ABSTRACT

In compliance with the Clean Air Act Section 211(b) for fuel and fuel additive registration, the petroleum industry and oxygenate manufacturers have conducted comparative chronic toxicology testing of evaporative emissions from unleaded gasoline alone, and unleaded gasoline containing the additive, methyl tertiary-butyl ether (gasoline MTBE vapor condensate [GMVC]) containing approximately 20% of the additive by mass. Groups of 50 male/50 female CDF(F344)CrlBR rats were exposed in H2000 whole-body inhalation chambers at GMVC vapor concentrations of 2 g/m^3 (low level), 10 g/m^3 (mid level), and 20 g/m^3 (high level) for 6 hours/day, 5 days/week for 104 weeks (520 exposure days). There were no clinical signs of toxicity attributable to GMVC inhalation. Survival of the GMVC-exposed rats was not significantly different from control rats. Body weights of high-level GMVC males and high- and mid-level females were significantly below control values for most of the study. At the final sacrifice, the body weights of male and female high-level rats were significantly below corresponding control values; weights were 91.5% and 91.8% of control, respectively. Brain weights of high-level males and females, adrenals of high-level females, and epididymides of mid-level males were significantly less than those of the corresponding controls. Significant increases in organ:body weight ratios were observed in males at final sacrifice; brain and lung in mid- and high-level groups and kidney in high-level males. Absolute kidney weight and percent kidney-to-brain weights of males from all GMVC exposure groups euthanized before the scheduled sacrifice were significantly increased compared to control values. No effects on total and differential white blood cell (WBC) counts could be attributed to GMVC inhalation. Differences in WBC counts and differential cell counts in males at the 12-month sampling time were not found in females at 12 months or at later sampling times. These findings suggest the differences may have been due to sampling technique than to GMVC inhalation.

The incidence of renal tubule adenomas in mid-level male rats (6/50; 12%) was significantly increased compared to controls (0%) by the Fisher's test. There was a non-statistically significant increase in renal tubule carcinomas in males (1/50; 2% in both low- and high-level groups). When incidences of adenomas and carcinomas were combined, a significant trend

toward increasing incidence with exposure concentration was found with the Cochran-Armitage test. However, only the incidence of combined adenomas and carcinomas in the mid-dose group remained significantly increased compared to the control group. The increases in combined renal adenomas and carcinomas may be attributed to GMVC treatment, based on similar findings in previous studies with MTBE and wholly vaporized gasoline (Bird et al., 1997; MacFarland et al., 1984). In the concurrently conducted study on Baseline Gasoline Vapor Condensate (LRRI Protocol FY01-027), the combined incidences of renal adenoma and carcinoma were significantly increased, but significant differences were not noted among individual dose groups. GMVC exposure caused increased severity, but not incidence of chronic progressive nephropathy among mid- and high-level males and high-level females compared to controls. Alpha-2u globulin nephropathy resulting in a sustained increase in tubule epithelial cell turnover is a plausible mechanism for GMVC-induced renal tubule adenomas and carcinomas in male rats and is substantiated by other MTBE and light hydrocarbon mechanistic study reports (Cruzan et al., 2007; Borghoff et al., 1991, 1992). However, it should be noted that for compounds inducing renal tumors in male rats through increased alpha-2u globulin accumulation and subsequent increased renal tubule cell turnover, alpha-2u globulin overload has been demonstrated to be a male rat specific mechanism with little or no relevance to human health risk assessment (Baetcke et al., 1991; Hard, 1998; Hard and Khan, 2004).

In contrast to findings in the parallel study with baseline gasoline vapor condensate, there was no GMVC treatment effect on the incidences of nasal squamous cell carcinoma and thyroid follicular cell adenomas and carcinomas in males.

There was a high background incidence of testicular interstitial adenomas in control male rats (43/50; 86%). The incidence among high-level males (50/50; 100%) was significantly greater than the control incidence (Fisher's exact test). The relationship between treatment and induction of testicular interstitial adenomas is considered to be equivocal, since background incidence of this tumor is extremely high and historical control incidence data from one 2-year bioassay conducted at LRRI for a commercial sponsor was 86% (n = 56). NTP control

incidence[1] for air inhalation controls: for NIH-07 diet avg. = 70.1% with range of 46% to 90%; for NTP-2000 diet avg. = 92.1% with range of 82% to 98%. Further, the control incidence in the concurrently run Baseline Gasoline Vapor Condensate chronic study (LRRI study number FY01-027) was 48/50 (96%), and no significant increase in the incidence of testicular interstitial adenoma with BGVC exposure was found in that study.

The incidences of mononuclear cell leukemia among males and females were high, as expected among a population of aged F344 rats. MCL incidence in low-level (31/39; 79%) and mid-level (31/38; 82%) males was significantly greater than controls (27/50; 54%), most likely due to protocol driven sampling bias (non-target organs in low- and mid-level groups were only examined histologically when gross lesions were observed). Overall, the incidence of MCL appeared unaffected by GMVC inhalation. The only other nonproliferative lesion attributable to GMVC exposure was hyaline degeneration of the olfactory epithelium (males) and respiratory epithelium (females) of the nose.

In summary, chronic GMVC inhalation suppressed body weight in males, and to a greater extent in females, increased the severity of chronic progressive nephropathy in mid- and high-level males and high-level females and caused epithelial (females) and olfactory (males) degeneration in the nasal passages of both sexes. The degenerative nasal effects were most likely caused by the test material. Chronic exposure to BGVC did not enhance the development of proliferative lesions (hyperplastic lesions, neoplasms) in female rats. In male rats, chronic exposure to BGVC did enhance the development of renal adenomas and carcinomas, when incidences were combined. Only the mid exposure group had significantly increased incidence of adenomas alone, or of combined adenomas and carcionomas compared to the control group. There was also an increase in testicular interstitial cell adenoma which was considered to be equivocal with respect to treatment. Consequently, due to treatment-related increases in renal adenomas and carcinomas in males, chronic inhalation of Gasoline MTBE Vapor Condensate was determined to be carcinogenic in male rats in this study. Gasoline MTBE Vapor Condensate was not determined to be carcinogenic in female rats in this study.

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[1] Reference - http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=92E61F1B-F1F6-975E-7D3BED551F07DC0A

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LABORATORY TITLE PAGE

211(b) CHRONIC CARCINOGENICITY STUDY

GASOLINE MTBE VAPOR CONDENSATE (GMVC)

LRRI Study Number: FY01-013

Laboratory:

Lovelace Respiratory Research Institute (LRRI)
2425 Ridgecrest Dr. SE
Albuquerque, NM 87108

Courier Address and Location of Laboratory:
Bldg. 9217, Area Y
Kirtland Air Force Base
Albuquerque, NM 87115

Sponsor:

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

September 2009

Total number of pages: ___

KEY STUDY PERSONNEL

Study Director	Janet M. Benson, PhD, DABT Lovelace Respiratory Research Institute
Veterinary Pathologist	Andrew P. Gigliotti DVM, PhD, DACVP Lovelace Respiratory Research Institute
Director of Quality (Until 2007)	Stephanie Taulbee, MSPH Lovelace Respiratory Research Institute
Quality Assurance Manager (Until 2006)	Dorothy L. Harris, MS, CRM, RQAP-GLP Lovelace Respiratory Research Institute
Quality Assurance Manager	Joan Gallis Lovelace Respiratory Research Institute
Aerosol Scientists	Quint H. Powell, MS Lovelace Respiratory Research Institute Edward B. Barr, MSEE Lovelace Respiratory Research Institute
Laboratory Animal Veterinarian	David G. Burt, DVM, DACLAM Lovelace Respiratory Research Institute Roger A. Van Andel, DVM, PhD, DACLAM Lovelace Respiratory Research Institute

SUBCONTRACTOR

Statistician	Betty Skipper, PhD Department of Family and Community Medicine, University of New Mexico

September 2009

LABORATORY SIGNATURE PAGE

Study Number FY01-013

211(b) Chronic Carcinogenicity Study

Gasoline MTBE Vapor Condensate (GMVC)

Janet M. Benson, PhD, DABT Study Director Lovelace Respiratory Research Institute	Date
Edward B. Barr, MSEE Aerosol Scientist Lovelace Respiratory Research Institute	Date
Andrew P. Gigliotti, DVM, PhD, DACVP Veterinary Pathologist (Clinical Pathology and Histopathology) Lovelace Respiratory Research Institute	Date
Betty Skipper, PhD Director, Biostatistics Department of Family and Community Medicine, University of New Mexico	Date

September 2009

LABORATORY QA STATEMENT

Study Title	211(b) Chronic Carcinogenicity Study – Gasoline MTBE Vapor Condensate (GMVC) and Baseline Vapor Condensate (BGVC)
LRRI Study Number	FY01-013
Sponsor Study Number	N/A
Study Director	J. Benson

This study was conducted under 21 CFR Part 58 Good Laboratory Practice (GLP). This study was inspected by the LRRI Quality Assurance Unit. The final report accurately reflects the raw data. Findings were reported to the Study Director and Test Facility Management as follows:

Study Phase	Performed by	Date(s) of Inspection or Audit	Testing Facility Management	Study Director
Afternoon servicing of chambers and animal body weights	R. Marr	29-May-01	4-June-01	4-June-01
Gasoline MTBE vapor condensate 20# tank receipt, 420# tank usage log, 20# tank usage and fill log	R. Marr	30-May-01	30-May-01	30-May-01
Body weights, clinical observations	R. Marr / D. Harris	3-Jul-01	9-Jul-01	9-Jul-01
Environmental data	R. Mar / D. Harris	7-Aug-01	9-Aug-01	9-Aug-01
Environmental data	R. Mar / D. Harris	9-Aug-01	9-Aug-01	9-Aug-01
TA management data, clinical observations and body weights, Miran calibration data, GC data	R. Marr	22-Oct-01	30-Oct-01	30-Oct-01
Environmental data, clinical observations, body weights, exposure data, GC data, Miran calibration data	R. Marr	5-Dec-01	18-Dec-01	18-Dec-01

Study Phase	Performed by	Date(s) of Inspection or Audit	Testing Facility Management	Study Director
Environmental data, exposure data, GC data, MTBE running exposure concentration summary	R. Marr	26-Dec-01	4-Jan-02	4-Jan-02
Environmental data, clinical observations, body weights, 1 point and 8 point performance qualifications	R. Marr	7-Mar-02	11-Mar-02	11-Mar-02
Environmental data, clinical observations, body weights, exposure data, GC data, Miran calibration data, TA usage forms	R. Marr	1-May-02	13-May-02	13-May-02
Environmental data, clinical observations, body weights, exposure chamber maps, exposure data, GC data, TA usage forms	R. Marr	6-Sept-02	12-Sept-02	12-Sept-02
Environmental data, clinical observations, body weights, TA characterization, 1 point and 8 point performance calibrations, chamber atmosphere characterization, individual animal removal records, blood smear/differential morphology, exposure data, TA usage forms	R. Marr	6-Nov-02	22-Nov-02	22-Nov-02

Study Phase	Performed by	Date(s) of Inspection or Audit	Testing Facility Management	Study Director
IANR forms, individual animal removal – chain of custody records, animal room exposure log, sick animal observations records, exposure chamber checklist, exposure chamber maps, 8 point performance calibration, environmental data, exposure data, Miran calibrations	R. Marr	17-Dec-02	19-Dec-02	19-Dec-02
Environmental data, GC data, exposure data, chamber profiles, Miran calibrations, body weights and observations	R. Marr	7-Jan-03	8-Jan-03	8-Jan-03
Exposure data, TA, body weight and observations, chamber atmosphere, environmental data, Miran calibrations, IANR forms, GC data, animal removal records	R. Marr	3-Feb-03	14-Feb-03	14-Feb-03
Exposure data, TA, body weight and observations, chamber atmosphere, environmental data, Miran calibrations, IANR forms, GC data, animal removal records	R. Marr	14-Mar-03	27-Mar-03	27-Mar-03
Terminal sacrifices	R. Marr	27-May-03	27-May-03	27-May-03
Gasoline MTBE vapor condensate 20# usage and fill log	R. Marr	27-May-03	14-Jun-03	14-Jun-03
Organ weight data, gross observations	R. Marr	5-Jun-03	6-Jun-03	6-Jun-03

Study Phase	Performed by	Date(s) of Inspection or Audit	Testing Facility Management	Study Director
Animal death dates against IANR	R. Marr	2-Jul-03	21-Jul-03	21-Jul-03
Histology specimen preparation	D. Harris	22-Jul-03	24-Jul-03	24-Jul-03
Histology lab	D. Harris	24-Jul-03	24-Jul-03	24-Jul-03
Exposure data, TA, Path Tox dead animal status list, body weight and observations, chamber atmosphere, environmental data, Miran calibrations, , GC data, 1 point and 8 point performance qualifications, IANR forms, animal removal records and chain of custody, additional animal observations, sick animal observations	R. Marr	29-Jul-03	19-Aug-03	19-Aug-03
TA records	D. Harris	3-Oct-03	6-Oct-03	6-Oct-03
Draft final report (report text/appendices, all report tabular data, pathologist report, statistics data/report	D. Harris	24-Jun-04	16-Jul-04	16-Jul-04
Final Report	D. Harris	July 2006	July 2006	July 2006
Final Report Text	C. Storch	June 2009	1-Jul-09	1-Jul-09
Final Report	C. Storch	30-Sept-09	30-Sept-09	30-Sept-09

Christa R. Storch, BS, RQAP-GLP
Senior Quality Assurance Specialist
LRRI Quality Assurance Unit

Date

September 2009

LABORATORY GLP COMPLIANCE STATEMENT

Study Number FY01-013

211(b) Chronic Carcinogenicity Study

Gasoline MTBE Vapor Condensate (GMVC)

This study was conducted in compliance with Alternative Tier 2 testing requirements under Section 211(b) of the Clean Air Act and the EPA Health Effects Test Guidelines OPPTS 870.4200 "Carcinogenicity." The stipulations of this protocol were implemented in conformance with EPA regulations as specified in 40 CFR 79.60 "Good Laboratory Practices (GLP) Standards for Inhalation Exposure Health Effects Testing."

It was the Sponsor's responsibility to maintain records of the method of synthesis, fabrication, or derivation of the test substance. The method of fabrication was not available at the time the study was initiated. It is however, presently available.

The following were not conducted under GLP Guidelines:

Serological assessments were conducted throughout the study. Sera were shipped to BioReliance Corporation, Rockville, MD. None of the assessments were conducted under GLP guidelines due to lack of specification by LRRI.

Light and noise measurements were not conducted under GLP guidelines. No Standard Operating Procedure was in place for conduct of these measurements.

Janet M. Benson, PhD, DABT Study Director Lovelace Respiratory Research Institute	Date
Thomas M. Gray, MS, DABT Sponsor Representative American Petroleum Institute	Date

SUMMARY

In compliance with the Clean Air Act Section 211(b) for fuel and fuel additive registration, the petroleum industry and oxygenate manufacturers have conducted comparative chronic toxicology testing of evaporative emissions from unleaded gasoline alone, and unleaded gasoline containing the additive, methyl tertiary-butyl ether (gasoline MTBE vapor condensate [GMVC]) containing approximately 20% of the additive by mass. Groups of 50 male/50 female CDF(F344)CrlBR rats were exposed in H2000 whole-body inhalation chambers at GMVC vapor concentrations of 2 g/m^3 (low level), 10 g/m^3 (mid level), and 20 g/m^3 (high level) for 6 hours/day, 5 days/week for 104 weeks (520 exposure days). There were no clinical signs of toxicity attributable to GMVC inhalation. Survival of the GMVC-exposed rats was not significantly different from control rats. Body weights of high-level GMVC males and high- and mid-level females were significantly below control values for most of the study. At the final sacrifice, the body weights of male and female high-level rats were significantly below corresponding control values; weights were 91.5% and 91.8% of control, respectively. Brain weights of high-level males and females, adrenals of high-level females, and epididymides of mid-level males were significantly less than those of the corresponding controls. Significant increases in organ:body weight ratios were observed in males at final sacrifice; brain and lung in mid- and high-level groups and kidney in high-level males. Absolute kidney weight and percent kidney-to-brain weights of males from all GMVC exposure groups euthanized before the scheduled sacrifice were significantly increased compared to control values. No effects on total and differential white blood cell (WBC) counts could be attributed to GMVC inhalation. Differences in WBC counts and differential cell counts in males at the 12-month sampling time were not found in females at 12 months or at later sampling times. These findings suggest the differences may have been due to sampling technique than to GMVC inhalation.

The incidence of renal tubule adenomas in mid-level male rats (6/50; 12%) was significantly increased compared to controls (0%) by the Fisher's test. There was a non-statistically significant increase in renal tubule carcinomas in males (1/50; 2% in both low- and high-level groups). When incidences of adenomas and carcinomas were combined, a significant trend

toward increasing incidence with exposure concentration was found with the Cochran-Armitage test. However, only the incidence of combined adenomas and carcinomas in the mid-dose group remained significantly increased compared to the control group. The increases in combined renal adenomas and carcinomas may be attributed to GMVC treatment, based on similar findings in previous studies with MTBE and wholly vaporized gasoline (Bird et al., 1997; MacFarland et al., 1984). In the concurrently conducted study on Baseline Gasoline Vapor Condensate (LRRI Protocol FY01-027), the combined incidences of renal adenoma and carcinoma were significantly increased, but significant differences were not noted among individual dose groups. GMVC exposure caused increased severity, but not incidence of chronic progressive nephropathy among mid- and high-level males and high-level females compared to controls. Alpha-2u globulin nephropathy resulting in a sustained increase in tubule epithelial cell turnover is a plausible mechanism for GMVC induced renal tubule adenomas and carcinomas in male rats and is substantiated by other MTBE and light hydrocarbon mechanistic study reports (Cruzan et al., 2007; Borghoff et al., 1991, 1992). However, it should be noted that for compounds inducing renal tumors in male rats through increased alpha-2u globulin accumulation and subsequent increased renal tubule cell turnover, alpha-2u globulin overload has been demonstrated to be a male rat specific mechanism with little or no relevance to human health risk assessment (Baetcke et al., 1991; Hard, 1998; Hard and Khan, 2004).

