FINAL REPORT

Immunotoxicological Evaluation of Gasoline Ethanol Vapor Condensate Using the Plaque-Forming Cell Assay

Test Substance:	Gasoline Ethanol Vapor Condensate
Protocol No:	HLS 00-6127
Subcontractor's Sponsor:	Huntingdon Life Sciences Mettlers Road East Millstone, NJ 08875 (732) 873-2550 *Phone* (732) 873-3992 *Fax*
Contractor's Study Director:	Gary M. Hoffman, B.A., D.A.B.T.
Sponsor:	American Petroleum Institute 1200 L Street, NW Washington, DC 20005
Sponsor's Representative:	Thomas Gray, M.S., D.A.B.T.
ImmunoTox®, Inc. Project Number:	ITI 701
Security:	Industrial Confidential
Date:	18 September 2009
Principal Investigator:	Kimber L. White, Jr., Ph.D.
Studies conducted at:	**ImmunoTox®, Inc.** Virginia Bio•Technology Research Park 800 East Leigh Street, Suite 209 Richmond, VA 23219-1534 (804) 828-6880 *Phone* (804) 828-6881 *Fax*

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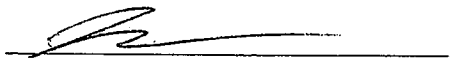
I. STATEMENT OF COMPLIANCE

This study was conducted in compliance with the United States Environmental Protection Agency's (EPA) Good Laboratory Practice Standards 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.



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

27 Jan 10
Date

II. QUALITY ASSURANCE STATEMENT

Test Substance: Gasoline Ethanol Vapor Condensate

Report Title: Immunotoxicological Evaluation of Gasoline Ethanol Vapor Condensate Using the Plaque-Forming Cell Assay

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

Huntingdon Life Sciences, Inc. Study No. 00-6127
Sponsor Study No. 211-EtOH-S

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 via the Principal Investigator.

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

Performed	Reported	Activity
June 20, 2001	June 20, 2001	AFC Assay
October 31-November 6, 2001	November 8, 2001	Data Audit
November 5-7, 2001	November 8, 2001	1st Draft Report

Approved and submitted by: _____ 16 September 09
Quality Assurance Manager — Date

Office of Research * Quality Assurance Unit * Box 980568 * Richmond, Virginia 23298 0568 * (804) 828-6587 * Fax (804) 828-5604

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
GLP Protocol Review	1 & 2 Feb 01	8 Feb 01
Dose Immunotoxicity Animals	15 Jun 01	15 Jun 01
Exposure & Monitoring	15 Jun 01	15 Jun 01
Immunotoxicity Necropsy	19 Jun 01	21 Jun 01

Fran Jannone — 26 Jan 10

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

Date

III. SIGNATURE OF PRINCIPALS

This report describes the results used to evaluate the relative immunotoxicological potential of the test substance, Gasoline Ethanol 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.

Date 18 Sep 09

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

Date 18 Sep 09

Approved:

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

27 Jan 10
Date

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IV. EXECUTIVE SUMMARY

The study was conducted as part of Huntingdon Life Sciences (HLS) Study No. 00-6127 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 Ethanol 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 Ethanol 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 resulted in no statistically significant changes in body weight for any exposure level. Furthermore, there were no statistically significant effects observed in either thymus or spleen weight following exposure to Gasoline Ethanol Vapor Condensate, when evaluated as either absolute or relative weight (% body weight), as compared to the air control.

Exposure to Gasoline Ethanol Vapor Condensate did result in a statistically significant dose-related decrease in the IgM antibody-forming cell (AFC) response to the T-dependent antigen, sheep erythrocytes, when evaluated as either specific activity (AFC/10^6 spleen cells) or as total spleen activity (AFC/spleen). The decrease reached the level of statistical significance at the high (20,000 mg/m^3) exposure level.

In conclusion, the results of this immunotoxicological evaluation demonstrate that, under the experimental conditions used, exposure to the Gasoline Ethanol 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	85%	20,000
IgM AFC/Spleen ($x10^3$)	Decrease	86%	20,000

V. INTRODUCTION

The purpose of this study was to provide evaluation of immunological parameters for Huntingdon Life Sciences (HLS) Study No. 00-6127. In this study, the test substance, Gasoline 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. The study was conducted in female animals because female rats have a more robust immune response than do the male animal of the species. Accordingly, female rats have a greater sensitivity for detecting an adverse effect of a compound should one occur. Routinely, immunotoxicology evaluations conducted by the National Toxicology Program (NTP) evaluate compounds only in female animals. 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 20 June 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 Ethanol 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]).

Kimber L. White, Jr., Ph.D., was the Principal Investigator for the immunological evaluation conducted by ImmunoTox®, Inc., and Gary M. Hoffman, B.A., D.A.B.T., 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 Ethanol Vapor Condensate on the immune system, the immunologic and toxicologic parameters evaluated were spleen and 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 Ethanol Vapor Condensate.

VI. METHODS OF PROCEDURE

EXPERIMENTAL DESIGN

The immunotoxicological satellite study consisted of a vehicle group, three exposure levels of Gasoline Ethanol 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 Ethanol 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. Cyclophosphamide (CAS #6055-19-2, lot number 108H0568, received 28 February 2001, expiration 30 June 2001, white powder, storage 2-8°C, purity 99.2%), was obtained from the Sigma Chemical Company (responsible for its characterization), and 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. The terminal body weights were obtained by Huntingdon Life Sciences PRC personnel. 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 (≤-70°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. 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].

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 number, following lysis of RBC, was performed on the 6-ml samples using a Model Z1 Coulter Counter. The spleen weight, cells/spleen, AFC/10^6 spleen cells, and AFC/spleen were determined. The plaques, which developed, were counted using a Bellco plaque viewer. For each spleen, 2 dilutions (1:50 and 1:150) were prepared. At the time of counting, each plate was examined. Routinely, the plate that had between 100-300 plaques was counted. When the number of plaques is in excess of 350 plaques per plate, it becomes difficult to obtain an accurate count using the Bellco 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 (SOP/CSA/006). 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 and charts, 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. Records maintained for this protocol include: study sheet, chemical preparation form, and authorized signatures and initials forms. 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. No statistically significant differences were observed in terminal body weights of the Gasoline Ethanol Vapor Condensate exposed animals at any exposure level as compared to the vehicle (air only) controls. Treatment with the positive control, Cyclophosphamide (CPS), produced a significant decrease (8%) in terminal body weight as compared to the vehicle control.

The organ weights of the control and Test Substance-exposed rats are shown in Table 1. No effect was observed, following exposure to Gasoline Ethanol 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 57% on absolute spleen weight and a significant decrease of 80% on absolute thymus weight, compared to the vehicle control. In addition, the positive control, cyclophosphamide, had a significant decrease of 53% 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 Ethanol Vapor Condensate.

