FINAL REPORT

TITLE: Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline with Methyl Tertiary Butyl Ether Vapor Condensate (G/MTBE) in CD-1® Mice

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Quality Assurance Statement

Study Title: Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD-1® Mice

Sponsor: American Petroleum Institute

Study Code: Mi04-HLS2

Protocol Number: RTI-909

This study was audited by the Regulatory and Quality Assurance (RQA) - Quality Assurance Unit and the results of the inspections and audits were reported to the Study Director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Protocol Audit	September 28; October 04, 2004	October 04, 2004
Necropsy	January 24, 2005	January 27, 2005
Data and Report Audit	April 1, 4-8, 11, 2005	April 11, 2005
Revised Report Audit	December 8, 9, 20, 21 & 29, 2009; January 4, 6, 8, 10, 11, 13, 14 & 18, 2010	January 19, 2010

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3/15/10
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3-15-10
Date

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GLP COMPLIANCE STATEMENT

This study was conducted in accordance with the U.S. EPA Good Laboratory Practice (GLP) standards for the 211(b) program (40 CFR 79; U.S. EPA, 1994), and performed according to the protocol (and two amendments) and following the SOPs of HLS and RTI. This study complied with all appropriate parts of the USDA Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, *Federal Register*, Vol. 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 55, No. 32, February 15, 1991, pp. 6426-6505, effective March 18, 1991.

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.

Rochelle W. Tyl, Ph.D., DABT
Study Director

03/18/2010
Date

Thomas M. Gray, M.S., DABT
Sponsor's Representative

1/16/2010
Date

ABSTRACT

This study was conducted to provide a robust evaluation of the developmental toxicity potential of an inhaled vapor condensate of gasoline/methyl tertiary butyl ether (G/MTBE) in mice. It was conducted in accordance with the U.S. EPA Good Laboratory Practice (GLP) standards for the 211(b) program (40 CFR 79; U.S. EPA, 1994). In the mouse study at ExxonMobil Biomedical Sciences, Inc. (EMBSI, 2009b), several uncommon ventral closure defects (malformations) were observed in a nonexposure responsive incidence. The study reported herein was conducted to confirm and/or extend the findings observed in that earlier mouse study.

Twenty-three plug-positive female CD-1 mice each were distributed on gestational day (gd) 0 into the 0, 2000, 10,000, and 20,000 mg/m^3 target concentration groups, and an additional 38 plug-positive CD-1 mice were distributed on gd 0 into the 30,000 mg/m^3 target concentration group. Exposures were for 6 hours/day on gd 5 through 16 for the 0-20,000 mg/m^3 groups and for 6 hours/day on gd 5 through 10 for the 30,000 mg/m^3 group. The females were weighed on gd 0 and daily on gd 5 through 17; feed consumption and clinical observations were also recorded daily. Clinical observations were recorded individually before and after each exposure period and recorded at least once, using general categories (e.g., few, some, most, all, etc.) during each exposure period. At scheduled necropsy on gd 17, all dams were euthanized, with body weight, gravid uterine weight, liver weight, paired adrenal gland weights, and paired kidney weights recorded. Ovarian corpora lutea were counted and uterine total implantations, resorptions, late fetal deaths, and live fetuses recorded for each pregnant dam. Each live fetus was euthanized by intraperitoneal injection of sodium pentobarbital, sexed, and examined externally for gross malformations and variations (including examination for cleft palate). Each fetus was then dissected by a ventral longitudinal cut; the thoracic and abdominal viscera were removed and retained in buffered neutral 10% formalin for possible subsequent visceral examination. The carcasses were skinned after blanching and retained in 70% ethanol for possible subsequent staining and skeletal examination.

Mean analytical exposure concentrations were 0, 2074, 9925, 20,342 and 29,250 mg/m^3. No females died or were sacrificed moribund; 1 female was removed due to a pre-existing condition. There were no differences across groups in maternal body weights or weight changes before, during, or after the exposure period, except for significant decreases in body weight

change from gd 12 to 13 at 2000 and 20,000 mg/m^3. These findings on gd 12-13 were considered incidental and unrelated to treatment since they were observed on only 1 day and not in a dose-response pattern. Clinical observations that appeared treatment related included labored breathing in 1 female each at 20,000 and 30,000 mg/m^3 and lacrimation in 1 female at 20,000 mg/m^3 and in 3 females at 30,000 mg/m^3. Absolute maternal feed consumption (g/day) was uniformly decreased at 20,000 and 30,000 mg/m^3 and increased at 10,000 mg/m^3 (and at 30,000 mg/m^3 for gd 13-14) in the exposure period, with sporadic increases and decreases in the postexposure period in all exposed groups. Relative maternal feed consumption (g/kg body weight/day) was reduced at 20,000 and 30,000 mg/m^3 during the exposure period (with significant increases during exposure at 10,000 mg/m^3 [gd 5-6 and 6-7] and at 30,000 mg/m^3 [gd 13-14]), variable during the postexposure period, and unaffected across all groups for the gestational period: gd 0-17. These decreased feed consumption effects at 20,000 and 30,000 mg/m^3 were considered related to exposure to the test material at these exposure concentrations, exacerbating effects from the procedures for inhalation exposures, per se (e.g., moving animals, removal of feed during exposures).

There were no differences in maternal gravid uterine weight or in absolute or relative paired adrenal gland weights across groups. Absolute maternal liver weight was significantly increased at 10,000 mg/m^3, and relative maternal liver weight was significantly increased at 2000, 10,000, and 20,000 mg/m^3, likely due to induction of metabolizing enzymes during gd 5-16 exposures (Conney, 1967). There were no differences across groups for the number of ovarian corpora lutea, uterine implantation sites, resorptions, late fetal deaths or live fetuses per litter, or percent preimplantation loss. There were also no statistically significant differences in the number (or %) of nonlive (resorptions plus late fetal deaths) or adversely affected (nonlive plus malformed) implantations/litter, although there were 3 (of 36) females with fully resorbed litters at 30,000 mg/m^3 (with 0, 1, 0, and 2 fully resorbed litters at 0, 2000, 10,000, and 20,000 mg/m^3, respectively).

For live litters, there were no differences across groups on the number of live fetuses/litter, % male fetuses/litter, number of male and female fetuses/litter, or on average fetal body weight per litter for all fetuses or by sexes separately. There were no statistically significant differences across groups for incidences of external malformation or variations by fetuses or by litter. External fetal malformations included encephalocoele in 1 fetus (in 1 litter)

at 2000 mg/m^3 and cleft palate in 2 fetuses (in 2 litters) at 0 mg/m^3, in 1 fetus (in 1 litter) each at 2000, 10,000, and 20,000 mg/m^3, and in 7 fetuses (in 4 litters) at 30,000 mg/m^3. The increased incidence of cleft palate at 30,000 mg/m^3 was not statistically significant; it is likely due to increased maternal stress and other toxicity at this exposure concentration during the 6-day exposure period, during the time of initial palatal formation (gd 5-10). Gastroschisis was also observed in 1 female fetus (in 1 litter) at 30,000 mg/m^3 (this female also had cleft palate). Fetal external variations included abnormal rugae in the palatal midline in 1 fetus (in 1 litter) each at 10,000 and 20,000 mg/m^3, and hematomas of the face, head, neck, and shoulder at 0-20,000 mg/m^3 (but not at 30,000 mg/m^3). The external variations are considered incidental and unrelated to treatment.

In conclusion, the current study did not confirm the presence of ectopia cordis observed in a previous EMBSI (2009b) study in any fetus in any litter of any group, and therefore this fetal finding is considered unlikely to be related to maternal exposure to the test material. In addition, the study did not confirm the presence of gastroschisis in fetuses at 10,000 mg/m^3 (observed in the EMBSI study) or at 2000 or 20,000 mg/m^3 (not observed in the EMBSI study or in the present study). Gastroschisis was observed in 1 female fetus in 1 litter at 30,000 mg/m^3; she also exhibited severely reduced body weight and cleft palate and was part of a litter with 2 other fetuses with cleft palate. In total, gastroschisis was observed in 1 fetus (out of 407 fetuses; 0.24%) in 1 litter (out of 33 litters; 3.03%) at 30,000 mg/m^3, and there was an increased incidence (not statistically significant) of cleft palate (7 fetuses in 4 litters), likely from maternal stress also at 30,000 mg/m^3. Cleft palate is the most common external malformation observed in mouse fetuses. Maternal treatment-related clinical signs of distress, consisting of labored breathing observed at 20,000 and 30,000 mg/m^3, likely produced an increased incidence of cleft palate at 30,000 mg/m^3. This increase in cleft palate is interpreted as likely to be secondary to maternal stress (during the gd 5-10 exposure period), which would likely result in increased corticosteroid synthesis (and cleft palate, as noted in the published literature; e.g., Carmichael et al., 2007; Senda et al., 2005; Pradat et al., 2003; Hemm et al., 1977). The increased corticosteroid levels likely would have resolved in this group by scheduled necropsy on gd 17. The results of this study indicate that the effects on fetuses at 30,000 mg/m^3 were most likely due to or exacerbated by maternal toxicity. The absence of gastroschisis in any of the 3,641 control CD-1 mouse fetuses, in 288 control litters, in RTI's historical control database (Table 9) lends

support to the conclusion that gastroschisis may have been treatment related, occurring in only 1 compromised fetus at 30,000 mg/m^3, with concomitant other fetotoxicity and some indication of maternal toxicity. Indication of slight fetotoxicity (gastroschisis in 1 compromised fetus) only at 30,000 mg/m^3 and of maternal toxicity (labored breathing) at 20,000 and 30,000 mg/m^3 in this study results in the following determinations: the maternal toxicity No Observable Adverse Effect Level (NOAEL) was 10,000 mg/m^3, and the developmental toxicity NOAEL was 20,000 mg/m^3.

INTRODUCTION

A very early inhalation toxicity study of MTBE for 6 hours/day on gd 6-15 in Sprague-Dawley rats and CD-1 mice showed no maternal or embryofetal effects at any exposure concentration in rats (Conaway et al., 1985). In mice, there were increases in fetal resorptions at the low (250 ppm) and high (2500 ppm) concentrations, attributed to 2 females in each group with a high number of resorptions. There were no treatment-related fetal external, visceral, or skeletal malformations or variations. Slightly increased sternebral fusions in the high concentration group were “attributed to fetotoxicity”.

A previous developmental toxicity study (EMBSI, 2009b) of G/MTBE vapor condensate by inhalation in mice was one of a series of tests required in accordance with the Alternative Tier 2 provisions of fuels and fuels additives health effects testing regulations (U.S. EPA, 1994, 40 C.F.R. § 79; Oge 1998). That study provided suggestive evidence of an increase (not statistically significant or exposure related) in midline defects among the offspring of dams exposed to G/MTBE by inhalation at the low and mid (but not high) vapor concentrations, reporting gastroschisis and ectopia cordis (2 very rare external malformations) in offspring at these exposure concentrations in the absence of dose-response patterns. That study involved whole-body inhalation exposure of timed-pregnant CD-1 mice for at least 6 hours/day, on gd 5 through 17, to baseline gasoline vapor condensate with 25.5% MTBE at target concentrations of 0, 2000, 10,000, and 20,000 mg/m^3 (the last is 50% of the lower explosive limit).

The purpose of the present study was to provide maternal and developmental toxicity data relative to a 6- or 12-day exposure regimen of inhaled G/MTBE during the period of early or major organogenesis in gravid mice in order to confirm and extend the findings observed in the EMBSI mouse study (2009b). The present study was conducted with the same exposure

concentrations used in the EMBSI study (2009b) for 6 hours/day on gd 5 through 16 (23 females/group) and an additional group of 38 timed-mated mice exposed to 30,000 mg/m^3 for 6 hours/day on gd 5 through 10. Fetal malformations of specific interest in this study (ventral wall closure defects) are formed early in the embryonic period of gestation; gd 7 through 9 in the mouse (e.g., Rugh, 1968), hence the shortened exposure period for this group. A range-finding study was previously conducted to evaluate the top exposure level and exposure duration to be used in this study (RTI, 2009).

MATERIALS AND METHODS

Designation of Responsibilities

RTI International was responsible for study design, protocol generation, designed the procedures and trained the Huntingdon Life Sciences (HLS) staff for pairing and detection of successful mating, assignment of plug-positive study females to groups, necropsy of the maternal and fetal animals on gd 17, generation of summary and individual data tables, and draft and final reports (with RTI QA oversight). RTI's Quality Assurance Unit performed a prestudy on-site inspection, reviewed the protocol and any amendments, and monitored all phases of the study in which RTI personnel participated. HLS was responsible for receipt of the test material, prestudy and study generation and analyses of the test vapors, receipt, quarantine and housing of the test females and breeder males, determining the successful mating and assignment of the study females, in-life observations, loading and unloading study females into and out of chambers, and submission of interim and final inhalation reports. The Quality Assurance Unit of HLS reviewed the protocol and monitored the facilities, equipment, personnel, methods, practices, records, raw data, draft and final inhalation reports, and controls used in this study to assure that they were in conformance with company standard operating procedures and the referenced GLP regulations.

Test Material

The test material, G/MTBE (MRD-00-713; "API 211BG with MTBE Vapor Condensate"), was a colorless liquid and identified by the supplier (Chevron Global Technology Services Company (CRTC; Richmond, CA) as Lot/Batch Number API 00-02. Methods of synthesis, fabrication, or derivation were documented by the Sponsor and located at API. Information on identity, strength, purity, and composition of G/MTBE was provided by the

Sponsor and documented in the raw data and in this final report (Appendix III, protocol attachment).

Two separate types of chemical analysis were performed on the test material. CRTC conducted proprietary characterization of all 120 components. EMBSI conducted a GLP-compliant characterization of 18 representative hydrocarbons and MTBE. In the CRTC method, the weight percent of each of the components was measured, whereas in the EMBSI method, the relative amount of each of the representative components was measured on area-percent basis.

EMBSI developed their method for API to share with CRTC and the laboratories performing toxicological studies on 211(b) testing program test substances. The objective of the method was to monitor and document the chemical and compositional stability of G/MTBE from manufacture through transportation, storage, and animal exposure using a standard method that all users could perform.

The test material was stable and stored under ambient conditions in an outside solvent shed except when in use in the inhalation laboratory. The test substance was handled as a flammable liquid. Detailed information on chemical handling is provided in the MSDS attached to the protocol (Appendix III).

Animals and Husbandry

The test animals were Caesarean-originated Virus Antibody Free (VAF) Crl:CD-1® (ICR) BR outbred albino mice supplied by Charles River Laboratories, Inc., Raleigh, NC. The use of live animals was requested by the Sponsor and required by U.S. EPA OPPTS Testing Guidelines (U.S. EPA, 1998). Alternative test systems are not available for the assessment of chemical effects on prenatal mammalian development. The Charles River CD-1® mouse has been the mouse strain of choice on developmental toxicology contracts at RTI since 1976. Large historical databases for reproductive performance and prevalence of spontaneous malformations in control mice are available from studies conducted at RTI (currently based on 288 control litters and over 3600 fetuses from 16 studies).

One hundred seventy (170) nulliparous female mice were ordered for this study and arrived at HLS on December 23, 2004. One hundred (100) male mice, 9-11 weeks old upon arrival at HLS (on August 31, 2004), of the same strain and from the same supplier, were received for the previous range-finding study, and the remaining 99 males were used as a male breeding colony for this study. The exact number of females ordered was received, so there were

no replacements available. However, there were no animals with clinical signs, injury, and/or reduced feed consumption during quarantine. The 99 males were used to generate timed-mated animals for this definitive developmental toxicity study, which required the mating of 170 female mice (1:1, with the subsequent addition of naïve females to males who inseminated their original females) to generate 130 plug-positive females. Females were 7-9 weeks old at arrival and 9-11 weeks of age and ~20-35 g in weight on gd 0. One hundred seventy (170) females were required to generate 130 plug-positive females in 11 consecutive days (the protocol indicated that we expected 130 plug-positive females in 4-5 days, but it took longer; Amendment 2); 130 plug-positive females (23/group for 4 groups and 38/group for the fifth group) were required to supply the optimal number (based on EPA's guidance; e.g., OPPTS 870.3600; U.S. EPA, 1996; for inhalation developmental toxicity studies) of pregnant animals and litters to assess any maternal and/or embryo/fetal toxicity to the test substance and to confirm and extend the fetal findings from the previous EMBSI study (2009b).

During an approximately 14-day quarantine/acclimation period at the HLS testing facility, animals were checked for viability twice daily. Prior to study assignment, all animals were examined to ascertain suitability for study. The HLS veterinarian formally released these animals for use by signature and date. Males and females were individually housed in stainless steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female were housed together. During cohabitation, male and female mice were housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). 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 weekly. After the gd 14 exposure (for Groups 1-4) or on the afternoon of gd 14 (Group 5), a stainless steel, perforated insert was placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. Females not undergoing daily exposures after gd 10 (Group 5) were removed from their home cage and placed in another suspended cage without feed to match as closely as possible the conditions of Group 1-4 females for the 6-hour exposure period. They were then returned to their home cage at the same time as the exposed females for feed measurement overnight. Feed (PMI 5002 Certified Meal) was available *ad libitum*, except during the daily 6-hour inhalation periods. Analytical certifications of batches of feed provided by the manufacturer are maintained

on file at the HLS testing facility, and there were no known contaminants found in the feed. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) was available *ad libitum* via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses were conducted by Elizabethtown Water Company to assure that water met standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analyses provided by the supplier are maintained on file at the HLS testing facility. There were no known contaminants that interfered with the objectives of this study. At all times, animals were housed, handled, and used according to the National Research Council Guide (NRC, 1996).

A 12-hour light/dark cycle was provided via automatic timer. Temperature and relative humidity were monitored in accordance with Testing Facility SOPs to ensure that the desired range of 18 to 26°C for temperature and 30 to 70% relative humidity was maintained to the maximum extent possible (NRC, 1996).

Each animal was assigned a temporary identification number (designated on each cage) upon receipt. During the second week of the quarantine/acclimation period, the 170 females received were tail tattooed with consecutive numbers 1 through 170. The 99 remaining males had been tail tattooed during the range-finding study with consecutive numbers 1 through 100 (except for No. 87). After selection for use on the study, mating, indication of copulation, and assignment to 1 of the five groups, each female was ear tagged with a number assigned by the HLS testing facility. This number, 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 and animal numbers.

It was anticipated that the concentrations employed would not result in irritation or corrosion to the respiratory tract of the test animals (based on previous studies with the test material; e.g., Conaway et al., 1985; Bevan et al., 1997a; EMBSI, 2008, 2009a,b). Animals were not subjected to undue pain or distress. All procedures used in this study were designed to avoid discomfort, distress, and pain to the animals. The HLS IACUC (Institutional Animal Care and Use Committee) Protocol Review Subcommittee and the RTI IACUC reviewed the protocol and found it to be in compliance with appropriate animal welfare regulations.

Immediately prior to pairing, each female was weighed and subjected to a clinical examination. For breeding, 1 male with 1 female pairing was employed since other pairing

patterns (e.g., 1 male with 2 females) may have resulted in an unacceptable number of plug-positive, nonpregnant females and/or sire effects. Individual females were placed in polycarbonate "shoebox" cages with stainless steel lids with singly-housed males. On the following morning and each morning thereafter, the females were examined for the presence of a vaginal copulation plug (Hafez, 1970). The day on which copulation plugs were found was designated as gd 0. Plug-positive females (dams) were individually housed until scheduled sacrifice on gd 17. Plug-negative females were retained in the same male's cage and checked for plugs on successive mornings until insemination occurred or the treatment groups were filled, whichever came first. HLS staff evaluated females for vaginal copulation plugs until all groups were filled and then completed the exposure schedule. When all treatment groups were filled, the remaining presumed plug-negative females were sacrificed by asphyxiation with CO_2 and examined for pregnancy status; many of the females were in fact pregnant (see Protocol Deviation No. 7). The males were also euthanized by HLS staff after the breeding period was completed. The fate of all animals is fully documented in the study records.

Study Schedule and Design

The actual dates of all major phases of the study are presented in Table A.

Table A. Study Schedule

Event	Dates
Females arrived at HLS:	December 23, 2004
Quarantine (14 days):	December 23, 2004 – January 5, 2005
Animals paired:	January 6-11, 2005
Dates of gd 0:	January 7-17, 2005
TSCA experimental start date:	January 12, 2005
Exposure dates: gd 5 through 10	January 12 – January 22, 2005
gd 5 through 16	January 12 – February 2, 2005
Terminal necropsy (gd 17)	January 24 – February 3, 2005
TSCA experimental termination date:	February 3, 2005
Submission of draft data on test atmospheres to Sponsor:	February 9, 2005 (within 1 week after the last exposure date, February 2, 2005)

This study was conducted with 4 treatment groups and 1 vehicle control group. Groups 1-4 were each comprised of 23 plug-positive female mice, and Group 5 was comprised of 38 plug-positive female mice (Table B).

Table B. Number of Animals Assigned to Study Groups

Group No.	No. Animals Exposed	No. Days Exposed	Exposure Period (gd)	Target Exposure Concentration (mg/m^3)
1	23	12	5 through 16	0
2	23	12	5 through 16	2000
3	23	12	5 through 16	10,000
4	23	12	5 through 16	20,000
5	38	6	5 through 10	30,000

The exposure period for Group 5, at 30,000 mg/m^3 G/MTBE on gd 5 through 10, was selected to reduce the number of days of generation of test atmosphere at a concentration that was 75% of the lower explosive limit. In addition, the fetal malformations of interest are formed early in the embryonic period of gestation; gd 7-9 in the mouse (e.g., Rugh, 1968), so extending the exposure period to gd 16 was considered unnecessary.

The test substance was administered as a vapor in the breathing air of the animals. The test atmosphere was generated by an appropriate procedure determined during prestudy trials. The prestudy trials were performed (at least two 6-hour periods) to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the target exposure levels and maintain uniform conditions throughout the exposure chambers. The 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 1 air change in 5 minutes (12 air changes/hour) and a T_{99} equilibrium time of at most 23 minutes. This chamber size and airflow rate was considered adequate to maintain the oxygen level at least 19% and the animal loading factor below 5%. At the end of each daily 6-hour exposure, all animals remained in the chamber for a minimum of the T_{99} equilibrium time. During this time, the chamber was operated at approximately the same flow rate using clean air only.

A nominal exposure concentration of G/MTBE was calculated. The flow of air through the chamber was monitored using appropriate calibrated equipment. The test substance consumed

during the exposure was 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 6-hour exposure, measurements of airborne concentrations were performed in the animals' breathing zone at least 4 times using an appropriate sampling procedure and IR analytical procedure. Specified airborne test material concentrations were within +/- 10% of the target concentrations. One sample per chamber during the trials period and the treatment period was analyzed by gas chromatography 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. During the treatment period, particle size determinations were performed once per chamber using a TSI Aerodynamic Particle Sizer to confirm the absence of particulate test substance condensate in the exposure atmosphere.

Chamber temperature, humidity, airflow rate, and static pressure were monitored continuously and recorded every 30 minutes during exposure. Chamber temperature and relative humidity were maintained, to the maximum extent possible, between 20 to 24°C and 40 to 60%, respectively. Chamber oxygen levels (maintained at least 19%) were measured pretest and at the beginning, middle, and end of the exposure period for the study. Air samples were taken in the vapor generation area pretest and at the beginning, middle, and end of the exposure period for the study. Light (maintained approximately 30 foot-candles at 1.0 meter above the floor) and noise levels (maintained below 85 decibels) in the exposure room were measured pretest and at the beginning, middle, and end of the exposure period for the study. The minimum frequency of chamber activity during the treatment period is summarized below:

Activity	Frequency/Chamber
Measured test substance concentration	4X/day
Measured test substance characterization	1X
Particle size	1X
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

Plug-positive female mice (dams) were assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights and equal distribution of females mated to the same male among dose groups using data from gd 0. A total of 23 plug-positive dams were assigned on gd 0 by stratified randomization (by body weight) to each of 4 groups (0 [Group 1], 2000 [Group 2], 10,000 [Group 3], and 20,000 [Group 4] mg/m^3), and 38 plug-positive dams were similarly assigned to Group 5 (30,000 mg/m^3). Because of the unexpectedly prolonged mating period, the decision was made at HLS (see Protocol Deviation No. 2) to use 130 plug-positive females rather than wait for 140 plug-positive females, and use 23/group (versus 25/group) for Group 4 and 38/group (versus 40/group) for Group 5. Plug-positive females were exposed to G/MTBE or air 6 hours per day from gd 5 through 16 for Groups 1-4 and for gd 5 through 10 for Group 5, 30,000 mg/m^3, since the malformations of interest (gastroschesis and ectopia cordis) result from ventral midline closure defects early in the embryologic period, and the shorter exposure period could possibly reduce the risk of prenatal deaths, which could limit the ability of the study to detect these early malformations. For each daily exposure, females were transferred to inhalation cages, and the cages were moved into the appropriate chambers for exposure. Following each daily exposure, females were transferred back to home caging for feed consumption measurements overnight.

Clinical observations of all animals were made once daily on gd 0 through 4 (prior to the start of the exposure period), twice daily (prior to and immediately after each daily exposure) throughout the exposure period (gd 5 through 10 or gd 5 through 16), and once daily on gd 11 through 17 or on gd 17 (after exposure period ended). In addition, during each daily exposure period, animals were observed at least once during each exposure. This was routinely performed near the middle of each exposure.

Dams were weighed in the mornings (prior to exposures for those days that exposures occurred) on gd 0 and 5-17. Maternal weight gains were calculated for gd 0-5 (pre-exposure period), gd 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14, 14-15,15-16, gd 5-10 or 5-16 (exposure period), gd 10-17 or 16-17 (postexposure period), and gd 0-17 (gestational period).

Maternal feed consumption was evaluated in the mornings from gd 0-5 (pre-exposure period), gd 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14, 14-15, 15-16, gd 5-10 or 5-16 (exposure period), 10-17 or 16-17 (postexposure period), and gd 0-17 (gestation period).

On gd 17, approximately 1 to 1½ days before expected parturition, all surviving maternal animals from all groups were killed by CO_2 asphyxiation at HLS by RTI staff. The thoracic and abdominal cavities and organs were examined, and pregnancy status was confirmed by uterine examination. Uteri that presented no visible implantation sites were stained with ammonium sulfide (10%) in order to visualize any implantation sites that may have undergone very early resorption (Salewski, 1964). At sacrifice, the body, liver, uterus, paired adrenal glands, and paired kidneys of each plug-positive female were weighed. Ovarian corpora lutea were counted and uterine contents (i.e., number of implantation sites, early and late resorptions, dead fetuses, live fetuses) recorded.

Live and dead fetuses were removed from the uterus, counted, weighed, sexed externally, and examined externally for gross malformations (including cleft palate) and variations by RTI staff. Each fetus was killed by intraperitoneal injection of sodium pentobarbital. Live and dead fetuses were dissected longitudinally, and the thoracic and abdominal viscera removed intact and retained individually in labeled scintillation vials in buffered neutral 10% formalin for possible subsequent visceral examination. The fetal carcasses were blanched, skinned, and retained in individually labeled scintillation vials in 70% ethanol for possible subsequent double staining (alizarin Red S and alcian blue) and skeletal evaluation. All maternal organs and carcasses were destroyed by incineration.

Statistics

The unit of comparison was the pregnant female or litter. Quantitative continuous data (e.g., maternal body weights, feed consumption, fetal body weights, etc.) were compared among the 4 treatment groups and 1 vehicle control group using either parametric ANOVA under the standard assumptions or robust regression methods (Zeger and Liang, 1986; Royall, 1986; Huber, 1967), which do not assume homogeneity of variance or normality. If the ANOVA test was statistically significant (i.e., if one or more of the pairwise comparisons, not necessarily to the vehicle control group, were statistically significant), then statistical pairwise comparisons were made (see below). The homogeneity of variance assumption was examined via Levene's Test (Levene, 1960), which is more robust to the underlying distribution of the data than the traditional Bartlett's Test. If Levene's Test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods were used to test all treatment effects. The heterogeneous variance

models (also known as robust regression methods) use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for overall treatment group differences (via Wald Chi-Square Tests), followed by individual *t*-tests for exposed vs. control group comparisons when the overall treatment effect was significant. At the time these methods were implemented in this study, the software did not have p-value adjustments for multiple pairwise comparisons. This problem was recognized, and to protect against spurious findings, the significance of individual pairwise comparisons to controls (repeated t-tests) were not reported for a given parameter unless the overall treatment effect was statistically significant at $p<0.05$ (Wald chi-square Test). The presence of linear trends was analyzed by robust regression methods for nonhomogenous data. Robust regression methods are available in the REGRESS procedure of SUDAAN® Release 8. (RTI, 2001).

If Levene's Test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® Release 8 was used to evaluate the overall effect of treatment and, when a significant treatment effect was present, to compare each exposed group to control via Dunnett's Test (Dunnett, 1955, 1964). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For the litter-derived percentage data, the ANOVA was weighted according to litter size. The presence of linear trends was analyzed by GLM procedures for homogenous data (SAS Institute Inc., 1999a, b, c, d, e; 2000; 2001). A one-tailed test (i.e., Dunnett's Test) was used for all pairwise comparisons to the vehicle control group, except that a two-tailed test was used for maternal body and organ weight parameters, maternal feed consumption, fetal body weight, and percent males per litter. Standard ANOVA methods, as well as Levene's Test, are available in the GLM procedure of SAS® Release 8 (SAS Institute Inc., 1999a, b, c, d, e; 2000; 2001).

Nominal scale measures were analyzed by Chi-Square Test for Independence for differences among treatment groups (Snedecor and Cochran, 1967) and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ($p<0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each treatment group and the control group.

A test for statistical outliers (SAS Institute, Inc., 1999b) was performed on female body weights, feed consumption (in g/day), and selected organ weights. Per RTI's SOPs, if examination of pertinent study data did not provide a plausible, biologically sound reason for inclusion of the data flagged as "outlier," then the data were excluded from summarization and analysis and designated as outliers. If there was a plausible, biologically sound reason to retain the flagged data, the data were included in the summarization and analysis. Unless otherwise specified, the level of significance used for the various tests was $p<0.05$.

Storage of Records

All data documenting experimental details and study procedures and observations were 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 RTI's secure archives for a period of 1 year after submission of the signed final report. The Sponsor will be contacted in order to determine the final disposition of these materials.

Personnel

This study was conducted by RTI under contract to the API (Mr. T.M. Gray, Sponsor's Representative) at HLS (Mr. G.M. Hoffman, Principal Investigator; Animal Research Facility Veterinarian, Dr. Teresa S. Kusznir; Animal Research Facility Director, Mr. I. Vanterpool; Necropsy Laboratory Supervisor, Ms. G.E. Baxter; Inhalation Laboratory Supervisor, Mr. S. Cracknell; Formulation Chemistry Services, Ms. K. Saladdin; Reproductive Consultant, Mr. K.P. Hazelden; and Quality Assurance, Ms. N.S. Iacono). Dr. R.W. Tyl of RTI served as Study Director. RTI Reproductive and Developmental Toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), Mr. W.P. Ross, Mr. C.G. Leach, Ms. L.L. Macdonald, Ms. N.M. Kuney, and Ms. A.J. Parham. RTI Quality Assurance personnel were Ms. D.A. Drissel (Manager), Ms. C.A. Ingalls, Ms. M.M. Oh, and Ms. S.C. Wade. Mr. W.P. Ross, Ms. N.M. Kuney, Mr. C.G. Leach, Ms. L.L. Macdonald, and Ms. M.C. Marr were present at HLS to perform the necropsy and external fetal evaluations. Ms. S.C. Wade was also present to observe the procedures and evaluations.

The final report was prepared by Dr. R.W. Tyl and Ms. M.C. Marr, with assistance from Ms. C.B. Myers for statistical analyses and generation of tables, and by Mr. T.W. Wiley for data

entry. Ms. M.C. Marr was responsible for all transfer of custody procedures for transfer of records and tissues from HLS to RTI, and for archiving the study records at RTI.

RESULTS

Test Chamber Analyses

Table 1, Appendix I

Prestudy chamber distribution analyses showed that the test substance was evenly distributed within the chamber. Prestudy and in-study chamber monitoring showed that the chamber oxygen levels were 20%.

The analytically measured exposure levels of the airborne test substance were reasonably close to the targeted exposure levels. The measured and nominal concentrations varied somewhat (less than 10%), but reasonably, from the expected 1:1 ratio for this type of vapor exposure. Chamber environmental conditions averaged 20.8ºC temperature and 29.6% (based on RTI calculations; 29.2% from HLS calculations) relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were essentially vapor only, as expected, since there was no substantial difference between the test substance chamber and the air control chamber for particle size distribution.

Analysis of the major components in the neat test substance and the test atmospheres showed a reasonably close concordance 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 proper proportions. The data were consistent between the prestudy and in-study analyses, indicating stability of the test substance and the atmosphere generation techniques.

The test atmospheres were generated to within 97.5 to 103.7% of the target (grand mean of daily means/chamber). There was no test material detected in the control chamber, with an estimated limit of quantification (LOQ) of 433 mg/m^3 (see HLS Study File Note in Appendix I; last page). The relative content of MTBE was 21.3%, as provided by the supplier (see Appendix III, attachments to protocol). The analytical profile of G/MTBE at HLS indicated 26-27% MTBE, confounded by coelution with 2,3-dimethylbutane, which could not be separated using gas chromatography with a flame ionization detector and a previously used Supelco Petrocol™

column for the range-finding study (range-finding final study report, Appendix I, HLS report), and ~23-25% MTBE (confounded by coelution with 3-methylpentane) using a new column for this endpoint-specific developmental toxicity study (see Appendix I, HLS report). Net MTBE concentrations were 21.66-22.72% (Appendix I, Table I).

Maternal Findings

Tables 2-5, Appendix II

One female (No. 3814) at 10,000 mg/m^3 was removed from study due to a pre-existing condition (right side undescended testis, seminal vesicle and prostate, left side ovary, oviduct, uterus, cervix and vagina). No females died or were euthanized moribund. The numbers of confirmed nonpregnant females (at scheduled sacrifice) were 0, 1, 3, 1, and 2 and fully resorbed litters were 0, 1, 0, 2, and 3 at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3, respectively. The number (and %) pregnant were 23 (100.0), 22 (95.7), 19 (86.4), 22 (95.7), and 36 (94.7) at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3, respectively (Table 2). There were no statistically or biologically significant differences between groups for % pregnant.

There were no effects of exposure across all groups on maternal body weights for gd 0, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 (in-life), and gd 17 (at sacrifice). Maternal body weight changes were also unaffected across all groups for the following intervals: gd 0-5 (pre-exposure period for all groups), gd 5-10 (exposure period for Group 5), gd 5-16 (exposure period for Groups 1-4), gd 10-17 (postexposure period for Group 5), gd 16-17 (postexposure period for Groups 1-4), and gd 0-17 (gestation period), except for decreased maternal body weight changes for gd 12-13 at 2000 ($p<0.01$) and 20,000 ($p<0.05$) mg/m^3 (with exposures on gd 5-16). These findings were only for 1 day at the lowest and next to the highest exposure concentrations (during the exposure period for Groups 1-4) and are considered incidental since they only occurred once and did not display a dose-response pattern. Maternal gestational weight change (gestational body weight gain minus gravid uterine weight) was unaffected across all groups (Table 2).

Maternal clinical observations for gd 0-4 (pre-exposure period), prior to and after each daily exposure period (gd 5-16 for Groups 1-4 or gd 5-10 for Group 5) and postexposure (gd 17 for Groups 1-4 or gd 10-17 for Group 5), are presented in Table 3. There were no clinical observations of interest recorded for any dam in any group during the daily exposures. Moderate

alopecia on extremities/snout was observed starting on gd 5 in 1 female (No. 4807) at 20,000 mg/m^3. Enophthalmos (eyeball sunk into orbital cavity), left, was observed in 1 female (No. 5829) at 30,000 mg/m^3. Labored breathing was observed on gd 9 postexposure for 1 female (No. 4823) at 20,000 mg/m^3 and on gd 10 postexposure for 1 female (No. 5838) at 30,000 mg/m^3. Lacrimation, either unilateral or bilateral, was observed for a total of 1 female (No. 4803) at 20,000 mg/m^3 and 3 females (Nos. 5802, 5805, and 5824) at 30,000 mg/m^3. Unilateral moderate lacrimation was observed in 1 female (No. 5824) at 30,000 mg/m^3 postexposure on gd 5, and 2 females (1 each at 20,000 [No. 4803] and at 30,000 [No. 5802] mg/m^3) postexposure on gd 6, possibly treatment and dose related. Bilateral moderate lacrimation was observed in 1 female (No. 5805) at 30,000 mg/m^3 postexposure on gd 6. Lacrimation and labored breathing, observed in more than one female at 20,000 and 30,000 mg/m^3, appeared to likely be treatment related. Also, red exudates were observed from the anogenital area of 2 females with resorptions: 1 female each at 10,000 mg/m^3 (No. 3808 with 2 mid resorptions) on gd 12 pre-exposure and at 20,000 mg/m^3 (No. 4814 with 1 mid resorption) on gd 11 postexposure (Table 3).

Maternal feed consumption (in g/day) was significantly reduced at 20,000 mg/m^3 for gd 0-5 (pre-exposure period), significantly increased at 10,000 mg/m^3 for gd 5-6, significantly increased at 2000 and 10,000 mg/m^3 for gd 6-7, significantly reduced at 20,000 mg/m^3 for gd 7-8, and significantly reduced at 20,000 and 30,000 mg/m^3 for gd 8-9. Feed consumption (in g/day) was also significantly reduced at 30,000 mg/m^3 for gd 10-11, significantly increased at 10,000 mg/m^3 for gd 12-13, and significantly increased at 30,000 mg/m^3 for gd 13-14. Feed consumption in g/day was equivalent across all groups for gd 9-10, 11-12, 14-15, 15-16, 16-17 (postexposure period, Groups 1-4), gd 5-10 (exposure period only for Group 5, 30,000 mg/m^3), gd 5-16 (exposure period for Groups 1-4), gd 10-17 (postexposure period for Group 5), and gd 0-17 (gestational period) (Table 4). None of these feed consumption changes are considered adverse or treatment related; all are likely incidental.

Maternal feed consumption (in g/kg body weight/day) was significantly reduced at 20,000 mg/m^3 for gd 0-5 (pre-exposure period), significantly increased at 10,000 mg/m^3 for gd 5-6, significantly increased at 2000 and 10,000 mg/m^3 for gd 6-7, significantly reduced at 20,000 mg/m^3 for gd 7-8, 8-9, 9-10, and 11-12, significantly reduced at 30,000 mg/m^3 for gd 8-9, 10-11, and 5-10 (exposure period for Group 5), and significantly increased at 30,000 mg/m^3 for gd 13-14. There were no differences across groups for feed consumption (in g/kg/day) for gd

12-13, 14-15, 15-16, 16-17 (postexposure period for Groups 1-4), gd 5-16 (exposure period, Groups 1-4), gd 10-17 (postexposure period for Group 5), and gd 0-17 (gestation period) (Table 4). Changes in relative feed consumption at 20,000 and 30,000 mg/m^3 during the exposure period were likely due to the processes for inhalation exposure; after the exposure period, there were essentially no effects on feed consumption.

At scheduled necropsy on gd 17, maternal absolute gravid uterine weight, paired adrenal gland weight, and paired kidney weight were unaffected across all groups. Maternal absolute liver weight was equivalent across 0, 2000, 20,000, and 30,000 mg/m^3 and was significantly increased (11%; $p<0.05$) at 10,000 mg/m^3. Maternal paired adrenal gland and paired kidney weights (relative to terminal body weights) were equivalent across all groups. Relative (to terminal body weight) maternal liver weight was significantly increased in a concentration-related manner at 2000 (6%; $p<0.01$), 10,000 (11%; $p<0.001$), and 20,000 (11%; $p<0.001$) mg/m^3, likely due to induction of metabolizing enzymes detected in dams exposed through gd 16 (observed in Groups 1-4, with exposures ending on gd 16); relative liver weight was unaffected at 30,000 mg/m^3, with exposures ending on gd 10, most likely due to down regulation of metabolizing enzymes postexposure (Conney, 1967) (Table 5).

Uterine and Embryofetal Findings

Tables 6-8, Appendix II

For all pregnant females, there were no effects across groups for any reproductive parameter, including no statistically significant effects on the number of ovarian corpora lutea/dam, number of uterine implantation sites/litter, percent preimplantation loss/litter, number (and %) of resorptions/litter, number (and %) of litters with resorptions, number (and %) of late fetal deaths/litter, number (and %) of litters with late fetal deaths, number (and %) of nonlive (late fetal deaths plus resorptions) implants/litter, number (and %) of litters with nonlive implants, number (and %) of litters with 100% nonlive implants (fully resorbed), number (or %) of adversely affected (nonlive plus malformed) implants/litter, and number (and %) of litters with adversely affected implants. There were 0, 1, 0, 2, and 3 fully resorbed litters (100% resorptions) at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3, respectively. These findings did not differ statistically across groups but might indicate maternal stress at 20,000 and 30,000 mg/m^3,

with slightly increased numbers of fully resorbed litters (full litter losses are almost always ascribed to effects on the maternal animal) (Table 6).

For live litters, there were no effects across groups for the number of live fetuses/litter, percent male fetuses/litter, number of male or female fetuses/litter, or for average fetal body weight/litter for all fetuses or separately by sex (Table 6).

Summary and statistical analysis of fetal external malformations and variations are presented in Table 7. Presentation of fetal external malformations and variations by defect type is in Table 8. The number of fetuses (litters) examined were 276 (23), 236 (21), 225 (19), 252 (20), and 407 (33) at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3, respectively. There were no statistically significant differences across groups for any of the parameters evaluated. These parameters included the number and percentage of fetuses with external malformations per litter (total and separately by sex), the number and percentage of fetuses (pooled by group) with external malformations, and of litters with at least 1 fetus with external malformations. Also, there were no differences across groups for the same parameters as above for fetal external variations. There were fetal external malformations and variations observed at low incidence in all 5 groups (Table 7).

The fetal external malformations included encephalocele in 1 fetus in 1 litter (Dam No. 2819, Implant No. 5) at 2000 mg/m^3, cleft palate in 2 fetuses (2 litters), 1(1), 1(1), 1(1), and 7(4) at 0, 2000, 10,000, 20,000, and 30,000 mg/m^3 respectively, and gastroschisis in 1 fetus in 1 litter (Dam No. 5810, Implant No. 7) at 30,000 mg/m^3. This apparent (nonstatistically significant) increase in the fetal (and litter) incidence of cleft palate at 30,000 mg/m^3 may be indirect evidence of maternal stress during exposures at this high concentration (see Discussion below).

The fetal external variations included abnormal rugae in the midline of the palate in 1 fetus in 1 litter each at 10,000 (Dam No. 3822, Implant No. 15) and 20,000 mg/m^3 (Dam No. 4822, Implant No. 3), not cleft palate, and hematomas at various locations (face, head, neck, and shoulder) in 4 fetuses (in 4 litters) at 0 and 2000 mg/m^3, in 1 fetus (in 1 litter) at 10,000 mg/m^3, in 2 fetuses (in 2 litters) at 20,000 mg/m^3, and in no fetuses at 30,000 mg/m^3 (Table 8). None of these external variations are considered related to treatment or exposure concentrations.

The historical control data for 288 litters of CD-1 mice at RTI International for governmental clients from 1997-2002 are presented in Table 9.

DISCUSSION

This study was designed and performed:

1. To confirm or refute the fetal malformation finding of ectopia cordis observed in 1 fetus at 2000 mg/m^3 and in 2 fetuses (in the same litter) at 10,000 mg/m^3 in the previous developmental toxicity study on this test material in CD-1® mice (EMBSI, 2009b);
2. To confirm or refute the fetal malformation finding of gastroschisis observed in 1 fetus at 10,000 mg/m^3 (but not at 2000 or 20,000 mg/m^3) in the previous developmental toxicity study at EMBSI (2009b) on this test material in CD-1® mice;
3. To extend the test atmospheric concentration range from 0, 2000, 10,000, and 20,000 mg/m^3 on gd 5 through 16 employed previously (EMBSI, 2009b), to 0, 2000, 10,000, 20,000, and 30,000 mg/m^3 (the last concentration at 75% of the lower explosive limit), with daily exposures on gd 5 through 16 for the 0-20,000 mg/m^3 groups and on gd 5 through 10 for the 30,000 mg/m^3 group. This last gestation interval was included to encompass the time of embryonic ventral wall closure, the failure of which is likely responsible for both ectopia cordis and gastroschisis. There were 23 plug-positive females/group at 0-20,000 mg/m^3 and 38 plug-positive females at 30,000 mg/m^3 to improve the possibility of detection of these rare fetal malformations.

There were no apparent treatment-related effects on maternal body weights or weight gains and no consistent treatment- or concentration-related effects on maternal feed consumption. The treatment-related increases in absolute (at 10,000 mg/m^3) and relative (at 2000, 10,000, and 20,000 mg/m^3) maternal liver weights are most likely due to the induction of hepatic metabolizing enzymes, with the concomitant increase in liver weight (Conney, 1967). This is not considered maternal toxicity, per se, but an adaptive metabolic response to exposure to a xenobiotic. The absence of an increased liver weight at 30,000 mg/m^3 might reflect the 7-day interval between the end of treatment and necropsy of animals in this group. It seems likely, given the increases seen in the other test-substance-treated groups, that liver weights were initially increased in all treatment groups.

