05	JD
TOTAL COLIFORM	0	PER 100 ML	EPA 600-R-00-013	383509	07/14/05	1308	07/15/05	MJG
E. COLI	0	PER 100 ML	EPA 600-R-00-013	383509	07/14/05	1308	07/15/05	MJG

SAMPLE: SITE #6, L-WING ROOM 458 TIME: 1617

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
PSEUDOMONAS *	<1	PER 100 ML	SM#19 9215 D	383512	07/14/05	1505	07/15/05	JD
STANDARD PLATE COUNT	<1	CFU/ML	SM#20 9215 B	383514	07/14/05	1105	07/16/05	JD
TOTAL COLIFORM	0	PER 100 ML	EPA 600-R-00-013	383513	07/14/05	1308	07/15/05	MJG
E. COLI	0	PER 100 ML	EPA 600-R-00-013	383513	07/14/05	1308	07/15/05	MJG

REMARKS:

THE ABOVE TEST RESULTS MEET ALL THE REQUIREMENTS OF NELAC AND RELATE ONLY TO THESE SAMPLES
*IS PARAMETER IS NOT CURRENTLY UNDER THE NELAC CERTIFICATION PROGRAM.

REVIEWED

MANAGER: Stephanie O'Hara DATE: July 25, 05

LAB ID# 39-401

BENCHMARK ANALYTICS
4777 SAUCON CREEK ROAD
CENTER VALLEY, PA 18034-9004

PHONE (610) 974-8100
FAX (610) 974-8104

SEND DATA TO:

NAME:	Terry Kusznir	INV #:	383516-383518
COMPANY:	Huntingdon Life Sciences		
ADDRESS:	PO Box 2360, Mettlers Road East Millstone, NJ 08875-2360	PAGE:	6 OF 6
		PO #:	
PHONE:	(732) 873-2550 x2100		
FAX:	(732) 873-3992 **TEST REPORT**	PWS ID#:	

PROJECT NAME:	ANIMAL DRINKING WATER	GRAB SAMPLES:	383516-383518
SAMPLED BY:	CARA KEIPER	COMPOSITE SAMPLES:	NONE
LOCATION:	SEE BELOW	DATE RECEIVED BY LAB:	07/14/05
DATE:	07/13/05	TIME RECEIVED BY LAB:	0819
TIME:	SEE BELOW	RECEIVED FOR LAB BY:	C. PASSOW

SAMPLE: SITE #7 PHARMACY 1 TIME: 1605

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
PSEUDOMONAS *	<1	PER 100 ML	SM#19 9215 B	383516	07/14/05	1505	07/15/05	JD
STANDARD PLATE COUNT	1,140	CFU/ML	SM#20 9215 B	383518	07/14/05	1105	07/16/05	JD
TOTAL COLIFORM	0	PER 100 ML	EPA 600-R-00-013	383517	07/14/05	1308	07/15/05	MJG
E. COLI	0	PER 100 ML	EPA 600-R-00-013	383517	07/14/05	1308	07/15/05	MJG

REMARKS:

THE ABOVE TEST RESULTS MEET ALL THE REQUIREMENTS OF NELAC AND RELATE ONLY TO HTESE SAMPLES

REVIEWED

MANAGER: Stephanie Clark DATE: July 25, 05

LAB ID# 39-401

BENCHMARK ANALYTICS
4777 SAUCON CREEK ROAD
CENTER VALLEY, PA 18034-9004

PHONE (610) 974-8100
FAX (610) 974-8104

SEND DATA TO:

NAME:	Terry Kusznir	INV #:	387033
COMPANY:	Huntingdon Life Sciences		
ADDRESS:	PO Box 2360, Mettlers Road East Millstone, NJ 08875-2360	PAGE:	1 OF 1
		PO #:	
PHONE:	(732) 873-2550 x2100		
FAX:	(732) 873-3992 **TEST REPORT**	PWS ID#:	

PROJECT NAME:	ANIMAL DRINKING WATER	GRAB SAMPLES:	NONE
SAMPLED BY:	TERRY KUSZNIR	COMPOSITE SAMPLES:	NONE
LOCATION:	SEE BELOW	DATE RECEIVED BY LAB:	08/05/05
DATE:	08/04/05	TIME RECEIVED BY LAB:	0838
TIME:	1455	RECEIVED FOR LAB BY:	C. PASSOW

SAMPLE: SITE #1, PHARMACY (DISTILLED WATER)

TEST	RESULT	UNITS	METHOD	INV #	START DATE	START TIME	END DATE	INIT.
STANDARD PLATE COUNT	3	CFU/ML	SM#20 9215 B	387033	08/05/05	1040	08/07/05	MJG

REVIEWED
19 Aug 05

REMARKS:
THE ABOVE TEST RESULTS MEET ALL THE REQUIREMENTS OF NELAC AND RELATE ONLY TO THESE SAMPLES.

MANAGER: Stephanie A Jeffers DATE: Aug 16, 2005

Elizabethtown Water Company
Physical & Chemical Analyses

							Date	August 9, 2005	
General Source	Raritan-Millstone Plant								
Sample No. 1	Plant Delivered Water - 8:10 a.m. 8-9-05 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	-	81							
Turbidity (NTU)	0.3 NTU	0.13							
Color	10 *	1.0							
Threshold / Odor 40 ° C	3 TON *	2.6/3cc							
Threshold Taste	3 TTN *	3.0							
Conductivity (micromhos / cm)	-	308							
Hardness, Total (as mg / l CaCO3)	250 mg / l *	94							
Alkalinity	-	36							
pH	6.5-8.5 *	6.7							
Chlorine, Free / Total (mg / l Cl)	-	0.67/.74							
Calcium (as mg / l CaCO3)	-	66							
Magnesium (as mg / l CaCO3)	-	28							
Iron, Total (mg / l Fe)	0.3 mg/ l *	ND							
Sulfates (mg / l SO4)	250 mg/ l *	46.3							
Chlorides (mg / l Cl)	250 mg/ l *	31.0							
Fluoride (mg / l F)	2 mg / l*	<0.1							
Total Dissolved Solids (mg / l)	500 mg / l *	204							
Total Suspended Solids (mg / l)	-	0.0							
Ammonia Nitrogen (mg / l N)	-	<0.05							
Nitrate Nitrogen (mg / l N)	10 mg / l	0.65							
Dissolved Oxygen (mg / l O2)	-	7.5							
BOD 5 (mg / l O2)	-	-							
Langelier Index	+/- 1.0	-2.10							
Surfactants (mg / l LAS)	0.5 mg / l *	<0.05							
Hydrogen Sulfide (mg / l H2S as S)	-	0							
Nitrite Nitrogen (mg / l N)	1 mg / l	<.01							
Phosphate (mg / l PO3)	-	<.05							
Manganese (mg / l MN)	0.05 mg / l *	ND							
Zinc	5 mg/l *	0.54							
T.O.C. (mg / l)	5 mg/l *	1.58							
						Laboratory Analyst: DSD			
* Secondary or Recommended MCL									
Any questions please call Water Quality Specialist- (732) 302-3192									

REVIEWED
24 Aug 05

Sterigenics * 305 Enterprise Drive * Westerville, Ohio 43081 * USA *
614-888-4077 * FAX 614-888-4223 * www.sterigenics.com

Sterigenics.