Figure 1

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

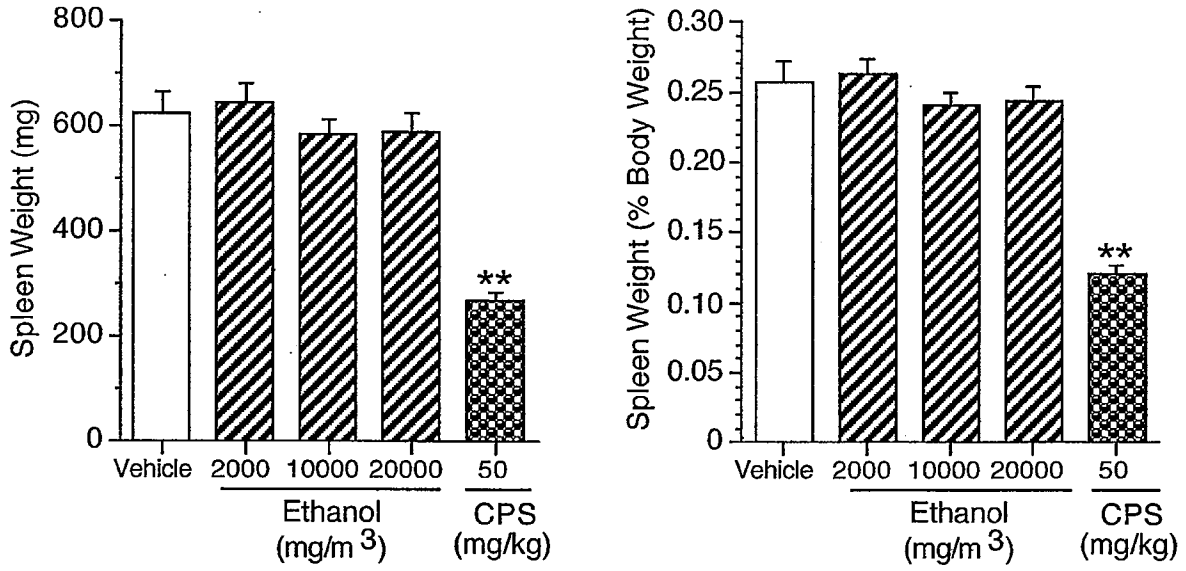
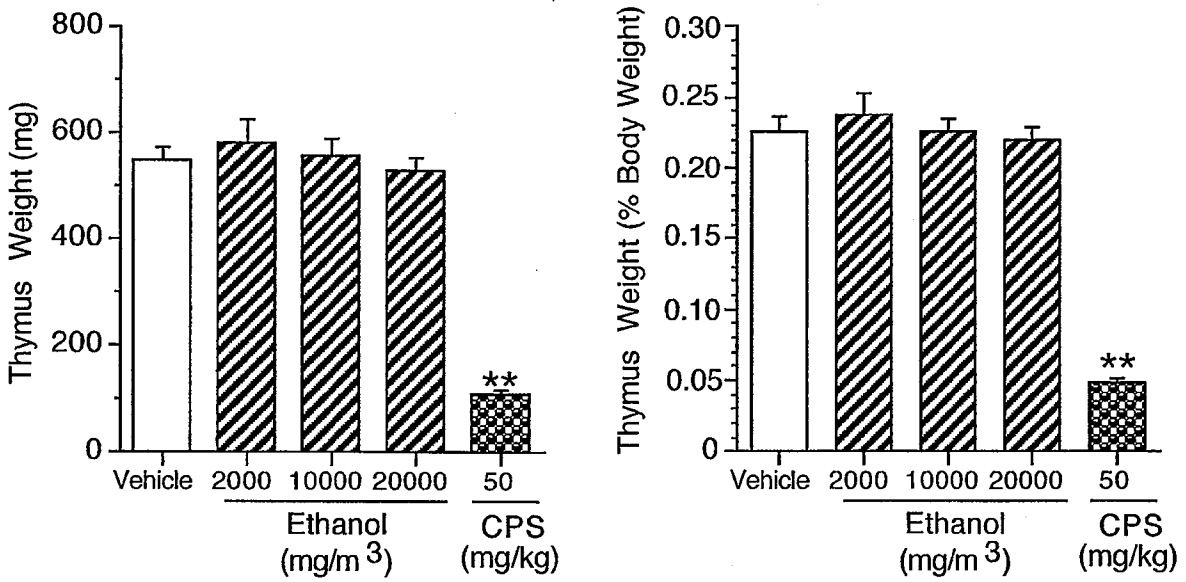


Figure 2

Absolute (mg) and Relative (%) Thymus Weight in Female Sprague Dawley Rats Exposed to Gasoline Ethanol 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. The viabilities from all samples were greater than 84%.

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 Ethanol 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 Ethanol Vapor Condensate as compared to the vehicle control group. As expected, the positive control, cyclophosphamide (CPS), produced an 84% decrease in spleen cell number when compared to the vehicle control group.

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 dose-related 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 Ethanol Vapor Condensate produced a reduced response at all exposure levels; however, only the high exposure level, 20,000 mg/m^3, reached the level of statistical significance. When evaluated as specific activity the response of the high dose group was suppressed 85% and 86% when the high dose group was evaluated as total spleen activity.

Figure 3

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

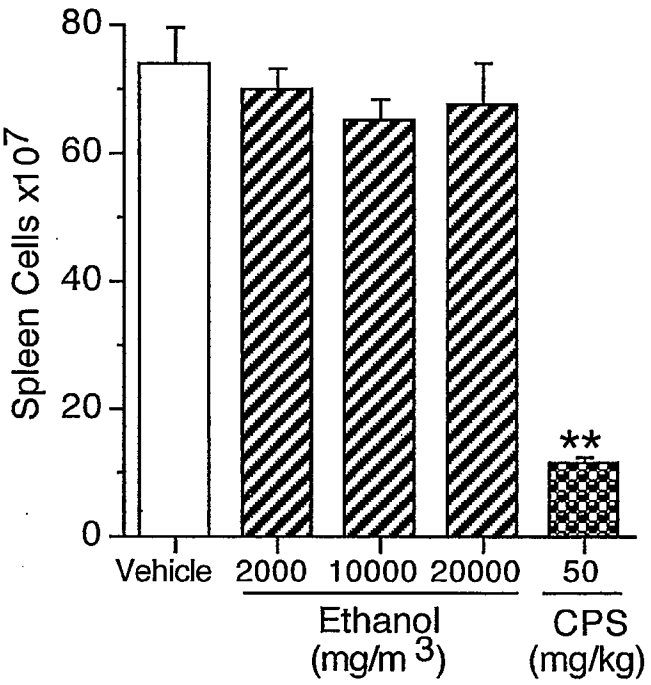
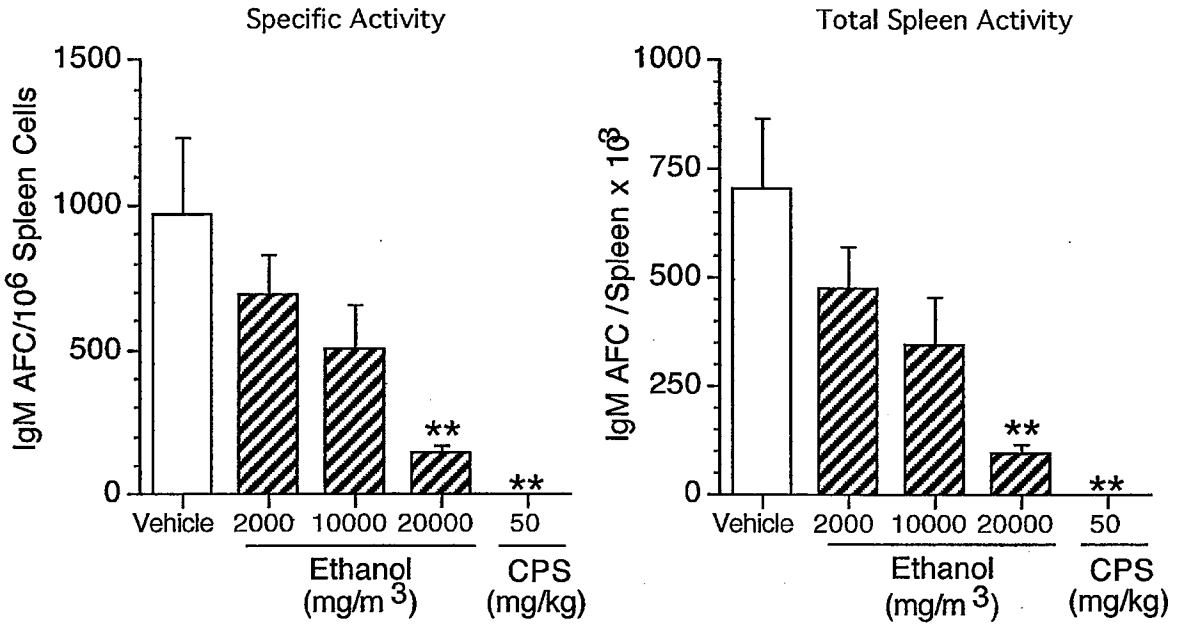


Figure 4

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



The suppression observed following Gasoline Ethanol Vapor Condensate exposure is not unexpected, for ethanol has been shown by numerous researchers to suppress the humoral immune response[10]. 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.