Terminal maternal adrenal gland weights were not changed across groups, although the current thinking is that there may be increased maternal production of corticosterone (shown to cause fetal cleft palate; Hemm et al., 1977; Pradat et al., 2003; Senda et al., 2005; Carmichael et al., 2007) in response to the stress of moving the animals in and out of chambers in all groups and in the high "dose" group also from the stress of the narcotic/lethargic effect of MTBE at this concentration (see Bevan et al., 1997a,b). It is possible (if not probable) that the adrenal glands of the dams exposed to 30,000 mg/m^3 on gd 5-10 were producing increased corticosterone (with temporary glandular enlargement) during the exposure period, with resolution of increased hormone output and glandular changes by scheduled termination on gd 17. Interestingly, lethargy was observed in the females at 30,000 mg/m^3 in the range-finding study but was not documented in this study during the daily exposures; it is likely the admittedly subjective effect was present in this study since it was present in the range-finding study at the same exposure concentration and duration. Clinical observations of the dams did indicate treatment-related findings, i.e., labored breathing only at 20,000 and 30,000 mg/m^3 postexposure on gd 9 and 10, respectively, in 1 female in each group, and lacrimation in 1 female on gd 6 at 20,000 mg/m^3 and in 3 females on gd 5 and 6 at 30,000 mg/m^3, early in the exposure period.

The CD-1® (Swiss) mice used in the previous study (EMBSI, 2009b) were from the Charles River, Portage, MI, facility; the CD-1® (Swiss) mice used in the current study were from the Charles River, Raleigh, NC, facility, because RTI International has a historical control database for developmental toxicity studies on this mouse strain from this source, and to preclude the possibility that the fetal findings from the EMBSI study were due to a different spontaneous rate of these two fetal malformations in the Portage colony (due to founder effects, genetic drift, etc.). Females like the pseudohermaphroditic adult female at 10,000 mg/m^3 (removed from study) have been observed at very low incidence in other studies with this mouse strain at RTI International from the Charles River, Raleigh, NC, facility.

1. This study did not confirm the presence of ectopia cordis in any mouse fetus at any exposure concentration out of a total 122 litters and 1396 fetuses. In the absence of a clear dose-response pattern to this finding in the EMBSI (2009b) study and the total absence of this finding in the present study, it is the Study Director's opinion that it is appropriate (and prudent) to conclude that this fetal finding is likely <u>not</u> related to maternal exposure to the test material.

2. This study did not confirm the presence of gastroschisis in fetuses at 2000, 10,000, or 20,000 mg/m^3; it was also not found at 2000 or 20,000 mg/m^3 at EMBSI (2009b). One fetus (out of 407) did exhibit gastroschisis at 30,000 mg/m^3 in the present study. This fetus (No. 6 female) was from Female No. 5810; her litter included 15 implants and 14 live fetuses. In her litter, fetus No. 5 female and fetus No. 12 male exhibited cleft palates, and fetus No. 6 female had cleft palate as well as gastroschisis. In this group at 30,000 mg/m^3, there were 7 fetuses in 4 litters with cleft palate (greater incidence relative to other 4 groups, but not statistically significantly different), with 3 of them in this index litter. The body weight of the single female fetus with gastroschisis and cleft palate was much lower (0.6057 g) than the body weights of the remaining fetuses in the litter: females 0.8034-0.9768 g; males 0.8406-0.8893 g (Table A-4 in Appendix II). Her body weight was also much lower than the mean female fetal body weight/litter for this group (1.0141±0.0239 [S.E.M.] g); i.e., she was classified as a "runt," with a body weight below 3 standard deviations (and 3 standard errors) from the litter mean by sex in this group (Table 6). Although there is a known relationship between fetal body weight and cleft palate (i.e., malformed fetuses tend to be lighter at term than normal fetuses; Ryan et al., 1991), there are not yet any data on the biological relationship between reduced fetal body weight and other malformations (i.e., is it cause and/or effect?). This 30,000 mg/m^3 group also contained 3 fully resorbed litters (out of 36 pregnant). At 20,000 mg/m^3, 2 litters were fully resorbed (out of 22 pregnant), and 1 fetus in 1 litter exhibited cleft palate (and no incidence of gastroschisis). There were no fully resorbed litters at 0 or 10,000 mg/m^3 and only 1 fully resorbed litter at 2000 mg/m^3 (Table 2). Cleft palate was present in 2 fetuses in 2 litters at 0 mg/m^3 and in 1 fetus in 1 litter each at 2000, 10,000, and 20,000 mg/m^3 (Table 8). The increase in fetal and litter incidence of cleft palate and the increase in fully resorbed litters at 30,000 mg/m^3 provide circumstantial evidence for maternal toxicity (i.e., stress from the exposure procedures, per se, and from a very high level of G/MTBE, resulting in additional stress and excessive corticosteroid production known to cause cleft palate; Hemm et al., 1977; Pradat et al., 2003; Senda et al., 2005; Carmichael et

al., 2007). Maternal toxicity (stress) is also known to cause full litter resorptions; it is ascribed to the dam, not the conceptuses, and exposure on gd 5-10 corresponded to periods of early implantation and palatal sensitivities. The presence of gastroschisis in 1 fetus at 30,000 mg/m^3 may also indicate a possible association with maternal stress and G/MTBE only at this very high atmospheric concentration.

In the present study, gastroschisis was observed in only 1 fetus, only at 30,000 mg/m^3 and only in the presence of profound developmental toxicity for that fetus (very low body weight and cleft palate). There was also general evidence of toxicity in this group (e.g., lethargy observed in dams in the range-finding study at 30,000 mg/m^3) and lower individual maternal body weight gain (1.9 g) for Dam No. 5810 with the fetus with gastroschisis, versus the 30,000 mg/m^3 mean (3.0 g) and the vehicle control group mean (3.3 g) for weight gain during gd 5-10, the exposure period. Historical control data from governmental studies with the Charles River CD-1® (Swiss) mouse at RTI (Table 9), with 288 litters and 3641 fetuses, indicates no fetuses with gastroschisis or ectopia cordis. There were 18 fetuses in 11 control litters (in 6 studies) with cleft palate (1 to 4 litters affected/affected study) and 2 fetuses in 2 control litters (1/litter) with exencephaly in 2 studies, 1 litter affected/affected study. The absence of gastroschisis in any of the 3,641 control CD-1 mouse fetuses in 288 control litters (Table 9) lends support to the conclusion that gastroschisis in this study may be treatment related, occurring in a compromised fetus at 30,000 mg/m^3 with likely concomitant maternal toxicity (see above and conclusion No. 4 below). No other historical control data on maternal and fetal findings in the Charles River CD-1® mouse could be found in the open literature.

Neither gastroschisis nor ectopia cordis was observed in CD-1® mouse fetuses from mothers exposed to 0, 1000, 4000, or 8000 ppm MTBE by whole-body inhalation (in the presence of maternal and embryofetal toxicity at 4000 and 8000 ppm MTBE; Bevan et al., 1997a), nor was either of these malformations observed in mice exposed to a vapor condensate of gasoline as part of this testing program (EMBSI, 2009a). It does not appear that exposure to either MTBE or gasoline vapor, at atmospheric concentrations $\leq$8000 ppm, causes ectopia cordis or gastroschisis in mice. Maternal ataxia, hypoactivity, prostration, labored breathing, and lacrimation were observed at 4000 and 8000 ppm MTBE, and the resultant stress was considered most likely responsible for (or at least exacerbated) the increased incidence of fetal cleft palate at

8000 ppm. Reduced fetal body weights and concomitant reduced fetal skeletal ossification were also observed at 4000 and 8000 ppm MTBE vapor, with 4 fully resorbed litters at 8000 ppm MTBE (Bevan et al., 1997a). Neither gastroschisis nor ectopia cordis was observed in CD® rat offspring in a 2-generation study of inhaled MTBE at 400, 3000, or 8000 ppm (Bevan et al., 1997b) or in rabbit fetuses from does exposed to 1000, 4000, or 8000 ppm MTBE in a developmental toxicity study (Bevan et al., 1997a). Ventral closure defects were also not observed in rat reproductive and developmental toxicity studies conducted with gasoline vapor condensate or G/MTBE (EMBSI, 2009a,b; Huntingdon Life Sciences, Inc., 2009a,b), nor with light ends of gasoline (Roberts et. al., 2001), nor in the mouse developmental toxicity study conducted with gasoline vapor condensate (EMBSI, 2009a).

The incidence of fetal cleft palate in the EMBSI study was only 1 fetus (in 1 litter), and only at 20,000 mg/m^3 (that study's highest concentration). In the present study, cleft palate was observed in all 5 groups, including the air control group (2 fetuses in 2 litters), at 2000-20,000 mg/m^3 (1 fetus in 1 litter in each group) and at 30,000 mg/m^3 (7 fetuses in 4 litters). The increased incidence of cleft palate observed at 30,000 mg/m^3, although not statistically significantly different from the control value, was considered to be biologically relevant. Cleft palate in fetal mice is inducible by increased corticosterone levels in the dam (and presumably transported to the fetuses; Carmichael et al., 2007; Senda et al., 2005; Pradat et al., 2003; Hemm et al., 1997). Maternal increased corticosterone levels may be attributed to increased maternal stress from inhalation exposures, per se (moving dams into and out of chambers, exposure to dynamic air flows, no feed or water during exposure periods, no solid flooring in exposure cages, etc.), and from test materials at anesthetic concentrations. In fact, maternal inhalation of MTBE has been shown to produce cleft palates in fetuses from CD-1 mouse dams which exhibited lethargy and apparent unconsciousness (Bevan et al., 1997a). Maternal lethargy during exposures was also observed by HLS staff during the daily exposure periods of G/MTBE at 30,000 mg/m^3 in the range-finding study at HLS (it was not noted by HLS staff during the daily exposure periods at any concentration in this definitive study). Therefore, the presence of fetal cleft palate in all groups (including the control group) was not unexpected, and the increased incidence at 30,000 mg/m^3 (from both inhalation procedures, per se, and the anesthetic qualities of the MTBE in the G/MTBE at this atmospheric concentration) was also anticipated. The increased cleft palate incidence at 30,000 mg/m^3 is interpreted as most likely secondary to

maternal stress effects. Maternal stress may have also played a role in the single case of gastroschisis in a vulnerable, compromised fetus developing in a dam exposed to anesthetic levels of MTBE. Since the maternal exposures to 30,000 mg/m^3 were from gd 5-10, it is highly likely that any indications of maternal stress or other toxicity (e.g., increased adrenal weights) would have resolved during the postexposure period (gd 10-17) and were therefore not present at scheduled necropsy on gd 17.

CONCLUSIONS

In conclusion, this study has demonstrated the following:

1. No confirmation of fetal ectopia cordis at any test atmospheric concentration employed;
2. No confirmation of fetal gastroschisis at 0-20,000 mg/m^3;
3. One fetus (out of 407 fetuses, 0.24%) in one litter (out of 33 litters with live fetuses, 3.03%) exhibited gastroschisis at 30,000 mg/m^3; this female fetus had very low body weight (designated a “runt”), also exhibited cleft palate, and was clearly compromised. The single incidence of gastroschisis and the increased incidence of cleft palate in this group may both be related to fetal toxicity, secondary to maternal stress, only at 30,000 mg/m^3.
4. Fetal cleft palate was present at a low incidence (1-2 fetuses/group) at 0-20,000 mg/m^3, with increased incidences (7 fetuses in 4 litters) at 30,000 mg/m^3, likely due to greater maternal stress from the anesthetic qualities of the test atmosphere at this concentration. See above for discussion on maternal stress causing elevated corticosteroids, which in turn cause offspring cleft palate. Since the dams at 30,000 mg/m^3 were exposed only on gd 5-10, the offspring palates would have been affected (Hemm et al., 1977; Pradat et al., 2003; Senda et al., 2005; Carmichael et al., 2007), but any elevated corticosteroid levels (and any effects on adrenal gland weights) would likely have resolved by gd 17 at the time of necropsy. Relatively minor maternal treatment-related clinical signs of distress were observed at 20,000 and 30,000 mg/m^3 in this study, with greater maternal clinical signs observed at 30,000 mg/m^3 in the range-finding study. The increased incidence of fetal cleft palate and the presence of gastroschisis in 1 fetus in this

group, in this study, may indicate effects on compromised fetuses in the presence of maternal stress (toxicity). The presence of gastroschisis at 30,000 mg/m^3 and its absence from all available CD-1 historical control databases lends some support to the possible conclusion that the presence of 1 fetus with gastroschisis may have been treatment related, secondary to maternal stress.

5. Therefore, in the Study Director's opinion, maternal exposure to the test chemical at 30,000 mg/m^3, an extremely high atmospheric concentration, in the presence of fetal and demonstrable maternal toxicity during the embryonic period of ventral body wall closure, may have resulted in a very low incidence of gastroschisis (1 out of 407 fetuses, 0.24%; 1 out of 33 litters with live fetuses, 3.03%) in vulnerable mouse fetuses in the highest concentration group. The one affected fetus also exhibited reduced body weight and cleft palate. With lower fetal and maternal toxicity at 20,000 mg/m^3 and below, there was no incidence of gastroschisis. The absence of any mouse fetus with gastroschisis in the performing laboratory's historical control database lends some support to the possible conclusion that the single case of gastroschisis at a very high atmosphere of G/MTBE (30,000 mg/m^3) in this study may have been treatment related. Since the one affected female fetus at 30,000 mg/m^3 was also very small (a "runt") and also exhibited cleft palate, her malformation findings may have been caused by (or exacerbated by) maternal exposure to G/MTBE. Alternatively, the fetal effects may have been caused by (or exacerbated by) maternal toxicity (stress) or may have been a spontaneous fetal malformation.

Under the conditions of this study, the NOAELs for maternal and developmental toxicity, based on the maternal effects observed at 20,000 and 30,000 mg/m^3 and the developmental effects observed at 30,000 mg/m^3, were determined by the Study Director to be 10,000 mg/m^3 for maternal toxicity and 20,000 mg/m^3 for developmental toxicity.

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LIST OF PROTOCOL DEVIATIONS

Study: Mi04-HLS2 Master Protocol No.: RTI-909 HLS Study Code: 04-4263

HLS

1. Due to technician error, food left weights were recorded for the Exposure Day 12-13 interval, but food fed weights for the Exposure Day 13-14 interval were not entered into the computer system for Animal Nos. 1805, 1806, 1807, 1808, 1809, 1810, 1811, 1812, 1814, 1815, 1816, 1818, 1819, 1820, 1823, 2804, 2806, 2808, 2814, 2815, 2817, 2818, 2819, 2821, 2822, and 2823. Since the feeders for these animals were returned to the cages after obtaining the food left weights and did not need to be changed, a series of edits was performed which provided a food fed weight equivalent to the food left weight.

2. Due to the unexpectedly extended mating period, only 130 mice were placed on test rather than 140. This resulted in the following group sizes: Group 1-4 had 23 mice and Group 5 had 38 mice.

3. The group means for MIRAN sampling were outside of the stated protocol range of ± 10% on Exposure Days 6, 9, 16 (Group 2), Exposure Days 16 and 19 (Group 3), and Exposure Day 2 and 16 (Group 4).

4. Individual MIRAN samples were outside the protocol specified range of ± 10% for the following Sample Nos.: 2001, 2002, 2020, 2021, 2022, 2032, 2034, 2035, 2036, 2037, 2044, 2052, 2063, 2064, 3061, 3062, 3063, 3064, 3065, 3066, 3077, 3078, 4001, 4004, 4005, 4406, 4016, 4024, 4063, 4064, 4070, 5001, 5005 and 5025. Chamber concentration values were confirmed as needed in accordance with Testing Facility SOP.

5. Due to technician oversight, a Nestlet® was given to Animal No. 5838 during the morning of Exposure Day 16 (removed after a total of 37 minutes) and then again in the afternoon. The protocol specifications were for afternoon only.

6. Due to technician oversight, animals in Groups 1-4 chambers and Group 5 chamber were not rotated on Exposure Days 11 and 13, respectively.

7. At RTI's request, with the Sponsor's approval, nonpregnant females were sacrificed and examined macroscopically in order to determine their actual state of pregnancy, although not required by protocol.

8. Due to a communication error with the Sponsor's Representative, Group 5 dams were removed from their cages with Nestlets® and inserts only from GD 14-16 and placed into cages without Nestlets® and inserts to simulate the Groups 1-4 exposure regimen. Per intent of Sponsor, the Group 5 dams should have been removed from their cages with Nestlets® and inserts from GD 10-16 and placed into cages without Nestlets® and inserts

to simulate the cage environment of the females in Groups 1-4 during the remaining exposure on gd 16.

9. Due to the HLS Principal Investigator's oversight, Protocol Amendment No. 2 was signed by one IACUC member instead of two, as designated by the protocol.

In the Study Director's professional opinion, these protocol deviations did not affect the study design, performance, or interpretation and are presented for completeness.

Table 1. Analysis of Test Atmospheres (page 1 of 1)

	Target Concentrations (mg/m^3)				
	0	2000	10,000	20,000	30,000
Mean analytical concentration ± SD (% of target)[a]	0 ± 0[b] (NA)	2074 ± 248 (103.7)	9925 ± 688 (99.25)	20,342 ± 1815 (101.7)	29,250 ± 1480 (97.5)
Particle Size Determination:[c]					
MMAD (μm)	2.179	5.699	9.319	3.845	1.144
GSD	1.676	2.117	2.071	1.955	2.910
TMC (mg/m^3)	2.56×10^{-2}	5.05×10^{-3}	3.41×10^{-3}	1.47×10^{-3}	1.54×10^{-2}
Mean temperature (°C ± SD)[d]	20.3 ± 0.9	20.8 ± 1.2	21.5 ± 0.9	20.7 ±0.9	20.7 ±0.8
Mean relative humidity (% ± SD)[d]	31.2 ± 4.9	32.0 ± 7.1	28.6 ± 4.2	28.4 ± 4.1	27.6 ± 4.7

[a] Mean of 4 assays/chamber/day (20 days for Group 1, 18 days for Group 2, 22 days for Group 3, 19 days for Group 4, and 12 days for Group 5) measured by infrared spectroscopy

[b] Estimated limit of quantification (LOQ) = 433 mg/m^3 (Appendix I)

[c] Measured 1 time/chamber

[d] Measured 13 times/chamber/day

SD = Standard deviation

MMAD = mass median aerodynamic diameter

GSD = geometric standard deviation

TMC = total mass concentration (measure of aerosol concentration)

Table 2. Summary and Statistical Analysis of the Maternal Body Weights and Body Weight Changes (page 1 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Subjects (No. Dams)					
No. on Study	23	23	23	23	38
No. Removed	0	0	1[a]	0	0
No. Dead or Euthanized	0	0	0	0	0
No. Nonpregnant	0	1	3	1	2
No. (%) Pregnant at Scheduled Sacrifice	23 (100.0)	22 (95.7)	19 (86.4)	22 (95.7)	36 (94.7)
No. (%) with 100% Resorptions	0 (0.0)	1 (4.5)	0 (0.0)	2 (9.1)	3 (8.3)
Maternal Body Weight (gd 0) (g)[b]					
	26.7 ± 0.4 N=23	27.2 ± 0.3 N=22	27.0 ± 0.3 N=19	27.3 ± 0.3 N=22	27.5 ± 0.2 N=36
Maternal Body Weight (gd 5) (g)[b]					
	27.6 ± 0.4 N=23	28.4 ± 0.4 N=22	27.8 ± 0.4 N=19	28.5 ± 0.3 N=22	28.3 ± 0.3 N=36
Maternal Body Weight (gd 6) (g)[b]					
	28.2 ± 0.4 N=23	29.1 ± 0.4 N=22	28.7 ± 0.4 N=19	29.0 ± 0.3 N=22	28.8 ± 0.3 N=36
Maternal Body Weight (gd 7) (g)[b]					
	28.7 ± 0.4 N=23	29.7 ± 0.4 N=22	29.1 ± 0.4 N=19	29.6 ± 0.3 N=22	29.3 ± 0.3 N=36
Maternal Body Weight (gd 8) (g)[b]					
	29.2 ± 0.4 N=23	30.2 ± 0.4 N=22	29.7 ± 0.4 N=19	30.0 ± 0.3 N=22	29.8 ± 0.3 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights and Body Weight Changes (page 2 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight (gd 9) (g)[b]					
	29.7 ± 0.4 N=23	30.6 ± 0.5 N=22	30.3 ± 0.5 N=19	30.6 ± 0.4 N=22	30.4 ± 0.4 N=36
Maternal Body Weight (gd 10) (g)[b]					
	30.9 ± 0.5 N=23	31.8 ± 0.6 N=22	31.6 ± 0.5 N=19	31.5 ± 0.4 N=22	31.3 ± 0.4 N=36
Maternal Body Weight (gd 11) (g)[b]					
	32.8 ± 0.5 N=23	33.9 ± 0.6 N=22	33.6 ± 0.6 N=19	33.2 ± 0.5 N=22	33.0 ± 0.5 N=36
Maternal Body Weight (gd 12) (g)[b]					
	34.9 ± 0.5 N=23	35.8 ± 0.8 N=22	35.5 ± 0.5 N=19	35.1 ± 0.6 N=22	35.3 ± 0.5 N=36
Maternal Body Weight (gd 13) (g)[b]					
	36.9 ± 0.5 N=23	37.2 ± 0.9 N=22	37.3 ± 0.6 N=19	36.7 ± 0.7 N=22	37.4 ± 0.6 N=36
Maternal Body Weight (gd 14) (g)[b]					
	39.2 ± 0.6 N=23	39.2 ± 1.0 N=22	39.4 ± 0.6 N=19	38.7 ± 0.9 N=22	39.7 ± 0.7 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights and Body Weight Changes (page 3 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight (gd 15) (g)[b]	42.0 ± 0.6 N=23	42.0 ± 1.1 N=22	42.2 ± 0.7 N=19	41.2 ± 1.0 N=22	42.4 ± 0.9 N=36
Maternal Body Weight (gd 16) (g)[b]	45.2 ± 0.7 N=23	45.1 ± 1.4 N=22	45.3 ± 0.7 N=19	44.0 ± 1.2 N=22	45.2 ± 1.1 N=36
Maternal Body Weight (gd 17) (g)[b]	48.4 ± 0.7 N=23	48.0 ± 1.5 N=22	48.3 ± 0.8 N=19	46.7 ± 1.4 N=22	48.3 ± 1.2 N=36
Maternal Body Weight (gd 17 at sacrifice) (g)[b]	47.14 ± 0.73 N=23	46.71 ± 1.50 N=22	47.52 ± 0.79 N=19	45.91 ± 1.43 N=22	47.44 ± 1.18 N=36
Maternal Body Weight Change (gd 0 to 5) (g)[b]	0.8 ± 0.2 N=23	1.2 ± 0.3 N=22	0.8 ± 0.3 N=19	1.2 ± 0.2 N=22	0.8 ± 0.2 N=36
Maternal Body Weight Change (gd 5 to 6) (g)[b]	0.6 ± 0.1 N=23	0.7 ± 0.1 N=22	0.9 ± 0.2 N=19	0.5 ± 0.1 N=22	0.5 ± 0.1 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights and Body Weight Changes (page 4 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight Change (gd 6 to 7) (g)[b]					
#	0.6 ± 0.1 N=23	0.6 ± 0.1 N=22	0.5 ± 0.1 N=19	0.6 ± 0.1 N=22	0.5 ± 0.1 N=36
Maternal Body Weight Change (gd 7 to 8) (g)[b]	0.5 ± 0.1 N=23	0.5 ± 0.1 N=22	0.6 ± 0.1 N=19	0.4 ± 0.1 N=22	0.5 ± 0.1 N=36
Maternal Body Weight Change (gd 8 to 9) (g)[b]	0.5 ± 0.1 N=23	0.4 ± 0.1 N=22	0.6 ± 0.1 N=19	0.5 ± 0.1 N=22	0.6 ± 0.1 N=36
Maternal Body Weight Change (gd 9 to 10) (g)[b]	1.2 ± 0.1 § N=23	1.2 ± 0.1 N=22	1.3 ± 0.2 N=19	0.9 ± 0.1 N=22	0.9 ± 0.1 N=36
Maternal Body Weight Change (gd 10 to 11) (g)[b]	1.9 ± 0.1 N=23	2.0 ± 0.2 N=22	2.0 ± 0.1 N=19	1.7 ± 0.2 N=22	1.7 ± 0.1 N=36
Maternal Body Weight Change (gd 11 to 12) (g)[b]	2.1 ± 0.1 § N=23	1.9 ± 0.2 N=22	1.8 ± 0.1 N=19	2.0 ± 0.2 N=22	2.3 ± 0.1 N=36

Table 2. Summary and Statistical Analysis of the Maternal Body Weights and Body Weight Changes (page 5 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight Change (gd 12 to 13) (g)[b]	2.1 ‡‡ ± 0.1 N=23	1.4 ** ± 0.2 N=22	1.9 ± 0.1 N=19	1.5 * ± 0.2 N=22	2.0 ± 0.1 N=36
Maternal Body Weight Change (gd 13 to 14) (g)[b]	2.3 ± 0.1 N=23	2.0 ± 0.2 N=22	2.1 ± 0.1 N=19	2.0 ± 0.2 N=22	2.3 ± 0.2 N=36
Maternal Body Weight Change (gd 14 to 15) (g)[b]	2.8 ± 0.1 N=23	2.8 ± 0.2 N=22	2.8 ± 0.1 N=19	2.5 ± 0.2 N=22	2.7 ± 0.3 N=36
Maternal Body Weight Change (gd 15 to 16) (g)[b]	3.2 ± 0.2 N=23	3.1 ± 0.2 N=22	3.0 ± 0.1 N=19	2.8 ± 0.3 N=22	2.9 ± 0.2 N=36
Maternal Body Weight Change (gd 16 to 17) (g)[b]	3.2 ± 0.2 N=23	2.9 ± 0.2 N=22	3.1 ± 0.1 N=19	2.7 ± 0.2 N=22	3.1 ± 0.1 N=36
Maternal Body Weight Change (gd 5 to 10) (g)[b,c]	3.3 ± 0.2 N=23				3.0 ± 0.2 N=36
Maternal Body Weight Change (gd 5 to 16) (g)[b,d]	17.6 ± 0.4 N=23	16.7 ± 1.2 N=22	17.5 ± 0.5 N=19	15.5 ± 1.2 N=22	

Table 2. Summary and Statistical Analysis of the Maternal Body Weights and Body Weight Changes (page 6 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Body Weight Change (gd 10 to 17) (g)[b,c]	17.5 ± 0.4 N=23				17.0 ± 0.9 N=36
Maternal Body Weight Change (gestation) (g)[b]	20.4 ± 0.6 N=23	19.6 ± 1.3 N=22	20.5 ± 0.7 N=19	18.6 ± 1.5 N=22	20.0 ± 1.1 N=36
Maternal Body Weight Change (corrected) (g)[b,e]	3.14 ± 0.25 N=23	3.98 ± 0.44 N=22	3.95 ± 0.51 N=19	2.88 ± 0.38 N=22	3.20 ± 0.32 N=36

[a]Female 3814 was removed from study due to a pre-existing condition. At necropsy she was found to have an undescended testis on the right and seminal vesicles and prostate to the right of the vagina and cervix.

[b]Includes all pregnant dams until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd=gestational day.

[c]This endpoint was only calculated for the 0 and 30,000 mg/m^3 dose groups.

[d]This endpoint was only calculated for the 0, 2000, 10,000, and 20,000 mg/m^3 dose groups.

[e]Weight change during gestation (gestational day 17 sacrifice weight minus gestational day 0 weight) minus gravid uterine weight.

[#]Levene's Test for homogeneity of variances was significant ($p<0.05$); therefore, robust regression methods were used to test all treatment effects.

[‡‡]$p<0.01$; ANOVA Test.

[§]$p<0.05$; Test for Linear Trend.

[*]$p<0.05$; Dunnett's Test.

[**]$p<0.01$; Dunnett's Test.

Table 3. Summary of the Maternal Clinical Observations (page 1 of 2)

A. Clinical Observations Summarized by Group

Observation	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Alopecia: extremities/snout, moderate				1	
Eye: enophthalmos[a], unilateral, left					1
Labored breathing				1	1
Lacrimation, bilateral, moderate					1
Lacrimation, unilateral, moderate				1	2
Red exudates from anogenital area			1	1	

B. Clinical Observations Summarized by Group, Day, and Time

Day[b]	Time[c]	Observation	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
			Dosed gd 5-16				Dosed gd 5-10
			0	2000	10,000	20,000	30,000
2	Prior	Eye: enophthalmos, unilateral, left					1
3	Prior	Eye: enophthalmos, unilateral, left					1
4	Prior	Eye: enophthalmos, unilateral, left					1
5	Prior	Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Lacrimation, unilateral, moderate					1
6	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Lacrimation, bilateral, moderate					1
		Lacrimation, unilateral, moderate				1	1
7	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1

Table 3. Summary of the Maternal Clinical Observations (page 2 of 2)

B. Clinical Observations Summarized by Group, Day, and Time

			Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
			Dosed gd 5-16				Dosed gd 5-10
Day[b]	Time[c]	Observation	0	2000	10,000	20,000	30,000
8	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
9	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Labored breathing				1	
10	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Labored breathing					1
11	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
	Post	Alopecia: extremities/snout, moderate				1	
		Red exudates from anogenital area				1	
12	Prior	Alopecia: extremities/snout, moderate				1	
		Eye: enophthalmos, unilateral, left					1
		Red exudates from anogenital area			1		
	Post	Alopecia: extremities/snout, moderate				1	
13	Prior	Eye: enophthalmos, unilateral, left					1
14	Prior	Eye: enophthalmos, unilateral, left					1
15	Prior	Eye: enophthalmos, unilateral, left					1
16	Prior	Eye: enophthalmos, unilateral, left					1
17	Prior	Eye: enophthalmos, unilateral, left					1

[a]A sinking of the eyeball into the orbital cavity.

[b]Gestational day.

[c]Time is prior to/post (after) exposures.

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 1 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Dams	23	22	19	22	36
Maternal Feed Consumption (gd 0 to 5) (g/day)[a]					
#	6.1 ††† ± 0.2 N=17[b]	6.5 ± 0.4 N=18[b,c]	6.2 ± 0.4 N=11[b]	5.3 ÞÞ ± 0.2 N=16[b,c]	6.8 ± 0.4 N=25[b,c]
Maternal Feed Consumption (gd 5 to 6) (g/day)[a]					
#	6.1 † ± 0.3 Ÿ N=20[b,d]	6.7 ± 0.3 N=21[b]	7.7 Þ ± 0.7 N=16[b]	6.2 ± 0.2 N=21[d]	5.9 ± 0.1 N=32[b,d]
Maternal Feed Consumption (gd 6 to 7) (g/day)[a]					
#	6.1 †† ± 0.2 N=18[b,d]	7.8 Þ ± 0.7 N=19[b,c,d]	7.7 ÞÞÞ ± 0.4 N=17[b]	6.2 ± 0.3 N=17[b,d]	6.3 ± 0.2 N=32[b,c]
Maternal Feed Consumption (gd 7 to 8) (g/day)[a]					
#	7.0 ††† ± 0.4 ŸŸŸ N=21[d]	7.4 ± 0.3 N=21[d]	8.2 ± 0.7 N=19	6.2 Þ ± 0.2 N=18[b,d]	6.4 ± 0.2 N=34[c,d]
Maternal Feed Consumption (gd 8 to 9) (g/day)[a]					
#	7.6 ††† ± 0.5 ŸŸŸ N=21[b]	6.8 ± 0.3 N=19[c,d]	7.5 ± 0.4 N=16[b,c]	6.1 ÞÞ ± 0.2 N=20[b,d]	6.2 ÞÞ ± 0.1 N=33[b,c]
Maternal Feed Consumption (gd 9 to 10) (g/day)[a]					
	6.7 ‡‡‡ ± 0.2 §§ N=23	7.0 ± 0.3 N=21[b]	7.3 ± 0.2 N=18[b]	6.1 ± 0.2 N=20[b,c]	6.3 ± 0.1 N=35[b]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 2 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Feed Consumption (gd 10 to 11) (g/day)[a]					
#	6.9 ††† ± 0.2 ŸŸŸ N=19[b]	7.6 ± 0.3 N=22	7.5 ± 0.2 N=18[b]	6.3 ± 0.2 N=20[d]	6.2 ÞÞ ± 0.1 N=33[c,d]
Maternal Feed Consumption (gd 11 to 12) (g/day)[a]					
	7.7 ‡ ± 0.4 N=23	7.1 ± 0.2 N=21[b]	7.7 ± 0.3 N=18[b]	6.7 ± 0.2 N=20[b,d]	7.8 ± 0.3 N=33[b,c,d]
Maternal Feed Consumption (gd 12 to 13) (g/day)[a]					
	7.1 ‡‡ ± 0.2 N=23	7.3 ± 0.3 N=21[c]	8.0 * ± 0.3 N=19	6.9 ± 0.2 N=22	7.8 ± 0.2 N=34[c,d]
Maternal Feed Consumption (gd 13 to 14) (g/day)[a]					
	7.3 ‡‡ ± 0.1 § N=23	7.5 ± 0.3 N=20[b,c]	7.9 ± 0.2 N=18[b]	7.1 ± 0.3 N=21[b]	8.0 * ± 0.2 N=36
Maternal Feed Consumption (gd 14 to 15) (g/day)[a]					
	7.4 ± 0.1 N=23	7.4 ± 0.3 N=21[b]	7.8 ± 0.2 N=19	7.1 ± 0.3 N=22	7.6 ± 0.2 N=36
Maternal Feed Consumption (gd 15 to 16) (g/day)[a]					
	7.5 ‡ ± 0.2 § N=23	7.8 ± 0.3 N=22	7.9 ± 0.3 N=19	6.9 ± 0.2 N=22	7.2 ± 0.2 N=35[c]
Maternal Feed Consumption (gd 16 to 17) (g/day)[a]					
	7.6 ± 0.2 N=23	7.4 ± 0.3 N=22	7.8 ± 0.2 N=19	7.3 ± 0.2 N=22	7.5 ± 0.2 N=35[c]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 3 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Maternal Feed Consumption (gd 5 to 10) (g/day)[a,e]					
#	6.9 ± 0.4 N=20[f]				6.2 ± 0.1 N=28[f]
Maternal Feed Consumption (gd 5 to 16) (g/day)[a,g]					
	6.9 ± 0.2 N=17[f]	7.0 ± 0.3 N=14[f]	7.7 ± 0.3 N=12[f]	6.8 ± 0.3 N=17[f]	
Maternal Feed Consumption (gd 10 to 17) (g/day)[a,e]					
	7.3 ± 0.2 N=19[f]				7.6 ± 0.2 N=31[f]
Maternal Feed Consumption (gd 0 to 17) (g/day)[a]					
	6.5 ‡ ± 0.1 N=14[f]	6.8 ± 0.3 N=13[f]	7.0 ± 0.3 N=8[f]	6.1 ± 0.2 N=14[f]	6.9 ± 0.2 N=20[f]
Relative Maternal Feed Consumption (gd 0 to 5) (g/kg/day)[a]					
#	224.8 ††† ± 10.3 N=17[b]	230.4 ± 11.3 N=18[b,c]	223.1 ± 13.5 N=11[b]	189.4 ÞÞ ± 5.5 N=16[b,c]	241.5 ± 14.3 N=25[b,c]
Relative Maternal Feed Consumption (gd 5 to 6) (g/kg/day)[a]					
#	216.1 † ± 8.3 Ÿ N=20[b,d]	233.1 ± 9.7 N=21[b]	270.7 Þ ± 24.6 N=16[b]	217.3 ± 8.9 N=21[d]	207.5 ± 4.6 N=32[b,d]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 4 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Relative Maternal Feed Consumption (gd 6 to 7) (g/kg/day)[a]					
#	214.6 †† ± 7.6 Ÿ N=18[b,d]	266.0 Þ ± 22.5 N=19[b,c,d]	264.7 ÞÞ ± 14.5 N=17[b]	213.3 ± 8.4 N=17[b,d]	218.1 ± 7.2 N=32[b,c]
Relative Maternal Feed Consumption (gd 7 to 8) (g/kg/day)[a]					
#	242.4 ††† ± 14.8 ŸŸŸ N=21[d]	247.6 ± 10.4 N=21[d]	275.4 ± 21.8 N=19	208.1 Þ ± 5.9 N=18[b,d]	216.5 ± 5.3 N=34[c,d]
Relative Maternal Feed Consumption (gd 8 to 9) (g/kg/day)[a]					
#	255.3 ††† ± 17.1 ŸŸŸ N=21[b]	224.2 ± 10.3 N=19[c,d]	249.3 ± 14.0 N=16[b,c]	202.6 ÞÞ ± 5.9 N=20[b,d]	204.2 ÞÞ ± 4.0 N=33[b,c]
Relative Maternal Feed Consumption (gd 9 to 10) (g/kg/day)[a]					
	222.9 ‡‡‡ ± 8.4 §§ N=23	225.2 ± 8.7 N=21[b]	234.9 ± 7.6 N=18[b]	198.8 * ± 4.7 N=20[b,c]	204.8 ± 3.4 N=35[b]
Relative Maternal Feed Consumption (gd 10 to 11) (g/kg/day)[a]					
	218.8 ‡‡‡ ± 10.0 §§§ N=19[b]	229.9 ± 8.3 N=22	230.3 ± 6.9 N=18[b]	197.4 ± 6.4 N=20[d]	193.4 * ± 2.8 N=33[c,d]
Relative Maternal Feed Consumption (gd 11 to 12) (g/kg/day)[a]					
	228.1 ‡ ± 11.6 N=23	203.7 ± 5.1 N=21[b]	222.9 ± 9.0 N=18[b]	197.0 * ± 4.6 N=20[b,d]	230.6 ± 8.0 N=33[b,c,d]
Relative Maternal Feed Consumption (gd 12 to 13) (g/kg/day)[a]					
	198.3 ‡‡ ± 5.9 N=23	198.8 ± 6.0 N=21[c]	221.2 ± 7.5 N=19	192.0 ± 5.9 N=22	213.4 ± 5.6 N=34[c,d]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 5 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Relative Maternal Feed Consumption (gd 13 to 14) (g/kg/day)[a]	191.7 ‡ ± 4.2 § N=23	195.8 ± 4.9 N=20[b,c]	205.9 ± 4.9 N=18[b]	189.8 ± 6.6 N=21[b]	209.4 * ± 4.1 N=36
Relative Maternal Feed Consumption (gd 14 to 15) (g/kg/day)[a]	182.2 ± 4.1 N=23	182.8 ± 4.9 N=21[b]	190.7 ± 5.8 N=19	177.8 ± 4.6 N=22	187.3 ± 5.0 N=36
Relative Maternal Feed Consumption (gd 15 to 16) (g/kg/day)[a]	171.5 ‡‡ ± 3.4 §§ N=23	179.0 ± 4.5 N=22	181.5 ± 6.1 N=19	162.0 ± 3.5 N=22	163.6 ± 2.6 N=35[c]
Relative Maternal Feed Consumption (gd 16 to 17) (g/kg/day)[a]	162.9 ± 4.7 N=23	160.3 ± 3.4 N=22	166.1 ± 3.4 N=19	161.2 ± 4.6 N=22	159.9 ± 2.4 N=35[c]
Relative Maternal Feed Consumption (gd 5 to 10) (g/kg/day)[a,e] #	235.6 † ± 12.1 Ÿ N=20[f]				209.5 Þ ± 3.7 N=28[f]
Relative Maternal Feed Consumption (gd 5 to 16) (g/kg/day)[a,g]	202.6 ± 5.7 N=17[f]	206.9 ± 5.3 N=14[f]	222.0 ± 6.7 N=12[f]	199.3 ± 7.0 N=17[f]	
Relative Maternal Feed Consumption (gd 10 to 17) (g/kg/day)[a,e]	189.6 ± 4.8 N=19[f]				194.5 ± 3.4 N=31[f]

Table 4. Summary and Statistical Analysis of the Maternal Feed Consumption (page 6 of 6)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Relative Maternal Feed Consumption (gd 0 to 17) (g/kg/day)[a]	187.1 ± 4.9 N=14[f]	197.0 ± 5.8 N=13[f]	198.2 ± 7.0 N=8[f]	178.6 ± 3.8 N=14[f]	194.3 ± 5.1 N=20[f]

[a]Includes all pregnant dams until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd = gestational day.

[b]Decrease in N is due to one or more feeders spilling, and therefore the feed weight was excluded.

[c]Decrease in N is due to the feed being contaminated for one or more animals, and therefore the feed weight was excluded.

[d]Decrease in N is due to the feed consumption value for one or more animals being a statistical outlier, and therefore they were excluded.

[e]This endpoint was only calculated for the 0 and 30,000 mg/m^3 dose groups.

[f]Decrease in N is due to interim feed consumption value(s) for one or more dams being missing, and therefore the overall feed consumption value could not be calculated.

[g]This endpoint was only calculated for the 0, 2000, 10,000, and 20,000 mg/m^3 dose groups.

[#]Levene's Test for homogeneity of variances was significant ($p<0.05$); therefore, robust regression methods were used to test all treatment effects.

[†]$p<0.05$; Wald Chi-square Test for overall treatment effect in robust regression model.

[††]$p<0.01$; Wald Chi-square Test for overall treatment effect in robust regression model.

[†††]$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.

[Ÿ]$p<0.05$; Linear trend test in robust regression model.

[ŸŸŸ]$p<0.001$; Linear trend test in robust regression model.

[Þ]$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model.

[ÞÞ]$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.

[ÞÞÞ]$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.

[‡]$p<0.05$; ANOVA Test.

[‡‡]$p<0.01$; ANOVA Test.

[‡‡‡]$p<0.001$; ANOVA Test.

[§]$p<0.05$; Test for Linear Trend.

[§§]$p<0.01$; Test for Linear Trend.

[§§§]$p<0.001$; Test for Linear Trend.

[*]$p<0.05$; Dunnett's Test.

Table 5. Summary and Statistical Analysis of the Maternal Absolute and Relative Organ Weights (page 1 of 2)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
Absolute Gravid Uterine Weight (g)[a]	17.2900 ± 0.4737 N=23	15.5703 ± 1.1217 N=22	16.5732 ± 0.3737 N=19	15.7215 ± 1.2039 N=22	16.7807 ± 0.9127 N=36
Absolute Maternal Liver Weight (g)[a]	2.4511 ‡ ±0.0498 N=23	2.5766 ±0.0889 N=22	2.7247 * ±0.0538 N=19	2.6308 ±0.0715 N=22	2.4253 ±0.0604 N=36
Absolute Maternal Paired Adrenal Gland Weight (g)[a]	0.0136 ±0.0006 N=22[b]	0.0144 ±0.0007 N=22	0.0132 ±0.0004 N=18[b]	0.0137 ±0.0005 N=22	0.0135 ±0.0003 N=36
Absolute Maternal Paired Kidney Weight (g)[a]	0.4277 ±0.0089 N=23	0.4454 ±0.0107 N=22	0.4394 ±0.0054 N=19	0.4376 ±0.0089 N=22	0.4311 ±0.0073 N=36
Relative Maternal Liver Weight (% sacrifice weight)[a] #	5.1961 ††† ± 0.0569 N=23	5.5269 ÞÞ ± 0.0865 N=22	5.7418 ÞÞÞ ± 0.0927 N=19	5.7610 ÞÞÞ ± 0.0754 N=22	5.1550 ± 0.0939 N=36
Relative Maternal Paired Adrenal Gland Weight (% sacrifice weight)[a]	0.0290 ± 0.0014 N=22[b]	0.0314 ± 0.0016 N=22	0.0279 ± 0.0009 N=18[b]	0.0306 ± 0.0017 N=22	0.0297 ± 0.0015 N=36
Relative Maternal Paired Kidney Weight (% sacrifice weight)[a]	0.9067 ± 0.0109 N=23	0.9783 ± 0.0451 N=22	0.9281 ± 0.0156 N=19	0.9817 ± 0.0496 N=22	0.9345 ± 0.0337 N=36

Table 5. Summary and Statistical Analysis of the Maternal Absolute and Relative Organ Weights (page 2 of 2)

[a]Includes all pregnant dams until terminal sacrifice on gestational day 17. Reported as the mean ± S.E.M.; gd=gestational day.

[b]Decrease in N is due to the paired adrenal weight for one animal being a statistical outlier and therefore it was excluded.

[#]Levene's Test for homogeneity of variances was significant ($p<0.05$); therefore, robust regression methods were used to test all treatment effects.

[‡]$p<0.05$; ANOVA Test.

[*]$p<0.05$; Dunnett's Test.

[†††]$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.

[ÞÞ]$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.

[ÞÞÞ]$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.