Certificate of Processing

Facility: Westerville
Cell: Production A
Customer Name: The Anderson Inc.
Customer PO #:
Run Date: 05/09/05
Date Format: MM/DD/YY
Run Number: **AND01 AND 6050006**
Customer Ref:
Total Pallets: 21

MATERIALS PROCESSED:

Qty	Product	Description	Lot Number	LOT #
1,680	BED'O COB 1/4" BAG		DC295	425RB
1	SAMPLE-506		DC295	425RB

1,681 Bags Total

Minimum Dose: 28.4 kGy
Maximum Dose: 52.1 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: 05/10/05

ISO 9001:2000 REGISTERED

Original

A-12 05-4287 JMO 28Feb06

NAMSA

PEOPLE > SCIENCE > SOLUTIONS

Confidential
MS005_00P

Ted Weaver
Andersons
P.O. Box 119
Maumee, OH 43537

Lab No. 05T_38013_01
P.O. No. TED WEAVER
Test Facility: NAMSA
6750 Wales Road
Northwood, OH 43619

STERILITY TEST

Test Article:	"425URB" - Bed O' Cobs; Lot #DC295
ID No.	Lot: DC295
Procedure/Test Method:	S-20431-01-00/Immersion
Test Article Received:	May 19, 2005
Test Start Date:	May 20, 2005
Test Termination Date:	June 3, 2005
Number of Products Tested:	1

STERILITY TEST RESULTS
Test Article Identity Maintained as Submitted by Client

Articles Tested	Number of Articles Tested	Type of Media	Incubation Temperature (Degrees C)	Number of Days Incubated	Number of Positive Articles
Product Section	1	SCDB-400 ml	20-25	14	0
Product Section	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 ISO 17025.

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 for a period of 5 years.

cal Date Completed 6-3-05 Approved By

Michelle R. Pierce
Manager, Microbiology

A-12 05-4287 smo 28 Feb 06

PROTOCOL

GASOLINE DIPE VAPOR CONDENSATE

EMBRYO-FETAL TOXICITY STUDY IN RATS BY INHALATION EXPOSURE

HLS Study No.:	**05-4287**
Protocol Version:	**Final Protocol**
Date:	**3 August 2005**

Huntingdon Life Sciences
Study No. 05-4287 **Final Protocol**

PROTOCOL SIGNATURES/PREFACE

Study Title: Gasoline DIPE Vapor Condensate: Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure

HLS Study No.: 05-4287
Sponsor Study No.: 211-DIPE-DEV

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

K. P. Hazelden 3 Aug 05

Keith P. Hazelden, BSc, CBiol, MIBiol — Date
Study Director
Huntingdon Life Sciences (HLS)
Address: 100 Mettlers Road
East Millstone, NJ 08875-2360
Phone No.: 732-873-2550 x2590
Fax No.: 732-873-3992
Email: hazeldek@princeton.huntingdon.com

Lorraine E. Twerdok for Tom Gray 3 August 05

Thomas M. Gray, M.S., D.A.B.T. — Date
Sponsor Representative
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.com

Ship Unused Test Substance and Empty Test Substance Containers to:

Name: Michael C. Henley
Supplier: ChevronTexaco Energy 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

HLS Study No.:	05-4287
Study Title:	Gasoline DIPE Vapor Condensate: Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure
Test Substance:	Gasoline DIPE Vapor Condensate
Testing Facility:	Huntingdon Life Sciences 100 Mettlers Road East Millstone, NJ 08875-2360

Purpose:

The study is designed to assess the potential maternal and/or developmental toxicity of Gasoline DIPE Vapor Condensate in the pregnant rat when administered by whole-body inhalation exposure. The study should permit detection of gross maternal organ changes and effects on the developing conceptus, including death, structural abnormalities or altered growth.

This particular study is a near replicate of a previous study conducted by ExxonMobil Biomedical Sciences, Inc. (EMBSI Study Number 171734). The previous study is incomplete due to technical errors made in evaluating the fetuses. The Sponsor needs to repeat the previous study to satisfy the Environmental Protection Agency's Alternative Tier 2 Testing Requirements under Sections 211(b) (2) of the Clean Air Act.

2. STUDY PERSONNEL

Study Director	Keith P. Hazelden, BSc, CBiol, MIBiol
Alternate Contact	Gary M. Hoffman, B.A., DABT Inhalation Toxicologist Tel.: 732-873-2550 x2920 Email: hoffmang@princeton.huntingdon.com

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

3. PROPOSED STUDY DATES

Study Initiation Date	Date Study Director Signs Protocol
Receipt of test animals:	4 August 2005 (GD 0, 1, 2 or 3)
Initiation of exposures:	6 August 2005 (Experimental Start Date) (First GD 5)
Termination of exposures:	24 August 2005 (Last GD 20)
Necropsy:	22-25 August 2005 (GD 21)
Experimental Termination:	November 2005 (Date of last data collection)
Submission of Draft Final Report:	30 December 2005
Study Completion Date:	Date Final Report signed by Study Director

4. EXPERIMENTAL DESIGN

Group	Group Designation	Exposure Levels (mg/m3)[a]	Treatment Gestation Days	Number of Animals				
				Time-Mated Females	Euthanized Gestation Day 21[b]	Fetal Evaluations (Fetuses/Litter)		
						External	Soft Tissue	Skeletal
1	Control	0 (air only)	5-20	24	All	All	~1/2	~1/2
2	Low	2000	5-20	24	All	All	~1/2	~1/2
3	Mid	10000	5-20	24	All	All	~1/2	~1/2
4	High	20000	5-20	24	All	All	~1/2	~1/2

[a]Exposures will be 6 hours per day for 7 consecutive days per week for gestation days 5-20. Exposure levels are expressed as mg/m3 of test substance. The exposures will be conducted via whole-body exposure. Control animals will be treated with air only with the same treatment regimen as the treated groups.

[b]Postmortem evaluations will also be performed on animals which are found dead or euthanized for humane reasons or in a moribund condition during the course of the study.