VIII. CONCLUSION

Exposure of female Sprague Dawley rats to Gasoline Ethanol Vapor Condensate for a period of 5 days per week for 4 weeks resulted in a dose-related decrease in the humoral immune response to the T-dependent antigen, sheep erythrocytes. The decrease was not unexpected based on the known immunotoxicity of ethanol. Although the 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 Ethanol Vapor Condensate adversely affected the humoral immune response of female Sprague Dawley rats.

IX. REFERENCES

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Table 1

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

00-6127

Parameter	Vehicle	Gasoline Ethanol Vapor (mg/m^3)			Cyclophosphamide	H/NH	Trend Analysis
		2000	10000	20000	50 mg/kg		
	(10)	(10)	(10)	(10)	(10)		
Body Wgt (g)	242.1 ± 4.4	243.8 ± 6.7	243.7 ± 4.9	240.1 ± 7.2	223.2 ± 7.6*	H	NS
Spleen (mg)	625 ± 39	645 ± 38	586 ± 28	591 ± 34	271 ± 14**	H	NS
% Body Wgt	0.257 ± 0.015	0.263 ± 0.010	0.240 ± 0.009	0.244 ± 0.010	0.121 ± 0.005**	H	$p \leq 0.05$
Thymus (mg)	549 ± 24	581 ± 44	557 ± 31	528 ± 24	110 ± 9**	H	NS
% Body Wgt	0.226 ± 0.010	0.238 ± 0.015	0.226 ± 0.009	0.219 ± 0.009	0.048 ± 0.003**	H	NS

Female Sprague Dawley rats were administered vehicle control (air only) or gasoline ethanol 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. 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:
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 Ethanol Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks - Day 4 Response

00-6127

Exposure	Body Wgt (g)	Spleen Wgt (mg)	Spleen Cells ($x10^7$)	IgM AFC/ 10^6 Spleen Cells	IgM AFC/Spleen ($x\ 10^3$)
Vehicle	242.1 ± 4.4 (10)	625 ± 39 (10)	74.34 ± 5.29 (10)	974 ± 259 (10)	705 ± 161 (10)
Gasoline Ethanol Vapor Condensate					
2000 mg/m^3	243.8 ± 6.7 (10)	645 ± 38 (10)	70.36 ± 2.86 (10)	692 ± 137 (10)	477 ± 93 (10)
10000 mg/m^3	243.7 ± 4.9 (10)	586 ± 28 (10)	65.44 ± 3.32 (10)	505 ± 148 (10)	347 ± 111 (10)
20000 mg/m^3	240.1 ± 7.2 (10)	591 ± 34 (10)	67.97 ± 6.29 (10)	143 ± 29** (10)	98 ± 18** (10)
Cyclophosphamide 50 mg/kg	223.2 ± 7.6* (10)	271 ± 14** (10)	11.60 ± 0.86** (10)	0 ± 0** (10)	0 ± 0** (10)
H/NH	H	H	H	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 ethanol 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 Ethanol Vapor Condensate
HLS Study No. 00-6127

Animal No.	Group	Dose	Sex	Body Weight (g)	Spleen (mg)	Thymus (mg)	Spleen / % Body Weight	Thymus / % Body Weight
1531	GI	AIR ONLY	F	259.0	529	509	0.200	0.200
1532	GI	AIR ONLY	F	243.6	516	544	0.210	0.220
1533	GI	AIR ONLY	F	228.8	608	663	0.270	0.290
1534	GI	AIR ONLY	F	264.0	779	658	0.300	0.250
1535	GI	AIR ONLY	F	247.2	663	553	0.270	0.220
1536	GI	AIR ONLY	F	220.6	489	540	0.220	0.240
1537	GI	AIR ONLY	F	242.8	705	568	0.290	0.230
1538	GI	AIR ONLY	F	237.7	743	401	0.310	0.170
1539	GI	AIR ONLY	F	227.8	444	501	0.190	0.220
1540	GI	AIR ONLY	F	249.2	769	548	0.310	0.220
2521	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	246.7	653	642	0.260	0.260
2522	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	217.4	536	560	0.250	0.260
2523	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	234.3	512	494	0.220	0.210
2524	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	275.6	886	746	0.320	0.270
2525	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	249.1	746	473	0.300	0.190
2526	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	265.2	665	468	0.250	0.180
2527	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	230.3	602	542	0.260	0.240
2528	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	207.8	487	425	0.230	0.200
2529	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	255.6	656	591	0.260	0.230
2530	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	256.3	708	873	0.280	0.340
3521	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	228.3	422	449	0.180	0.200
3522	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	225.9	471	508	0.210	0.220
3523	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	244.8	581	499	0.240	0.200
3524	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	248.0	731	624	0.290	0.250
3525	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	270.6	636	791	0.240	0.290
3526	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	228.0	603	483	0.260	0.210
3527	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	253.3	576	507	0.230	0.200
3528	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	254.3	625	565	0.250	0.220
3529	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	227.2	572	547	0.250	0.240
3530	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	256.4	644	601	0.250	0.230
4531	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	239.9	691	536	0.290	0.220
4532	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	235.7	657	561	0.280	0.240
4533	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	279.0	704	622	0.250	0.220
4534	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	218.3	433	486	0.200	0.220
4535	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	265.0	698	565	0.260	0.210
4536	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	214.6	438	339	0.200	0.160
4537	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	224.9	606	557	0.270	0.250
4538	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	231.7	536	586	0.230	0.250
4539	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	268.7	653	522	0.240	0.190
4540	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	222.9	492	503	0.220	0.230

Individual Animal Data – Organ Weights (CONT'D.)
Gasoline Ethanol Vapor Condensate
HLS Study No. 00-6127

Animal No.	Group	Dose	Sex	Body Weight (g)	Spleen (mg)	Thymus (mg)	Spleen / % Body Weight	Thymus / % Body Weight
5531	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	215.0	290	104	0.130	0.050
5532	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	206.5	264	110	0.130	0.050
5533	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	268.7	319	160	0.120	0.060
5534	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	260.4	294	150	0.110	0.060
5535	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	226.8	195	84	0.090	0.040
5536	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	226.4	324	126	0.140	0.060
5537	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	218.5	279	95	0.130	0.040
5538	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	199.0	197	88	0.100	0.040
5539	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	197.4	278	64	0.140	0.030
5540	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	213.1	266	114	0.120	0.050

KEY:

G=GRAMS, MG=MILLIGRAMS, M^3=CUBIC METER OF AIR, KG=KILOGRAMS, WT=WEIGHT

Individual Animal Data – AFC
Gasoline Ethanol Vapor Condensate
HLS Study No. 00-6127