Table 6. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 1 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
ALL LITTERS[a]:	23	22	19	22	36
No. Corpora Lutea per Dam[b]	12.96 ± 0.38 N=23	12.18 ± 0.57 N=22	12.78 ± 0.36 N=18[c]	13.18 ± 0.56 N=22	13.19 ± 0.47 N=36
No. Implantation Sites per Litter[b]	12.74 ± 0.35 N=23	12.00 ± 0.61 N=22	12.68 ± 0.33 N=19	13.23 ± 0.46 N=22	12.89 ± 0.37 N=36
%Preimplantation Loss per Litter[b]	2.06 ± 0.93 N=23	5.99 ± 3.74 N=22	3.93 ± 1.52 N=18[c]	2.61 ± 0.99 N=22	4.81 ± 1.24 N=36
No. Resorptions per Litter[b]	0.61 ± 0.16 N=23	1.27 ± 0.52 N=22	0.74 ± 0.18 N=19	1.68 ± 0.86 N=22	1.56 ± 0.48 N=36
% Resorptions per Litter[b]	4.88 ± 1.31 N=23	11.03 ± 4.90 N=22	5.57 ± 1.40 N=19	12.91 ± 6.21 N=22	13.75 ± 4.52 N=36
No. Litters with Resorptions	11	9	10	10	20
% Litters with Resorptions	47.83	40.91	52.63	45.45	55.56

Table 6. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 2 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Late Fetal Deaths per Litter[b]	0.13 ± 0.07 N=23	0.00 ± 0.00 N=22	0.11 ± 0.07 N=19	0.09 ± 0.06 N=22	0.03 ± 0.03 N=36
%Late Fetal Deaths per Litter[b]	1.02 ± 0.56 N=23	0.00 ± 0.00 N=22	0.70 ± 0.49 N=19	0.65 ± 0.45 N=22	0.20 ± 0.20 N=36
No. Litters with Late Fetal Deaths	3	0	2	2	1
% Litters with Late Fetal Deaths	13.04	0.00	10.53	9.09	2.78
No. Nonlive Implants per Litter[b,d]	0.74 ± 0.19 N=23	1.27 ± 0.52 N=22	0.84 ± 0.22 N=19	1.77 ± 0.86 N=22	1.58 ± 0.48 N=36
% Nonlive Implants per Litter[b,d]	5.90 ± 1.52 N=23	11.03 ± 4.90 N=22	6.28 ± 1.59 N=19	13.56 ± 6.16 N=22	13.95 ± 4.52 N=36
No. Litters with Nonlive Implants[d]	11	9	10	12	20
% Litters with Nonlive Implants[d]	47.83	40.91	52.63	54.55	55.56
No. Litters with 100% Nonlive Implants[d]	0	1	0	2	3
% Litters with 100% Nonlive Implants[d]	0.00	4.55	0.00	9.09	8.33

Table 6. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 3 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Adversely Affected Implants per Litter[b,e]	0.83 ± 0.20 N=23	1.36 ± 0.52 N=22	0.89 ± 0.23 N=19	1.82 ± 0.85 N=22	1.78 ± 0.48 N=36
% Adversely Affected Implants per Litter[b,e]	6.69 ± 1.64 N=23	11.66 ± 4.85 N=22	6.63 ± 1.63 N=19	13.87 ± 6.15 N=22	15.38 ± 4.50 N=36
No. Litters with Adversely Affected Implants[e]	12	11	10	12	21
% Litters with Adversely Affected Implants[e]	52.17	50.00	52.63	54.55	58.33
LIVE LITTERS[f]:	23	21	19	20	33
No. Live Fetuses per Litter[b]	12.00 ± 0.39 N=23	11.24 ± 0.68 N=21	11.84 ± 0.27 N=19	12.60 ± 0.49 N=20	12.33 ± 0.26 N=33
% Male Fetuses per Litter[b]	50.03 ± 3.11 N=23	46.46 ± 3.82 N=21	52.95 ± 3.70 N=19	44.67 ± 3.14 N=20	45.58 ± 2.99 N=33
No. **Male** Fetuses per Litter[b]	6.09 ± 0.47 N=23	5.38 ± 0.45 N=21	6.21 ± 0.41 N=19	5.65 ± 0.48 N=20	5.61 ± 0.36 N=33

Table 6. Summary and Statistical Analysis of Ovarian Corpora Lutea, Uterine Contents, Live Fetal Sex and Live Fetal Body Weight (page 4 of 4)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. **Female** Fetuses per Litter[b]	5.91 ± 0.37 N=23	5.86 ± 0.52 N=21	5.63 ± 0.48 N=19	6.95 ± 0.47 N=20	6.73 ± 0.39 N=33
Average Fetal Body Weight (g) per Litter[b]	0.9994 ±0.0265 N=23	1.0114 ±0.0212 N=21	0.9471 ±0.0241 N=19	0.9492 ±0.0288 N=20	1.0248 ±0.0249 N=33
Average **Male** Fetal Body Weight (g) per Litter[b]	1.0114 ±0.0309 N=23	1.0088 ±0.0166 N=20[g]	0.9605 ±0.0254 N=19	0.9722 ±0.0311 N=20	1.0367 ±0.0261 N=33
Average **Female** Fetal Body Weight (g) per Litter[b]	0.9961 ±0.0246 N=23	0.9937 ±0.0227 N=21	0.9344 ±0.0231 N=19	0.9343 ±0.0281 N=20	1.0141 ±0.0239 N=33

[a]Includes all dams pregnant at terminal sacrifice on gestational day 17; litter size = no. implantation sites per dam.

[b]Reported as the mean ± S.E.M.

[c]Decrease in N is due to the right ovary for one female inadvertently being lost prior to the corpora lutea being counted.

[d]Nonlive = late fetal deaths plus resorptions.

[e]Adversely affected = nonlive plus malformed.

[f]Includes only dams with live fetuses; litter size = no. live fetuses per dam.

[g]Decrease in N is due to one litter having female fetuses only.

Table 7. Summary and Statistical Analysis of Fetal External Malformations and Variations (page 1 of 3)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Fetuses Examined[a]	276	236	225	252	407
No. Litters Examined[b]	23	21	19	20	33
No. Fetuses with External Malformations per Litter[c,d]	0.09 ± 0.06 N=23	0.10 ± 0.07 N=21	0.05 ± 0.05 N=19	0.05 ± 0.05 N=20	0.21 ± 0.11 N=33
No. **Male** Fetuses with External Malformations per Litter[c,d]	0.04 ± 0.04 N=23	0.05 ± 0.05 N=20	0.05 ± 0.05 N=19	0.00 ± 0.00 N=20	0.09 ± 0.05 N=33
No. **Female** Fetuses with External Malformations per Litter[c,d]	0.04 ± 0.04 N=23	0.05 ± 0.05 N=21	0.00 ± 0.00 N=19	0.05 ± 0.05 N=20	0.12 ± 0.07 N=33
% Fetuses with External Malformations per Litter[c,d]	0.85 ± 0.61 N=23	0.66 ± 0.46 N=21	0.38 ± 0.38 N=19	0.36 ± 0.36 N=20	1.64 ± 0.85 N=33
% **Male** Fetuses with External Malformations per Litter[c,d]	0.62 ± 0.62 N=23	0.83 ± 0.83 N=20	0.88 ± 0.88 N=19	0.00 ± 0.00 N=20	1.89 ± 1.16 N=33
% **Female** Fetuses with External Malformations per Litter[c,d]	0.72 ± 0.72 N=23	0.95 ± 0.95 N=21	0.00 ± 0.00 N=19	1.25 ± 1.25 N=20	1.29 ± 0.74 N=33

Table 7. Summary and Statistical Analysis of Fetal External Malformations and Variations (page 2 of 3)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
No. Fetuses with External Malformations[d]	2	2	1	1	7
% Fetuses with External Malformations[d]	0.72	0.85	0.44	0.40	1.72
No. Litters with External Malformations[e]	2	2	1	1	4
% Litters with External Malformations[e]	8.70	9.52	5.26	5.00	12.12
No. Fetuses with External Variations per Litter[c,d]	0.13 ± 0.07 N=23	0.14 ± 0.10 N=21	0.11 ± 0.07 N=19	0.15 ± 0.08 N=20	0.00 ± 0.00 N=33
No. **Male** Fetuses with External Variations per Litter[c,d]	0.09 ± 0.06 N=23	0.05 ± 0.05 N=20	0.05 ± 0.05 N=19	0.05 ± 0.05 N=20	0.00 ± 0.00 N=33
No. **Female** Fetuses with External Variations per Litter[c,d]	0.04 ± 0.04 N=23	0.10 ± 0.10 N=21	0.05 ± 0.05 N=19	0.10 ± 0.07 N=20	0.00 ± 0.00 N=33
% Fetuses with External Variations per Litter[c,d]	1.34 ± 0.76 N=23	1.27 ± 0.99 N=21	0.81 ± 0.56 N=19	1.26 ± 0.69 N=20	0.00 ± 0.00 N=33
% **Male** Fetuses with External Variations per Litter[c,d]	1.45 ± 1.00 N=23	0.56 ± 0.56 N=20	0.66 ± 0.66 N=19	1.00 ± 1.00 N=20	0.00 ± 0.00 N=33

Table 7. Summary and Statistical Analysis of Fetal External Malformations and Variations (page 3 of 3)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
% **Female** Fetuses with External Variations per Litter[c,d]	0.72 ± 0.72 N=23	1.59 ± 1.59 N=21	1.05 ± 1.05 N=19	1.39 ± 0.98 N=20	0.00 ± 0.00 N=33
No. Fetuses with External Variations[d]	3	3	2	3	0
% Fetuses with External Variations[d]	1.09	1.27	0.89	1.19	0.00
No. Litters with External Variations[e]	3	2	2	3	0
% Litters with External Variations[e]	13.04	9.52	10.53	15.00	0.00

[a]Only live fetuses were examined for malformations and variations.

[b]Includes only litters with live fetuses.

[c]Reported as the mean ± S.E.M.

[d]Fetuses with one or more malformations or variations.

[e]Litters with one or more fetuses with malformations or variations.

Table 8. Summary of Morphological Abnormalities in CD-1 Mouse Fetuses: Listing by Defect Type[a]
(page 1 of 1)

	Gasoline MTBE Vapor Condensate (mg/m^3, inhaled)				
	Dosed gd 5-16				Dosed gd 5-10
	0	2000	10,000	20,000	30,000
EXTERNAL MALFORMATIONS					
Total No. of Fetuses Examined for External Malformations[b]	276	236	225	252	407
No. of Fetuses with External Malformations[c]	2	2	1	1	7
% Fetuses with External Malformations	0.7%	0.8%	0.4%	0.4%	1.7%
Total No. of Litters Examined for External Malformations[d]	23	21	19	20	33
No. of Litters with External Malformations[e]	2	2	1	1	4
% Litters with External Malformations	8.7%	9.5%	5.3%	5.0%	12.1%
Encephalocele		1(1)			
Cleft Palate	2(2)	1(1)	1(1)	1(1)	7(4)
Gastroschisis					1(1)
EXTERNAL VARIATIONS					
Total No. of Fetuses Examined for External Variations[b]	276	236	225	252	407
No. of Fetuses with External Variations[c]	3	3	2	3	0
% Fetuses with External Variations	1.1%	1.3%	0.9%	1.2%	0.0%
Total No. of Litters Examined for External Variations[d]	23	21	19	20	33
No. of Litters with External Variations[e]	3	2	2	3	0
% Litters with External Variations	13.0%	9.5%	10.5%	15.0%	0.0%
Abnormal Rugae in Midline of Palate			1(1)	1(1)	
Hematoma: Face		2(1)			
Hematoma: Head	2(2)	1(1)			
Hematoma: Neck	1(1)	1(1)	1(1)	2(2)	
Hematoma: Shoulder	1(1)				

[a]A single fetus may be represented more than once in listing individual defects. Data are presented as the number of fetuses (number of litters).

[b]Only live fetuses were included.

[c]Fetuses with one or more malformations/variations.

[d]Includes only litters with live fetuses.

[e]Litters with one or more malformed/variant fetuses.

Table 9. External Malformations in Control CD-1® Mouse Litters From Studies Performed for Governmental Clients at RTI From 1997 to 2002 (page 1 of 1)

Study Code	Year	Study Type[a]	Control Group		With Gastroschisis[b]		Incidence of External Malformations No. Fetuses (No. Litters)
			No. Dams	No. Fetuses	No. Fetuses	No. Litters	
A	2002	RF	10	138	0	0	1 (1) cleft palate
B	2002	RF	9	123	0	0	1 (1) exencephaly
C	1999	RF	15	184	0	0	0
D	1999	RF	15	200	0	0	0
E	2000	RF	14	172	0	0	0
F	1999	RF	15	187	0	0	0
G	2000	RF	12	165	0	0	0
H	1999	D	24	302	0	0	0
I	2000	D	24	326	0	0	2 (1) cleft palate
J	1999	D	23	291	0	0	0
K	1997	D	22	271	0	0	1 (1) cleft palate
L	2000	D	23	283	0	0	1 (1) exencephaly
M	2001	D	24	270	0	0	1 (1) cleft palate
N	2002	D	20	254	0	0	6 (4) cleft palate
O	2002	D	22	279	0	0	7 (3) cleft palate
P	2000	D	16	196	0	0	0
TOTAL			288	3641	0	0	18 (11) cleft palate 2 (2) exencephaly

[a] RF = range-finding study D = definitive study

[b] There were also no fetuses with ectopia cordis

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INHALATION REPORT

STUDY NO. 04-4263

ENDPOINT-SPECIFIC DEVELOPMENTAL TOXICITY EVALUATION OF INHALED GASOLINE WITH METHYL TERTIARY BUTYL ETHER (MTBE) VAPOR CONDENSATE IN CD-1® MICE

Principal Investigator: Gary M. Hoffman, B.A., D.A.B.T.

Performed by: Huntingdon Life Sciences (HLS)
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Washington, D.C. 20005

Date: 3 June 2009

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STATEMENT OF COMPLIANCE

The portion of the study conducted at Huntingdon Life Sciences was performed in accordance with the protocol, Huntingdon Life Sciences' Standard Operating Procedures (SOPs) and the United States Environmental Protection Agency's Good Laboratory Practice Standards for the 211(b) program (40 C.F.R. 79.60).

3 June 09

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

Date

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SIGNATURE PAGE

SCIENTISTS

The following Scientists were responsible for the overall conduct of this study:

3 Jun 09

Gary M. Hoffman, B.A., D.A.B.T. Date
Principal Investigator

3 JUN 09

Robert M. Parker, Ph.D., D.A.B.T. Date
Reproductive Consultant

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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 Principal Investigator, Study Director and their Management.

Type of Inspection	Date(s) of Inspection	Reported to Principal Investigator and Management	Reported to Study Director and Management
GLP Protocol Review	4 Oct 04	4 Oct 04	11 Apr 05
Exposure, Monitoring & Equipment Records	12 Jan 05	12 Jan 05	11 Apr 05
GD 14 Body Weights & Equipment Records	21 Jan 05	21 Jan 05	11 Apr 05
Final Inhalation Report & Inhalation Study Data	17 – 21 Mar 05	22 Mar 05	9 May 05
Analytical Sample Analysis Data & Report	24 & 25 Mar 05	25 Mar 05	22 Apr 05
Non-Exposure Related Data (Study File, Body/Feeder Weight, Physical Observations Data, Pharmacy Data)	1-7 Apr 05	8 Apr 05	9 May 05
Protocol Amendments Nos. 1 & 2	15 Apr 05	18 Apr 05	22 Apr 05

Sonya Gray
Senior Quality Assurance Auditor

3 Jun 09
Date

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1. INTRODUCTION

This appendix presents the methodology for exposure, atmosphere generation, monitoring and results of a 6 hours/day exposure regimen of inhaled Gasoline Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate during the period of early (Gestation Days 5-10) or major (Gestation Days 5-16) organogenesis in gravid CD-1® mice.

2. MATERIALS AND METHODS

2.1. HUSBANDRY DURING EXPOSURE PERIODS

2.1.1. HOUSING

Animals were individually housed in 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 monitored continuously and recorded every half-hour during exposure and maintained, to the maximum extent possible, within the ranges presented on the next page. Excursions outside the specified range were not considered to have affected the integrity of the study.

Temperature

Desired: 20 to 24°C
Actual: 18 to 23°C

Relative Humidity

Desired: 40 to 60%
Actual: 20 to 58%

2.2. TEST SUBSTANCE ADMINISTRATION AND CHAMBER OPERATIONS

2.2.1. ROUTE OF ADMINISTRATION

Inhalation as a vapor, via whole-body exposures

2.2.2. TEST SUBSTANCE ADMINISTRATION

The test substance was administered 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 (at least two 6-hour periods) to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the target exposure levels and maintain uniform conditions throughout the exposure chambers. During this time, samples were taken to determine the distribution of the test substance in the exposure chamber.

2.2.3. TARGET EXPOSURE LEVELS

Group 1 – 0 mg/m^3
Group 2 – 2,000 mg/m^3
Group 3 – 10,000 mg/m^3
Group 4 – 20,000 mg/m^3
Group 5 – 30,000 mg/m^3

2.2.4. DURATION AND FREQUENCY OF ADMINISTRATION

Plug-positive female mice were exposed to Gasoline MTBE Vapor Condensate once daily, at either 6 hours/day for 12 days (25 animals) from Gestation Days 5 through 16 (Groups 1-4) or 6 hours/day for 6 days (40 animals) from Gestation Days 5 through 10 (Group 5).

2.2.5. CHAMBER OPERATIONS

The whole-body exposure chambers each had a volume of 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 23 minutes. Initial settings for each group were as follows:

Group	Airflow Rate (Lpm)	Air Change (min)	T_{99} (min)
1	207	4.8	22
2	202	4.9	23
3	210	4.7	22
4	211	4.7	22
5	207	4.8	22

The chamber size and airflow rates were considered adequate to maintain the animal loading factor below 5% and the oxygen level at 19% or higher. At the end of the exposure, all animals remained in chamber for a minimum of 22 minutes. During this time, the chamber was operated at the same flow rate as used during the exposure using clean air only. Recordings of airflow rate and static pressure were monitored continuously and recorded every 30 minutes during exposure. Chamber oxygen levels were measured pretest and at the beginning, middle and end of the study.

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.

See Figures 1 and 2 and Table III (Inhalation Report) for equipment details.

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 directed to the turret of the 1 m^3 glass and stainless steel exposure chamber where it was mixed with room air as it was drawn into the chamber.

Groups 2-5

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

The nitrogen for the test substance pressurization flow was directed through a metering valve, attached to a back pressure 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 disconnect fitting (equipped with a toggle valve) and through a filter to prevent equipment contamination. From the filter, the test substance flowed to a liquid flowmeter via 1/8" tubing. The outlet of the flowmeter was regulated by a metering valve. From this metering valve, the test substance flowed via 1/8" tubing onto the glass helix of a counter current volatilization chamber. This glass helix was heated by a nichrome wire which was controlled by a variable autotransformer

and inserted in the center of the glass tube that supported the helix external to volatilization chamber.

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 via ½" tubing to the turret of the chamber where it was mixed with room air.

See Figures 1 and 2 and Table III (Inhalation Report) 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 test substance consumed during the exposure was divided by the total volume of air passing through the chamber (flowrate multiplied by total exposure time).

Calculation

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

See Table III (Inhalation Report) for equipment list.

2.3.2. CHAMBER SAMPLING

During each 6-hour exposure, measurements of airborne concentrations were performed in the animals' breathing zone at least 4 times 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. Airborne test substance concentrations were within +/- 10% of the target concentration.

One charcoal tube sample drawn (15 minutes for Groups 1 and 2, 3 minutes for Group 3, 2 minutes for Group 4 and 1 minute for Group 5, at a rate of 0.200 Lpm) per chamber during the trials and treatment period was analyzed by gas chromatography 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.

See Table III for equipment list and Table V for MIRAN calibration (Inhalation Report).

2.3.3. PARTICLE SIZE DISTRIBUTION

Particle size samples were drawn (for 5 seconds at a rate of 5.0 Lpm) from each chamber once during the study using a TSI Aerodynamic Particle Sizer to confirm the absence of particulate test substance condensate in the exposure atmosphere. Particle size

samples were also drawn twice (once from each room) during the study from room air. The mass median aerodynamic diameter, geometric standard deviation and total mass concentration were calculated. 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.

See Table III (Inhalation Report) for equipment list.

2.3.4. CHAMBER AND EXPOSURE ROOM ENVIRONMENT

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

See Table III (Inhalation Report) for equipment list.

3. PROTOCOL DEVIATIONS

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

1. Due to technician error, food left weights were recorded for the Exposure Day 12-13 interval, but food fed weights for the Exposure Day 13-14 interval were not entered into the computer system for Animal Nos. 1805, 1806, 1807, 1808, 1809, 1810, 1811, 1812, 1814, 1815, 1816, 1818, 1819, 1820, 1823, 2804, 2806, 2808, 2814, 2815, 2817, 2818, 2819, 2821, 2822, and 2823. Since the feeders for these animals were returned to the cages after obtaining the food left weights and did not need to be changed, a series of edits was performed which provided a food fed weight equivalent to the food left weight.

2. Due to the unexpectedly extended mating period, only 130 mice were placed on test rather than 140. This resulted in the following group sizes: Group 1-4 had 23 mice and Group 5 had 38 mice.

3. The group means for MIRAN sampling were outside of the stated protocol range of ± 10% on Exposure Days 6, 9, 16 (Group 2), Exposure Days 16 and 20 (Group 3), and Exposure Day 2 and 16 (Group 4).

4. Individual MIRAN samples were outside the protocol specified range of ± 10% for the following Sample Nos.: 2001, 2002, 2020, 2021, 2022, 2032, 2034, 2035, 2036, 2037, 2044, 2052, 2063, 2064, 3061, 3062, 3063, 3064, 3065, 3066, 3077, 3078, 4001, 4004, 4005, 4406, 4016, 4024, 4063, 4064, 4070, 5001, 5005 and 5025. Chamber concentration values were confirmed as needed in accordance with Testing Facility SOP.

5. Due to technician oversight, a Nestlet® was given to Animal No. 5838 during the morning of Exposure Day 16 (removed after a total of 37 minutes) and then again in the afternoon. The protocol specifications were for afternoon only.

6. Due to technician oversight, animals in Groups 1-4 chambers and Group 5 chamber were not rotated on Exposure Days 11 and 13, respectively.

7. At the Sponsor's approval, non-pregnant females were sacrificed and examined macroscopically in order to determine their actual state of pregnancy, although not required by protocol.

8. Due to a communication error with the Sponsor representative, Group 5 dams were removed from their cages with Nestlets® and inserts only from GD 14-16 and placed into cages without Nestlets® and inserts to simulate the Groups 1-4 exposures regimen. Per intent of Sponsor, the Group 5 dams should have been removed from their cages with Nestlets® and inserts from GD 10-16 and placed into cages without Nestlets® and inserts to simulate the Groups 1-4 exposures regimen.

9. Due to the Principal Investigator's oversight, Protocol Amendment No. 2 was signed by one IACUC member instead of two, as designated by the protocol.

4. RESULTS AND DISCUSSION

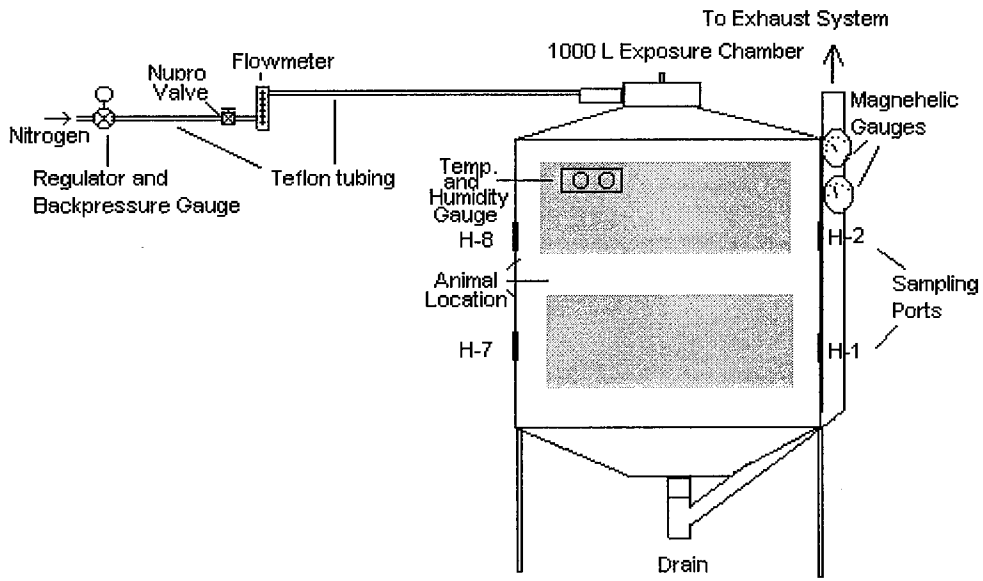
Prestudy chamber distribution analyses showed that the test substance was evenly distributed within the chamber. Pre-study and on-study chamber monitoring showed that the chamber oxygen levels were 20%. Pre-study and on-study chamber room monitoring showed that no test substance was present in the room and that the sound and light levels were acceptable.

The analytically measured exposure levels of the airborne test substance were reasonably close to the targeted exposure level. The measured and nominal concentrations varied somewhat (less than 10%), but reasonably, from the expected 1:1 ratio for this type of vapor exposure. Chamber environmental conditions averaged 20.8°C temperature and 29.2% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were essentially vapor only, as expected, since there was no substantial difference between the test substance chamber and the air control chamber.

Analysis of the major components in the neat test substance and the test atmospheres showed a reasonably close comparison between the neat test substance and the vaporized test substance. This data demonstrated that the test animals were exposed, as expected, to all of the major components of the test substance in their proper proportion. The data was consistent between the pre-study and on-study indicating stability of the test substance and the atmosphere generation techniques.

Group 1	Inhalation Report	Appendix I

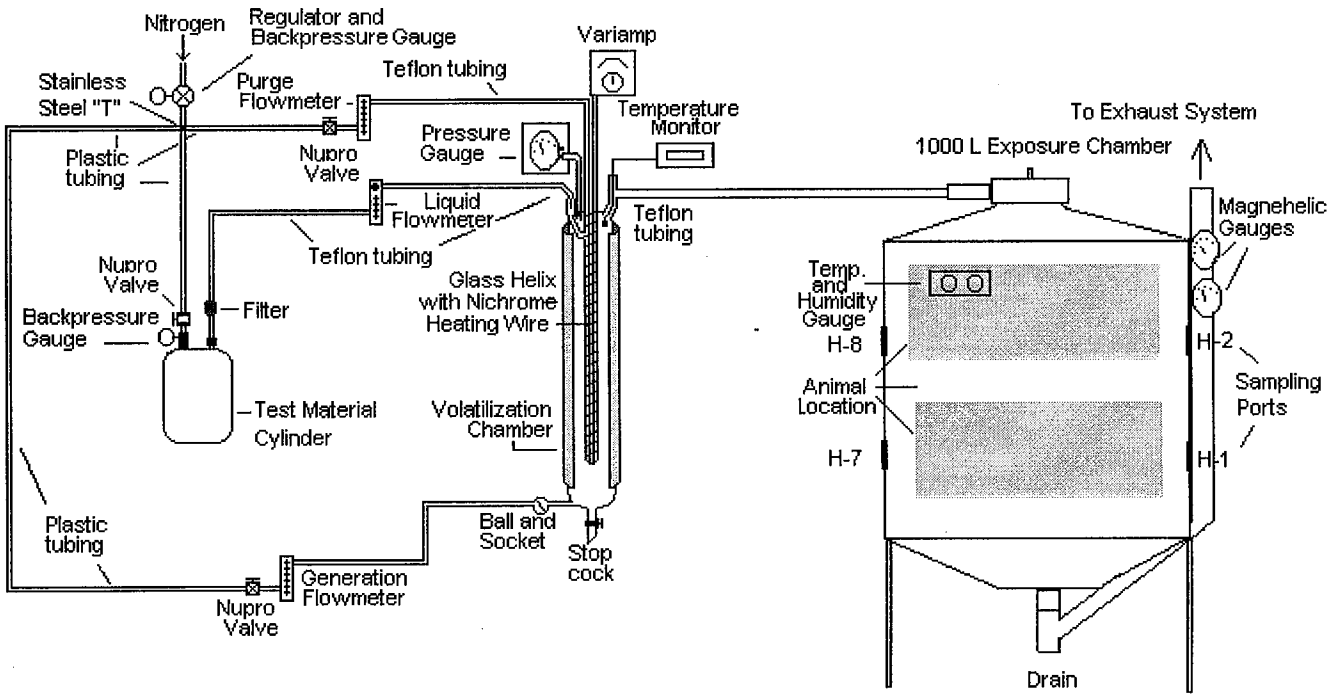
Figure 1
Chamber Generation System
and Whole-Body Exposure Chamber



Note: Animals were individually housed on the mid-level of the exposure chamber.

Groups 2-5	Inhalation Report	Appendix I

Figure 2
Chamber Generation System
and Whole-Body Exposure Chamber



Note: Animals were individually housed on the mid-level of the exposure chamber. Sampling Ports H-11 (left-bottom), H-12 (left-top), H-13 (right-bottom) and H-14 (right-top) used for pretest distribution sampling, were located on the back wall of the chambers.

Table I	Inhalation Report Summary of In-Chamber Observations													Appendix I
Exposure Day	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
Group 1 – 0 mg/m^3														
Number of Animals in Chamber	3	6	11	16	19	20	22	22	23	23	23	23	20	17
Within Normal Limits	All	All	All	All	All	All	All	All	All	All	All	All	All	All
Group 2 – 2000 mg/m^3														
Number of Animals in Chamber	3	6	12	17	20	21	23	23	23	23	23	23	20	17
Within Normal Limits	All	All	All	All	All	All	All	All	All	All	All	All	All	All
Group 3 – 10,000 mg/m^3														
Number of Animals in Chamber	3	6	12	17	19	20	21	22	22	22	23	23	20	17
Within Normal Limits	All	All	All	All	All	All	All	All	All	All	All	All	All	All
Group 4 – 20,000 mg/m^3														
Number of Animals in Chamber	4	7	13	18	20	21	22	23	23	23	23	23	19	16
Within Normal Limits	All	All	All	All	All	All	All	All	All	All	All	All	All	All
Group 5 – 30,000 mg/m^3														
Number of Animals in Chamber	6	11	23	30	34	37	32	27	15	8	4	1		
Within Normal Limits	All	All	All	All	All	All	All	All	All	All	All	All		

All = 100% of the animals exhibiting a given observation.
Note: In-chamber observations are based on all animals present in the exposure chamber at the time.

Table I	Inhalation Report Summary of In-Chamber Observations							Appendix I
Exposure Day	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Group 1 – 0 mg/m^3								
Number of Animals in Chamber	12	7	4	3	1	1		
Within Normal Limits	All	All	All	All	All	All		
Group 2 – 2,000 mg/m^3								
Number of Animals in Chamber	11	6	3	2				
Within Normal Limits	All	All	All	All				
Number of Animals in Chamber Group 3 – 10,000 mg/m^3								
Number of Animals in Chamber	11	6	4	3	2	1	1	1
Within Normal Limits	All	All	All	All	All	All	All	All
Group 4 – 20,000 mg/m^3								
Number of Animals in Chamber	10	5	3	2	1			
Within Normal Limits	All	All	All	All	All			

All = 100% of the animals exhibiting a given observation.
Note: In-chamber observations are based on all animals present in the exposure chamber at the time.

	Inhalation Report	Appendix I

Table II
Chamber Monitoring Results
Preface

Key To Abbreviations:

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

Table II	Inhalation Report Chamber Monitoring Results	Appendix I

Cumulative Exposure Record

Group 1 - 0 mg/m³ (Air Control)

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
0	12-Jan-05	1	0	0.00	0.00	0.00	0.00	0.00				20	42
1	13-Jan-05	2	0	0.00	0.00	0.00	0.00	0.00				20	44
2	14-Jan-05	3	0	0.00	0.00	0.00	0.00	0.00				21	34
3	15-Jan-05	4	0	0.00	0.00	0.00	0.00	0.00				20	27
4	16-Jan-05	5	0	0.00	0.00	0.00	0.00	0.00				19	30
5	17-Jan-05	6	0	0.00	0.00	0.00	0.00	0.00				20	25
6	18-Jan-05	7	0	0.00	0.00	0.00	0.00	0.00				18	29
7	19-Jan-05	8	0	0.00	0.00	0.00	0.00	0.00	2.179	1.676	2.56E-02	19	28
8	20-Jan-05	9	0	0.00	0.00	0.00	0.00	0.00				20	33
9	21-Jan-05	10	0	0.00	0.00	0.00	0.00	0.00				20	27
10	22-Jan-05	11	0	0.00	0.00	0.00	0.00	0.00				20	31
11	23-Jan-05	12	0	0.00	0.00	0.00	0.00	0.00				20	34
12	24-Jan-05	13	0	0.00	0.00	0.00	0.00	0.00				21	31
13	25-Jan-05	14	0	0.00	0.00	0.00	0.00	0.00				21	32
14	26-Jan-05	15	0	0.00	0.00	0.00	0.00	0.00				21	34
15	27-Jan-05	16	0	0.00	0.00	0.00	0.00	0.00				21	27
16	28-Jan-05	17	0	0.00	0.00	0.00	0.00	0.00				21	27
17	29-Jan-05	18	0	0.00	0.00	0.00	0.00	0.00				21	27
18	30-Jan-05	19	0	0.00	0.00	0.00	0.00	0.00				21	32
19	31-Jan-05	20	0	0.00	0.00	0.00	0.00	0.00				21	30
		Mean	**0**		**0.00**				**2.179**	**1.676**	**2.56E-02**	**20.3**	**31.2**
		S.D.	**0**		**0.00**				**-**	**-**	**-**	**0.9**	**4.9**

Table II	Inhalation Report Chamber Monitoring Results	Appendix I

Cumulative Exposure Record

Group 2 - 2,000 mg/m³

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
0	12-Jan-05	1	2100	1800	1600	1700	2000	1900				19	45
1	13-Jan-05	2	2300	1900	1800	1800	1800	2200				20	52
2	14-Jan-05	3	2300	2100	2100	2100	2000	2200				21	37
3	15-Jan-05	4	2200	2050	1800	2200	2100	2100				19	33
4	16-Jan-05	5	2300	2200	2000	1900	2000	2900				20	33
5	17-Jan-05	6	2400	2230	2400	2600	2100	1800				20	32
6	18-Jan-05	7	2100	1930	1800	1900	1800	2200				19	29
7	19-Jan-05	8	2100	2100	2000	2000	2000	2400	5.699	2.117	5.05E-03	20	26
8	20-Jan-05	9	2400	2380	2000	2400	2500	2600				22	28
9	21-Jan-05	10	2200	2180	2600	2000	2100	2000				21	27
10	22-Jan-05	11	2000	2100	1900	2000	2000	2500				21	32
11	23-Jan-05	12	2000	1930	2000	2000	1900	1800				21	34
12	24-Jan-05	13	1900	2050	2100	2100	1900	2100				21	30
13	25-Jan-05	14	2100	1950	1800	1900	2000	2100				22	32
14	26-Jan-05	15	2100	2030	2100	2000	2000	2000				23	32
15	27-Jan-05	16	2400	2230	1800	2100	2800	2200				22	24
16	28-Jan-05	17	2200	2050	2200	1900	2200	1900				22	26
17	29-Jan-05	18	2200	2150	2200	2000	2200	2200				22	24
		Mean	**2183**			**2074**			**5.699**	**2.117**	**5.05E-03**	**20.8**	**32.0**
		S.D.	**147**			**248**			**-**	**-**	**-**	**1.2**	**7.1**

Table II	Inhalation Report Chamber Monitoring Results	Appendix I

Cumulative Exposure Record

Group 3 - 10,000 mg/m³

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
0	12-Jan-05	1	9700	9800	9500	9800	10000	9900				21	34
1	13-Jan-05	2	9900	9680	9500	9600	9600	10000				22	39
2	14-Jan-05	3	10000	10000	10000	10000	10000	10000				22	32
3	15-Jan-05	4	9800	9930	9700	10000	10000	10000				21	27
4	16-Jan-05	5	9500	9900	10000	9600	10000	10000				20	26
5	17-Jan-05	6	9800	9930	10000	10000	9800	9900				21	25
6	18-Jan-05	7	9600	9880	10000	10000	9900	9600				20	24
7	19-Jan-05	8	9900	10100	11000	10000	9700	9700	9.319	2.071	3.41E-03	19	24
8	20-Jan-05	9	9500	9750	10000	10000	9000	10000				21	30
9	21-Jan-05	10	9600	10000	10000	10000	10000	10000				22	24
10	22-Jan-05	11	10000	10100	10000	10000	9400	11000				21	25
11	23-Jan-05	12	9600	10100	10000	11000	10000	9400				21	28
12	24-Jan-05	13	9800	10300	10000	10000	11000	10000				22	24
13	25-Jan-05	14	9300	10000	10000	10000	10000	10000				22	28
14	26-Jan-05	15	9900	10000	10000	10000	10000	10000				23	31
15	27-Jan-05	16	8400	8250	8200	6800	8900	9100				22	24
16	28-Jan-05	17	9700	9300	8600	8700	9900	10000				22	23
17	29-Jan-05	18	9300	9880	11000	9100	9500	9900				22	22
18	30-Jan-05	19	9700	9900	11000	9000	9900	9700				22	27
19	31-Jan-05	20	10000	11300	12000	12000	11000	10000				22	26
20	1-Feb-05	21	9700	9930	10000	9700	10000	10000				22	24
21	2-Feb-05	22	9800	10500	11000	11000	9800	10000				22	23
		Mean	**9659**		**9925**				**9.319**	**2.071**	**3.41E-03**	**21.5**	**26.8**
		S.D.	**346**		**688**				**-**	**-**	**-**	**0.9**	**4.2**

Table II	Inhalation Report Chamber Monitoring Results	Appendix I

Cumulative Exposure Record

Group 4 - 20,000 mg/m³

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
0	12-Jan-05	1	22000	18500	16000	19000	20000	19000				21	34
1	13-Jan-05	2	23000	22800	25000	23000	22000	21000				21	39
2	14-Jan-05	3	21000	19500	18000	20000	20000	20000				22	33
3	15-Jan-05	4	20000	19500	21000	20000	18000	19000				20	28
4	16-Jan-05	5	18000	19500	21000	19000	19000	19000				20	27
5	17-Jan-05	6	19000	21000	19000	21000	20000	24000				20	26
6	18-Jan-05	7	20000	20000	20000	20000	20000	20000				19	25
7	19-Jan-05	8	20000	19500	20000	20000	19000	19000	3.845	1.955	1.47E-03	19	25
8	20-Jan-05	9	21000	21500	21000	21000	22000	22000				20	31
9	21-Jan-05	10	20000	18800	18000	18000	19000	20000				21	25
10	22-Jan-05	11	20000	19500	19000	20000	20000	19000				20	26
11	23-Jan-05	12	21000	20500	21000	21000	20000	20000				21	31
12	24-Jan-05	13	21000	21500	22000	21000	22000	21000				21	25
13	25-Jan-05	14	19000	19500	22000	18000	19000	19000				22	29
14	26-Jan-05	15	19000	19800	20000	22000	19000	18000				22	32
15	27-Jan-05	16	22000	23500	20000	20000	28000	26000				21	25
16	28-Jan-05	17	21000	21300	22000	21000	22000	20000				21	24
17	29-Jan-05	18	19000	20300	20000	20000	21000	20000				21	24
18	30-Jan-05	19	20000	20300	21000	21000	20000	19000				22	30
		Mean	**20316**		**20342**				**3.845**	**1.955**	**1.47E-03**	**20.7**	**28.4**
		S.D.	**1250**		**1815**				**-**	**-**	**-**	**0.9**	**4.1**

Table II	Inhalation Report Chamber Monitoring Results	Appendix I

Cumulative Exposure Record

Group 5 - 30,000 mg/m³

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
0	12-Jan-05	1	30000	29500	31000	30000	30000	27000				21	33
1	13-Jan-05	2	29000	28500	26000	29000	29000	30000				21	38
2	14-Jan-05	3	30000	29300	30000	28000	29000	30000				22	33
3	15-Jan-05	4	30000	28300	27000	30000	29000	27000				21	26
4	16-Jan-05	5	29000	28500	27000	29000	29000	29000				21	25
5	17-Jan-05	6	31000	29500	28000	32000	28000	30000				21	24
6	18-Jan-05	7	31000	29500	26000	33000	29000	30000				20	24
7	19-Jan-05	8	30000	29500	29000	30000	30000	29000	1.144	2.910	1.54E-02	19	24
8	20-Jan-05	9	31000	30000	29000	30000	30000	31000				21	29
9	21-Jan-05	10	32000	29500	29000	30000	30000	29000				21	23
10	22-Jan-05	11	31000	29800	30000	27000	30000	32000				20	25
11	23-Jan-05	12	31000	29300	31000	28000	30000	28000				20	27
		Mean	**30417**		**29250**				**1.144**	**2.910**	**1.54E-02**	**20.7**	**27.6**
		S.D.	**900**		**1480**				**-**	**-**	**-**	**0.8**	**4.7**

	Inhalation Report	Appendix I

Table III
Equipment List

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

Compound Generator
Counter-Current Volatilization Unit, with coiled glass rod insert and Nichrome wire (Crown Glass Co., Inc.).
T° Sentry Digital Alarm Module, Model 110 (Hampshire Controls Corporation).

Compound Reservoir
5 and 100 Gallon Cylinders (American Petroleum Institute provided).

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

Flowmeters
Flowmeter, size 0 –4, 0 – 5, 0 – 20, 0 – 40 Lpm (Dwyer® Instruments Inc.).
Liquid Flowmeter with built in metering valve, size 0 – 65 mm, Model 6G02/6G03/6G04 (Key Instruments).
Top Trak ™ Mass Flowmeter, size 0 – 1 Lpm, Model 821-4 (Sierra Instruments), calibrated prestudy with Gilibrator® Bubble Generator, S/N 6688-S, flow cell assembly P/N D800286.

Pressure/Vacuum Gauges
U.S. Gauge backpressure gauge, P/N 126172.
Ashcroft backpressure gauge, P/N 733-47.
Matheson® backpressure gauge, P/N 63-3161.
Norgreen backpressure gauge, P/N 9892K23.
Magnehelic gauge (Dwyer® Instruments Inc.).
Union Carbide backpressure gauge, PN SG-6383

	Inhalation Report	Appendix I

Table III
Equipment List

Regulators
Union Carbide, Model P/N SG 3800 30.
Norgreen, P/N 9892K23.
Stainless Steel Purge/Vent System (MG Industries).

Valves
Metering Valve, Model SS-4L Series (Nupro® Co.).
Metering Valve, Model SS-1RM4-S4, (Whitey®).

Tubing
Plastic, size 1/4", 1/2", 3/16" (Norton and Baxter).
Teflon®/Tygon®, size 1/8", 1/4", 1/2".
T-Tube, plastic (Crown Glass Co.).
Stainless steel "cross" (Swage).
1/2" stainless steel.

Filters
Balston® Microfibre™ Disposable Filter Units Grade DQ, No. L9933-05.

Timers
Gralab Universal Timer, Model 171.

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

Absorbent Tubes
Charcoal Tubes, Lot No. 2000, Model ORBO-32 (Supelco).

Balances
Pelouze, No. 4010
Mettler PM30000K (Mettler Instrument Corporation).

	Inhalation Report	Appendix I

Table III
Equipment List

Air Analyzer
MIRAN 1A Ambient Air Analyzer (Wilks) with a Cole Parmer strip chart recorder, Model 201 and a Micronta® LCD Benchtop Digital Multimeter No. 22-195.
Syringe, size 0 – 25 and 0 – 250 µL, Nos. 1702 and 1725 (Hamilton).

Particle Sizer/Analyzer
TSI Aerodynamic Particle Sizer, Model 331001, with a DELL computer, Model 486P/25, equipped with an Epson LQ-570+ Dot matrix printer, Model P630B.

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

Chamber Air-flow
Dwyer® Magnehelic® gauge (Dwyer® Instruments Inc.), calibrated prestudy with a Dry Gas Meter , Model 2M (Singer).