The first day of gestation (Gestation Day 0 = day of detection of positive sign of mating) will be defined as Day 0 of the study for each animal.

4.1. JUSTIFICATION FOR ROUTE, DURATION AND FREQUENCY OF ADMINISTRATION

The inhalation route was used for the EMBSI study and is one of the potential routes of human exposure to this test substance. The duration and frequency of the exposures are as for the EMBSI study and as recommended in the relevant OECD and EPA test guidelines, ensuring adequate exposure of post-implantation embryo-fetal development.

4.2. JUSTIFICATION FOR TEST ANIMAL SELECTION

The rat was used for the EMBSI study and is used as a surrogate for humans in the detection of embryo-fetal toxicity and is the rodent species that is recommended by the relevant OECD and EPA test guidelines. Historical control data are also available with this strain of rat for comparative evaluation, if necessary.

4.3. JUSTIFICATION FOR NUMBER OF ANIMALS

The number of animals in this study is considered the minimum necessary to allow for meaningful interpretation of the data as required by OECD and EPA test guidelines. Some of the endpoints of principal interest (*eg* embryo-fetal death, fetal malformations) are low-frequency events and a group size of ~20 litters tends to provide the necessary degree of consistency between studies. In the expectation of pregnancy rates of 80-100%, the group size of 24 mated female rats is considered appropriate for this study and should provide a sufficient number of litters for evaluation in each group. Also, the number of animals is comparable to the number in the EMBSI study (25/group).

4.4. EXPOSURE LEVEL SELECTION AND JUSTIFICATION

The exposure levels were as used in the EMBSI study and as proscribed by the Sponsor based on the lower explosive limit of the test substance (low, intermediate and high levels were ~ 5, 25, and 50% of the LEL).

5. TEST SUBSTANCE

5.1. TEST SUBSTANCE

Gasoline DIPE (di-isopropyl ether) Vapor Condensate

TEST SUBSTANCE CATEGORY: gasoline product

Description, lot number, storage, expiration date (if available) and handling procedures, as well as other pertinent information will be

documented in the study data and final report. The test substance is supplied by the Sponsor and will be stored (ambient conditions) in an outside solvent shed except when in use in the inhalation laboratory.

5.2. 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 the signature page of this protocol. The Sponsor has conducted a purity analysis of the test substance by GC prior to the start of this study. The Testing Facility will conduct a stability analysis of the test substance by GC prior to the start of this study.

5.3. 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.

5.4. 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 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.

6. TEST ANIMALS

6.1. SPECIES

Albino Rats (Outbred), Sprague Dawley - derived
[Crl: CD® IGS BR, VAF/Plus®]

6.2. SUPPLIER

Charles River Laboratories (Raleigh, NC): Documentation will be maintained in the study file and included in the final report. This supplier and facility are the same as in the EMBSI study.

6.3. ANIMAL REQUIREMENTS/SPECIFICATIONS

6.3.1. NUMBER

Order: 100 time-mated rats.

Place on study: 96 time-mated rats.

6.3.2. AGE AND WEIGHT

Time-mated female rats 70-84 days in age and weighing 210-250 grams on Gestation Day 0 will be purchased. Animals will be received on GD 0, 1, 2 or 3.

6.4. ACCLIMATION PERIOD

Animals will be acclimated for a minimum of 2 days from time of receipt until initiation of exposures (GD 5).

6.5. ANIMAL CARE AND HUSBANDRY

6.5.1. 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 Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

6.5.2. 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. The staff veterinarian will approve the animals for inclusion on test.

6.5.3. ENVIRONMENTAL CONDITIONS

Light/Dark Cycle

Twelve-hour light/dark cycle provided by an automatic timer.

Temperature

Temperature will be monitored in accordance with HLS SOPs to ensure that the desired range of 18-26°C is maintained. Temperature will be maintained within this range to the maximum extent possible. The actual temperature range will be included in the final report.

Humidity

Humidity will be monitored in accordance with HLS SOPs to ensure that the desired range of 30 to 70% is maintained. Humidity will be maintained within this range to the maximum extent possible. The actual humidity range will be included in the final report.

Air Changes

Animal quarters will have 10-15 air changes per hour. The actual number of air changes per hour in each animal room is recorded at least twice each year and the Testing Facility retains these records.

6.5.4. HOUSING

All animals will be housed individually in stainless steel suspended cages with wire mesh floors and fronts. Cages will be arranged in such a way that possible effects due to placement are minimized (as per EPA guideline). Each cage will be fitted to secure a glass feeder jar with a stainless steel lid. Clean feed jars and fresh feed will be provided at least every 7 days for periods when feed consumption is not being recorded and at least every 7 days when feed consumption will be recorded.

All dams after exposure on GD20 will be transferred to solid bottom cages with bedding material (ground corncobs).

6.5.5. FEED

Certified Rodent Diet, No. 5002; (Meal) (PMI Feeds, Inc., St. Louis, MO) *ad libitum* except during exposures.

6.5.6. FEED ANALYSIS

Analytical certification of batches of feed provided by the manufacturer will be maintained on file at the Testing Facility and included in the final report. There are no known contaminants in the feed that are expected to interfere with the objectives of this study.

6.5.7. WATER

Facility water is supplied by Elizabethtown Water Company, (Westfield, NJ) and will be provided *ad libitum* except during exposures to individual animal cages through an automated watering system.

6.5.8. WATER ANALYSIS

Water analyses are conducted monthly by the 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 and included in the final report. 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 and included in the final report. There are no known contaminants in the water that are expected to interfere with this study.

6.6. ANIMAL HUSBANDRY DURING EXPOSURE

Housing: Individually in stainless steel wire mesh cages.

Feed: None.

Water: None.

6.7. ANIMAL IDENTIFICATION

Each animal will be assigned a temporary identification number upon receipt. After selection for study, each animal will be ear-tagged by the Testing Facility with a number assigned by the Testing Facility. The number assigned by the Testing Facility plus the study number will comprise the unique animal number for each animal. If the tag is lost, it will be replaced or the animal will be tattooed for identification. Each cage will be provided with a cage card that will be color-coded for exposure level identification and will contain the study number and animal number.

6.8. ASSIGNMENT OF ANIMALS INTO GROUPS

Animals will be placed into study groups using a computerized randomization program. This ranks Gestation Day 4 body weights into blocks and randomly assigns each animal within each block into one of the study groups. Animals may be replaced based on physical examinations and/or body weight changes up to GD 5, prior to exposures. Disposition of all animals not utilized in the study will be maintained in the study file.

7. TEST SUBSTANCE ADMINISTRATION

7.1. ROUTE OF ADMINISTRATION

Inhalation by whole-body exposures.

