I. GLP COMPLIANCE STATEMENT

This study was conducted in compliance with EPA Good Laboratory Practices as set forth in 79.60, CFR Vol. 59, No. 122, 27 June 1994 with the following exceptions:

1. It was the Sponsor's responsibility to maintain the methods of synthesis, fabrication, or derivation of the test fuel. This had not been completed when the study initiated but is currently with the Sponsor.

2. The identity, strength, purity and composition or other characteristics to define the positive control article have not been determined by the Testing Facility. The positive control article has not been characterized as per the Certificate of analysis on file with the Testing Facility. The stability of the positive control article has not been determined by the Testing Facility. Analyses to determine the uniformity (as applicable) or concentration of the positive control mixture were not performed by the Testing Facility. The stability of the positive control article mixture has not been determined by the Testing Facility.

Kimber L. White, Jr., Ph.D
Principal Investigator
ImmunoTox®, Inc.

13 Aug 10
Date

Gary M. Hoffman, B.A., DABT
Study Director
Huntingdon Life Sciences

20 Aug 10
Date

II. QUALITY ASSURANCE STATEMENT

Test Substance: Gasoline ETBE Vapor Condensate

Report Title: Immunological Evaluation of Gasoline ETBE Vapor Condensate in Female Sprague Dawley Rats Using the Plaque-Forming Cell Assay

Protocol Title: Gasoline ETBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments And 4-Week *In Vivo* Genotoxicity and Immunotoxicity Assessments

Protocol No.: HLS Study No. 00-6129

The final report for the indicated protocol has been reviewed by the Quality Assurance Unit of Virginia Commonwealth University. Furthermore, the Quality Assurance Unit has conducted the following inspections and reported to the ImmunoTox®, Inc. Principal Investigator, and then has submitted written reports of said inspections to the Study Director and Management.

Inspection/Audits were performed and reported on the following dates:

Performed	Reported	Activity
21 November 01	28 November 01	AFC Assay
3-5 May 02	6 May 02	Data Audit
4-5 May 02	6 May 02	1st Draft Report Audit
14 April 07	19 April 07	2nd Draft Report Audit

Approved and submitted by: _____ 13 August 10
Quality Assurance Manager Date

HUNTINGDON LIFE SCIENCES QUALITY ASSURANCE STATEMENT

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

Type of Inspection	Date(s) of Inspection	Reported to Study Director and Management
General Facility Inspection	26 Sep 00	5 Dec 00[a]
GLP Protocol Review	24, 29 Aug 01	29 Aug 01
Exposure, Monitoring and Equipment Records	23 Oct 01	23 Oct 01
GC Characterization	09 Nov 01	09 Nov 01
Immunotoxicity and Positive Control Dosing	16 Nov 01	16 Nov 01
Immunotoxicity Necropsy	20 Nov 01	20 Nov 01

Fran Jannone

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

20 Oct 10
Date

[a]Date reported to Testing Facility Management

III. SIGNATURE OF PRINCIPALS

This report describes the results used to evaluate the relative immunotoxicological potential of the test substance, Gasoline ETBE Vapor Condensate, which was administered by inhalation via whole-body exposure to female Sprague Dawley rats.

Kimber L. White, Jr., Ph.D., Principal Investigator, was responsible for the overall conduct of the immunotoxicity evaluations in this study. Vanessa L. Peachee, M.S., served as the Assistant Principal Investigator and was responsible for the day-to-day activities of the immunotoxicity evaluations in this study.

Kimber L. White, Jr., Ph.D.
Principal Investigator
ImmunoTox®, Inc.

Kimber L White Date 13 Aug 10

Vanessa L. Peachee, M.S.
Assistant Principal Investigator
ImmunoTox®, Inc.

Vanessa L Peachee Date 17 Aug 10

Approved:

Gary M. Hoffman, B.A., DABT
Study Director
Huntingdon Life Sciences

20 Oct 10
Date

TABLE OF CONTENTS

I. GLP Compliance Statement 2
II. Quality Assurance Statements
ImmunoTox®, Inc. 3
Huntingdon Life Sciences 4
III. Signature of Principals 5
IV. Executive Summary 8
V. Introduction 10
VI. Methods of Procedure 12
Experimental Design 12
Variables Assessed 12
Terminal Body and Organ Weights 12
Splenocyte Preparation 12
Spleen IgM Antibody Response to the T-dependent Antigen, sRBC. Day 4 Response .. 13
Data 13
Data Handling and Statistical Analysis 13
Data Retention 14
VII. Results 15
Terminal Body and Organ Weights 15
Spleen IgM Antibody Response to the T-dependent Antigen, sRBC. Day 4 Response 17
VIII. Conclusion 21
IX. References 22
X. List of Figures
1. Absolute (mg) and Relative (%) Spleen Weight in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks 16
2. Absolute (mg) and Relative (%) Thymus Weight in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks 17
3. Spleen Cell Number in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks 19

4. IgM Antibody-Forming Cell Response to Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks 20

XI. List of Tables

ES1 Summary Table for Toxicology and Immunology Studies 9

1 Body Weight (g) and Organ Weights (mg) in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks 23

2. Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks 24

APPENDICES

A Individual Animal Data

B Contracting Sponsor's Exposure and Animal Data

IV. EXECUTIVE SUMMARY

The study was conducted as part of Huntingdon Life Sciences (HLS) Study No. 00-6129 at ImmunoTox®, Inc., Richmond, Virginia. The Principal Investigator was Kimber L. White, Jr., Ph.D., and Vanessa L. Peachee, M.S., served as the Assistant Principal Investigator. The study was conducted to provide evaluation of immunological parameters for Huntingdon Life Sciences.

The objective of the study was to determine the potential effects of Gasoline ETBE Vapor Condensate for its ability to affect the humoral immune component of the immune system, when evaluated in the antibody-forming cell response to the T-dependent antigen, sheep erythrocytes. Female Sprague Dawley rats were administered Gasoline ETBE Vapor Condensate for 5 days per week for 4 weeks by inhalation via whole body exposure by Huntingdon Life Sciences (HLS) Princeton Research Center (PRC) personnel. Three exposure levels of 2,000, 10,000 and 20,000 mg/m^3 of the test substance were used in the study. The in-life phase of the study was conducted by HLS, East Millstone, NJ, and the immunological evaluation was conducted by ImmunoTox®, Inc., Richmond, VA. On the day of sacrifice, spleens were placed in tubes containing media, placed on ice, and shipped to ImmunoTox®, Inc. in Richmond, VA, for assay evaluation on the following day.

Executive Summary Table ES-1 shows a summary of the selected toxicology and immunology parameters evaluated. Exposure to Gasoline ETBE Vapor Condensate resulted in no statistically significant changes in terminal body weight for any exposure level. Furthermore, there were no statistically significant effects observed in either thymus or spleen weight following exposure to Gasoline ETBE Vapor Condensate, when evaluated as either absolute or relative weight (% body weight), as compared to the air control.

Exposure to Gasoline ETBE Vapor Condensate did result in a statistically significant decrease in the humoral immune response when evaluated in the IgM antibody-forming cell (AFC) response to the T-dependent antigen, sheep erythrocytes. When evaluated as specific activity (AFC/10^6 spleen cells), a 76% decrease was observed at the middle dose group and a 72% decrease at the high dose group. When evaluated as total spleen activity (AFC/spleen), there was a 74% decrease at the middle dose group and a 70% decrease at the high dose group. A no effect level was observed at the 2000 mg/m^3 exposure level. The positive control, CPS, produced the anticipated results in the various parameters evaluated.

In conclusion, the results of this immunotoxicological evaluation demonstrate that, under the experimental conditions used, exposure to the Gasoline ETBE Vapor Condensate test substance adversely affected the functional ability of the humoral immune component of the immune system.

Table ES-1

SUMMARY TABLE FOR TOXICOLOGY AND IMMUNOLOGY STUDIES

Parameter	Result	Maximum Effect	Exposure Level (mg/m^3)
Terminal Body Weight			
Day 29	No Effect		
Organ Weights (Absolute and Relative)			
Spleen	No Effect		
Thymus	No Effect		
Spleen IgM Antibody-Forming Cell Response to Sheep Erythrocytes			
IgM AFC/10^6 Spleen Cells	Decrease	76%	10,000 [a]
IgM AFC/Spleen ($x10^3$)	Decrease	74%	10,000 [a]

[a] The 20000 mg/m^3 is statistically significant data even though it is slightly lower than the 10000 mg/m^3 data.

V. INTRODUCTION

The purpose of this study was to provide evaluation of immunological parameters for Huntingdon Life Sciences (HLS) Study No. 00-6129. In this study, the ability of the test substance, Gasoline ETBE Vapor Condensate, was evaluated for its ability to affect the humoral immune component of the immune system, when evaluated in the antibody-forming cell response to the T-dependent antigen sheep erythrocytes. Four days prior to sacrifice, ImmunoTox®, Inc. personnel sensitized the rats by intravenous administration of sheep erythrocytes at the HLS facility. On the day of sacrifice, HLS Princeton Research Center (PRC) personnel aseptically removed the spleen from each animal. The spleens were weighed, placed in tubes containing media, and sent to ImmunoTox®, Inc. in Richmond, VA, on ice for evaluation the following day. Spleens were received on 21 November 2001 and the immunological evaluation was conducted on the same day. The IgM antibody-forming cell (AFC) response to the T-dependent antigen sheep erythrocytes, also referred to as the plaque assay, was the immunological assay conducted to evaluate the effect of Gasoline ETBE Vapor Condensate on the immune response. This assay has been shown to be the most predictive functional assay for determining the immunotoxicological potential of a compound (Luster *et al.*[1]).

As background, sheep erythrocytes (sRBC) are a T-dependent antigen and, thus, T cells, B cells, and macrophages are required to function properly in order to obtain an antibody-forming cell (AFC) response. If the test article affects any of these cell types to a significant degree, an altered response will be observed. As a result, the T-dependent IgM response to sRBC is one of the most sensitive immunotoxicological assays currently in use. A significant modulation in the IgM AFC response, when appropriately compared to vehicle controls, indicates that the test agent is capable of modifying the humoral immune response in the whole animal and, thus, has the potential for immunotoxicity. This assay is one of the Tier I assays used by the NTP[2].