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

Miscellaneous
Quick-disconnect fitting with toggle valve (Rego®)

	Inhalation Report	Appendix I

Table IV
Chamber Distribution Records

Group (target)	Date	Port	IR Conc (mg/m^3)	Ratio to H-1
2 (2,000 mg/m^3)	15 November 2004	H-1	2100	1.00
		H-2	2200	1.05
		H-7	2200	1.05
		H-1	2100	1.00
		H-8	2000	0.95
	16 November 2004	H-1	1900	1.00
		H-14	1900	1.00
		H-13	1800	0.95
		H-1	2000	1.00
		H-12	2000	1.00
		H-11	1900	0.95
3 (10,000 mg/m^3)	15 November 2004	H-1	9100	1.00
		H-2	8900	0.98
		H-1	9200	1.00
		H-2	9300	1.01
		H-7	9300	1.01
		H-8	9300	1.01
	16 November 2004	H-1	9900	1.00
		H-14	9700	0.98
		H-13	9500	0.96
		H-1	9700	1.00
		H-12	9800	1.01
		H-11	9700	1.00

	Inhalation Report	Appendix I

Table IV
Chamber Distribution Records

Group (target)	Date	Port	IR Conc (mg/m^3)	Ratio to H-1
4 (20,000 mg/m^3)	15 November 2004	H-1	20000	1.00
		H-2	20000	1.00
		H-7	22000	1.10
		H-1	20000	1.00
		H-8	22000	1.10
	16 November 2004	H-1	20000	1.00
		H-14	21000	1.05
		H-13	22000	1.10
		H-1	20000	1.00
		H-12	19000	0.95
		H-11	21000	1.05
5 (30,000 mg/m^3)	15 November 2004	H-1	30000	1.00
		H-2	30000	1.00
		H-7	30000	1.00
		H-1	29000	1.00
		H-8	29000	1.00
	16 November 2004	H-1	29000	1.00
		H-14	29000	1.00
		H-13	29000	1.00
		H-1	30000	1.00
		H-12	30000	1.00
		H-11	30000	1.00

	Inhalation Report	Appendix I

Table V
MIRAN Calibration

Methodology for Gasoline MTBE Vapor Condensate

Settings: The instrument settings for the MIRAN 07 Unit are summarized below:

wavelength, microns	10.3
pathlength, dial setting	4.00
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)
8.4	998	0.0179	0.0191	0.0185
17	2020	0.0444	0.0467	0.0456
85	10098	0.2222	0.2263	0.2243
170	20195	0.415	0.455	0.435
250	29699	0.626	0.670	0.648
295	35044	0.770	0.789	0.780

[1]Calculated Concentration (mg/m^3) = $\frac{\text{Injection volume } (\mu L) \text{ X density } (mg/\mu L)}{\text{Volume of MIRAN closed-loop (L) X } 1000 L/m^3}$

where: density = 0.67 mg/μL
volume of MIRAN closed-loop = 5.64 L

	Inhalation Report	Appendix I

Table V
MIRAN Calibration

Calibration Check: A four-point calibration check of the MIRAN was performed for each exposure prior to sampling the chambers. The parameters are shown below:

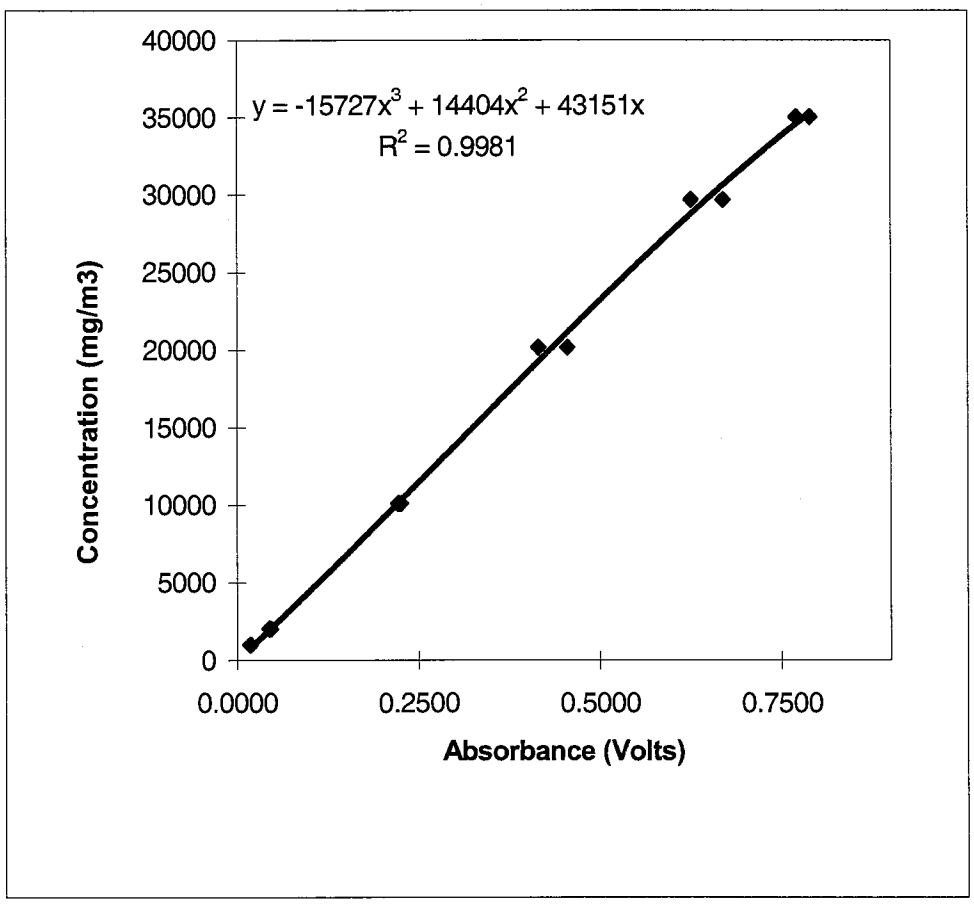
Injection Volume (µL)	Calculated Concentration (mg/m^3)	Expected Absorbance Reading (volts)	Acceptable Absorbance Range (volts)
17	2020	0.0456	0.0388 – 0.0524
85	10098	0.2243	0.1907 – 0.2579
170	20195	0.435	0.370 – 0.500
250	29699	0.648	0.551 – 0.745

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 Principal Investigator decided if a new graph was to be prepared.

	Inhalation Report	Appendix I

Table V
MIRAN Calibration

Calibration Curve for Gasoline MTBE Vapor Condensate (Groups 1 - 5)



	Inhalation Report	Appendix I

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	38.0	63.5	-	[b]$1.33E \times 10^{-1}$
	Room 813 - Back	0	37.8	62.4	-	
	Group 1 Chamber	-	-	-	20	$1.21E \times 10^{-1}$
	Group 2 Chamber	-	-	-	20	$1.30E \times 10^{-1}$
	Group 3 Chamber	-	-	-	20	$1.03E \times 10^{-1}$
	Group 4 Chamber	-	-	-	20	$7.37E \times 10^{-2}$
	Group 5 Chamber	-	-	-	20	$7.66E \times 10^{-2}$
Exposure Day 2	Room 813 - Front	0	35.2	64.4	-	
	Room 813 - Back	0	36.4	65.8	-	
	Group 1 Chamber	-	-	-	20	
	Group 2 Chamber	-	-	-	20	
	Group 3 Chamber	-	-	-	20	
	Group 4 Chamber	-	-	-	20	
	Group 5 Chamber	-	-	-	20	
Exposure Day 9	Room 813 - Front	0	34.4	65.0	-	
	Room 813 - Back	0	36.7	65.3	-	
	Group 1 Chamber	-	-	-	20	
	Group 2 Chamber	-	-	-	20	
	Group 3 Chamber	-	-	-	20	
	Group 4 Chamber	-	-	-	20	
	Group 5 Chamber	-	-	-	20	

[a]Pre-test results for 16Nov04 presented above. For on-test results, see CMR (Appendix I, Table II).
[b]Room air sample; front or back location not specified in raw data.

	Inhalation Report	Appendix I

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]
Exposure Day 16	Room 813 - Front	0	34.5	65.9	-	
	Room 813 - Back	0	36.0	66.5	-	
	Group 1 Chamber	-	-	-	20	
	Group 2 Chamber	-	-	-	20	
	Group 3 Chamber	-	-	-	20	
	Group 4 Chamber	-	-	-	20	
	Group 5 Chamber	-	-	-	20	

[a]Pretest results presented above. For Exposure Day 8 results, see CMR (Appendix I, Table II).

	Analytical Report	Appendix I

STUDY TITLE

Endpoint-SpecificDevelopmental Toxicity Evalution of Inhaled Gasoline with Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD-1 ® Mice

AUTHOR

Yonggang Wang

REPORT DATE

3 June 2009

STUDY NUMBER

04-4263

	Analytical Report	Appendix I

SIGNATURES

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	Analytical Report	Appendix I

1. Summary

Samples of the test substance (Gasoline MTBE Vapor Condensate) exposures to CD-1® mice were analyzed to confirm that the relative concentrations of the test substance's major components were appropriate under the study conditions. The analytical method was validated at Huntingdon Life Sciences (HLS). The method involved the extraction of Gasoline MTBE Vapor Condensate from charcoal tubes with Carbon Disulfide (CS_2). The test substance's major components were then quantified (relative area percent) utilizing Gas Chromatography with a Flame Ionization Detection (FID).

2. Experimental Procedures

The analytical method (HLS-001-01R1) was validated by the Formulation Chemistry Department at HLS. Details of the analytical methods and their validation are maintained in the study files for Study No. 00-6126.

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 MTBE 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 Turbochrom installed on a personal computer was used for data collection and processing.

Date of sample receipt and analysis is listed as follows:

Interval	Date of Exposures	Date Received	Date Analyzed
Pretest	13 Dec 04	13 Dec 04	14-15 Dec 04
Exposure 9	20 Jan 05	20 Jan 05	20-21 Jan 05

	Analytical Report	Appendix I

3. Results and Discussion

During the trials and exposures, Gasoline MTBE Vapor Condensate was analyzed to determine the area percent of the test substance's major components in the chamber. The results of the trial and animal exposures are presented in Tables I and II. Typical chromatograms of groups 1-5 are presented in Figures I to V.

	Analytical Report	Appendix I

Table I. Chamber Components Confirmation
Area Percent of Gasoline MTBE Vapor Condensate
Trials (Groups 1-5)
Area %

Compound	Spiked Control 1 006_003	TM Standard 1 006_002	Sample 101 (Group 1) 006_004	Sample 201 (Group 2) 006_005	Sample 301 (Group 3) 006_006	Sample 401 (Group 4) 006_007	Sample 501 (Group 5) 006_008	Spiked Control 2 006_009	TM Standard-2 006_010
Isobutane	1.61	1.59	ND	1.77	1.72	1.78	1.81	1.42	1.51
N-Butane	9.17	9.14	ND	9.95	9.67	9.83	9.94	8.00	8.85
3-Methyl-1-butene	0.44	0.40	ND	0.36	0.39	0.36	0.39	0.33	0.32
Isopentane	31.78	31.94	ND	31.37	31.01	31.23	31.53	29.98	30.55
N-Pentane	8.85	9.59	ND	9.29	9.12	9.10	9.48	8.98	9.41
Trans-2-pentene	2.09	2.07	ND	2.06	2.05	2.07	2.06	2.05	2.06
2,3-Dimethylbutane	1.44	1.39	ND	1.36	1.36	1.36	1.25	1.37	1.28
2-Methylpentane	4.86	4.71	ND	4.68	4.63	4.63	4.61	4.68	4.51
MTBE	21.91	21.66	ND	21.89	21.98	22.09	22.03	22.64	22.72
3-Methylpentane	2.95	3.44	ND	3.08	2.97	3.06	2.91	2.91	2.98
N-Hexane	2.61	2.48	ND	2.54	2.55	2.52	2.49	2.64	2.56
Methylcyclopentane	1.34	1.25	ND	1.22	1.25	1.24	1.27	1.35	1.26
2,4-Dimethylpentane	1.07	1.13	ND	1.09	1.04	1.05	1.06	1.12	1.06
Benzene	2.04	1.83	ND	1.94	2.05	1.94	1.95	2.11	2.10
2-Methylhexane	1.21	1.13	ND	1.18	1.14	1.15	1.13	1.32	1.17
2,3-Dimethylpentane	1.22	1.21	ND	1.21	1.16	1.19	1.20	1.45	1.26
3-Methylhexane	1.35	1.27	ND	1.33	1.37	1.31	1.26	1.38	1.49
Isooctane	1.46	1.21	ND	1.29	1.41	1.40	1.18	1.59	1.50
Toluene	2.59	2.53	ND	2.39	2.72	2.68	2.44	2.77	2.84
Total	99.99	99.97	0.00	100.00	99.59	99.99	99.99	98.09	99.16

ND = none detected. [a]3-Methylpentane co-eluted with MTBE.

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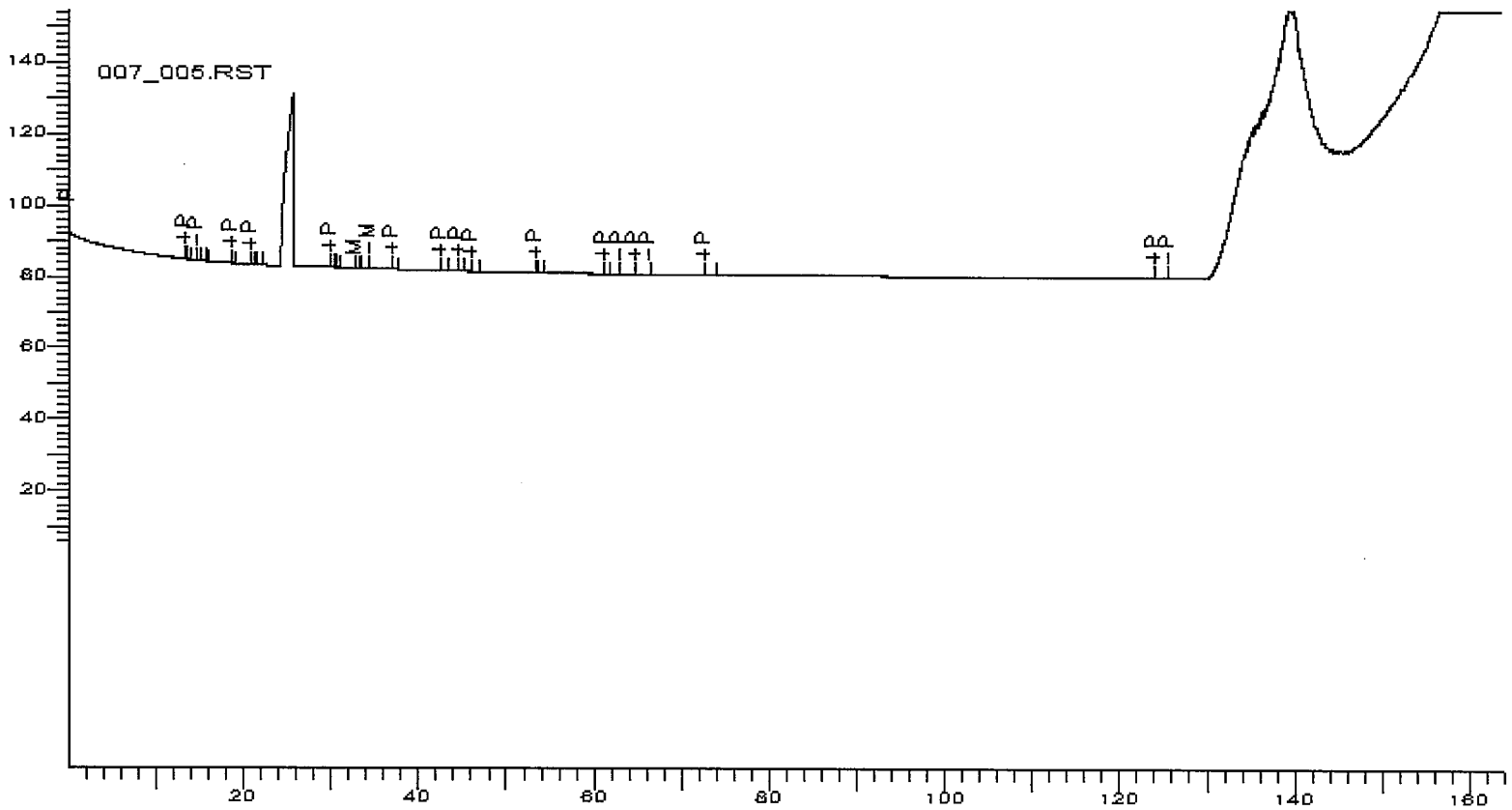
Table II-A. Chamber Components Confirmation
Area Percent of Gasoline MTBE Vapor Condensate
Exposure 9 (Groups 1-5)
Area %

Compound	Control Sample 007_002	Spiked Control 1 007_004	TM Standard-1 007_003	Sample 101 (Group 1) 007_005	Sample 201 (Group 2) 007_006	Sample 301 (Group 3) 007_007	Sample 401 (Group 4) 007_008	Sample 501 (Group 5) 007_009	Spiked Control 2 007_010	TM Standard-2 007_011
Isobutane	ND	1.54	1.54	ND	1.83	1.80	1.84	1.82	1.46	1.48
N-Butane	ND	8.79	8.87	ND	9.82	9.78	9.88	9.81	8.40	8.56
3-Methyl-1-butene	ND	0.55	0.58	ND	0.57	0.65	0.65	0.65	0.58	0.58
Isopentane	ND	30.83	30.94	ND	31.57	31.66	31.45	31.54	30.54	30.47
N-Pentane	ND	9.48	9.49	ND	9.57	9.58	9.47	9.52	9.43	9.38
Trans-2-pentene	ND	2.13	2.07	ND	2.10	2.10	2.08	2.10	2.07	2.06
2, 3-Dimethylbutane	ND	1.32	1.44	ND	1.29	1.38	1.22	1.39	1.45	1.42
2-Methylpentane	ND	4.84	4.80	ND	4.72	4.69	4.59	4.68	4.93	4.82
MTBE + 3-Methylpentane[a]	ND	24.61	24.33	ND	23.34	23.34	24.34	23.33	24.69	25.28
N-hexane	ND	2.66	2.65	ND	2.55	2.56	2.56	2.61	2.76	2.70
Methylcyclopentane	ND	1.36	1.36	ND	1.32	1.30	1.28	1.31	1.38	1.33
2,4-Dimethylpentane	ND	1.15	1.15	ND	1.09	1.07	1.04	1.07	1.16	1.11
Benzene	ND	2.14	2.18	ND	2.05	2.09	1.98	2.04	2.29	2.13
2-Methylhexane	ND	1.24	1.22	ND	1.17	1.13	1.11	1.15	1.26	1.22
2,3-Dimethylpentane	ND	1.30	1.28	ND	1.23	1.21	1.15	1.19	1.30	1.24
3-Methylhexane	ND	1.48	1.43	ND	1.41	1.31	1.31	1.38	1.50	1.44
Isooctane	ND	1.30	1.32	ND	1.19	1.38	1.11	1.29	1.25	1.36
Toluene	ND	2.82	2.89	ND	2.75	2.56	2.53	2.68	3.11	2.99
Total	0.00	99.54	99.54	0.00	99.57	99.59	99.59	99.56	99.56	99.57

ND = none detected. [a]3-Methylpentane co-eluted with MTBE.

	Analytical Report	Appendix I

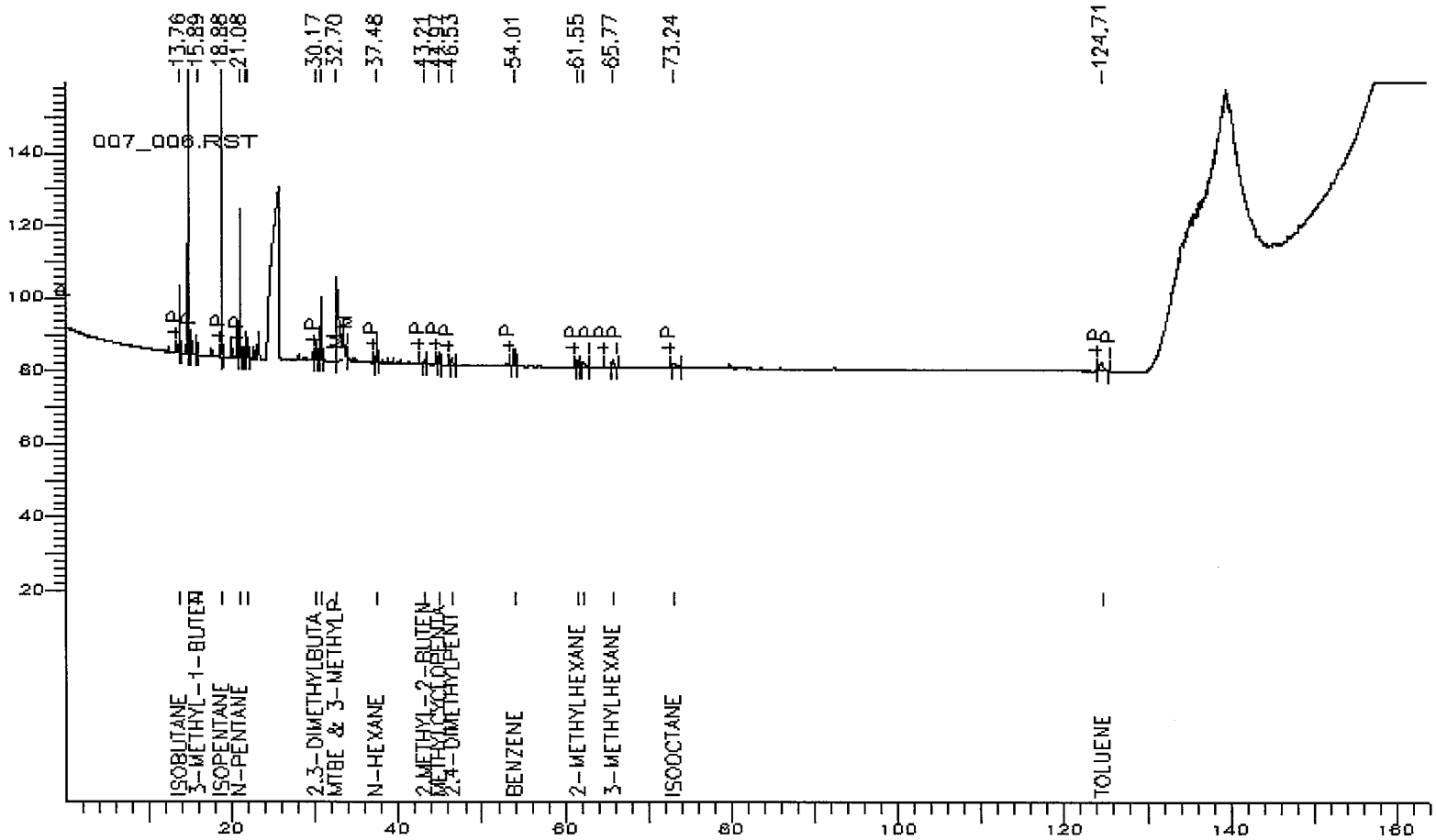
Figure I. Gas Chromatogram of Sample 1001 (Group 1) Charcoal Tube



Response (mV) vs. Time (minutes)

	Analytical Report	Appendix I

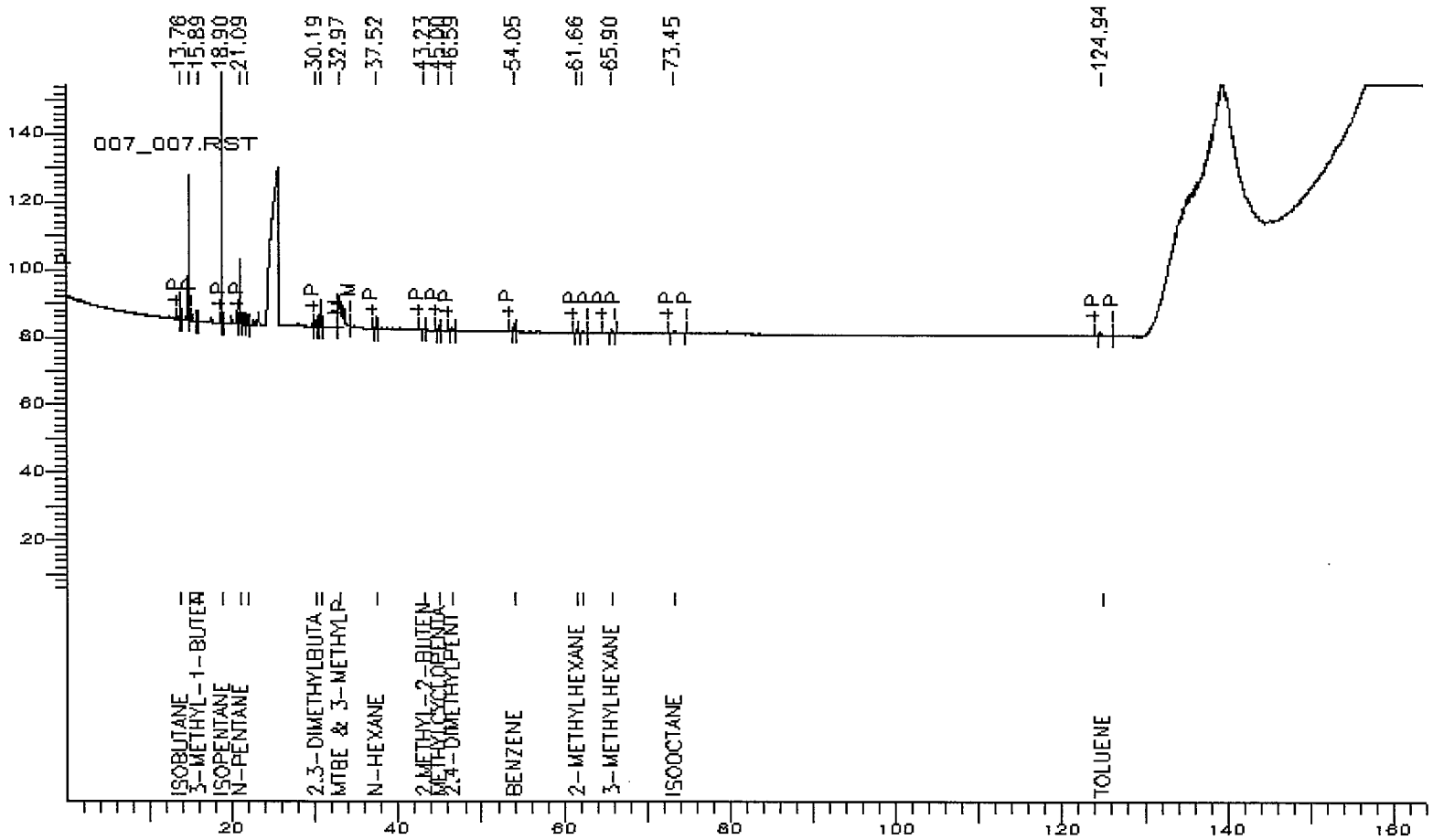
Figure II. Gas Chromatogram of Sample 2001 (Group 2) Charcoal Tube



Response (mV) vs. Time (minutes)

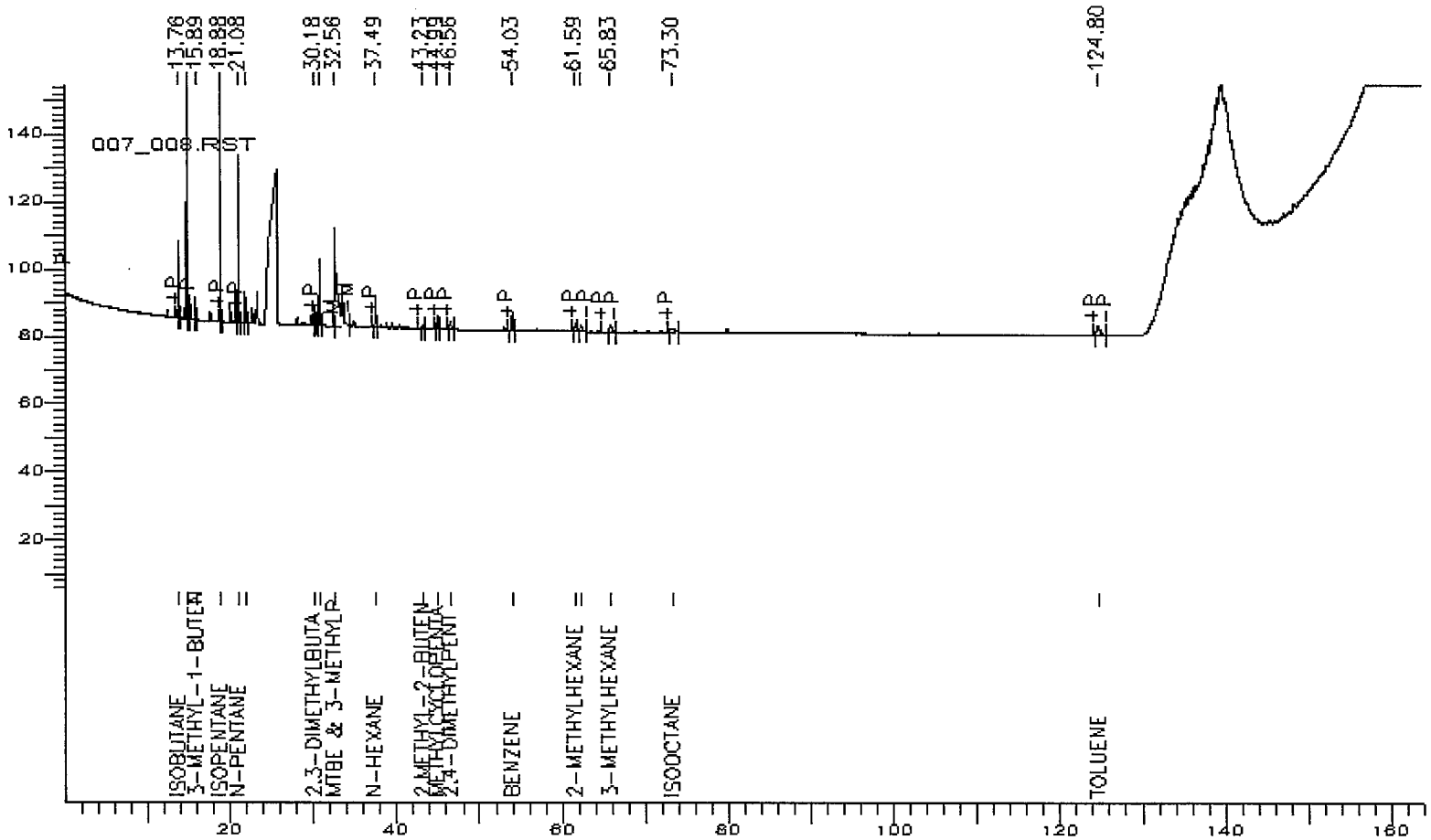
	Analytical Report	Appendix I

Figure III. Gas Chromatogram of Sample 3001 (Group 3) Charcoal Tube



	Analytical Report	Appendix I

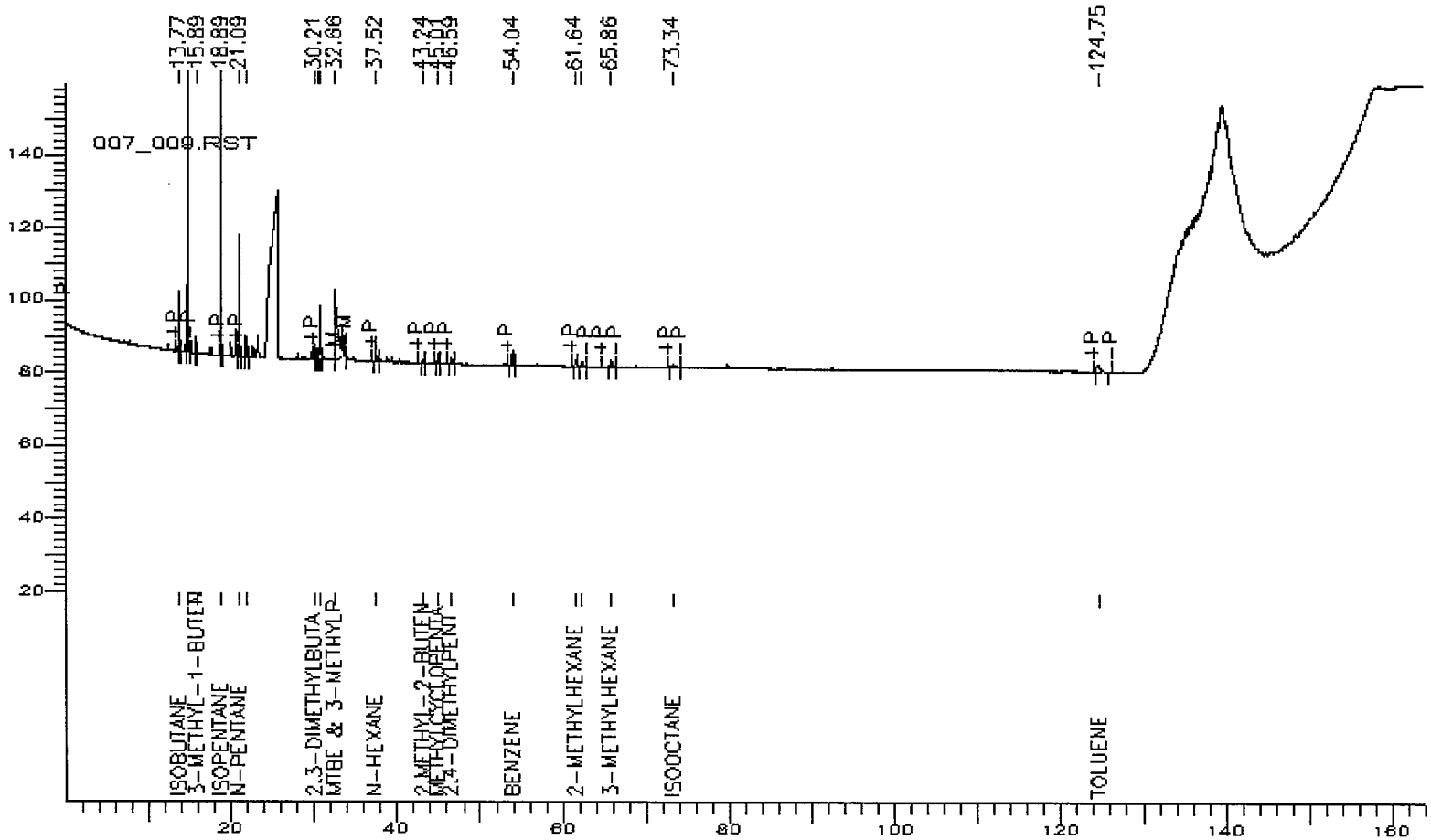
Figure IV. Gas Chromatogram of Sample 4001 (Group 4) Charcoal Tube



Response (mV) vs. Time (minutes)

	Analytical Report	Appendix I

Figure IV. Gas Chromatogram of Sample 5001 (Group 5) Charcoal Tube



Response (mV) vs. Time (minutes)

STUDY FILE NOTE

Study Number: 04-4262/4263 Effective Date: 9Dec08

cc: GMH

Subject: MIRAN LOQ

The control chamber limit of quantification was estimated at 433 mg/m^3 based on the extrapolated value for an absorbance reading of 0.01 which is considered the lowest practical reading above the baseline. Since the nominal to measured ratio for even the low exposure level for the main study was close to 1:1, we did not see need to change the calibration curve.

Prepared by and
Principal Investigator
Approval: _____ Date: 9Dec08

Appendix II:

Individual Animal Data Tables

Table of Contents

Table A-1. Individual Maternal Body Weights and Organ Weights (g) (page 1 of 4)

		Gestational Day																		
Dose[a]	Dam ID	0	5	6	7	8	9	10	11	12	13	14	15	16	17	17[b]	Gravid Uterine Wt.	Liver Wt.	Paired Adrenal Gland Wt.	Paired Kidney Wt.
0	1801	23.7	25.3	25.6	26.9	27.8	29.5	29.4	31.0	33.4	35.7	37.4	40.2	42.7	45.7	44.04	14.4811	2.3692	0.0136	0.4460
	1802	27.4	28.6	29.3	30.1	30.7	31.0	32.3	34.7	37.2	40.0	41.5	44.1	49.0	52.3	51.46	19.7607	2.6311	0.0158	0.4819
	1803	27.5	27.3	28.6	28.6	29.3	29.6	29.9	31.9	33.1	34.5	36.4	39.0	41.8	44.2	41.65	13.6798	2.1015	0.0115	0.3775
	1804	24.0	24.5	24.9	25.6	26.2	26.3	27.4	29.6	31.8	34.3	36.3	40.2	43.3	46.1	45.15	17.7023	2.3577	0.0165	0.4112
	1805	27.2	27.3	27.6	28.8	30.0	29.9	30.7	32.9	34.9	36.3	38.2	41.8	44.4	47.7	46.02	16.6375	2.2176	0.0115	0.3908
	1806	27.2	27.5	28.3	29.2	29.5	30.0	31.0	32.9	35.6	37.4	39.6	43.5	46.2	49.3	48.23	18.2375	2.4033	0.0164	0.4395
	1807	23.9	26.2	25.7	26.9	27.3	27.9	29.9	31.7	33.1	35.9	37.2	39.9	43.8	48.4	47.32	18.3623	2.4555	.[c]	0.4020
	1808	26.4	26.7	26.9	27.6	27.7	27.6	28.7	30.3	32.8	35.1	38.3	40.1	43.6	47.8	46.10	17.9075	2.3041	0.0141	0.4291
	1809	26.5	28.4	29.2	29.0	29.6	30.2	31.1	32.4	34.6	36.2	38.0	39.4	41.1	44.4	43.49	14.7802	2.2250	0.0127	0.3379
	1810	27.8	28.4	29.6	30.3	29.7	30.5	31.6	33.7	34.3	36.0	39.0	41.1	43.4	46.2	44.21	13.6742	2.3782	0.0128	0.3950
	1811	27.9	27.6	28.4	28.6	29.5	29.8	31.3	34.0	37.3	40.1	41.2	43.7	48.6	53.1	52.05	20.7503	2.9608	0.0135	0.5055
	1812	25.0	25.0	25.5	25.9	26.7	26.5	28.1	29.3	31.5	33.5	36.2	39.2	42.4	45.3	44.26	17.3355	2.2345	0.0135	0.4018
	1813	27.2	28.3	29.2	30.2	30.7	30.9	32.5	33.5	35.0	36.8	39.1	42.3	44.8	48.3	46.90	15.3242	2.6188	0.0211	0.4432
	1814	27.2	27.7	28.5	29.3	30.1	30.6	32.2	33.3	35.8	37.9	40.9	43.6	46.8	50.3	48.78	17.9133	2.7351	0.0106	0.4211
	1815	28.3	28.6	29.3	30.3	30.9	31.2	32.9	35.3	36.6	38.4	40.6	42.8	45.9	49.2	47.46	16.4148	2.7521	0.0135	0.4091
	1816	28.7	30.0	31.9	32.3	32.5	33.1	34.4	36.8	38.9	40.5	43.5	46.3	48.2	52.4	51.34	19.6942	2.6059	0.0132	0.4968
	1817	23.9	24.6	24.5	24.6	25.1	25.4	26.3	27.8	29.6	31.5	33.6	36.0	38.9	40.5	40.07	13.4026	2.0213	0.0130	0.3758
	1818	28.5	29.9	31.0	30.5	31.1	32.2	33.9	36.8	38.7	40.8	43.2	46.8	49.9	52.5	51.75	20.6578	2.5824	0.0112	0.4805
	1819	28.8	30.0	31.2	30.9	31.2	32.5	34.0	36.1	38.1	40.4	43.0	46.1	49.4	51.8	51.28	20.1298	2.5947	0.0192	0.4735
	1820	25.2	26.8	27.5	28.1	28.8	29.7	30.5	33.2	35.0	36.6	40.0	43.0	46.2	49.7	48.71	18.7321	2.3504	0.0088	0.4119
	1821	24.3	25.4	25.7	26.5	26.6	27.2	28.0	29.3	31.6	33.6	35.5	38.0	41.5	43.9	43.40	15.6224	2.1432	0.0149	0.4096
	1822	28.9	30.7	30.9	31.5	31.5	32.1	33.1	34.3	36.9	39.2	41.1	44.5	48.9	52.4	51.15	18.5153	2.6422	0.0099	0.4761
	1823	28.8	29.0	28.9	29.3	29.6	29.7	31.5	34.0	36.3	38.7	41.6	44.9	48.9	51.8	49.47	17.9535	2.6913	0.0119	0.4204
2000	2801	25.0	27.6	27.9	28.4	28.5	28.9	28.9	29.7	30.2	30.1	30.5	32.0	32.4	33.5	31.58	2.3762	1.8423	0.0139	0.4669
	2802	27.0	29.1	29.9	31.1	31.7	32.3	33.2	34.5	35.8	36.9	38.6	41.0	44.6	46.7	45.48	13.0643	2.4958	0.0162	0.4533
	2803	27.5	27.1	27.3	28.6	28.2	28.5	30.1	34.4	36.3	37.8	40.6	45.8	49.6	52.9	50.08	21.7074	2.1868	0.0110	0.3865
	2804	24.3	25.3	25.4	26.5	27.0	26.8	27.9	29.7	32.3	33.9	36.5	39.5	42.6	44.8	43.98	15.4744	2.4670	0.0114	0.4041
	2805	26.7	26.0	26.2	27.0	28.4	28.3	29.1	30.7	33.1	34.8	36.8	40.2	43.7	45.5	44.36	16.1582	2.2875	0.0159	0.3687
	2806	27.4	27.6	28.8	29.1	29.6	30.2	31.4	34.1	36.1	37.5	40.3	43.9	46.2	50.2	49.83	17.2433	2.7615	0.0162	0.4452
	2807	24.6	24.8	26.4	27.7	27.5	28.0	29.3	31.2	32.9	34.8	36.0	38.4	41.6	44.6	43.51	14.5031	2.6123	0.0154	0.4026
	2808	26.4	28.8	30.3	30.7	30.7	31.2	32.5	34.4	35.9	37.7	39.3	40.8	42.5	45.0	44.13	12.2719	2.5176	0.0146	0.4944
	2809	26.8	30.4	31.0	31.5	32.6	32.9	34.7	36.5	38.8	40.0	40.9	44.1	47.6	49.5	48.54	15.9050	2.9708	0.0161	0.4968
	2810	27.7	30.5	30.1	30.3	31.7	32.0	32.6	35.1	36.4	37.6	38.9	42.0	45.1	48.1	46.70	14.7170	2.5925	0.0142	0.4062
	2811	28.0	28.6	29.0	29.6	30.0	30.7	31.8	33.7	35.4	37.6	39.8	42.1	45.3	47.8	46.65	17.7486	2.3583	0.0141	0.3872
	2812	29.8	30.7	31.9	32.0	32.3	33.6	34.8	37.4	40.7	42.5	45.5	48.7	52.7	56.9	55.09	20.6185	2.9510	0.0177	0.4590
	2813	25.2	25.3	26.0	26.0	26.7	26.6	26.3	26.1	25.9	24.9	24.4	25.1	24.7	26.5	24.98	0.2012	1.4584	0.0094	0.4166
	2814	27.0	26.7	27.6	28.0	28.5	29.0	30.2	32.7	34.6	36.6	37.7	40.1	43.3	46.5	45.18	14.7611	2.5448	0.0227	0.4334

Table A-1. Individual Maternal Body Weights and Organ Weights (g) (page 2 of 4)

		Gestational Day																		
Dose[a]	Dam ID	0	5	6	7	8	9	10	11	12	13	14	15	16	17	17[b]	Gravid Uterine Wt.	Liver Wt.	Paired Adrenal Gland Wt.	Paired Kidney Wt.
20000	2815	27.5	31.4	32.4	32.7	33.4	33.9	36.0	38.0	40.3	41.5	44.1	47.4	51.5	54.2	53.35	18.9077	3.1841	0.0163	0.4782
	2816	28.3	27.8	29.1	30.4	30.6	30.5	32.1	34.6	37.8	39.0	41.8	43.9	48.0	51.4	49.86	18.2901	2.6670	0.0133	0.4295
	2817	28.8	29.8	29.7	30.0	30.6	31.0	32.2	34.0	35.4	36.7	38.7	41.1	43.8	46.8	46.53	17.2023	2.2908	0.0141	0.4120
	2818	25.6	27.7	29.1	30.1	30.1	30.9	32.9	34.9	37.6	39.7	42.4	45.6	49.4	52.8	52.35	21.5024	2.8345	0.0151	0.5062
	2819	28.2	30.7	31.5	31.9	32.5	33.3	34.7	37.2	39.5	42.0	44.1	47.2	50.7	55.0	53.58	18.9426	3.0684	0.0129	0.5620
	2820	29.1	30.3	31.4	31.6	32.7	33.3	35.3	38.4	39.9	41.2	44.5	47.5	50.9	54.7	53.60	18.8395	2.9602	0.0172	0.5350
	2821	.[d]																		
	2822	27.3	27.0	27.7	28.6	28.7	28.8	30.1	31.9	33.9	35.1	38.5	41.2	44.7	47.7	46.32	15.2662	2.5073	0.0069	0.4186
	2823	29.3	30.8	31.5	32.4	32.7	33.3	34.3	36.1	38.3	39.9	42.3	46.2	50.4	54.0	51.95	16.8460	3.1252	0.0127	0.4374
10000	3801	25.4	26.5	26.8	27.3	27.5	28.4	29.6	31.8	33.7	35.5	37.1	39.8	42.3	44.4	44.62	14.7301	2.7557	0.0115	0.4576
	3802	.[d]																		
	3803	27.5	28.0	28.7	29.2	30.3	30.4	31.3	33.4	35.5	37.2	39.3	41.7	44.1	47.1	47.34	16.9253	2.4204	0.0121	0.4191
	3804	25.4	27.5	28.4	29.0	30.2	30.7	33.5	35.1	36.7	38.3	40.7	43.8	46.9	49.7	48.87	16.1002	2.6020	0.0127	0.4318
	3805	26.5	28.2	28.7	29.0	30.1	31.1	32.5	35.6	37.3	38.9	41.5	44.7	46.9	49.3	48.51	17.6251	2.5656	0.0140	0.4162
	3806	27.6	29.4	30.1	31.0	32.0	33.0	34.9	37.6	39.7	42.2	45.0	48.0	51.9	55.2	53.77	19.7811	2.9632	0.0135	0.4945
	3807	25.0	24.8	25.3	25.7	26.7	27.3	28.4	30.5	32.1	33.7	35.2	37.4	41.0	44.3	43.27	14.5264	2.8788	0.0106	0.4709
	3808	26.2	26.3	26.3	27.0	27.3	27.3	28.1	30.1	32.7	34.3	35.8	38.1	40.0	42.5	41.96	13.4726	2.4245	0.0157	0.4236
	3809	26.9	27.4	27.6	28.5	28.8	29.2	30.4	32.1	34.0	35.1	37.0	39.6	42.7	45.2	44.35	16.7280	2.4484	0.0138	0.4345
	3810	27.5	28.9	29.8	30.1	30.5	30.9	31.5	34.2	36.0	38.3	41.1	42.6	45.7	48.9	47.29	16.8350	2.5713	0.0152	0.4403
	3811	28.1	26.1	27.2	27.2	27.9	27.7	28.8	30.3	32.2	34.4	36.5	39.3	41.7	44.3	43.58	14.9477	2.5122	0.0122	0.4060
	3812	29.1	29.1	29.5	30.3	30.7	31.1	32.4	34.3	36.3	38.1	40.7	43.9	47.2	50.3	48.94	18.1387	2.7584	0.0129	0.4366
	3813	25.5	24.2	27.3	27.1	27.1	27.6	29.3	30.5	32.0	34.4	35.7	39.4	41.8	44.8	43.89	15.3821	2.6385	0.0116	0.4457
	3814	.[e]																		
	3815	27.7	29.0	30.5	31.2	31.8	32.7	35.1	37.0	38.4	40.5	42.1	45.7	48.9	52.8	52.65	17.5735	2.904[f]	0.0141	0.4550
	3816	28.2	28.0	29.0	29.6	30.2	30.3	32.0	34.5	36.3	38.7	41.3	43.9	48.0	51.5	50.10	18.6991	2.9257	0.0135	0.4536
	3817	.[d]																		
	3818	25.8	27.3	28.8	28.7	28.5	30.5	30.3	32.4	34.1	35.4	37.2	40.5	43.4	46.1	45.33	14.8345	2.5348	0.0141	0.4111
	3819	28.2	28.8	29.4	29.6	29.9	30.4	32.0	32.9	35.3	37.2	38.6	41.1	44.2	47.6	46.66	16.0488	2.9220	0.0151	0.4295
	3820	26.9	30.3	32.1	32.4	33.6	34.7	35.4	37.9	39.0	41.0	43.2	45.6	48.7	53.2	52.04	16.8824	3.1119	0.0104	0.4274
	3821	27.3	29.7	30.1	30.8	30.6	31.4	33.5	34.6	37.2	38.3	41.2	45.0	48.1	51.3	50.73	18.0837	3.2004	0.0141	0.4744
	3822	28.1	29.1	29.4	29.9	30.8	31.3	31.4	33.7	35.1	37.7	39.8	42.5	46.7	50.1	48.90	17.5758	2.6313	.[c]	0.4213
	3823	.[d]																		
20000	4801	25.7	26.3	26.7	27.7	28.0	28.1	28.6	30.4	32.5	33.8	36.7	39.0	42.6	45.0	44.82	16.7765	2.6705	0.0084	0.4149
	4802	26.4	29.1	29.1	30.3	30.3	31.3	32.0	34.0	36.8	38.8	40.6	43.6	46.3	48.6	48.83	17.0931	2.8105	0.0141	0.5266
	4803	28.0	30.0	30.4	31.3	31.4	31.4	32.2	34.7	36.9	38.6	41.4	43.9	47.3	50.8	50.04	18.8684	2.4162	0.0127	0.4472