7.2. FREQUENCY OF ADMINISTRATION

Once daily.

7.3. DURATION OF ADMINISTRATION

The test substance will be administered for 6 hours/day, 7 days per week for Gestation Days (GD) 5-20. The duration of administration was chosen to be the same as in the EMBSI study.

7.4. ADMINISTRATION OF TEST SUBSTANCE

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 (at least three 6-hour periods) to evaluate the optimal set of conditions and equipment to generate a stable and uniform atmosphere at the target exposure levels. 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 1000 liters. Each chamber will be operated at a minimum flow rate of 200 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 T99 equilibrium time of at most 23 minutes. This chamber size and airflow rate is considered adequate to maintain the oxygen level at least 19% and the animal loading factor below 5%. At the end of the exposure, all animals will remain in the chamber for a minimum of the T_{99} equilibrium time. During this time, the chamber will be operated at approximately the same flow rate using clean air only.

7.5. 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).

7.6. PARTICLE SIZE DISTRIBUTION ANALYSIS

During each week of exposure, particle size determinations will be performed using a TSI Aerodynamic Particle Sizer to demonstrate the absence of any condensation aerosol.

7.7. CHAMBER AND EXPOSURE ROOM 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 30-40 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.

7.8. 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/day
Loading/Unloading Verification	1X/day

8. IN-LIFE EXPERIMENTAL OBSERVATIONS

8.1. CLINICAL OBSERVATIONS

8.1.1. VIABILITY OBSERVATIONS (cage-side)

Observations for mortality, morbidity, and signs of severe toxicity will be made at least twice daily. Animals in extremely poor health or in a possibly moribund condition will be identified for further monitoring and possible euthanasia.

8.1.2. DETAILED PHYSICAL EXAMINATIONS

In-Chamber: All animals will be observed as a group at least once during each exposure. This will routinely be performed near the middle of each exposure and may be performed more frequently if significant signs of toxicity are noted. Pertinent behavioral changes and all signs of toxicity, including mortality, should be recorded. These signs should include time of onset, degree and duration.

Out-of-Chamber: Animals on study will be examined daily from day after receipt through to terminal euthanasia on GD 21. Examinations will include observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) will be recorded as well as evaluations of respiration. During the treatment period, these evaluations will be performed after exposures.

8.2. BODY WEIGHTS

Body weights will be recorded on GD 4 (for randomization into test groups), 5, 8, 11, 14, 17, 20 and 21 (day of scheduled sacrifice). Weight gain will be calculated for intervals as found appropriate, including (but not limited to) GD 5-14, GD 14-21 and 5-21.

8.3. FEED CONSUMPTION

Feed consumption will be recorded and reported for these intervals: GD 4-5, 5-8, 8-11, 11-14, 14-17, 17-20, 20-21 and 5-21.

9. TERMINAL OBSERVATIONS

9.1. METHOD OF EUTHANASIA

All dams will be euthanized (by noon) by an overdose of carbon dioxide inhalation. All live fetuses will be euthanized by an intraperitoneal overdose of sodium pentobarbital.

9.2. MACROSCOPIC POSTMORTEM EXAMINATIONS

A macroscopic postmortem examination will be performed on all the dams, including those dying spontaneously or euthanized for humane reasons or in either a moribund condition or after prematurely delivering a litter. Macroscopic lesions or tissues with significant findings will be saved (10% Neutral Buffered Formalin).

Females that are found dead or sacrificed prior to scheduled termination will have implantation data (*ie* number of fetuses and early/late embryo-fetal deaths) determined at necropsy. Corpora lutea will be counted for these animals.

Females that do not have fetuses when caesarean-sectioned will have their uterus' examined for implantation sites and resorptions, including confirmation of apparent non-pregnant status, by means of staining with ammonium sulphide (modified Salewski test).

Dams showing signs of premature delivery will be euthanized on the day such evidence is observed. Reproductive tracts will be examined for implantation data (implantation sites, live/dead fetuses and early/late embryo-fetal deaths). Fetuses obtained earlier than Gestation Day (GD) 21 will be counted, sexed externally, evaluated for external malformations, euthanized and discarded. Corpora lutea will be counted for these animals.

Any dams initiating delivery on GD 21 will be immediately euthanized and the fetuses processed with the other GD 21 fetuses.

Dams surviving on GD 21 will have the following examinations of their pregnancies:

9.2.1. Reproductive System

The intact uteri (ovaries attached) will be removed from the abdominal cavity. The gravid uteri, including the cervix, will be weighed. Corpora lutea will be counted and the number per ovary recorded. The number and location of the following will be recorded for each uterine horn:

- live fetuses
- dead fetuses (no significant degeneration)
- late embryo-fetal deaths (recognizable dead fetus undergoing degeneration, regardless of size)
- early embryonic deaths (evidence of implantation but no recognizable fetus)

Each placenta will be examined macroscopically. The maternal carcass, uterus, ovaries and placenta will then be discarded.

9.3. FETAL EVALUATIONS

9.3.1. External Evaluations

All live fetuses will be weighed and individually identified within cassettes. Each live and dead retained fetus will be given a macroscopic external examination for defects including observation of the palate.

9.3.2. Fetal Soft Tissue (Visceral) Evaluation

Approximately one-half of the fetuses in each litter (alternating fetuses within the litter, nominally) will be placed in Modified Davidson's fixative for preservation and decalcification. These fetuses will be subjected to soft tissue examination by gross dissection of the torso and a razor blade sectioning technique for the head (derived from that of Wilson, J.S., Warkany, J. (1965), Teratology: Principles and Techniques. The University of Chicago Press. Chicago and London. Page 271). All malformations and variations will be recorded. Particular attention will be paid to the reproductive tract for altered signs of development. Unusual malformations or developmental findings will be photographed. During the dissection process, the sex of each fetus will be confirmed by internal inspection of the gonads. Following complete dissection of the fetuses, all carcasses and sections will be preserved in 10% neutral buffered formalin.

9.3.3. Fetal Skeletal Evaluations

Approximately one-half of the fetuses in each litter (alternating fetuses within the litter, nominally) will be eviscerated, placed in 70% isopropyl alcohol for preservation and processed for staining of the skeleton using Alizarin Red S. Subsequently, these fetuses will be evaluated for skeletal malformations and ossification variations. These skeletons will then be stored in 100% glycerin with a mold inhibitor. During the dissection process, the sex of each fetus will be confirmed by internal inspection of the gonads.

9.3.4. Dead Fetuses

These will be removed from the uterus and examined for external malformations. Only those with obvious external malformations will be preserved (10% neutral buffered formalin) for possible future examination. All other dead fetuses, and early embryonic deaths, will be discarded.