Kimber L. White, Jr., Ph.D., was the Principal Investigator for the immunological evaluation conducted by ImmunoTox®, Inc., and Gary M. Hoffman, B.A., DABT, was the HLS Study Director. Vanessa L. Peachee, M.S., served as the Assistant Principal Investigator for ImmunoTox®, Inc. and was responsible for carrying out the IgM antibody-forming cell assay.

In evaluating the effects of Gasoline ETBE Vapor Condensate on the immune system, the immunologic and toxicologic parameters evaluated were spleen weights, thymus weights, and the spleen IgM antibody response to the T-dependent antigen (sheep erythrocytes, sRBC).

To the best of our knowledge, no significant protocol or standard operating procedure deviations occurred during the study, which affected the quality of the data and the ability to interpret the data with respect to the immunotoxicology of Gasoline ETBE Vapor Condensate.

VI. METHODS OF PROCEDURE

EXPERIMENTAL DESIGN

The immunotoxicological satellite study consisted of a vehicle group, three exposure levels of Gasoline ETBE Vapor Condensate, and a positive control group. There were 10 female Sprague Dawley rats in each of the groups. Animals were exposed by Huntingdon Life Sciences Princeton Research Center (PRC) personnel to either vehicle (air only) or Gasoline ETBE Vapor Condensate at exposure levels of 2,000, 10,000 or 20,000 mg/m^3 via inhalation for 4 weeks (5 days per week). Cyclophosphamide (CPS) was given as the positive control and was obtained from the Sigma Chemical Company (responsible for its characterization). Cyclophosphamide was dissolved and diluted in phosphate buffered saline at Huntingdon Life Sciences to stock concentrations of 5.0 mg/mL for use as the positive control for this study. The positive control animals received 50 mg/kg @ 10 mL/kg of CPS, a known immunosuppressive agent, administered intraperitoneally (i.p.) on the last 4 days of exposure. These animals were not chamber exposed. On the day of sacrifice, one day after the last exposure, PRC personnel aseptically removed the spleen from each animal, weighed it, placed it in a collecting tube containing Earle's Balanced Salt Solution (EBSS) with HEPES and Gentamicin solution (prepared at PRC), and shipped the spleens on ice in individual shipping containers at 2-8°C by carrier to ImmunoTox®, Inc. for overnight delivery. Upon receipt, spleens were further processed for determination of IgM antibody response.

VARIABLES ASSESSED

Terminal Body and Organ Weights. Huntingdon Life Sciences PRC personnel collected blood (serum) samples (orbital collection anesthetized via carbon dioxide/oxygen inhalation) and then sacrificed (carbon dioxide inhalation) the animals on the day after the final exposure. The serum samples were frozen (≤-20°C). The thymuses were removed, weighed, and preserved (formalin) for possible histopathology. Spleens were removed, weighed, and shipped at the time of sacrifice by PRC personnel to ImmunoTox®, Inc. for immunotoxicological evaluation.

Splenocyte Preparation. Upon arrival at the ImmunoTox®, Inc. testing facility, spleens were accessioned in accordance with the SOP for receipt of biological samples. Single-cell suspensions were prepared from each spleen using a Stomacher® 80 Lab Blender in accordance with the SOP for rat spleens. Cell suspensions were then centrifuged and resuspended in Earle's Balanced Salt

Solution with HEPES. Viability of splenocytes was determined using propidium iodide (PI) and the Coulter EPICS XL-MCL Flow Cytometer.

Spleen IgM Antibody Response to the T-dependent Antigen, sRBC. Day 4 Response. The primary IgM response to sheep erythrocytes was measured using a modified hemolytic plaque assay of Jerne[3]. Rats were exposed to the test article for 5 days per week for 4 weeks. Rats were sensitized by ImmunoTox®, Inc. personnel with $2x10^8$ sRBC i.v. four days prior to sacrifice and, on the day after the last exposure, animals were sacrificed by PRC personnel. Spleen cell suspensions were prepared as described above. The cells were centrifuged and resuspended in a 6-ml volume, and 1:50 and 1:150 dilutions were prepared. An 0.1-ml aliquot of spleen cells from each suspension was added to separate test tubes, each containing 25 µl guinea pig complement, 25 µl sRBC, and 0.5 ml of warm agar (0.5%). After thoroughly mixing, each test tube mixture was plated onto a separate petri dish, covered with a microscope cover slip, and incubated at approximately 36-38°C for 3 hours. One dilution per animal was evaluated. Spleen cell counts were performed on the 6-ml samples using a Model Z1 Coulter Counter. The cells/spleen, AFC/10^6 spleen cells, and AFC/spleen were determined. The plaques, which developed, were counted using a Bellco plaque viewer. A plaque, occurring from the lysis of sRBC, is elicited as a result of the interaction of complement and antibodies (produced in response to the i.v. sensitization) directed against sRBC. Each plaque is generated from a single IgM antibody-producing B cell, permitting the number of AFC present in the whole spleen to be calculated. The data are expressed as specific activity (AFC/10^6 spleen cells) and total spleen activity (AFC/spleen).

DATA

Data Handling and Statistical Analysis. The data obtained in this study were analyzed in accordance with standard operating procedure. Data were first tested for homogeneity of variances using the Bartlett's Chi Square Test[4]. Homogeneous data were evaluated by a parametric one-way analysis of variance[5]. When significant differences occur, exposed groups were compared to the vehicle control group using the Dunnett's t Test[6]. Non-homogeneous data were evaluated using a non-parametric analysis of variance[5]. When significant differences occur, exposed groups were compared to vehicle control group using the Gehan-Wilcoxon Test[7] when appropriate. The Jonckheere's Test[8] was used to test for exposure level-related trends across the vehicle and exposed groups. The positive control was compared to the vehicle control group using the Student t Test[9]. The criteria for accepting the results of the positive

control in the assay was a statistically significant ($p \leq 0.05$) decrease in the response as compared to the vehicle control group.

P values of 0.05 or less, as compared to the vehicle control group, were considered statistically significant and are indicated in the tables and in the figures with a single asterisk (*). A double asterisk (**) was used to indicate a p value of 0.01 or less. In the text, the word significant indicates that the response was statistically significant at $p \leq 0.05$. In the tables, the abbreviation NS is used to indicate "Not Significant" for p values greater than 0.05.

Data Retention. All data and records were returned to the Contracting Sponsor following acceptance of the final report. Upon completion of this study, the report and raw data for this study will be maintained in the archives of Huntingdon Life Sciences.

VII. RESULTS

TERMINAL BODY AND ORGAN WEIGHTS

The terminal body weight data from the study are shown in Table 1 for the control and Test Substance-exposed groups. The terminal body weights were obtained by Huntingdon Life Sciences PRC personnel. No statistically significant differences were observed in terminal body weights of the Gasoline ETBE Vapor Condensate-exposed animals at any exposure level as compared to the vehicle (air only) controls.

The organ weights of the control and Test Substance-exposed rats are shown in Table 1. No effect was observed, following exposure to Gasoline ETBE Vapor Condensate, on spleen or thymus weights when evaluated either as absolute or relative weight. Treatment with the positive control, cyclophosphamide, had a significant decrease of 50% on absolute spleen weight and a significant decrease of 79% on absolute thymus weight, compared to the vehicle control. In addition, the positive control, cyclophosphamide, had a significant decrease of 48% on relative spleen weight and a 79% decrease on relative thymus weight, compared to the vehicle control. Shown graphically in Figures 1 and 2 is the lack of effect on spleen and thymus weights following exposure to Gasoline ETBE Vapor Condensate.

Figure 1

Absolute (mg) and Relative (%) Spleen Weight in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks

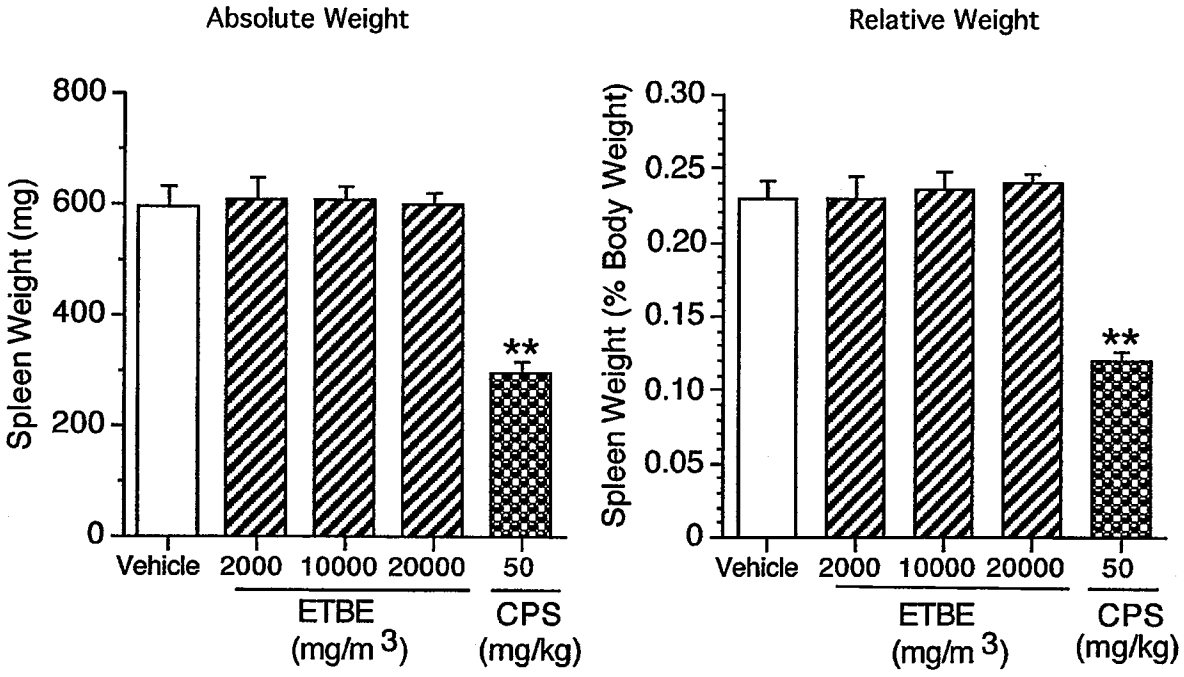
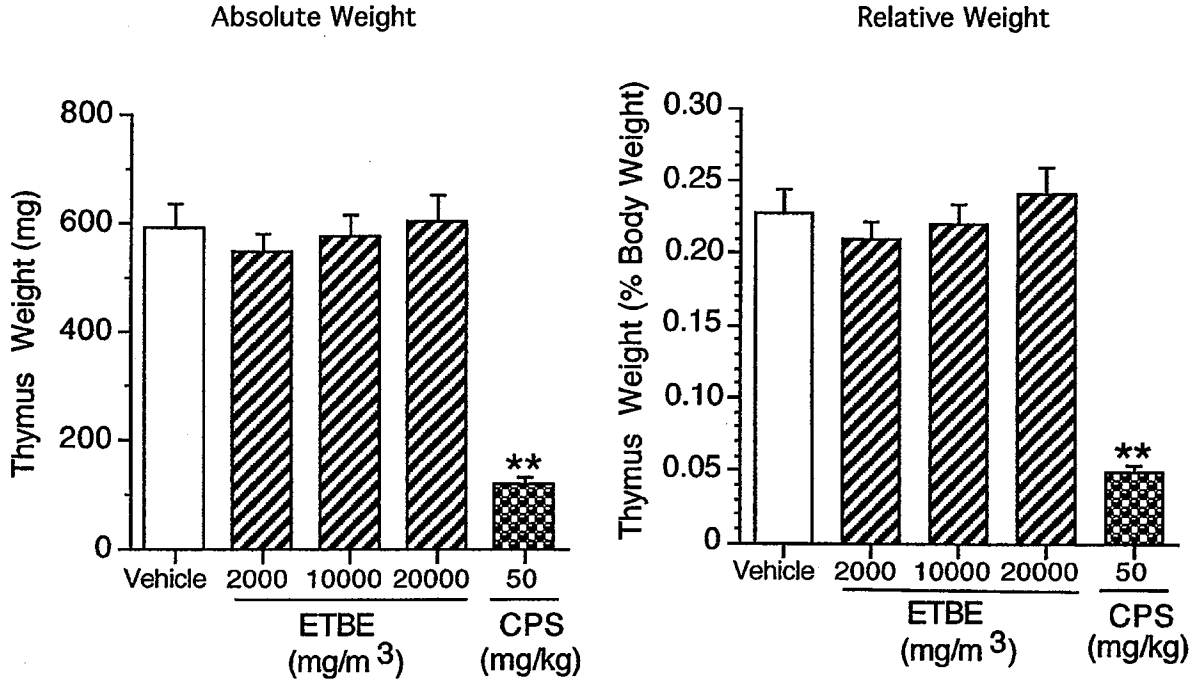


Figure 2

Absolute (mg) and Relative (%) Thymus Weight in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks



SPLEEN IGM ANTIBODY RESPONSE TO THE T-DEPENDENT ANTIGEN, SRBC. DAY 4 RESPONSE

The spleen IgM antibody-forming cell response, i.e. plaque assay, was evaluated on spleens removed 1 day after the last exposure, which was Day 4 after antigen sensitization. Day 4 after antigen sensitization is the peak day for the sRBC IgM AFC response in rats. Viabilities were conducted on all cell suspensions using propidium iodide (PI) and the Coulter EPICS XL-MCL Flow Cytometer. With the exception of three animals the viabilities from all samples were greater than 84%, which is consistent with the spleen cell viability routinely obtained with Sprague Dawley female rats.

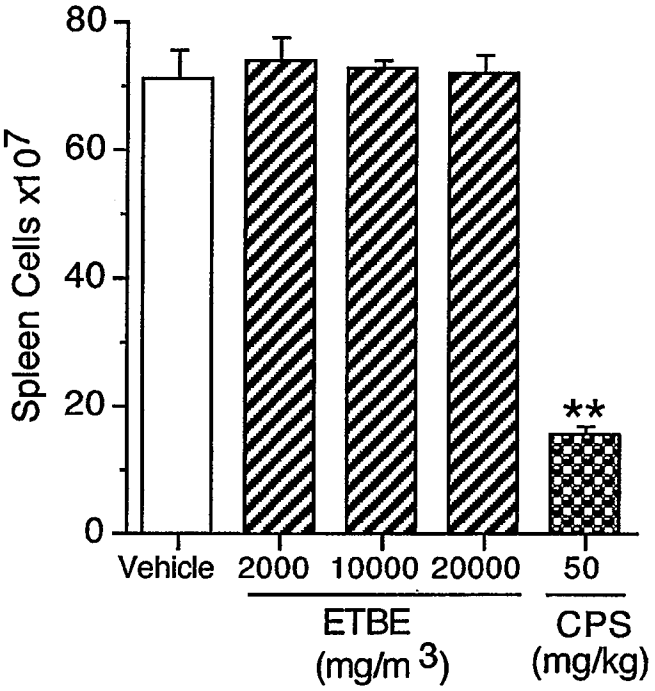
In the plaque-forming cell (PFC) assays conducted by our laboratory and at the National Toxicology Program (NTP) Immunotoxicology Laboratory of the National Institute of Environmental Health Sciences, the PFC assay results are not adjusted for spleen cell viability. The reasons for this are as follows. In *in vitro* studies, which utilize a single population of cells, e.g. YAC-1 cells, correcting for viability is biologically meaningful. These cells, being of identical type, respond to stimuli in a similar manner and will die off at a similar rate. When spleens are

utilized as the source of cells, this represents a heterogeneous mixture of cells, including neutrophils, lymphocytes, and macrophages. Each of these cell types will respond differently to stimuli under *in vitro* conditions, i.e., neutrophils will die off at a faster rate than lymphocytes. Accordingly, conducting viability determinations on total spleen cells is of little biological value when one is evaluating antigen specific antibody production by plasma cells. More specifically, once the structural integrity of the spleen is compromised, as occurs in preparing a single cell suspension, the cells now in an *in vitro* environment begin to die with the polymorphonuclear cells dying off at a much faster rate than will either lymphocytes or macrophages. The procedure utilized in our laboratory, and by the NTP Immunotoxicology Laboratory, minimizes the time it takes from preparing the single cell suspension of spleen cells to having them incubating in the assay petri dishes. By minimizing this preparation time, we also minimize the loss of viability, which occurs the longer the cells sit in the *in vitro* cell culture conditions. The decrease in viability, which does occur during this time, is predominately due to the dying off of the more fragile polymorphonuclear cells and not the lymphocytes, particularly those antibody-forming cells (plasma cells) making antibody to sheep erythrocytes. This is due in part to the fact that cells undergoing high metabolic activities, such as rapidly proliferating cells or cells synthesizing antibody, are less susceptible to compounds which produce cell death than are quiescent cells. It is for these reasons that there is no correlation between viability of individual spleen cell preparations and their ability to produce antibodies to sheep erythrocytes. Correcting for viability for a homogenous population in *in vitro* cultures is scientifically sound; however, as indicated above, using this procedure for mixed cell populations such as those present in the spleen, will result in artificially inflated PFC values.

The results of the AFC response are shown in Table 2 and in Figures 3 and 4. As indicated above, exposure to Gasoline ETBE Vapor Condensate did not result in spleen weights that were significantly different from the vehicle control group. Furthermore, as shown graphically in Figure 3, there were no significant differences in spleen cell number following exposure to Gasoline ETBE Vapor Condensate as compared to the vehicle control group.
As expected, the positive control, cyclophosphamide (CPS), produced a 78% decrease in spleen cell number when compared to the vehicle control group.

Figure 3

Spleen Cell Number in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks

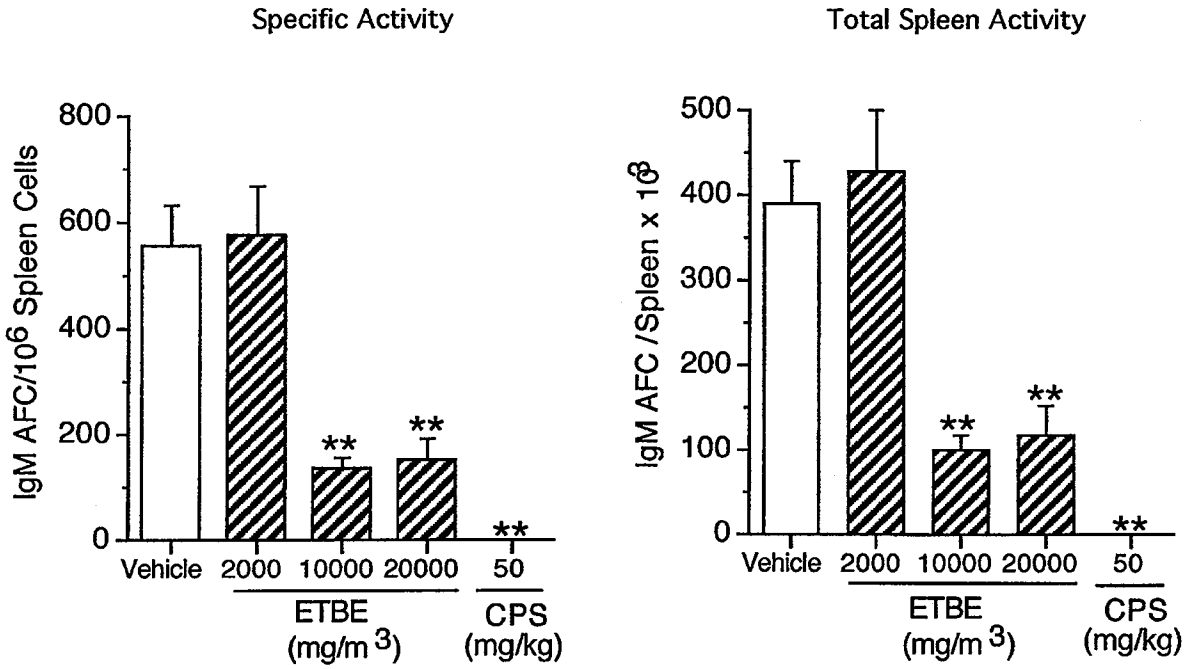


Shown in Table 2 and Figure 4 are the functional results from the IgM antibody-forming cell (AFC) assay. Shown in the left panel are the results when the data are expressed as specific activity and the results of the total spleen activity are shown in the right panel. As can be seen, a decrease in the IgM-antibody-forming cell response to the T-dependent antigen, sRBC, was observed when the data were evaluated as either specific activity (AFC/10^6 spleen) or as total spleen activity (AFC/spleen). For both parameters, exposure to Gasoline ETBE Vapor Condensate reached the level of statistical significance at the middle and high exposure levels. When evaluated as specific activity, the response was suppressed 76% for the middle exposure level and 72% for the high exposure level. When evaluated as total spleen activity, the response was suppressed 74% for the middle exposure level and 70% for the high exposure level.