In contrast to findings in the parallel study with baseline gasoline vapor condensate, there was no GMVC treatment effect on the incidences of nasal squamous cell carcinoma and thyroid follicular cell adenomas and carcinomas in males.

There was a high background incidence of testicular interstitial adenomas in control male rats (43/50; 86%). The incidence among high-level males (50/50; 100%) was significantly greater than the control incidence (Fisher's test). The relationship between treatment and induction of testicular interstitial adenomas is considered to be equivocal, since background incidence of this tumor is extremely high and historical control incidence data from one 2-year bioassay

conducted at LRRI for a commercial sponsor was 86% (n = 56). NTP control incidence[2] for air inhalation controls: for NIH-07 diet avg. = 70.1% with range of 46% to 90%; for NTP-2000 diet avg. = 92.1% with range of 82% to 98%. Further, the control incidence in the concurrently run Baseline Gasoline Vapor Condensate chronic study (LRRI study number FY01-027) was 48/50 (96%), and no significant increase in testicular interstitial adenoma among BGVC males was found in that study.

The incidences of mononuclear cell leukemia among males and females were high, as expected among a population of aged F344 rats. MCL incidence in low-level (31/39; 79%) and mid-level (31/38; 82%) males was significantly greater than controls (27/50; 54%), most likely due to protocol driven sampling bias (non-target organs in low- and mid-level groups were only examined histologically when gross lesions were observed). Overall, the incidence of MCL appeared unaffected by GMVC inhalation. The only other nonproliferative lesion attributable to GMVC exposure was hyaline degeneration of the olfactory epithelium (males) and respiratory epithelium (females) of the nose.

In summary, chronic GMVC inhalation suppressed body weight in males, and to a greater extent in females, increased the severity of chronic progressive nephropathy in high- and mid-level males and high-level females and caused epithelial (females) and olfactory (males) degeneration in the nasal passages of both sexes. The degenerative nasal effects were most likely caused by the test material. Chronic exposure to BGVC did not enhance the development of proliferative lesions (hyperplastic lesions, neoplasms) in female rats. In male rats, chronic exposure to BGVC did enhance the development of renal adenomas and carcinomas, when incidences were combined; only the mid-exposure group had significantly increased incidence of adenomas or combined adenomas and carcionomas compared to the control group. There was also an increase in testicular interstitial cell adenoma which was considered to be equivocal with respect to treatment. Consequently, due to treatment-related increases in renal adenomas and carcinomas in males chronic inhalation of Gasoline MTBE Vapor Condensate was determined to be carcinogenic in male rats in this study. Gasoline MTBE Vapor Condensate was not determined to be carcinogenic in female rats in this study.

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[2] Reference - http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=92E61F1B-F1F6-975E-7D3BED551F07DC0A

INTRODUCTION

The purpose of this study was to evaluate the chronic toxicity, potential carcinogenicity, and exposure concentration-response relationships associated with inhalation of gasoline vapor condensate containing the fuel additive methyl tertiary-butyl ether (gasoline MTBE) vapor condensate (GMVC). The study was conducted in compliance with Alternative Tier 2 testing requirements under Section 211(b) of the Clean Air Act and the EPA Health Effects Test Guidelines OPPTS 870.4200 "Carcinogenicity." The stipulations of this protocol were implemented in conformance with EPA regulations as specified in 40 CFR 79.60 "Good Laboratory Practices (GLP) Standards for Inhalation Exposure Health Effects Testing." The study protocol and amendments are provided in Appendix A.

MATERIALS, METHODS

TEST SUBSTANCE

Identification, Source, and Storage

Gasoline MTBE vapor condensate (GMVC; Lot Number API 00-02) was prepared from gasoline containing 20% MTBE and supplied in 420-pound and 20-pound gas cylinders by Chevron Research and Technology Center (CRTC; Richmond, CA). Original characterization of the test substance was performed by CRTC and ExxonMobil Biomedical Sciences, Inc. (EMBSI), Annandale, NJ. The reference gas chromatographic profile of the 19 key components was provided by EMBSI.

Twenty-pound cylinders and some 420-pound cylinders were stored at ambient temperature in a storage building dedicated for that purpose at the Lovelace Respiratory Research Institute (LRRI). The remaining 420-pound cylinders were stored in an outside, controlled area at ambient temperature. The test substance was transferred, as needed, from the 420-pound to the 20-pound cylinders. Only authorized personnel were allowed access to the test substance. Receipt, use, and inventory of this test substance were documented.

Analysis

The analytical results are provided in Appendix B.

Before dispensing GMVC from each 420-pound tank, a sample was removed from the tank and analyzed by gas chromatography at LRRI using a Shimadzu Model GC-17A/FID (Columbia, MD). The gas chromatographic profile of the 19 major peaks (retention time and relative peak area) was compared with that originally determined for the GMVC by EMBSI. This was done to show that all tanks were similar in profile and that the test material was stable throughout the testing period. Results are provided in Appendix B.

Expiration Date

An expiration date is not available. The test material is stable per MSDS. The test substance stability was tested concurrently with the study with the analysis of the exposure chamber

atmospheres and each #420 pound tank before its use. Results, provided in Appendix E, showed it to be stable for the duration of the study.

Reserve Sample

The Sponsor-contracted archives have retained samples of the test substance.

ANIMALS AND ANIMAL ASSIGNMENTS

Animal Receipt, Housing, and Quarantine

Four hundred-forty CDF(F344)CrlBR rats (5–6 weeks old when received) were purchased from Charles River Laboratories (Raleigh, NC). They were examined by a veterinarian upon their arrival. All animals were quarantined and acclimated to whole-body inhalation chambers for 23 days. Healthy animals were randomly assigned by weight to the core exposure groups (400 total; 50 rats/sex/exposure level). The weight range for males the day before exposures began was 134.2–211 g. The weight range for females on the day before exposures began was 108.5–138.9 g. Following randomization, the rats assigned to the study were identified by tail tattoo. Five male and five female rats were assigned as sentinels and housed in the control chamber. Five unassigned male and female rats were sacrificed before exposures began to evaluate their health status as an indicator of the health status of the population on study. The remaining unassigned rats were euthanized. Receipt and initiation of exposures of male and female rats were staggered by one week.

Animal Disease Screening Program

Five male and five female rats not assigned to study were sacrificed, bled and received a complete necropsy. No gross lesions were found, and tissues were subsequently discarded because there were no subsequent questions regarding the health stats of the animals on study. Sera from these rats were submitted to BioReliance, Gaithersburg, MD, for analysis of antibodies common to rodents. The following tests were run: Cilia-Associated Respiratory Bacillus (CARB), Mycoplasma pulmonis (M. PUI), Pneumonia virus of mice (PVM), Rat Coronavirus/ Sialodacryoadenitis Virus (RCV/SDA), Reovirus (Reo), Sendai Virus (Sendai), Lymphocytic Choriomenengitis Virus (LCM), Parvovirus (Parvo), Toolan's H-1 Virus (H-1), and Kilham rat

Virus (KRV). The five male and five female sentinel rats in the control chamber were bled retroorbitally after 26, 52, and 79 weeks of exposure. Blood was also obtained from sentinels surviving to 104 weeks. Sera were analyzed by BioReliance, as described above.

Justification of Test Animals

Rats were used in this study because of the large database available on the inhalation toxicity and carcinogenicity of toxic materials in rats. The study design was justified because it provided exposure concentration-response information on the possible carcinogenicity associated with repeated inhalation of GMVC.

Environmental Conditions

The rats were housed 24 hours per day during quarantine and exposure in Hazleton 2000 whole-body inhalation chambers. Initially, all rats were housed separately in 3.8-inch wide by 11-inch long by 8-inch high compartments within stainless steel baskets. When male rats reached 400 g they were transferred to baskets with 5.7-inch by 11-inch by 8-inch compartments. Each chamber contained six baskets.

The chambers and cage racks were washed weekly. The cage racks were rotated clockwise weekly when the rats were transferred from the dirty to the clean chamber.

The chambers were held at approximately 1 inch of water negative pressure with respect to the exposure room, and the chamber flow rates were maintained at 12 to 15 air changes per hour (400–500 liters per minute [LPM]). Chamber temperatures were maintained at 20° to 24°C. Temperature, relative humidity, and chamber air flow rates were continuously monitored, 24 hours per day. Values for the three parameters were recorded at 30-minute intervals. Oxygen concentration in the chambers was maintained at 19%. This parameter was monitored but not recorded.

A 12-hour light cycle was maintained with lights on at 0600. Light levels in the exposure room and noise levels in the chambers were determined periodically.

Diet and Drinking Water

Unlimited municipal tap water was available at all times. Rats were fed Teklad Certified Rodent Diet (8728C; Harlan Teklad, Madison, WI). Food was available at all times except during the daily exposure period. Certified diet was analyzed for heavy metals, aflatoxin, organophosphates and chlorinated hydrocarbons, and values present were below maximum allowable values established by the manufacturer. Water was analyzed by an independent laboratory for metals, ions, microbes, and pesticides. Analytes were either present at non-detectable concentrations or sufficiently low concentrations as to not interfere with the outcome of the study. Results of feed and water analyses are provided in Appendix D.

EXPERIMENTAL DESIGN

Group Assignment

The experimental design is shown in Table 2-1.

The Laboratory Animal Veterinarian visually examined all rats before they were placed on study. Only animals judged to be of acceptable health were used. Animals were weighed and randomly assigned to a test group using a computerized data acquisition system (Path-Tox®; Xybion, Cedar Knolls, NJ) operated according to LRRI Standard Operating Procedures (SOPs).

Table 2-1. Experimental Design

Rat Strain and Sex	CDF(F344)CrlBR; 200 males/200 females assigned to core study
Animal Source	Charles River Laboratories, Raleigh, NC
Time Held Before Exposure	23 days
Age When Placed on Study	9–10 weeks
Study Dates	
Study Initiation Date	May 22, 2001
Initiation of Exposures (Males)	May 23, 2001
Initiation of Exposure (Females)	May 30, 2001
Last Exposure Day (Males)	May 23, 2003
Last Exposure Day (Females)	May 30, 2003
Final Sacrifice (Males)	May 27–30, 2003
Final Sacrifice (Females)	June 2–6, 2003

Table 2-1. Experimental Design (Concluded)

Target Exposure Concentrations	
Control	0 g/m^3 GMVC
Low Level	2 g/m^3 GMVC
Mid Level	10 g/m^3 GMVC
High Level	20 g/m^3 GMVC
Animal Identification Scheme (by tail tattoo)	
Control	E400-E450 (M); E451-E500 (F)
Low Level	F501-E550 (M); F551-E600 (F)
Mid Level	G601-G650 (M); G651-G700 (F)
High Level	H701-H750 (M); H751-H800 (F)
Exposure Duration	6 hours/day, 5 days/week for 104 weeks (520 exposure days)
In-Life Monitoring	Daily observations for morbidity, mortality. Weekly body weights for first 13 weeks, then every 4th week. Weekly detailed clinical observations.
Sentinel Animal Bleeds for Serology	Prior to study start and at six month intervals thereafter, including prior to final sacrifice.
Hematology Evaluations (Control and High Dose groups)	At 12 and 18 months and at final sacrifice.
Histopathology	All protocol-required tissues collected

Exposure System

A schematic of the vapor exposure system is shown in Figure 2-1.

The daily supply of GMVC for each exposure chamber was contained in 20-pound gas storage cylinders. Exposure atmospheres were generated by controlling the flow of pressurized GMVC through a rotameter, into a heated stainless steel transfer line where the GMVC was completely vaporized. Chamber concentrations were controlled by adjusting the flow rates of the GMVC and dilution air rate. Chamber exhaust was carried to an oxidizer on the roof of the exposure facility where it was burned.

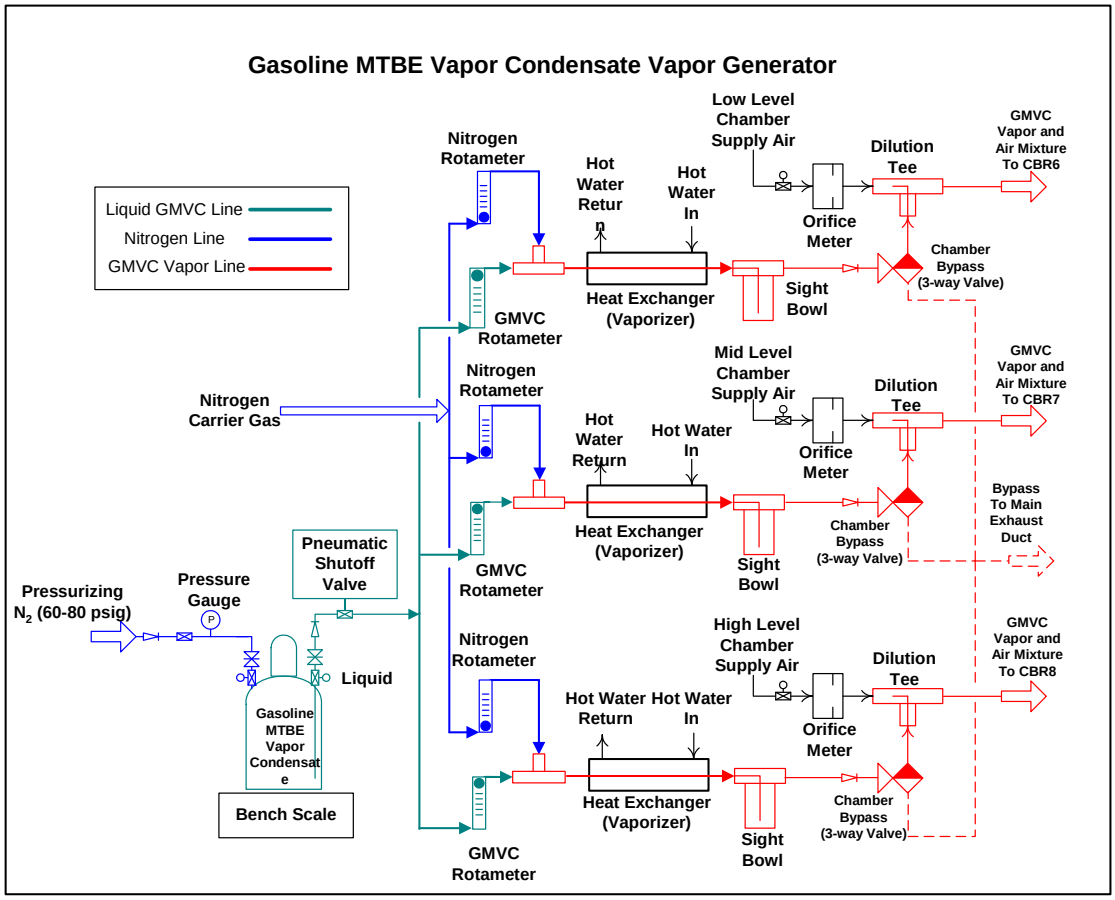

Figure 2-1. Vapor Generator for GMVC

Pre-Test Characterization. The exposure system was tested prior to animal exposures. Characterization included the following: 1) uniformity of the distribution of total vapor within each chamber; 2) within-day and between-day stability of vapor concentration; 3) within-day and between-day consistency of the hydrocarbon profile as determined by gas chromatography; and 4) determination of the time for vapor concentration to achieve 90% of the equilibrium target value (T90). The exposure atmosphere in the animals' breathing zone was also examined for the presence of aerosol particles using a TSI Scanning Mobility Particle Sizer (TSI Industries, Shoreview, MN).

Once, prior to the initiation of animal exposures, the concentration of the test substance in the generator containment hood and in the exposure room were measured to ensure that the generator containment hood was operating satisfactorily.

Chamber Distribution Evaluation During Exposures. During the second week of exposure, the uniformity of vapor distribution was re-evaluated to determine the distribution in the presence of the test animals.

Quantitation of Exposure Atmospheres. Vapor concentrations were continuously monitored using Miran 1A infrared analyzers (Foxboro Wilks, Foxboro, CT). The high-, mid-, and low-level exposure chambers were each monitored with their own analyzer. The analyzers for the high-, mid-, and low-level chambers underwent weekly five-point calibrations and daily one-point calibration checks using test substance. A fourth analyzer was devoted to monitoring the control chamber, the room air, and the hood enclosing the 20-pound tank of test substance. This Miran underwent a three-point calibration approximately quarterly until December 2001, and then once before exposures ended. The Miran 1A analyzers dedicated to the mid- and high-level chambers were calibrated over a range of 6–35 g/m^3. The Miran 1A analyzers for the control chamber and the low-level chamber were calibrated over a concentration range of 1–7 g/m^3.

The absorbance in each chamber was monitored continuously. Data were recorded every 3 seconds and the mean of these values was calculated and saved every 120 minutes. Average values were obtained for the first, second, and third two-hour segments of each exposure day. The mean of the three 120 minute average value of values from each exposure chamber was reported as the day's exposure concentration for that chamber.

Qualitative Assessment of Exposure Atmospheres. The qualitative composition of the exposure atmosphere in each chamber was determined weekly by gas chromatography using a Shimadzu Model GC-17A/FID (Columbia, MD). The percent peak area of each of 19 components was determined and recorded. The retention times of eight representative components were verified against EMBSI values each week, and one-point performance qualifications were performed on any day in which chamber profiles were analyzed. The one-point check was performed using a certified standard of 840 ppm butane in N_2 (Matheson Tri-Gas, Irving, TX).

