FINAL REPORT

Immunological Evaluation of Gasoline TBA Vapor Condensate in Female Sprague Dawley Rats Using the Plaque Forming Cell Assay

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Test Substance:	Gasoline TBA Vapor Condensate
Protocol No:	HLS 00-6131
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., DABT
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Immunotox's Project Number:	ITI 1101
Date:	07 January 2013
Principal Investigator:	Kimber L. White, Jr., Ph.D.
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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:

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 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.

07 JAN 13
Date

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

7 JAN 2013
Date

II. QUALITY ASSURANCE STATEMENT - IMMUNOTOX®, INC.

Test Substance: Gasoline TBA Vapor Condensate

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

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

Huntingdon Life Sciences, Inc. Study No. 00-6131
Sponsor Study No. 211-TBA-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 to ImmunoTox®, Inc. P.I.	Reported to S.D. and Management	Activity
July 24, 20022	July 24, 2002	January 8, 2003	AFC Assay
January 4-5, 2003	January 8, 2003	January 8, 2003	Data Audit
January 6-7, 2003	January 8, 2003	January 8, 2003	1st Draft Report Audit

Approved and submitted by: _____ 1/10/2013
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	05 Dec 00[a]
GLP Protocol Review	9 Apr 02	9 Apr 02
Positive Control Immunotoxicity Dose Administration	19 Jul 02	19 Jul 02
Immunotoxicity Blood Collection & Necropsy	23 Jul 02	24 Jul 02
Draft Immuno Report & In-Life Study Data	15-19, 22 Aug 03 & 2 Dec 03	2 Dec 03

Kathleen Stilwell, AS, BS, LATg, SRS
Quality Assurance Auditor

5MAR13
Date

[a]General Facility Inspection 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 TBA 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, Ph.D., 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. Date 07 Jan 13
Principal Investigator
ImmunoTox®, Inc.

Vanessa L. Peachee, Ph.D. Date 07 Jan 13
Assistant Principal Investigator
ImmunoTox®, Inc.

Approved:

Gary M. Hoffman, B.A., DABT Date 8 March 2013
Study Director
Huntingdon Life Sciences

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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. 00-6131 at ImmunoTox®, Inc., Richmond, Virginia. The Principal Investigator was Kimber L. White, Jr., Ph.D., and Vanessa L. Peachee, Ph.D., 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 TBA 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 TBA 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 2000, 10000 and 20000 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 wet 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 TBA 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 TBA Vapor Condensate, when evaluated either as absolute weight or relative weight, as compared to the air control.

Exposure to Gasoline TBA Vapor Condensate did not result in any statistically significant effect 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 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 TBA Vapor Condensate test substance did not adversely affect 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 (Absolute)	No Effect		
Spleen (Relative)	No Effect		
Thymus (Absolute)	No Effect		
Thymus (Relative)	No Effect		
Spleen IgM Antibody-Forming Cell Response to Sheep Erythrocytes			
IgM AFC/10^6 Spleen Cells	No Effect		
IgM AFC/Spleen ($x10^3$)	No Effect		

V. INTRODUCTION

The purpose of this study was to provide evaluation of immunological parameters for Huntingdon Life Sciences (HLS) Study No. 00-6131. In this study, the test substance, Gasoline TBA 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 wet ice for evaluation the following day. Spleens were received on 24 July 2002 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 TBA 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., DABT, was the HLS Study Director. Vanessa L. Peachee, Ph.D., 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 TBA 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 TBA Vapor Condensate.

VI. METHODS OF PROCEDURE

EXPERIMENTAL DESIGN

The immunotoxicological satellite study consisted of a vehicle group, three exposure levels of Gasoline TBA 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 TBA Vapor Condensate at exposure levels of 2000, 10000 or 20000 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 091K1176, received 12 July 2002, expiration September 2004, white powder, storage 2-8ºC, purity 99.5%), 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.) once 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 wet ice in individual shipping containers at 2-8°C by carrier to ImmunoTox 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 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[3].

The primary IgM response to sheep erythrocytes was measured using a modified hemolytic plaque assay of Jerne[2]. 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. Spleen cell counts were 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. 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 in tables are presented as means ± SE (standard error). Individual animal data were rounded and the original (unrounded) data were used for mean and standard error calculations. 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. The mean ending weight for the vehicle-exposed rats was 262.1 ± 5.1 grams. No statistically significant differences were observed in terminal body weights of the Gasoline TBA 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 TBA Vapor Condensate, on spleen or thymus weights when evaluated as absolute weight or as relative weight. Treatment with the positive control, cyclophosphamide, had a significant decrease of 55% on absolute spleen weight and a significant decrease of 77% on absolute thymus weight, compared to the vehicle control. In addition, the positive control, cyclophosphamide, had a significant decrease of 52% on relative spleen weight and a 75% 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 TBA Vapor Condensate.