As anticipated, the positive control, CPS, produced a significant decrease in specific activity (100%) and total spleen cell activity (100%) when compared to the vehicle control animals.

Figure 4

IgM Antibody-Forming Cell Response to Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks



VIII. CONCLUSION

Exposure of female Sprague Dawley rats to Gasoline ETBE Vapor Condensate for a period of 5 days per week for 4 weeks resulted in a statistically significant decrease in the humoral immune response to the T-dependent antigen, sheep erythrocytes, at the middle (10,000 mg/m^3) and high (20,000 mg/m^3) exposure levels. A no effect level was observed at the 2000 mg/m^3 exposure level. Although the humoral immune functional ability of the animals was reduced, there was no statistically significant effect on body weight, spleen weight, thymus weight, or spleen cell number. Based on the immunological parameters evaluated, under the experimental conditions of the study, exposure to Gasoline ETBE Vapor Condensate adversely affected the humoral immune response of female Sprague Dawley rats.

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Table 1

Body Weight (g) and Organ Weights (mg) in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks

Parameter	Vehicle	Gasoline ETBE Vapor (mg/m^3)			Cyclophosphamide	H/NH	Trend
		2000	10000	20000	50 mg/kg		Analysis
	(10)	(10)	(10)	(10)	(10)		
Body Wgt (g)	259.0 ± 5.0	263.0 ± 3.2	259.4 ± 4.0	250.3 ± 3.5	247.0 ± 4.0	H	NS
Spleen (mg)	597 ± 37	608 ± 41	608 ± 25	603 ± 17	299 ± 17**	H	NS
% Body Wgt	0.230 ± 0.011	0.230 ± 0.014	0.235 ± 0.012	0.240 ± 0.006	0.120 ± 0.006**	H	NS
Thymus (mg)	593 ± 45	549 ± 31	579 ± 40	605 ± 48	123 ± 9**	H	NS
% Body Wgt	0.228 ± 0.016	0.209 ± 0.013	0.220 ± 0.013	0.241 ± 0.018	0.049 ± 0.004**	H	NS

Female Sprague Dawley rats were administered vehicle control (air only) or gasoline ETBE vapor condensate by inhalation via whole-body exposure for 5 days per week for 4 weeks. The positive control, cyclophosphamide, was administered i.p. on the last 4 days of exposure. On the day of sacrifice, spleens were placed in tubes containing media and sent to Richmond, VA, on ice for next day cell preparation. The rats were necropsied and indicated organs weighed. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. The positive control was compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p \leq 0.05$ are indicated by an asterisk, while those significant at $p \leq 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Key:
mg = milligrams; m^3 = cubic meter of air; kg = kilograms; Wgt = weight; NS = not significant for p values greater than 0.05.

Table 2

Spleen Antibody-Forming Cell Response to T-dependent Antigen Sheep Erythrocytes in Female Sprague Dawley Rats Exposed to Gasoline ETBE Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks - Day 4 Response

Exposure	Body Wgt (g)	Spleen Wgt (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x\ 10^3$)
Vehicle	259.0 ± 5.0 (10)	597 ± 37 (10)	71.48 ± 4.48 (10)	556 ± 76 (10)	391 ± 48 (10)
Gasoline ETBE Vapor Condensate					
2000 mg/m^3	263.0 ± 3.2 (10)	608 ± 41 (10)	74.21 ± 3.69 (10)	576 ± 94 (10)	427 ± 72 (10)
10000 mg/m^3	259.4 ± 4.0 (10)	608 ± 25 (10)	72.95 ± 1.27 (10)	136 ± 23** (10)	100 ± 17** (10)
20000 mg/m^3	250.3 ± 3.5 (10)	603 ± 17 (10)	72.34 ± 2.78 (10)	153 ± 39** (10)	117 ± 35** (10)
Cyclophosphamide					
50 mg/kg	247.0 ± 4.0 (10)	299 ± 17** (10)	15.79 ± 1.21** (10)	0 ± 0** (10)	0 ± 0** (10)
H/NH	H	H	NH	NH	NH
Trend Analysis	NS	NS	NS	$p \leq 0.01$	$p \leq 0.01$

Female Sprague Dawley rats were administered vehicle control (air only) or gasoline ETBE vapor condensate by inhalation via whole-body exposure for 5 days per week for 4 weeks. The positive control, cyclophosphamide, was administered i.p. the last 4 days of exposure. Four days prior to sacrifice, the rats were immunized (iv) with $2x10^8$ sRBC. On the day of sacrifice, spleens were placed in tubes containing media and sent to Richmond, VA, on ice for next day cell preparation. Spleens were prepared into single cell suspensions and the number of IgM sRBC antibody-forming cells was determined. Values represent the mean ± SE derived from the number of animals indicated in parentheses. H = homogeneous data and NH = non-homogeneous data using the Bartlett's Test for homogeneity. Homogeneous data were evaluated using a parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Dunnett's t Test. Non-homogeneous data were evaluated using a non-parametric analysis of variance. When significant differences occurred, exposed groups were compared to the vehicle control group using the Wilcoxon Rank Test. The positive control was compared to the vehicle control using the Student's t Test. Values significantly different from vehicle control at $p \leq 0.05$ are indicated by an asterisk, while those significant at $p \leq 0.01$ are noted by a double asterisk. The Jonckheere's Test was used to test for dose-related trends among the vehicle and exposed groups.

Key:
g = grams; mg = milligrams; m^3 = cubic meter of air; kg = kilograms; Wgt = weight; NS = not significant for p values greater than 0.05.

APPENDIX A

INDIVIDUAL ANIMAL DATA

INDIVIDUAL ANIMAL DATA
ORGAN WEIGHTS
GASOLINE ETBE VAPOR CONDENSATE
00-6129

ANIMAL NO	GROUP	DOSE	SEX	BODY WGT (G)	SPLEEN WGT (MG)	THYMUS WGT (MG)	SPLEEN WGT / % BODY WGT	THYMUS WGT / % BODY WGT
1581	GI	AIR ONLY	F	265.0	702	437	0.260	0.160
1582	GI	AIR ONLY	F	272.4	521	685	0.190	0.250
1583	GI	AIR ONLY	F	267.1	535	757	0.200	0.280
1584	GI	AIR ONLY	F	247.0	637	644	0.260	0.260
1585	GI	AIR ONLY	F	243.0	581	364	0.240	0.150
1586	GI	AIR ONLY	F	247.4	483	652	0.200	0.260
1587	GI	AIR ONLY	F	241.9	534	672	0.220	0.280
1588	GI	AIR ONLY	F	281.6	821	765	0.290	0.270
1589	GI	AIR ONLY	F	279.5	701	495	0.250	0.180
1590	GI	AIR ONLY	F	245.0	455	459	0.190	0.190
2571	GII	2,000 MG/M3 ETBE	F	261.6	650	556	0.250	0.210
2572	GII	2,000 MG/M3 ETBE	F	248.9	595	625	0.240	0.250
2573	GII	2,000 MG/M3 ETBE	F	273.6	664	551	0.240	0.200
2574	GII	2,000 MG/M3 ETBE	F	255.3	607	530	0.240	0.210
2575	GII	2,000 MG/M3 ETBE	F	262.7	615	715	0.230	0.270
2576	GII	2,000 MG/M3 ETBE	F	254.2	464	653	0.180	0.260
2577	GII	2,000 MG/M3 ETBE	F	279.1	895	525	0.320	0.190
2578	GII	2,000 MG/M3 ETBE	F	275.1	491	522	0.180	0.190
2579	GII	2,000 MG/M3 ETBE	F	256.7	447	440	0.170	0.170
2580	GII	2,000 MG/M3 ETBE	F	263.1	656	375	0.250	0.140
3571	GIII	10,000 MG/M3 ETBE	F	249.8	504	487	0.200	0.190
3572	GIII	10,000 MG/M3 ETBE	F	272.1	677	624	0.250	0.230
3573	GIII	10,000 MG/M3 ETBE	F	264.8	560	605	0.210	0.230
3574	GIII	10,000 MG/M3 ETBE	F	257.2	595	520	0.230	0.200
3575	GIII	10,000 MG/M3 ETBE	F	248.2	766	598	0.310	0.240
3576	GIII	10,000 MG/M3 ETBE	F	244.2	627	534	0.260	0.220
3577	GIII	10,000 MG/M3 ETBE	F	246.8	536	481	0.220	0.190
3578	GIII	10,000 MG/M3 ETBE	F	278.7	571	599	0.200	0.210
3579	GIII	10,000 MG/M3 ETBE	F	256.5	685	446	0.270	0.170
3580	GIII	10,000 MG/M3 ETBE	F	275.3	564	894	0.200	0.320
4581	GIV	20,000 MG/M3 ETBE	F	259.3	647	602	0.250	0.230
4582	GIV	20,000 MG/M3 ETBE	F	239.9	512	483	0.210	0.200
4583	GIV	20,000 MG/M3 ETBE	F	246.9	612	661	0.250	0.270
4584	GIV	20,000 MG/M3 ETBE	F	260.0	579	402	0.220	0.150
4585	GIV	20,000 MG/M3 ETBE	F	264.4	612	760	0.230	0.290
4586	GIV	20,000 MG/M3 ETBE	F	251.1	577	500	0.230	0.200
4587	GIV	20,000 MG/M3 ETBE	F	253.8	644	601	0.250	0.240
4588	GIV	20,000 MG/M3 ETBE	F	236.3	549	613	0.230	0.260
4589	GIV	20,000 MG/M3 ETBE	F	258.6	698	928	0.270	0.360
4590	GIV	20,000 MG/M3 ETBE	F	232.9	597	501	0.260	0.210
5551	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	246.1	328	133	0.130	0.050
5552	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	259.8	371	104	0.140	0.040
5553	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	229.1	299	165	0.130	0.070
5554	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	251.7	232	132	0.090	0.050
5555	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	242.1	220	100	0.090	0.040
5556	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	266.8	360	139	0.130	0.050
5557	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	260.9	266	104	0.100	0.040
5558	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	238.7	337	74	0.140	0.030
5559	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	232.4	314	134	0.140	0.060
5560	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	242.0	262	148	0.110	0.060