Determination of Nominal Concentration. Daily nominal or "anticipated" usage was calculated by multiplying the average GMVC concentration in each chamber (low, mid, high; g/m^3) by the total flow through each respective chamber ([L/min * min]/[1000 m^3]) and then summing the

values for all three chambers. This value was compared to the actual GMVC usage determined by taking the difference between the weight of the 20-pound cylinder before and after each exposure.

Concentration of Test Substance in the Exposure Room. Concentration of the test substance in the exposure room was determined periodically during the study (at approximately 60-day intervals) using a Miran 1A Infrared Spectrometer (Foxboro Wilks, Foxboro, CT) operated using the same settings as used to monitor the low-level exposure chamber.

In-Life Endpoints

Rats were exposed 6 hours/day (plus 14 minutes, the time for the vapor concentration to reach T90), 5 days/week for 104 weeks (520 exposure days). The following observations and measurements were made during the dosing phase:

Mortality and Morbidity. Laboratory animal technicians observed the rats twice daily throughout the study. Examinations were oriented toward identifying dead, weak, or moribund animals and documenting the onset and progression of any abnormal clinical signs. Appropriate actions were taken to minimize the loss of animals from study (e.g., necropsy or refrigeration of any rats found dead and sacrifice of weak or moribund animals).

Body Weight. All animals were individually weighed using the Path-Tox® data acquisition system (Xybion, Cedar Knolls, NJ). Males and females on this study were initially weighed for randomization on May 7, 2001 and May 14, 2001, respectively. Due to problems with setup and validation of the exposure system, the initiation of the study was delayed by approximately 10 days. This decision was made by mutual agreement between LRRI and the Sponsor, after the animals had already been weighed, randomized, and identified. Day -1 weights, obtained on the day prior to initiation of exposures, were taken on May 22 and May 29, for males and females, respectively. Therefore, the Day -1 weights were obtained 16 days after randomization occurred. After initiation of exposures, weights were obtained and weekly for 13 weeks and then every 4 weeks thereafter.

Clinical Signs of Toxicity. Thorough clinical examinations were made at randomization, on Day 1, and weekly thereafter. Observations were detailed and carefully recorded using Path-Tox® software. Observations included evaluations of skin and fur; eyes and mucous membranes; respiratory and circulatory effects; autonomic effects such as salivation and central nervous system effects including tremors and convulsions; and changes in the level of activity, gait, posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behavior (e.g., self-mutilation, walking backward).

Hematology. At 12 months, 18 months and at final sacrifice, blood smears were prepared and manual differential cell counts were made. For the 12- and 18-month time points, blood was obtained by tail nick. At final sacrifice, blood was obtained by cardiac puncture and collected into Vacutainer tubes containing ethylenediamine tetra-acetic acid (EDTA). Evaluated parameters included total leukocyte estimates (white blood cell [WBC]); nucleated red blood cell counts (cells/100 WBC), anisocytosis (0 to 4+), polychromasia (1+ to 4+) and; relative differential leukocyte counts for segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, basophils, atypical lymphocytes and blastocytes. Absolute leukocyte counts were calculated for segmented neutrophils, band neutrophils, lymphocytes, monocytes, eosinophils, basophils, atypical lymphocytes and blastocytes using the formula WBC * relative percent/100.

Post-Exposure Endpoints

Gross Necropsy. A complete gross examination was performed on all animals at final sacrifice and on those animals that died naturally or were euthanized in a moribund condition. Sacrifices of rats surviving 520 days of exposure occurred during the week following the last exposure day for each sex. Animals were randomly assigned to a sacrifice day. Samples collected at necropsy are listed in Table 2-2.

Table 2-2. Organs and Representative Samples Taken for Examination

Cardiovascular/Hematopoietic System
1. Aorta
2. Bone marrow (and/or fresh aspirate)
3. Heart
4. Lymph nodes (mandibular, mesenteric, bronchial, mediastinal)
5. Spleen

Table 2-2. Organs and Representative Samples Taken for Examination (Concluded)

Digestive System
1. Cecum
2. Colon
3. Duodenum
4. Esophagus
5. Ileum
6. Jejunum
7. Liver
8. Pancreas
9. Rectum
10. Salivary glands
11. Stomach

Glandular System
1. Adrenals
2. Parathyroid
3. Thyroid
4. Mammary gland (male[a] and female)

Nervous System
1. Brain (including sections of medulla/pons, cerebellum, and cerebrum)
2. Eyes (retina, optic nerve)
3. Peripheral nerve (sciatic or tibial, preferably in close proximity to the muscle)
4. Pituitary
5. Spinal cord (three levels: cervical, mid-thoracic, and lumbar)

Other
1. All gross lesions and masses
2. Skin
3. Tail (for identification)
4. Skeletal muscle[a]
5. Femur[a]

Respiratory System
1. Larynx
2. Lung (infused with fixative)
3. Nose
4. Paranasal sinuses
5. Pharynx (reviewed with larynx section)
6. Trachea

Urogenital System
1. Epididymis
2. Kidneys[a]
3. Ovaries
4. Prostate
5. Seminal vesicle(s)
6. Testes
7. Urinary bladder (infused with fixative)
8. Uterus

[a]Examined microscopically but not required by protocol.

All study animals received a complete necropsy. Animals were euthanized with an overdose of intraperitoneally injected barbiturate anesthetic (Euthasol®, Virbac AH Inc., Fort Worth, TX). Body weights and fresh organ weights were collected on lungs, liver, kidneys, adrenals, testes, epididymis, ovaries, uterus, spleen, brain, and heart of final sacrifice and moribund sacrifice animals. Animals found dead received a complete necropsy with tissue collection, but blood and organ weight data were not routinely collected. Cardiac blood was collected from animals at final sacrifice for determination of total and differential WBC counts. Gross lesions, body weights, and organ weights were entered on pre-prepared forms, and then the information was recorded on the Path-Tox® database (Version 4.2.2, Module P, Xybion Medical Systems, Cedar Knolls, NJ).

Lungs were gently instilled via the trachea with 10% neutral buffered formalin (NBF) to approximate normal volume. Organs/tissues were immersion fixed in 10% NBF for subsequent histopathologic examination. Tissues were trimmed, processed routinely, paraffin embedded, sectioned at 5 μm, and stained with hematoxylin and eosin for histopathologic examination.

Histopathology. All collected tissues and lesions were examined histologically in control (0 g/m^3) animals, high-level (20 g/m^3) animals, and dead or moribund animals of all groups. Respiratory tissues (lungs, larynx, trachea, and nasal turbinate sections), potential target tissues (testes, kidneys of males) and gross lesions were examined histologically from final sacrifice low- (2 g/m^3) and mid- (10 g/m^3) level animals. Nomenclature of proliferative lesions was based on the international harmonized nomenclature recommended by the Rat Nomenclature Reconciliation Subcommittee of the Society of Toxicologic Pathologists (see http://www.toxpath.org; Standardized Rat Nomenclature). Nomenclature of other lesions was routine, widely understood usage (see Boorman et al., 1990a; Gopinath et al., 1987).

Standard, subjective severity scoring of most nonproliferative lesions was based on the extent of tissue affected by the change and the severity of the change within affected areas. Typically, a score of 0 (none) = essentially no tissue affected; 1 (minimal) = 1 to 10% affected, 2 (mild) = 11 to 25% affected, 3 (moderate) = 26 to 50% affected, 4 (marked) = 51 to 100% affected. Severity

scores may have been adjusted up or down (usually by 1 point) based on the severity of the change within the affected areas.

The kidney is an expected target tissue for toxicity in this study, especially for development of chronic progressive nephropathy or hyaline droplet nephropathy. The diagnosis of chronic progressive nephropathy encompasses a large constellation of changes; severity scoring for this diagnosis was somewhat more complex and is described in Table 2-3.

Table 2-3. Severity Scoring of Chronic Progressive Nephropathy

Score	Histologic Findings
0	Essentially no changes of chronic progressive nephropathy. May have rare scattered protein casts in tubules, mineral concretions.
1	Increased number and size of tubular protein casts; primarily within tubules at corticomedullary junction, some within collecting ducts. Minor/scattered foci of basophilic, regenerative tubules. Changes affect less than 5% of tissue component.
2	Homogeneous protein casts relatively abundant within tubules and collecting ducts (especially at corticomedullary junction). Increased size and frequency of tubular basophilia. Accompanying changes present, may include small foci of tubular basement membrane thickening and/or mineralization, interstitial fibrosis, tubular atrophy and dilatation, minor associated mononuclear inflammatory infiltrates, basophilia changes (including mesangial proliferation, synechiae, proliferation of parietal epithelium). Changes may affect up to 25% of component.
3	As 2 with increased tubular atrophy/dilatation/regeneration, interstitial fibrosis, increased inflammatory infiltrates, increased thickening of basement membranes of tubules and glomeruli.
4	As 3 with increased severity and extent of changes. Senescent glomeruli relatively common. Typically combined changes affect well over 50% of kidney.

Statistics

Body and Organ Weights. Group mean body weight, organ weight, percent organ-to-body weight data, and percent organ to brain weight data, were tested for statistical significance using Path-Tox® software. After testing for an overall trend among test groups by an analysis of variance, Bartlett's test was used to establish the homogeneity of the data. If the data were

homogeneous, significant differences between the control and exposed groups were evaluated using a modified Dunnett's test. If data were nonhomogeneous, a modified t test was used. Significance levels were set at $p \leq 0.05$.

Survival Analysis. The number of days of survival was calculated for each animal in the study. Two female rats that died subsequent to accidental nose injuries early in the study were deleted from the analysis. The probability of survival was estimated by the Kaplan-Meier product-limit method using PROC LIFETEST in SAS Version 8.2. Mean numbers of survival days and time to 25% mortality were estimated for each dose group. Log-rank tests were used to test the hypothesis that there are differences among the four groups for each sex. The significance level was set at $p = 0.05$. All reported p-values for the survival analysis are two sided.

Analysis of White Blood Cell Total and Differential Cell Counts. For the hematology data, medians and ranges are presented since the distributions are highly skewed for some variables. Preliminary analyses were done using the generalized estimating equation approach for longitudinal data analysis since there were three time points. For some variables these analyses showed significant interactions between time and group. Therefore, analyses were done to compare dose groups at each time point and to compare time points within each dose group. The Krusal-Wallis test was used for these comparisons.

Histopathology. The incidences of all neoplastic and non-neoplastic lesions are given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. Three statistical evaluations were performed on the histopathology lesion incidence data: 1) Cochran-Armitage test, which tests whether the incidence of lesions shows a trend across dose groups; 2) logistic regression that takes death date into account when assessing the presence of a dose-dependent trend; and 3) the Fisher's exact test to compare incidences among the four dose groups. The two-sided significance level was set at $p = 0.05$. If a significant difference was detected by the Fisher's test, six possible pairwise comparisons were calculated. Using the Bonferroni correction for pairwise comparisons, each pairwise comparison would be considered significant if $p < 0.008$.

Fisher's exact test and the Cochran-Armitage test do not use survival information and are appropriate in situations where survival is similar among exposure group as is the case for this study. The Fisher's exact tests the null hypothesis of equality of prevalences across dose groups against the alternate hypothesis that the prevalences are not equal while the Cochran-Armitage tests the null hypothesis of equality across doses against the alternate hypothesis of a monotonic increasing or decreasing trend.

In the case of renal tubule nephropathy, statistical evaluation of group differences in severity scores was performed using the Kolmogorov-Smirnov test within the Path-Tox® software. The significance level was $p \leq 0.05$.

RESULTS

EXPOSURE ATMOSPHERE

Pre-Exposure Characterization

Three-Day Stability Evaluation and Nominal GMVC Usage. Before exposures were initiated the system was operated for 6 hours/day for 3 consecutive days. The concentration of vapor in each chamber was close to target and constant within each day, and the concentrations were reproducible from day to day (Table 3-1). Gas chromatographic analysis of the chamber atmospheres indicated that the relative percentages of the major components were constant over the 3-day period. The percentages of actual GMVC usage/anticipated usage (nominal usage) on the 3 days of stability testing were 95, 97, and 90 for days 1, 2, and 3, respectively. Pre-test chamber trial data are presented in Appendix E.

Table 3-1. Results of 3-Day Stability Evaluations

Target Exposure Concentration (g/m^3)/ Chamber Number	Achieved Concentration ± SD (n = 3)		
	Day 1 (5/17/01)	Day 2 (5/18/01)	Day 3 (5/19/01)
2 (Chamber 6)	2.06 ± 0.04	1.97 ± 0.10	1.93 ± 0.05
10 (Chamber 7)	9.45 ± 0.23	9.37 ± 0.15	9.37 ± 0.28
20 (Chamber 8)	18.8 ± 0.28	18.9 ± 1.14	19.5 ± 0.37

Homogeneity of Vapor Concentration. The homogeneity of vapor concentration throughout the exposure chambers was determined by conducting a chamber distribution study. The concentrations of GMVC were measured from four sampling ports located in front and four sampling ports located in the back of the chambers during an exposure day and compared to the concentration obtained in a reference location within the chamber. The spatial variations measured in Chambers 6 (2 g/m^3), 7 (10 g/m^3), and 8 (20 g/m^3) were 3.0%, 1.3%, and 0.8%, respectively. These results indicate the vapor was distributed evenly within each of the chambers. Homogeneity of vapor concentration data is presented in Appendix E.

T90 Determination. The amounts of time required to reach T90 were 13, 14.1, and 14.2 minutes for Chambers 6, 7, and 8, respectively. A T90 of 14 minutes was chosen for use with this study. Daily exposure periods to GMVC were then 6 hours and 14 minutes.

Confirmation of the Absence of Aerosols. The particle concentrations of air inside Chamber 8, operated at a target concentration 20 g GMVC/m^3, and the control chamber (Chamber 5) were determined using a TSI Aerodynamic Particle Sizer. There was less than 1 particle per cubic centimeter air in the control and high-level chambers, indicating that there were no GMVC aerosols at the highest target vapor concentration used in this study.

In-Study Data

Chamber Concentrations and Nominal Usage. The overall study means of the daily vapor concentrations achieved for the 2, 10, and 20 g GMVC/m^3 vapors are provided in Table 3-2. The overall achieved means were within 2% of target for each exposure concentration. Thus, all future references to exposure concentration are in terms of target concentration. Concentrations of vapor in the control chamber were below the lowest concentration on the standard curve used to calibrate the control chamber Miran 1A (1 g GMVC/m^3). The overall average of the daily percent nominal usage was 99% ± 4%, indicating excellent agreement between anticipated and actual GMVC usage. Daily vapor concentrations and nominal usage are provided in Appendix E.

Table 3-2. Summary of GMVC Vapor Concentrations[a]

Target g GMVC/m^3	Achieved Concentrations	Percent of Target
0	NA[b]	NA
2	2.02 ± 0.07	101
10	10.0 ± 0.37	100
20	20.3 ± 0.74	101

[a]Results are the mean ± SD of vapor concentrations obtained in 520 exposure days.
[b]Values were below 1 g GMVC/m^3, the lowest concentration on the standard curve used to calibrate the Miran 1A used to make the measurements.

Homogeneity of Vapor Concentration. An evaluation of the homogeneity of vapor concentration in each GMVC chamber was conducted after animal exposures began. The spatial variations measured in Chambers 6 (2 g/m^3), 7 (10 g/m^3), and 8 (20 g/m^3) were 2.8%, 0.86%, and 1.8%, respectively. These results indicate the vapor is distributed evenly within each of the chambers. Homogeneity of vapor concentration data are presented in Appendix E.

Gas Chromatographic Profiles of GMVC Exposure Atmospheres. Gas chromatographic profiles were obtained from each exposure chamber weekly to assess whether the animals were exposed to the relative amounts of the same 19 major components throughout the study. The peak areas of the 19 major components were summed to provide a "total peak area" for those components. The peak area of each of the components was divided by the total peak area for the components to obtain a relative peak area (in percent) for each of the components. These were compared to the reference values provided by EMBSI. Profiles remained acceptably constant throughout the study. Weekly results are provided in Appendix F.

Chamber Environmental Data. The temperature, relative humidity, chamber flow rate and pressures were maintained within acceptable ranges with occasional excursions outside acceptable limits that are described in Section 4. Environmental data are provided in Appendix G.

Survival Analysis. All groups had 50 animals except the mid-level group for females. Two animals in that group were omitted from the analysis because they were euthanized following accidental nose injuries early in the study. Figures 3-1 and 3-2 show the survival curves for male and female animals, respectively. The differences among the groups are not statistically significant for male ($p = 0.46$) or female animals ($p = 0.07$). Table 3-3 shows means, standard errors, and day of 25% mortality for male and female animals under each experimental condition. For the calculation of mean and standard errors of survival times, the last day of the study was used as the value of the survival time for animals that underwent final sacrifice. Therefore, the means and standard errors may be underestimated since some of the animals may have lived longer if the protocol had included following them for a longer period of time. The results indicate that chronic GMVC inhalation did not shorten the lifespan of the exposed rats compared to the controls.