10. ARCHIVING 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.

11. STATISTICAL EVALUATIONS

Analysis will be performed by Graham Healey (Principal Investigator) of Huntingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. The Testing Facility will be responsible for the GLP compliance and the archiving of the raw data and original final report.

11.1 CONTINUOUS DATA

The following mean measures will be analyzed as described below:

maternal body weight values and body weight changes during gestation (Note: body weight measurements will only be compared to GD 5 values or subsequent relevant values)

maternal feed consumption values (presented as grams of feed/kg of body weight/day)

gravid uterine weights

corpora lutea

implantation data

pre-implantation loss

early embryonic deaths (evidence of implantation but no recognizable fetus)

live fetuses

dead fetuses (no significant degeneration)

late embryo-fetal deaths (recognizable dead fetus undergoing degeneration, regardless of size)

total embryo-fetal deaths and as % of implant sites

number of males and females per litter

Mean values of all exposure groups will be compared to the mean value for the control group at each time interval. Evaluation of equality of group means will be made by the appropriate statistical method (either parametric or non-parametric methods), followed by a multiple comparison test if needed. Bartlett's test (Bartlett, 1937; Sokal and Rohlf, 1995; Snedecor and Cochran, 1967) will be performed to determine if groups have equal variances. For all parameters if the variances are equal, parametric procedures will be used; if not, nonparametric procedures will be used.

The parametric method will be the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means are indicated Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964) test will be used to determine which means are significantly different from the control. The nonparametric method will be the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953) and if differences are indicated, Steel's test (Steel, 1959) will be used to determine which means differ from control. Bartlett's test for equality of variance will be conducted at the 1% significance level; all other statistical tests will be conducted at the 5% and 1% significance levels.

References for these procedures are:

Armitage, P. 1971. Statistical Methods in Medical Research. Oxford, UK: Blackwell Scientific Publications.

Bartlett, M.S. 1937. Properties of sufficiency and statistical tests. Proceedings of the Royal Society, Series A 160:268-282.

Dunlap, W.P. and Duffy, J.A. 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., 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.

Snedecor, G.W. and Cochran, W.G. (1989) Statistical Methods. 8th edition Iowa State University Press, Iowa, USA.

Sokal, R.R. and Rohlf, F.J. 1995. Biometry. 3rd Edition. San Francisco: W.H. Freeman, pp. 369-371.

Steel, R.G.D. 1959. A multiple comparison rank sum test: treatment versus control. Biometrics 15: 560-572.

11.1.1 FETAL BODY WEIGHT

The fetal body weight (by sex and as a composite for both sexes) will be analyzed by a mixed model analysis of variance that provides an accurate statistical model of the biology. The analysis uses the litter as the basis for analysis and effectively uses the litter size as a covariate. The model considers dose group, litter size, and fetal sex as explanatory variables. If the dose group effect in the model is statistically significant, the dose group least squares means will be tested pairwise vs the control group using t-tests associated with least squares means. The least squares means allows comparisons that account for differences in litter size and sex. The mathematical model is based on a paper by Chen et. al. (1996). Statistical

significance of differences from control will be recognized at the 5% or 1%, two-sided levels.

Reference:

Chen, J.J., Gaylor, D.W. and Laborde, J.B. 1996. Dose-response modeling of growth for developmental toxicity. Environmetrics 7:135-144.

11.2 INCIDENCE DATA

premature deliveries

total pregnancy loss (no live fetuses)

maternal necropsy findings

external fetal defects

skeletal malformations and variations

soft tissue malformations and variations

These data will be analyzed based on a Generalized Estimating Equation (GEE) application of the linearized model (Ryan, 1992). For litter endpoints, the model uses the litter as the basis for analysis and considers correlation among littermates by incorporating an estimated constant correlation and the litter size as a covariate. If the dose group effect in the model is statistically significantly the dose group least squares means will be tested pairwise vs. the control group using t-tests associated with least squares means. The least squares means allows comparisons that account for differences in litter size. Statistical significance of differences from control will be recognized at the 5% or 1%, two-sided levels.

Reference:

Ryan, L. 1992. The use of generalized estimating equations for risk assessment in developmental toxicity. Risk Analysis, 12:439-447.

12. REPORTING

12.1. STATUS REPORTS

Periodic verbal and written updates on study progress will be provided by the Study Director. In general, a written status report will be submitted weekly and following completion of the GD21 terminations.

12.2. FINAL REPORT

One unbound hard copy and one electronic copy of an audited draft report will be submitted following termination of the study. After receipt and review of the Sponsor's comments, appropriate changes will be made and an unbound hard copy and electronic copy of the revised report will be forwarded to the Sponsor for a final review. Upon receipt of the Sponsor's final 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 report will minimally include:

12.2.1. Body of Report

- Compliance Statement (including Sponsor signature line)
- Quality Assurance Statement
- Abstract
- Introduction
- Experimental Design (including justifications for exposure levels)
- Materials and Methods
- Results and Discussion
- Conclusion and No-Observed-Effect-Level (NOEL) or No-Observed-Adverse-Effect-Level (NOAEL) Statement
- Statistical Procedures
- Protocol Deviations and Study Impact Statements

12.2.2. Summary Tables

- Exposure data
- Mortality data
- Pregnancy data
- Summary of maternal physical in-life observations
- Maternal body weight and weight change data during gestation
- Maternal feed consumption data
- Mean number of corpora lutea and implantation sites
- Mean number and percent of resorptions and live fetuses
- Summary of maternal macroscopic postmortem examination data including gravid uterine weights
- Pre-implantation loss
- Post-implantation loss
- Mean fetal weight
- Total number of male and female fetuses per group and mean percent male/percent female per group

- Incidence of litters and fetuses with observations (external, visceral and skeletal)

12.2.3. Appendix Tables (Individual data)

- Individual animal termination history
- Individual clinical observations
- Individual maternal body weight data and weight change data
- Individual maternal feed consumption data
- Individual uterine implantation data and corpora lutea data
- Maternal macroscopic postmortem observations
- Individual fetal observations (external, visceral and skeletal)

12.2.4. Appendices

- Inhalation report
- Analytical results
- Feed and water analysis
- Personnel involved in the study
- Protocol and Amendment(s)

12.3. ROBUST SUMMARY

This report will be separately provided by the Testing Facility's UK office in both IUCLID and WORD® electronic formats.

13. REGULATORY REFERENCES

13.1. TEST GUIDELINES

This study is designed to meet or exceed the pertinent requirements of:

US EPA Vehicle Emissions Inhalation Exposure Guideline 79.61, CFR Vol. 59, No. 122, 27 June 1994.