Table A-1. Individual Maternal Body Weights and Organ Weights (g) (page 3 of 4)

		Gestational Day																		
Dose[a]	Dam ID	0	5	6	7	8	9	10	11	12	13	14	15	16	17	17[b]	Gravid Uterine Wt.	Liver Wt.	Paired Adrenal Gland Wt.	Paired Kidney Wt.
20000	4804	29.7	29.8	30.5	31.0	31.3	31.7	32.6	34.6	37.6	39.0	41.3	43.5	47.0	49.3	49.19	17.6044	2.6289	0.0172	0.4266
	4805	25.7	26.8	27.6	28.3	28.7	28.7	29.3	30.9	33.5	35.4	37.1	40.3	42.7	46.6	45.87	16.9954	2.6732	0.0150	0.4320
	4806	26.4	28.8	29.2	29.8	30.9	31.0	32.4	33.5	35.6	38.2	39.2	42.5	44.9	47.4	47.48	17.0330	2.8556	0.0132	0.4188
	4807	27.7	29.0	28.9	30.1	30.8	30.7	31.6	34.3	36.2	38.3	41.0	44.2	46.9	50.4	49.18	17.5032	2.8624	0.0165	0.4116
	4808	25.1	26.7	27.6	28.3	28.5	28.9	30.2	32.1	34.3	36.2	38.8	41.8	45.9	49.4	48.53	18.8523	2.8895	0.0168	0.4153
	4809	26.0	29.3	29.6	30.8	31.6	32.3	34.0	36.7	39.6	41.8	44.9	47.5	52.5	54.8	55.12	22.6107	2.9528	0.0137	0.4431
	4810	27.0	26.6	27.5	28.2	28.3	28.8	30.0	31.6	33.5	34.2	37.0	39.7	43.2	46.2	45.12	16.4734	2.7402	0.0161	0.3932
	4811	27.5	28.2	29.9	30.9	31.3	32.5	34.3	36.0	38.2	39.1	42.4	43.6	46.4	51.0	49.15	19.6011	2.8417	0.0131	0.3939
	4812	28.2	27.7	28.2	28.2	28.5	29.1	29.8	30.9	33.2	34.3	36.5	38.7	41.0	43.5	42.80	15.8511	2.2713	0.0131	0.3702
	4813	29.0	29.1	29.5	30.5	30.4	31.8	32.9	36.5	39.2	41.1	44.2	47.4	51.8	55.3	53.28	21.9836	2.9297	0.0106	0.4795
	4814	25.6	27.3	27.7	27.8	28.1	28.4	29.7	31.1	32.1	33.6	34.7	37.5	38.2	40.0	38.65	10.0827	2.2418	0.0152	0.4031
	4815	.[d]																		
	4816	27.7	29.2	30.3	30.2	31.2	31.3	32.0	34.0	35.3	37.4	39.6	43.2	45.7	49.0	47.99	17.4621	2.6378	0.0105	0.4726
	4817	28.2	28.0	29.0	29.5	30.3	30.9	32.0	33.6	35.7	37.5	39.6	42.5	45.9	49.2	48.70	17.1257	2.8046	0.0149	0.4653
	4818	30.1	30.6	30.9	31.3	31.3	32.3	31.9	32.0	31.3	31.9	30.6	30.5	30.9	31.4	29.96	0.1257	1.8489	0.0149	0.4931
	4819	26.0	26.5	26.2	26.3	27.2	27.4	27.3	27.6	27.7	27.0	27.7	27.3	27.3	27.0	26.27	0.1142	1.7097	0.0124	0.4325
	4820	27.9	28.6	29.1	29.0	29.4	30.5	31.7	33.2	35.7	36.7	39.3	41.6	44.4	47.7	46.95	15.0383	2.7616	0.0165	0.4797
	4821	27.3	29.3	29.8	30.3	31.2	31.8	32.4	34.6	36.6	38.9	40.8	44.3	47.7	50.5	50.09	18.7231	2.8499	0.0104	0.3662
	4822	27.4	28.7	29.2	29.7	30.1	30.1	31.0	31.5	34.1	35.9	37.4	40.8	44.4	47.4	45.92	15.4136	2.7219	0.0139	0.4855
	4823	28.1	31.5	31.4	32.2	32.1	33.5	34.0	35.6	37.3	39.3	40.7	43.3	44.7	46.7	46.09	14.5411	2.7599	0.0112	0.4560
30000	5801	.[d]																		
	5802	26.2	27.5	26.8	28.1	28.6	29.5	30.4	32.0	35.1	37.6	40.6	43.6	47.1	50.0	49.36	18.8132	2.2720	0.0147	0.4919
	5803	26.3	27.4	28.0	28.5	29.5	30.0	30.3	33.3	35.3	37.1	39.9	43.5	45.3	48.6	46.62	18.7458	1.9859	0.0092	0.3940
	5804	28.2	29.5	29.5	30.3	31.2	32.0	32.7	34.5	36.5	39.6	42.3	45.8	48.7	50.6	51.20	18.2262	2.4269	0.0115	0.5063
	5805	28.2	28.4	29.3	29.5	30.3	30.9	30.9	32.7	34.5	36.8	39.1	36.0	42.6	46.5	45.64	18.1812	2.0216	0.0131	0.4078
	5806	29.6	30.7	31.0	32.7	33.0	34.2	34.8	36.7	38.5	40.9	44.7	48.6	51.8	55.1	54.57	22.0056	2.3285	0.0131	0.4011
	5807	25.9	26.4	27.1	27.8	29.0	29.6	30.0	31.9	34.7	36.8	40.3	42.8	45.4	48.6	47.78	18.3769	2.3540	0.0126	0.3456
	5808	25.9	26.6	26.7	27.9	29.0	28.9	29.7	32.1	34.1	36.7	39.5	42.0	45.3	47.7	47.72	17.6901	2.3910	0.0138	0.4082
	5809	26.3	26.6	27.2	28.3	29.1	29.3	30.8	32.7	35.4	37.1	40.8	43.6	46.0	49.8	48.81	18.6972	2.2420	0.0139	0.4398
	5810	29.2	29.9	29.9	30.1	31.1	30.7	31.8	33.8	36.7	38.7	41.9	44.6	48.4	51.0	49.73	19.1084	2.5462	0.0119	0.4036
	5811	31.3	31.9	32.1	32.6	33.6	33.8	35.1	37.3	40.3	43.0	47.0	50.6	53.8	57.0	55.58	21.4731	2.8532	0.0148	0.4761
	5812	25.6	27.5	27.3	28.0	28.4	29.4	30.3	31.7	34.2	36.9	38.9	42.0	44.8	47.6	46.86	16.2375	2.5839	0.0155	0.5004
	5813	25.7	26.7	27.0	27.8	27.3	26.5	26.2	26.5	27.1	27.6	27.3	26.7	25.9	27.3	26.21	0.1443	1.7424	0.0151	0.4138
	5814	25.8	28.4	28.4	29.1	29.6	30.1	31.1	32.7	35.7	38.4	40.8	43.5	47.4	50.9	50.04	17.4432	2.8111	0.0134	0.4814
	5815	25.9	26.4	27.3	28.5	28.5	28.8	29.7	31.8	34.2	36.5	38.2	41.2	44.9	47.7	46.86	17.2691	2.5748	0.0122	0.3722
	5816	27.1	28.3	29.4	31.0	31.2	32.3	34.1	35.6	38.4	40.9	43.4	46.8	49.3	51.7	51.43	20.6765	2.6542	0.0140	0.4346
	5817	.[d]																		

Table A-1. Individual Maternal Body Weights and Organ Weights (g) (page 4 of 4)

		Gestational Day																		
Dose[a]	Dam ID	0	5	6	7	8	9	10	11	12	13	14	15	16	17	17[b]	Gravid Uterine Wt.	Liver Wt.	Paired Adrenal Gland Wt.	Paired Kidney Wt.
30000	5818	27.3	27.0	28.1	27.6	27.7	28.7	30.5	32.0	34.6	37.5	39.7	41.4	44.7	48.9	47.49	19.4554	2.1213	0.0136	0.4744
	5819	27.4	29.0	29.6	29.6	30.1	31.2	32.5	34.3	36.4	39.2	42.1	44.9	48.2	51.8	49.80	18.6413	2.5928	0.0122	0.4614
	5820	28.3	28.6	28.8	29.5	29.8	29.9	31.3	32.3	35.3	37.1	39.3	42.6	45.4	48.9	47.34	16.7165	2.3715	0.0106	0.4088
	5821	28.3	29.5	30.5	32.0	32.1	33.3	34.2	35.8	38.2	40.5	41.6	44.5	48.7	51.5	50.76	17.7549	2.8188	0.0123	0.4731
	5822	28.4	28.4	28.3	29.0	28.2	28.5	29.3	30.5	32.8	34.2	36.8	39.3	41.4	44.9	43.85	16.2522	2.0635	0.0116	0.3750
	5823	28.7	29.1	30.0	30.7	31.6	33.1	34.1	36.2	39.2	41.3	44.6	49.0	51.5	55.1	55.01	22.3565	2.3108	0.0144	0.3464
	5824	25.8	24.9	25.5	24.2	23.4	24.0	23.8	24.0	25.2	25.4	25.5	25.2	25.0	25.7	25.39	0.1097	1.4946	0.0172	0.3639
	5825	26.2	26.8	28.5	28.6	29.0	29.8	30.1	31.8	34.4	35.3	36.9	40.3	43.0	46.1	45.25	14.9805	2.3879	0.0143	0.4199
	5826	26.6	27.1	28.3	28.5	28.7	29.2	30.3	32.3	35.8	38.1	41.0	44.1	47.2	50.7	50.20	19.2251	2.7888	0.0131	0.4647
	5827	27.7	26.4	27.2	27.1	27.8	28.1	29.0	31.1	33.7	35.4	37.0	39.9	42.9	46.6	45.81	15.3833	2.3213	0.0143	0.3784
	5828	27.8	28.5	29.3	28.9	29.1	29.9	30.9	32.9	36.5	37.6	39.1	42.7	44.2	47.7	46.68	15.6981	2.2852	0.0151	0.4336
	5829	28.1	28.7	29.1	29.0	29.5	30.1	31.1	33.0	35.4	36.2	38.2	41.1	44.1	47.0	45.62	14.9195	2.4199	0.0111	0.4803
	5830	30.3	33.4	33.1	33.0	33.5	34.3	35.0	37.2	40.1	42.8	45.4	50.5	53.5	58.0	56.48	20.2425	3.0487	0.0150	0.4365
	5831	26.3	28.0	28.2	28.7	29.0	29.5	30.1	33.4	35.4	37.1	39.8	42.9	46.4	49.5	48.76	18.3770	2.6777	0.0132	0.4656
	5832	26.9	30.0	30.2	31.4	32.2	33.2	35.0	37.4	39.3	41.4	44.0	47.3	51.1	54.7	54.13	20.3232	3.2612	0.0151	0.4721
	5833	27.0	27.5	27.6	28.4	28.7	29.2	30.4	32.1	34.5	36.0	38.5	41.3	44.6	47.8	47.02	16.3016	2.7776	0.0161	0.4644
	5834	27.9	27.3	28.0	28.2	29.5	29.1	31.2	32.5	33.6	36.2	38.0	39.7	42.6	45.9	44.84	14.7283	2.3049	0.0131	0.4299
	5835	27.7	30.2	30.4	31.3	31.9	33.4	35.7	37.9	39.7	42.8	45.4	49.3	51.9	56.8	56.05	22.1501	2.7876	0.0131	0.4039
	5836	28.1	28.4	29.3	30.1	30.5	31.6	33.1	34.4	36.6	38.5	41.1	44.9	48.9	52.4	51.13	19.7858	2.3708	0.0158	0.4420
	5837	28.3	29.1	30.0	30.0	30.2	29.9	30.1	29.7	29.9	30.3	30.2	29.7	30.1	31.2	29.40	0.1544	1.8195	0.0131	0.4512
	5838	28.0	28.1	28.7	28.9	30.0	31.3	31.5	32.8	35.1	37.7	39.7	43.1	46.7	49.2	48.33	17.4607	2.5002	0.0145	0.3965

[a]Mg/m^3 of gasoline MTBE vapor condensate.
[b]Body weight at sacrifice.
[c]Paired adrenal gland weight was a statistical outlier and therefore it was excluded.
[d]Female was not pregnant.
[e]Female was removed due to a preexisting condition. At necropsy she was found to have an undescended testis on the right and seminal vesicles and prostate to the right of the vagina and cervix.
[f]Liver weight was inadvertently recorded to only 3 decimal places.

Table A-2. Individual Maternal Clinical Observations (page 1 of 1)

Dose[a]	Dam ID	Day[b]	Time[c]	Observation
10000	3808	12	Prior	Red exudate from anogenital area
20000	4803	6	Post	Lacrimation, unilateral, moderate
	4807	5	Post	Alopecia: extremities/snout, moderate
		6	Prior	Alopecia: extremities/snout, moderate
			Post	Alopecia: extremities/snout, moderate
		7	Prior	Alopecia: extremities/snout, moderate
			Post	Alopecia: extremities/snout, moderate
		8	Prior	Alopecia: extremities/snout, moderate
			Post	Alopecia: extremities/snout, moderate
		9	Prior	Alopecia: extremities/snout, moderate
			Post	Alopecia: extremities/snout, moderate
		10	Prior	Alopecia: extremities/snout, moderate
			Post	Alopecia: extremities/snout, moderate
		11	Prior	Alopecia: extremities/snout, moderate
			Post	Alopecia: extremities/snout, moderate
		12	Prior	Alopecia: extremities/snout, moderate
			Post	Alopecia: extremities/snout, moderate
	4814	11	Post	Red exudate from anogenital area
	4823	9	Post	Labored breathing
30000	5802	6	Post	Lacrimation, unilateral, moderate
	5805	6	Post	Lacrimation, bilateral, moderate
	5824	5	Post	Lacrimation, unilateral, moderate
	5829	2	Prior	Eye: enophthalmos, unilateral, left
		3	Prior	Eye: enophthalmos, unilateral, left
		4	Prior	Eye: enophthalmos, unilateral, left
		5	Prior	Eye: enophthalmos, unilateral, left
			Post	Eye: enophthalmos, unilateral, left
		6	Prior	Eye: enophthalmos, unilateral, left
			Post	Eye: enophthalmos, unilateral, left
		7	Prior	Eye: enophthalmos, unilateral, left
			Post	Eye: enophthalmos, unilateral, left
		8	Prior	Eye: enophthalmos, unilateral, left
			Post	Eye: enophthalmos, unilateral, left
		9	Prior	Eye: enophthalmos, unilateral, left
			Post	Eye: enophthalmos, unilateral, left
		10	Prior	Eye: enophthalmos, unilateral, left
			Post	Eye: enophthalmos, unilateral, left
		11	Prior	Eye: enophthalmos, unilateral, left
		12	Prior	Eye: enophthalmos, unilateral, left
		13	Prior	Eye: enophthalmos, unilateral, left
		14	Prior	Eye: enophthalmos, unilateral, left
		15	Prior	Eye: enophthalmos, unilateral, left
		16	Prior	Eye: enophthalmos, unilateral, left
		17	Prior	Eye: enophthalmos, unilateral, left
	5838	10	Post	Labored breathing

[a]Mg/m^3 of gasoline MTBE vapor condensate.
[b]Gestational day.
[c]Time is prior to dosing (Prior) or after dosing (Post).

Table A-3. Individual Maternal Feed Consumption (g/day)[a] (page 1 of 5)

		Gestational Days																
Dose[b]	Dam ID	0-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	5-10[c]	5-16[d]	10-17[c]	0-17
0	1801	.[e]	5.6	6.4	6.8	7.4	6.8	8.8	8.6	7.3	7.8	8.0	8.0	8.8	6.6	7.4	8.2	.[f]
	1802	5.5	6.0	6.2	6.2	6.8	6.9	7.2	7.6	7.5	7.3	7.4	8.7	8.5	6.4	7.1	7.7	6.7
	1803	.[e]	.[e]	.[e]	.[g]	10.4	8.5	6.8	6.6	6.7	8.6	7.4	7.4	9.4	.[f]	.[f]	7.6	.[f]
	1804	5.6	5.1	5.8	5.8	6.8	5.8	9.4	8.2	7.0	6.8	7.2	7.9	7.9	5.9	6.9	7.8	6.6
	1805	7.3	6.2	6.4	6.5	6.5	6.5	.[e]	9.8	6.9	6.8	7.1	7.3	7.4	6.4	.[f]	.[f]	.[f]
	1806	4.9	4.9	5.9	5.7	5.7	5.6	6.1	6.0	6.5	6.2	7.5	7.1	7.1	5.6	6.1	6.6	5.8
	1807	6.0	5.8	7.9	7.3	9.8	6.3	6.7	6.7	7.0	7.4	7.1	7.0	6.8	7.4	7.2	7.0	6.8
	1808	5.4	5.2	5.4	5.5	5.6	5.9	5.2	5.5	5.7	6.6	6.2	6.1	6.6	5.5	5.7	6.0	5.7
	1809	5.0	5.4	5.5	6.0	5.4	6.1	5.7	6.2	6.1	6.2	6.4	6.2	6.4	5.7	5.9	6.2	5.7
	1810	6.5	8.7	7.1	6.1	6.6	6.7	7.7	6.6	6.4	7.9	7.3	7.0	7.9	7.0	7.1	7.3	7.0
	1811	.[e]	.[g]	.[g]	6.9	14.5	8.0	7.3	11.3	7.6	7.0	7.3	8.2	8.5	10.4	9.1	8.2	.[f]
	1812	.[e]	4.1	5.1	.[g]	.[e]	6.7	.[e]	5.6	5.8	7.5	8.2	6.6	7.1	.[f]	.[f]	.[f]	.[f]
	1813	5.3	6.6	6.1	8.8	8.3	6.0	6.3	5.8	6.8	8.4	6.9	8.2	8.0	7.2	7.1	7.2	6.6
	1814	.[e]	8.4	.[g]	9.0	9.7	9.7	.[e]	8.3	8.2	8.1	7.9	7.9	8.1	10.6	.[f]	.[f]	.[f]
	1815	.[e]	6.0	6.8	7.2	8.2	7.1	7.3	6.9	7.1	7.9	6.9	7.5	10.2	7.1	7.2	7.7	.[f]
	1816	7.4	8.1	.[g]	9.7	10.1	8.6	.[e]	10.2	7.3	7.6	8.2	6.5	7.9	10.8	.[f]	.[f]	.[f]
	1817	7.5	.[e]	.[e]	12.2	.[e]	8.3	8.1	11.0	9.2	6.5	7.9	7.6	5.6	.[f]	.[f]	8.0	.[f]
	1818	7.4	7.1	5.4	6.0	5.6	5.8	6.4	10.8	9.5	7.9	7.1	8.4	7.8	6.0	7.3	8.3	7.3
	1819	5.0	6.1	5.5	6.7	5.2	6.0	6.6	7.2	8.1	7.1	8.2	8.9	7.2	5.9	6.9	7.6	6.3
	1820	5.2	6.0	6.3	5.8	6.5	5.7	6.3	7.4	7.1	7.1	7.1	7.6	6.9	6.1	6.6	7.1	6.2
	1821	7.7	4.4	6.5	7.3	7.8	6.1	6.5	7.9	6.2	6.3	8.0	7.2	6.6	6.4	6.7	7.0	7.0
	1822	5.7	5.5	5.2	5.8	5.4	6.0	6.5	5.8	6.3	7.3	7.3	6.9	7.3	5.6	6.2	6.8	6.1
	1823	6.2	6.3	5.7	5.7	6.9	5.6	6.1	7.0	7.0	7.0	6.8	7.5	7.0	6.0	6.5	6.9	6.5
2000	2801	5.8	6.1	5.3	6.4	7.6	6.3	6.2	6.2	6.3	6.1	6.2	5.6	5.9		6.2		6.1
	2802	6.3	6.9	9.5	8.0	6.5	8.3	7.0	6.9	7.1	7.0	6.0	7.8	8.0		7.4		7.1
	2803	5.3	5.8	6.2	9.5	4.5	6.0	7.5	6.7	6.2	.[e]	.[e]	7.0	7.4		.[f]		.[f]
	2804	.[e]	5.5	11.2	6.6	7.7	7.5	6.5	8.4	7.6	7.4	6.9	6.3	7.2		7.4		.[f]
	2805	4.7	4.8	5.5	6.2	6.5	6.2	6.1	6.7	6.9	6.6	6.9	7.3	6.7		6.3		5.9
	2806	.[h]	7.7	16.0	8.2	.[h]	6.8	7.5	8.0	6.1	.[h]	6.8	7.9	7.3		.[f]		.[f]
	2807	.[h]	8.1	.[g]	10.9	.[g]	10.6	10.6	.[e]	7.5	7.1	6.3	8.2	6.9		.[f]		.[f]
	2808	7.9	9.5	8.2	7.5	6.9	7.2	9.1	8.1	7.7	8.1	9.0	8.5	7.9		8.2		8.1
	2809	7.4	5.9	5.6	6.7	5.7	7.2	8.4	7.3	6.9	6.1	8.8	6.9	7.7		6.9		7.1
	2810	7.5	6.0	7.1	7.7	6.8	6.9	7.6	7.2	8.0	7.1	9.3	8.1	7.6		7.4		7.5

Table A-3. Individual Maternal Feed Consumption (g/day)[a] (page 2 of 5)

		Gestational Days																
Dose[b]	Dam ID	0-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	5-10[c]	5-16[d]	10-17[c]	0-17
2000	2811	5.2	5.5	6.3	5.8	6.3	6.0	6.0	6.2	6.3	6.3	7.1	9.8	6.0		6.5		6.1
	2812	8.2	.[e]	12.8	9.8	11.4	8.0	8.1	7.8	11.4	11.2	8.8	9.0	9.3		.[f]		.[f]
	2813	4.3	5.2	5.7	5.5	4.3	5.2	4.6	4.8	4.3	4.4	4.4	4.9	4.3		4.8		4.6
	2814	6.6	8.2	6.0	6.2	6.7	6.8	7.5	7.9	7.4	7.3	7.5	8.1	8.6		7.2		7.1
	2815	9.5	9.0	.[e]	8.2	8.3	7.5	9.3	8.8	7.0	9.0	8.0	8.4	7.3		.[f]		.[f]
	2816	7.7	7.2	7.6	7.7	7.9	7.0	7.3	7.6	7.5	9.0	7.7	9.0	8.8		7.8		7.8
	2817	4.7	5.3	5.2	5.9	5.5	5.5	5.4	5.9	5.7	6.8	5.5	5.9	6.3		5.7		5.4
	2818	4.9	6.5	6.2	7.2	6.3	.[e]	8.6	6.9	7.3	7.8	7.5	7.5	7.3		.[f]		.[f]
	2819	5.5	7.0	6.9	7.1	7.2	6.9	10.2	7.3	8.7	8.4	8.3	8.5	8.4		7.9		7.2
	2820	8.2	8.2	9.5	7.4	7.4	7.9	8.4	7.7	8.6	8.2	8.5	8.7	8.7		8.2		8.3
	2821	.[i]																
	2822	.[h]	5.0	7.0	6.5	6.5	5.5	7.3	5.9	.[h]	7.9	6.6	8.3	6.8		.[f]		.[f]
	2823	7.8	6.7	.[h]	.[g]	.[g]	7.9	7.4	7.1	8.6	7.8	9.3	9.1	9.0		.[f]		.[f]
10000	3801	5.5	5.6	5.8	4.9	6.9	5.7	6.8	6.8	7.2	7.6	7.3	6.9	8.8		6.5		6.4
	3802	.[i]																
	3803	.[e]	.[e]	8.4	10.8	.[h]	6.1	6.7	.[e]	12.0	8.0	7.9	7.3	8.1		.[f]		.[f]
	3804	.[e]	5.9	9.4	6.8	6.6	8.1	7.4	7.3	8.3	10.3	8.2	8.2	8.4		7.9		.[f]
	3805	.[e]	.[e]	10.0	8.1	7.7	7.3	8.9	8.1	7.7	7.7	7.8	8.0	7.0		.[f]		.[f]
	3806	6.7	6.8	11.4	8.2	8.0	7.2	8.1	7.7	8.3	8.9	7.4	10.2	8.3		8.4		7.9
	3807	4.9	5.8	6.5	6.0	6.2	6.0	6.3	6.8	7.0	7.1	6.1	7.3	7.1		6.5		6.0
	3808	.[e]	6.0	8.1	6.9	9.3	8.4	6.3	10.7	6.9	6.9	7.2	6.6	6.9		7.6		.[f]
	3809	6.0	4.9	5.6	5.3	6.5	7.7	6.6	7.9	8.4	.[e]	6.6	7.3	6.7		.[f]		.[f]
	3810	8.9	13.0	8.8	7.4	7.2	7.0	7.2	7.6	8.3	7.7	8.6	7.6	8.7		8.2		8.5
	3811	7.9	10.9	.[e]	9.7	7.0	8.5	7.1	6.9	7.2	7.3	7.4	7.1	7.4		.[f]		.[f]
	3812	5.4	6.0	5.7	6.0	5.9	6.4	6.4	6.5	7.0	7.4	8.1	7.5	7.2		6.6		6.3
	3813	.[e]	9.0	9.2	6.9	.[e]	7.4	7.7	7.5	7.3	8.0	7.5	7.6	7.0		.[f]		.[f]
	3814	.[j]																
	3815	.[e]	14.5	8.7	12.4	11.8	8.1	8.6	8.9	8.4	9.0	9.1	9.0	8.7		9.9		.[f]
	3816	5.8	6.6	6.0	6.4	10.4	6.2	9.5	7.3	6.7	8.3	6.8	7.4	7.3		7.4		6.9
	3817	.[i]																
	3818	.[e]	.[e]	.[e]	11.9	.[h]	.[e]	9.5	9.1	8.4	7.6	10.5	11.7	7.9		.[f]		.[f]
	3819	5.2	5.4	5.8	5.9	5.2	6.7	.[e]	6.2	7.7	6.8	6.7	6.8	7.1		.[f]		.[f]
	3820	.[e]	9.3	8.1	17.8	8.9	8.9	7.7	9.1	8.7	7.7	7.9	8.9	9.2		9.4		.[f]

Table A-3. Individual Maternal Feed Consumption (g/day)[a] (page 3 of 5)

		Gestational Days																
Dose[b]	Dam ID	0-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	5-10[c]	5-16[d]	10-17[c]	0-17
10000	3821	6.5	7.6	7.2	6.4	7.0	8.0	7.5	7.6	7.6	9.4	9.4	8.7	8.3		7.9		7.5
	3822	5.1	5.3	5.8	7.3	5.8	7.0	6.8	6.0	9.6	6.9	7.2	6.8	7.4		6.8		6.3
	3823	.[i]																
20000	4801	4.2	5.2	6.2	5.9	5.3	6.1	5.9	5.7	6.1	6.4	7.2	6.5	8.0		6.0		5.6
	4802	5.7	6.1	6.5	6.0	6.6	6.5	7.1	7.5	7.8	7.7	8.4	6.8	8.3		7.0		6.7
	4803	.[e]	.[g]	.[g]	.[g]	8.4	6.7	.[g]	.[g]	8.8	10.2	7.7	7.4	9.9		10.2		.[f]
	4804	5.1	5.0	6.1	5.6	5.5	5.5	5.5	6.4	6.2	6.6	6.9	7.1	8.9		6.0		5.9
	4805	5.4	5.9	6.3	6.0	5.7	5.6	6.0	6.3	6.5	6.9	6.1	7.1	6.8		6.2		6.0
	4806	.[e]	6.0	.[g]	6.1	6.4	6.8	6.2	.[e]	7.1	7.2	6.4	7.7	6.2		.[f]		.[f]
	4807	.[e]	7.8	9.3	.[g]	.[g]	5.7	.[g]	6.7	7.4	7.9	7.6	8.4	7.0		8.6		.[f]
	4808	4.8	5.6	6.0	5.7	5.6	5.5	5.7	6.7	6.7	6.5	7.2	7.0	7.0		6.2		5.8
	4809	6.2	5.9	6.8	7.5	5.8	6.6	6.8	6.8	7.1	6.9	9.1	7.1	6.4		6.9		6.7
	4810	.[e]	7.3	5.0	7.1	5.2	7.3	6.3	7.1	7.3	6.8	8.4	6.3	6.9		6.7		.[f]
	4811	4.4	5.8	6.0	6.3	6.5	5.7	5.8	6.1	6.1	6.3	6.7	5.5	7.7		6.1		5.7
	4812	4.0	4.9	4.7	5.1	5.0	5.1	5.1	5.0	5.2	5.3	7.7	5.9	6.5		5.4		5.0
	4813	5.6	6.1	6.0	6.7	6.7	6.6	7.4	7.4	7.5	7.9	9.7	8.4	8.1		7.3		6.8
	4814	5.3	9.8	.[e]	5.8	6.7	6.2	6.3	6.8	6.1	7.7	6.1	6.3	6.8		.[f]		.[f]
	4815	.[i]																
	4816	5.4	7.1	6.7	6.2	6.5	6.4	6.9	7.2	6.9	8.8	7.6	7.8	8.0		7.1		6.7
	4817	5.4	5.7	7.0	6.4	5.9	6.0	5.7	6.8	6.3	8.2	6.4	7.2	7.1		6.5		6.2
	4818	5.8	6.7	6.7	5.1	6.5	5.2	6.4	6.1	6.2	7.6	4.2	5.7	6.4		6.0		6.0
	4819	4.9	5.0	4.3	5.3	5.2	5.6	6.7	5.5	7.0	5.2	4.4	4.9	4.1		5.4		5.2
	4820	.[h]	6.2	.[e]	.[e]	.[e]	.[e]	8.3	8.6	8.3	.[e]	8.1	7.4	7.1		.[f]		.[f]
	4821	.[e]	5.5	5.6	6.1	5.3	.[h]	5.2	7.1	5.1	6.9	6.9	6.7	7.1		.[f]		.[f]
	4822	6.1	6.5	6.8	8.0	7.8	6.2	7.7	6.9	7.6	6.9	7.2	7.8	8.7		7.2		7.0
	4823	6.0	6.6	.[g]	.[e]	6.2	7.6	5.7	7.3	7.6	5.6	7.0	6.1	6.9		.[f]		.[f]
30000	5801	.[i]																
	5802	5.4	4.7	5.5	5.4	5.6	6.7	6.5	11.9	7.3	7.7	6.9	7.1	7.7	5.6		7.9	6.5
	5803	5.3	5.9	5.9	5.7	5.6	6.1	6.1	6.7	6.6	7.2	7.1	6.7	8.2	5.8		6.9	6.1
	5804	5.2	5.4	6.5	5.8	5.6	6.9	6.1	7.2	7.9	7.4	7.6	7.7	7.4	6.0		7.3	6.3
	5805	.[h]	5.7	5.8	5.7	5.5	5.8	5.2	6.0	8.1	7.2	3.9	6.8	7.6	5.7		6.4	.[f]

Table A-3. Individual Maternal Feed Consumption (g/day)[a] (page 4 of 5)

		Gestational Days																
Dose[b]	Dam ID	0-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	5-10[c]	5-16[d]	10-17[c]	0-17
30000	5806	.[h]	5.9	7.0	6.2	6.2	6.6	.[h]	.[e]	8.3	7.2	6.4	7.0	8.0	6.4		.[f]	.[f]
	5807	7.6	6.0	6.3	6.8	6.6	6.2	5.9	7.9	7.4	7.4	8.1	7.2	7.1	6.4		7.3	7.1
	5808	8.3	5.3	6.4	.[h]	5.0	6.8	6.2	7.1	6.2	7.4	8.1	6.5	.[h]	.[f]		.[f]	.[f]
	5809	.[h]	6.1	5.7	6.1	.[h]	6.1	5.5	8.4	.[h]	10.3	8.0	7.8	8.1	.[f]		.[f]	.[f]
	5810	5.4	6.5	5.0	5.3	5.7	6.9	6.7	6.5	7.6	7.3	7.3	6.8	7.9	5.9		7.2	6.3
	5811	5.6	6.3	7.9	7.0	7.0	6.0	6.9	7.3	8.2	8.1	8.3	8.6	9.0	6.8		8.1	7.0
	5812	9.4	5.7	5.6	6.4	7.0	6.4	6.4	9.7	7.8	8.5	10.0	8.8	8.8	6.2		8.6	8.1
	5813	.[e]	.[g]	6.2	7.3	6.5	6.8	6.3	7.2	5.9	6.5	5.0	3.7	4.8	7.6		5.6	.[f]
	5814	5.3	5.7	5.2	6.5	6.4	5.8	6.5	7.0	9.6	8.0	7.4	8.2	8.2	5.9		7.8	6.5
	5815	10.4	6.0	6.2	5.8	6.2	5.7	5.9	6.9	7.9	7.8	7.8	7.0	6.5	6.0		7.1	7.7
	5816	6.2	6.5	8.9	7.1	6.1	6.5	6.1	7.4	6.8	8.2	8.3	7.0	7.1	7.0		7.3	6.9
	5817	.[i]																
	5818	4.7	6.3	4.6	6.3	5.8	5.7	6.7	7.2	6.6	7.7	5.6	.[h]	6.8	5.7		.[f]	.[f]
	5819	6.4	.[e]	.[e]	7.4	6.4	6.2	.[g]	7.6	7.9	8.3	8.4	8.1	7.6	.[f]		8.4	.[f]
	5820	9.0	5.1	5.4	6.9	5.3	5.6	5.6	6.3	11.4	8.5	7.2	7.2	7.1	5.7		7.6	7.4
	5821	11.1	6.8	6.5	7.7	7.2	8.0	7.2	.[g]	7.3	7.8	8.1	8.6	8.5	7.2		8.9	9.0
	5822	5.2	5.4	4.6	7.0	6.5	6.5	4.9	5.4	5.3	6.9	6.7	6.2	6.6	6.0		6.0	5.8
	5823	5.7	6.7	5.3	6.6	5.8	6.1	6.2	7.2	6.9	8.7	8.8	8.1	8.3	6.1		7.7	6.7
	5824	.[e]	4.7	4.7	3.9	5.0	5.2	4.7	6.8	6.2	6.3	6.6	3.7	4.2	4.7		5.5	.[f]
	5825	5.1	5.9	6.7	6.2	5.9	5.5	5.8	6.4	7.7	7.1	9.0	7.4	6.7	6.0		7.2	6.2
	5826	.[e]	6.2	7.4	5.8	6.2	6.6	6.3	8.7	8.6	9.4	9.1	7.7	7.5	6.4		8.2	.[f]
	5827	4.5	4.5	5.6	5.0	4.9	5.6	5.3	6.4	6.8	7.5	7.4	6.9	7.3	5.1		6.8	5.6
	5828	5.3	6.0	6.8	6.2	6.4	7.1	6.3	8.0	8.3	8.4	8.3	7.0	7.5	6.5		7.7	6.6
	5829	10.0	5.7	.[e]	.[g]	7.0	7.4	7.0	9.2	10.2	8.8	8.8	8.8	8.6	.[f]		8.8	.[f]
	5830	7.2	6.6	.[e]	9.3	6.6	6.5	6.6	13.8	9.1	9.0	8.5	8.8	8.3	.[f]		9.2	.[f]
	5831	.[e]	6.7	6.5	6.0	6.2	5.7	6.5	8.6	7.5	8.5	9.2	7.1	8.2	6.2		7.9	.[f]
	5832	7.5	7.7	8.4	7.7	6.9	6.8	6.8	8.8	8.5	8.1	9.2	8.8	8.9	7.5		8.4	7.9
	5833	.[e]	.[g]	10.3	8.3	.[e]	.[e]	.[g]	9.6	9.4	9.6	7.9	6.9	6.8	.[f]		9.5	.[f]
	5834	.[h]	4.5	.[h]	7.7	.[h]	6.0	6.0	.[h]	.[g]	10.1	6.0	6.2	8.5	.[f]		.[f]	.[f]
	5835	6.3	5.0	6.4	5.7	8.8	6.9	7.6	9.6	8.3	8.8	7.9	7.8	7.2	6.6		8.2	7.2
	5836	.[e]	8.1	6.8	5.8	5.9	6.4	6.6	8.2	7.9	8.4	8.7	7.2	7.1	6.6		7.7	.[f]
	5837	6.6	6.3	5.8	6.1	5.5	5.2	6.1	6.6	5.5	6.8	6.0	4.6	4.9	5.8		5.8	6.0
	5838	.[e]	.[e]	6.1	5.3	5.7	6.4	5.7	6.9	8.5	7.7	7.4	7.5	7.9	.[f]		7.4	.[f]

Table A-3. Individual Maternal Feed Consumption (g/day)[a] (page 5 of 5)

[a]These data represent the difference between the new feed jar weights (beginning weight; full feed jar) and the old feed jar (end weight; after the animal has fed for the measurement interval) weights divided by the number of days in the measurement interval.
[b]Mg/m^3 of gasoline MTBE vapor condensate.
[c]This endpoint was only calculated for the 0 and 30000 mg/m^3 dose groups.
[d]This endpoint was only calculated for the 0, 2000, 10000 and 20000 mg/m^3 dose groups.
[e]Feed spilled therefore the feed weight was excluded.
[f]Interim feed consumption value(s) are missing and therefore the overall feed consumption value could not be calculated.
[g]Feed consumption value was a statistical outlier and therefore it was excluded.
[h]Feed was contaminated therefore the feed weight was excluded.
[i]Female was not pregnant.
[j]Female was removed due to a preexisting condition. At necropsy she was found to have an undescended testis on the right and seminal vesicles and prostate to the right of the vagina and cervix.