Figure 1

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

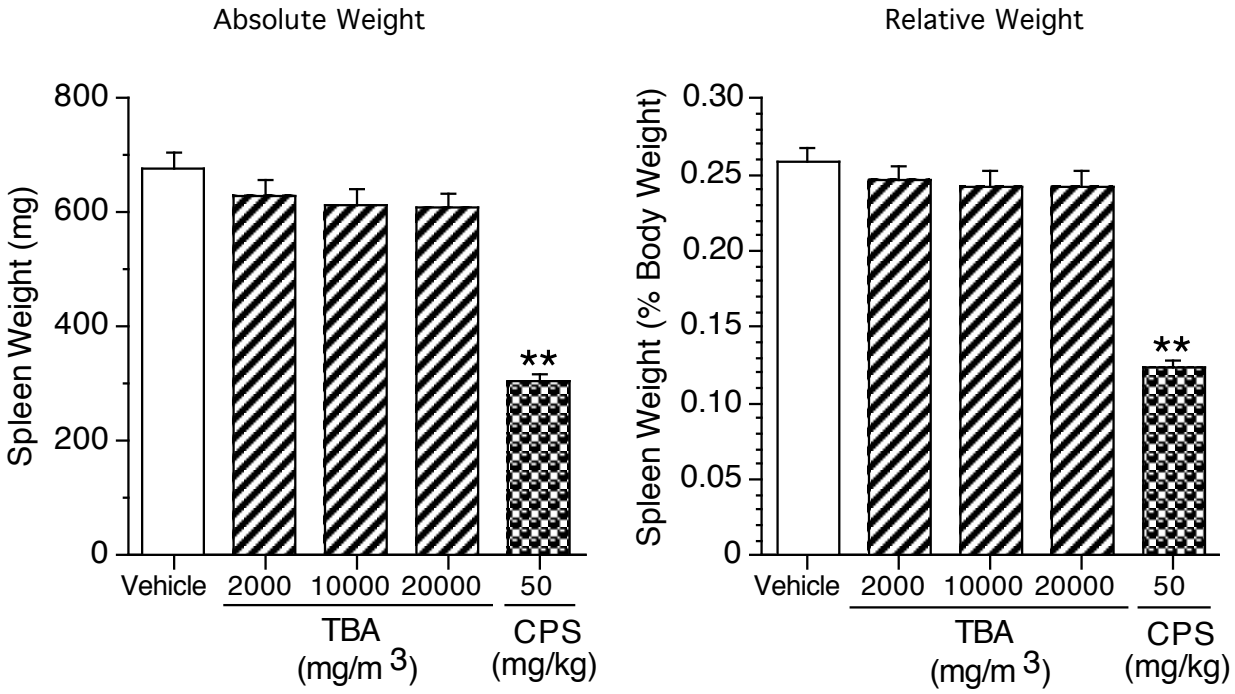
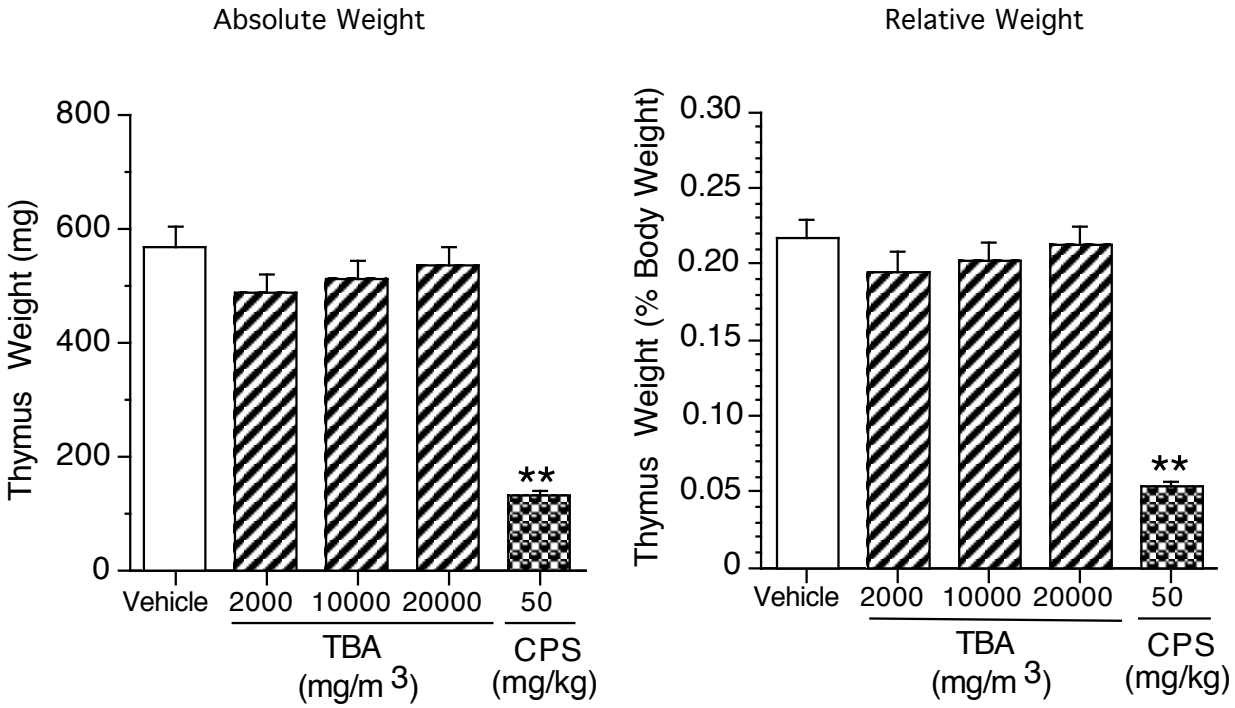