Organization for Economic Cooperation and Development (22 January 2001). OECD Guidelines for Testing of Chemicals; OECD Guideline 414: Prenatal Developmental Toxicity Study.

US EPA OPPTS Health Effects Test Guidelines 870.3700, Prenatal Developmental Toxicity Study EPA 712-C-98-207, August 1998.

13.2. GOOD LABORATORY PRACTICES

This study will be conducted in accordance with US EPA 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.

13.3. 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 this protocol documents for the study described, 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 anaesthetics 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 the test substance, injury or illness may be treated by the Testing Facility's veterinary staff with approved analgesics or agents to relieve pain after consultation with the Study Director/Sponsor. However, in emergency situations, 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.

13.4. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)

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

14. QUALITY ASSURANCE MONITORING

The Quality Assurance Unit of Huntingdon Life Sciences (East Millstone, NJ) will monitor the facilities, equipment, personnel, methods, practices, records, protocols, study conduct, raw data, draft and final reports and controls used/produced at the Testing Facility in this study, to assure that they are in conformance with this protocol, company Standard Operating Procedures, and the appropriate Good Laboratory Practice regulations.

15. ALTERATION OF PROTOCOL AND STUDY DESIGN

Alterations to 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. The Study Director and a Sponsor representative will sign all protocol modifications. Two members of the Institutional Animal Care and Use Committee prior to the modification's implementation will also sign any modifications potentially affecting animal welfare.

Protocol Amendment No.: 1

Study Title: Gasoline DIPE Vapor Condensate: Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure

No. of Pages in Amendment: 1

Change(s)

Protocol Page 2:

The date for experimental termination (date of last data collection) is amended from November to December 2005.
The date for issue of the Draft Final Report (audited) is amended from 30 December 2005 to 12 January 2006.

Reason(s) for Change(s)

To assist with the logistics of fetal examinations, and report production, audit and review, prior to issue of the draft report.

Amendment approved by:

K. P. Hazelden — 23 Aug 05

Keith P Hazelden
Study Director
Huntingdon Life Sciences
East Millstone, New Jersey

Date

For Sponsor — 1 Sept. 05

Date

Protocol Amendment No.: 2

Study Title: Gasoline DIPE Vapor Condensate: Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure

No. of Pages in Amendment: 2

Changes (inserted text underlined)

Protocol Page 15:

9.3.3. Fetal Skeletal Evaluations

Approximately one-half of the fetuses in each litter (alternating fetuses within the litter, nominally) will be eviscerated, placed in 70% isopropyl alcohol for preservation and processed for staining of the skeleton using Alizarin Red S. Subsequently, these fetuses will be evaluated for skeletal malformations and ossification variations by an approved subcontractor, Michael Mercieca (Principal Investigator) of Pathology Associates International (PAI), 15 Worman's Mill Court, Suite 1, Frederick, MD 21701, USA. The skeletons will then be stored in 100% glycerin with a mold inhibitor. During the dissection process, the sex of each fetus will be confirmed by internal inspection of the gonads.

The Testing Facility will be responsible for the GLP compliance and archiving of raw data produced by the subcontractor.

Reason for Changes

To assist with the logistics of fetal examinations and ensure timely report production, the skeletal evaluations are being subcontracted to an approved contractor.

There are no additional cost implications associated with this change.

Protocol Amendment No.: 2

Amendment approved by:

K. P. Hazelden

28 October 2005

Keith P Hazelden
Study Director
Huntingdon Life Sciences
East Millstone, New Jersey

Date

For Sponsor

November 2 2005

Date

Protocol Amendment No.: 3

Study Title: Gasoline DIPE Vapor Condensate:
Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure

Change(s)

Protocol Signatures/Preface Page and Page 1, Section 2 Study Personnel, update Study Director
From:
Keith P. Hazelden, BSc, CBiol, MIBiol

To:
Gary M. Hoffman, BA, DABT
Tel: 732-873-2550 x 2920
Email: HoffmanG@Princeton.Huntingdon.com

Reason(s) for Change(s)

Keith P. Hazelden was responsible for the conduct of the study and submission of the draft report although is no longer employed with Huntingdon Life Sciences. Gary Hoffman will assume Study Director responsibility for finalization of the report. Robert M. Parker will be Alternate Contact (x2389) and will provide reproductive toxicology consultation.

Amendment approved by:

Gary M. Hoffman, BA, DABT
Study Director

7 NOV 08
Date

Thomas M. Gray, MS, DABT
Sponsor Representative

11/07/08
Date

Protocol Amendment No.: 4

Study Title: Gasoline DIPE Vapor Condensate:
Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure

Change(s)

Statistical Evaluations, page 15:

Revise from: Analysis will be performed by Graham Healey (Principal Investigator) of Huntingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. The Testing Facility will be responsible for the GLP compliance and the archiving of the raw data and original final report.

Revise to: Analysis will be **initially** performed by Graham Healey (Principal Investigator) of Huntingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon, Cambridgeshire, PE28 4HS, England. The Testing Facility will be responsible for the GLP compliance and the archiving of the raw data and original final report. **Further statistical analysis (gravid uterus weight and net body weight change minus gravid uterine weight) will be performed by Gareth Thomas (Principal Investigator) and expert report prepared for Testing Facility auditing. This additional phase of the study will also be conducted in compliance with principles of Good Laboratory Practice Standards as set forth in: The UK Good Laboratory Practice Regulations (Statutory Instrument 1999 No 3106 as amended by Statutory Instrument 2004 No. 994). OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17. EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No L 50/44). Test Site-UK QA will audit the study protocol and any subsequent protocol amendments pertaining to the statistical phase of work conducted by the Principal Investigator. The dates of inspection will be specified on the QA Statement of the final report. The SAS 9.1.3 [SAS INSTITUTE (2002) SAS OnlineDoc® *Version Nine*. SAS Institute Inc., Cary, NC, USA.] or Quasar 1.1. [QUASAR 1.1 User Guide (Stand-Alone) version 1.1 (2009). Huntingdon Life Sciences Internal Document.] computer system will be used for the statistical analysis.**

Reason(s) for Change(s)

Graham Healey has retired from HLS and this study was reassigned to Gareth Thomas to assume the responsibilities of Principal Investigator that had belonged to Graham Healey. Also, added clarification of the GLP regulations, auditing and computer systems relevant to the statistical evaluations.