Animal No.	Group	Dose	Sex	IgM AFC/ 10^6 Spleen Cells	IgM AFC/ Spleen x 10^3	Cells/ Spleen x10^7	Spleen (mg)	Body Weight (g)
1531	GI	AIR ONLY	F	566	339	59.88	529	259.0
1532	GI	AIR ONLY	F	226	141	62.52	516	243.6
1533	GI	AIR ONLY	F	411	342	83.22	608	228.8
1534	GI	AIR ONLY	F	1428	1323	92.64	779	264.0
1535	GI	AIR ONLY	F	1010	687	68.04	663	247.2
1536	GI	AIR ONLY	F	2962	1674	56.52	489	220.6
1537	GI	AIR ONLY	F	573	546	95.28	705	242.8
1538	GI	AIR ONLY	F	1473	1116	75.78	743	237.7
1539	GI	AIR ONLY	F	403	213	52.86	444	227.8
1540	GI	AIR ONLY	F	692	669	96.66	769	249.2
2521	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	378	294	77.70	653	246.7
2522	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	270	189	70.08	536	217.4
2523	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	505	315	62.40	512	234.3
2524	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	890	750	84.30	886	275.6
2525	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	872	645	73.98	746	249.1
2526	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	1358	879	64.74	665	265.2
2527	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	666	375	56.28	602	230.3
2528	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	1377	903	65.58	487	207.8
2529	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	511	339	66.36	656	255.6
2530	GII	2000 MG/M^3 GASOLINE ETOH VAPOR	F	99	81	82.20	708	256.3
3521	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	217	108	49.68	422	228.3
3522	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	367	168	45.72	471	225.9
3523	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	60	39	65.22	581	244.8
3524	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	212	141	66.60	731	248.0
3525	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	734	570	77.64	636	270.6
3526	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	274	192	70.02	603	228.0
3527	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	181	120	66.42	576	253.3
3528	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	466	354	75.90	625	254.3
3529	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	937	594	63.42	572	227.2
3530	GIII	10000 MG/M^3 GASOLINE ETOH VAPOR	F	1598	1179	73.80	644	256.4
4531	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	49	42	85.62	691	239.9
4532	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	158	135	85.20	657	235.7
4533	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	133	123	92.16	704	279.0
4534	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	13	6	45.12	433	218.3
4535	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	107	102	95.40	698	265.0
4536	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	43	18	42.18	438	214.6
4537	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	249	147	58.98	606	224.9
4538	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	221	129	58.38	536	231.7
4539	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	279	180	64.44	653	268.7
4540	GIV	20000 MG/M^3 GASOLINE ETOH VAPOR	F	178	93	52.26	492	222.9

Individual Animal Data – AFC (CONT'D.)
Gasoline Ethanol Vapor Condensate
HLS Study No. 00-6127

Animal No.	Group	Dose	Sex	IgM AFC/ 10^6 Spleen Cells	IgM AFC/ Spleen x 10^3	Cells/ Spleen x10^7	Spleen (mg)	Body Weight (g)
5531	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	14.16	290	215.0
5532	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	11.76	264	206.5
5533	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	15.66	319	268.7
5534	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	11.52	294	260.4
5535	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	7.26	195	226.8
5536	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	14.22	324	226.4
5537	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	11.64	279	218.5
5538	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	7.62	197	199.0
5539	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	11.70	278	197.4
5540	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	10.50	266	213.1

KEY:

G=GRAMS, MG=MILLIGRAMS, M^3=CUBIC METER OF AIR, KG=KILOGRAMS

APPENDIX B

CONTRACTING SPONSOR'S EXPOSURE AND ANIMAL DATA

	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:	8 May 2001
Experimental Initiation Date:	22 May 2001 (in-life)
Experimental Completion Date:	19 June 2001 (in-life)

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.

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Table A GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE BODY INHALATION TOXICITY STUDY IN RATS 00-6127

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
35	22-May-01	1	0	0	0	0	0	0				24	50
36	23-May-01	2	0	0	0	0	0	0				24	50
37	24-May-01	3	0	0	0	0	0	0	0.8431	1.531	3.16E-03	24	49
38	25-May-01	4	0	0	0	0	0	0				24	50
41	28-May-01	5	0	0	0	0	0	0				24	52
42	29-May-01	6	0	0	0	0	0	0				25	50
43	30-May-01	7	0	0	0	0	0	0				25	47
44	31-May-01	8	0	0	0	0	0	0	8.759	2.355	2.40E-03	25	47
45	1-Jun-01	9	0	0	0	0	0	0				25	46
48	4-Jun-01	10	0	0	0	0	0	0				25	45
49	5-Jun-01	11	0	0	0	0	0	0				24	48
50	6-Jun-01	12	0	0	0	0	0	0				23	51
51	7-Jun-01	13	0	0	0	0	0	0	2.067	2.172	2.13E-03	24	44
52	8-Jun-01	14	0	0	0	0	0	0				24	45
55	11-Jun-01	15	0	0	0	0	0	0				24	48
56	12-Jun-01	16	0	0	0	0	0	0				26	50
57	13-Jun-01	17	0	0	0	0	0	0				25	50
58	14-Jun-01	18	0	0	0	0	0	0	0.8592	1.676	5.00E-02	25	50
59	15-Jun-01	19	0	0	0	0	0	0				25	50
62	18-Jun-01	20	0	0	0	0	0	0				25	47
		Mean	0		0				3.132	1.934	1.44E-02	24.5	48.5
		S.D.	0		0				3.795	0.393	2.37E-02	0.7	2.2

Table A GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE BODY INHALATION TOXICITY STUDY IN RATS 00-6127

Chamber Monitoring Results
Cumulative Exposure Record
Group IB - 0 mg/m³ (Air Control)

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
				Mean	Individual								Mean Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)					MMAD (μm)	GSD	TMC (mg/m³)	(°C)	(%)
35	22-May-01	1	0	0	0	0	0	0					25	48
36	23-May-01	2	0	0	0	0	0	0					25	47
37	24-May-01	3	0	0	0	0	0	0		0.8692	1.676	3.48E-03	25	47
38	25-May-01	4	0	0	0	0	0	0					24	47
41	28-May-01	5	0	0	0	0	0	0					24	49
42	29-May-01	6	0	0	0	0	0	0					24	49
43	30-May-01	7	0	0	0	0	0	0					24	44
44	31-May-01	8	0	0	0	0	0	0		1.557	1.915	7.43E-04	24	45
45	1-Jun-01	9	0	0	0	0	0	0					25	44
48	4-Jun-01	10	0	0	0	0	0	0					24	44
49	5-Jun-01	11	0	0	0	0	0	0					25	46
50	6-Jun-01	12	0	0	0	0	0	0					24	49
51	7-Jun-01	13	0	0	0	0	0	0		1.434	1.602	1.49E-03	24	43
52	8-Jun-01	14	0	0	0	0	0	0					25	44
55	11-Jun-01	15	0	0	0	0	0	0					25	46
56	12-Jun-01	16	0	0	0	0	0	0					25	49
57	13-Jun-01	17	0	0	0	0	0	0					25	48
58	14-Jun-01	18	0	0	0	0	0	0		0.8688	1.854	5.01E-02	25	49
59	15-Jun-01	19	0	0	0	0	0	0					24	50
62	18-Jun-01	20	0	0	0	0	0	0					25	46
		Mean	0		0					1.182	1.762	1.40E-02	24.6	46.7
		S.D.	0		0					0.365	0.147	2.41E-02	0.5	2.2

Table A GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE BODY INHALATION TOXICITY STUDY IN RATS 00-6127