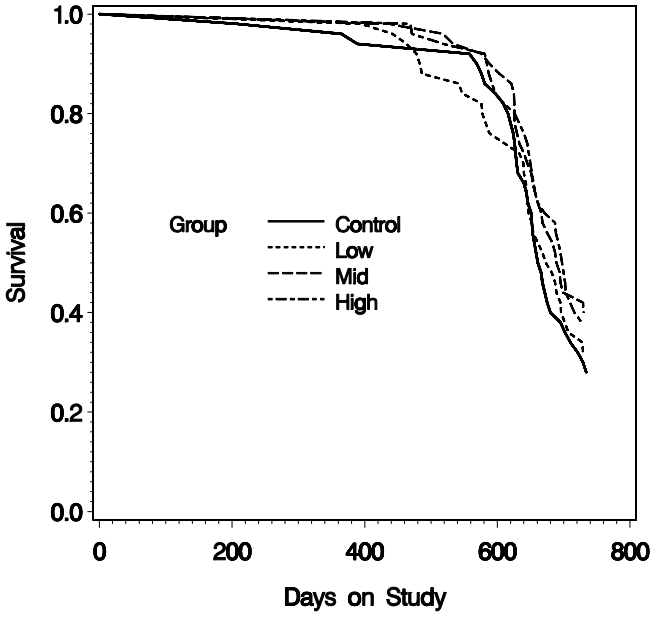

Figure 3-1. Survival of Male Animals

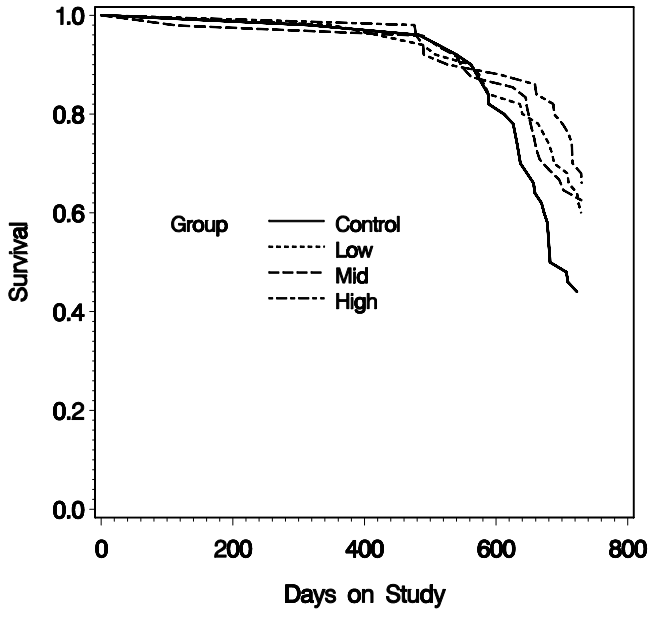

Figure 3-2. Survival of Female Animals

Table 3-3. Mean Survival Days and Day of 25% Mortality for Male and Female Rats

	Male		Female	
Group	Mean Days of Survival (SE)	Estimate Day of 25% Mortality	Mean Days of Survival (SE)	Estimated Day of 25% Mortality
Control	652.6 (14.4)	626	665.9 (11.5)	632
Low	651.6 (12.8)	633	685.5 (11.9)	680
Mid	675.3 (9.4)	633	680.9 (15.5)	660
High	674.1 (9.5)	645	697.3 (10.6)	715

Animal Disposition. The disposition of all animals on study is summarized in Table 3-4. A small percentage of animals died naturally. The majority of animals not surviving 104 weeks on study underwent moribund sacrifices. Individual animal disposition is provided in Appendix H.

Table 3-4. Disposition of Rats on Study

	Control		2 g GMVC/m^3		10 g GMVC/m^3		20 g GMVC/m^3	
Death Type	Male	Female	Male	Female	Male	Female	Male	Female
Moribund Sacrifice	35	24	29	18	27	17	29	15
Natural Death	1	4	5	2	3	3	2	2
Terminal Sacrifice	14	22	16	30	20	30	19	33
Total	50	50	50	50	50	50	50	50

Body Weight Gain. Mean group body weights of male and female rats are provided in Tables 3-5 and 3-6, respectively. Growth curves are provided in Figures 3-3 and 3-4 for males and females, respectively.

Table 3-5. Body Weight Summary for Male Rats[a]

Study Day	Control		2 g/m^3			10 g/m^3			20 g/m^3		
	Body Weight (g)		Body Weight (g)		Percent Control	Body Weight (g)		Percent Control	Body Weight (g)		Percent Control
	Mean	SD	Mean	SD		Mean	SD		Mean	SD	
-1	184.4	7.8	182.1	9.8	98.8	184.1	10.9	99.8	***177.9***	11.6	96.5
7	188.2	11.5	***193.8***	11.6	103.0	***193.4***	12.9	102.8	184.2	20.2	97.9
14	207.5	12.9	207.8	16.2	100.1	208.0	14.3	100.2	***199.5***	14.5	96.1
21	226.5	14.3	226.1	15.6	99.8	225.3	15.3	99.5	***217.4***	14.3	96.0
28	239.7	16.1	240.0	15.6	100.1	237.7	16.5	99.2	***229.5***	14.3	95.7
35	251.2	17.3	250.9	16.4	99.9	250.7	17.4	99.8	***241.7***	15.5	96.2
42	259.1	18.2	260.7	16.6	100.6	260.5	17.8	100.5	252.2	15.8	97.3
49	272.7	17.8	273.7	15.9	100.4	271.0	17.9	99.4	***263.4***	16.6	96.6
56	283.1	17.5	282.2	15.7	99.7	281.6	18.3	99.5	***275.0***	16.2	97.1
63	290.5	18.3	296.5	18.5	102.1	288.6	18.3	99.3	282.0	17.3	97.1
70	297.6	18.4	297.1	16.7	99.8	296.6	18.7	99.7	291.2	17.8	97.8
77	309.1	19.0	306.1	17.0	99.0	305.9	19.6	99.0	***299.6***	17.6	96.9
84	314.7	18.8	312.5	16.6	99.3	312.3	20.2	99.2	***305.8***	17.8	97.2
91	319.3	18.9	316.9	16.8	99.2	313.5	18.4	98.2	***309.2***	17.2	96.8
119	340.8	18.1	338.5	15.8	99.3	338.8	19.8	99.4	***329.9***	18.5	96.8
147	355.0	17.9	353.3	15.0	99.5	351.1	21.1	98.9	***343.8***	18.5	96.8
175	368.6	18.3	363.0	15.8	98.5	363.5	21.9	98.6	***358.8***	18.8	97.3
203	379.4	19.9	375.5	16.2	99.0	377.9	23.0	99.6	***369.4***	19.7	97.4
231	396.3	21.0	389.5	16.5	98.3	390.2	23.9	98.5	***381.4***	21.1	96.2
259	397.0	19.4	394.5	16.4	99.4	390.3	22.8	98.3	***383.4***	19.2	96.6
287	405.3	22.5	400.2	17.7	98.7	398.3	22.7	98.3	***389.0***	19.6	96.0
315	415.8	22.5	408.6	18.1	98.3	406.5	24.6	97.8	***395.5***	22.1	95.1
343	426.1	21.9	419.1	19.7	98.4	417.0	26.3	97.9	***408.2***	23.2	95.8
371	424.8	27.9	420.5	19.5	99.0	419.3	25.5	98.7	***406.7***	22.2	95.7
399	435.7	25.8	424.4	20.9	97.4	***422.9***	24.9	97.1	***408.1***	22.8	93.7
427	437.0	26.2	***424.4***	20.4	97.1	***419.6***	24.6	96.0	***406.5***	23.0	93.0
455	441.8	26.1	***424.9***	23.8	96.2	***424.4***	25.0	96.1	***407.8***	24.9	92.3
487	445.8	26.6	***431.8***	21.6	96.9	***425.2***	24.0	95.4	***412.3***	23.6	92.5
515	444.2	25.0	***430.8***	22.1	97.0	***423.4***	24.8	95.3	***409.7***	23.9	92.2
539	433.6	24.6	***420.1***	22.9	96.9	***415.0***	22.3	95.7	***397.3***	23.1	91.6
567	431.3	25.9	***417.8***	23.0	96.9	***410.2***	21.2	95.1	***389.7***	25.5	90.4
595	425.7	26.1	***413.2***	19.4	97.1	***405.4***	19.7	95.2	***383.0***	29.3	90.0
623	416.6	32.5	408.4	19.7	98.0	***392.9***	27.0	94.3	***381.6***	22.6	91.6
651	401.6	34.7	401.1	26.2	99.9	390.5	22.8	97.2	***376.0***	29.1	93.6
679	402.7	27.6	400.9	26.9	99.6	389.0	15.4	96.6	***369.8***	23.2	91.8
707	391.8	35.7	383.9	27.6	98.0	381.7	12.6	97.4	***362.6***	24.7	92.5

[a]Bolded and italicized values are significantly different from controls at that time point; $p \leq 0.05$. Group mean body weight, organ weight, and percent organ-to-body weight data were tested for statistical significance using Path-Tox® software. After testing for an overall trend among test groups by an analysis of variance, Bartlett's test was used to establish the homogeneity of the data. If the data were homogeneous, group differences were evaluated using a modified Dunnett's test. If data were non-homogeneous, group differences were assessed using a modified t-test. Significance levels were set at $p \leq 0.05$.

Table 3-6. Body Weight Summary in Female Rats[a]

Study Day	Control		2 g/m^3			10 g/m^3			20 g/m^3		
	Body Weight (g)		Body Weight (g)		Percent	Body Weight (g)		Percent	Body Weight (g)		Percent
	Mean	SD	Mean	SD	Control	Mean	SD	Control	Mean	SD	Control
-1	125.4	5.3	123.6	4.0	98.6	***122.8***	6.6	97.9	***121.8***	4.9	97.1
7	135.4	5.9	135.2	4.6	99.9	133.8	7.4	98.8	***130.1***	5.4	96.1
14	145.6	7.1	144.8	4.9	99.5	***141.9***	8.4	97.5	***136.2***	6.3	93.5
21	153.0	8.1	153.1	6.6	100.1	***147.5***	8.9	96.4	***142.3***	6.7	93.0
28	156.6	9.6	155.8	7.9	99.5	152.7	9.2	97.5	***148.7***	7.2	95.0
35	161.3	9.9	160.8	6.7	99.7	***156.1***	9.7	96.8	***151.7***	6.9	94.0
42	165.3	8.3	165.3	6.7	100.0	***160.9***	10.1	97.3	***154.5***	7.5	93.5
49	170.1	8.8	170.8	6.7	100.4	***164.5***	10.5	96.7	***159.5***	7.4	93.8
56	172.1	8.9	173.8	6.9	101.0	168.5	10.4	97.9	***162.6***	7.6	94.5
63	176.1	9.3	178.9	7.2	101.6	173.7	10.5	98.6	***168.1***	7.8	95.5
70	181.6	9.2	181.3	7.3	99.8	***175.9***	10.3	96.9	***169.8***	7.8	93.5
77	183.7	9.5	183.7	7.3	100.0	***177.2***	10.4	96.5	***170.9***	7.8	93.0
84	185.1	9.8	185.2	8.0	100.1	***179.5***	11.0	97.0	***172.9***	7.5	93.4
91	186.5	10.1	188.0	7.6	100.8	***180.7***	11.4	96.9	***174.9***	7.7	93.8
119	196.2	9.9	197.0	7.8	100.4	***189.4***	10.6	96.5	***182.1***	8.2	92.8
147	199.9	10.5	202.4	7.5	101.3	***193.1***	10.9	96.6	***186.0***	8.5	93.0
175	207.1	10.6	207.3	7.8	100.1	***199.4***	10.9	96.3	***194.0***	9.1	93.7
203	210.5	10.8	209.9	8.8	99.7	***204.2***	11.3	97.0	***196.4***	8.4	93.3
231	212.2	10.5	212.8	8.5	100.3	***206.9***	11.0	97.5	***198.1***	8.6	93.4
259	214.2	10.2	214.9	9.0	100.3	***206.8***	11.0	96.5	***199.1***	8.1	93.0
287	219.0	10.7	220.8	9.5	100.8	***212.1***	11.5	96.8	***201.9***	9.2	92.2
315	221.5	12.2	222.0	10.9	100.2	***214.7***	12.0	96.9	***204.7***	8.7	92.4
343	225.8	12.9	225.2	11.4	99.7	***215.8***	13.0	95.6	***207.3***	8.6	91.8
371	232.6	15.5	232.7	12.8	100.0	***220.6***	13.8	94.8	***208.7***	9.8	89.7
399	237.4	17.6	236.5	14.8	99.6	***226.4***	14.0	95.4	***215.0***	10.4	90.6
427	243.2	19.6	241.8	16.3	99.4	***229.3***	15.1	94.3	***216.2***	11.0	88.9
459	255.9	22.0	255.8	18.1	100.0	***239.6***	18.3	93.6	***226.3***	13.0	88.4
487	260.5	22.4	259.7	19.6	99.7	***241.8***	19.4	92.8	***229.6***	15.5	88.1
512	258.1	24.4	258.3	17.7	100.1	***241.0***	17.8	93.4	***228.6***	12.7	88.6
539	259.5	22.5	261.9	18.6	100.9	***243.3***	20.9	93.8	***229.6***	13.2	88.5
567	263.7	21.5	261.5	16.8	99.2	***245.5***	19.3	93.1	***229.4***	12.7	87.0
595	266.1	21.6	266.3	15.2	100.1	***250.9***	18.6	94.3	***234.1***	12.8	88.0
623	264.1	25.4	269.7	14.8	102.1	***249.7***	21.2	94.5	***233.6***	11.7	88.5
651	268.8	26.2	273.3	15.2	101.7	***252.5***	22.1	93.9	***237.3***	13.6	88.3
679	269.1	27.6	274.7	15.9	102.1	***255.6***	24.2	95.0	***238.4***	13.0	88.6
707	268.4	30.4	273.6	16.7	101.9	256.1	25.2	95.4	***239.3***	16.1	89.2

[a]Bolded and italicized values are significantly different from controls at that time point; $p \leq 0.05$. Group mean body weight, organ weight, and percent organ-to-body weight data were tested for statistical significance using Path-Tox® software. After testing for an overall trend among test groups by an analysis of variance, Bartlett's test was used to establish the homogeneity of the data. If the data were homogeneous, group differences were evaluated using a modified Dunnett's test. If data were non-homogeneous, group differences were assessed using a modified t-test. Significance levels were set at $p \leq 0.05$.