Table A-4. Individual Embryo/Fetal Data (page 1 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
0	1801	11	1	A	L	1	F	1.0230			
			2	A	L	2	M	0.8182			
			3	A	L	3	M	0.9328			
			4	D	L			0.7402			
			5	E	R						
			6	A	R	4	M	0.9507			
			7	A	R	5	M	0.9290			
			8	A	R	6	F	0.9828			
			9	A	R	7	F	1.0268			
			10	A	R	8	M	0.9286			
			11	A	R	9	M	0.9976	External	Variation	Hematoma: Head
									External	Variation	Hematoma: Neck
	1802	13	1	A	L	1	F	1.0746			
			2	A	L	2	M	1.0913			
			3	A	L	3	F	1.0557			
			4	A	L	4	M	1.0646			
			5	A	R	5	M	1.0013			
			6	A	R	6	F	1.0405			
			7	A	R	7	F	0.9931			
			8	A	R	8	F	1.0460			
			9	A	R	9	M	1.1404			
			10	A	R	10	M	1.1569			
			11	A	R	11	M	1.0598			
			12	A	R	12	F	1.0618			
			13	A	R	13	F	1.0505			
	1803	9	1	A	L	1	F	1.2866			
			2	A	L	2	F	1.4021			
			3	E	L						
			4	A	R	3	M	1.3324			
			5	A	R	4	F	1.2360			
			6	A	R	5	F	0.9690	External	Malformation	Cleft Palate
			7	A	R	6	F	1.1523	External	Variation	Hematoma: Head
			8	A	R	7	F	1.2401			
			9	A	R	8	M	1.3432			
	1804	14	1	A	L	1	F	1.0263			
			2	A	L	2	M	1.0068			
			3	A	L	3	M	0.9753			
			4	A	L	4	F	0.8615			
			5	A	L	5	F	0.9291			
			6	A	R	6	M	1.0035			
			7	A	R	7	M	0.9158			
			8	A	R	8	M	0.9942			
			9	A	R	9	F	0.9321			
			10	A	R	10	F	0.9239			
			11	A	R	11	M	0.9972			
			12	A	R	12	F	0.9071			
			13	A	R	13	F	0.9282			
	1805	13	1	A	L	1	M	1.0623			
			2	A	L	2	M	1.0939			
			3	A	L	3	M	1.0992			
			4	A	L	4	M	1.0797			
			5	A	L	5	F	1.0217			
			6	A	R	6	F	1.0826			
			7	A	R	7	F	1.0288			
			8	A	R	8	M	1.0531			
			9	A	R	9	F	1.0119			
			10	A	R	10	M	1.0624			
			11	A	R	11	F	0.8523			
			12	A	R	12	F	1.0232			

Table A-4. Individual Embryo/Fetal Data (page 2 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
0	1806	14	1	A	L	1	F	1.0370			
			2	A	L	2	M	1.0681			
			3	A	L	3	M	0.8841			
			4	A	L	4	M	0.9099			
			5	A	L	5	M	0.9751			
			6	A	L	6	M	0.7932			
			7	A	L	7	M	0.9739			
			8	A	L	8	M	1.0688			
			9	A	R	9	M	1.1047			
			10	A	R	10	M	0.9852			
			11	A	R	11	F	0.9760			
			12	A	R	12	M	1.0491			
			13	A	R	13	F	1.0917			
	1807	15	1	A	L	1	M	0.8745			
			2	A	L	2	F	0.9240			
			3	A	L	3	M	0.8271			
			4	A	L	4	F	0.9596			
			5	A	L	5	M	0.8050			
			6	A	L	6	M	0.9051			
			7	A	L	7	M	0.8972			
			8	A	L	8	M	0.9840			
			9	A	R	9	M	0.9552			
			10	A	R	10	M	0.9733			
			11	E	R						
			12	A	R	11	M	1.0288			
			13	A	R	12	F	0.9371			
			14	A	R	13	F	0.8945			
			15	A	R	14	M	0.9640			
	1808	15	1	A	L	1	F	0.8116			
			2	A	L	2	M	0.8444			
			3	A	L	3	M	0.8401			
			4	A	L	4	M	0.7738			
			5	A	L	5	F	0.7672			
			6	A	L	6	F	0.8215			
			7	A	R	7	F	0.8106			
			8	A	R	8	M	0.7823			
			9	A	R	9	M	0.7679			
			10	A	R	10	M	0.6397			
			11	A	R	11	F	0.7671			
			12	A	R	12	M	0.8271			
			13	A	R	13	M	0.8018			
			14	A	R	14	M	0.7566			
			15	A	R	15	M	0.8540			
	1809	11	1	A	L	1	F	0.9641			
			2	A	L	2	F	1.0304			
			3	A	R	3	M	0.6548			
			4	A	R	4	M	0.8226			
			5	A	R	5	F	0.8699			
			6	A	R	6	F	0.8221			
			7	A	R	7	M	0.8814			
			8	A	R	8	M	0.9828			
			9	A	R	9	F	0.8574			
			10	A	R	10	F	0.8405			
			11	A	R	11	F	0.8094			

Table A-4. Individual Embryo/Fetal Data (page 3 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
0	1810	13	1	A	L	1	M	1.1877			
			2	A	L	2	M	0.9171			
			3	A	L	3	M	1.1916			
			4	A	L	4	F	1.1147			
			5	E	R						
			6	E	R						
			7	A	R	5	F	1.1019			
			8	A	R	6	F	1.2054			
			9	A	R	7	F	1.0496			
			10	A	R	8	F	1.1505			
			11	E	R						
			12	A	R	9	M	1.0525			
	1811	14	1	A	L	1	M	1.1393			
			2	A	L	2	F	1.0167			
			3	A	L	3	M	1.0642			
			4	A	L	4	M	1.0878			
			5	A	L	5	F	1.0595			
			6	A	L	6	M	1.0034			
			7	A	L	7	M	0.9544			
			8	A	L	8	M	0.9429			
			9	A	L	9	M	1.0414			
			10	A	R	10	M	1.1475			
			11	A	R	11	M	1.0808			
			12	A	R	12	F	1.0640			
			13	A	R	13	F	1.0829			
			14	A	R	14	M	1.0891			
	1812	13	1	A	L	1	F	0.9006			
			2	A	L	2	M	0.8712			
			3	A	L	3	F	0.8832			
			4	A	R	4	F	0.8264			
			5	A	R	5	F	0.8169			
			6	A	R	6	F	0.7940			
			7	A	R	7	M	0.8558			
			8	A	R	8	M	0.8438			
			9	A	R	9	F	0.8537			
			10	A	R	10	F	0.7710			
			11	A	R	11	F	0.8807			
			12	A	R	12	M	0.8740			
			13	A	R	13	M	0.8937			
	1813	11	1	A	L	1	F	1.0101			
			2	A	L	2	M	1.1114			
			3	A	L	3	M	1.1098			
			4	A	L	4	M	1.0110			
			5	A	L	5	M	0.9292			
			6	A	L	6	F	1.0585			
			7	A	L	7	M	1.0834			
			8	A	R	8	M	1.1358			
			9	E	R						
			10	A	R	9	F	1.0958			
			11	A	R	10	F	1.0459			

Table A-4. Individual Embryo/Fetal Data (page 4 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
0	1814	14	1	A	L	1	F	0.9528			
			2	A	L	2	F	0.7076			
			3	A	L	3	F	0.9589			
			4	A	L	4	F	1.0443			
			5	A	L	5	M	1.0409			
			6	A	L	6	F	1.0072			
			7	A	R	7	M	0.9022			
			8	A	R	8	F	0.9146			
			9	A	R	9	F	0.8631			
			10	D	R			0.6018			
			11	A	R	10	F	0.9350			
			12	A	R	11	M	0.9835			
			13	E	R						
			14	A	R	12	F	1.0240			
	1815	11	1	A	L	1	M	1.0836			
			2	A	L	2	F	0.9789			
			3	E	L						
			4	A	L	3	M	1.0703			
			5	A	L	4	M	0.9151			
			6	A	L	5	M	1.0118			
			7	A	L	6	F	1.1307			
			8	A	R	7	F	1.0776			
			9	A	R	8	M	1.0655			
			10	A	R	9	F	1.1202			
			11	A	R	10	F	0.9812			
			12	A	R	11	M	1.1028			
	1816	14	1	A	L	1	M	0.9686	External	Malformation	Cleft Palate
			2	A	L	2	M	1.0881			
			3	A	L	3	M	1.0086			
			4	A	L	4	F	0.9104			
			5	A	L	5	F	0.9647			
			6	A	L	6	M	1.1311			
			7	A	L	7	F	1.1062			
			8	A	R	8	F	1.0862			
			9	A	R	9	F	1.0008			
			10	A	R	10	F	1.0492			
			11	A	R	11	F	1.0072			
			12	A	R	12	M	1.1214			
			13	A	R	13	M	1.1400			
			14	A	R	14	M	1.0859			
	1817	11	1	A	L	1	M	0.8516			
			2	A	L	2	M	0.8587			
			3	A	L	3	M	0.8686			
			4	A	L	4	F	0.8578			
			5	A	L	5	F	0.9120			
			6	A	L	6	F	0.8655			
			7	A	R	7	F	0.8314			
			8	A	R	8	F	0.9139			
			9	A	R	9	F	1.0210			
			10	E	R						
			11	A	R	10	M	0.7935			

Table A-4. Individual Embryo/Fetal Data (page 5 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
0	1818	17	1	A	L	1	M	1.3283			
			2	A	L	2	M	1.4374			
			3	A	L	3	F	1.2843			
			4	A	L	4	F	1.3751			
			5	A	L	5	M	1.4098			
			6	E	R						
			7	A	R	6	F	1.3769			
			8	A	R	7	F	1.1389			
			9	D	R			0.8652			
			10	A	R	8	M	1.2370			
			11	A	R	9	F	0.9656			
			12	A	R	10	F	1.1105			
			13	A	R	11	F	1.1893			
			14	A	R	12	F	1.3430			
	1819	13	1	A	L	1	F	1.2033			
			2	A	L	2	F	1.2014			
			3	A	L	3	M	1.2734			
			4	A	L	4	M	1.2249			
			5	A	L	5	M	1.2560			
			6	A	L	6	F	1.2349			
			7	A	L	7	M	1.3133			
			8	A	L	8	M	1.2207			
			9	A	R	9	M	1.1921			
			10	A	R	10	M	1.2161			
			11	A	R	11	M	1.2267			
			12	A	R	12	F	1.1668			
			13	A	R	13	F	1.2489			
	1820	15	1	A	L	1	M	1.1172			
			2	A	L	2	M	0.9227			
			3	A	L	3	F	0.9747			
			4	E	L						
			5	E	L						
			6	A	L	4	F	0.8975			
			7	A	L	5	F	0.9357			
			8	A	R	6	M	0.9353	External	Variation	Hematoma: Shoulder
			9	A	R	7	M	0.9363			
			10	A	R	8	M	0.9574			
			11	A	R	9	F	0.9115			
			12	A	R	10	M	0.9384			
			13	A	R	11	F	0.9389			
			14	A	R	12	F	0.8823			
			15	A	R	13	F	0.9283			
			16	A	R	14	F	0.7267			
	1821	11	1	A	L	1	M	0.8618			
			2	A	L	2	M	0.9622			
			3	A	L	3	F	0.9537			
			4	A	R	4	M	0.7376			
			5	A	R	5	M	0.8339			
			6	A	R	6	F	0.8517			
			7	A	R	7	M	0.8706			
			8	A	R	8	M	0.9207			
			9	A	R	9	F	0.8109			
			10	A	R	10	M	0.7936			
			11	A	R	11	F	0.9996			

Table A-4. Individual Embryo/Fetal Data (page 6 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
0	1822	12	1	A	L	1	F	0.9341			
			2	A	L	2	F	0.9481			
			3	A	L	3	M	0.9897			
			4	A	L	4	M	1.0243			
			5	A	L	5	M	1.0367			
			6	A	R	6	F	0.9537			
			7	A	R	7	F	0.8929			
			8	A	R	8	M	0.8955			
			9	A	R	9	F	0.8876			
			10	A	R	10	F	0.9153			
			11	A	R	11	M	0.9270			
			12	A	R	12	F	0.9892			
	1823	14	1	A	L	1	F	0.7610			
			2	A	L	2	M	0.9371			
			3	A	L	3	F	1.0011			
			4	E	L						
			5	A	L	4	F	0.9726			
			6	A	R	5	F	0.8709			
			7	A	R	6	F	0.9728			
			8	A	R	7	F	0.9841			
			9	A	R	8	M	0.9371			
			10	A	R	9	F	0.9246			
			11	A	R	10	F	0.7944			
			12	A	R	11	M	0.9476			
			13	A	R	12	M	0.8361			
			14	A	R	13	M	0.9707			
2000	2801	5	1	A	R	1	F	1.3441			
	2802	8	1	A	L	1	M	1.2708			
			2	A	L	2	F	1.0697			
			3	E	L						
			4	E	L						
			5	E	L						
			6	A	R	3	M	1.2216			
			7	A	R	4	M	1.2408			
			8	E	R						
			9	A	R	5	F	1.0764			
			10	A	R	6	M	1.1545			
			11	A	R	7	F	1.0262			
			12	E	R						
			13	A	R	8	F	1.0773			
	2803	15	1	A	L	1	M	0.9750			
			2	A	L	2	M	1.0125			
			3	A	L	3	F	1.0308			
			4	A	L	4	M	1.0001			
			5	A	L	5	M	1.0323			
			6	A	L	6	F	0.9611			
			7	A	R	7	M	1.0954	External	Variation	Hematoma: Head
									External	Variation	Hematoma: Neck
			8	A	R	8	M	1.1073			
			9	A	R	9	F	0.9910			
			10	A	R	10	M	0.9514			
			11	A	R	11	M	0.9946			
			12	A	R	12	M	1.0013			
			13	A	R	13	F	1.0459			
			14	A	R	14	F	0.9746			
			15	A	R	15	F	1.0812			

Table A-4. Individual Embryo/Fetal Data (page 7 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
2000	2804	12	1	A	L	1	F	0.9064			
			2	A	L	2	M	0.9034			
			3	A	R	3	M	0.9715			
			4	A	R	4	F	0.8306			
			5	A	R	5	F	0.7990			
			6	A	R	6	F	0.8979			
			7	A	R	7	M	0.7550			
			8	A	R	8	M	0.8960			
			9	A	R	9	F	0.7931			
			10	A	R	10	F	0.7344			
			11	A	R	11	F	0.8566			
			12	A	R	12	M	0.9062			
	2805	12	1	A	L	1	M	0.9858			
			2	A	L	2	F	0.9488			
			3	A	L	3	F	0.7516			
			4	A	L	4	M	0.9525			
			5	A	R	5	F	0.8988			
			6	A	R	6	M	0.9142			
			7	A	R	7	M	1.0188			
			8	A	R	8	F	0.8397			
			9	A	R	9	M	0.7913			
			10	A	R	10	M	0.8964			
			11	A	R	11	F	0.8909			
			12	A	R	12	M	1.0129			
	2806	12	1	A	L	1	M	1.0891			
			2	A	L	2	M	1.0178			
			3	A	L	3	M	1.0216			
			4	A	R	4	F	0.9649			
			5	A	R	5	M	0.9381			
			6	A	R	6	M	0.9603			
			7	A	R	7	F	0.8702			
			8	A	R	8	M	1.0281			
			9	A	R	9	F	0.9887			
			10	A	R	10	M	1.0687			
			11	A	R	11	M	1.0483			
			12	A	R	12	M	0.9483			
	2807	12	1	A	L	1	M	1.0771			
			2	A	L	2	M	0.8771			
			3	E	L						
			4	A	L	3	M	1.1134			
			5	A	L	4	M	1.0197			
			6	A	R	5	M	1.0057			
			7	A	R	6	F	0.8473			
			8	A	R	7	F	0.8830			
			9	A	R	8	M	1.0132			
			10	A	R	9	F	0.8436			
			11	E	R			.			
			12	A	R	10	M	1.0088			
	2808	13	1	A	L	1	M	0.8984			
			2	M	L						
			3	A	L	2	M	0.9015			
			4	A	L	3	F	0.9677			
			5	A	L	4	M	0.9335			
			6	M	L						
			7	A	L	5	M	0.9938			
			8	A	L	6	M	0.9303			
			9	M	R						
			10	A	R	7	M	1.1087			
			11	E	R						
			12	E	R						
			13	A	R	8	F	1.0222			

Table A-4. Individual Embryo/Fetal Data (page 8 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
2000	2809	10	1	A	L	1	M	1.0041			
			2	A	L	2	F	1.0047			
			3	E	L						
			4	A	L	3	F	0.9698			
			5	A	L	4	M	0.9814			
			6	A	L	5	M	0.9031			
			7	A	R	6	M	1.0715			
			8	E	R						
			9	A	R	7	F	1.0581			
			10	A	R	8	F	1.0225			
			11	A	R	9	F	0.8569			
			12	A	R	10	F	0.9546			
			13	A	R	11	M	0.9146			
	2810	10	1	A	L	1	F	1.0928			
			2	A	L	2	M	1.0999			
			3	A	L	3	F	1.0128			
			4	A	R	4	M	1.1283			
			5	A	R	5	F	1.0145			
			6	A	R	6	F	0.9308			
			7	A	R	7	M	1.0088			
			8	A	R	8	F	0.9268			
			9	A	R	9	M	1.0164			
			10	A	R	10	M	0.9720			
	2811	14	1	A	L	1	F	1.0482			
			2	A	L	2	F	0.9048			
			3	A	L	3	F	0.9513			
			4	A	L	4	F	0.9646			
			5	A	L	5	M	0.9417			
			6	A	L	6	M	0.7961			
			7	A	L	7	M	0.9538			
			8	A	L	8	F	0.8648			
			9	A	R	9	F	0.8361			
			10	A	R	10	F	0.9768			
			11	A	R	11	M	0.9976			
			12	A	R	12	M	0.9588			
			13	A	R	13	M	0.9767			
	2812	14	1	A	L	1	M	1.1579			
			2	A	L	2	F	1.0919			
			3	A	L	3	F	1.0422			
			4	A	L	4	F	1.0483			
			5	A	L	5	M	0.9578			
			6	A	L	6	M	1.0596			
			7	A	L	7	F	1.0260			
			8	A	L	8	M	1.1331			
			9	A	R	9	F	1.0548			
			10	A	R	10	M	1.1496			
			11	A	R	11	F	1.0310			
			12	A	R	12	M	1.0993			
			13	A	R	13	F	1.0189			
			14	A	R	14	F	1.0590			
	2813	10	1	I	L						
			2	I	L						
			3	I	L						
			4	I	R						
			5	I	R						
			6	I	R						
			7	I	R						
			8	I	R						
			9	I	R						
			10	I	R						

Table A-4. Individual Embryo/Fetal Data (page 9 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
2000	2814	11	1	A	L	1	M	1.0360			
			2	A	L	2	M	0.9772			
			3	A	L	3	M	0.8825			
			4	A	L	4	F	0.9772			
			5	A	L	5	M	0.9588			
			6	E	L						
			7	A	L	6	F	1.0073			
			8	A	R	7	F	0.9526			
			9	A	R	8	F	1.0928			
			10	A	R	9	F	1.0102			
			11	A	R	10	F	1.0398			
	2815	16	1	A	L	1	F	1.0205			
			2	A	L	2	F	1.0379			
			3	A	L	3	F	0.9504			
			4	A	L	4	M	1.0584			
			5	A	L	5	F	1.1114			
			6	A	L	6	F	0.8976			
			7	A	L	7	F	1.0575			
			8	A	L	8	F	1.0156			
			9	A	L	9	M	1.0038			
			10	A	R	10	F	0.9891			
			11	A	R	11	M	1.1001			
			12	A	R	12	M	1.0271			
			13	A	R	13	M	1.1058			
	2816	12	1	A	L	1	M	1.1100			
			2	A	L	2	F	1.0718			
			3	A	L	3	M	1.0676			
			4	A	L	4	F	1.0803			
			5	A	L	5	M	1.1129			
			6	A	R	6	F	1.1096			
			7	A	R	7	F	0.9815			
			8	A	R	8	F	1.0332			
			9	A	R	9	F	0.9981			
			10	A	R	10	F	1.1400			
			11	A	R	11	F	0.9800			
			12	A	R	12	M	0.9989			
	2817	15	1	A	L	1	F	1.0393			
			2	A	L	2	F	0.9766			
			3	A	L	3	M	1.1003			
			4	A	L	4	F	1.0763			
			5	A	R	5	F	1.0411			
			6	A	R	6	F	0.9787			
			7	A	R	7	F	0.9803			
			8	A	R	8	M	1.0243			
			9	A	R	9	M	1.0327			
			10	A	R	10	F	0.9913			
			11	A	R	11	F	1.0011			
			12	A	R	12	F	0.9916			

Table A-4. Individual Embryo/Fetal Data (page 10 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
2000	2818	17	1	A	L	1	F	0.9018			
			2	A	L	2	M	0.9189			
			3	A	L	3	M	1.0189			
			4	A	L	4	F	0.9339			
			5	A	L	5	F	0.7564			
			6	A	L	6	F	0.9032			
			7	A	L	7	M	0.8642	External	Malformation	Cleft Palate
			8	A	L	8	F	0.9189			
			9	A	L	9	M	1.0005			
			10	A	L	10	M	0.9558			
			11	A	R	11	F	0.9258			
			12	A	R	12	M	0.9915			
			13	A	R	13	F	0.9614			
			14	A	R	14	F	0.8635			
			15	A	R	15	F	0.8936			
			16	A	R	16	F	0.8928			
	2819	13	1	A	L	1	F	1.0275			
			2	A	L	2	F	1.1046			
			3	A	L	3	M	1.0580			
			4	A	R	4	M	1.0291			
			5	A	R	5	F	0.7359	External	Malformation	Encephalocele
			6	A	R	6	M	0.8896			
			7	A	R	7	M	0.9365			
			8	A	R	8	M	0.9639			
			9	A	R	9	F	0.9304			
			10	A	R	10	M	1.0039			
			11	A	R	11	M	1.0009			
			12	A	R	12	F	0.9925			
			13	A	R	13	M	1.0325			
	2820	14	1	A	L	1	F	1.1862			
			2	A	L	2	M	1.0684			
			3	A	L	3	M	0.9071			
			4	A	L	4	F	1.2382			
			5	A	R	5	F	1.0283			
			6	A	R	6	F	1.0491			
			7	A	R	7	F	1.0592			
			8	E	R						
			9	A	R	8	F	0.9977			
			10	A	R	9	F	1.0505			
			11	A	R	10	F	0.8839			
			12	A	R	11	M	0.9944			
			13	A	R	12	M	0.7045			
			14	A	R	13	F	0.9805			
	2821	.[g]									
	2822	11	1	A	L	1	F	0.9312			
			2	A	L	2	F	0.9493	External	Variation	Hematoma: Face
			3	A	L	3	F	1.0554			
			4	A	L	4	F	1.0212			
			5	A	R	5	M	1.0618			
			6	E	R						
			7	A	R	6	F	1.0410	External	Variation	Hematoma: Face
			8	A	R	7	M	1.0158			
			9	A	R	8	F	1.0331			
			10	A	R	9	M	1.0376			
			11	A	R	10	M	0.9412			

Table A-4. Individual Embryo/Fetal Data (page 11 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
2000	2823	12	1	A	L	1	F	0.9744			
			2	A	L	2	F	1.0834			
			3	A	L	3	M	1.0539			
			4	E	L						
			5	A	L	4	F	1.0137			
			6	A	R	5	M	1.1048			
			7	A	R	6	F	0.9936			
			8	A	R	7	M	1.0680			
			9	A	R	8	M	1.0025			
			10	A	R	9	M	1.0029			
			11	A	R	10	M	1.0112			
			12	A	R	11	F	0.9788			
10000	3801	13	1	A	L	1	M	1.0190			
			2	A	L	2	M	0.9014			
			3	A	L	3	M	0.9821			
			4	A	L	4	M	1.0398			
			5	A	L	5	M	1.0434			
			6	A	L	6	F	0.8964			
			7	A	L	7	F	0.9460			
			8	A	R	8	M	1.0947			
			9	A	R	9	M	1.0239			
			10	A	R	10	M	0.9811			
			11	E	R						
			12	A	R	11	M	0.9961			
	3802	.[g]									
	3803	10	1	A	L	1	F	1.1517			
			2	A	L	2	M	1.0631			
			3	A	L	3	F	1.1021			
			4	E	L						
			5	A	L	4	F	1.1125			
			6	A	L	5	M	1.2200			
			7	A	R	6	F	1.0961			
			8	A	R	7	F	1.0464			
			9	A	R	8	F	1.0687			
			10	A	R	9	M	1.2010			
			11	A	R	10	F	1.1963			
			12	A	R	11	M	1.2033			
			13	E	R						
	3804	14	1	A	L	1	M	0.9328			
			2	A	L	2	M	0.9306			
			3	A	L	3	F	0.8937			
			4	A	L	4	F	0.9386			
			5	L	R						
			6	A	R	5	M	0.9326			
			7	A	R	6	M	0.9338			
			8	A	R	7	M	0.8966			
			9	A	R	8	M	0.9382			
			10	A	R	9	M	0.9291			
			11	A	R	10	M	0.9202			
			12	A	R	11	F	0.9163			

Table A-4. Individual Embryo/Fetal Data (page 12 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
10000	3805	16	1	A	L	1	F	0.8832			
			2	A	L	2	F	0.9752			
			3	A	L	3	M	1.0583			
			4	A	L	4	M	0.8836			
			5	E	R						
			6	D	R			0.7638			
			7	A	R	5	F	0.9635			
			8	A	R	6	M	0.8553			
			9	A	R	7	F	0.8790			
			10	A	R	8	F	0.8643			
			11	A	R	9	M	0.9115			
			12	A	R	10	M	0.9509			
			13	A	R	11	F	0.8660			
			14	A	R	12	F	0.8833			
	3806	13	1	A	L	1	F	1.1007			
			2	A	L	2	M	1.0647			
			3	A	L	3	F	1.0466			
			4	A	L	4	M	1.0508			
			5	A	L	5	F	1.1125			
			6	A	L	6	M	1.1215			
			7	A	L	7	M	1.0254			
			8	A	L	8	F	1.1635			
			9	A	R	9	M	1.2134			
			10	A	R	10	F	1.1405			
			11	A	R	11	M	1.2279			
			12	A	R	12	F	1.1768			
			13	A	R	13	F	1.2030			
	3807	12	1	A	L	1	F	0.8125			
			2	A	L	2	F	0.8706			
			3	A	L	3	F	0.7146			
			4	A	L	4	F	0.7455			
			5	A	L	5	F	0.6843			
			6	A	R	6	M	0.7882			
			7	A	R	7	F	0.7540			
			8	A	R	8	F	0.7794			
			9	A	R	9	F	0.7171			
			10	A	R	10	M	0.7736			
			11	A	R	11	M	0.7202			
			12	A	R	12	M	0.7913			
	3808	11	1	A	L	1	M	0.9736			
			2	A	L	2	F	0.9106			
			3	A	L	3	M	0.9582			
			4	A	L	4	M	0.9339			
			5	A	R	5	M	0.9355			
			6	A	R	6	F	0.8832			
			7	A	R	7	M	0.9395			
			8	A	R	8	F	0.9138			
			9	M	R						
			10	A	R	9	M	0.8939			
			11	M	R						

Table A-4. Individual Embryo/Fetal Data (page 13 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
10000	3809	13	1	A	L	1	F	0.7510			
			2	A	L	2	F	0.8011			
			3	A	L	3	M	0.8512			
			4	A	L	4	F	0.9113			
			5	A	L	5	M	0.8740			
			6	A	R	6	F	0.8957			
			7	A	R	7	M	0.8702			
			8	A	R	8	M	0.9312	External	Variation	Hematoma: Neck
			9	A	R	9	M	0.9074			
			10	A	R	10	F	0.8759			
			11	A	R	11	M	0.8169			
			12	A	R	12	M	0.8717			
			13	A	R	13	M	0.8925			
	3810	12	1	A	L	1	F	0.9400			
			2	A	L	2	F	0.8910			
			3	A	L	3	M	1.0533			
			4	A	L	4	M	0.9740			
			5	A	L	5	F	0.9930			
			6	A	L	6	F	0.9413			
			7	A	R	7	F	0.9848			
			8	A	R	8	M	1.0769			
			9	A	R	9	F	0.9693			
			10	A	R	10	M	1.0352			
			11	A	R	11	M	1.0835			
			12	A	R	12	F	0.9862			
	3811	11	1	A	L	1	M	0.9645			
			2	A	L	2	M	1.0063			
			3	A	L	3	F	0.9305			
			4	A	L	4	F	0.8759			
			5	A	R	5	M	0.9009			
			6	A	R	6	M	0.8406			
			7	A	R	7	M	0.8920			
			8	A	R	8	M	0.5587			
			9	A	R	9	F	0.8932			
			10	A	R	10	F	0.9297			
			11	A	R	11	M	0.9312			
	3812	13	1	A	L	1	M	1.0739			
			2	A	L	2	M	0.9977			
			3	A	L	3	F	1.0568			
			4	A	L	4	F	0.9341			
			5	A	L	5	F	0.8089			
			6	A	L	6	F	1.0368			
			7	A	L	7	M	1.0440			
			8	A	R	8	F	1.0519			
			9	A	R	9	F	0.9655			
			10	A	R	10	F	1.0877			
			11	A	R	11	F	1.0286			
			12	A	R	12	F	1.0178			
			13	A	R	13	M	0.9960			
	3813	12	1	A	L	1	M	0.9396			
			2	A	L	2	M	0.9545			
			3	E	L						
			4	A	L	3	M	0.9843			
			5	A	L	4	F	0.9474			
			6	A	L	5	M	0.9391			
			7	A	R	6	M	0.9248			
			8	A	R	7	F	0.9039			
			9	A	R	8	F	0.9342			
			10	A	R	9	F	0.9293			
			11	A	R	10	F	0.9427			
			12	A	R	11	F	0.9326			

Table A-4. Individual Embryo/Fetal Data (page 14 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
10000	3814	.[h]									
	3815	11	1	A	L	1	F	1.1360			
			2	A	L	2	M	1.1999			
			3	A	L	3	F	1.1700			
			4	A	L	4	M	1.1825			
			5	A	L	5	M	1.1779			
			6	A	L	6	M	1.1906			
			7	A	R	7	M	1.1756			
			8	A	R	8	M	1.1893			
			9	A	R	9	M	1.2653			
			10	A	R	10	M	1.1124			
			11	A	R	11	M	1.1893			
	3816	15	1	A	L	1	M	0.8593			
			2	A	L	2	F	0.8340			
			3	A	L	3	F	0.8706			
			4	A	L	4	F	0.8719			
			5	A	L	5	F	0.8671			
			6	A	L	6	M	0.8532			
			7	A	L	7	F	0.8176			
			8	A	R	8	M	0.9453			
			9	A	R	9	M	0.8392	External	Malformation	Cleft Palate
			10	A	R	10	M	0.8336			
			11	A	R	11	F	0.8685			
			12	A	R	12	F	0.8623			
			13	A	R	13	M	0.9595			
			14	M	R						
			15	A	R	14	F	0.8344			
	3817	.[g]									
	3818	14	1	A	L	1	F	0.8906			
			2	A	L	2	F	0.8837			
			3	A	L	3	M	1.0012			
			4	A	R	4	M	0.9925			
			5	A	R	5	F	0.9106			
			6	A	R	6	F	0.8052			
			7	A	R	7	M	0.9135			
			8	A	R	8	M	1.0314			
			9	A	R	9	M	0.9259			
			10	A	R	10	F	0.8523			
			11	A	R	11	F	0.8937			
	3819	14	1	A	L	1	M	0.8560			
			2	A	L	2	F	0.8368			
			3	A	L	3	F	0.8758			
			4	A	L	4	F	0.9340			
			5	A	L	5	M	0.9200			
			6	A	L	6	F	0.8570			
			7	A	L	7	M	0.9478			
			8	A	L	8	M	0.9177			
			9	E	R						
			10	A	R	9	F	0.9978			
			11	A	R	10	F	0.9447			
			12	A	R	11	M	1.0698			
			13	A	R	12	F	0.9054			

Table A-4. Individual Embryo/Fetal Data (page 15 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
10000	3820	13	1	A	L	1	F	0.7350			
			2	A	L	2	M	0.7557			
			3	A	L	3	F	0.8625			
			4	A	L	4	M	0.9689			
			5	A	L	5	F	0.8864			
			6	A	L	6	F	0.9464			
			7	A	L	7	M	0.9343			
			8	A	R	8	F	0.9803			
			9	A	R	9	F	0.8394			
			10	A	R	10	M	1.0069			
			11	A	R	11	M	0.9770			
			12	A	R	12	F	0.9931			
	3821	.[i]	1	D	L			0.7329			
			2	A	L	1	M	0.9617			
			3	A	L	2	M	0.8419			
			4	A	L	3	M	0.8902			
			5	A	L	4	F	0.9598			
			6	A	L	5	M	0.9075			
			7	E	R						
			8	A	R	6	F	0.9211			
			9	A	R	7	F	0.9019			
			10	A	R	8	M	0.7350			
			11	A	R	9	M	0.8225			
			12	L	R						
			13	A	R	10	M	0.7931			
			14	A	R	11	M	0.8594			
			15	A	R	12	M	0.8169			
			16	A	R	13	F	0.9058			
	3822	13	1	A	L	1	M	0.8384			
			2	E	L						
			3	A	L	2	M	0.8672			
			4	A	L	3	F	0.8362			
			5	A	L	4	F	0.9295			
			6	E	L						
			7	A	R	5	M	0.9220			
			8	A	R	6	M	0.7331			
			9	A	R	7	F	0.8611			
			10	A	R	8	M	0.8935			
			11	A	R	9	M	0.8065			
			12	A	R	10	M	0.9002			
			13	A	R	11	M	0.8964			
			14	A	R	12	F	0.9067			
			15	A	R	13	F	0.7866	External	Variation	Abnormal Rugae in Midline of Palate
	3823	.[g]									
20000	4801	15	1	A	L	1	M	0.7756	External	Variation	Hematoma: Neck
			2	A	L	2	M	0.9303			
			3	A	L	3	F	0.8679			
			4	A	L	4	F	0.8982			
			5	A	L	5	M	0.8411			
			6	A	L	6	F	0.9145			
			7	A	L	7	M	0.9662			
			8	A	L	8	M	0.9387			
			9	A	L	9	F	0.7659			
			10	A	L	10	F	0.8141			
			11	A	R	11	F	0.9080			
			12	A	R	12	F	0.9201			
			13	A	R	13	F	0.8216			

Table A-4. Individual Embryo/Fetal Data (page 16 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
20000	4802	13	1	A	L	1	M	1.0719			
			2	A	L	2	M	1.0172			
			3	E	L						
			4	A	L	3	F	0.9307	External	Variation	Hematoma: Neck
			5	A	L	4	F	0.7838			
			6	A	L	5	F	1.0347			
			7	A	R	6	F	0.9325			
			8	A	R	7	F	0.9266			
			9	A	R	8	F	1.0504			
			10	A	R	9	F	0.9771			
			11	A	R	10	F	1.0003			
			12	A	R	11	M	0.9209			
			13	A	R	12	F	1.0126			
	4803	12	1	A	L	1	F	1.1729			
			2	A	L	2	M	1.1987			
			3	A	L	3	M	1.0925			
			4	A	L	4	M	1.1984			
			5	A	L	5	F	1.1336			
			6	A	L	6	F	1.1188			
			7	A	L	7	F	1.1314			
			8	A	R	8	F	1.1490			
			9	A	R	9	F	1.0659			
			10	A	R	10	F	1.1253			
			11	A	R	11	M	1.1589			
			12	A	R	12	M	1.0840			
			13	A	R	13	M	1.1359			
	4804	15	1	A	L	1	M	1.1320			
			2	A	L	2	M	1.0061			
			3	A	L	3	M	1.0438			
			4	A	L	4	F	1.0220			
			5	A	L	5	M	1.0451			
			6	A	R	6	F	0.9857			
			7	A	R	7	M	0.9875			
			8	A	R	8	M	0.9954			
			9	A	R	9	F	0.9630			
			10	A	R	10	F	0.9950			
			11	A	R	11	F	0.7918			
			12	A	R	12	M	1.0053			
			13	M	R						
			14	A	R	13	M	0.9716			
	4805	13	1	A	L	1	M	0.8523			
			2	A	L	2	F	0.8314			
			3	A	L	3	F	0.9040			
			4	A	L	4	F	0.8392			
			5	A	L	5	M	0.7989			
			6	A	L	6	M	0.8065			
			7	A	L	7	M	0.8913			
			8	A	R	8	M	0.9045			
			9	A	R	9	M	0.9049			
			10	A	R	10	F	0.9584			
			11	A	R	11	M	0.9496			
			12	A	R	12	F	0.8322			
			13	A	R	13	M	0.9007			

Table A-4. Individual Embryo/Fetal Data (page 17 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
20000	4806	13	1	A	L	1	F	0.9073			
			2	A	L	2	M	0.8205			
			3	A	L	3	F	0.9315			
			4	A	L	4	M	0.9512			
			5	A	L	5	F	0.6950			
			6	A	L	6	M	0.9079			
			7	A	L	7	F	0.9127			
			8	A	L	8	F	0.8739			
			9	A	R	9	M	0.9375			
			10	A	R	10	M	0.9311			
			11	A	R	11	M	0.8928			
			12	A	R	12	M	0.8862			
			13	A	R	13	F	0.8459			
	4807	13	1	A	L	1	M	0.8860			
			2	A	L	2	F	0.9351			
			3	A	L	3	F	0.9293			
			4	A	L	4	M	0.9260			
			5	A	L	5	F	0.9085			
			6	A	R	6	F	0.8282			
			7	A	R	7	M	0.8468			
			8	A	R	8	F	0.7430			
			9	A	R	9	F	0.7945			
			10	A	R	10	F	0.8475			
			11	A	R	11	M	0.9188			
			12	A	R	12	M	0.8337			
			13	A	R	13	F	0.8254			
	4808	14	1	A	L	1	M	0.8765			
			2	A	L	2	M	0.8406			
			3	A	L	3	F	0.7655			
			4	A	L	4	M	0.8192			
			5	A	L	5	M	0.8560			
			6	A	L	6	M	0.9250			
			7	A	L	7	F	0.8788			
			8	A	L	8	F	0.8942			
			9	A	R	9	F	0.8507			
			10	A	R	10	F	0.8718			
			11	A	R	11	M	0.8882			
			12	A	R	12	F	0.9554			
			13	A	R	13	M	0.9473			
			14	A	R	14	M	0.8788			
	4809	19	1	A	L	1	M	0.8810			
			2	A	L	2	M	0.8340			
			3	A	L	3	F	0.7542			
			4	A	L	4	M	0.8357			
			5	A	L	5	M	0.8400			
			6	A	L	6	F	0.8336			
			7	A	L	7	M	0.8674			
			8	A	L	8	M	0.8963			
			9	A	R	9	F	0.7561			
			10	A	R	10	F	0.7887			
			11	A	R	11	M	0.7948			
			12	A	R	12	F	0.8452			
			13	E	R						
			14	A	R	13	F	0.8102			
			15	A	R	14	F	0.8396			
			16	A	R	15	M	0.8383			
			17	A	R	16	F	0.7813			
			18	A	R	17	F	0.7973			
			19	A	R	18	F	0.7978			

Table A-4. Individual Embryo/Fetal Data (page 18 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
20000	4810	13	1	A	L	1	F	0.7567			
			2	A	L	2	M	0.8425			
			3	A	L	3	M	0.8637			
			4	A	L	4	M	0.9020			
			5	A	L	5	F	0.8044			
			6	A	L	6	F	0.8863			
			7	A	L	7	M	0.9203			
			8	A	R	8	F	0.7931			
			9	A	R	9	M	0.8795			
			10	A	R	10	F	0.8196			
			11	A	R	11	F	0.8692			
			12	A	R	12	M	0.8416			
			13	A	R	13	F	0.8331			
	4811	13	1	A	L	1	F	1.1239			
			2	A	L	2	F	1.1188			
			3	A	L	3	F	1.1692			
			4	A	L	4	M	1.2309			
			5	A	L	5	F	1.1065			
			6	D	L			0.8750			
			7	A	L	6	F	1.1848			
			8	A	R	7	F	1.2355			
			9	A	R	8	F	1.2401			
			10	A	R	9	F	1.1965			
			11	A	R	10	F	1.1526			
			12	A	R	11	M	1.2364			
			13	A	R	12	M	1.2652			
	4812	14	1	A	L	1	M	0.9838			
			2	A	L	2	F	0.9007			
			3	A	L	3	F	0.8484			
			4	A	L	4	M	0.9993			
			5	A	L	5	F	0.8709			
			6	A	R	6	M	0.9528			
			7	A	R	7	F	0.8973			
			8	A	R	8	F	0.8975			
			9	A	R	9	F	0.9014			
			10	A	R	10	F	0.8079			
			11	A	R	11	F	0.7973			
			12	A	R	12	M	1.0232			
	4813	16	1	A	L	1	M	1.1349			
			2	A	L	2	M	1.0482			
			3	A	L	3	M	1.1813			
			4	A	L	4	F	1.1449			
			5	E	L						
			6	A	R	5	M	1.1067			
			7	A	R	6	F	1.0247			
			8	A	R	7	F	0.9778			
			9	A	R	8	F	0.9348			
			10	A	R	9	F	0.9969			
			11	A	R	10	F	0.9783			
			12	A	R	11	F	1.0145			
			13	A	R	12	F	1.0523			
			14	A	R	13	F	0.9525			
			15	A	R	14	F	0.9687			
			16	A	R	15	F	0.9585			

Table A-4. Individual Embryo/Fetal Data (page 19 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
20000	4814	9	1	M	L						
			2	A	L	1	F	1.2799			
			3	A	L	2	F	1.3151			
			4	A	L	3	M	1.3496			
			5	E	L						
			6	A	L	4	F	1.1992			
			7	A	R	5	M	1.3070			
			8	A	R	6	M	1.2653			
			9	E	R						
	4815	.[g]									
	4816	13	1	A	L	1	M	0.8639			
			2	A	L	2	M	0.9429			
			3	A	L	3	F	0.8220			
			4	A	L	4	M	0.8379			
			5	A	L	5	M	0.9266			
			6	A	L	6	F	0.9481			
			7	A	L	7	F	0.9420			
			8	A	L	8	F	0.9747			
			9	A	R	9	F	1.0099			
			10	A	R	10	F	0.8801			
			11	A	R	11	F	0.9603			
			12	A	R	12	F	0.9483			
			13	A	R	13	M	0.9909			
	4817	14	1	A	L	1	M	1.0301			
			2	E	L						
			3	A	L	2	M	1.0935			
			4	A	L	3	M	1.0339			
			5	A	L	4	M	1.0177			
			6	A	L	5	M	0.9907			
			7	A	L	6	M	0.9161			
			8	A	L	7	F	1.0390			
			9	A	R	8	M	1.0381			
			10	A	R	9	F	1.0077			
			11	A	R	10	M	1.0401			
			12	A	R	11	F	0.9537			
			13	A	R	12	F	0.9633			
	4818	14	1	I	L						
			2	I	L						
			3	I	L						
			4	I	L						
			5	I	L						
			6	I	L						
			7	I	R						
			8	I	R						
			9	I	R						
			10	I	R						
			11	I	R						
			12	I	R						
			13	I	R						
			14	I	R						
			15	I	R						
			16	I	R						
			17	I	R						

Table A-4. Individual Embryo/Fetal Data (page 20 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
20000	4819	5	1	I	L						
			2	I	L						
			3	I	L						
			4	I	R						
			5	I	R						
			6	I	R						
			7	I	R						
			8	I	R						
			9	I	R						
			10	I	R						
	4820	13	1	A	L	1	F	0.9712			
			2	A	L	2	F	0.9335			
			3	A	L	3	F	0.9432			
			4	A	L	4	F	0.9313			
			5	A	L	5	F	0.7709			
			6	A	L	6	M	1.0237			
			7	A	L	7	F	0.9602			
			8	A	L	8	M	0.9823			
			9	A	R	9	M	0.9386			
			10	E	R						
			11	A	R	10	F	0.9344			
			12	A	R	11	F	0.9133			
	4821	15	1	A	L	1	M	0.8292			
			2	A	L	2	F	0.8331			
			3	A	L	3	F	0.7993			
			4	A	R	4	M	0.8301			
			5	A	R	5	M	0.7856			
			6	A	R	6	M	0.6891			
			7	A	R	7	F	0.7089	External	Malformation	Cleft Palate
			8	A	R	8	M	0.5875			
			9	A	R	9	M	0.7899			
			10	D	R			0.6560			
			11	A	R	10	M	0.8427			
			12	A	R	11	M	0.8427			
			13	A	R	12	M	0.8158			
			14	A	R	13	F	0.7604			
			15	A	R	14	M	0.7674			
	4822	12	1	A	L	1	F	0.9253			
			2	A	L	2	M	0.9153			
			3	A	L	3	F	0.9479	External	Variation	Abnormal Rugae in Midline of Palate
			4	A	L	4	M	0.9499			
			5	A	R	5	F	0.9415			
			6	A	R	6	F	0.8508			
			7	A	R	7	M	0.9713			
			8	A	R	8	M	0.9437			
			9	A	R	9	F	0.8754			
			10	A	R	10	M	0.8642			
			11	A	R	11	F	0.8712			
	4823	12	1	A	L	1	F	0.7820			
			2	A	L	2	F	0.8031			
			3	A	L	3	M	0.8638			
			4	E	L						
			5	A	L	4	M	0.9099			
			6	A	L	5	M	0.6567			
			7	A	R	6	F	0.9373			
			8	A	R	7	F	0.8679			
			9	A	R	8	F	0.9426			
			10	A	R	9	F	0.9749			
			11	A	R	10	F	0.8428			
			12	A	R	11	M	0.9194			

Table A-4. Individual Embryo/Fetal Data (page 21 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	5801	.[g]									
	5802	13	1	A	L	1	M	0.9804			
			2	A	L	2	M	1.1120			
			3	A	L	3	M	1.0901			
			4	A	R	4	M	1.0564			
			5	A	R	5	M	1.0590			
			6	A	R	6	F	0.9079			
			7	A	R	7	F	0.9483			
			8	A	R	8	F	0.9521			
			9	A	R	9	F	0.9818			
			10	A	R	10	F	0.9639			
			11	A	R	11	M	1.0117			
			12	L	R						
			13	A	R	12	M	0.9321			
	5803	13	1	A	L	1	F	1.1267			
			2	A	L	2	M	1.1515			
			3	A	L	3	M	1.0179			
			4	A	L	4	M	1.1013			
			5	A	L	5	M	1.0478			
			6	A	L	6	M	1.1700			
			7	A	R	7	F	1.1515			
			8	A	R	8	M	1.2303			
			9	A	R	9	F	1.1074			
			10	A	R	10	F	1.2296			
			11	M	R						
			12	A	R	11	M	1.0830			
			13	A	R	12	M	1.1170			
	5804	15	1	A	L	1	M	1.1464			
			2	A	L	2	M	1.0855			
			3	A	L	3	M	0.7745	External	Malformation	Cleft Palate
			4	A	L	4	M	1.0201			
			5	A	L	5	F	1.1951			
			6	A	R	6	F	0.9255			
			7	A	R	7	M	1.2088			
			8	M	R						
			9	A	R	8	M	1.1263			
			10	A	R	9	F	1.0717			
			11	A	R	10	M	1.1267			
			12	A	R	11	M	1.0228			
			13	A	R	12	F	1.1004			
	5805	13	1	A	L	1	M	1.0774			
			2	A	L	2	F	0.9531			
			3	A	L	3	F	0.9565			
			4	A	L	4	M	0.9550			
			5	A	L	5	F	0.8124			
			6	A	L	6	M	1.0683			
			7	A	L	7	F	1.0170			
			8	A	L	8	M	1.0931			
			9	A	R	9	F	1.0089			
			10	A	R	10	M	1.0940			
			11	A	R	11	F	1.0375			
			12	A	R	12	M	1.0142			
			13	A	R	13	M	1.0256			

Table A-4. Individual Embryo/Fetal Data (page 22 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	5806	18	1	A	L	1	M	0.9533			
			2	A	L	2	F	0.9073			
			3	A	L	3	M	0.9066			
			4	A	L	4	F	0.9136			
			5	A	L	5	F	1.0194			
			6	L	L						
			7	A	L	6	F	0.9655			
			8	A	L	7	M	1.0357			
			9	A	L	8	F	0.9715			
			10	A	L	9	M	0.9788			
			11	A	L	10	M	1.0303			
			12	A	R	11	F	1.0017			
			13	A	R	12	F	0.9086			
			14	A	R	13	M	1.0746			
			15	A	R	14	M	1.1079			
			16	E	R						
			17	A	R	15	F	0.9868			
			18	A	R	16	F	0.9189			
	5807	13	1	A	L	1	F	0.9649			
			2	A	L	2	F	0.9225			
			3	A	L	3	F	1.0054			
			4	A	L	4	F	1.0138			
			5	A	L	5	M	1.0280			
			6	A	L	6	F	1.0273			
			7	A	R	7	F	0.9359			
			8	A	R	8	F	0.9338			
			9	A	R	9	F	0.9303			
			10	A	R	10	M	1.0376			
			11	A	R	11	F	0.9072			
			12	A	R	12	F	0.8520			
			13	A	R	13	M	0.9406			
	5808	13	1	A	L	1	M	0.9483			
			2	A	L	2	F	1.0104			
			3	A	L	3	M	1.0077			
			4	A	R	4	M	0.9191			
			5	A	R	5	M	0.8189			
			6	A	R	6	M	0.9226			
			7	A	R	7	M	0.7506			
			8	A	R	8	M	0.8968			
			9	A	R	9	M	0.9360			
			10	A	R	10	F	0.9469			
			11	A	R	11	F	0.9655			
			12	A	R	12	F	0.9790			
			13	A	R	13	M	0.9588			
	5809	16	1	A	L	1	M	0.8884			
			2	A	L	2	M	0.8127			
			3	A	L	3	M	0.8462			
			4	A	L	4	M	0.9215			
			5	A	L	5	F	0.9343			
			6	E	L						
			7	A	R	6	F	0.8106			
			8	A	R	7	F	0.8068			
			9	A	R	8	M	0.8612			
			10	L	R						
			11	A	R	9	F	0.9078			
			12	A	R	10	F	0.8592			
			13	A	R	11	M	0.9218			
			14	L	R						
			15	A	R	12	F	0.8428			
			16	A	R	13	M	0.8899			