Chamber Monitoring Results
Cumulative Exposure Record
Group IIA - 2,000 mg/m³

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
35	22-May-01	1	2990	1978	2070	1990	1960	1890				23	50
36	23-May-01	2	2940	2018	2180	1920	1910	2060				23	50
37	24-May-01	3	3010	1978	2050	1740	1930	2190	0.8657	1.945	5.90E-03	23	51
38	25-May-01	4	3160	1948	2020	1860	1720	2190				23	52
41	28-May-01	5	3060	1913	1670	2070	1870	2040				23	52
42	29-May-01	6	3030	2085	2390	1910	2090	1950				24	50
43	30-May-01	7	3000	2065	2150	2200	1920	1990				24	45
44	31-May-01	8	2820	2318	2680	2070	2330	2190	2.020	1.763	1.49E-03	24	46
45	1-Jun-01	9	3000	2043	1870	1900	2050	2350				24	44
48	4-Jun-01	10	2900	2135	2360	1870	2240	2070				24	44
49	5-Jun-01	11	3010	2083	1900	2190	2170	2070				24	47
50	6-Jun-01	12	3030	2083	1930	2070	2260	2070				23	51
51	7-Jun-01	13	3140	2035	1870	1980	2170	2120	2.037	2.179	2.23E-03	23	44
52	8-Jun-01	14	3050	2078	2090	2000	2060	2160				24	46
55	11-Jun-01	15	3240	2180	2060	2180	2180	2300				24	48
56	12-Jun-01	16	2850	2125	1920	2320	2360	1900				25	49
57	13-Jun-01	17	2930	1898	1710	1750	2150	1980				24	48
58	14-Jun-01	18	3200	2023	2090	1960	1960	2080	0.8687	1.992	5.26E-02	24	49
59	15-Jun-01	19	3150	2165	2020	2160	2270	2210				23	51
62	18-Jun-01	20	3200	2025	2070	1790	1980	2260				24	47
		Mean	3036		2059				1.448	1.970	1.56E-02	23.7	48.2
		S.D.	117		178				0.671	0.171	2.48E-02	0.6	2.7

Table A GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE BODY INHALATION TOXICITY STUDY IN RATS 00-6127

Chamber Monitoring Results
Cumulative Exposure Record
Group IIB - 2,000 mg/m³

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
35	22-May-01	1	2990	1963	1720	2070	2070	1990				23	49
36	23-May-01	2	2940	2035	2180	1920	1970	2070				23	48
37	24-May-01	3	3010	2005	2190	1890	2140	1800	0.7828	1.492	4.50E-03	23	49
38	25-May-01	4	3160	2025	1840	2210	1790	2260				23	49
41	28-May-01	5	3060	1805	1680	1830	1820	1890				23	51
42	29-May-01	6	3030	2123	2400	2020	2110	1960				24	49
43	30-May-01	7	3000	2155	2190	2040	2150	2240				24	46
44	31-May-01	8	2820	2375	2770	2250	2300	2180	1.726	1.913	1.02E-03	24	46
45	1-Jun-01	9	3000	2043	1890	1890	2070	2320				24	44
48	4-Jun-01	10	2900	2163	2160	1900	2350	2240				24	44
49	5-Jun-01	11	3010	1968	1730	2080	2100	1960				24	46
50	6-Jun-01	12	3030	2010	1710	1820	2330	2180				23	51
51	7-Jun-01	13	3140	2103	1850	1960	2200	2400	1.979	1.925	2.29E-03	24	43
52	8-Jun-01	14	3050	2113	2090	2060	2090	2210				24	44
55	11-Jun-01	15	3240	2128	2200	2130	2170	2010				24	46
56	12-Jun-01	16	2850	2200	2180	2110	2400	2110				25	49
57	13-Jun-01	17	2930	1863	1940	1850	1940	1720				25	48
58	14-Jun-01	18	3200	2213	2320	2090	2110	2330	2.246	2.712	9.40E-02	24	50
59	15-Jun-01	19	3150	2210	2120	2130	2310	2280				24	51
62	18-Jun-01	20	3200	1985	1850	1970	1990	2130				25	46
		Mean	3036		2074				1.683	2.011	2.55E-02	23.9	47.5
		S.D.	117		198				0.637	0.509	4.57E-02	0.7	2.5

Table A GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE BODY INHALATION TOXICITY STUDY IN RATS 00-6127

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIA - 10,000 mg/m³

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
35	22-May-01	1	12500	10170	9920	9950	10600	10200				24	50
36	23-May-01	2	12600	10160	9950	9690	10400	10600				24	49
37	24-May-01	3	12400	10230	9720	10200	10300	10700	0.7874	1.582	5.47E-03	24	50
38	25-May-01	4	12600	10210	9820	10200	10400	10400				24	49
41	28-May-01	5	12600	9310	7950	9950	9490	9850				24	51
42	29-May-01	6	11900	10380	10300	10500	10200	10500				24	49
43	30-May-01	7	12200	10780	10900	10400	10600	11200				24	46
44	31-May-01	8	10400	11120	13000	11200	10500	9790	2.164	1.913	1.58E-03	24	45
45	1-Jun-01	9	11800	9905	8490	11500	9130	10500				24	45
48	4-Jun-01	10	12000	10060	10100	10300	9620	10200				24	44
49	5-Jun-01	11	12300	10040	9790	10300	10100	9950				25	47
50	6-Jun-01	12	12100	9930	9690	9950	10200	9880				24	49
51	7-Jun-01	13	12300	10350	10600	10300	10200	10300	1.591	1.735	2.40E-03	24	43
52	8-Jun-01	14	12400	10180	10400	10000	10100	10200				24	45
55	11-Jun-01	15	12500	10450	9690	10600	10400	11100				24	47
56	12-Jun-01	16	11700	10550	11300	9790	10100	11000				25	48
57	13-Jun-01	17	12000	9808	9320	9790	9620	10500				25	48
58	14-Jun-01	18	12500	9908	10700	9720	9690	9520	0.8862	1.735	4.44E-02	24	50
59	15-Jun-01	19	12200	10180	10200	10500	10000	10000				24	50
62	18-Jun-01	20	12300	10070	10600	10200	9360	10100				25	45
		Mean	12170		10190				1.357	1.741	1.35E-02	24.2	47.5
		S.D.	496		640				0.646	0.135	2.07E-02	0.4	2.4

Table A GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE BODY INHALATION TOXICITY STUDY IN RATS 00-6127

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIB - 10,000 mg/m³

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
35	22-May-01	1	12500	10480	10200	11000	10300	10400				24	49
36	23-May-01	2	12600	10400	10100	10100	10800	10600				24	49
37	24-May-01	3	12400	10900	10000	10800	11800	11000	0.7889	1.540	5.63E-03	24	50
38	25-May-01	4	12600	10090	10300	9850	10200	10000				24	49
41	28-May-01	5	12600	9680	9825	9590	9490	9820				24	50
42	29-May-01	6	11900	10110	10100	10400	9820	10100				25	49
43	30-May-01	7	12200	10020	9490	10100	10400	10100				24	46
44	31-May-01	8	10400	10600	12800	10600	10100	8900	2.518	2.423	2.13E-03	25	46
45	1-Jun-01	9	11800	9710	8050	10700	9490	10600				25	45
48	4-Jun-01	10	12000	10380	10500	10700	9620	10700				25	44
49	5-Jun-01	11	12300	10630	10600	10700	10800	10400				24	46
50	6-Jun-01	12	12100	10040	9590	9850	10600	10100				24	49
51	7-Jun-01	13	12300	10220	10200	10700	10000	9980	2.076	1.926	2.14E-03	24	42
52	8-Jun-01	14	12400	10240	10200	9950	10400	10400				24	43
55	11-Jun-01	15	12500	10510	9130	10800	10600	11500				24	47
56	12-Jun-01	16	11700	10700	11800	10100	10300	10600				26	49
57	13-Jun-01	17	12000	10250	10200	10100	10600	10100				25	49
58	14-Jun-01	18	12500	9993	10200	10100	9820	9850	0.8732	1.657	4.46E-02	25	50
59	15-Jun-01	19	12200	10080	9790	9920	10100	10500				24	51
62	18-Jun-01	20	12300	10360	9850	10100	10400	11100				25	46
		Mean	12170		10270				1.564	1.887	1.36E-02	24.5	47.5
		S.D.	496		631				0.866	0.392	2.07E-02	0.6	2.6