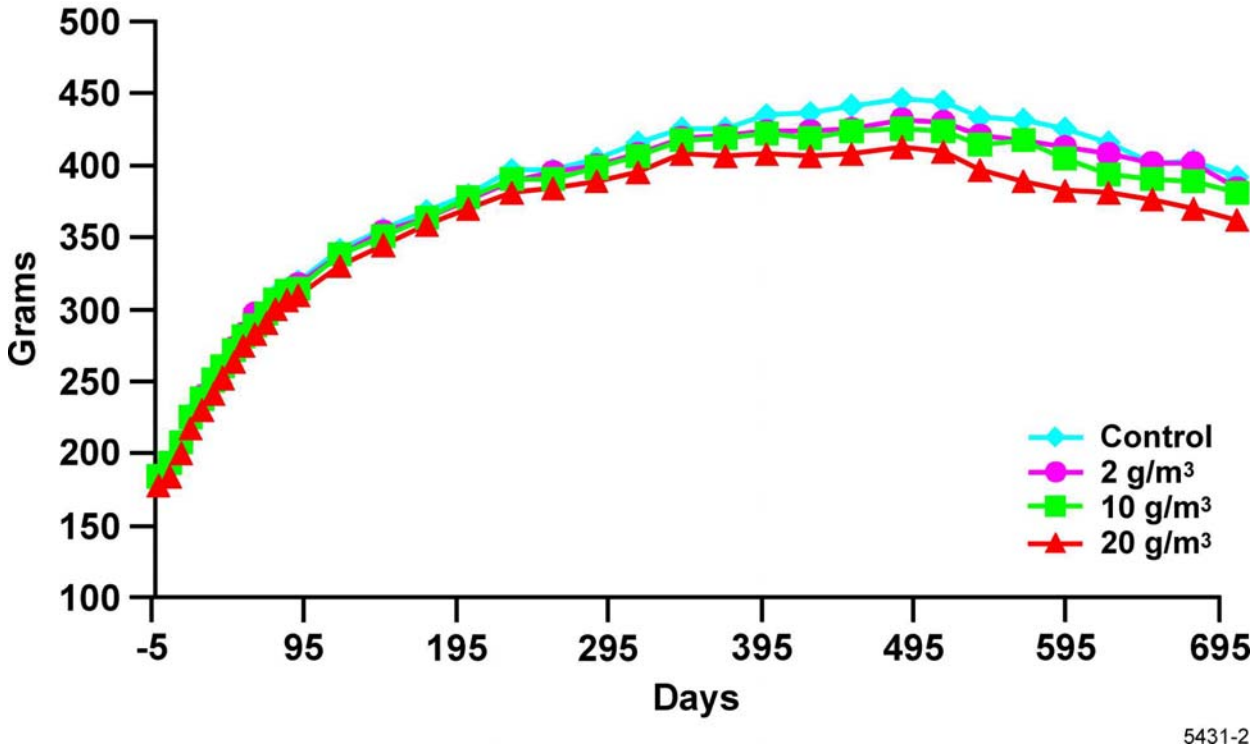

Figure 3-3. Body Weights of Male Rats Exposed to GMVC

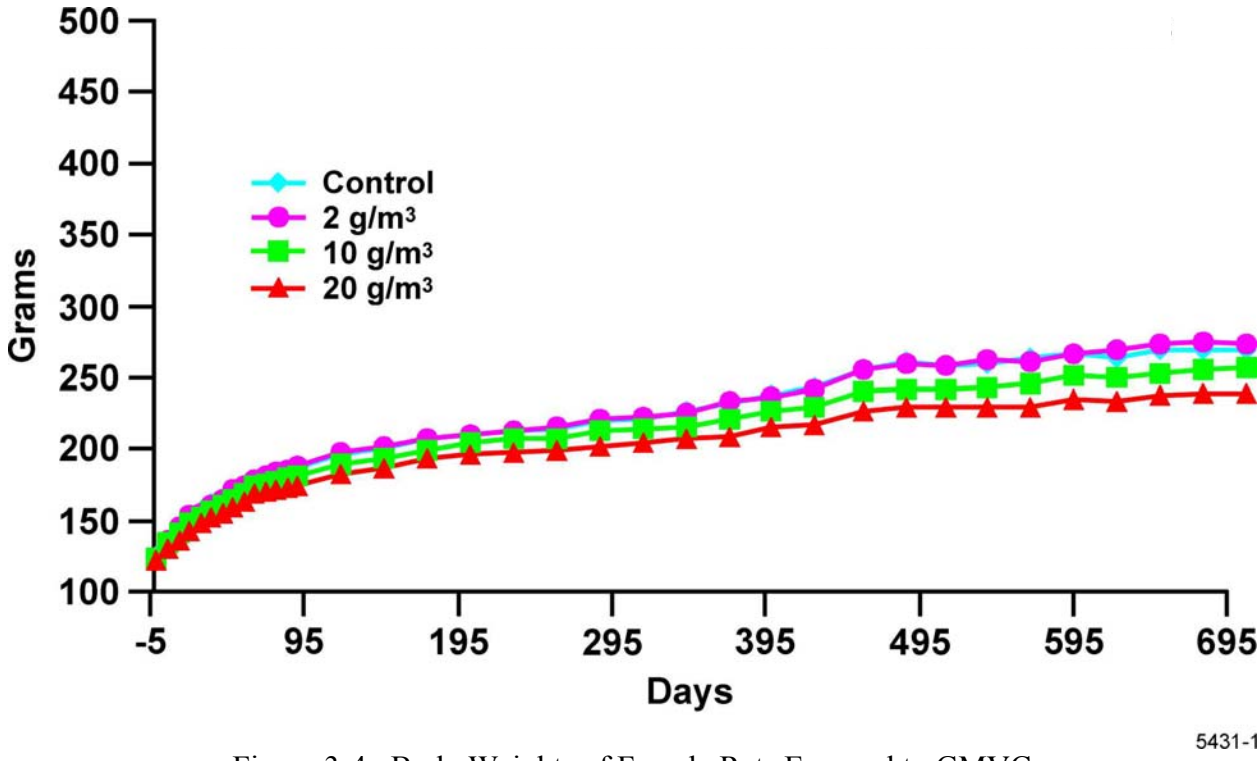

Figure 3-4. Body Weights of Female Rats Exposed to GMVC

On Day -1, male rats in the high-dose group had a group mean body weight (177.9 g) significantly below that of the control rats (184.4g). Due to recently identified problems with the vapor exposure system, the animals had been randomized approximately two weeks before initiation of dosing. Review of the body weight gain from the time of randomization to the day before initiation of exposures (Day -1) indicated that the control males gained an average of 4.99 g/day. The lowest weight gain among control males was 3.9 g/day. Six of 50 males in the high-dose group gained less than 3.9 g/day, with one of these gaining only 1.9 g/day. The rats gaining less weight were not clustered in one location within the chamber, suggesting the low weight gain was not due to a basket feeder issue. Clinical observations on these rats reported them to be normal. Because the rats had already been tattooed, re-randomization was not an option.

At Day -1, mean body weights of the mid- and high-level females at were significantly below controls. Review of the randomization weight and Day -1 weight data indicated the control rats gained an average of 2.27 g/day, with the lowest weight gain in this group being 1.52 g/day. Three of the 50 mid-dose and 5 of 50 high-dose females had gains of less than 1.5 g/day. As with the males, there was no obvious clustering of animal with low weight gain, indicating that the lower weight gain was not a feeder issue.

The matter of significantly different body weights among males and females before the study start was brought to the attention of the Sponsor and the EPA. Comments from the EPA dated July 6, 2001, indicated the agency did not feel the group differences in body weight were large enough to warrant restarting the study.

All animals gained weight during the study; however, body weights were reduced in both males and females exposed to GMVC compared to control values. Body weights of high-level male and female rats remained statistically significantly below control values for most of the study (with the exception of days 63 and 70 for males and days 35, 42, and 49 for females). From Days 399–623, body weights of mid-level males and, occasionally, low-level males were also significantly below control values. Mid-level female body weights were significantly below control values except on Day 7, Days 28–63, and Day 707. Individual animal in-life body weights are provided in Appendix I.

Clinical Signs of Toxicity. Rats exposed to GMVC displayed no clinical signs of toxicity related to GMVC exposure. As this was a chronic toxicity evaluation, many of the observations observed during the second year of the study were related to aging (mammary masses, jaundice). A summary of clinical observations by exposure group is provided in Appendix J.

Body Weights at Final Sacrifice

Final sacrifice body weights of male and female rats exposed to 20 g GMVC/m^3 were significantly below control values (Table 3-7); weights were 91.5% and 91.8 % of control, respectively. Individual animal data are provided in Appendix K.

Table 3-7. Summary Statistics for Final Sacrifice Body Weights of Male and Female Rats

	Control	2 g GMVC/m^3	10 g GMVC/m^3	20 g GMVC/m^3
Weight in grams	Males			
Mean ± SD	390.0 ± 18.2	375.5 ± 35.2	368.7 ± 28.5	357.0 ± 28.4[a]
n	14	16	20	19
	Females			
Mean ± SD	265.4 ± 33.3	273.5 ± 20.4	256.3 ± 21.9	243.6 ± 15.7[b]
n	22	30	30	33

[a]Mean significantly different from controls. Data homogeneous by Bartlett's test. Means compared using Dunnett's t test; $p \leq 0.05$.
[b]Mean significantly different from controls. Data nonhomogeneous by Bartlett's test. Means compared using a Modified t test; $p \leq 0.05$.

Absolute Organ Weight at Final Sacrifice

Organ weight for adrenal glands, brain, epididymis (males), heart, kidneys (pair), liver, lungs, ovaries (females), spleen, testes (males) and uterus (females) were recorded. At terminal sacrifice, mean group brain weights of high-level males and females were significantly below corresponding control values. The epididymis weight of mid-level males and adrenal weight of high-level females were significantly below that of the corresponding control values. Summary statistics for males and females are provided in Table 3-8. Individual animal data are provided in Appendix K, pages K-2 through K-16.

Table 3-8. Absolute Organ Weight at Terminal Sacrifice (g ± SD)

Exposure Group	Number in Group	Adrenal Glands	Brain	Epididymis/ Uterus	Heart	Kidneys	Liver	Lungs	Spleen	Testes/ Ovaries
					Male					
1	14	0.098 ± 0.099	2.100 ± 0.051	0.549 ± 0.200	1.197 ± 0.101	2.906 ± 0.230	16.081 ± 4.838	1.917 ± 0.281	2.568 ± 1.831	5.183 ± 3.133
2	16	0.175 ± 0.418	2.062 ± 0.042	0.436 ± 0.081	1.196 ± 0.099	3.053 ± 0.598	14.765 ± 2.589	2.237 ± 0.566	3.993 ± 4.794	5.378 ± 1.948
3	20	0.075 ± 0.014	2.072 ± 0.048	0.416 ± 0.081[a]	1.192 ± 0.140	2.960 ± 0.236	14.162 ± 1.268	2.103 ± 0.411	2.995 ± 2.489	5.996 ± 2.419
4	19	0.072 ± 0.010	2.054 ± 0.058[b]	0.429 ± 0.092	1.134 ± 0.082	3.054 ± 0.365	15.475 ± 2.832	2.128 ± 0.570	4.776 ± 6.159	6.324 ± 2.448
					Female					
1	22	0.066 ± 0.008	1.894 ± 0.060	0.843 ± 0.718	0.922 ± 0.085	1.951 ± 0.348	9.243 ± 1.452	1.562 ± 0.503	2.110 ± 3.251	0.117 ± 0.099
2	30	0.063 ± 0.008	1.875 ± 0.052	0.771 ± 0.232	0.894 ± 0.072	1.900 ± 0.171	9.668 ± 2.759	1.366 ± 0.145	1.083 ± 0.842	0.126 ± 0.062
3	30	0.066 ± 0.022	1.871 ± 0.043	0.972 ± 0.632	0.883 ±0.081	1.908 ± 0.171	9.297 ± 2.322	1.360 ± 0.305	0.987 ± 0.714	0.141 ± 0.174
4	33	0.058 ± 0.009[a]	1.857 ± 0.046[b]	0.826 ± 0.356	0.884 ±0.086	1.933 ± 0.155	9.036 ± 1.452	1.374 ± 0.278	1.681 ± 2.486	0.105 ± 0.022

[a]Mean significantly different from control. Data are nonhomogeneous by Bartlett's test; $p \leq 0.05$. Means were compared using a Modified t test; $p \leq 0.05$.
[b]Mean significantly different from control. Data are homogeneous by Bartlett's test; $p \leq 0.05$. Means were compared using Dunnett's t test; $p \leq 0.05$.

Percent Organ-to-Body Weight at Final Sacrifice

Significant change occurred only among male rats. Percent brain-to-body weight and lung-to-body weight were significantly increased in mid and high GMVC-exposed males compared to controls. The lung results are likely a result of the treatment-related depression in body weight, rather than effects on the organs weights since there were no changes in absolute lung weights in males. For brain there was an apparent trend for the effects to be exposure concentration dependent. The percent kidney-to-body weight was increased only in high-level GMVC-exposed males. Summary statistics are provided in Table 3-9 for males and females. Individual animal data are provided in Appendix K pages K-17 through K-31.

Percent Organ-to-Brain Weight at Final Sacrifice

There were few effects on organ-to-brain weights. The percent kidney-to-brain weight was significantly increased for high-level GMVC-exposed male rats. The percent epididymis-to-brain weight was significantly decreased in mid-level males. The percent adrenal glands-to-brain weight was significantly decreased in high-level GMVC-exposed female rats. Summary statistics are provided in Table 3-10 for males and females. Individual animal data are provided in Appendix K pages K-32 through K-46.

Terminal Body Weight and Organ Weight of Euthanized (Moribund) Rats

As the rats reached about 20 months of age (18 months on study), mortality due to effects of aging increased. Most animals not surviving to the final sacrifice were euthanized and terminal body and organ weights were recorded. Because the animals were euthanized over a period of several months, interpretation of the statistical significance of differences among exposed and control groups is not as straightforward as with data obtained at the final sacrifice. Summary statistics and individual animal terminal body and organ weight data are provided in Appendix K pages K-47 through K-98. Of particular note, the absolute kidney and percent kidney-to-brain weight of all GMVC-exposed groups were significantly increased compared to control values. Kidney-to-body weight was only significantly increased among males exposed to the mid and high levels of GMVC. The epididymis weight and epididymis-to-body weight was significantly decreased among mid-level males. Liver-to-body weight ratios for high-level males were statistically significantly increased. Among females, the absolute brain weight was significantly decreased in high-level GMVC females. The liver-to-body weight was significantly decreased in the low group females and the lung-to-body weight was significantly increased among the high-level females.

Table 3-9. Percent Organ-to-Body Weight at Terminal Sacrifice (% ± SD)

Exposure Group	Number in Group	Adrenal Glands	Brain	Epididymis/ Uterus	Heart	Kidneys	Liver	Lungs	Spleen	Testes/ Ovaries
					Male					
1	14	0.025 ± 0.025	0.539 ± 0.020	0.141 ± 0.052	0.308 ± 0.03	0.747 ± 0.074	4.143 ± 1.302	0.494 ± 0.085	0.666 ± 0.489	1.318 ± 0.774
2	16	0.046 ± 0.106	0.554 ± 0.056	0.117 ± 0.024	0.32 ± 0.029	0.818 ± 0.167	3.965 ± 0.793	0.609 ± 0.209	1.119 ± 1.451	1.412 ± 0.47
3	20	0.02 ± 0.005	0.565 ± 0.047[a]	0.114 ± 0.029	0.324 ± 0.032	0.810 ± 0.122	3.861 ± 0.443	0.577 ± 0.142[a]	0.832 ± 0.72	1.616 ± 0.617
4	19	0.02 ± 0.004	0.579 ± 0.048[a]	0.120 ± 0.026	0.319 ± 0.023	0.860 ± 0.118[a]	4.378 ± 0.991	0.602 ± 0.181[a]	1.437 ± 2.046	1.762 ± 0.667
					Female					
1	22	0.025 ± 0.005	0.725 ± 0.100	0.319 ± 0.258	0.353 ± 0.056	0.763 ± 0.299	3.506 ± 0.542	0.606 ± 0.252	0.852 ± 1.419	0.043 ± 0.032
2	30	0.023 ± 0.002	0.689 ± 0.049	0.282 ± 0.080	0.328 ± 0.031	0.697 ± 0.067	3.548 ± 1.069	0.502 ± 0.068	0.395 ± 0.293	0.047 ± 0.024
3	30	0.026 ± 0.011	0.735 ± 0.063	0.391 ± 0.293	0.346 ± 0.036	0.747 ± 0.072	3.617 ± 0.726	0.534 ± 0.135	0.383 ± 0.275	0.058 ± 0.083
4	33	0.024 ± 0.004	0.766 ± 0.056	0.341 ± 0.152	0.364 ± 0.036	0.795 ± 0.058	3.706 ± 0.540	0.566 ± 0.123	0.689 ± 1.003	0.043 ± 0.010

[a]Mean significantly different from control. Data are nonhomogeneous by Bartlett's test; $p \leq 0.05$. Means were compared using a Modified t test; $p \leq 0.05$.

Table 3-10. Percent Organ-to-Brain Weight at Terminal Sacrifice (% ± SD)

Exposure Group	Number in Group	Adrenal Glands	Brain	Epididymis/ Uterus	Heart	Kidneys	Liver	Lungs	Spleen	Testes/ Ovaries
					Male					
1	14	4.66 ± 4.69	100.00 ± 0.00	26.14 ± 9.39	56.99 ± 4.13	138.45 ± 11.17	766.95 ± 234.40	91.38 ± 13.82	122.91 ± 88.49	246.49 ± 148.82
2	16	8.40 ± 19.92	100.00 ± 0.00	21.13 ± 3.98	57.95 ± 4.39	148.07 ± 29.12	716.34 ± 127.37	108.57 ± 27.97	194.18 ± 233.92	260.53 ± 94.67
3	20	3.61 ± 0.70	100.00 ± 0.00	20.07 ± 3.90[a]	57.47 ± 6.34	142.89 ± 11.80	683.24 ± 57.03	101.56 ± 20.17	144.77 ± 120.69	287.93 ± 113.07
4	19	3.51 ± 0.51	100.00 ± 0.00	20.87 ± 4.37	55.19 ± 3.45	148.57 ± 16.10[a]	753.74 ± 140.92	103.39 ± 26.35	234.10 ± 305.08	308.98 ± 122.09
					Female					
1	22	3.48 ± 0.41	100.00 ± 0.00	44.58 ± 38.18	48.67 ± 4.35	103.38 ± 21.41	487.79 ± 73.46	82.44 ± 26.00	110.78 ± 167.42	6.11 ± 4.94
2	30	3.34 ± 0.39	100.00 ± 0.00	41.16 ± 12.32	47.70 ± 4.04	101.39 ± 9.04	515.51 ± 145.29	72.83 ± 7.33	57.41 ± 43.75	6.74 ± 3.32
3	30	3.51 ± 1.17	100.00 ± 0.00	52.14 ± 34.44	47.18 ± 4.23	101.96 ± 8.70	496.78 ± 123.14	72.62 ± 15.69	52.58 ± 37.59	7.55 ± 9.51
4	33	3.13 ± 0.48[a]	100.00 ± 0.00	44.41 ± 18.72	47.62 ± 4.63	104.21 ± 8.96	486.95 ± 77.87	73.99 ± 14.99	90.45 ± 133.07	5.68 ± 1.15

[a]Mean significantly different from control. Data are nonhomogeneous by Bartlett's test; $p \leq 0.05$. Means were compared using a Modified t test; $p \leq 0.05$.

Hematology

A summary of the effects of exposure concentration and exposure time on WBC estimates and absolute numbers of WBC types is provided in Table 3-11. Individual animal data are located in Appendix L. Statistical analyses were conducted on continuous variables (WBC estimates and percentage and absolute differential cell count data), and are presented in the Statistician's Report.

Statistically significant differences were found in the WBC estimate, and in absolute numbers of neutrophils, band neutrophils, lymphocytes, monocytes, and eosinophils at the 12-month point. In all cases, values for the high-level male group were greater than the controls. The changes were influenced by both differences in absolute numbers of WBCs and in the percentage of the cell types found. These differences were not found among females at 12 months, nor were they as pronounced in males or present in females at the 18- and 24-month sacrifices. The results in 12-month males possibly were affected by the tail blood sampling technique, which improved by 18 months. At 12 months we had difficulty sampling blood from the tail, especially for the male rats. This may have resulted in artifactually high WBC counts.

By 24 months, when blood was taken by cardiac puncture, few differences in WBC parameters were observed between high-level and control rats of either sex. Only the percentage and absolute numbers of monocytes in high-level males were significantly different (lower than controls). In females the percentage and absolute numbers of atypical lymphocytes were significantly lower in high-level animals compared to controls. The presence of atypical lymphocytes is compatible with the high incidence of leukemia in rats at this age. Since controls and high-level females had similar incidences of leukemia, the biological significance of the increased number of atypical lymphocytes in high-level females is unknown.

The high incidence of leukemia in the aged F344 rats on study, other physiologic changes with aging, and the limitations of the estimate of total WBC from a blood smear indicate that the hematology results should be interpreted with caution.