INDIVIDUAL ANIMAL DATA
IGM AFC ASSAY
GASOLINE ETBE VAPOR CONDENSATE
00-6129

ANIMAL NO	GROUP	DOSE	SEX	IGM AFC/10^6 SP.C.	IGM AFC/SPLEEN 10^3	CELLS/SPLEEN 10^7	SPLEEN WGT (MG)	BODY WGT (G)
1581	GI	AIR ONLY	F	607	483	79.56	702	265.0
1582	GI	AIR ONLY	F	292	147	50.28	521	272.4
1583	GI	AIR ONLY	F	216	156	72.12	535	267.1
1584	GI	AIR ONLY	F	750	549	73.20	637	247.0
1585	GI	AIR ONLY	F	670	456	68.10	581	243.0
1586	GI	AIR ONLY	F	377	252	66.90	483	247.4
1587	GI	AIR ONLY	F	718	501	69.78	534	241.9
1588	GI	AIR ONLY	F	492	480	97.50	821	281.6
1589	GI	AIR ONLY	F	428	366	85.44	701	279.5
1590	GI	AIR ONLY	F	1005	522	51.96	455	245.0
2571	GII	2,000 MG/M^3 ETBE	F	1119	870	77.76	650	261.6
2572	GII	2,000 MG/M^3 ETBE	F	358	288	80.40	595	248.9
2573	GII	2,000 MG/M^3 ETBE	F	965	654	67.80	664	273.6
2574	GII	2,000 MG/M^3 ETBE	F	840	675	80.34	607	255.3
2575	GII	2,000 MG/M^3 ETBE	F	557	408	73.20	615	262.7
2576	GII	2,000 MG/M^3 ETBE	F	562	324	57.66	464	254.2
2577	GII	2,000 MG/M^3 ETBE	F	407	387	95.10	895	279.1
2578	GII	2,000 MG/M^3 ETBE	F	300	204	67.92	491	275.1
2579	GII	2,000 MG/M^3 ETBE	F	335	195	58.20	447	256.7
2580	GII	2,000 MG/M^3 ETBE	F	315	264	83.70	656	263.1
3571	GIII	10,000 MG/M^3 ETBE	F	186	141	75.90	504	249.8
3572	GIII	10,000 MG/M^3 ETBE	F	151	123	81.72	677	272.1
3573	GIII	10,000 MG/M^3 ETBE	F	70	48	68.58	560	264.8
3574	GIII	10,000 MG/M^3 ETBE	F	122	90	73.50	595	257.2
3575	GIII	10,000 MG/M^3 ETBE	F	49	36	73.56	766	248.2
3576	GIII	10,000 MG/M^3 ETBE	F	236	174	73.80	627	244.2
3577	GIII	10,000 MG/M^3 ETBE	F	129	96	74.16	536	246.8
3578	GIII	10,000 MG/M^3 ETBE	F	47	33	70.08	571	278.7
3579	GIII	10,000 MG/M^3 ETBE	F	256	177	69.12	685	256.5
3580	GIII	10,000 MG/M^3 ETBE	F	117	81	69.06	564	275.3
4581	GIV	20,000 MG/M^3 ETBE	F	464	402	86.64	647	259.3
4582	GIV	20,000 MG/M^3 ETBE	F	100	60	60.00	512	239.9
4583	GIV	20,000 MG/M^3 ETBE	F	30	21	70.92	612	246.9
4584	GIV	20,000 MG/M^3 ETBE	F	106	72	68.10	579	260.0
4585	GIV	20,000 MG/M^3 ETBE	F	131	96	73.32	612	264.4
4586	GIV	20,000 MG/M^3 ETBE	F	180	138	76.50	577	251.1
4587	GIV	20,000 MG/M^3 ETBE	F	32	24	73.92	644	253.8
4588	GIV	20,000 MG/M^3 ETBE	F	128	84	65.82	549	236.3
4589	GIV	20,000 MG/M^3 ETBE	F	211	180	85.26	698	258.6
4590	GIV	20,000 MG/M^3 ETBE	F	148	93	62.88	597	232.9
5551	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	19.74	328	246.1
5552	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	16.44	371	259.8
5553	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	17.58	299	229.1
5554	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	15.66	232	251.7
5555	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	9.66	220	242.1
5556	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	20.22	360	266.8
5557	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	10.50	266	260.9
5558	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	19.98	337	238.7
5559	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	12.54	314	232.4
5560	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	15.54	262	242.0

	Animal Exposure and Animal Data Preface	Appendix B

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

STUDY DATES:	Date of Animal Receipt:	4 October 2001
	Experimental Initiation Date:	23 October 2001 (in-life)
	Experimental Completion Date:	20 November 2001 (in-life)
	Draft Report Date:	19 June 2002

EXPOSURES AND IN-LIFE SUMMARY: The actual measured results during the exposures were comparable to the targeted exposure levels. There were no exposure-related effects seen in the test animals with regards to body weights and feed consumption.

TABLE OF CONTENTS

TABLES

A. Chamber Monitoring Results 1227
B. Summary of Clinical Observations (pretest only) 1235
C. Mean Body Weights (grams) 1236
D. Mean Body Weight Change (grams) 1237
E. Mean Feed Consumption Values (grams/kg/day) 1238
F. Individual Clinical Observations (pretest only) 1239
G. Individual Body Weights (grams) 1244
H. Individual Body Weight Change (grams) 1249
I. Individual Feed Consumption Values (grams/kg/day) 1254
J. Animal Termination History 1259

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

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

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
0	23-Oct-01	1	0	0	0	0	0	0					25	53
1	24-Oct-01	2	0	0	0	0	0	0					25	50
2	25-Oct-01	3	0	0	0	0	0	0					25	50
3	26-Oct-01	4	0	0	0	0	0	0					25	54
6	29-Oct-01	5	0	0	0	0	0	0		1.482	1.752	1.72E-03	25	50
7	30-Oct-01	6	0	0	0	0	0	0					24	54
8	31-Oct-01	7	0	0	0	0	0	0					24	53
9	1-Nov-01	8	0	0	0	0	0	0		0.9534	2.306	6.33E-03	24	52
10	2-Nov-01	9	0	0	0	0	0	0					24	55
13	5-Nov-01	10	0	0	0	0	0	0					24	52
14	6-Nov-01	11	0	0	0	0	0	0					25	53
15	7-Nov-01	12	0	0	0	0	0	0					25	53
16	8-Nov-01	13	0	0	0	0	0	0		0.9473	2.112	1.95E-03	25	52
17	9-Nov-01	14	0	0	0	0	0	0					25	52
20	12-Nov-01	15	0	0	0	0	0	0					25	52
21	13-Nov-01	16	0	0	0	0	0	0					24	53
22	14-Nov-01	17	0	0	0	0	0	0					24	52
23	15-Nov-01	18	0	0	0	0	0	0		0.8686	2.044	4.56E-03	24	52
24	16-Nov-01	19	0	0	0	0	0	0					25	49
27	19-Nov-01	20	0	0	0	0	0	0					24	49
		Mean	**0**		**0**					**1.063**	**2.054**	**3.64E-03**	**24.6**	**52.0**
		S.D.	**0**		**0**					**0.282**	**0.230**	**2.21E-03**	**0.5**	**1.7**

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

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

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
				Mean	Individual								Mean	
			(mg/m^3)	(mg/m^3)	(mg/m^3)					MMAD (µm)	GSD	TMC (mg/m^3)	Temperature (°C)	Humidity (%)
0	23-Oct-01	1	0	0	0	0	0	0					24	56
1	24-Oct-01	2	0	0	0	0	0	0					24	53
2	25-Oct-01	3	0	0	0	0	0	0					23	53
3	26-Oct-01	4	0	0	0	0	0	0					24	57
6	29-Oct-01	5	0	0	0	0	0	0		5.659	2.488	2.88E-03	24	52
7	30-Oct-01	6	0	0	0	0	0	0					24	53
8	31-Oct-01	7	0	0	0	0	0	0					24	54
9	1-Nov-01	8	0	0	0	0	0	0		0.8546	2.214	5.97E-03	24	53
10	2-Nov-01	9	0	0	0	0	0	0					24	55
13	5-Nov-01	10	0	0	0	0	0	0					24	52
14	6-Nov-01	11	0	0	0	0	0	0					24	54
15	7-Nov-01	12	0	0	0	0	0	0					24	56
16	8-Nov-01	13	0	0	0	0	0	0		1.738	2.383	5.12E-03	24	54
17	9-Nov-01	14	0	0	0	0	0	0					24	53
20	12-Nov-01	15	0	0	0	0	0	0					24	54
21	13-Nov-01	16	0	0	0	0	0	0					24	55
22	14-Nov-01	17	0	0	0	0	0	0					24	53
23	15-Nov-01	18	0	0	0	0	0	0		0.9337	2.189	6.73E-03	24	54
24	16-Nov-01	19	0	0	0	0	0	0					24	51
27	19-Nov-01	20	0	0	0	0	0	0					25	50
		Mean	**0**		**0**					**2.296**	**2.319**	**5.18E-03**	**24.0**	**53.6**
		S.D.	**0**		**0**					**2.277**	**0.142**	**1.67E-03**	**0.3**	**1.7**