Table A GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE BODY INHALATION TOXICITY STUDY IN RATS 00-6127

Chamber Monitoring Results
Cumulative Exposure Record
Group IVA - 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)	(%)
35	22-May-01	1	25900	20400	19300	20600	21800	19900				24	52
36	23-May-01	2	26100	21300	22700	20600	20600	21300				24	49
37	24-May-01	3	25700	20350	21400	19600	20400	20000	4.411	2.361	2.14E-02	24	51
38	25-May-01	4	26500	21030	21400	21300	20800	20600				24	50
41	28-May-01	5	25600	18430	17500	18100	19000	19100				24	53
42	29-May-01	6	26800	20630	21200	21000	20400	19900				25	51
43	30-May-01	7	26600	20000	20100	19800	20000	20100				25	52
44	31-May-01	8	22900	21830	25100	21000	20300	20900	1.555	1.642	1.49E-03	25	48
45	1-Jun-01	9	23900	18430	17000	18100	19000	19600				25	46
48	4-Jun-01	10	26200	20650	20100	20800	20800	20900				25	46
49	5-Jun-01	11	26700	21150	20100	21600	22300	20600				24	49
50	6-Jun-01	12	27000	20780	19200	20600	21900	21400				23	54
51	7-Jun-01	13	25600	20980	21400	20900	20500	21100	1.392	1.555	1.10E-03	24	45
52	8-Jun-01	14	25300	21300	22400	22000	20400	20400				24	45
55	11-Jun-01	15	24900	20400	21100	20100	19600	20800				24	50
56	12-Jun-01	16	23600	20980	22700	19800	21300	20100				26	51
57	13-Jun-01	17	24600	20550	20200	21100	20800	20100				25	51
58	14-Jun-01	18	25000	20150	20000	19600	20200	20800	0.8922	1.727	4.61E-02	25	54
59	15-Jun-01	19	25200	20780	20800	21600	20100	20600				25	53
62	18-Jun-01	20	25000	19380	17400	19900	20500	19700				25	48
		Mean	25460		20470				2.063	1.821	1.75E-02	24.5	49.9
		S.D.	1109		1204				1.591	0.367	2.13E-02	0.7	2.8

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

Chamber Monitoring Results
Cumulative Exposure Record
Group IVB - 20,000 mg/m³

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
35	22-May-01	1	25900	20830	20300	21300	21600	20100				25	56
36	23-May-01	2	26100	20500	21600	19600	20800	20000				25	54
37	24-May-01	3	25700	20230	20800	19600	20600	19900	0.7816	1.437	4.78E-03	25	54
38	25-May-01	4	26500	20400	20900	20800	20100	19800				25	54
41	28-May-01	5	25600	19430	19000	19100	19800	19800				25	54
42	29-May-01	6	26800	20380	21400	20300	19400	20400				25	55
43	30-May-01	7	26600	20680	21100	20700	20100	20800				24	47
44	31-May-01	8	22900	21650	23000	21800	20800	21000	1.786	2.141	1.16E-03	25	48
45	1-Jun-01	9	23900	19230	18700	18100	19800	20300				25	49
48	4-Jun-01	10	26200	20450	20300	20600	20500	20400				25	48
49	5-Jun-01	11	26700	20750	19700	21000	21300	21000				25	53
50	6-Jun-01	12	27000	21400	20300	21600	21900	21800				25	56
51	7-Jun-01	13	25600	20430	20400	20300	20000	21000	1.618	1.703	1.63E-03	25	48
52	8-Jun-01	14	25300	20430	19800	21200	20100	20600				25	49
55	11-Jun-01	15	24900	21580	21400	20100	22600	22200				25	53
56	12-Jun-01	16	23600	21230	23200	21300	20100	20500				26	54
57	13-Jun-01	17	24600	20380	20000	21000	20600	19900				25	54
58	14-Jun-01	18	25000	19750	19200	19300	20000	20500	0.9184	2.066	4.89E-02	25	57
59	15-Jun-01	19	25200	19950	19700	20900	19100	20100				24	56
62	18-Jun-01	20	25000	20550	21800	19900	20400	20100				25	51
		Mean	25460		20510				1.276	1.837	1.41E-02	25.0	52.5
		S.D.	1109		917				0.500	0.328	2.32E-02	0.4	3.2

Huntingdon Life Sciences 00-6127I
Immunotoxicity Sub-Group

TABLE B

GASOLINE ETHANOL 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 -8	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 ETHANOL 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
EXPOSURE LEVEL(mg/m3):		0	2,000	10,000	20,000	POSITIVE CONTROL
WEEK -1	MEAN	118	118	118	118	118
	S.D.	6.6	6.3	8.0	7.8	7.2
	N	10	10	10	10	10
WEEK 0	MEAN	159	162	163	159	162
	S.D.	6.3	9.7	10.6	8.0	9.8
	N	10	10	10	10	10
WEEK 1	MEAN	181	184	187	182	184
	S.D.	7.4	12.5	11.1	14.8	14.7
	N	10	10	10	10	10
WEEK 2	MEAN	204	211	211	206	205
	S.D.	8.8	18.5	13.0	17.7	21.0
	N	10	10	10	10	10
WEEK 3	MEAN	224	231	229	225	226
	S.D.	10.9	24.7	14.5	17.5	22.5
	N	10	10	10	10	10
WEEK 4	MEAN	242	244	244	240	223
	S.D.	13.8	21.3	15.6	22.8	24.0
	N	10	10	10	10	10

No statistically significant differences

TABLE D

GASOLINE ETHANOL 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 (GRAMS)

DOSE GROUP:		I	II	III	IV	V
EXPOSURE LEVEL(mg/m3):		0	2,000	10,000	20,000	POSITIVE CONTROL
WEEK 0 TO 1	MEAN	22	21	24	23	22
	S.D.	5.5	6.8	3.5	9.0	7.7
	N	10	10	10	10	10
WEEK 0 TO 2	MEAN	45	49	48	47	43
	S.D.	6.1	11.1	6.0	10.6	13.6
	N	10	10	10	10	10
WEEK 0 TO 3	MEAN	65	69	66	66	64
	S.D.	7.8	21.2	8.6	10.9	14.4
	N	10	10	10	10	10
WEEK 0 TO 4	MEAN	83	82	80	82	61**
	S.D.	10.4	13.8	8.8	16.9	16.7
	N	10	10	10	10	10

Statistical key: ** = p<0.01

TABLE E

GASOLINE ETHANOL 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
EXPOSURE LEVEL(mg/m3):			0	2,000	10,000	20,000	POSITIVE CONTROL
WEEK	0	MEAN	124	129	127	124	132
		S.D.	7.3	6.0	6.2	8.6	9.2
		N	10	10	10	10	10
WEEK	1	MEAN	100	104	105	104	110**
		S.D.	4.1	6.4	5.5	5.1	4.8
		N	10	10	10	10	10
WEEK	2	MEAN	89	95*	95*	95**	99**
		S.D.	4.7	3.5	4.7	4.9	4.9
		N	10	10	10	10	9
WEEK	3	MEAN	85	84	89	91	92*
		S.D.	5.7	6.6	4.0	5.0	5.9
		N	10	9	10	9	9
WEEK	4	MEAN	79	80	80	83	73*
		S.D.	4.1	6.0	3.7	5.2	4.6
		N	10	10	9	10	10