Figure 2

Absolute (mg) and Relative (%) Thymus Weight in Female Sprague Dawley Rats Exposed to Gasoline TBA 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.

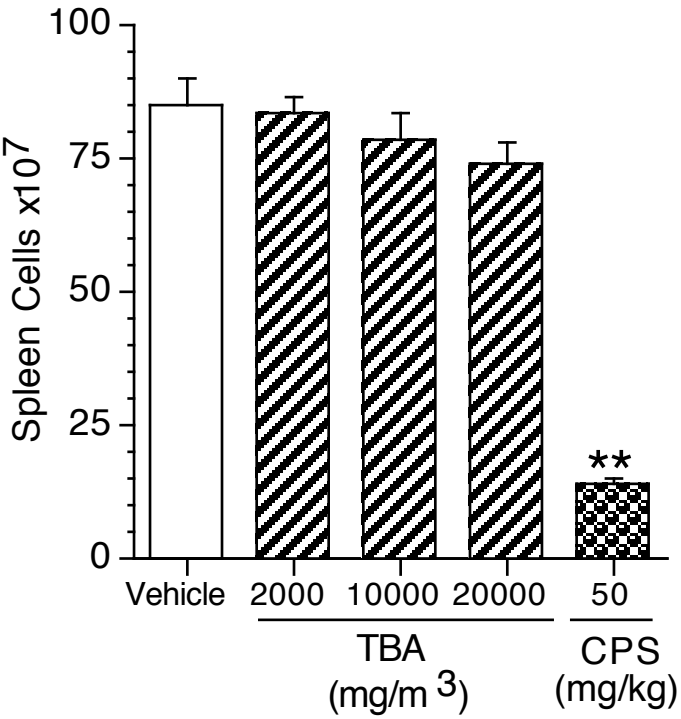
The spleen IgM antibody-forming cell response, i.e. plaque assay, was evaluated on spleens removed 1 day after the last exposure, which was Day 4 after antigen sensitization. Day 4 after antigen sensitization is the peak day for the sRBC IgM AFC response in rats. Viabilities were conducted on all cell suspensions using propidium iodide (PI) and the Coulter EPICS XL-MCL Flow Cytometer. The viabilities from all samples were greater than 71% with the exception of four samples.

The results of the AFC response are shown in Table 2 and in Figures 3 and 4. As indicated above, exposure to Gasoline TBA 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 TBA Vapor Condensate as compared to the vehicle control group. As expected, the

positive control, cyclophosphamide (CPS), produced an 83% decrease in spleen cell number when compared to the vehicle control group.