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

Chamber Monitoring Results
Cumulative Exposure Record
Group IIA - 2,000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
0	23-Oct-01	1	2090	2038	2280	2160	1960	1750					23	50
1	24-Oct-01	2	2160	2015	1880	2110	2050	2020					23	47
2	25-Oct-01	3	2270	2160	2020	1960	2350	2310					23	47
3	26-Oct-01	4	2090	2023	2010	2060	2060	1960					23	50
6	29-Oct-01	5	2110	1988	1850	2030	2040	2030		2.045	2.243	2.28E-03	24	46
7	30-Oct-01	6	2030	2020	2170	2050	1900	1960					24	49
8	31-Oct-01	7	2080	1990	1910	1950	2090	2010					24	50
9	1-Nov-01	8	2060	1940	1860	1760	2180	1960		0.7630	1.745	3.75E-03	24	48
10	2-Nov-01	9	2120	2010	2040	1980	2000	2020					24	50
13	5-Nov-01	10	2030	2025	2170	2020	1990	1920					24	48
14	6-Nov-01	11	2080	1995	1860	1960	2150	2010					24	49
15	7-Nov-01	12	2100	2063	2060	2090	2040	2060					24	45
16	8-Nov-01	13	2220	2195	2270	2270	1840	2400		0.8737	1.804	2.31E-03	24	47
17	9-Nov-01	14	2310	2293	2180	2030	2540	2390					24	47
20	12-Nov-01	15	2140	2048	2060	1960	2140	2030					24	49
21	13-Nov-01	16	2060	1918	1950	1920	1860	1940					23	50
22	14-Nov-01	17	2130	2055	2060	1930	2200	2030					24	48
23	15-Nov-01	18	2120	2043	2230	1970	2110	1860		0.8069	1.867	4.75E-03	24	48
24	16-Nov-01	19	2100	2053	1970	1800	2160	2280					24	47
27	19-Nov-01	20	1970	1935	1690	1730	1970	2350					24	46
		Mean	**2114**		**2040**					**1.122**	**1.915**	**3.27E-03**	**23.8**	**48.1**
		S.D.	**80**		**161**					**0.617**	**0.224**	**1.20E-03**	**0.4**	**1.5**

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

Chamber Monitoring Results
Cumulative Exposure Record
Group IIB - 2,000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
0	23-Oct-01	1	2090	1875	1620	1980	1880	2020					23	54
1	24-Oct-01	2	2160	2043	2060	2060	2010	2040					23	51
2	25-Oct-01	3	2270	2160	2070	1990	2320	2260					23	50
3	26-Oct-01	4	2090	2020	2030	1990	1920	2140					23	53
6	29-Oct-01	5	2110	1995	2160	1980	1880	1960		12.38	3.012	6.67E-03	23	50
7	30-Oct-01	6	2030	2000	2030	1970	2030	1970					23	51
8	31-Oct-01	7	2080	1983	2220	1990	1690	2030					23	53
9	1-Nov-01	8	2090	2030	1990	1820	2280	2030		0.8035	2.139	7.47E-03	23	52
10	2-Nov-01	9	2120	2048	2140	2040	1970	2040					23	53
13	5-Nov-01	10	2030	1983	1890	2020	2010	2010					23	49
14	6-Nov-01	11	2080	2058	1960	1850	2280	2140					23	53
15	7-Nov-01	12	2100	1935	1990	1890	1930	1930					23	52
16	8-Nov-01	13	2220	2088	2190	2300	1740	2120		0.7894	1.578	2.04E-03	24	51
17	9-Nov-01	14	2310	1875	1640	1910	1920	2030					23	51
20	12-Nov-01	15	2140	2013	1940	1890	2020	2200					23	52
21	13-Nov-01	16	2060	1948	1980	1960	2010	1840					22	52
22	14-Nov-01	17	2130	1935	2120	1790	1890	1940					22	50
23	15-Nov-01	18	2120	2143	2160	1980	2170	2260		0.8338	2.476	7.31E-03	23	50
24	16-Nov-01	19	2100	2050	2010	1960	2190	2040					23	48
27	19-Nov-01	20	1970	1958	1730	2030	2200	1870					23	47
		Mean	**2115**		**2007**					**3.702**	**2.301**	**5.87E-03**	**23.0**	**51.1**
		S.D.	**79**		**145**					**5.786**	**0.601**	**2.58E-03**	**0.4**	**1.8**

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIA - 10,000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
0	23-Oct-01	1	11600	10240	9720	10700	9820	10700					23	50
1	24-Oct-01	2	11000	9938	9920	10500	9230	10100					24	47
2	25-Oct-01	3	9890	8895	9430	8040	9130	8980					23	47
3	26-Oct-01	4	11600	10080	9000	10600	10600	10100					24	46
6	29-Oct-01	5	11300	10280	10400	10500	10100	10100		2.117	2.220	2.09E-03	24	45
7	30-Oct-01	6	11000	10180	10100	10100	10400	10100					24	48
8	31-Oct-01	7	10200	9153	9990	8710	8930	8980					24	49
9	1-Nov-01	8	11000	10300	9790	10500	10400	10500		0.9487	2.649	5.23E-03	24	48
10	2-Nov-01	9	11300	10450	10500	9790	10800	10700					24	52
13	5-Nov-01	10	11000	10380	10700	9530	10800	10500					24	47
14	6-Nov-01	11	10800	9788	10100	9490	10100	9460					24	47
15	7-Nov-01	12	10200	10040	10100	10500	9060	10500					24	47
16	8-Nov-01	13	9970	9823	10000	10800	8900	9590		3.553	2.083	1.22E-02	24	46
17	9-Nov-01	14	11300	10600	9890	10800	11100	10600					24	45
20	12-Nov-01	15	10900	10220	10500	10100	10400	9860					24	45
21	13-Nov-01	16	10500	9645	9890	9130	10100	9460					24	48
22	14-Nov-01	17	11000	9945	10100	10100	9790	9790					23	49
23	15-Nov-01	18	10200	9690	9790	9790	9790	9390		0.7951	2.370	1.06E-02	24	49
24	16-Nov-01	19	10400	9413	8230	9460	10100	9860					24	47
27	19-Nov-01	20	11500	10520	9790	11000	10500	10800					24	46
		Mean	**10833**		**9978**					**1.853**	**2.331**	**7.53E-03**	**23.9**	**47.4**
		S.D.	**541**		**632**					**1.278**	**0.243**	**4.69E-03**	**0.4**	**1.8**

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIB - 10,000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
0	23-Oct-01	1	11600	10780	11200	10700	10100	11100					24	47
1	24-Oct-01	2	11000	10080	10800	10500	9560	9460					24	47
2	25-Oct-01	3	9890	9985	10100	8850	9890	11100					24	46
3	26-Oct-01	4	11600	10150	9790	10100	10600	10100					24	46
6	29-Oct-01	5	11300	10030	10100	9960	9960	10100		5.488	2.816	3.57E-03	24	44
7	30-Oct-01	6	11000	10530	11000	10100	10800	10200					24	44
8	31-Oct-01	7	10200	9230	10300	8910	8950	8760					24	46
9	1-Nov-01	8	11000	10020	10500	9990	9860	9720		0.7852	1.929	3.90E-03	24	47
10	2-Nov-01	9	11300	10400	10500	10400	10500	10200					24	48
13	5-Nov-01	10	11000	10120	10100	9460	10500	10400					24	44
14	6-Nov-01	11	10800	9728	10100	9020	10000	9790					24	46
15	7-Nov-01	12	10200	9790	9990	10100	8970	10100					24	47
16	8-Nov-01	13	9970	9483	10000	10200	8110	9620		0.8095	2.085	4.71E-03	24	46
17	9-Nov-01	14	11300	10330	9530	10800	10300	10700					24	45
20	12-Nov-01	15	10900	10030	9620	10300	10200	10000					24	44
21	13-Nov-01	16	10500	9293	10100	8350	9030	9690					23	46
22	14-Nov-01	17	11000	10370	10100	11100	9990	10300					23	46
23	15-Nov-01	18	10200	9795	9720	10000	10000	9460		0.7347	2.097	1.04E-02	23	45
24	16-Nov-01	19	10400	9755	9460	9460	10000	10100					24	44
27	19-Nov-01	20	11500	10160	9920	10500	10100	10100					23	45
		Mean	**10833**		**10002**					**1.954**	**2.232**	**5.65E-03**	**23.8**	**45.7**
		S.D.	**541**		**604**					**2.356**	**0.397**	**3.21E-03**	**0.4**	**1.2**

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

Chamber Monitoring Results
Cumulative Exposure Record
Group IVA - 20,000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
0	23-Oct-01	1	19100	19000	21000	18100	18400	18500					25	52
1	24-Oct-01	2	19900	19980	19300	19700	21500	19400					25	52
2	25-Oct-01	3	19600	20250	22300	19000	19700	20000					25	50
3	26-Oct-01	4	20300	19530	19600	19600	19600	19300					25	49
6	29-Oct-01	5	19600	20250	18500	20400	22300	19800		1.051	1.750	7.17E-04	25	48
7	30-Oct-01	6	20500	20030	19700	20900	20100	19400					25	50
8	31-Oct-01	7	19900	19630	19400	19800	19300	20000					24	50
9	1-Nov-01	8	20200	19580	19600	20100	19100	19500		0.8223	1.803	4.14E-03	25	49
10	2-Nov-01	9	19200	19800	19700	19700	20100	19700					24	54
13	5-Nov-01	10	20200	20250	20400	19400	19800	21400					24	50
14	6-Nov-01	11	19600	19650	20100	19400	19500	19600					26	48
15	7-Nov-01	12	20200	19480	19400	19400	19700	19400					25	51
16	8-Nov-01	13	20700	19680	17100	21400	21400	18800		0.9253	1.866	2.15E-03	25	49
17	9-Nov-01	14	20200	19330	19700	16000	21200	20400					26	47
20	12-Nov-01	15	20400	19630	18900	21400	19300	18900					25	47
21	13-Nov-01	16	19900	19900	18500	20300	20000	20800					24	49
22	14-Nov-01	17	19700	19630	20200	19000	19400	19900					24	51
23	15-Nov-01	18	19100	18880	18000	18800	19300	19400		0.8504	2.121	4.80E-03	24	51
24	16-Nov-01	19	20000	20680	20000	21600	20100	21000					25	48
27	19-Nov-01	20	19400	19050	19500	19400	19700	17600					25	48
		Mean	**19885**		**19708**					**0.912**	**1.885**	**2.95E-03**	**24.8**	**49.7**
		S.D.	**465**		**1044**					**0.102**	**0.164**	**1.87E-03**	**0.6**	**1.8**