Table A-4. Individual Embryo/Fetal Data (page 23 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	5810	15	1	A	L	1	F	0.8985			
			2	A	L	2	F	0.9065			
			3	A	L	3	F	0.9001			
			4	A	L	4	F	0.9440			
			5	E	L						
			6	A	L	5	F	0.9382	External	Malformation	Cleft Palate
			7	A	L	6	F	0.6057	External	Malformation	Cleft Palate
									External	Malformation	Gastroschisis
			8	A	L	7	M	0.8893			
			9	A	R	8	F	0.8080			
			10	A	R	9	F	0.8684			
			11	A	R	10	F	0.8034			
			12	A	R	11	M	0.8844			
			13	A	R	12	M	0.8406	External	Malformation	Cleft Palate
			14	A	R	13	F	0.8456			
			15	A	R	14	F	0.9768			
	5811	16	1	A	L	1	M	0.9681			
			2	A	L	2	F	0.9535			
			3	L	L						
			4	A	L	3	F	1.0411			
			5	A	L	4	F	0.9981			
			6	A	L	5	F	1.0156			
			7	E	L						
			8	A	L	6	M	1.1714			
			9	A	L	7	M	1.1660			
			10	A	R	8	F	1.1421			
			11	A	R	9	M	1.1165			
			12	A	R	10	M	1.0938			
			13	A	R	11	M	1.1929			
			14	A	R	12	F	1.1948			
			15	A	R	13	M	1.2758			
			16	A	R	14	F	1.2000			
	5812	11	1	A	L	1	M	1.0452			
			2	A	L	2	M	0.9058			
			3	A	L	3	F	0.9278			
			4	A	L	4	F	1.0021			
			5	A	L	5	M	0.9996			
			6	A	L	6	M	0.9291			
			7	A	L	7	M	1.0208			
			8	A	R	8	M	1.0233			
			9	A	R	9	M	1.0146			
			10	A	R	10	F	0.9597			
			11	A	R	11	M	1.0220			
	5813	4	1	I	L						
			2	I	L						
			3	I	L						
			4	I	L						
			5	I	L						
			6	I	R						
			7	I	R						
			8	I	R						
			9	I	R						
			10	I	R						
			11	I	R						
			12	I	R						

Table A-4. Individual Embryo/Fetal Data (page 24 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	5814	12	1	A	L	1	M	0.9903			
			2	A	L	2	F	0.9792			
			3	A	L	3	M	1.0668			
			4	A	L	4	F	1.0714			
			5	A	L	5	F	1.0852			
			6	A	R	6	F	0.8905			
			7	A	R	7	M	0.8522			
			8	A	R	8	F	0.8659			
			9	A	R	9	M	1.0235			
			10	A	R	10	F	0.9251			
			11	A	R	11	F	1.0418			
			12	A	R	12	M	1.0593			
	5815	12	1	A	L	1	M	1.1130			
			2	A	L	2	F	1.0309			
			3	A	L	3	M	1.0280			
			4	A	L	4	F	1.0562			
			5	A	L	5	F	1.0166			
			6	A	L	6	F	1.0871			
			7	A	R	7	F	1.0025			
			8	A	R	8	M	0.7835			
			9	A	R	9	F	1.0818			
			10	A	R	10	F	0.9947			
			11	A	R	11	F	1.0768			
			12	A	R	12	F	0.9457			
	5816	14	1	A	L	1	M	1.3655			
			2	A	L	2	F	1.2790			
			3	D	L			0.8132			
			4	A	L	3	F	1.2630			
			5	A	L	4	F	1.1545			
			6	A	L	5	F	1.2992			
			7	A	L	6	M	1.2512			
			8	A	R	7	M	1.3040			
			9	A	R	8	M	1.2248			
			10	A	R	9	F	1.2875			
			11	A	R	10	M	1.3026			
			12	E	R						
			13	A	R	11	F	1.2087			
			14	A	R	12	F	1.3550			
	5817	.[g]									
	5818	16	1	A	L	1	F	0.8573			
			2	A	L	2	F	0.8314			
			3	A	L	3	M	0.8302			
			4	A	L	4	F	0.8590			
			5	A	L	5	F	0.7868			
			6	A	L	6	F	0.9320			
			7	A	L	7	F	0.9167			
			8	A	L	8	F	0.8636			
			9	A	R	9	F	0.8699			
			10	A	R	10	F	0.8666			
			11	A	R	11	M	0.7944			
			12	A	R	12	M	0.8529			
			13	A	R	13	F	0.8398			
			14	A	R	14	M	0.8448			
			15	A	R	15	M	0.8584			

Table A-4. Individual Embryo/Fetal Data (page 25 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
30000	5819	14	1	A	L	1	F	1.3938			
			2	A	L	2	M	1.4852			
			3	A	R	3	F	1.2505			
			4	A	R	4	M	1.3148			
			5	A	R	5	M	1.1664			
			6	A	R	6	F	0.8750			
			7	A	R	7	M	1.3726			
			8	E	R						
			9	A	R	8	M	1.1908			
			10	A	R	9	M	1.4493			
			11	A	R	10	M	1.3387			
			12	A	R	11	M	1.3200			
	5820	13	1	L	L						
			2	A	L	1	F	0.9656			
			3	A	L	2	M	1.0512			
			4	A	L	3	F	0.9488			
			5	A	L	4	F	0.9156			
			6	A	L	5	F	1.0114			
			7	A	R	6	F	0.9614			
			8	E	R						
			9	A	R	7	F	0.9043			
			10	A	R	8	F	0.8433			
			11	A	R	9	F	0.8728			
			12	A	R	10	F	0.9826			
			13	M	R						
			14	A	R	11	F	0.9827			
	5821	17	1	A	L	1	F	1.0300			
			2	A	L	2	F	0.9014			
			3	A	L	3	M	0.9976			
			4	A	L	4	M	1.0325			
			5	E	L						
			6	A	L	5	F	0.8470			
			7	A	L	6	M	1.0474			
			8	E	L						
			9	A	R	7	M	1.0702			
			10	A	R	8	F	1.0475			
			11	A	R	9	F	0.9996			
			12	A	R	10	M	1.0595			
			13	A	R	11	F	0.9625			
			14	A	R	12	F	1.0545			
	5822	11	1	A	L	1	F	1.0140			
			2	A	L	2	F	1.0250			
			3	A	R	3	F	0.9620			
			4	A	R	4	M	0.9893			
			5	A	R	5	F	0.8717			
			6	A	R	6	M	0.9655			
			7	A	R	7	M	0.9312			
			8	A	R	8	F	1.0055			
			9	A	R	9	M	1.0297			
			10	A	R	10	F	0.9577			
			11	A	R	11	F	0.9520			

Table A-4. Individual Embryo/Fetal Data (page 26 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	5823	13	1	A	L	1	M	1.5475			
			2	A	L	2	F	1.3667			
			3	A	L	3	F	1.2577			
			4	A	L	4	M	1.4539			
			5	A	L	5	M	1.3758			
			6	A	L	6	F	1.4071			
			7	A	L	7	F	1.3181			
			8	A	L	8	F	1.3916			
			9	A	R	9	F	1.4486			
			10	A	R	10	M	1.3247			
			11	A	R	11	F	1.2217			
			12	A	R	12	M	1.4355			
			13	A	R	13	M	1.4881			
	5824	5	1	I	R						
			2	I	R						
			3	I	R						
			4	I	R						
	5825	11	1	A	L	1	M	1.0203			
			2	A	L	2	F	0.9395			
			3	A	L	3	F	0.9975			
			4	A	L	4	F	0.9981			
			5	A	L	5	F	1.0025			
			6	A	L	6	M	0.8743			
			7	A	L	7	F	0.9600			
			8	A	R	8	F	1.1533			
			9	E	R						
			10	A	R	9	M	1.0698			
			11	A	R	10	M	1.0630			
	5826	16	1	A	L	1	M	0.8428			
			2	A	L	2	M	0.9350			
			3	A	L	3	F	0.9731			
			4	A	L	4	M	1.0226			
			5	A	L	5	M	0.9213			
			6	A	L	6	M	0.8604			
			7	A	R	7	F	0.8411			
			8	A	R	8	F	0.9784			
			9	A	R	9	M	0.8939			
			10	A	R	10	F	0.8954			
			11	A	R	11	F	0.8163			
			12	A	R	12	M	0.9026			
			13	A	R	13	M	0.8072			
			14	A	R	14	F	0.8687			
	5827	14	1	A	L	1	M	0.9385			
			2	A	L	2	F	0.9648			
			3	A	L	3	M	0.9358			
			4	A	L	4	F	0.7844			
			5	A	R	5	F	0.9408			
			6	A	R	6	F	0.9805			
			7	A	R	7	F	1.0502			
			8	A	R	8	F	0.8167			
			9	A	R	9	M	1.0225			
			10	A	R	10	F	0.9819			
			11	A	R	11	F	0.9894			

Table A-4. Individual Embryo/Fetal Data (page 27 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	5828	11	1	A	L	1	F	0.9467			
			2	A	L	2	F	0.9957			
			3	A	L	3	F	0.8725			
			4	A	L	4	F	0.8279			
			5	A	L	5	F	0.9775			
			6	A	L	6	F	1.0164	External	Malformation	Cleft Palate
			7	A	L	7	M	0.8595			
			8	A	L	8	F	0.9852			
			9	A	R	9	F	0.8664			
			10	A	R	10	F	0.9977			
			11	E	R						
			12	A	R	11	F	0.9024			
	5829	14	1	A	L	1	F	1.1204			
			2	E	L						
			3	A	L	2	F	1.1703			
			4	A	L	3	F	1.1694			
			5	A	R	4	M	1.1893			
			6	A	R	5	M	1.1602			
			7	A	R	6	M	1.1989			
			8	A	R	7	M	1.1962			
			9	A	R	8	F	1.1228			
			10	E	R						
			11	A	R	9	M	1.1192			
	5830	15	1	A	L	1	M	1.0950			
			2	A	L	2	F	1.1618			
			3	A	L	3	M	1.0445			
			4	A	L	4	F	1.0395			
			5	A	R	5	F	1.2304			
			6	A	R	6	F	1.0734			
			7	A	R	7	M	0.9946	External	Malformation	Cleft Palate
			8	A	R	8	M	1.1270			
			9	A	R	9	M	0.9994			
			10	A	R	10	F	1.1301			
			11	A	R	11	F	1.0525	External	Malformation	Cleft Palate
			12	A	R	12	M	0.9878			
			13	A	R	13	F	1.0706			
	5831	14	1	A	L	1	F	0.9588			
			2	A	L	2	M	1.0149			
			3	A	L	3	M	0.9975			
			4	A	L	4	F	0.8350			
			5	A	L	5	F	0.9145			
			6	A	L	6	M	1.0151			
			7	A	R	7	M	0.9522			
			8	A	R	8	F	0.9081			
			9	A	R	9	F	0.8420			
			10	A	R	10	M	0.9452			
			11	A	R	11	F	0.8850			
			12	A	R	12	M	0.9097			
			13	A	R	13	M	0.9792			

Table A-4. Individual Embryo/Fetal Data (page 28 of 29)

Dose[a]	Dam ID#	NCL[b]	Implant #	Implant Type[c]	Implant Position[d]	Fetus #	Fetus Sex	Fetus Wt.[e]	Defect[f] Exam	Defect[f] Type	Defect[f] Description
30000	5832	14	1	A	L	1	M	1.1667			
			2	A	L	2	F	1.0426			
			3	A	L	3	F	1.0582			
			4	A	L	4	F	0.9516			
			5	A	L	5	F	0.9912			
			6	A	L	6	F	0.9265			
			7	A	L	7	M	1.0659			
			8	A	L	8	M	1.1146			
			9	A	L	9	F	1.0422			
			10	A	R	10	F	1.0611			
			11	A	R	11	M	1.1725			
			12	A	R	12	M	1.1148			
			13	A	R	13	F	1.1144			
			14	A	R	14	M	1.0441			
	5833	16	1	A	L	1	M	0.9488			
			2	A	L	2	F	0.8791			
			3	E	L						
			4	A	L	3	F	0.8688			
			5	A	L	4	F	0.7998			
			6	E	L						
			7	A	L	5	M	0.8976			
			8	A	L	6	F	0.9030			
			9	A	R	7	F	0.8906			
			10	A	R	8	F	0.8321			
			11	A	R	9	F	0.8715			
			12	A	R	10	F	0.8348			
			13	A	R	11	M	0.8665			
			14	A	R	12	F	0.9051			
	5834	10	1	A	L	1	M	0.9527			
			2	A	L	2	F	0.9792			
			3	A	L	3	M	0.9705			
			4	A	L	4	M	0.8064			
			5	A	L	5	M	1.0083			
			6	A	L	6	M	0.9922			
			7	A	L	7	F	0.9961			
			8	A	R	8	M	1.0352			
			9	A	R	9	M	0.9722			
			10	A	R	10	F	1.0271			
	5835	12	1	A	L	1	F	1.5416			
			2	E	L						
			3	A	L	2	M	1.5103			
			4	A	L	3	M	1.4931			
			5	A	L	4	M	1.1562			
			6	A	R	5	M	1.2581			
			7	A	R	6	F	1.4263			
			8	A	R	7	F	1.5269			
			9	A	R	8	F	1.4091			
			10	A	R	9	M	1.5567			
			11	A	R	10	F	1.3408			
			12	A	R	11	F	1.3742			
			13	A	R	12	M	1.5313			

Table A-4. Individual Embryo/Fetal Data (page 29 of 29)

			Implant			Fetus			Defect[f]		
Dose[a]	Dam ID#	NCL[b]	#	Type[c]	Position[d]	#	Sex	Wt.[e]	Exam	Type	Description
30000	5836	13	1	A	L	1	F	0.9538			
			2	A	L	2	M	0.9240			
			3	A	L	3	F	0.8944			
			4	A	L	4	M	0.9606			
			5	A	L	5	F	0.9417			
			6	A	L	6	M	0.8582			
			7	A	L	7	M	0.9532			
			8	A	R	8	F	0.8749			
			9	A	R	9	F	1.0230			
			10	A	R	10	F	0.9640			
			11	A	R	11	M	0.8196			
			12	A	R	12	M	0.9105			
			13	A	R	13	F	0.8231			
			14	A	R	14	M	1.0398			
	5837	15	1	I	L						
			2	I	L						
			3	I	L						
			4	I	L						
			5	I	L						
			6	I	R						
			7	I	R						
			8	I	R						
			9	I	R						
			10	I	R						
			11	I	R						
			12	I	R						
			13	I	R						
	5838	13	1	A	L	1	M	1.0087			
			2	A	L	2	F	0.9374			
			3	A	L	3	M	0.8882			
			4	A	L	4	M	0.9084			
			5	A	L	5	F	0.8730			
			6	L	L						
			7	A	L	6	M	0.9249			
			8	A	L	7	M	0.9987			
			9	A	L	8	F	0.9615			
			10	A	L	9	F	0.9365			
			11	A	R	10	M	1.0784			
			12	A	R	11	F	1.0335			
			13	A	R	12	F	1.0152			
			14	E	R						

[a]Mg/m^3 of gasoline MTBE vapor condensate.
[b]Number of corpora lutea.
[c]Implant type codes are as follows: A - Live Fetus; D - Dead Fetus; F - Full Resorption; L - Late Resorption; M - Middle Resorption; E - Early Resorption and I - Implantation Site.
[d]Position refers to uterine horn (R - right, L - left).
[e]Weight is in grams.
[f]Absence of entries under "Exam", "Type" and "Description" for "Defect" indicates no external malformation or variation observed for that fetus.
[g]Female was not pregnant.
[h]Female was removed due to a preexisting condition. At necropsy she was found to have an undescended testis on the right and seminal vesicles and prostate to the right of the vagina and cervix.
[i]Right ovary was inadvertently lost prior to the corpora lutea being counted.

Appendix III:

Protocol and 2 Amendments

RTI Project No.: 09189.000
RTI Study Code: Mi04-HLS2
RTI Master Protocol No.: RTI-909
HLS Study No.: 04-4263

TITLE: Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD-1® Mice

SPONSOR: American Petroleum Institute (API)
1220 L Street, NW
Washington, DC 20005

TESTING FACILITY: Huntingdon Life Sciences (HLS)
Princeton Research Center
100 Mettlers Road
East Millstone, NJ 08875-2360

SITE OF POSTMORTEM EVALUATIONS AND ANALYSES: RTI International (RTI)
Center for Life Sciences and Toxicology
Health Sciences Unit
Post Office Box 12194, 3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

PROPOSED IN-LIFE STUDY DATES: November 18 – December 17, 2004

AMENDMENTS:

No.	Date	Section(s)	Page(s)
1	12/16/04	Title page, Secr 2.5.1, 3.1	1, 7, 10
2	2/23/05	2.5.1, 3.1, 3.8, 3.9, 3.10.c	(Am 1 p4), 9, 12, 13
3			
4			

APPROVED BY:

Thomas M. Gray, M.S., DABT — 10/25/04 Date
American Petroleum Institute
Sponsor's Representative

Rochelle W. Tyl, Ph.D., DABT — 10/27/04 Date
Study Director/Research Director
Life Sciences and Toxicology
RTI International

Gary M. Hoffman, B.A., DABT — 12 Oct 04 Date
Senior Toxicologist and Inhalation Specialist
Principal Investigator
Huntingdon Life Sciences

ADDITIONAL APPROVALS

RTI Quality Assurance Review by: Carrie Ingalls / 10/25/04
Carrie A. Ingalls, B.S. Date
Quality Assurance Specialist, Quality Assurance Unit

HLS Quality Assurance Review by: M Elliott for NSI / 28 OCT 04
Nicki S. Iacono, B.S. Date
Director, Quality Assurance

RTI Institutional Management: / 10/26/04
Alan H. Staple, M.Sc. Date
Vice President, Health Sciences

HLS Institutional Management: / 28 Oct 04
Teresa S. Kusznir, V.M.D. Date
Animal Facility Veterinarian

/ 28 Oct 04
Kay Saladdin, B.S. Date
Assoc. Director of Formulation Chemistry Services

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1.0 Introduction and Objective

The purpose of this study is to provide maternal and developmental toxicity data relative to a 6- or 12-day exposure regimen of inhaled gasoline methyl tertiary butyl ether (MTBE) vapor condensate during early or major organogenesis in gravid mice. A developmental toxicity evaluation of Gasoline MTBE Vapor Condensate by inhalation to mice was one of series tests required in accordance with the Alternative Tier 2 provisions of fuels and fuels additives health effects testing regulations (40 C.F.R. § 79; Oge 1998). The study involved whole-body inhalation exposure of timed-pregnant CD-1 mice for at least 6 hours/day, on gestational day (gd) 5 through 17, to baseline gasoline vapor condensate with 21.5% MTBE at target concentrations of 0, 2000, 10,000 and 20,000 mg/m^3 (the last is 50% of the lower explosive limit; ExxonMobil Biological Sciences Institute [EMBSI], 2002). The present study is being conducted with the same exposure concentrations for gd 5 through 16, plus 30,000 mg/m^3 for 6 hours/day on gd 5 through 10 in order to confirm and extend the findings observed in the EMBSI study (2002). See Section 3.1 for justification of exposure concentrations and durations.

Alterations to this study protocol may be made as the study progresses. No changes in the protocol will be made without the Sponsor's consent. In the event the Sponsor verbally authorizes a protocol change, such change will be honored by the Testing Facilities and will be followed by written verification and a protocol amendment. All protocol amendments will be signed and reviewed by the HLS and RTI QA Managers. Any modifications potentially affecting animal welfare will also be signed by 2 members of the Institutional Animal Care and Use Committee (IACUC) of the HLS Testing Facility and of the RTI IACUC prior to the modification's implementation.

RTI is responsible for study design, protocol generation, necropsy of the maternal and fetal animals on gestational day (gd) 17, any postmortem evaluations, generation of summary and individual data tables, and study draft and final report generation (with RTI QA oversight). HLS is responsible for receipt of the test substance, prestudy and study generation and analyses of the test vapors, receipt, quarantine, and housing of the test females and breeder males, mating and assignment of the study animals, in-life observations, loading and unloading study females into and out of chambers, and submission of interim and final inhalation reports.

2.0 Materials and Methods

2.1 Test Substance

Chemical Name:	Gasoline MTBE Vapor Condensate (MRD-00-713; "API 211BG with MTBE Vapor Condensate")
CAS Registry Number:	None
Specific Gravity:	0.66 to 0.68 g/ml @ 15.6°C
Molecular Formula:	Not applicable
Average Molecular Weight:	Not applicable
Supplier:	Chevron Global Technology Services Company, Richmond, CA
Lot/Batch Number:	API 00-02

Identity, Strength, Purity and Composition:	Information on identity, strength, purity, and composition of Gasoline MTBE Vapor Condensate will be provided by the Sponsor and documented in the raw data and final report (see Attachment for MSDS and Test Substance Characterization and below for stability assessments at HLS).
Methods of Synthesis:	Methods of synthesis, fabrication, or derivation will be documented by the Sponsor, and documents will be located at API.
Appearance:	Colorless liquid
Solubility:	Soluble in hydrocarbons, insoluble in water
Stability and Storage:	Stable. The test substance will be stored (under ambient conditions) in an outside solvent shed except when in use in the inhalation laboratory. The test substance will be handled as a flammable liquid (see MSDS in Attachment). The stability of the test substance will be assessed at HLS by comparing results of the pretest liquid and vapor analyses with the results of the liquid and vapor analyses during the study. An archive sample of the test substance will be retained at HLS with appropriate documentation.

2.2 Chemical Safety and Handling

Detailed information on chemical handling is provided in the MSDS in the Attachment.

2.3 Analysis of Chamber Vapor Atmosphere

See Section 3.4.

2.4 Animals

2.4.1 Species and Supplier

The proposed test animals will be Caesarean-originated Virus Antibody Free (VAF) Crl:CD-1® (ICR) BR outbred albino mice supplied by Charles River Laboratories, Inc., Raleigh, NC.

2.4.2 Live Animals and Species Justification

The use of live animals has been requested by the Sponsor and required by U.S. EPA OPPTS Testing Guidelines (U.S. EPA, 1998). Alternative test systems are not available for the assessment of chemical effects on prenatal mammalian development. The Charles River CD-1® mouse has been the subject of choice on developmental toxicology contracts at RTI since 1976. Large historical databases for reproductive performance and prevalence of spontaneous malformations in control mice are available from studies conducted at RTI (currently based on over 348 control litters).

2.4.3 Total Number, Age, and Weight

One hundred seventy (170) nulliparous female mice will be ordered for this study. One hundred (100) male mice, 9-11 weeks old upon arrival at HLS (on August 31, 2004), of the same strain and from the same supplier, were received for the previous range-finding study, and the

remaining 99 males will be used as a male breeding colony for this study. If more than the ordered number of females is received, any extra animals will be used to replace any animals with clinical signs, injury, and/or reduced feed consumption. If none of the animals has indicators during quarantine, then the animal(s) with the lowest or highest body weight(s) will not be used on study. The 99 males will be used to generate timed-mated animals for this definitive developmental toxicity study which will require the mating of 170 female mice (1:1, with the subsequent addition of naïve females to males who inseminated their original females) to generate 140 plug-positive females. Females will be 7-9 weeks old at arrival. Female mice will be 9-11 weeks of age and ~20-35 g in weight on gd 0. One hundred seventy (170) females are required to generate 140 plug-positive females in 4 to 5 consecutive days; 140 plug-positive females (25/group for 4 groups and 40/group for the fifth group) are required to supply the optimal number (based on EPA's guidance; e.g., OPPTS 870.3600; U.S. EPA, 1996; for inhalation developmental toxicity studies) of pregnant animals and litters to assess any maternal and/or embryo/fetal toxicity to the test substance and to confirm and extend the fetal findings from the previous EMBSI study.

2.5 Animal Husbandry

2.5.1 Acclimation, Housing, Feed, and Water

During an approximately 7-day quarantine period at the HLS Testing Facility, animals will be checked for viability twice daily. Prior to study assignment, all animals will be examined to ascertain suitability for study. The HLS veterinarian (or designate) will formally release these animals for use by signature and date. Males and females will be individually housed in stainless steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female will be housed together. During cohabitation, male and female mice will be housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). 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 weekly for periods when feed consumption is not being recorded and at each interval when feed consumption will be recorded. After the gd 14 exposure period (for Groups 1-4) or on the afternoon of gd 14 (Group 5; see Section 3.0), a stainless steel, perforated insert will be placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. For females still undergoing daily exposures after gd 14 (Groups 1-4), the floor insert and Nestlet® will be removed before each daily exposure and replaced after each exposure. Feed (PMI 5002 Certified Meal) will be available ad libitum, except during the daily 6-hour inhalation periods. Analytical certification of batches of feed provided by the manufacturer will be maintained on file at the HLS Testing Facility. There are no known contaminants in the feed that are expected to interfere with the objectives of this study. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) will be available ad libitum via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses are conducted by Elizabethtown Water Company to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis provided by the supplier will be maintained on file at the HLS Testing Facility. There are no known contaminants that are expected to interfere with the objectives of this study. At all times, animals will be housed, handled, and used according to the National Research Council Guide (NRC, 1996).

2.5.2 Environmental Conditions

A 12-hour light/dark cycle is provided via automatic timer. Temperature and relative humidity will be monitored in accordance with Testing Facility SOPs to ensure that the desired range of 18 to 26°C for temperature and 30 to 70% relative humidity is maintained to the maximum extent possible (NRC, 1996).

2.5.3 Animal Identification

Each animal will be assigned a temporary identification number (designated on each cage) upon receipt. During the second week of the quarantine/acclimation period, the 170 ordered females (and any extras supplied) will be tail tattooed with consecutive numbers, 1 through 170. The 99 remaining males have already been tail tattooed with consecutive numbers, 1 through 100 (except 87). After selection for use on the study, mating, indication of copulation, and assignment to 1 of the 5 groups, each female will be ear tagged with a number assigned by the HLS Testing Facility. If an eartag is lost, it will not be replaced, but the number will be placed on the female's tail with a marking pen and documented. This number, plus the study number, will comprise the unique animal number for each animal. Each cage will be provided with a cage card that will be color coded for exposure level identification and will contain the study and animal numbers.

2.5.4 Limitation of Discomfort

Some adult toxicity (e.g., narcosis) may be caused by exposure to the higher concentrations. It is anticipated that the concentrations employed will not result in irritation or corrosion to the respiratory tract of the test animals. Discomfort or injury to animals will be limited in that if any animal becomes severely debilitated or moribund, it will be humanely terminated by CO_2 asphyxiation. All necropsies will be performed after terminal CO_2 anesthesia. Animals will not be subjected to undue pain or distress.

2.5.5 Breeding

Immediately prior to pairing, each female will be weighed and subjected to a clinical examination. Any females that have clinical signs, sore(s), wound(s), or that have very low or very high body weights, relative to the rest of the females, will not be paired unless absolutely necessary. If used, the animals with extreme body weights (high or low) will be used before any animals with clinical signs, etc. For breeding, a 1 male with 1 female pairing will be employed, since other pairing patterns (e.g., 1 male with 2 females) may result in an unacceptable number of plug-positive, nonpregnant females and/or sire effects. Individual females will be placed in polycarbonate "shoebox" cages with stainless steel lids (and Alpha-Dri® bedding) with singly-housed males. The males will be placed in the polycarbonate breeding cages at least 24 hours prior to the addition of the females. On the following morning and each morning thereafter, the females will be examined for the presence of a vaginal or dropped copulation plug (Hafez, 1970). The day on which copulation plugs are found will be designated as gd 0. Plug-positive females (dams) will be individually housed in stainless steel, wire-mesh, hanging cages until scheduled sacrifice on gd 17. Stainless steel, perforated inserts with Nestlets® (Ancare, Bellmore, NY) will be placed on the wire-mesh floor of each study female's cage after the gd 14 exposure period (for Groups 1-4) or in the afternoon of gd 14 (Group 5; see Section 3.0) to scheduled sacrifice. Plug-negative females will be retained in the same male's cage and checked for plugs on successive mornings until insemination occurs or the treatment groups are

filled, whichever comes first. Once a female is found plug/sperm positive and removed from the male's cage, a naïve (not yet paired) female will be added to the successful male's cage until 140 inseminated females are found. The plug-positive females will be distributed across all 5 groups so that no group will contain a disproportionate number of females mated to the same male. HLS staff will examine the paired females to detect vaginal copulation plugs and then set up the exposure schedule, based on the gd 0 dates. When all treatment groups are filled, remaining plug-negative and plug-positive females will be sacrificed by asphyxiation with CO_2. The breeding males will also be euthanized by CO_2 asphyxiation after the breeding period is completed. The fate of all animals will be fully documented.

3.0 Experimental Design

3.1 Study Design

This study will be conducted with 4 treatment groups and 1 vehicle control group, with Groups 1-4 each comprised of 25 plug-positive female mice and Group 5 comprised of 40 plug-positive female mice (Table 1).

Table 1
Endpoint-Specific Developmental Toxicity
Number of Animals Assigned to Study Groups

Group No.	No. Animals Exposed	No. Days Exposed	Exposure Period (gd)	Target Exposure Concentration (mg/m^3)
1	25	12	5 through 16	0
2	25	12	5 through 16	2000
3	25	12	5 through 16	10,000
4	25	12	5 through 16	20,000
5	40	6	5 through 10	30,000

The size of Group 5 (40 females vs. 25 each in the other 4 groups) was increased to optimize the production and detection of the fetal malformations of interest (if they occur) previously observed in the EMBSI study at 2000 and 10,000 mg/m^3 but not at 20,000 mg/m^3. The exposure period for dams at 0 through 20,000 mg/m^3 in the EMBSI (2002) study was gd 5 through 17, with necropsy on gd 18. The present study specifies an exposure period, at these same exposure concentrations, of gd 5 through 16 (and necropsy on gd 17) for 3 reasons:

1. CD-1® mice begin delivering their litters on gd 18 but do not normally deliver on gd 17.

2. The Study Director and her staff have extensive experience in reproductive and developmental toxicity studies (and historical data) in the CD-1® mouse, with scheduled necropsy on gd 17.

3. The fetal malformations of interest are formed early in the embryonic period of gestation; gd 7-9 in the mouse (e.g., Rugh, 1968), so extending the exposure period to gd 17 is unnecessary.

The exposure period for Group 5 at 30,000 mg/m^3 of gd 5 through 10 was selected based on reason #3 above and to reduce the number of days of generation of test atmosphere at a concentration that is 75% of the lower explosive limit.

Tentative Study Schedule:

Females arrive at HLS:	November 18, 2004
Quarantine (7 days):	November 18-25, 2004
Animals paired:	November 25-30, 2004
Dates of gd 0:	November 26-30, 2004
TSCA experimental start date:	December 1, 2004
Exposure dates: (gd 5 through 10):	December 1-10, 2004
(gd 5 through 16):	December 1-16, 2004
Scheduled termination (gd 17)	December 13-17, 2004
TSCA experimental termination date:	December 17, 2004
Submission of draft data on test atmospheres to Sponsor:	December 23, 2004 (within 1 week after the last exposure date, December 16, 2004)
Submission of interim data report:	January 14, 2005 (within 4 weeks of last necropsy)
Submission of audited draft final report:	February 17, 2005 (within 2 months of last necropsy date)
Submission of final report:	Within 1 month of receipt of Sponsor's comments on the audited draft report

3.2 Exposure Selection

The 0, 2000, 10,000, and 20,000 mg/m^3 exposure concentrations for gd 5 through 16 are the same as those employed in the original developmental toxicity study (EMBSI, 2002), in an attempt to confirm their fetal findings. In addition, 30,000 mg/m^3 (at 75% of the lower explosive limit) will be employed, with exposures for 6 hours/day on gd 5 through 10, to extend the original exposure concentrations and to attempt to confirm and extend the original fetal findings.

3.3 Test Substance Administration and Analysis

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 prestudy trials. The trials will be performed (at least two 6-hour periods) to evaluate the optimal set of conditions and equipment to generate a stable atmosphere at the target exposure levels and maintain uniform

conditions throughout the exposure chambers. The method will be described in the raw data of the study and in the final report.

The whole-body exposure chambers will each have a volume of approximately 1000 liters. The chambers will be operated at a minimum flow rate of 200 liters per minute. The final airflow will be set to provide at least 1 air change in 5 minutes (12 air changes/hour) and a T_{99} 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 each daily 6-hour 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.

3.4 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 6-hour 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. Airborne test material concentrations will be within +/- 10% of the target concentration. One sample per chamber during the trials period and the treatment period will be analyzed by gas chromatography 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 (at additional cost).

3.5 Particle Size Distribution Analysis

During the treatment period, particle size determinations will be performed once per chamber using a TSI Aerodynamic Particle Sizer to confirm the absence of particulate test substance condensate in the exposure atmosphere.

3.6 Chamber Environment

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

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

3.7 Summary of Chamber Activity

The minimum frequency of chamber activity during the treatment period is summarized below:

Activity	Frequency/Chamber
Measured Test Substance Concentration	4X/day
Measured Test Substance Characterization	1X
Particle Size	1X
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

3.8 Allocation and Exposure of Maternal Animals

Plug-positive female mice (dams) will be assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights and equal distribution of females mated to the same male among dose groups on gd 0. Females will be exposed to gasoline MTBE vapor condensate or air 6 hours per day from gd 5 through 16 for Groups 1-4 and for gd 5 through 10 for Group 5. Inhalation was chosen by the Sponsor as the route of administration.

For each daily exposure, females will be transferred to inhalation cages, and the cages will be moved into the appropriate chambers for exposure. Following each daily exposure, females will be transferred back to home caging for feed consumption measurements overnight.

3.9 Observation of Maternal Animals

3.9.1 Clinical Observations

Clinical observations of all animals will be made once daily on gd 0 through 4 (prior to exposure period), on gd 11 through 17 or gd 16 through 17 (after the exposure period), and twice daily (prior to and immediately after each daily exposure) throughout the exposure period (gd 5 through 10 or gd 5 through 16). In addition, during each daily exposure period, animals will be observed at least once during each exposure. This will be routinely performed near the middle of each exposure and may be performed more frequently if significant signs of toxicity are noted.

3.9.2 Maternal Body Weights

Dams will be weighed in the mornings (prior to exposures for those days that exposures occur) on gd 0 and 5 through 17. Maternal weight gains will be calculated for gd 0-5 (pre-exposure period), 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14, 14-15, 15-16, 16-17, 5 through 10 or 5 through 16 (exposure period), 10 through 17 or 16 through 17 (postexposure period), and 0 through 17 (gestational period).

3.9.3 Maternal Feed Consumption

Maternal feed consumption will be evaluated in the mornings from gd 0-5 (pre-exposure period), 5-6, 6-7, 7-8, 8-9, 9-10, 10-11, 11-12, 12-13, 13-14, 14-15, 15-16, 16-17, 5 through 10 or 5 through 16 (exposure period), 10 through 17 or 16 through 17 (postexposure period), and 0 through 17 (gestation period).

3.10 Postmortem Evaluation

3.10.1 Maternal

Maternal animals that die during the course of the study will be necropsied in an attempt to determine cause of death. No organs will be weighed or saved. Females that appear moribund will be humanely euthanized by CO_2 asphyxiation and necropsied to determine the cause of the morbidity, if possible, with no organs weighed or retained. Females showing signs of abortion or premature delivery will also be sacrificed, as described above, as soon as the event is detected and subjected to a gross necropsy with no organs weighed or saved. On gd 17, approximately 1 to 1½ days before expected parturition, all surviving maternal animals will be killed by CO_2 asphyxiation by RTI staff, thoracic and abdominal cavities and organs examined, and pregnancy status confirmed by uterine examination. Uteri which present no visible implantation sites will be stained with ammonium sulfide (10%) in order to visualize any implantation sites that may have undergone very early resorption (Salewski, 1964). At sacrifice, the body, liver, uterus, paired adrenal glands, and paired kidneys of each plug-positive female will be weighed. Ovarian corpora lutea will be counted and uterine contents (i.e., number of implantation sites, early and late resorptions, dead fetuses, live fetuses) will be recorded.

3.10.2 Fetal

Live fetuses will be removed from the uterus, counted, weighed, sexed externally, and examined externally for gross malformations (including cleft palate) and variations by RTI staff. Each fetus will be killed by intraperitoneal injection of sodium pentobarbital, dissected longitudinally, and the thoracic and abdominal viscera removed intact and retained individually in labeled scintillation vials in buffered neutral 10% formalin for possible subsequent visceral examination. The fetal carcass will be blanched, skinned, and retained in individually labeled scintillation vials in 70% ethanol for possible subsequent double staining (alizarin Red S and alcian blue) and skeletal evaluation. All maternal organs and carcasses will be destroyed by incineration.

4.0 Statistics

The unit of comparison will be the pregnant female or litter. Quantitative continuous data (e.g., maternal body weights, feed consumption, fetal body weights, etc.) will be compared among the 4 treatment groups and one vehicle control group using either parametric ANOVA under the standard assumptions or robust regression methods (Zeger and Liang, 1986; Royall, 1986; Huber, 1967), which do not assume homogeneity of variance or normality. The homogeneity of variance assumption will be examined via Levene's Test (Levene, 1960), which is more robust to the underlying distribution of the data than the traditional Bartlett's Test. If Levene's Test indicates lack of homogeneity of variance ($p<0.05$), robust regression methods will be used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They will be used to test for overall treatment group differences (via Wald Chi-Square Tests), followed by individual *t*-tests for exposed vs. control group comparisons when the overall treatment effect is significant. The presence of linear trends will be analyzed by robust regression methods for nonhomogenous data. Robust regression methods are available in the REGRESS procedure of SUDAAN® Release 8. (RTI, 2001).

If Levene's Test does not reject the hypothesis of homogeneous variances, standard ANOVA techniques will be applied for comparing the treatment groups. The GLM procedure in SAS® Release 8 will be used to evaluate the overall effect of treatment and, when a significant treatment effect is present, to compare each exposed group to control via Dunnett's Test (Dunnett, 1955, 1964). Prior to GLM analysis, an arcsine-square root transformation will be performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For the litter-derived percentage data, the ANOVA will be weighted according to litter size. The presence of linear trends will be analyzed by GLM procedures for homogenous data (SAS Institute Inc., 1999a,b,c,d,e; 2000; 2001). A one-tailed test (i.e., Dunnett's Test) will be used for all pairwise comparisons to the vehicle control group, except that a two-tailed test will be used for maternal body and organ weight parameters, maternal feed consumption, fetal body weight, and percent males per litter. Standard ANOVA methods, as well as Levene's Test, are available in the GLM procedure of SAS® Release 8 (SAS Institute Inc., 1999a,b,c,d,e; 2000; 2001).

Nominal scale measures will be analyzed by Chi-Square Test for Independence for differences among treatment groups (Snedecor and Cochran, 1967) and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square reveals significant ($p<0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, will be used for pairwise comparisons between each treatment group and the control group.

A test for statistical outliers (SAS Institute, Inc., 1999b) will be performed on female body weights, feed consumption (in g/day), and selected organ weights. If examination of pertinent study data does not provide a plausible, biologically sound reason for inclusion of the data flagged as "outlier," then the data will be excluded from summarization and analysis and will be designated as outliers.

5.0 Storage of Records

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 RTI's secure archives for a period of 1 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 1 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.

6.0 Good Laboratory Practices, Animal Welfare Act Compliance, IACUC, and Quality Assurance Monitoring

This study will be conducted in accordance with the U.S. EPA's GLP standards for the 211(b) program (40 C.F.R. 79.60). This study will be performed according to the protocol and HLS' and RTI's SOPs. This study will comply with all appropriate parts of the USDA Animal Welfare Act regulations: 9 CFR Parts 1 and 2 Final Rules, *Federal Register*, Vol. 54, No. 168, August 31, 1989, pp. 36112-36163, effective October 30, 1989, and 9 CFR Part 3 Animal Welfare Standards; and the Final Rule, *Federal Register*, Vol. 55, No. 32, February 15, 1991, pp. 6426-6505, effective March 18, 1991. The Sponsor should make particular note of the following:

- The Sponsor's signature on this study protocol documents that there are no generally accepted non-animal alternatives, and the study does not unnecessarily duplicate previous experiments.

- 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.

- Any procedures outlined in this study protocol that are expected to cause more than momentary or slight pain/distress (there are no procedures in this study protocol that are expected to cause more than momentary or slight pain or distress to the animals other than some adult toxicity; e.g., narcosis) to the animals will be performed with appropriate analgesics or anesthetics unless the withholding of these agents is justified for scientific reasons, in writing, by the Sponsor and the Study Director and approved by the IACUC, in which case the procedure will continue for the minimum time necessary. Documentation of the justification for withholding treatment for pain or distress and IACUC approval of the procedures will be made prior to study initiation on the IACUC Protocol Review form.

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

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

The HLS IACUC Protocol Review Subcommittee and the RTI IACUC have both reviewed this protocol and found it to be in compliance with appropriate animal welfare regulations.

The Quality Assurance Unit of HLS (East Millstone, NJ) will monitor the facilities, equipment, personnel, methods, practices, records, raw data, draft and final inhalation reports, and controls used in their portion of this study to assure that they are in conformance with this protocol, company standard operating procedures, and the referenced Good Laboratory Practice (GLP) regulations. RTI's Quality Assurance Unit will perform a prestudy on-site inspection, review the protocol and any amendments, and monitor all phases of the study in which RTI personnel participate, the data, and the draft final study report. The HLS report on Generation and Analysis of Test Atmospheres (which will be an appendix to the final study report) will contain its own GLP Compliance Statement and QA Statement. The RTI final study report will contain a GLP Compliance Statement and a QA Statement to cover all other aspects of the study.

7.0 Reports

7.1 Draft Data Report on Test Atmospheres

A draft data report which tabulates chamber concentration and environmental values will be provided within 1 week of the completion of the exposure phase. The Principal Investigator will provide an explanation and interpretation of the data (where appropriate).

7.2 Interim Data Report

An interim, unaudited data report will be submitted within 4 weeks of the last necropsy date. This interim report will include summary and analysis of all in-life maternal data and all necropsy (and any postnecropsy) fetal data. The Study Director will provide an explanation and/or interpretation of the data, as appropriate.

7.3 Final Report

An audited draft report will be provided within 2 months of the last sacrifice date. A final report will be issued within 30 days of receiving the Sponsor's comments. The final report for the tolerance range-finding study (performed previously) will be submitted as an appendix to this endpoint-specific, definitive, developmental toxicology study of inhaled Gasoline MTBE Vapor Condensate in CD-1® mice.

8.0 Personnel

Sponsor's Representative: Mr. Thomas M. Gray, M.S. DABT, American Petroleum Institute

Study Director: Dr. Rochelle W. Tyl., Ph.D., DABT, RTI

Principal Investigator: Mr. Gary M. Hoffman, B.A., DABT, HLS

HLS Personnel:

Animal Research Facility Veterinarian: Dr. Teresa S. Kusznir, V.M.D.

Animal Research Facility Director: Mr. Ian Vanterpool, F.I.A.T.

Necropsy Laboratory Supervisor: Ms. G. Elizabeth Baxter, B.S.

Inhalation Laboratory Supervisor: Mr. Stuart Cracknell, C.Bio., M.I.Biol.

Analytical Chemistry: Ms. Kay Saladdin, B.S.

Quality Assurance: Ms. Nicki S. Iacono, B.S.

RTI Personnel:

Reproductive and Developmental Laboratory Supervisor: Ms. Melissa C. Marr, B.A., LATG

Data Specialist: Ms. Christina B. Myers, M.S.

Quality Assurance Unit Manager: Ms. Debra A. Drissel, B.S.

Additional study team members to be determined.

9.0 Study Records to be Maintained

Protocol and any amendments

List of standard operating procedures

Animal requisition and receipt records

Acclimation records

Temperature and humidity records for the treatment room(s)

Animal Research Facility room log(s)

Water analysis

Feed type, source, lot number, dates used, certification, contaminants

Mating records

Randomization records

Assignment to study records

Bulk chemical receipt, storage and use records

Test chemical generation and analysis records

Analytical chemistry records

Shipment of bulk chemical to supplier after the definitive study

Exposure records, clinical signs, maternal weight, feed consumption

Necropsy records (in the event of maternal mortality)

Teratology sacrifice records- external examination sheets

Transfer of custody of retained fetal viscera and carcasses

Statistical printouts

Correspondence

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ATTACHMENT

MATERIAL SAFETY DATA SHEET

AND

TEST SUBSTANCE CHARACTERIZATION

Material Safety Data Sheet

1. CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

API 211BG w/MTBE Vapor Condensate

COMPANY IDENTIFICATION

Chevron Global Technology Services Co.
Rm. 51-2126
100 Chevron Way
Richmond, CA. 94802-9627

EMERGENCY TELEPHONE NUMBERS

HEALTH (24 hr): (800)231-0623 or
(510)231-0623 (International)
TRANSPORTATION (24 hr): CHEMTREC
(800)424-9300 or (703)527-3887
Emergency Information Centers
are located in U.S.A.
Int'l collect calls accepted

PRODUCT INFORMATION: (510) 242-3062

2. COMPOSITION/INFORMATION ON INGREDIENTS

100.0 % API 211BG w/MTBE Vapor Condensate

CONTAINING

COMPONENTS	AMOUNT	LIMIT/QTY	AGENCY/TYPE
GASOLINE (GENERIC)			
		890 mg/m3	ACGIH TWA
		1480 mg/m3	ACGIH STEL
		2000 mg/m3	OSHA PEL
WHICH MAY CONTAIN			
BENZENE Chemical Name: BENZENE CAS71432	< 2.00%	0.5 ppm	ACGIH TWA
		2.5 ppm	ACGIH STEL
		1 ppm	OSHA PEL

		5 ppm	OSHA CEILING
		10 LBS	CERCLA 302.4 RQ
TOLUENE			
Chemical Name: TOLUENE			
CAS108883	< 5.00%	50 ppm	ACGIH TWA
		200 ppm	OSHA PEL
		300 ppm	OSHA CEILING
		1,000 LBS	CERCLA 302.4 RQ
N-BUTANE			
Chemical Name: N-BUTANE			
CAS106978	< 8.00%	800 ppm	ACGIH TWA
N-HEXANE			
Chemical Name: N-HEXANE			
CAS110543	< 2.00%	50 ppm	ACGIH TWA
		500 ppm	OSHA PEL
		5,000 LBS	CERCLA 302.4 RQ
HEXANE ISOMERS (OTHER THAN N)			
HEXANES			
	< 20.00%	500 ppm	ACGIH TWA
		1000 ppm	ACGIH STEL
PENTANE (ALL ISOMERS)			
PENTANES			
	< 35.00%	600 ppm	ACGIH TWA
		750 ppm	ACGIH STEL
		1000 ppm	OSHA PEL
2,2,4-TRIMETHYLPENTANE			
Chemical Name: 2,2,4-TRIMETHYLPENTANE			
CAS540841	< 2.00%	1,000 LBS	CERCLA 302.4 RQ
ETHYL BENZENE			
Chemical Name: BENZENE, ETHYL-			
CAS100414	< 1.00%	100 ppm	ACGIH TWA
		125 ppm	ACGIH STEL
		100 ppm	OSHA PEL
		1,000 LBS	CERCLA 302.4 RQ
METHYL TERT BUTYL ETHER (MTBE)			
Chemical Name: 2-METHOXY-2-METHYL PROPANE			
CAS1634044	< 26.00%	40 ppm	ACGIH TWA
		50 ppm	Chevron STEL
		1,000 LBS	CERCLA 302.4 RQ

COMPOSITION COMMENT:
Refer to the OSHA Benzene Standard (29 CFR 1910.1028) and Table Z-2 for detailed training, exposure monitoring, respiratory protection and medical surveillance requirements before using this product.