Table 3-11. Summary of Hematological Findings in Rats Inhaling GMVC; Median (range)

	Males				Females			
	Cells × 10^3/µL				Cells × 10^3/µL			
White Blood Cell Type	12 Months[a]	18 Months[b]	24 Months[c]	Trend in Group Over Time	12 Months[a]	18 Months[b]	24 Months[c]	Trend in Group Over Time
WBC Estimate								
Control	10.0 (4.0, 20.0)	12.0 (6.8, 29.6)	3.7 (2.0, 68.8)	+	8.6 (4.4, 13.6)	4.4 (2.0, 9.2)	3.7 (1.8, 130.0)	+
High-dose	16.8 (8.8, 29.6)[d]	12.4 (4.4, 36.4)	3.6 (1.6, 362.0)	+	8.8 (4.8, 12.0)	4.6 (1.8, 8.2)	3.0 (1.4, 40.0)	+
Absolute Neutrophils								
Control	3.3 (1.0, 8.9)	4.1 (1.2, 17.8)	1.9 (1.1, 5.4)	+	2.0 (0.4, 5.0)	1.2 (0.3, 2.8)	1.1 (0.6, 6.5)	+
High-dose	6.5 (2.5, 13.2)[d]	3.8 (1.6, 24.0)	1.8 (0.7, 25.3)	+	2.1 (1.1, 4.3)	1.3 (0.4, 3.3)	1.1 (0.4, 2.9)	+
Absolute Band Neutrophils								
Control	0.0 (0.0, 0.1)	0.0 (0.0, 0.2)	0.0 (0.0, 0.0)	–	0.0 (0.0, 0.0)	0.0 (0.0, 0.1)	0.0 (0.0, 1.3)	+
High-dose	0.0 (0.0, 0.4)[d]	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	+	0.0 (0.0, 0.2)	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	–
Absolute Lymphocytes								
Control	6.3 (2.7, 12.2)	7.3 (4.1, 22.7)	1.6 (0.6, 51.6)	+	6.3 (4.0, 10.5)	3.1 (1.3, 6.9)	1.8 (0.8, 97.5)	+
High-dose	9.2 (4.4, 15.3)[d]	6.2 (2.3, 15.4)	1.4 (0.8, 307.7)	+	5.8 (3.3, 8.1)	3.1 (1.3, 5.1)	1.3 (0.7, 31.6)	+
Absolute Monocytes								
Control	0.3 (0.0, 1.0)	0.5 (0.1, 2.7)	0.2 (0.0, 1.4)	+	0.3 (0.0, 0.8)	0.1 (0.0, 0.3)	0.1 (0.0, 0.8)	+
High-dose	0.6 (0.1, 1.8)[d]	0.4 (0.0, 1.9)	0.0 (0.0, 0.4)[d]	+	0.3 (0.0, 0.9)	0.1 (0.0, 0.5)[d]	0.0 (0.0, 0.6)	+
Absolute Eosinophils								
Control	0.1 (0.0, 0.7)	0.3 (0.0, 0.9)	0.0 (0.0, 0.2)	+	0.1 (0.0, 0.5)	0.1 (0.0, 0.3)	0.0 (0.0, 0.2)	+
High-dose	0.3 (0.0, 1.3)[d]	0.3 (0.0, 0.9)	0.0 (0.0, 0.1)	+	0.1 (0.0, 0.5)	0.0 (0.0, 0.2)[d]	0.0 (0.0, 0.1)	+
Absolute Basophils								
Control	0.0 (0.0, 0.2)	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	–	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	+
High-dose	0.0 (0.0, 0.2)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	+	0.0 (0.0, 0.1)[d]	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	–
Absolute Atypical Lymphs								
Control	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.2 (0.0, 13.1)	+	0.0 (0.0, 0.0)	0.0 (0.0, 0.3)	0.3 (0.1, 20.8)	+
High-dose	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.2 (0.1, 25.3)	+	0.0 (0.0, 0.0)	0.0 (0.0, 0.1)	0.1 (0.0, 4.4)	+
Absolute Blasts								
Control	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	–	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 3.9)	–
High-dose	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 3.6)	+	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 1.2)	+

[a]n = 48 for control males and females; n = 50 for high-level males and females.
[b]n = 47 for control and high-level males; n = 45 for control females; n = 43 for high-level females.
[c]n = 14 for control males, n = 19 for high-level males; n = 22 for control females, and n = 33 for high-level males.
[d]Median significantly different from control at that time point; $p \leq 0.05$.

Histopathology

Proliferative Lesions Attributable to GMVC Exposure. Proliferative lesions include hyperplasias and neoplasias (benign and malignant). Male rats exposed to 10 g GMVC/m^3 demonstrated significant increases in the incidence of renal tubule cell adenomas (Table 3-12 and Appendices M, N, O, and P). There was also a non-statistically significant increase in renal tubule carcinomas in the males (1/50; 2% in low and 1/50; 2% in high-level males). Combining adenomas and carcinomas for statistical analysis maintained the statistically significant increase in tumors in the mid-level animals, and additionally showed a significant trend toward increased incidence with increasing exposure concentration (Table 3-12). It should also be noted that development of renal tumors is a late-life event, and it is possible that the incidence of renal tumors in males may have been higher if more than 40% of the males had survived to terminal sacrifice. These renal tumors are considered to be treatment induced based on previous studies with wholly vaporized unleaded gasoline and neat MTBE (MacFarland et al., 1984; Bird et al., 1997).

There was a high background incidence of testicular interstitial adenomas in control male rats (43/50; 86%). The incidence among high-level males (50/50; 100%) was significantly greater than the control incidence. The relationship between treatment and induction of testicular interstitial adenomas is considered to be equivocal, since background incidence of this tumor is extremely high (Boorman et al., 1990b; Gopinath et al., 1987) and historical incidence data from one 2-year bioassay conducted at LRRI for a commercial sponsor was 86% (n = 56). NTP control incidence[3] for air inhalation controls: for NIH-07 diet avg. = 70.1% with range of 46% to 90%; for NTP-2000 diet avg. = 77.2% with range of 66% to 84%). Further, the control incidence in the concurrently run Baseline Gasoline Vapor Condensate chronic study (LRRI study number FY01-027) was 48/50 (96%). It has been hypothesized that MTBE disruption of hormonal homeostasis could account for increased testicular interstitial cell adenomas (Cruzan et al., 2007). Mechanistic work to date to evaluate this hypothesis has been conducted in *in vitro* studies with high doses of MTBE; only one *in vivo* study has been reported and this study found decreases in leuteinizing hormone and testosterone in a single time point measurement. Consequently, this data should be interpreted with caution.

[3] Reference - http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=92E61F1B-F1F6-975E-7D3BED551F07DC0A

Table 3-12. Incidences of Proliferative (Neoplastic and Hyperplastic) Lesions in Rats

		Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
		MALES			
Kidney	*No. examined*	*50*	*50*	*50*	*50*
	Adenoma, renal tubule	0 (0%)	0 (0%)	6 (12%)[a]	2 (4%)
	Carcinoma, renal tubule	0 (0%)	1 (2%)	0 (0%)	1 (2%)
	Renal tubule adenoma and carcinoma, combined	0 (0%)	1 (2%)	6 (12%)[b]	3 (6%)
Nasal Sections (Note that the same tumor may appear in more than one level.)	Turbinate Level 1				
	No. examined	*50*	*50*	*50*	*50*
	Hyperplasia	3 (6%)	0 (0%)	3 (6%)	4 (8%)
	Avg. severity	0.2	0.0	0.1	0.2
	Turbinate Level 2				
	No. examined	*50*	*50*	*50*	*50*
	Hyperplasia	0 (0%)	2 (4%)	2 (4%)	3 (6%)
	Avg. severity	0.0	0.1	0.1	0.2
	Carcinoma, squamous cell	1 (2%)	0 (0%)	0 (0%)	0 (0%)
	Turbinate Level 3				
	No. examined	*50*	*50*	*50*	*50*
	Carcinoma, squamous cell	0 (0%)	1 (2%)	0 (0%)	1 (2%)
	Turbinate Level 4				
	No. examined	*50*	*50*	*50*	*50*
	Carcinoma, squamous cell	0 (0%)	2 (4%)	0 (0%)	2 (4%)
Thyroid	*No. examined*	*50*	*35*	*31*	*50*
	Hyperplasia, follicular cell	0 (0%)	0 (0%)	1 (3%)	0 (0%)
	Avg. severity	0.0	0.0	0.1	0.0
	Adenoma, follicular cell	2 (4%)	0 (0%)	0 (0%)	3 (6%)
	Carcinoma, follicular cell	1 (2%)	0 (0%)	1 (3%)	3 (6%)
	Follicular cell adenoma and carcinoma, combined	3 (6%)	0 (0%)	1 (3%)	5 (10%)
Testes	*No. examined*	*50*	*50*	*50*	*50*
	Adenoma, interstitial cell[c]	43 (86%)	47 (94%)	48 (96%)	50 (100%)[d]
	Mesothelioma	0 (0%)	1 (2%)	1 (2%)	2 (4%)
Spleen	*No. examined*	*50*	*39*	*38*	*50*
	Leukemia, mononuclear[e]	27 (54%)	31 (79%)[d]	31 (82%)[d]	31 (62%)

Table 3-12. Incidences of Proliferative (Neoplastic and Hyperplastic) Lesions in Rats (Concluded)

		Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
		FEMALES			
Spleen	*No. examined*	*50*	*22*	*24*	*50*
	Leukemia, mononuclear	27 (54%)	12 (55%)	11 (46%)	23 (46%)
Mammary Gland	*No. examined*	*49*	*20*	*22*	*47*
	Fibroadenoma[e]	0 (0%)	3 (15%)[d]	2 (9%)	5 (11%)[d]

[a]Incidence is significantly different from controls and from the 2 g/m^3 group (Fisher's test).
[b]Incidence is significantly different from controls (Fisher's test). A positive trend toward increased incidence with increasing dose was found with the Cochran-Armitage test.
[c]A positive trend with increasing dose was found with the Cochran-Armitage and Logistic tests. A trend was also detected with Fisher's test with the incidence among the 20 g/m^3 group being significantly increased compared to the control incidence.
[d]Incidence significantly different from controls (Fisher's test).
[e]Trend detected with Fisher's Exact test.

Squamous cell carcinoma occurred in nasal sections with an animal incidence of 1/50 (2%), 2/50 (4%), 0/50 (0%) and 2/50 (4%) for control, low-, mid- and high-dose animals respectively with no statistically significant differences or trends. It was not appropriate to combine carcinoma incidence of nasal turbinate levels for statistical analysis in the low- and high-dose groups because the lesion spanned two levels for one of the two animals. By comparison, in the concurrent Baseline Gasoline Vapor Condensate study, the incidence of squamous cell carcinoma in the nasal pasages (turbinate levels 2–4) was elevated in high-dose males, with tumor incidences of 1/50, 3/50, 3/50 for levels 2, 3, and 4, respectively. The incidences in turbiante level 3 were statistically significantly increased with exposure concentration (Cochran-Armitae test).

There is an increased incidence (see Table 3-12) of thyroid follicular cell adenoma and carcinoma male rats with combined incidences of 3/50 (6%), 0/35 (0%), 1/31 (3%) and 5/50 (10%) for control, low-, mid- and high-level groups, respectively. However, the incidences of adenomas, carcinomas and the combined incidences of adenomas and carcinomas was not found to be statistically significant. In the concurrent BGVC study, there was a statistically increased

incidence of thyroid follicular cell carcinomas in males. However the combined incidences of follicular cell adenomas and carcionomas were not statistically inceased in that study.

Proliferative Lesions Not Attributable to GMVC Exposure. Leukemia, a well-documented feature of old age in F344 rats, did not appear to be enhanced by GMVC exposure. Results for spleen, the defining organ for this disease, are shown for males and females in Table 3-12. In males, the incidences of leukemia in the low- and mid-GMVC levels are greater than controls (Fisher's test); however, the diagnoses were made on animals where gross lesions in the spleen were present, and therefore some sampling bias was involved. There was no overall trend with exposure concentration as determined by the Cochran-Armitage and logistic tests, and the incidence of leukemia in high-level males was not different from controls (Fisher's test). The incidences of leukemia in GMVC-exposed females were not different from the incidence among control females.

Mammary gland fibroadenoma was significantly increased in low- and high-level females (Table 3-12). Interpretation of this finding is problematic due to the atypically low control incidence of 0% in control females. Control incidences for this lesion have been reported to range from 27–40% (Boorman et al., 1990c). The control incidence in the parallel BGVC study (LRRI study number FY01-027) was 4/49 (8%). Additionally, increases in this lesion have not been observed in previous studies with neat MTBE or wholly vaporized gasoline (Cruzan et al., 2007; MacFarland et al., 1984). Therefore, it is unlikely that the increased incidence of female mammary gland fibroadenoma was treatment-related.

Nonproliferative Lesions Attributable to GMVC Exposure. Nonproliferative lesions included degenerative and inflammatory changes. Only lesions showing a positive trend with exposure concentration and lesions in kidney, an expected target tissue are discussed here.

Chronic progressive nephropathy is a lesion of particular interest to this study, as this lesion is expected to occur in rats, especially male rats exposed to hydrocarbon mixtures. In this study, the incidence of chronic progressive nephropathy among control male and female rats was 44/59 (88%) in males and 42/50 (84%) in females (Table 3-13 and Appendices M, N, O, and P). The incidences among male and female rats inhaling GMVC were not significantly increased over

the incidence in the respective controls. However, the severity of this lesion was significantly increased in mid- and high-level males and in high-level females, compared to the corresponding control values. This finding in males is consistent with male-rat-specific light hydrocarbon-induced alpha-2u globulin overload nephropathy that has been reported previously in studies with wholly vaporized unleaded gasoline and neat MTBE (MacFarland et al., 1984; Bird et al., 1997). It should be noted that in F344 male rats, after approximately 1 year of age, alpha-2u globulin overload nephropathy is essentially masked by age-related chronic progressive nephropathy, since both forms of nephropathy share similar if not identical diagnostic hallmarks. Therefore. the effect of alpha-2u globulin overload nephropathy typically manifests itself in increased severity, rather than incidence, since essentially all males surviving past 1 year of age will develop CPN. The increased severity of nephropathy in females at the highest dose must be attributed to a different, likely non-specific mechanism since alpha-2u globulin overload is male rat specific.

Table 3-13. Nonproliferative (Non-Neoplastic) Lesion Incidences and Severity Scores

		Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
		Males			
Kidney	*No. examined*	*50*	*50*	*50*	*50*
	Nephropathy, chronic	44 (88%)[a]	47 (94%)	50 (100%)	46 (92%)
	Avg. severity	1.9	2.1	2.6[b]	2.7[b]
Nose/Turbinate 2	*No. examined*	*50*	*50*	*50*	*50*
	Degeneration, hyaline - olfactory epithelium	0 (0%)	5 (10%)	7[c] (14%)	6[c] (12%)
	Average severity	0.0	0.2	0.2	0.2
Nose/Turbinate 3	*No. examined*	*50*	*50*	*50*	*50*
	Degeneration, hyaline - olfactory epithelium	2 (4%)	1 (2%)	7 (14%)	7 (14%)[d]
	Average severity	0	0	0.2	0.3
Nose/Turbinate 4	*No. examined*	*50*	*50*	*50*	*50*
	Degeneration, hyaline - olfactory epithelium	0	0	1 (2%)	1 (2%)
	Average severity	0	0	0	0

Table 3-13. Nonproliferative (Non-Neoplastic) Lesion Incidences and Severity Scores (Concluded)

		Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
		Females			
Kidney	*No. examined*	*50*	*20*	*21*	*50*
	Nephropathy, chronic	42 (84%)	17 (85%)	15 (71%)	43 (86%)
	Average severity	1.0	1.0	1.0	1.5[b]
Nose/Turbinate 2	*No. examined*	*50*	*50*	*50*	*50*
	Degeneration, hyaline - respiratory epithelium	0 (0%)	1 (2%)	7[c] (14%)	6[c] (12%)[d]
	Average severity	0.0	0.0	0.3	0.2

[a]Percentage affected is in parentheses adjacent to incidence.
[b]Significantly higher than controls at 0.05 level using Kolmogorov-Smirnov one-tailed test.
[c]Significantly different from controls at 0.05 level using Fisher's exact two-tailed test.
[d]Positive concentration-dependent trend by Cochran-Armitage and logistic tests. p = 0.054 by Fisher's exact (no group comparisons made).

The only other nonproliferative lesion attributable to GMVC inhalation is hyaline degeneration in the nasal epithelium, with significant changes in the mid- and high-level GMVC animals of both sexes. In males, this lesion occurred in the olfactory epithelium of turbinates at levels 2, 3, and 4, while in females, the lesion occurred in the respiratory epithelium of turbinates at level 2. The severity scores for both lesions were quite low, ranging from 0.0 to 0.3 on a scale of 0 to 4.

CONCLUSIONS

The overall purpose of the current study was to evaluate the long-term toxicity, including carcinogenicity, of MTBE inhaled as a component of unleaded gasoline vapor for 2 years.

In this study, rats were exposed to 0, 2, 10, and 20 g GMVC/m^3. The GMVC mixture contained 20% MTBE by mass, so the corresponding MTBE concentrations were 0, 0.4, 2, and 4 g/m^3. Body weights were affected in males and to a greater extent in females in a concentration-dependent manner, but survival rates were not affected by GMVC exposure in either sex. Exposure to GMVC did not adversely affect WBC parameters. Effects seen at 12 months compared to at the 18- and 24-month sampling times were more likely due to difficulties in

blood sampling than to GMVC inhalation. Chronic exposure to GMVC increased the severity of chronic progressive nephropathy in mid- and high- level males and high-level females and caused epithelial (females) and olfactory (males) degeneration in the nasal passages of both sexes. The degenerative nasal effects were most likely caused by toxicant effects of the test material (Boorman et al., 1990d; Gopinath et al., 1987).

Exposure to GMVC resulted in an increase in renal tubule adenomas and combined adenomas and carcinomas in males exposed to GMVC. Testicular interstitial cell (Leydig cell) adenomas showed an increasing trend with exposure concentration, with the incidence in the high-level GMVC group (50/50; 100%) significantly greater than controls (43/50; 86%). The relationship of the testicular interstitial cell adenomas with treatment is less clear due to the high background incidence of this lesion in aged male F344 rats. However, GMVC induction of testicular interstitial cell adenomas in the 20 g/m^3 group cannot be ruled out, particularly in light of similar equivocal observations reported in previous studies with neat MTBE (Cruzan et al., 2007). Chronic exposure to GMVC did not enhance the development of proliferative lesions (hyperplastic lesions, neoplasms) in female rats. Consequently, due to treatment-related increases in renal adenomas and combined andenomas and carcinomas in males, chronic inhalation of Gasoline MTBE Vapor Condensate was determined to be carcinogenic in male rats in this study. Gasoline MTBE Vapor Condensate was determined not to be carcinogenic in female rats in this study.