Table A GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6129

Chamber Monitoring Results
Cumulative Exposure Record
Group IVB - 20,000 mg/m^3

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(µm)		(mg/m^3)	(°C)	(%)
0	23-Oct-01	1	19100	20630	17700	20500	22700	21600				24	51
1	24-Oct-01	2	19900	19150	20800	18800	18200	18800				24	51
2	25-Oct-01	3	19600	18750	17200	18800	18900	20100				24	51
3	26-Oct-01	4	20300	19330	18500	20100	19600	19100				24	50
6	29-Oct-01	5	19600	18930	20400	17500	19500	18300	8.942	3.141	2.73E-03	24	49
7	30-Oct-01	6	20500	20030	19600	20800	20400	19300				25	49
8	31-Oct-01	7	19900	19500	19100	19800	19200	19900				25	49
9	1-Nov-01	8	20200	19850	19700	20100	19700	19900	0.7851	2.273	5.82E-03	25	48
10	2-Nov-01	9	19200	19750	19700	20200	20200	18900				25	53
13	5-Nov-01	10	20200	20750	20800	20800	21200	20200				25	48
14	6-Nov-01	11	19600	19080	17300	19900	19900	19200				24	48
15	7-Nov-01	12	20200	19880	19900	19400	20000	20200				24	51
16	8-Nov-01	13	20700	21280	18400	22000	23900	20800	0.8337	1.690	2.09E-03	24	49
17	9-Nov-01	14	20200	20050	20800	16000	22000	21400				24	49
20	12-Nov-01	15	20400	20300	19500	20400	20800	20500				24	49
21	13-Nov-01	16	19900	19750	19700	20100	19500	19700				25	49
22	14-Nov-01	17	19700	19600	18200	19700	19900	20600				25	49
23	15-Nov-01	18	19100	20480	20100	20200	20800	20800	0.9512	3.722	1.36E-02	25	49
24	16-Nov-01	19	20000	19080	20500	17400	19000	19400				25	47
27	19-Nov-01	20	19400	18930	17200	20100	20100	18300				25	48
		Mean	**19885**		**19753**				**2.878**	**2.707**	**6.06E-03**	**24.5**	**49.4**
		S.D.	**465**		**1268**				**4.043**	**0.902**	**5.28E-03**	**0.5**	**1.4**

Huntingdon Life Sciences 00-6129I
Immunotoxicity Sub-Group

TABLE B

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

FEMALES — SUMMARY OF CLINICAL OBSERVATIONS

	GROUP#	DAY OF STUDY -11	TOTAL
# OF ANIMALS EXAMINED	1	10	
	2	10	
	3	10	
	4	10	
	5	10	
NORMAL			
WITHIN NORMAL LIMITS	1	10	10
	2	10	10
	3	10	10
	4	10	10
	5	10	10

TABLE C

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

FEMALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV	V
DOSE LEVEL (MG/M3):		0	2000	10000	20000	POSITIVE CONTROL
WEEK -1	MEAN	124	125	125	125	124
	S.D.	5.4	5.6	5.1	5.2	6.3
	N	10	10	10	10	10
WEEK 0	MEAN	189	188	187	187	191
	S.D.	13.9	8.9	7.1	8.2	8.5
	N	10	10	10	10	10
WEEK 1	MEAN	210	210	208	206	217
	S.D.	17.8	8.8	8.4	11.2	10.6
	N	10	10	10	10	10
WEEK 2	MEAN	230	232	231	224	237
	S.D.	17.8	7.1	10.7	10.0	11.6
	N	10	10	10	10	10
WEEK 3	MEAN	250	249	246	238	254
	S.D.	15.8	7.5	9.1	12.8	13.4
	N	10	10	10	10	10
WEEK 4	MEAN	259	263	259	250	247
	S.D.	15.8	10.0	12.6	10.9	12.6
	N	10	10	10	10	10

No statistically significant differences

TABLE D

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

FEMALES — MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

DOSE GROUP:		I	II	III	IV	V
DOSE LEVEL (MG/M3):		0	2000	10000	20000	POSITIVE CONTROL
WEEK 0 TO 1	MEAN	20	22	21	19	26*
	S.D.	4.6	5.1	3.8	5.4	4.9
	N	10	10	10	10	10
WEEK 0 TO 2	MEAN	41	44	44	37	46
	S.D.	5.3	6.1	5.2	5.5	7.7
	N	10	10	10	10	10
WEEK 0 TO 3	MEAN	61	61	59	51*	64
	S.D.	8.1	6.0	5.3	7.3	7.5
	N	10	10	10	10	10
WEEK 0 TO 4	MEAN	70	75	72	63	56**
	S.D.	4.7	9.3	9.1	5.3	7.1
	N	10	10	10	10	10

Statistical key: * = p<0.05 ** = p<0.01

TABLE E

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

FEMALES — MEAN FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

	DOSE GROUP:	I	II	III	IV	V
	DOSE LEVEL (MG/M3):	0	2000	10000	20000	POSITIVE CONTROL
WEEK 0	MEAN	129	132	131	129	132
	S.D.	4.8	10.6	9.3	7.9	8.0
	N	7	10	8	10	10
WEEK 1	MEAN	95	101	103*	98	105**
	S.D.	4.2	7.5	3.3	7.4	4.6
	N	10	10	8	10	10
WEEK 2	MEAN	90	99	97	90	98*
	S.D.	5.1	16.9	6.2	3.2	5.3
	N	10	10	10	10	9
WEEK 3	MEAN	85	87	91	86	91
	S.D.	6.5	4.7	7.5	3.0	8.3
	N	10	10	10	10	10
WEEK 4	MEAN	77	85*	84*	82	73
	S.D.	5.7	5.4	6.2	4.5	5.3
	N	10	9	10	9	10

Statistical key: * = p<0.05 ** = p<0.01

TABLE F

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP I 0 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 1 1
1581	WITHIN NORMAL LIMITS	P
1582	WITHIN NORMAL LIMITS	P
1583	WITHIN NORMAL LIMITS	P
1584	WITHIN NORMAL LIMITS	P
1585	WITHIN NORMAL LIMITS	P
1586	WITHIN NORMAL LIMITS	P
1587	WITHIN NORMAL LIMITS	P
1588	WITHIN NORMAL LIMITS	P
1589	WITHIN NORMAL LIMITS	P
1590	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP II 2000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY 1 1
2571	WITHIN NORMAL LIMITS	P
2572	WITHIN NORMAL LIMITS	P
2573	WITHIN NORMAL LIMITS	P
2574	WITHIN NORMAL LIMITS	P
2575	WITHIN NORMAL LIMITS	P
2576	WITHIN NORMAL LIMITS	P
2577	WITHIN NORMAL LIMITS	P
2578	WITHIN NORMAL LIMITS	P
2579	WITHIN NORMAL LIMITS	P
2580	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP III 10000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY -1 1
3571	WITHIN NORMAL LIMITS	P
3572	WITHIN NORMAL LIMITS	P
3573	WITHIN NORMAL LIMITS	P
3574	WITHIN NORMAL LIMITS	P
3575	WITHIN NORMAL LIMITS	P
3576	WITHIN NORMAL LIMITS	P
3577	WITHIN NORMAL LIMITS	P
3578	WITHIN NORMAL LIMITS	P
3579	WITHIN NORMAL LIMITS	P
3580	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP IV 20000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 1 1
4581	WITHIN NORMAL LIMITS	P
4582	WITHIN NORMAL LIMITS	P
4583	WITHIN NORMAL LIMITS	P
4584	WITHIN NORMAL LIMITS	P
4585	WITHIN NORMAL LIMITS	P
4586	WITHIN NORMAL LIMITS	P
4587	WITHIN NORMAL LIMITS	P
4588	WITHIN NORMAL LIMITS	P
4589	WITHIN NORMAL LIMITS	P
4590	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP V POSITIVE CONTROL

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 1 1
5551	WITHIN NORMAL LIMITS	P
5552	WITHIN NORMAL LIMITS	P
5553	WITHIN NORMAL LIMITS	P
5554	WITHIN NORMAL LIMITS	P
5555	WITHIN NORMAL LIMITS	P
5556	WITHIN NORMAL LIMITS	P
5557	WITHIN NORMAL LIMITS	P
5558	WITHIN NORMAL LIMITS	P
5559	WITHIN NORMAL LIMITS	P
5560	WITHIN NORMAL LIMITS	P

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

TABLE G

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP I 0 MG/M3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
1581	130	191	210	239	261	265
1582	128	198	220	247	261	272
1583	125	202	226	241	249	267
1584	117	172	193	209	233	247
1585	118	181	194	216	238	243
1586	124	178	195	218	254	247
1587	122	176	194	214	230	242
1588	127	206	232	249	271	282
1589	133	209	238	256	271	280
1590	121	178	196	212	233	245
MEAN	124	189	210	230	250	259
S.D.	5.4	13.9	17.8	17.8	15.8	15.8
N	10	10	10	10	10	10

TABLE G

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP II 2000 MG/M3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
2571	127	195	220	231	249	262
2572	119	184	201	220	239	249
2573	128	194	217	239	261	274
2574	123	176	206	228	243	255
2575	123	191	209	240	250	263
2576	129	184	210	227	249	254
2577	127	202	228	243	261	279
2578	116	179	201	228	249	275
2579	121	178	204	227	239	257
2580	135	196	210	232	250	263
MEAN	125	188	210	232	249	263
S.D.	5.6	8.9	8.8	7.1	7.5	10.0
N	10	10	10	10	10	10

Huntingdon Life Sciences 00-6129I
Immunotoxicity Sub-Group

TABLE G

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP III 10000 MG/M3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
3571	130	178	204	222	235	250
3572	129	192	217	241	260	272
3573	119	181	201	229	245	265
3574	126	184	208	225	248	257
3575	122	180	198	224	238	248
3576	127	184	204	217	240	244
3577	117	183	198	224	234	247
3578	123	194	219	242	255	279
3579	133	195	213	242	248	257
3580	121	198	219	248	256	275
MEAN	125	187	208	231	246	259
S.D.	5.1	7.1	8.4	10.7	9.1	12.6
N	10	10	10	10	10	10