Figure 3

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

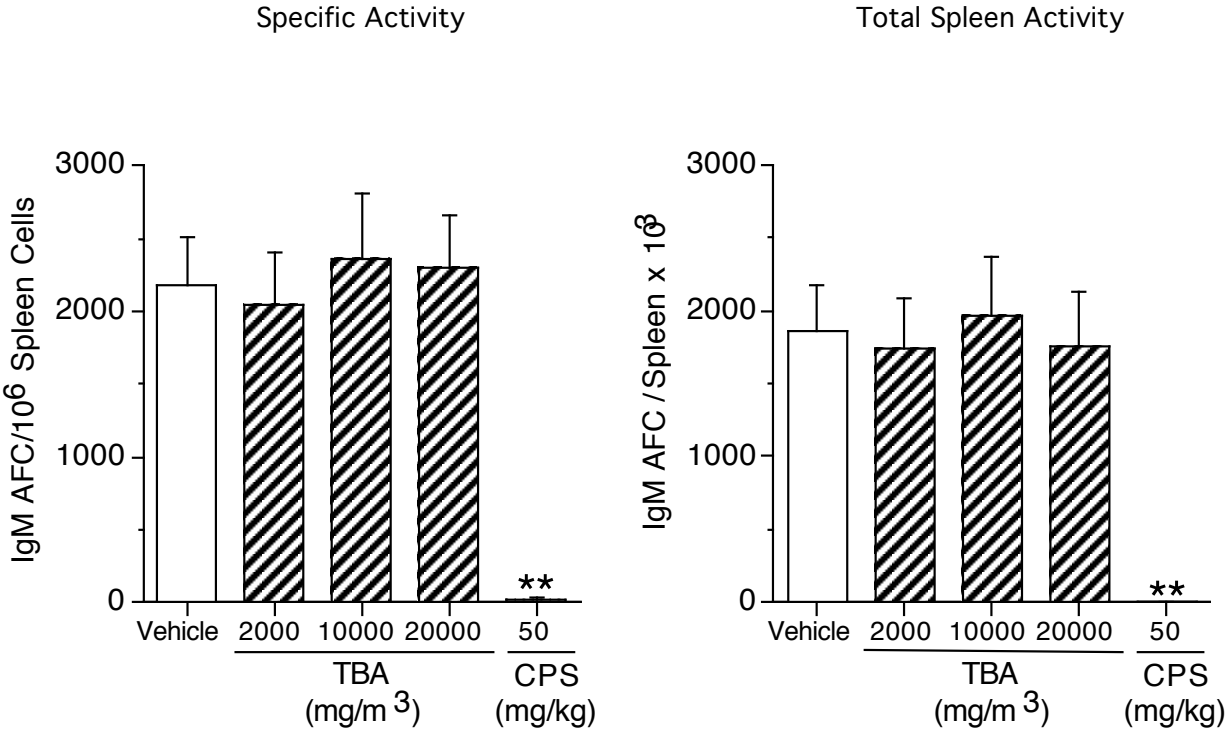


Shown in Table 2 and Figure 4 are the functional results from the IgM antibody-forming cell (AFC) assay. Shown in the left panel are the results when the data are expressed as specific activity and the results of the total spleen activity are shown in the right panel. No effect was observed, following exposure to Gasoline TBA Vapor Condensate, when evaluated as either Specific Activity (AFC/10^6 spleen) or as Total Spleen Activity (AFC/Spleen).

As anticipated, the positive control, CPS, produced a significant decrease in Specific Activity (99%) and Total Spleen 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 TBA Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks



VIII. CONCLUSION

Exposure of female Sprague Dawley rats to Gasoline TBA Vapor Condensate for a period of 5 days per week for 4 weeks resulted in no statistically significant effects in the humoral immune response to the T-dependent antigen, sheep erythrocytes. There were no statistically significant effects on body weight, spleen or thymus weights (absolute or relative), spleen cell number, Specific Activity (AFC/10^6 spleen), or Total Spleen Activity (AFC/Spleen). Based on the immunological parameters evaluated, under the experimental conditions of the study, exposure to Gasoline TBA Vapor Condensate did not adversely affect 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 TBA Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks

Parameter	Vehicle	Gasoline TBA Vapor (mg/m^3)			Cyclophosphamide	H/NH	Trend
		2000	10000	20000	50 mg/kg		Analysis
	(10)	(10)	(10)	(10)	(10)		
Body Wgt (g)	262.1 ± 5.1	255.4 ± 6.7	252.6 ± 6.8	254.2 ± 5.2	243.4 ± 5.0*	H	NS
Spleen (mg)	676 ± 31	630 ± 27	614 ± 27	611 ± 24	304 ± 12**	H	NS
% Body Wgt	0.259 ± 0.009	0.247 ± 0.008	0.242 ± 0.011	0.242 ± 0.010	0.124 ± 0.004**	H	$p \leq 0.05$
Thymus (mg)	571 ± 33	490 ± 33	513 ± 32	537 ± 33	134 ± 9**	H	NS
% Body Wgt	0.217 ± 0.012	0.194 ± 0.014	0.202 ± 0.012	0.212 ± 0.012	0.054 ± 0.003**	H	NS