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

TABLE F

GASOLINE ETHANOL 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 - 8
1531	WITHIN NORMAL LIMITS	P
1532	WITHIN NORMAL LIMITS	P
1533	WITHIN NORMAL LIMITS	P
1534	WITHIN NORMAL LIMITS	P
1535	WITHIN NORMAL LIMITS	P
1536	WITHIN NORMAL LIMITS	P
1537	WITHIN NORMAL LIMITS	P
1538	WITHIN NORMAL LIMITS	P
1539	WITHIN NORMAL LIMITS	P
1540	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETHANOL 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 2,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
2521	WITHIN NORMAL LIMITS	P
2522	WITHIN NORMAL LIMITS	P
2523	WITHIN NORMAL LIMITS	P
2524	WITHIN NORMAL LIMITS	P
2525	WITHIN NORMAL LIMITS	P
2526	WITHIN NORMAL LIMITS	P
2527	WITHIN NORMAL LIMITS	P
2528	WITHIN NORMAL LIMITS	P
2529	WITHIN NORMAL LIMITS	P
2530	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETHANOL 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 10,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
3521	WITHIN NORMAL LIMITS	P
3522	WITHIN NORMAL LIMITS	P
3523	WITHIN NORMAL LIMITS	P
3524	WITHIN NORMAL LIMITS	P
3525	WITHIN NORMAL LIMITS	P
3526	WITHIN NORMAL LIMITS	P
3527	WITHIN NORMAL LIMITS	P
3528	WITHIN NORMAL LIMITS	P
3529	WITHIN NORMAL LIMITS	P
3530	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETHANOL 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 20,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
4531	WITHIN NORMAL LIMITS	P
4532	WITHIN NORMAL LIMITS	P
4533	WITHIN NORMAL LIMITS	P
4534	WITHIN NORMAL LIMITS	P
4535	WITHIN NORMAL LIMITS	P
4536	WITHIN NORMAL LIMITS	P
4537	WITHIN NORMAL LIMITS	P
4538	WITHIN NORMAL LIMITS	P
4539	WITHIN NORMAL LIMITS	P
4540	WITHIN NORMAL LIMITS	P

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

TABLE F

GASOLINE ETHANOL 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 - 8
5531	WITHIN NORMAL LIMITS	P
5532	WITHIN NORMAL LIMITS	P
5533	WITHIN NORMAL LIMITS	P
5534	WITHIN NORMAL LIMITS	P
5535	WITHIN NORMAL LIMITS	P
5536	WITHIN NORMAL LIMITS	P
5537	WITHIN NORMAL LIMITS	P
5538	WITHIN NORMAL LIMITS	P
5539	WITHIN NORMAL LIMITS	P
5540	WITHIN NORMAL LIMITS	P

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

TABLE G

GASOLINE ETHANOL 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
1531	128	162	197	218	237	259
1532	123	167	183	203	221	244
1533	105	150	172	194	214	229
1534	119	168	182	217	243	264
1535	113	156	179	210	225	247
1536	119	150	171	193	208	221
1537	121	160	185	204	229	243
1538	125	163	187	205	225	238
1539	117	158	177	197	213	228
1540	112	156	181	199	227	249
MEAN	118	159	181	204	224	242
S.D.	6.6	6.3	7.4	8.8	10.9	13.8
N	10	10	10	10	10	10

TABLE G

GASOLINE ETHANOL 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 2,000 mg/m3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
2521	108	157	186	214	278	247
2522	115	155	172	192	206	217
2523	119	160	177	202	216	234
2524	124	175	200	241	241	276
2525	115	159	185	215	229	249
2526	127	177	191	225	246	265
2527	119	152	176	200	214	230
2528	111	151	160	178	192	208
2529	125	173	198	229	247	256
2530	120	163	192	216	240	256
MEAN	118	162	184	211	231	244
S.D.	6.3	9.7	12.5	18.5	24.7	21.3
N	10	10	10	10	10	10

TABLE G

GASOLINE ETHANOL 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 10,000 mg/m3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
3521	118	158	177	198	213	228
3522	115	158	183	201	212	226
3523	123	170	195	221	234	245
3524	119	164	187	211	233	248
3525	129	179	208	240	258	271
3526	101	141	167	194	214	228
3527	125	167	193	212	231	253
3528	110	165	186	212	241	254
3529	117	157	182	205	219	227
3530	124	173	192	216	231	256
MEAN	118	163	187	211	229	244
S.D.	8.0	10.6	11.1	13.0	14.5	15.6
N	10	10	10	10	10	10

TABLE G

GASOLINE ETHANOL 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 20,000 mg/m3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
4531	120	156	184	202	220	240
4532	102	149	175	195	216	236
4533	126	167	205	230	249	279
4534	113	147	164	186	207	218
4535	125	167	197	227	242	265
4536	117	152	172	188	202	215
4537	109	159	172	206	229	225
4538	118	163	174	207	219	232
4539	127	170	203	230	252	269
4540	121	154	171	187	211	223
MEAN	118	159	182	206	225	240
S.D.	7.8	8.0	14.8	17.7	17.5	22.8
N	10	10	10	10	10	10

TABLE G

GASOLINE ETHANOL 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
5531	122	161	180	197	213	215
5532	109	151	169	185	210	207
5533	126	171	211	241	267	269
5534	130	183	203	232	264	260
5535	118	162	181	205	223	227
5536	112	159	184	206	221	226
5537	117	159	181	210	227	219
5538	120	158	183	203	214	199
5539	108	149	159	167	198	197
5540	123	166	185	202	224	213
MEAN	118	162	184	205	226	223
S.D.	7.2	9.8	14.7	21.0	22.5	24.0
N	10	10	10	10	10	10

Huntingdon Life Sciences 00-6127I
Immunotoxicity Sub-Group

TABLE H

GASOLINE ETHANOL 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 (GRAMS)

FEMALES GROUP I 0 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
1531	34	56	75	97
1532	16	36	54	76
1533	22	43	64	79
1534	14	49	75	96
1535	23	54	68	91
1536	21	43	58	71
1537	24	44	69	83
1538	25	42	62	75
1539	19	39	55	70
1540	24	43	71	93
MEAN	22	45	65	83
S.D.	5.5	6.1	7.8	10.4
N	10	10	10	10

TABLE H

GASOLINE ETHANOL 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 (GRAMS)

FEMALES GROUP II 2,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
2521	29	57	121	90
2522	18	38	52	63
2523	17	42	57	75
2524	26	66	66	101
2525	26	56	70	90
2526	14	48	69	88
2527	24	48	62	78
2528	9	27	41	57
2529	24	56	73	82
2530	29	53	77	93
MEAN	21	49	69	82
S.D.	6.8	11.1	21.2	13.8
N	10	10	10	10

TABLE H

GASOLINE ETHANOL 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 (GRAMS)

FEMALES GROUP III 10,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
3521	18	40	54	70
3522	26	44	55	68
3523	25	52	65	75
3524	22	47	69	84
3525	29	61	79	92
3526	26	53	74	87
3527	26	44	64	86
3528	21	47	76	89
3529	25	47	62	70
3530	19	43	59	84
MEAN	24	48	66	80
S.D.	3.5	6.0	8.6	8.8
N	10	10	10	10

TABLE H

GASOLINE ETHANOL 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 (GRAMS)

FEMALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
4531	27	45	63	84
4532	26	46	66	86
4533	38	63	82	112
4534	17	39	61	72
4535	30	60	75	98
4536	19	36	50	62
4537	13	47	70	66
4538	11	44	56	69
4539	33	60	81	98
4540	17	32	57	69
MEAN	23	47	66	82
S.D.	9.0	10.6	10.9	16.9
N	10	10	10	10

Huntingdon Life Sciences 00-6127I
Immunotoxicity Sub-Group

TABLE H

GASOLINE ETHANOL 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 (GRAMS)

FEMALES GROUP V POSITIVE CONTROL

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
5531	19	35	51	54
5532	19	34	59	56
5533	40	71	96	98
5534	20	49	81	78
5535	19	43	61	65
5536	25	47	63	68
5537	22	51	68	60
5538	25	45	57	41
5539	10	18	49	48
5540	19	36	58	47
MEAN	22	43	64	61
S.D.	7.7	13.6	14.4	16.7
N	10	10	10	10

TABLE I

GASOLINE ETHANOL 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
1531	117	100	87	77	81
1532	131	99	90	91	87
1533	124	99	90	84	75
1534	132	103	96	86	80
1535	124	97	86	85	79
1536	115	94	86	83	75
1537	121	103	97	87	79
1538	116	98	82	82	73
1539	119	96	86	81	77
1540	136	109	87	98	81
MEAN	124	100	89	85	79
S.D.	7.3	4.1	4.7	5.7	4.1
N	10	10	10	10	10

TABLE I

GASOLINE ETHANOL 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 2,000 mg/m3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
2521	131	103	96	68	68
2522	126	104	93	84	74
2523	125	102	98	83	78
2524	134	108	101	SF	85
2525	135	118	96	90	82
2526	130	102	94	86	81
2527	123	101	92	86	82
2528	117	93	89	83	76
2529	135	106	97	90	88
2530	132	108	93	86	85
MEAN	129	104	95	84	80
S.D.	6.0	6.4	3.5	6.6	6.0
N	10	10	10	9	10

SF=Spilled Feeder

TABLE I

GASOLINE ETHANOL 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 10,000 mg/m3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
3521	122	101	89	86	78
3522	121	96	90	84	73
3523	130	109	97	87	84
3524	123	104	95	92	SF
3525	126	102	92	86	80
3526	139	116	102	93	86
3527	122	105	93	88	81
3528	136	109	103	96	80
3529	125	108	93	84	80
3530	126	103	92	91	80
MEAN	127	105	95	89	80
S.D.	6.2	5.5	4.7	4.0	3.7
N	10	10	10	10	9

SF=Spilled Feeder

TABLE I

GASOLINE ETHANOL 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 20,000 mg/m3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
4531	118	103	88	90	86
4532	128	112	99	98	84
4533	132	111	95	94	87
4534	114	100	93	87	79
4535	132	107	100	SF	87
4536	108	105	90	86	74
4537	133	96	101	91	90
4538	123	98	92	87	79
4539	127	106	92	87	79
4540	128	104	102	99	89
MEAN	124	104	95	91	83
S.D.	8.6	5.1	4.9	5.0	5.2
N	10	10	10	9	10

SF=Spilled Feeder

TABLE I

GASOLINE ETHANOL 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
5531	122	103	94	88	77
5532	126	106	102	95	70
5533	132	114	97	88	71
5534	137	109	SF	SF	80
5535	126	103	96	86	75
5536	129	116	98	92	69
5537	127	111	98	87	65
5538	155	117	111	92	76
5539	134	109	99	103	71
5540	133	111	101	99	76
MEAN	132	110	99	92	73
S.D.	9.2	4.8	4.9	5.9	4.6
N	10	10	9	9	10

SF=Spilled Feeder

TABLE J

GASOLINE ETHANOL 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
1531	TERMINAL SACRIFICE	19-JUN-01	4	28
1532	TERMINAL SACRIFICE	19-JUN-01	4	28
1533	TERMINAL SACRIFICE	19-JUN-01	4	28
1534	TERMINAL SACRIFICE	19-JUN-01	4	28
1535	TERMINAL SACRIFICE	19-JUN-01	4	28
1536	TERMINAL SACRIFICE	19-JUN-01	4	28
1537	TERMINAL SACRIFICE	19-JUN-01	4	28
1538	TERMINAL SACRIFICE	19-JUN-01	4	28
1539	TERMINAL SACRIFICE	19-JUN-01	4	28
1540	TERMINAL SACRIFICE	19-JUN-01	4	28

TABLE J

GASOLINE ETHANOL 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 2,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2521	TERMINAL SACRIFICE	19-JUN-01	4	28
2522	TERMINAL SACRIFICE	19-JUN-01	4	28
2523	TERMINAL SACRIFICE	19-JUN-01	4	28
2524	TERMINAL SACRIFICE	19-JUN-01	4	28
2525	TERMINAL SACRIFICE	19-JUN-01	4	28
2526	TERMINAL SACRIFICE	19-JUN-01	4	28
2527	TERMINAL SACRIFICE	19-JUN-01	4	28
2528	TERMINAL SACRIFICE	19-JUN-01	4	28
2529	TERMINAL SACRIFICE	19-JUN-01	4	28
2530	TERMINAL SACRIFICE	19-JUN-01	4	28

TABLE J

GASOLINE ETHANOL 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 10,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3521	TERMINAL SACRIFICE	19-JUN-01	4	28
3522	TERMINAL SACRIFICE	19-JUN-01	4	28
3523	TERMINAL SACRIFICE	19-JUN-01	4	28
3524	TERMINAL SACRIFICE	19-JUN-01	4	28
3525	TERMINAL SACRIFICE	19-JUN-01	4	28
3526	TERMINAL SACRIFICE	19-JUN-01	4	28
3527	TERMINAL SACRIFICE	19-JUN-01	4	28
3528	TERMINAL SACRIFICE	19-JUN-01	4	28
3529	TERMINAL SACRIFICE	19-JUN-01	4	28
3530	TERMINAL SACRIFICE	19-JUN-01	4	28

TABLE J

GASOLINE ETHANOL 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 20,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4531	TERMINAL SACRIFICE	19-JUN-01	4	28
4532	TERMINAL SACRIFICE	19-JUN-01	4	28
4533	TERMINAL SACRIFICE	19-JUN-01	4	28
4534	TERMINAL SACRIFICE	19-JUN-01	4	28
4535	TERMINAL SACRIFICE	19-JUN-01	4	28
4536	TERMINAL SACRIFICE	19-JUN-01	4	28
4537	TERMINAL SACRIFICE	19-JUN-01	4	28
4538	TERMINAL SACRIFICE	19-JUN-01	4	28
4539	TERMINAL SACRIFICE	19-JUN-01	4	28
4540	TERMINAL SACRIFICE	19-JUN-01	4	28

TABLE J

GASOLINE ETHANOL 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
5531	TERMINAL SACRIFICE	19-JUN-01	4	28
5532	TERMINAL SACRIFICE	19-JUN-01	4	28
5533	TERMINAL SACRIFICE	19-JUN-01	4	28
5534	TERMINAL SACRIFICE	19-JUN-01	4	28
5535	TERMINAL SACRIFICE	19-JUN-01	4	28
5536	TERMINAL SACRIFICE	19-JUN-01	4	28
5537	TERMINAL SACRIFICE	19-JUN-01	4	28
5538	TERMINAL SACRIFICE	19-JUN-01	4	28
5539	TERMINAL SACRIFICE	19-JUN-01	4	28
5540	TERMINAL SACRIFICE	19-JUN-01	4	28