COMPARATIVE CHRONIC TOXICITY AND CARCINOGENICITY BETWEEN GMVC AND BGVC

Background

The current study on GMVC was conducted in parallel with chronic study on BGVC. Both studies were conducted in compliance with the Clean Air Act Section 211(b) for fuel and fuel additive registration. The 211b program was designed to evaluate the comparative toxicity of evaporative emissions of unleaded gasoline with and without oxygenates, such as MTBE. A comparison of the components of the two test materials is presented in Table 3-14. Below, results from the two studies are compared to determine if addition of MTBE affected the chronic toxicity of BGVC alone.

Table 3-14. Analytical Comparison of Test Materials

Compound	BGVC Lot # API 99-01[a] (area percent)	GMVC Lot # API 00-02 (area percent)
Isobutane	3.6	2.2
n-Butane	15.2	11.1
Isopentane	35.1	31.0
n-Pentane	13.2	9.1
Trans-2-pentene	2.5	2.0
2-Methyl-2-butene	3.8	2.9
MTBE	--	21.3
2,3-Dimethylbutane	1.6	0.9
2-Methylpentane	6.3	4.5
3-Methylpentane	3.6	2.6
n-Hexane	3.0	2.1
Methylcyclopentane	1.5	1.1
2,4-Dimethylpentane	1.0	0.9
Benzene	2.1	1.5
2-Methylhexane	1.1	1.0
2,3-Dimethylpentane	1.1	1.0
3-Methylhexane	1.3	1.1
Isooctane	1.3	1.2
Toluene	3.0	2.5

[a]A second lot of BGVC test material was used for the last 10 - 11 weeks of the BGVC chronic study. See Section 2.1 of the BGVC Chronic Study Report for a comparison of the two lots of BGVC test material.

Methyl tertiary-butyl ether (MTBE) has been used as an octane enhancing additive for gasoline and as part of the oxygenated fuels program to reduce carbon monoxide in automotive emissions. Concerns regarding the carcinogenicity of MTBE have been raised. In previous chronic inhalation studies, F344 rats were exposed to 0, 400 (~1.4 g/m^3), 3000 (~10.8 g/m^3), and 8000 (~28.8 g/m^3) ppm MTBE (Bird et al., 1997; Mennear, 1997). An increased incidence of renal tubular adenoma and/or carcinoma was observed in males exposed to 8000 ppm (28.8 g/m^3), and an increased incidence in testicular tumors was observed among rats exposed to 3000 and 8000 ppm (10.8–28.8 g/m^3). Belpoggi et al. (1995) reported that 1000 mg MTBE/kg administered by gavage over a 2-year period increased the incidences of Leydig cell (testicular) tumors in males and lymphoma and leukemia (combined) in females.

Results – General

The effects of treatment on survival (none), clinical observations (none), body weights (decreases), organ weights (few), organ:body weight ratios (increases likely to be an artifact of decreased body weight), organ:brain weight ratios (few and not dose dependent), and hematology (changes at 12 months attributable to blood collection technical difficulties) were comparable between the two studies.

Results – Proliferative Lesions

The male rat incidences of proliferative lesions are compared in Table 3-15 to help assess the role of MTBE in development of these findings. In both GMVC- and BGVC-exposed males, the incidence of renal tubule adenomas or combined incidences of adenomas and carcinomas peaks at the 10 g vapor/m^3 level and was very similar for the two studies. At the 20 g vapor/m^3 level, the incidence of adenomas and combined adenomas and carcinomas is higher in the GMVC study.

In testes, there was a high, not unexpected, incidence of adenomas in males in all dose groups (86%–100% for the GMVC study and 94%–100% for the BGVC study). Inhalation of BGVC did not significantly increase the numbers of tumors above control values (48%). However, the incidence of adenomas in the high exposure level GMVC males was significantly increased compared to controls, but this is likely attributable to the low incidence among controls in that study (43%). Malignant testicular mesothelioma was observed in both studies, but a significant positive trend was only observed in the BGVC study, and only by the Fisher's test.

Nasal squamous cell carcinoma (turbinate levels 2–4 combined taking into account tumors spanning two or more levels) was observed in the low- and high-level groups in both studies. Incidences were generally similar; however the incidence in the high BGVC exposure group was greater than in the high GMVC exposure group.

Similar to the renal tumor findings, thyroid follicular cell adenomas and carcinomas were observed in both studies. However, there was a significant trend toward increase incidence of

thyroid follicular carcinomas only in the BGVC study. And in this case, group comparisons showed no significant increases among the individual groups.

Table 3-15. Comparison of Incidences of Renal, Nasal Turbinate, Testicular, and Thyroid Tumors in Male Rats Inhaling GMVC and BGVC

Tissue	Diagnosis	Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
		GMVC			
Kidney	*No. examined*	*50*	*50*	*50*	*50*
	Adenoma, renal tubule	0 (0%)	0 (0%)	6[a] (12%)	2 (4%)
	Carcinoma, renal tubule	0 (0%)	1 (2%)	0 (0%)	1 (2%)
	Renal tubule adenoma and carcinoma, combined	0 (0%)	1 (2%)	6[b] (12%)	3 (6%)[b]
Nasal Turbinates	*No. examined*	*50*	*50*	*50*	*50*
	Carcinoma, squamous cell, animal incidence (any level)	1 (2%)	2 (4%)	0 (0%)	2 (4%)
Testes	*No. examined*	*50*	*50*	*50*	*50*
	Adenoma, interstitial cell[c]	43 (86%)	47 (94%)	48 (96%)	50[d] (100%)
	Mesothelioma, malignant	0 (0%)	1 (2%)	1 (2%)	2 (4%)
Thyroid	*No. examined*	*50*	*35*	*31*	*50*
	Adenoma, follicular cell	2 (4%)	0 (0%)	0 (0%)	3 (6%)
	Carcinoma, follicular cell	1 (2%)	0 (0%)	1 (3%)	3 (6%)
	Follicular cell adenoma and carcinoma, combined	3 (6%)	0 (0%)	1 (3%)	5 (10%)
		BGVC			
Kidney	*No. examined*	*50*	*50*	*50*	*50*
	Adenoma, renal tubule	1 (2%)	1 (2%)	4 (8%)	0 (0%)
	Carcinoma, renal tubule	0 (0%)	0 (0%)	3 (6%)	0 (0%)
	Renal tubule adenoma and carcinoma, combined[e]	1 (2%)	1 (2%)	7 (14%)	0 (0%)
Nasal Turbinates	*No. examined*	*50*	*50*	*49-50*[f]	*50*
	Carcinoma, squamous cell, animal incidence (any level)[g]	0 (0%)	1 (2%)	0 (0%)	3 (6%)
Testes	*No. examined*	*50*	*49*	*50*	*50*
	Adenoma, interstitial cell	48 (96%)	46 (94%)	50 (100%)	49 (98%)
	Mesothelioma, malignant[h]	0 (0%)	0 (0%)	4 (8%)	0 (0%)

Table 3-15. Comparison of Incidences of Renal, Nasal Turbinate, Testicular, and Thyroid Tumors in Male Rats Inhaling GMVC and BGVC (Concluded)

Tissue	Diagnosis	Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
	BGVC (Concluded)				
Thyroid	*No. examined*	*50*	*29*	*27*	*50*
	Adenoma, follicular cell	0 (0%)	2 (7%)	0 (0%)	2 (4%)
	Carcinoma, follicular cell[i]	0 (0%)	0 (0%)	2 (7%)	0 (0%)
	Follicular cell adenoma and carcinoma, combined[j]	0 (0%)	2 (7%)	2 (7%)	2 (4%)

[a]Incidence is significantly different from controls and from the 2 g/m^3 group (Fisher's test).
[b]Incidence is significantly different from controls (Fisher's test). A positive trend toward increased incidence with increasing dose was found with the Cochran-Armitage test.
[c]A positive trend with increasing dose was found with the Cochran-Armitage and Logistic tests. A trend was also detected with Fisher's test with the incidence among the 20 g/m^3 group being significantly increased compared to the control incidence.
[d]Incidence significantly different from controls (Fisher's test).
[e]A positive trend was found with the Fisher's exact test, but pair-wise comparisons are not significant ($p > 0.008$).
[f]Forty nine for turbinates 3 and 4; fifty for turbinate 2.
[g]A trend toward increased incidence with exposure level found for nasal turbinate level 3 only by the Cochran Armitage test.
[h]Significant trend for increased incidence by FE test ($p = 0.014$), although pairwise comparisons are not significant ($p > 0.008$).
[i]Incidences are different than expected by chance ($p = 0.029$, overall FE test), although pair-wise comparisons are not significant ($p > 0.008$, FE tests).
[j]Incidences are not different than expected by chance ($p = 0.14$, overall FE test).

The incidences of leukemias in both studies were high, but incidences did not increase with vapor exposure concentration in either study (Table 3-16). In the cases where incidences in the low- and mid-vapor level groups are significantly increased compared to controls, it is possible that some sampling bias occurred because for these exposure groups, only tissues with gross abnormalities were examined histologically. Based on the incidences of leukemias in the GMVC and BGVC studies, it does not appear that MTBE-containing unleaded fuel vapors increased the incidence of leukemias over the already high incidence found in aged rats.

Table 3-16. Comparison of Leukemia Incidences in Spleens of Rats inhaling GMVC and BGVC

	Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
		GMVC		
Males	27/50 (54%)	31/39 (79%)[a]	31/38 (82%)[a]	31/50 (62%)
Females	27/50 (54%)	12/22 (55%)	11/24 (46%)	23/50 (46%)
		BGVC		
Males	32/50 (64%)	23/34 (68%)	25/38 (66%)	32/50 (64%)
Females[b]	13/50 (26%)	14/25 (56%)	18/32 (56%)	15/50 (30%)

[a]Significantly different from controls, Fisher's test; $p \leq 0.05$.
[b]Incidences are different than *expected by chance* ($p = 0.007$, overall FE test), although pair-wise comparisons are not significant ($p > 0.008$, FE tests).

Overall, it is difficult to draw strong conclusions about the role of MTBE in inducing proliferative lesions, especially in regard to inducing renal or testicular tumors in male rats. The predictive value of renal tumors and testicular interstitial cell adenomas for humans has also been questioned (Baetcke et al., 1991; Hard, 1998; Hard and Khan, 2004; Cook et al., 1999; Prentice and Meikle, 1995).

Non-Proliferative Lesions

The incidence of chronic progressive nephropathy was high among control males and females in both studies and not significantly increased with GMVC or BGVC exposure (Table 3-17). The severity of male chronic progressive nephropathy was significantly increased in mid- and high-level males inhaling GMVC and BGVC. The increased severity of chronic progressive nephropathy in high-level GMVC females is likely due to non-specific protein accumulation rather than treatment.

Table 3-17. Comparison of Chronic Progressive Nephropathy in Rats Inhaling GMVC and BGVC for 2 Years

	Control (0 g/m^3)	Low (2 g/m^3)	Mid (10 g/m^3)	High (20 g/m^3)
		GMVC		
Males				
Incidence	44/50 (88%)[a]	47/50 (94%)	50/50 (100%)	46/50 (92%)
Avg. severity	1.9	2.1	2.6[b]	2.7[b]
Females				
Incidence	42/50 (84%)	17/20 (85%)	15/21 (71%)	43/50 (86%)
Avg. severity	1.0	1.0	1.0	1.5[b]
		BGVC		
Males				
Incidence	49/50 (98%)	49/50 (98%)	50/50 (100%)	50/50 (100%)
Avg. severity	2.4	2.5	2.9[c]	2.9[c]
Females				
Incidence	27/50 (54%)	12/24 (50%)	16/25 (64%)	32/50 (64%)
Avg. severity	0.7	1.0	0.9	0.9

[a]Percentage affected is in parentheses adjacent to incidence.
[b]Significantly higher than controls at 0.05 level using Kolmogorov-Smirnov one-tailed test.
[c]Overall severity/incidence significantly higher than control at 0.05 level using Kolmogorov-Smirnov one-tailed test.

For both GMVC- and BGVC-treated male rats, the increased incidence of renal tumors and increased severity is likely due in part to male rat specific alpha-2u globulin accumulation and overload in the kidney during the first year of life. Other mechanisms may also contribute, as evidenced by increased severity of nephropathy in high-level GMVC females, lacking renal adenomas and carcinomas.

The respiratory epithelial degeneration of the respiratory and olfactory epithelium is a common, nonspecific finding in aged rats (Boorman et al., 1990d; Gopinath et al., 1987). Mid- and high-level males and females exposed to GMVC had an increased incidence of these lesions in nasal turbinate 2. High-level males also had an increased incidence of degeneration in turbinate 3. The incidences of this lesion were also significantly increased among male and female rats exposed to the mid and high levels of BGVC. Interestingly, the respiratory epithelial

degeneration in BGVC-exposed male and female rats extended to turbinate level 3, so it was more extensive than that caused by GMVC. Therefore, the increased incidence of the epithelial degeneration is not attributable to MTBE, but more likely due to the irritant properties of the fuel vapor mixture.

In summary, we have conducted a side by side comparison of the toxicity and carcinogenicity of Baseline Gasoline Vapor Condensate and Condensate containing approximately 20% MTBE. The inhalation exposure systems were identical. Animals were obtained from the same source, were the same age, and housed under identical conditions. The health effects resulting in F344 rats following 2 years of GMVC or BGVC inhalation were unequivocally comparable in the production of renal adenomas and carcinomas in male rats. Methyl tertiary butyl ether was not identified as enhancing the production of these renal adenomas or adenomas and carcinomas compared to those induced by gasoline vapor condensate alone.

LOCATION OF SPECIMENS, RAW DATA, AND FINAL REPORT

Specimens were identified by test system, study, nature, and date of collection. All raw data and records and specimens that are required to reconstruct the study will be maintained in the LRRI archives for 10 years. The Sponsor will be notified and will authorize in writing the destruction or transfer of any specimens, raw data, and study records to the Sponsor or to an archive specified by the Sponsor's Contracting Representative.

PROTOCOL DEVIATIONS

Delayed Sponsor Signature on Amendment 1

Amendment 1 to the study protocol was signed by the Study Director on August 22, 2001 and submitted to the Sponsor for signature. A signature was not obtained after many requests. The operative LRRI SOP at the time stated, "The study director signs and dates the amendment prior to implementing the changes and the dated signature of the Sponsor is obtained as soon as reasonably possible." Good Laboratory Practice guidelines do not require Sponsor signature. Thereafter, LRRI no longer sought Sponsor signature on Amendment 1 and considered it approved on August 22, 2001.

Personnel

On page 3 of the study protocol, key personnel were listed as Quint H. Powell, MS, as Inhalation Engineer, Thomas H. March, DVM, PhD, DACVP, as Veterinary and Clinical Pathologist, David G. Burt, DVM, DACLAM, as Laboratory Animal Veterinarian, and Justin Kubatko, MS, Statistician. During the study, Mr. Powell left the Institute and was replaced by Mr. Edward B. Barr, MSEE, an inhalation engineer with over 25 years experience at Lovelace. Dr. Burt left the Institute and was replaced by Roger van Andel, DVM, PhD, DACLAM. Mr. Kubatko also left the Institute. Betty Skipper, PhD, University of New Mexico, was subcontracted to perform the statistics. Dr. Thomas March took over responsibilities for the Baseline Gasoline Vapor Condensate carcinogenicity study pathology and was replaced on this study by Dr. Andrew Gigiliotti, DVM, PhD, DACVP.

There was no impact on the quality of the study as the above listed personnel were replaced by individuals of equal or greater experience.

Calculation of Nominal Concentration

Section IX.C.6. states, "Nominal concentration will be determined daily and will reflect the concentration generated for all three exposure systems combined. Therefore, the total mass of test substance generated will be divided by the total flow distributed to all three exposure chambers. This value will be compared to the sum of the mean concentrations achieved in the three chambers throughout that exposure day."

The actual and nominal (anticipated) usage were calculated as follows:

Daily nominal or "anticipated" usage was calculated by multiplying the average vapor condensate concentration in each chamber (low, mid, high; g/m^3) by the total flow through each respective chamber ([L/min * min/1000L/m^3]) and then summing the values for all three chambers. This value was compared to the actual BGVC usage determined by taking the difference between the weight of the 20-pound cylinder before and after the exposure.

There was no adverse impact on the study. The way nominal concentration was calculated and compared to actual gasoline MTBE vapor condensate usage provides the same answer as the method described in the protocol.

Statistics

Proposed statistical methods were presented in the protocol. In several cases the actual evaluations differed from those originally proposed. This occurred in three specific cases. First, the Hoel Walburg test was not used to analyze tumor data although originally listed as a possible statistic in the protocol. Second, hematology results were analyzed as a function of time as well as a function of dose. Finally, in the case of chronic progressive nephropathy, where the incidence of lesions was similar among control and dosed groups, the Kolmogorov-Smirnov test was used to assess differences in severity scores. This evaluation was done within the P module of the Path-Tox® software, and not by the Study Statistician.

The actual statistics used have been described in the Statistics section of the report. The fact that the statistics proposed in the protocol were not always those used for data analysis has no major impact on the quality of the study. In most cases the analysis were more thorough and appropriate than those originally proposed. Since GMVC inhalation did not affect animal survival, the Hoel Walburg analysis, that assumes that treatment affects animal survival, was not necessary. Since the hematology data did not have normal distributions, alternative statistical approaches were warranted. Since repeated measurements were made on the same rats over time, evaluation of the effect of time and exposure concentration on the hematology parameters was appropriate. The use of the Kolmogorov-Smirnov statistic in the Path-Tox® database was appropriate because nephropathy was an important endpoint in this study. Statistical analysis of the severity of the lesion as a function of exposure concentration in the absence of a concentration-response effect on lesion incidence provided more information than would have been gained by analysis of incidence alone.