Female Sprague Dawley rats were administered vehicle control (air only) or gasoline TBA 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: g = grams; 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 TBA 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	262.1 ± 5.1 (10)	676 ± 31 (10)	85.01 ± 5.36 (10)	2175 ± 339 (10)	1863 ± 308 (10)
Gasoline TBA Vapor Condensate					
2000 mg/m^3	255.4 ± 6.7 (10)	630 ± 27 (10)	83.78 ± 3.20 (10)	2049 ± 357 (10)	1734 ± 350 (10)
10000 mg/m^3	252.6 ± 6.8 (10)	614 ± 27 (10)	78.71 ± 5.28 (10)	2363 ± 451 (10)	1960 ± 408 (10)
20000 mg/m^3	254.2 ± 5.2 (10)	611 ± 24 (10)	74.30 ± 4.03 (10)	2296 ± 359 (10)	1759 ± 368 (10)
Cyclophosphamide 50 mg/kg	243.4 ± 5.0* (10)	304 ± 12** (10)	14.46 ± 0.64** (10)	27 ± 9** (10)	4 ± 1** (10)
H/NH	H	H	H	H	H
Trend Analysis	NS	NS	NS	NS	NS

Female Sprague Dawley rats were administered vehicle control (air only) or gasoline TBA 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. 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

INIDIVIDUAL ANIMAL DATA
ORGAN WEIGHTS
GASOLINE TBA VAPOR CONDENSATE
00-6131

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	276.9	662	491	0.240	0.180
1582	GI	AIR ONLY	F	229.6	522	527	0.230	0.230
1583	GI	AIR ONLY	F	268.7	787	789	0.290	0.290
1584	GI	AIR ONLY	F	265.8	712	548	0.270	0.210
1585	GI	AIR ONLY	F	273.3	861	549	0.320	0.200
1586	GI	AIR ONLY	F	250.0	599	636	0.240	0.250
1587	GI	AIR ONLY	F	251.8	647	514	0.260	0.200
1588	GI	AIR ONLY	F	266.6	709	587	0.270	0.220
1589	GI	AIR ONLY	F	285.0	621	659	0.220	0.230
1590	GI	AIR ONLY	F	253.1	640	411	0.250	0.160
2571	GII	2000 MG/M^3 TBA	F	284.6	612	541	0.220	0.190
2572	GII	2000 MG/M^3 TBA	F	232.6	530	611	0.230	0.260
2573	GII	2000 MG/M^3 TBA	F	270.1	770	533	0.290	0.200
2574	GII	2000 MG/M^3 TBA	F	240.8	561	365	0.230	0.150
2575	GII	2000 MG/M^3 TBA	F	258.1	642	440	0.250	0.170
2576	GII	2000 MG/M^3 TBA	F	248.6	721	423	0.290	0.170
2577	GII	2000 MG/M^3 TBA	F	232.5	561	411	0.240	0.180
2578	GII	2000 MG/M^3 TBA	F	253.2	604	697	0.240	0.280
2579	GII	2000 MG/M^3 TBA	F	293.0	739	408	0.250	0.140
2580	GII	2000 MG/M^3 TBA	F	240.6	556	473	0.230	0.200
3571	GIII	10000 MG/M^3 TBA	F	256.2	599	494	0.230	0.190
3572	GIII	10000 MG/M^3 TBA	F	233.3	671	654	0.290	0.280
3573	GIII	10000 MG/M^3 TBA	F	241.8	444	409	0.180	0.170
3574	GIII	10000 MG/M^3 TBA	F	248.9	680	427	0.270	0.170
3575	GIII	10000 MG/M^3 TBA	F	269.9	640	448	0.240	0.170
3576	GIII	10000 MG/M^3 TBA	F	276.2	728	566	0.260	0.200
3577	GIII	10000 MG/M^3 TBA	F	226.0	606	456	0.270	0.200
3578	GIII	10000 MG/M^3 TBA	F	221.6	520	475	0.230	0.210
3579	GIII	10000 MG/M^3 TBA	F	271.3	690	717	0.250	0.260
3580	GIII	10000 MG/M^3 TBA	F	280.4	564	490	0.200	0.170
4581	GIV	20000 MG/M^3 TBA	F	280.7	653	559	0.230	0.200
4582	GIV	20000 MG/M^3 TBA	F	242.8	651	467	0.270	0.190
4583	GIV	20000 MG/M^3 TBA	F	223.9	582	535	0.260	0.240
4584	GIV	20000 MG/M^3 TBA	F	248.3	504	492	0.200	0.200
4585	GIV	20000 MG/M^3 TBA	F	250.1	534	594	0.210	0.240
4586	GIV	20000 MG/M^3 TBA	F	262.3	644	365	0.250	0.140
4587	GIV	20000 MG/M^3 TBA	F	273.3	621	640	0.230	0.230
4588	GIV	20000 MG/M^3 TBA	F	257.1	507	608	0.200	0.240
4589	GIV	20000 MG/M^3 TBA	F	243.1	696	410	0.290	0.170
4590	GIV	20000 MG/M^3 TBA	F	260.0	716	702	0.280	0.270
5551	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	238.6	290	107	0.120	0.040
5552	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	277.7	394	135	0.140	0.050
5553	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	248.3	317	173	0.130	0.070
5554	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	225.4	297	120	0.130	0.050
5555	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	226.9	302	122	0.130	0.050
5556	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	240.3	307	133	0.130	0.060
5557	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	251.1	281	186	0.110	0.070
5558	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	239.0	310	105	0.130	0.040
5559	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	230.9	235	108	0.100	0.050
5560	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	255.3	311	150	0.120	0.060