TABLE G

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP IV 20000 MG/M3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
4581	127	194	210	224	248	259
4582	121	182	193	217	228	240
4583	126	193	209	222	229	247
4584	132	197	215	236	255	260
4585	131	197	222	237	252	264
4586	128	186	216	234	246	251
4587	124	183	202	221	232	254
4588	121	175	192	212	223	236
4589	118	190	211	231	248	259
4590	117	175	189	208	223	233
MEAN	125	187	206	224	238	250
S.D.	5.2	8.2	11.2	10.0	12.8	10.9
N	10	10	10	10	10	10

TABLE G

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP V POSITIVE CONTROL

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
5551	123	192	217	236	253	246
5552	128	199	224	241	264	260
5553	118	181	199	216	237	229
5554	125	184	214	236	258	252
5555	122	182	206	223	236	242
5556	131	208	233	250	274	267
5557	133	198	230	252	270	261
5558	128	190	218	239	260	239
5559	120	187	221	247	249	232
5560	113	188	210	230	241	242
MEAN	124	191	217	237	254	247
S.D.	6.3	8.5	10.6	11.6	13.4	12.6
N	10	10	10	10	10	10

Huntingdon Life Sciences 00-6129I
Immunotoxicity Sub-Group

TABLE H

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP I 0 MG/M3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
1581	19	48	70	74
1582	22	48	63	74
1583	23	39	47	65
1584	21	37	61	75
1585	13	36	57	62
1586	17	39	75	69
1587	18	38	54	66
1588	26	42	64	75
1589	29	47	62	70
1590	18	34	54	67
MEAN	20	41	61	70
S.D.	4.6	5.3	8.1	4.7
N	10	10	10	10

TABLE H

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP II 2000 MG/M3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
2571	25	36	53	66
2572	16	36	55	65
2573	23	46	67	80
2574	30	51	66	79
2575	18	49	59	72
2576	26	43	65	70
2577	25	41	58	77
2578	21	49	70	96
2579	26	49	62	79
2580	14	36	55	67
MEAN	22	44	61	75
S.D.	5.1	6.1	6.0	9.3
N	10	10	10	10

TABLE H

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP III 10000 MG/M3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
3571	26	44	57	72
3572	25	49	68	80
3573	20	48	64	84
3574	24	41	64	73
3575	17	44	58	68
3576	19	33	56	60
3577	15	41	51	64
3578	25	48	61	85
3579	18	47	53	61
3580	20	49	58	77
MEAN	21	44	59	72
S.D.	3.8	5.2	5.3	9.1
N	10	10	10	10

TABLE H

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP IV 20000 MG/M3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
4581	16	30	54	65
4582	11	36	46	58
4583	16	29	36	54
4584	18	39	59	63
4585	25	40	55	68
4586	30	47	60	65
4587	19	38	49	71
4588	17	37	48	62
4589	21	40	57	68
4590	14	33	47	58
MEAN	19	37	51	63
S.D.	5.4	5.5	7.3	5.3
N	10	10	10	10

TABLE H

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP V POSITIVE CONTROL

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
5551	25	44	61	54
5552	25	42	65	61
5553	18	35	56	48
5554	30	52	74	67
5555	24	41	55	60
5556	25	42	66	59
5557	32	55	73	63
5558	28	49	71	49
5559	34	61	63	46
5560	22	42	53	54
MEAN	26	46	64	56
S.D.	4.9	7.7	7.5	7.1
N	10	10	10	10

Huntingdon Life Sciences 00-6129I
Immunotoxicity Sub-Group

TABLE I

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP I 0 MG/M3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
1581	SF	90	87	86	73
1582	137	102	95	85	79
1583	132	92	88	83	83
1584	130	101	94	93	82
1585	127	93	92	91	84
1586	SF	94	92	94	69
1587	SF	100	96	81	80
1588	130	95	85	78	73
1589	125	93	80	73	68
1590	123	93	91	87	77
MEAN	129	95	90	85	77
S.D.	4.8	4.2	5.1	6.5	5.7
N	7	10	10	10	10

SF=Spilled Feeder

TABLE I

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP II 2000 MG/M3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
2571	142	111	91	87	88
2572	140	100	121	94	89
2573	148	107	98	91	81
2574	144	113	101	94	88
2575	128	96	91	88	SF
2576	117	94	89	82	79
2577	121	90	86	81	74
2578	126	99	85	82	87
2579	129	105	137	90	87
2580	126	97	95	87	88
MEAN	132	101	99	87	85
S.D.	10.6	7.5	16.9	4.7	5.4
N	10	10	10	10	9

SF=Spilled Feeder

TABLE I

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP III 10000 MG/M3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
3571	118	SF	89	103	76
3572	SF	103	93	90	83
3573	146	107	108	105	98
3574	122	98	91	84	79
3575	SF	SF	106	90	83
3576	129	101	92	87	78
3577	133	104	98	90	90
3578	133	104	98	85	86
3579	124	99	95	84	85
3580	140	106	100	89	86
MEAN	131	103	97	91	84
S.D.	9.3	3.3	6.2	7.5	6.2
N	8	8	10	10	10

SF=Spilled Feeder

TABLE I

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP IV 20000 MG/M3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
4581	123	92	87	84	79
4582	120	91	93	85	83
4583	123	95	85	86	86
4584	136	93	88	82	77
4585	147	116	92	87	81
4586	127	98	88	83	79
4587	125	97	91	92	91
4588	127	103	95	90	SF
4589	133	99	92	85	79
4590	128	94	90	84	79
MEAN	129	98	90	86	82
S.D.	7.9	7.4	3.2	3.0	4.5
N	10	10	10	10	9

SF=Spilled Feeder

TABLE I

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP V POSITIVE CONTROL

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
5551	127	104	97	89	71
5552	140	111	107	94	75
5553	143	104	103	107	71
5554	135	110	99	104	76
5555	125	110	97	89	83
5556	128	101	100	91	70
5557	124	100	91	79	66
5558	122	97	91	86	68
5559	132	103	98	87	67
5560	143	104	SF	87	78
MEAN	132	105	98	91	73
S.D.	8.0	4.6	5.3	8.3	5.3
N	10	10	9	10	10

SF=Spilled Feeder

TABLE J

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

ANIMAL TERMINATION HISTORY

FEMALES GROUP I 0 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1581	TERMINAL SACRIFICE	20-NOV-01	4	28
1582	TERMINAL SACRIFICE	20-NOV-01	4	28
1583	TERMINAL SACRIFICE	20-NOV-01	4	28
1584	TERMINAL SACRIFICE	20-NOV-01	4	28
1585	TERMINAL SACRIFICE	20-NOV-01	4	28
1586	TERMINAL SACRIFICE	20-NOV-01	4	28
1587	TERMINAL SACRIFICE	20-NOV-01	4	28
1588	TERMINAL SACRIFICE	20-NOV-01	4	28
1589	TERMINAL SACRIFICE	20-NOV-01	4	28
1590	TERMINAL SACRIFICE	20-NOV-01	4	28

TABLE J

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

ANIMAL TERMINATION HISTORY

FEMALES GROUP II 2000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2571	TERMINAL SACRIFICE	20-NOV-01	4	28
2572	TERMINAL SACRIFICE	20-NOV-01	4	28
2573	TERMINAL SACRIFICE	20-NOV-01	4	28
2574	TERMINAL SACRIFICE	20-NOV-01	4	28
2575	TERMINAL SACRIFICE	20-NOV-01	4	28
2576	TERMINAL SACRIFICE	20-NOV-01	4	28
2577	TERMINAL SACRIFICE	20-NOV-01	4	28
2578	TERMINAL SACRIFICE	20-NOV-01	4	28
2579	TERMINAL SACRIFICE	20-NOV-01	4	28
2580	TERMINAL SACRIFICE	20-NOV-01	4	28

TABLE J

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

ANIMAL TERMINATION HISTORY

FEMALES GROUP III 10000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3571	TERMINAL SACRIFICE	20-NOV-01	4	28
3572	TERMINAL SACRIFICE	20-NOV-01	4	28
3573	TERMINAL SACRIFICE	20-NOV-01	4	28
3574	TERMINAL SACRIFICE	20-NOV-01	4	28
3575	TERMINAL SACRIFICE	20-NOV-01	4	28
3576	TERMINAL SACRIFICE	20-NOV-01	4	28
3577	TERMINAL SACRIFICE	20-NOV-01	4	28
3578	TERMINAL SACRIFICE	20-NOV-01	4	28
3579	TERMINAL SACRIFICE	20-NOV-01	4	28
3580	TERMINAL SACRIFICE	20-NOV-01	4	28

TABLE J

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

ANIMAL TERMINATION HISTORY

FEMALES GROUP IV 20000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4581	TERMINAL SACRIFICE	20-NOV-01	4	28
4582	TERMINAL SACRIFICE	20-NOV-01	4	28
4583	TERMINAL SACRIFICE	20-NOV-01	4	28
4584	TERMINAL SACRIFICE	20-NOV-01	4	28
4585	TERMINAL SACRIFICE	20-NOV-01	4	28
4586	TERMINAL SACRIFICE	20-NOV-01	4	28
4587	TERMINAL SACRIFICE	20-NOV-01	4	28
4588	TERMINAL SACRIFICE	20-NOV-01	4	28
4589	TERMINAL SACRIFICE	20-NOV-01	4	28
4590	TERMINAL SACRIFICE	20-NOV-01	4	28

TABLE J

GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

ANIMAL TERMINATION HISTORY

FEMALES GROUP V POSITIVE CONTROL

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
5551	TERMINAL SACRIFICE	20-NOV-01	4	28
5552	TERMINAL SACRIFICE	20-NOV-01	4	28
5553	TERMINAL SACRIFICE	20-NOV-01	4	28
5554	TERMINAL SACRIFICE	20-NOV-01	4	28
5555	TERMINAL SACRIFICE	20-NOV-01	4	28
5556	TERMINAL SACRIFICE	20-NOV-01	4	28
5557	TERMINAL SACRIFICE	20-NOV-01	4	28
5558	TERMINAL SACRIFICE	20-NOV-01	4	28
5559	TERMINAL SACRIFICE	20-NOV-01	4	28
5560	TERMINAL SACRIFICE	20-NOV-01	4	28

GFAP Levels in Specific Rat Brain Areas Following a 13-Week Whole-Body Inhalation Exposure to Gasoline ETBE Vapor Condensate

HLS Study No.: **00-6129**
Sponsor Study No.: 211-ETBE-S
Date: 28 July 2010