Analysis of Tank 16 (420 pound tank)

The protocol required a gas chromatographic profile to be performed on all 420 pound tanks prior to use. Tank 16 was inadvertently not analyzed prior to being used for exposures. However, profiles of the exposure atmosphere were determined when Tank 16 was used May 17, 2001 through June 28, 2001. Therefore these data are included in Appendix B to the report. Because profiles were obtained on the tank during its use, and there was no indication that the hydrocarbon composition of this tank was different from that of the other tanks, there is no significant impact on the outcome of the study.

Analysis of Exposure Atmosphere Profiles

During the weeks of July 23, 2001 and September 10, 2001, the gas chromatograph used to qualitatively analyze the hydrocarbon profiles of the exposure atmospheres was not working properly. Therefore profiles were not obtained during these weeks. The technician responsible for the analyses worked with the Lovelace electrician and the instrument vendor to troubleshoot and resolve the problems. Profiles were obtained on previous and subsequent weeks. Since hydrocarbon profiles in the exposure atmospheres were relatively stable for these lots of fuel, the

loss of the chamber atmosphere profile data during the 2 weeks of the 104-week study did not significantly affect the outcome of the study or interpretation of results.

Exhaust System Failure During Conditioning/Quarantine Period Affecting Chamber Pressure

On August 2, 2001, a subcontractor was at the Institute to repair a motor that had burned out on the backup exhaust system for the exposure wing. While here, he was asked to change the filters on the main system. To do this, he had to temporarily shut off the main blower. After the filters had been changed, the blower motor could not be restarted. This occurred about 12:30 p.m. The Study Director, Dr. Benson and LRRI Animal Care Operations were notified because the study animals were being quarantined in chambers in that wing. Since air was still being supplied to the chambers, the corks in the doors of the chamber were taken out to allow air flow. During this time the pressure in the chambers changed from negative (approximately -1 inch of water) to positive (about 1 inch of water). The maintenance crew attempted to restart the motor all afternoon. At 4:00 p.m., the exhaust system was still not working, so the exhaust lines were totally disconnected from the chambers housing the study animals. All environmental parameters continued to be monitored by computer as before the exhaust system went down. Surveillance personnel also checked the conditions in the room twice per shift and were directed to open the chamber doors in the event that temperatures exceeded 24°C. A new motor was delivered and installed early August 3, 2001. The exhaust system was up and running by 8:30 a.m.

The impact to the study was small because animals had air, and were monitored by personnel throughout the time the exhaust system was down.

Loss of Supply Air to Exposure System

On November 27, 2001, at approximately 10:30 a.m., supply airflow to the animal chambers dropped, resulting in low exposure chamber flows and pressures. Surveillance personnel were summoned to the room. They monitored the room closely and made certain Facility Engineeering and Exposure personnel were aware of the situation. It was detemined that the flows and pressures were in a range that would not adversely affect the health of the animals and therefore the animals remained within the chambers during the night.

During this time, chamber pressures did not exceed -3" water (within normal range of LRRI SOP). Chamber flows did not drop below 350 lpm (10 changes per hour), above the minimum stated in EPA OPPTS 870.4200 guidelines, but below those specified in the study protocol. Chamber temperatures remained within protocol-defined limits.

On the following morning, November 28, 2001, the Study Engineer and Facility Engineering personnel examined the problem. Flows to the chamber were adjusted to within normal operating parameters. Facility Engineering personnel examined the supply air blower and could not find anything wrong with the supply air system.

At this time, the Study Engineer determined that it was ok to begin exposures. Approximately 30 minutes into the exposure, Facility Engineering discovered that the chamber supply air blower was not working properly. Exposures were terminated after 40 minutes of exposure were completed so that repairs could be made. Exposure concentrations achieved during the 40 minute run were 1.53 g/m^3, 7.88 g/m^3 and 13.62 g/m^3 for the 2, 10 and 20 g/m^3 chambers, respectively. Flow through the chambers was maintained above 10 air changes per hour and chamber temperatures were withn limits while the blower was being repaired. The environment in the exposure chamber was safe for animal occupancy at all times. Repairs to the blower were completed aby 12:00 p.m. Exposures were not continued that day. One exposure day was missed but this was not considered significant because there were over 500 exposure days during the study.

Environmental Conditions

Throughout the gasoline MTBE vapor condensate exposure study, environmental conditions in the animal exposure chambers deviated from protocol required ranges, or data were not acquired for short periods because of problems with the monitoring system. Most excursions of temperature and pressure or periods of data loss were of short duration (~2% per month). These deviations were judged to have no significant effect on the outcome of the study. Tables summarizing deviations from protocol-specified environmental conditions are included in the Study File.

On a few occasions, environmental data were not acquired due to equipment failure or because monitoring devices were not plugged in. These instances are listed below.

On November 23, 2001 RH sensors were not plugged into the chamber from 6:59–14:29. Any data acquired were listed as erroneous and not included in the data summary for that day.

The Relative Humidity meter on chamber 6 (2 mg/m^3) came loose and data were missing for all or parts of May 24–May 28, 2002. Apparently the sensor became disconnected on Friday (possibly after the chambers were serviced) and this was not noticed until the following Tuesday). Part of the reason for the delay in detecting this was that RH was not set to alarm when out of the acceptable range and that the disconnect occurred over a weekend, when exposure personnel were not on duty.

A similar occurrence happened July 11–15, 2002, where RH data were not recorded.

Temperature, airflow and relative humidity were not recorded for approximately 0.5–1 hour on August 30, September 6, and September 12, 2002. This was likely due to the fact that the computer monitoring system was shut down and rebooted to shorten data backup time. The data would have been logged when the computer was restarted and monitoring resumed, but since the data were not necessarily logged on the half hour, they were not included in the data summaries.

From August 31–September 3, 2002, humidity data for chamber 5 were not recorded because a sensor wire was loose and not repaired.

The impact of the data loss is not great. The RH in the remaining chambers was recorded. The RH in the missed chambers may be inferred from the data acquired in the other three.

Temporary Loss of Environmental Monitoring System Operation

On May 20, 2003 a major power outage occurred at 12:20 a.m. Normally, in power outage events, emergency backup diesel generators are turned on within 10–15 seconds. During this particular outage, a breaker was tripped and emergency power was not provided to the environmental monitoring system and the system was on uninterrupted power supply battery power until they were drained. During this outage, the supply air fan stopped operating, but due

to the lack of environmental monitoring capability, this was not detected until morning. All systems were restored by approximately 6:30 a.m. The animals appeared healthy. Estimated flow rates through the chamber were approximately 200–300 L/minute. While supply air was not available, the vacuum system was still operational and pulled air from the room into the chambers. Several actions, described in a memo to the study file, were taken to prevent similar situations in the future.

On August 28, 2002 (14:29) to August 29, 2002 (06:29) no environmental data were acquired or saved to file. This was because on August 28, 2002, the LRRI network domain configuration modifications were performed on the monitoring PC in the study control room. The software program controlling and recording environmental data was shut down and inadvertently not restarted.

Light and Sound Measurements

According to the study protocol, light and sound measurements in the animal exposure room were to be measured quarterly. In 2001, measurements were not made for the July–September quarter. In 2002, no measurement was made between January and March. In 2003, no measurement was made between July and the end of the study in August. The impact on the outcome of the study is considered minimal because light and sound levels were similar among locations and did not vary greatly between measurements that were made.

Gas Chromatographic Analysis

The original version of the SOP ASP-1166.0, Calibration of the Shimadzu GC-17A/FID for Analysis of Gasoline Vapor Condensate, indicated GC calibration for monitoring the exposure atmosphere and test article hydrocarbon composition profile may be calibrated daily. However, calibrations were performed only on the days when analyses were performed. The SOP was modified and signed by management on September 25, 2002, changing the requirement for calibration to only days when analyses are performed. The impact of this SOP deviation is minimal because the GC was calibrated on days when analyses were performed.

Body Weights and Observations Not According to Protocol Defined Schedule

Body weights were performed at erratic intervals. Body weights were generally performed every fourth week on the same day of the week (the day that chambers are not changed in the exposure room). The body weights obtained between 9/1/02 and 10/14/02 were not performed at exactly weekly intervals. This was because we were investigating the feasibility of going to a weekend weigh schedule. This would allow study personnel to perform other tasks with the animals on the day chambers are not changed, such as tattoo touch ups after exposure. In trying the new schedule, weights were collected 3–4 days off from the previous schedule. This had minimal effect on the study, since the rats were weighed only a few days later than "normal" and all groups were weighed on the same day, so statistical comparisons were still valid.

The same explanation holds true for the associated observations. The impact was minimal. The observations were still made, but 3–4 days later than the previous schedule.

Cage Board Usage/Availability

In December 2002, we ran out of untreated cage board used to line the excreta pans in the H2000 chambers. Although additional cage board was ordered, it was on "back order" and was estimated to arrive December 20, 2002. Until we received permission from the Sponsor to switch to treated (with neomycin) cage board, no cage board was used and pans were washed twice daily instead of the usual once per week. Dr. Benson contacted the Sponsor and received permission to use treated cage board on December 13, 2002. Use of untreated cage board continued when it arrived.

There should be no adverse effect on the study associated with not using cage board (with twice daily pan washes to keep ammonia levels down) or associated with use of neomycin-treated cage board. The latter type is used throughout the institute for short-term and long-term inhalation studies.

Animal Tattoo Requirements for Identification

During the period between April and August 2002, several animals were repeatedly noted as needing tattoo touch ups to aid in identification and fulfill SOP requirements. Tattoos were

reapplied or touched up from August 2002 on and attempts were made to tattoo more frequently. In addition, new equipment was purchased so that tattoos applied were more vivid and lasted longer. There was no adverse effect.

Animal Misidentification

Animal S14 (a sentinel in the control chamber) was designated as moribund and authorized for euthanasia to be conducted on December 20, 2002. A different moribund animal, F514 was incorrectly brought to necropsy, where the staff failed to verify the tattoo number. F514 underwent a complete necropsy that day. The error was noted and S14 was euthanized and underwent necropsy on December 24, 2002. The affected Individual Animal Necropsy Record forms were corrected and error coded. Removal of F514 instead of sentinel S14 did not adversely affect the integrity of the study, as F514 was not healthy when euthanized. Lesions of spleen and testes were observed in both S14 and F514.

Tissue Collection at Necropsy

In collecting mediastinal lymph nodes, small portions of adjacent thymic tissue was collected because mediastinal lymph nodes are easily missed during sectioning in the absence of a supporting stroma. Data from thymus collected with these specific sections were not reported. Thymus was collected and data reported for that particular tissue.

Collection of Organ Weights at Necropsy

The protocol states that lung, liver, kidneys, testes, epididymis, ovaries, uterus, spleen, brain and heart will be weighed at necropsy. It also states that these weights are not to be recorded on animals found dead. The brain weight was not recorded for E402. The seminal vesicle and prostate were weighed for E402, even though these tissues weights are not required by the study protocol. The impact of these deviations was minimal. Only one brain weight was lost.

Contrary to protocol, organ weights were recorded for rats found dead. Organ weight summaries have been prepared to exclude natural death animals, so their weights do not skew the group means for the final sacrifice and euthanized animal reports. There is no adverse effect on the outcome of the study.

Hematology

Blood smears were to be evaluated for differential white blood cell counts, and an estimate of total white blood cells was made. At the 12 month time point, a white blood cell estimate was not made on E419 (control male) because the smear was too thick and for high-level female H772. Poor smear quality prevented obtaining a white blood cell estimate for E478 (control female) at the 18 month time point. Because these were the only samples for which a white blood cell estimate were not obtained, the impact on the outcome of the study is small.

LIST OF ABBREVIATIONS

BGVC	Baseline gasoline vapor condensate
CFR	Code of Federal Regulations
CPN	Chronic progressive nephropathy
CRTC	Chevron Research and Technology Center
EDTA	Ethylenediamine tetra-acetic acid
EMBSI	Exxon Mobile Biomedical Sciences, Inc.
FID	Flame ionization detector
GC	Gas chromatograph
GLP	Good Laboratory Practices
GMVC	Gasoline MTBE vapor condensate
HN	Hydrocarbon nephropathy
KRV/H-1	Kilharn rat virus/H-1 virus
LPM	Liters per minute
LRRI	Lovelace Respiratory Research Institute
MTBE	Methyl tertiary-butyl ether
NBF	Neutral buffered formalin
NFPA	National Fire Protection Association
OPPTS	Office of Prevention, Pesticides, and Toxic Substances (U.S. EPA)
PVM	Pneumonia virus of mice
RCV/SDAV	Rat corona virus/sialodacryoadenitis
SOP	Standard operating procedure
SD/Stdev	Standard deviation
SE	Standard error
WBC	White blood cells

REFERENCE LIST

Baetcke, K. P., Hard, G. C., Rodgers, I. S., McGaughy, R. E., and Tahan, L. M. (1991). Alpha$_{2u}$-Globulin: Association with Chemically Induced Renal Toxicity and Neoplasia in the Male Rat. (EPA/625/3-91/019F) Risk Assessment Forum, U.S. Environmental Protection Agency (U.S. EPA), Washington, D.C.

Belpoggi, F., Soffritti, M., and Maltoni, C. (1995). Methyl-Tertiary-Butyl Ether (MTBE)—A Gasoline Additive—Causes Testicular and Lymphohaematopoietic Cancers in Rats. *Toxicol. Ind. Health 11(2)*: 119-149.

Bird, M. G., Burleigh-Flayer, H. D., Chun, J. S., Douglas, J. F., Kneiss, J. J., and Andrews, L. S. (1997). Oncogenicity Studies of Inhaled Methyl-Tertiary-Butyl Ether (MTBE) in CD-1 Mice and F-344 Rats. *J. Appl. Toxicol. 17(Suppl. 1)*: S45-S55.

Boorman, G. A., Eustis, S. L., Elwell, M. R., and Leininger, J. R., eds. (1990a). *Pathology of the Fischer Rat*, 1st Ed., Academic Press, San Diego, CA.

Boorman, G. A., Chapin, R. E., and Mitsumori, K. (1990b). Testis and Epididymis. In *Pathology of the Fischer Rat* (Boorman, G. A., Eustis, S. L., Elwell, M. R., and Leininger, J. R., eds.), 1st Ed., pp. 405-418, Academic Press, San Diego, CA.

Boorman, G. A., Wilson, J. T., van Zwieten, M. J., and Eutis, S. L. (1990c). Mammary Gland. In *Pathology of the Fischer Rat* (Boorman, G. A., Eustis, S. L., Elwell, M. R., and Leininger, J. R., eds.), 1st Ed., pp. 295-314, Academic Press, San Diego, CA.

Boorman, G. A., Morgan, K. T., and Uriah, L. C. (1990d). Nose, Larynx, and Trachea. In *Pathology of the Fischer Rat* (Boorman, G. A., Eustis, S. L., Elwell, M. R., and Leininger, J. R., eds.), 1st Ed., pp. 315-338, Academic Press, San Diego, CA.

Borghoff, S. J., Miller, A. B., Bowen, J. P., and Swenbert, J. A. (1991). Characteristics of Chemical Binding to α2u-globulin *In Vitro* – Evaluating Structure-Activity Relationships. *Toxicol. Appl. Pharmacol. 107*: 228-238.

Borghoff, S. J., Youtsey, N. L., and Swenbert, J. A. (1992). A Comparison of European High Test Gasoline and PS-6 Unleaded Gasoline in Their Abilities to Induce α2u-Globulin Nephropathy and Renal Cell Proliferation. *Toxicol. Lett. 63*: 21-33.

Cruzan, G., Borghoff, S. J., dePeyster, A., Hard, G. C., McClain, M., McGregor, D. G., and Thomas, M. G. (2007). Methyl *Tertiary*-Butyl Ether Mode of Action for Cancer Endpoints in Rodents. *Reg. Toxicol. Pharmacol. 47*: 156-165.

Cook, J. C., Klinefelter, G. R., Hardisty, J. F., Sharpe, R. M., and Foster, P. M. (1999). Rodent Leydig Cell Tumorigenesis: A Review of the Physiology, Pathology, Mechanisms, and Relevance to Humans. *Crit. Rev. Toxicol. 29(2)*: 169-261.

Gopinath, C., Prentice, D. E., and Lewis, D. J. (1987). *Atlas of Experimental Toxicological Pathology* (Gresham, G. A., ed.), MTP Press Limited, Norwell, MA.

Hard, G. C. (1998). Mechanisms of Chemically Induced Renal Carcinogenesis in the Laboratory Rodent. *Toxicol. Pathol. 26(1)*: 104-112.

Hard, G. C. and Khan, K. N. (2004). A Contemporary Overview of Chronic Progressive Nephropathy in the Laboratory Rat, and its Significance for Human Risk Assessment. *Toxicol. Pathol. 32(2)*: 171-180.

MacFarland, H. N., Ulrich, C. E., Holdsworth, C. E., Ketchen, D. N., Halliwell, W. H., and Blum, S. C. (1984). A Chronic Inhalation Study wth Unleaded Gasoline Vapor. *J. Am. Coll. Toxicol. 3(4):* 231-248.

Mennear, J. H. (1997). Carcinogenicity Studies on MTBE: Critical Review and Interpretation. *Risk Anal. 17(6)*: 673-681.

Prentice, D. E. and Meikle, A. W. (1995). A Review of Drug-Induced Leydig Cell Hyperplasia and Neoplasia in the Rat and Some Comparisons with Man. *Hum. Exp. Toxicol. 14(7)*: 562-572.