Protocol Amendment No.: 4

Amendment approved by:

Gary M. Hoffman, BA, DABT
Study Director

24 May 11
Date

Thomas M. Gray, MS, DABT
Sponsor Representative

May 23, 2011
Date

Protocol Amendment No.: 5

Study Title: Gasoline DIPE Vapor Condensate:
Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure

Change(s)

Statistical Evaluations, page 15:

CONTINUOUS DATA

The following mean measures will be analyzed as described below:

maternal body weight values and body weight changes during gestation (Note: body weight measurements will only be compared to GD 5 values or subsequent relevant values)

maternal feed consumption values (presented as grams of feed/kg of body weight/day)

gravid uterine weights

corpora lutea

implantation data

pre-implantation loss

early embryonic deaths (evidence of implantation but no recognizable fetus)

live fetuses

dead fetuses (no significant degeneration)

late embryo-fetal deaths (recognizable dead fetus undergoing degeneration, regardless of size)

total embryo-fetal deaths and as % of implant sites

number of males and females per litter

Mean values of all exposure groups will be compared to the mean value for the control group at each time interval. Evaluation of equality of group means will be made by the appropriate statistical method (either parametric or non-parametric methods), followed by a multiple comparison test if needed. Bartlett's test (Bartlett, 1937; ~~Sokal and Rohlf, 1995; Snedecor and Cochran, 1967~~) will be performed to determine if groups have equal variances. For all parameters if the variances are equal, parametric procedures will be used; if not, nonparametric procedures will be used.

The parametric method will be the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, **Berry and Matthews 2002** ~~1971; Dunlap and Duffy, 1975~~). If significant differences among the means are indicated Dunnett's (~~Dunlap et al., 1981~~; Dunnett, 1955, 1964) test will be used to determine

which means are significantly different from the control. The nonparametric method will be the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953) and if differences are indicated, Steel's test (Steel, 1959) will be used to determine which means differ from control. Bartlett's test for equality of variance will be conducted at the 1% significance level; all other statistical tests will be conducted at the 5% and 1% significance levels.

References for these procedures are:

Armitage, P., Berry, G. and Matthews, J.N.S. (2002) *Statistical Methods in Medical Research, 4th edition*. Blackwell Science, Oxford, UK, pp 208-215.

~~Armitage, P. 1971. Statistical Methods in Medical Research. Oxford, UK: Blackwell Scientific Publications.~~

Bartlett, M.S. 1937. Properties of sufficiency and statistical tests. Proceedings of the Royal Society, Series A 160:268-282.

~~Dunlap, W.P. and Duffy, J.A. 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., 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.

~~Snedecor, G.W. and Cochran, W.G. (1989) Statistical Methods. 8th edition Iowa State University Press, Iowa, USA.~~

~~Sokal, R.R. and Rohlf, F.J. 1995. Biometry. 3rd Edition. San Francisco: W.H. Freeman, pp. 369-371.~~

Steel, R.G.D. 1959. A multiple comparison rank sum test: treatment versus control. Biometrics 15: 560-572.

Protocol Amendment No.: 5

FETAL BODY WEIGHT

The fetal body weight (by sex and as a composite for both sexes) will be analyzed by a mixed model analysis of variance **(Proc Mixed, SAS Institute 1999)** that provides an accurate statistical model of the biology. The analysis uses the litter as the basis for analysis and effectively uses the litter size as a covariate. The model considers dose group, litter size, and fetal sex as explanatory variables **and will include a random animal effect. The Satterthwaite method will be used for the fixed effects denominator degrees of freedom.** If the dose group effect in the model is statistically significant, the dose group least squares means will be ~~tested pairwise vs~~ **compared with the** control group using ~~t tests associated with least squares means~~ **Dunnett's test with the means being adjusted for the litter size**. The least squares means allows comparisons that account for differences in litter size and sex. ~~The mathematical model is based on a paper by Chen et. al. (1996).~~ Statistical significance of differences from control will be recognized at the 5% or 1%, two-sided levels.

Reference:

~~Chen, J.J., Gaylor, D.W. and Laborde, J.B. 1996. Dose-response modeling of growth for developmental toxicity. Environmetrics 7:135-144.~~

SAS INSTITUTE (1999) SAS OnlineDoc® *Version Eight*. SAS Institute Inc., Cary, NC, USA.

INCIDENCE DATA

premature deliveries

total pregnancy loss (no live fetuses)

maternal necropsy findings

external fetal defects

skeletal malformations and variations

soft tissue malformations and variations

For maternal parameters:

These parameters will be analyzed using non-parametric methods. If the dose group effect is statistically significant, each dose group will be compared with the control group using pairwise Wilcoxon rank sum tests (Wilcoxon 1945).

For fetal findings:

Protocol Amendment No.: 5

These data will be analyzed based on a Generalized Estimating Equation (GEE) application of the linearized model (~~Ryan, 1992~~ **Proc Genmod, SAS Institute 1999**). For litter endpoints, the model uses the litter as the basis for analysis and considers correlation among littermates by incorporating an estimated constant correlation and the litter size as a covariate. If the dose group effect in the model is statistically **significant,** ~~significantly~~ the dose group least squares means will be tested pairwise vs. the control group using t-tests associated with least squares means. The least squares means allows comparisons that account for differences in litter size. Statistical significance of differences from control will be recognized at the 5% or 1%, two-sided levels. **If this method does not converge, then non-parametric methods will be applied. The Kruskal-Wallis test (Kruskal and Wallis 1952, 1953) will be applied. If this is significant at the 5% level then each treatment group will be compared with the control using Wilcoxon rank sum tests (Wilcoxon 1945).**

References:

SAS INSTITUTE (1999) SAS OnlineDoc® *Version Eight.* SAS Institute Inc., Cary, NC, USA

~~Ryan, L. 1992. The use of generalized estimating equations for risk assessment in developmental toxicity. Risk Analysis, 12:439-447.~~

Wilcoxon, F. (1945) Individual comparisons by ranking methods. Biometrics Bulletin, 1, 80-83.

Reason(s) for Change(s)

Added clarifications and corrections of the statistical evaluations as conducted by HLS UK Statistics Department.