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********

Colorless liquid

- **EXTREMELY FLAMMABLE**
- **HARMFUL OR FATAL IF SWALLOWED - CAN ENTER LUNGS AND CAUSE DAMAGE**
- **VAPOR HARMFUL**
- **MAY CAUSE EYE AND SKIN IRRITATION**
- **LONG-TERM EXPOSURE TO VAPOR HAS CAUSED CANCER IN LABORATORY ANIMALS**

****

IMMEDIATE HEALTH EFFECTS

EYE:
Contact with the eyes causes irritation. Eye contact with the vapors, fumes, or spray mist from this substance could also cause similar signs and symptoms.

SKIN:
Contact with the skin causes irritation. Not expected to be harmful to internal organs if absorbed through the skin. Prolonged or frequently repeated contact may cause the skin to become cracked or dry from the defatting action of this material.

INGESTION:
Because of the low viscosity of this substance, it can directly enter the lungs if it is swallowed (this is called aspiration). This can occur during the act of swallowing or when vomiting the substance. Once in the lungs, the substance is very difficult to remove and can cause severe injury to the lungs and death.

INHALATION:
May be harmful if inhaled. Breathing the vapors at concentrations above the recommended exposure standard can cause central nervous system effects. The vapor or fumes from this material may cause respiratory irritation.

SIGNS AND SYMPTOMS OF EXPOSURE:
Eye damage or irritation: may include pain, tearing, reddening, swelling, and impaired vision. Skin injury: may include pain, discoloration, swelling, and blistering. Respiratory irritation: may include coughing and difficulty breathing. Central nervous system effects may include headache, dizziness, nausea, vomiting, weakness, loss of coordination, blurred vision, drowsiness, confusion, or disorientation. At extreme exposures, central nervous system effects may include respiratory depression, tremors or convulsions, loss of consciousness, coma or death.

CARCINOGENICITY:
Risk depends on duration and level of exposure. See Section 11 for additional information. Gasoline has been classified as a Group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC). Contains chemical(s) known to the State of California to cause cancer. Contains benzene, which has been classified

as a carcinogen by the National Toxicology Program (NTP), and a Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer (IARC). Contains ethylbenzene which has been classified as a Group 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC).

4. FIRST AID MEASURES

EYE:
Flush eyes with water immediately while holding the eyelids open. Remove contact lenses, if worn, after initial flushing, and continue flushing for at least 15 minutes. Get medical attention if irritation persists.
SKIN:
Wash skin immediately with soap and water and remove contaminated clothing and shoes. Get medical attention if irritation persists. Discard contaminated clothing and shoes or thoroughly clean before reuse.
INGESTION:
If swallowed, give water or milk to drink and telephone for medical advice. DO NOT make person vomit unless directed to do so by medical personnel. If medical advice cannot be obtained, then take the person and product container to the nearest medical emergency treatment center or hospital.
INHALATION:
Move the exposed person to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if breathing difficulties continue.
NOTE TO PHYSICIANS:
Ingestion of this product or subsequent vomiting can result in aspiration of light hydrocarbon liquid which can cause pneumonitis.

5. FIRE FIGHTING MEASURES

FIRE CLASSIFICATION:
OSHA Classification (29 CFR 1910.1200): Flammable liquid. See section 7 for appropriate handling and storage conditions.
FLAMMABLE PROPERTIES:
FLASH POINT: (TCC) < -49F (<-45C)
AUTOIGNITION: NDA
FLAMMABILITY LIMITS (% by volume in air): Lower: NDA Upper: NDA
EXTINGUISHING MEDIA:
Dry Chemical, CO2, Alcohol Resistant (AR) AFFF.
NFPA RATINGS: Health 1; Flammability 3; Reactivity 0.
FIRE FIGHTING INSTRUCTIONS:
Use water spray to cool fire-exposed containers and to protect personnel. For fires involving this material, do not enter any enclosed or confined fire space without proper protective equipment, including self-contained breathing apparatus.
COMBUSTION PRODUCTS:
Normal combustion forms carbon dioxide and water vapor; incomplete combustion can produce carbon monoxide.

6. ACCIDENTAL RELEASE MEASURES

CHEMTREC EMERGENCY NUMBER (24 hr): (800)424-9300 or (703)527-3887
International Collect Calls Accepted
ACCIDENTAL RELEASE MEASURES:
Eliminate all sources of ignition in the vicinity of the spill or released vapor.

Stop the source of the leak or release. Clean up releases as soon as possible, observing precautions in Exposure Controls/Personal Protection. Contain liquid to prevent further contamination of soil, surface water or groundwater. Clean up small spills using appropriate techniques such as sorbent materials or pumping. Where feasible and appropriate, remove contaminated soil. Follow prescribed procedures for reporting and responding to larger releases. Place contaminated materials in disposable containers and dispose of in a manner consistent with applicable regulations. Contact local environmental or health authorities for approved disposal of this material.

Release of this product should be prevented from contaminating soil and water and from entering drainage and sewer systems. U.S.A. regulations require reporting spills of this material that could reach any surface waters. The toll free number for the U.S. Coast Guard National Response Center is (800) 424-8802.

7. HANDLING AND STORAGE

This product presents an extreme fire hazard. Liquid very quickly evaporates, even at low temperatures, and forms vapor (fumes) which can catch fire and burn with explosive violence. Invisible vapor spreads easily and can be set on fire by many sources such as pilot lights, welding equipment, and electrical motors and switches.

Electrostatic charge may accumulate and create a hazardous condition when handling this material. To minimize this hazard, bonding and grounding may be necessary but may not, by themselves, be sufficient. Review all operations which have the potential of generating an accumulation of electrostatic charge and/or a flammable atmosphere (including tank and container filling, splash filling, tank cleaning, sampling, gauging, switch loading, filtering, mixing, agitation, and vacuum truck operations) and use appropriate mitigating procedures. For more information, refer to OSHA Standard 29 CFR 1910.106, "Flammable and Combustible Liquids", National Fire Protection Association (NFPA) 77, "Recommended Practice on Static Electricity", and/or the American Petroleum Institute (API) Recommended Practice 2003, "Protection Against Ignitions Arising Out of Static, Lightning, and Stray Currents".

Never siphon gasoline by mouth. Use only as a motor fuel. Do not use for cleaning, pressure appliance fuel, or any other such use. DO NOT USE OR STORE near heat, sparks or open flames. USE AND STORE ONLY IN WELL

VENTILATED AREA. Keep container closed when not in use. READ AND OBSERVE ALL PRECAUTIONS ON PRODUCT LABEL.

Empty containers retain product residue (solid, liquid, and/or vapor) and can be dangerous. Do not pressurize, cut, weld, braze, solder, drill, grind, or expose such containers to heat, flame, sparks, static electricity, or other sources of ignition. They may explode and cause injury or death. Empty containers should be completely drained, properly closed, and promptly returned to a drum reconditioner, or properly disposed of.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

GENERAL CONSIDERATIONS:
Consider the potential hazards of this material (see Section 3), applicable exposure limits, job activities, and other substances in the work place when designing engineering controls and selecting personal protective equipment. If engineering controls or work practices are not adequate to prevent exposure to harmful levels of this material, the personal protective equipment listed below is recommended. The user should read and understand all instructions and limitations supplied with the equipment since protection is usually provided for a limited time or under certain circumstances.

ENGINEERING CONTROLS
Use process enclosures, local exhaust ventilation, or other engineering controls to control airborne levels below the recommended exposure limits.

PERSONAL PROTECTIVE EQUIPMENT
EYE/FACE PROTECTION:
No special eye protection is normally required. Where splashing is possible, wear safety glasses with side shields as a good safety practice.
SKIN PROTECTION:
No special protective clothing is normally required. Where splashing is possible, select protective clothing depending on operations conducted, physical requirements and other substances. Suggested materials for protective gloves include: <Nitrile> <Polyurethane> <Viton> <Chlorinated Polyethylene (or Chlorosulfonated Polyethylene or CPE)>
RESPIRATORY PROTECTION:
Determine if airborne concentrations are below the recommended exposure limits. If not, wear a NIOSH approved respirator that provides adequate protection from measured concentrations of this material. Use the following respirators: Organic Vapor. Use a positive pressure, air-supplying respirator if there is potential for uncontrolled release, exposure levels are not known, or other circumstances where air-purifying respirators may not provide adequate protection.

9. PHYSICAL AND CHEMICAL PROPERTIES

PHYSICAL DESCRIPTION:
Colorless liquid

pH:	NA
VAPOR PRESSURE:	<20 PSI @ 100F (REID)
VAPOR DENSITY (AIR=1):	3-4
BOILING POINT:	31 - 300F
FREEZING POINT:	NDA
MELTING POINT:	NDA
SOLUBILITY:	Soluble in hydrocarbons; insoluble in water.
SPECIFIC GRAVITY:	0.66 - 0.68 @ 15.6/15.6C

10. STABILITY AND REACTIVITY

HAZARDOUS DECOMPOSITION PRODUCTS:
None known
CHEMICAL STABILITY:
Stable.
CONDITIONS TO AVOID:
See section 7.
INCOMPATIBILITY WITH OTHER MATERIALS:
May react with strong oxidizing agents, such as chlorates, nitrates, peroxides, etc.
HAZARDOUS POLYMERIZATION:
Polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

EYE EFFECTS:
No product toxicology data available.
SKIN EFFECTS:
No product toxicology data available.
ACUTE ORAL EFFECTS:
No product toxicology data available.
ACUTE INHALATION EFFECTS:
No product toxicology data available.
ADDITIONAL TOXICOLOGY INFORMATION:
When vapor exposures are low, or short duration and infrequent, such as during refuelling and tanker loading/unloading, neither total hydrocarbon nor components such as benzene are likely to result in any adverse health effects. In situations such as accidents or spills where exposure to gasoline vapor and liquid is potentially high, attention should be paid to potential toxic effects of specific components in addition to those of total hydrocarbons. Information about specific components in gasoline are found in Section 1 and Section 15 of this MSDS. More detailed information on the health hazard of specific gasoline components can be obtain from the Chevron Emergency Information Center (see Section 1 for telephone numbers).

A study was done in which ten volunteers were exposed for 30 minutes to about 200, 500 or 1000 ppm concentrations of the vapor of three different unleaded gasolines. Irritation of the eyes was the only significant effect observed, based on both subjective and objective assessments.

An inhalation study with rats exposed to 0, 400 and 1600 ppm of wholly vaporized unleaded gasoline, 6 hours per day on day 6 through 16 of gestation, showed no teratogenic effects nor indication of toxicity to either the mother or the fetus (sex ratio, embryotoxicity, fetal growth and development).

An inhalation study with pregnant rats exposed to 0, 1000, 3000, and 9000 ppm of unleaded gasoline vapor, 6 hours per day on days 6 through 20 of gestation, showed no teratogenic effects nor indications of toxicity to either the mother or the fetus.

In an inhalation study, groups of 6 Fischer rats (3 male, 3 female) were exposed to 2056 ppm of wholly vaporized unleaded gasoline for 6 hours per day, 5 day per week for up to 18 months. Histopathology of the peripheral nervous system and spinal cord revealed no distal axonal neuropathy of the type associated with exposure to n-hexane even though gasoline contained 1.9% n-hexane. The authors concluded that gasoline treatment may have amplified the incidence and prominence of some naturally occurring age related changes in the nervous system.

Wholly vaporized unleaded gasoline was used in a 3 month inhalation study. Groups of 40 rats (20 males, 20 female) and 8 squirrel monkeys (4 male, 4 female) were exposed 6 hours per day and 5 days per week for 13 weeks to 384 or 1552 ppm gasoline. One group of each species served as unexposed controls. The initial conclusion of this study was that inhalation of gasoline at airborne concentrations of up to 1522 ppm caused no toxicity in rats or monkeys. However, further histopathological examination of male rat kidneys on the highest dose group revealed an increased incidence and severity of regenerative epithelium and dilated tubules containing proteinaceous deposits.

Rabbits were exposed to unleaded gasoline 24 hour per day, 5 days per week for two weeks; 0, 2.5, 5 or 8 ml were applied to the skin under an occlusive dressing. Applied in such a way, this motor gasoline was corrosive to the rabbit skin and animals in all dose groups had decreased bodyweights. The slight and/or isolated systemic effects noted in the study were judged to be not significant.

Unleaded gasoline was assayed for mutagenic and cytogenetic activity. Gasoline was not mutagenic, either with or without activation, in Ames assay (Salmonella typhimurium), Saccharamyces cerevisesae, or mouse lymphoma assays. In addition, point mutations were not induced in human lymphocytes exposed to gasoline in vivo. The gasoline was not mutagenic when tested in the mouse dominant lethal assay. Administration of gasoline to rats did not cause chromosomal aberrations in their bone marrow cells.

In a lifetime skin painting study, 50 male Swiss mice were treated with 0.05 ml of unleaded gasoline three times per week. Positive control groups were treated with benzo(a)pyrene in acetone; an untreated negative control group was also included. The repeated exposure to gasoline caused severe skin irritation, ulceration, hyperkeratosis and abscesses. There was no statistically significant increase in the incidence of skin tumors. Histopathology at the end of the study showed that unleaded gasoline did

not increase the incidence of tumors in other organs.

Lifetime inhalation of wholly vaporized unleaded gasoline at 2056 ppm has caused increased liver tumors in female mice. The mechanism of this response is still being investigated but is thought to be an epigenetic process unique to the female mouse. This exposure also caused kidney damage and eventually kidney cancer in male rats. No other animal model studied has shown these adverse kidney effects and there is no physiological reason to believe that they would occur in man. EPA has concluded that the mechanism by which wholly vaporzied unleaded gasoline causes kidney damage is unique to the male rat. The response in that species (kidney damage and cancer) should not be used in human risk assessment.

In their 1988 review of carcinogenic risk from gasoline, The Internatioal Agency for Research on Cancer (IARC) noted that, because published epidemiology studies did not include any exposure data, only occupations where gasoline exposure may have occurred were reviewed. These included gasoline service station attendants and automobile mechanics. IARC also noted that there was no opportunity to separate effects of combustion products from those of gasoline itself. Although IARC allocated gasoline a final overall classification of Group 2B, i.e. possibly carcinogenic to humans, this was based on limited evidence in experimental animals plus supporting evidence including the presence in gasoline of benzene and 1, 3-butadiene. The actual evidence for cacinogenicity in humans was considered inadequate.

To explore the health effects of workers potentially exposed to gasoline vapors in the marketing and distribution sectors of the petroleum industry, the American Petroleum Institute sponsored a cohort mortality, a nested case-control, and an exposure assessment study. Histories of exposure to gasoline were reconstructed for a cohort of more than 18,000 employees from four companies for the time period between 1946 and 1985. Data were analyzed based on length of employment, length of exposure, job category, age at first exposure and estimated cumulative and peak exposures. Cumulative exposure was defined as the sum of products of TWA exposure and duration of exposure of each job in an employee's work history. Amoung cohort members, cumulative exposure ranged from 2 to 8,000 ppm-years. In general, long-term drivers at small terminals had the highest exposures, and short-term workers with "other terminal jobs" had the lowest. A peak exposure was defined as an episode in excess of 500 ppm lasting 15 to 90 minutes.

The results of the cohort study indicated that there was no increased mortality from either kidney cancer or leukemia among marketing and marine distribution employees who were exposed to gasoline in the petroleum industry, when compared to the general population. More importantly, based on internal comparisons, there was no association between mortality from kidney cancer or leukemia and various indices of gasoline exposure.

For acute myeloid leukemia (AML), a non-significant mortality increase was found in land-based terminal employees, but no trend was detected when the data were analyzed by various gasoline exposure indices. This non-significant excess was limited to land-based terminal employees hired prior to 1948. On the other hand, a deficit of mortality from AML was

observed among marine employees.

In addition to the cohort study, a subsequent nested case-control study was also conducted. Four diseases were selected for analysis in the case-control study: Leukemia (all cell types), AML, kidney cancer and multiple myeloma. For each case, five individually matched controls were randomly selected from the cohort. In the original cohort study, broad generic job categories were used as part of exposure assessment. In the case-control study, a finer and more homogeneous job classification was developed. In addition to job category, several quantitative gasoline exposure indices were used in the case-control analysis: length of exposure, cumulative exposure (ppm-years in terms of total hydrocarbons) and frequency of peak exposure. Time period of first exposure to gasoline (1948 or before and 1949 or after) was also included as an exposure index. Results of the nested case-control study confirmed the findings of the original cohort study. That is, exposure to gasoline at the levels experienced by this cohort of distribution workers is not a significant risk factor for leukemia (all cell types), acute myeloid leukemia, kidney cancer or multiple myeloma.

12. ECOLOGICAL INFORMATION

ECOTOXICITY:
Gasoline studies have been conducted in the laboratory under a variety of test conditions with a range of fish and invertebrate species. An even more extensive database is available on the aquatic toxicity of individual aromatic constituents. The majority of published studies do not identify the type of gasoline evaluated, or even provide distinguishing characteristics such as aromatic content or presence of lead alkyls. As a result, comparison of results among studies using open and closed vessels, different ages and species of test animals and different gasoline types, is difficult.

ENVIRONMENTAL FATE:
Following spillage, the more volatile components of gasoline will be rapidly lost, with concurrent dissolution of these and other constituents into the water. Factors such as local environmental conditions (temperature, wind, mixing or wave action, soil type, etc), photo-oxidation, biodegradation and adsorption onto suspended sediments, can contribute to the weathering of spilled gasoline. The aqueous solubility of non-oxygenated unleaded gasoline, based on analysis of benzene, toluene, ethylbenzene+xylenes and naphthalene, is reported to be 112 mg/l. Solubility data on individual gasoline constituents also available.

13. DISPOSAL CONSIDERATIONS

Use material for its intended purpose or recycle if possible.

This material, if it must be discarded, may meet the criteria of a

hazardous waste as defined by USEPA under RCRA (40CFR261) or other State and local regulations. Measurement of certain physical properties and analysis for regulated components may be necessary to make a correct determination. If this material is classified as a hazardous waste, federal law requires disposal at a licensed hazardous waste disposal facility.

14. TRANSPORT INFORMATION

The description shown may not apply to all shipping situations. Consult 49CFR, or appropriate Dangerous Goods Regulations, for additional description requirements (e.g., technical name) and mode-specific or quantity-specific shipping requirements.

DOT SHIPPING NAME: Hydrocarbon gas mixture, liquefied, N.O.S.(isopentane, n-butane)
DOT HAZARD CLASS: 2.1 (FLAMMABLE GAS)
DOT IDENTIFICATION NUMBER: UN1965
DOT PACKING GROUP: n/a

15. REGULATORY INFORMATION

SARA 311 CATEGORIES:

1. Immediate (Acute) Health Effects: YES
2. Delayed (Chronic) Health Effects: YES
3. Fire Hazard: YES
4. Sudden Release of Pressure Hazard: NO
5. Reactivity Hazard: NO

REGULATORY LISTS SEARCHED:

01=SARA 313
02=MASS RTK
03=NTP Carcinogen
04=CA Prop 65-Carcin
05=CA Prop 65-Repro Tox
06=IARC Group 1
07=IARC Group 2A
08=IARC Group 2B
09=SARA 302/304
10=PA RTK
11=NJ RTK
12=CERCLA 302.4
13=MN RTK
14=ACGIH TWA
15=ACGIH STEL
16=ACGIH Calc TLV
17=OSHA PEL
18=DOT Marine Pollutant
19=Chevron TWA
20=EPA Carcinogen
22=TSCA Sect 5(a)(2)
23=TSCA Sect 6
24=TSCA Sect 12(b)
25=TSCA Sect 8(a)
26=TSCA Sect 8(d)
27=TSCA Sect 4(a)
28=Canadian WHMIS
29=OSHA CEILING
30=Chevron STEL

The following components of this material are found on the regulatory lists indicated.

BENZENE, ETHYL-
is found on lists: 01,02,08,10,11,12,13,14,15,17,26,28,
N-BUTANE
is found on lists: 02,10,11,13,14,28,
TOLUENE
is found on lists: 01,02,05,10,11,12,13,14,17,26,28,29,
N-HEXANE

is found on lists: 01,02,10,11,12,13,14,17,27,28,
2-METHOXY-2-METHYL PROPANE
is found on lists: 01,02,10,11,12,14,24,26,27,30,
2,2,4-TRIMETHYLPENTANE
is found on lists: 02,10,11,12,26,
BENZENE
is found on lists: 01,02,03,04,05,06,10,11,12,13,14,15,17,20,28,29,
GASOLINE (GENERIC)
is found on lists: 04,08,14,15,17,
PENTANES
is found on lists: 14,15,17,
HEXANES
is found on lists: 14,15,

16. OTHER INFORMATION

NFPA RATINGS: Health 1; Flammability 3; Reactivity 0;
(0-Least, 1-Slight, 2-Moderate, 3-High, 4-Extreme, PPE:- Personal Protection Equipment Index recommendation, *- Chronic Effect Indicator). These values are obtained using the guidelines or published evaluations prepared by the National Fire Protection Association (NFPA) or the National Paint and Coating Association (for HMIS ratings).

REVISION STATEMENT:
This is a new Material Safety Data Sheet.

ABBREVIATIONS THAT MAY HAVE BEEN USED IN THIS DOCUMENT:

TLV - Threshold Limit Value	TWA - Time Weighted Average
STEL - Short-term Exposure Limit	TPQ - Threshold Planning Quantity
RQ - Reportable Quantity	PEL - Permissible Exposure Limit
C - Ceiling Limit	CAS - Chemical Abstract Service Number
A1-5 - Appendix A Categories	() - Change Has Been Proposed
NDA - No Data Available	NA - Not Applicable

Prepared according to the OSHA Hazard Communication Standard (29 CFR 1910.1200) and the ANSI MSDS Standard (Z400.1) by the Toxicology and Health Risk Assessment Unit, CRTC, P.O. Box 1627, Richmond, CA 94804

****

The above information is based on the data of which we are aware and is believed to be correct as of the date hereof. Since this information may be applied under conditions beyond our control and with which we may be unfamiliar and since data made available subsequent to the date hereof may suggest modification of the information, we do not assume any responsibility for the results of its use. This information is furnished upon condition that the person receiving it shall make his own determination of the suitability of the material for his particular purpose.

****
THIS IS THE LAST PAGE OF THIS MSDS

****

THIRD PARTY
Limited Distribution

ExxonMobil Biomedical Sciences, Inc.

Memorandum
DRAFT Not QA audited

To T. M. Gray
American Petroleum Institute
1220 L Street, Northwest
Washington, DC 20005-4070

Re 01TP 20

Study 167490 Interim Report 4
"Results of MRD-00-713 (Gasoline MTBE Vapor Condensate) Test Substance Characterization"

From D. J. Letinski

cc J. J. Freeman
Archives
QA Unit

Date February 28, 2001

This memo is the fourth in a series of interim reports in support of ExxonMobil Biomedical Sciences, Inc (EMBSI) study 167490 "Gasoline Vapor Condensate Characterization". This report, along with subsequent memos documenting the results of characterization analysis of a series of vapor condensate test substances, will be summarized in a single final report submitted at the conclusion of this study and coinciding with the completion of the API 211(b) Vapor Condensate test program.

This report has been subjected to a single review by EMBSI's Quality Assurance Unit.

Approval Signatures

_____ _____
J.J. Freeman, Ph.D., D.A.B.T — Date
Director - Laboratory Operations

_____ _____
D.J. Letinski, M.S. — Date
Study Director

Test Substance	MRD-00-713	Gasoline MTBE Vapor Condensate (Lot Number API -00-02)
EMBSI Receipt Date	Test Substance Received by EMBSI 11 Dec 00	
Analysis Date	12, 13 December 2000	
Method	Described in 167490 Interim Report 1 (00TP165) and Interim Report 3 (01TP 19)	
Results	See attached Table 1	

DJL:dew
Attachment

DRAFT

TABLE 1

Results of MRD-00-713 Analysis

(Gasoline MTBE Vapor Condensate - Lot Number API 00-02)

Compound	Area-Percent
Isobutane	2.2
n-butane	11.1
Isopentane	31.0
n-pentane	9.1
trans-2-pentene	2.0
2-methyl 2-butene	2.9
MtBE	21.3
2,3-dimethylbutane	0.9
2-methylpentane	4.5
3-methylpentane	2.6
n-hexane	2.1
Methylcyclopentane	1.1
2,4-dimethylpentane	0.9
Benzene	1.5
2-methylhexane	1.0
2,3-dimethylpentane	1.0
3-methylhexane	1.1
Isooctane	1.2
Toluene	2.5

RTI Project No.: 09189.000
RTI Study Code: Mi04-HLS2
RTI Master Protocol No.: RTI-909
HLS Study No.: 04-4263

AMENDMENT 1

TITLE: Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD-1® Mice

SPONSOR: American Petroleum Institute (API)
1220 L Street, NW
Washington, DC 20005

TESTING FACILITY: Huntingdon Life Sciences (HLS)
Princeton Research Center
100 Mettlers Road
East Millstone, NJ 08875-2360

SITE OF POSTMORTEM EVALUATIONS AND ANALYSES: RTI International (RTI)
Center for Life Sciences and Toxicology
Health Sciences Unit
Post Office Box 12194, 3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

PROPOSED IN-LIFE STUDY DATES: December 23, 2004 – January 29, 2005

APPROVED BY:

Thomas M. Gray, M.S., DABT / 12/10/04 Date
American Petroleum Institute
Sponsor's Representative

Rochelle W. Tyl, Ph.D., DABT / 12/16/04 Date
Study Director/Research Director
Life Sciences and Toxicology
RTI International

Gary M. Hoffman, B.A., DABT / 22 Dec 04 Date
Senior Toxicologist and Inhalation Specialist
Principal Investigator
Huntingdon Life Sciences

ADDITIONAL APPROVALS

RTI Quality Assurance Review by: Carrie Ingalls / 12/16/04
Carrie A. Ingalls, B.S. Date
Quality Assurance Specialist, Quality Assurance Unit

HLS Quality Assurance Review by: Nicki Iacono for NSI / 21 Dec 04
Nicki S. Iacono, B.S. Date
Director, Quality Assurance

RTI Institutional Management: / 12/16/04
Alan H. Staple, M.Sc. Date
Vice President, Health Sciences

HLS Institutional Management: / 22 Dec 04
Teresa S. Kusznir, V.M.D. Date
Animal Facility Veterinarian

K A. Saladdin / 21 Dec 04
Kay Saladdin, B.S. Date
Assoc. Director of Formulation Chemistry Services

IACUC 22 Dec 04
IACUC 22 Dec 04

The protocol, signed by the Study Director on October 27, 2004, is amended as follows (changes are in ***bold italics*** for clarity).

1. Location of protocol change: title page (page 1)

FROM:

PROPOSED IN-LIFE STUDY DATES: November 18 – December 17, 2004

TO:

PROPOSED IN-LIFE STUDY DATES: ***December 23, 2004 – January 29, 2005***

RATIONALE:

Due to problems associated with analyses of the test material and receipt of animals from the incorrect Charles River production facility (both now resolved), this study will be run beginning with animal receipt at HLS on December 23, 2004, and ending with the last proposed day of necropsy on January 29, 2005.

2. Location of protocol change: 2.0 Materials and Methods, 2.5 Animal Husbandry, 2.5.1 Acclimation, Housing, Feed, and Water (page 7)

FROM:

2.5 Animal Husbandry

2.5.1 Acclimation, Housing, Feed, and Water

During an approximately 7-day quarantine period at the HLS Testing Facility, animals will be checked for viability twice daily. Prior to study assignment, all animals will be examined to ascertain suitability for study. The HLS veterinarian (or designate) will formally release these animals for use by signature and date. Males and females will be individually housed in stainless steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female will be housed together. During cohabitation, male and female mice will be housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). 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 weekly for periods when feed consumption is not being recorded and at each interval when feed consumption will be recorded. After the gd 14 exposure period (for Groups 1-4) or on the afternoon of gd 14 (Group 5; see Section 3.0), a stainless steel, perforated insert will be placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. For females still undergoing daily exposures after gd 14 (Groups 1-4), the floor insert and Nestlet® will be

removed before each daily exposure and replaced after each exposure. Feed (PMI 5002 Certified Meal) will be available ad libitum, except during the daily 6-hour inhalation periods. Analytical certification of batches of feed provided by the manufacturer will be maintained on file at the HLS Testing Facility. There are no known contaminants in the feed that are expected to interfere with the objectives of this study. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) will be available ad libitum via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses are conducted by Elizabethtown Water Company to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis provided by the supplier will be maintained on file at the HLS Testing Facility. There are no known contaminants that are expected to interfere with the objectives of this study. At all times, animals will be housed, handled, and used according to the National Research Council Guide (NRC, 1996).

TO:

2.5 Animal Husbandry

2.5.1 Acclimation, Housing, Feed, and Water

During an approximately ***14-day quarantine/acclimation*** period at the HLS Testing Facility, animals will be checked for viability twice daily. Prior to study assignment, all animals will be examined to ascertain suitability for study. The HLS veterinarian (or designate) will formally release these animals for use by signature and date. Males and females will be individually housed in stainless steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female will be housed together. During cohabitation, male and female mice will be housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). 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 weekly for periods when feed consumption is not being recorded and at each interval when feed consumption will be recorded. After the gd 14 exposure period (for Groups 1-4) or on the afternoon of gd 14 (Group 5; see Section 3.0), a stainless steel, perforated insert will be placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. For females still undergoing daily exposures after gd 14 (Groups 1-4), the floor insert and Nestlet® will be removed before each daily exposure and replaced after each exposure. Feed (PMI 5002 Certified Meal) will be available ad libitum, except during the daily 6-hour inhalation periods. Analytical certification of batches of feed provided by the manufacturer will be maintained on file at the HLS Testing Facility. There are no known contaminants in the feed that are expected to interfere with the objectives of this study. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) will be available ad libitum via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses are conducted by Elizabethtown Water Company to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis provided by the supplier will be maintained on file at the HLS Testing Facility. There are no known contaminants that are expected to interfere with the objectives of this study. At all times, animals will be housed, handled, and used according to the National Research Council Guide (NRC, 1996).

RATIONALE:

See Rationale for No. 1. Since the study will now start on December 23, 2004, there is adequate time for a 14-day quarantine/acclimation period prior to mating (standard for HLS).

3. Location of protocol change: 3.0 Experimental Design, 3.1 Study Design (page 10)

FROM:

The exposure period for Group 5 at 30,000 mg/m^3 of gd 5 through 10 was selected based on reason #3 above and to reduce the number of days of generation of test atmosphere at a concentration that is 75% of the lower explosive limit.

Tentative Study Schedule:

Females arrive at HLS:	November 18, 2004
Quarantine (7 days):	November 18-25, 2004
Animals paired:	November 25-30, 2004
Dates of gd 0:	November 26-30, 2004
TSCA experimental start date:	December 1, 2004
Exposure dates: (gd 5 through 10):	December 1-10, 2004
(gd 5 through 16):	December 1-16, 2004
Scheduled termination (gd 17):	December 13-17, 2004
TSCA experimental termination date:	December 17, 2004
Submission of draft data on test atmospheres to Sponsor:	December 23, 2004 (within 1 week after the last exposure date, December 16, 2004)
Submission of interim data report:	January 14, 2005 (within 4 weeks of last necropsy)
Submission of audited draft final report:	February 17, 2005 (within 2 months of last necropsy date)
Submission of final report:	Within 1 month of receipt of Sponsor's comments on the audited draft report

TO:

The exposure period for Group 5 at 30,000 mg/m^3 of gd 5 through 10 was selected based on reason #3 above and to reduce the number of days of generation of test atmosphere at a concentration that is 75% of the lower explosive limit.

Tentative Study Schedule:

Females arrive at HLS:	***December 23, 2004***
Quarantine (***14*** days):	***December 23, 2004 – January 5, 2005***
Animals paired:	***January 6-11, 2005***
Dates of gd 0:	***January 7-12, 2005***
TSCA experimental start date:	***January 12, 2005***
Exposure dates: (gd 5 through 10):	***January 12-22, 2005***
(gd 5 through 16):	***January 12-28, 2005***
Scheduled termination (gd 17):	***January 24-29, 2005***
TSCA experimental termination date:	***January 29, 2005***
Submission of draft data on test atmospheres to Sponsor:	***February 4, 2005*** (within 1 week after the last exposure date, ***January 28, 2005***)
Submission of interim data report:	***February 28, 2005*** (within 4 weeks of last necropsy date)
Submission of audited draft final report:	***March 28, 2005*** (within 2 months of last necropsy date)
Submission of final report:	Within 1 month of receipt of Sponsor's comments on the audited draft report

RATIONALE:

See Rationale for Nos. 1 and 2. The tentative schedule is predicated on the assumption that it will take 6 breeding days to generate 140 plug-positive females. The final schedule will reflect the actual number of breeding days required.

RTI Project No.: 09189.000
RTI Study Code: Mi04-HLS2
RTI Master Protocol No.: RTI-909
HLS Study No.: 04-4263

AMENDMENT 2

TITLE: Endpoint-Specific Developmental Toxicity Evaluation of Inhaled Gasoline With Methyl Tertiary Butyl Ether (MTBE) Vapor Condensate in CD-1® Mice

SPONSOR: American Petroleum Institute (API)
1220 L Street, NW
Washington, DC 20005

TESTING FACILITY: Huntingdon Life Sciences (HLS)
Princeton Research Center
100 Mettlers Road
East Millstone, NJ 08875-2360

SITE OF POSTMORTEM EVALUATIONS AND ANALYSES: RTI International (RTI)
Center for Life Sciences and Toxicology
Health Sciences Unit
Post Office Box 12194 ■ 3040 Cornwallis Road
Research Triangle Park, NC 27709-2194

PROPOSED IN-LIFE STUDY DATES: December 23, 2004 – January 29, 2005

APPROVED BY:

12-24-05
Thomas M. Gray, M.S., DABT — Date
American Petroleum Institute
Sponsor's Representative

12-23-05
Rochelle W. Tyl, Ph.D., DABT — Date
Study Director/Research Director
Life Sciences and Toxicology
RTI International

1/10/2005
Gary M. Hoffman, B.A., DABT — Date
Senior Toxicologist and Inhalation Specialist
Principal Investigator
Huntingdon Life Sciences

ADDITIONAL APPROVALS

RTI Quality Assurance Review by: Carrie A. Ingalls / 02/23/05
Carrie A. Ingalls, B.S. — Date
Quality Assurance Specialist, Quality Assurance Unit

HLS Quality Assurance Review by: NS Iacono / 2/28/05
Nicki S. Iacono, B.S. — Date
Director, Quality Assurance

RTI Institutional Management: AH Staple / 02/23/05
Alan H. Staple, M.Sc. — Date
Vice President, Health Sciences

HLS Institutional Management: Teresa S. Kusznir / 1 Mar 05
Teresa S. Kusznir, V.M.D. — Date
Animal Facility Veterinarian

KA Saladdin / 1 Mar 05
Kay Saladdin, B.S. — Date
Assoc. Director of Formulation Chemistry Services

The protocol as signed by the Study Director on October 27, 2004, and amended on December 16, 2004 (Amendment 1), is further amended as follows (changes are in ***bold italics*** for clarity).

1. Location of protocol change: 2.0 Materials and Methods, 2.5 Animal Husbandry, 2.5.1 Acclimation, Housing, Feed, and Water (Amendment 1, Page 4)

FROM:

2.5 Animal Husbandry

2.5.1 Acclimation, Housing, Feed, and Water

During an approximately 14-day quarantine/acclimation period at the HLS Testing Facility, animals will be checked for viability twice daily. Prior to study assignment, all animals will be examined to ascertain suitability for study. The HLS veterinarian (or designate) will formally release these animals for use by signature and date. Males and females will be individually housed in stainless steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female will be housed together. During cohabitation, male and female mice will be housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). 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 weekly for periods when feed consumption is not being recorded and at each interval when feed consumption will be recorded. After the gd 14 exposure period (for Groups 1-4) or on the afternoon of gd 14 (Group 5; see Section 3.0), a stainless steel, perforated insert will be placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. For females still undergoing daily exposures after gd 14 (Groups 1-4), the floor insert and Nestlet® will be removed before each daily exposure and replaced after each exposure. Feed (PMI 5002 Certified Meal) will be available ad libitum, except during the daily 6-hour inhalation periods. Analytical certification of batches of feed provided by the manufacturer will be maintained on file at the HLS Testing Facility. There are no known contaminants in the feed that are expected to interfere with the objectives of this study. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) will be available ad libitum via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses are conducted by Elizabethtown Water Company to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis provided by the supplier will be maintained on file at the HLS Testing Facility. There are no known contaminants that are expected to interfere with the objectives of this study. At all times, animals will be housed, handled, and used according to the National Research Council Guide (NRC, 1996).

TO:

2.5 Animal Husbandry

2.5.1 Acclimation, Housing, Feed, and Water

During an approximately 14-day quarantine/acclimation period at the HLS Testing Facility, animals will be checked for viability twice daily. Prior to study assignment, all animals will be examined to ascertain suitability for study. The HLS veterinarian (or designate) will formally

release these animals for use by signature and date. Males and females will be individually housed in stainless steel suspended cages with wire mesh floors and fronts, except for the mating period when 1 male and 1 female will be housed together. During cohabitation, male and female mice will be housed in polycarbonate "shoebox" cages with stainless steel lids and Alpha-Dri® bedding (Shepherd Specialty Papers, Watertown, TN). 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 weekly. ~~***for periods when feed consumption is not being recorded and at each interval when feed consumption will be recorded***~~. After the gd 14 exposure ***period*** (for Groups 1-4) or on the afternoon of gd 14 (Group 5; see Section 3.0), a stainless steel, perforated insert will be placed on the wire-mesh floor of the stainless steel suspended cage of each female and 1 Nestlet® (Ancare, Bellmore, NY) added to each cage until scheduled sacrifice on gd 17. ~~***For***~~ Females ~~***still***~~ ***not*** undergoing daily exposures after gd ***10*** **(Group 5)** ***will be removed from their home cage and placed in another suspended cage without feed to match as closely as possible the conditions of Group 1-4 females for the 6-hour exposure period. They will then be returned to their home cage at the same time as the exposed females for feed measurement overnight.*** Feed (PMI 5002 Certified Meal) will be available ad libitum, except during the daily 6-hour inhalation periods. Analytical certification of batches of feed provided by the manufacturer will be maintained on file at the HLS Testing Facility. There are no known contaminants in the feed that are expected to interfere with the objectives of this study. Facility water (supplied by Elizabethtown Water Company, Westfield, NJ) will be available ad libitum via the automatic watering system or water bottles (during mating), except during the daily 6-hour inhalation periods. Water analyses are conducted by Elizabethtown Water Company to assure that water meets standards specified under the EPA Federal Safe Drinking Water Act Regulations (40 CFR Part 141). Water analysis provided by the supplier will be maintained on file at the HLS Testing Facility. There are no known contaminants that are expected to interfere with the objectives of this study. At all times, animals will be housed, handled, and used according to the National Research Council Guide (NRC, 1996).

RATIONALE:

Fresh feed in clean jars was not required for each daily weighing interval but will be provided for at least weekly intervals. In order to provide the same housing conditions for all females, the Group 5 females were housed in suspended cages without access to feed, water, insert or Nestlet® for the 6-hour exposure period in order to match as closely as possible the conditions of the Groups 1-4 females being exposed through gd 16. This was effective January 11, 2005.

2. Location of protocol change: 3.0 Experimental Design, 3.1 Study Design, Table 1 (Page 9)

FROM:

Table 1
Endpoint-Specific Developmental Toxicity
Number of Animals Assigned to Study Groups

Group No.	No. Animals Exposed	No. Days Exposed	Exposure Period (gd)	Target Exposure Concentration (mg/m^3)
1	25	12	5 through 16	0
2	25	12	5 through 16	2000
3	25	12	5 through 16	10,000
4	25	12	5 through 16	20,000
5	40	6	5 through 10	30,000

TO:

Table 1
Endpoint-Specific Developmental Toxicity
Number of Animals Assigned to Study Groups

Group No.	No. Animals Exposed	No. Days Exposed	Exposure Period (gd)	Target Exposure Concentration (mg/m^3)
1	***23***	12	5 through 16	0
2	***23***	12	5 through 16	2000
3	***23***	12	5 through 16	10,000
4	***23***	12	5 through 16	20,000
5	***38***	6	5 through 10	30,000

RATIONALE:

After 11 days of mating, most of the remaining mated females appeared to be pregnant. As only 2 plug-positive females had been found on 2 out of 3 days on January 15-17, 2005, the Principal Investigator at Huntingdon suggested that mating be terminated; the Study Director concurred. The reduction of 2 females assigned to each of the 5 groups would not affect the statistical power. This was effective January 18, 2005.

3. Location of protocol change: 3.8 Allocation and Exposure of Maternal Animals (Page 12)

FROM:

3.8 Allocation and Exposure of Maternal Animals

Plug-positive female mice (dams) will be assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights and equal distribution of females mated to the same male among dose groups on gd 0. Females will be exposed to gasoline MTBE vapor condensate or air 6 hours per day from gd 5 through 16 for Groups 1-4 and for gd 5 through 10 for Group 5. Inhalation was chosen by the Sponsor as the route of administration.

TO:

3.8 Allocation and Exposure of Maternal Animals

Plug-positive female mice (dams) will be assigned to treatment groups by a stratified randomization method designed to provide uniform mean body weights and equal distribution of females mated to the same male among dose groups ***using data from*** gd 0. Females will be exposed to gasoline MTBE vapor condensate or air 6 hours per day from gd 5 through 16 for Groups 1-4 and for gd 5 through 10 for Group 5. Inhalation was chosen by the Sponsor as the route of administration.

RATIONALE:

Determination of representation of females mated to the same male could not be made until mating was completed.

4. Location of protocol change: 3.0 Experimental Design, 3.9 Observation of Maternal Animals (Page 12)

FROM:

3.9 Observation of Maternal Animals

3.9.1 Clinical Observations

Clinical observations of all animals will be made once daily on gd 0 through 4 (prior to exposure period), on gd 11 through 17 or gd 16 through 17 (after the exposure period), and twice daily (prior to and immediately after each daily exposure) throughout the exposure period (gd 5 through 10 or gd 5 through 16). In addition, during each daily exposure period, animals will be observed at least once during each exposure. This will be routinely performed near the middle of each exposure and may be performed more frequently if significant signs of toxicity are noted.

TO:

3.9 Observation of Maternal Animals

3.9.1 Clinical Observations

Clinical observations of all animals will be made once daily on gd 0 through 4 (prior to exposure period), on gd 11 through 17 or gd ~~***16 through***~~ 17 (after the exposure period), and twice daily (prior to and immediately after each daily exposure) throughout the exposure period (gd 5 through 10 or gd 5 through 16). In addition, during each daily exposure period, animals will be observed at least once during each exposure. This will be routinely performed near the middle of each exposure and may be performed more frequently if significant signs of toxicity are noted.

RATIONALE:

Groups 1-4 females will be observed 2 times per day during the exposure period (gd 5 through 16). Therefore, a once daily clinical observation was necessary only on gd 17 for these females. This was effective January 11, 2005.

5. Location of protocol change: 3.0 Experimental Design, 3.10 Postmortem Evaluation, 3.10.2 Fetal (Page 13)

FROM:

Live fetuses will be removed from the uterus, counted, weighed, sexed externally, and examined externally for gross malformations (including cleft palate) and variations by RTI staff. Each fetus will be killed by intraperitoneal injection of sodium pentobarbital, dissected longitudinally, and the thoracic and abdominal viscera removed intact and retained individually in labeled scintillation vials in buffered neutral 10% formalin for possible subsequent visceral examination. The fetal carcass will be blanched, skinned, and retained in individually labeled scintillation vials in 70% ethanol for possible subsequent double staining (alizarin Red S and alcian blue) and skeletal evaluation. All maternal organs and carcasses will be destroyed by incineration.

TO:

Live ***and dead fetuses*** will be removed from the uterus, counted, weighed, sexed externally, and examined externally for gross malformations (including cleft palate) and variations by RTI staff. Each ***live*** fetus will be killed by intraperitoneal injection of sodium pentobarbital, ***live and dead*** dissected longitudinally, and the thoracic and abdominal viscera removed intact and retained individually in labeled scintillation vials in buffered neutral 10% formalin for possible subsequent visceral examination. The fetal carcass will be blanched, skinned, and retained in individually labeled scintillation vials in 70% ethanol for possible subsequent double staining (alizarin Red S and alcian blue) and skeletal evaluation. All maternal organs and carcasses will be destroyed by incineration.

RATIONALE:

To assure that any malformations were recorded for any late term fetus, live or dead, the dead fetuses were included in the evaluation at necropsy. This was effective January 24, 2005.