Amendment approved by:

6 NOV 2012
Gary M. Hoffman, BA, DABT Date
Study Director

11/2/12
Thomas M. Gray, MS, DABT Date
Sponsor Representative

	Historical Control Data Fetal Skeletal Observations	Appendix P

Rat Strain: Sprague-Dawley [Crl: CD IGS BR]
Supplier: Charles River Laboratories

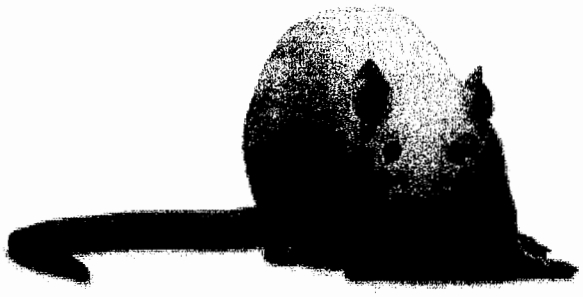
Fetal Variation: Rib(s) 14th Rudimentary

				Fetuses			Litters		
Study	Route	1st GD 6	Last GD 20	No. Examined	No. with Finding	% with Finding	No. Examined	No. with Finding	% with Finding
ALL		**Sep-02**	**Jul-05**	**902**	**79**	**8.76**	**134**	**45**	**33.58**
02-4235	Oral gavage	Sep-02	Oct-02	160	11	6.88	22	6	27.27
03-4253	Inhalation	Feb-05	Mar-05	167	12	7.19	24	7	29.17
03-4255	Oral gavage	Jun-03	Sep-03	148	23	15.54	22	15	68.18
04-4261	Inhalation	Aug-04	Sep-04	150	22	14.67	22	9	40.91
04-4267	Subcutaneous	Nov-04	Dec-04	135	6	4.44	22	4	18.18
05-4292	Intravenous	Jun-05	Jul-05	142	5	3.52	22	4	18.18

charles river
accelerating drug development. exactly.

Reproductive Toxicology

Historical Control Data in Rats



905 Sheehy Drive, Bldg. A, Horsham, PA 19044 • 215.443.8710 • FAX 215.443.8587

CHARLES RIVER LABORATORIES, PRECLINICAL SERVICES, PENNSYLVANIA
SUMMARY OF REPRODUCTIVE INDICES
Crl:CD(SD) RATS
DAY 21 CAESAREAN-SECTION

PERIOD: JUNE 2008 - JUNE 2010

	FULL STUDIES	DOSAGE-RANGE STUDIES
NUMBER OF STUDIES	48	11
NUMBER OF RATS:		
TESTED	1195	86
PREGNANT	1106	79
FOUND DEAD	7*	1
ABORTED	1	0
DELIVERED	13	0
NUMBER OF RATS PREGNANT AT CAESAREAN-SECTIONING	1089	78
NUMBER OF RATS WITH SINGLE CONCEPTUS LITTER:		
LIVE	1	0
RESORBED	0	0
ABORTED	0	0

	MEAN or %	RANGE/STUDY MEAN or %	MEAN or %	RANGE/STUDY MEAN or %
% PREGNANT	92.9	(76.0-100)	93.6	(62.5-100)
AVERAGE NO. CORPORA LUTEA	15.8	(13.6-17.9)	15.6	(14.0-16.5)
AVERAGE NO. IMPLANTATIONS	14.8	(13.0-17.0)	14.5	(13.3-15.5)
AVERAGE % PREIMPLANTATION LOSS	5.4	(1.8-11.3)	5.1	(3.0-10.2)
AVERAGE LITTER SIZE				
AVERAGE NO. LIVE FETUSES	14.1	(12.5-16.1)	14.0	(12.8-15.2)
AVERAGE NO. DEAD FETUSES	0.0	---	0.0	---
AVERAGE NO. RESORPTIONS	0.7	(0.3-1.2)	0.6	(0.1-1.0)
AVERAGE NO. EARLY RESORPTIONS	0.7	(0.3-1.2)	0.6	(0.1-1.0)
AVERAGE NO. LATE RESORPTIONS	0.0	---	0.0	(0-0.2)

* Two were unscheduled sacrifices and one was euthanized due to an intubation accident

CHARLES RIVER LABORATORIES, PRECLINICAL SERVICES, PENNSYLVANIA
SUMMARY OF REPRODUCTIVE INDICES
Crl:CD(SD) RATS
DAY 21 CAESAREAN-SECTION

	FULL STUDIES		DOSAGE-RANGE STUDIES	
	MEAN or %	RANGE/STUDY MEAN or %	MEAN or %	RANGE/STUDY MEAN or %
AVERAGE % POSTIMPLANTATION LOSS	4.7	(2.1-8.8)	4.6	(1.4-7.4)
AVERAGE % DAMS WITH ANY RESORPTIONS	46.1	(24.0-75.0)	44.9	(12.5-80.0)
AVERAGE % DAMS WITH ALL CONCEPTUSES RESORBED	0.0	---	0.0	---
AVERAGE % DAMS WITH ONE OR MORE LIVE FETUSES	100.0	---	100.0	---
AVERAGE SEX RATIO (% MALES/LITTER)	49.8	(42.6-55.9)	49.3	(42.0-55.4)
AVERAGE FETAL BODY WEIGHT (G)	5.47	(5.16-5.75)	5.39	(5.04-5.65)
AVERAGE FOR MALES (G)	5.61	(5.27-5.91)	5.53	(5.19-5.86)
AVERAGE FOR FEMALES (G)	5.32	(4.97-5.57)	5.25	(4.92-5.52)
AVERAGE % DEAD OR RESORBED CONCEPTUSES/LITTER	4.8	(2.1-8.8)	4.2	(1.2-7.4)

	Testing Facility Personnel	Appendix Q

TITLE/DEPARTMENT	NAME/DEGREE
SENIOR VICE PRESIDENT, SAFETY ASSESSMENT	Sylvie J. Gosselin, D.V.M., Ph.D., Diplomate A.C.V.P.
DIRECTOR, TOXICOLOGY OPERATIONS	Ian Vanterpool, F.I.A.T.
DIRECTOR, QUALITY ASSURANCE	Nicki S. Iacono, B.S.
STUDY DIRECTOR[a]	Keith P. Hazelden, B.Sc., C.Biol., M.I.Biol. Gary M. Hoffman, B.A., D.A.B.T.
INHALATION TOXICOLOGIST	Gary M. Hoffman, B.A., D.A.B.T.
VETERINARIAN	Teresa S. Kusznir, V.M.D.
REPORT PRODUCTION	Janet E. Scimone, B.A.
MANAGER/SUPERVISOR	
Reproductive Toxicology	Sally Wilcox, B.Sc.
Inhalation Toxicology	Stuart Cracknell, CBiol, MIBiol.
Analytical Services	Yonggang Wang, M.A.
Pharmacy	Michael S. McCarthy
Necropsy and Fetal Pathology	G. Elizabeth Baxter, B.S.
STATISTICIAN	Gareth D. Thomas, B.Sc. (Hons). Principal Investigator
SUBCONTRACTOR	Michael Mercieca, Principal Investigator Pathology Associates International (PAI)

[a]Keith Hazeldon was responsible for the conduct of the study and submission of the draft report; however he is no longer employed with Huntingdon Life Sciences. Gary Hoffman assumes responsibility for finalization of the study report.

	Report Amendments	Appendix R

There are no amendments for this report at this time.