INDIVIDUAL ANIMAL DATA
IGM AFC ASSAY
GASOLINE TBA VAPOR CONDENSATE
00-6131

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	2613	2007	76.80	662	276.9
1582	GI	AIR ONLY	F	946	582	61.50	522	229.6
1583	GI	AIR ONLY	F	1139	1206	105.90	787	268.7
1584	GI	AIR ONLY	F	4602	3672	79.80	712	265.8
1585	GI	AIR ONLY	F	2722	3204	117.72	861	273.3
1586	GI	AIR ONLY	F	1151	1008	87.54	599	250.0
1587	GI	AIR ONLY	F	2562	2223	86.76	647	251.8
1588	GI	AIR ONLY	F	1993	1755	88.08	709	266.6
1589	GI	AIR ONLY	F	2224	1800	80.94	621	285.0
1590	GI	AIR ONLY	F	1799	1170	65.04	640	253.1
2571	GII	2000 MG/M^3 TBA	F	2237	1584	70.80	612	284.6
2572	GII	2000 MG/M^3 TBA	F	1945	1431	73.56	530	232.6
2573	GII	2000 MG/M^3 TBA	F	928	954	102.84	770	270.1
2574	GII	2000 MG/M^3 TBA	F	608	474	77.94	561	240.8
2575	GII	2000 MG/M^3 TBA	F	2322	1998	86.04	642	258.1
2576	GII	2000 MG/M^3 TBA	F	4807	4635	96.42	721	248.6
2577	GII	2000 MG/M^3 TBA	F	2117	1665	78.66	561	232.5
2578	GII	2000 MG/M^3 TBA	F	1573	1332	84.66	604	253.2
2579	GII	2000 MG/M^3 TBA	F	1703	1512	88.80	739	293.0
2580	GII	2000 MG/M^3 TBA	F	2248	1755	78.06	556	240.6
3571	GIII	10000 MG/M^3 TBA	F	3679	2457	66.78	599	256.2
3572	GIII	10000 MG/M^3 TBA	F	3053	2907	95.22	671	233.3
3573	GIII	10000 MG/M^3 TBA	F	468	306	65.34	444	241.8
3574	GIII	10000 MG/M^3 TBA	F	3201	2889	90.24	680	248.9
3575	GIII	10000 MG/M^3 TBA	F	4015	3339	83.16	640	269.9
3576	GIII	10000 MG/M^3 TBA	F	3272	3375	103.14	728	276.2
3577	GIII	10000 MG/M^3 TBA	F	3286	2583	78.60	606	226.0
3578	GIII	10000 MG/M^3 TBA	F	1610	945	58.68	520	221.6
3579	GIII	10000 MG/M^3 TBA	F	629	576	91.62	690	271.3
3580	GIII	10000 MG/M^3 TBA	F	414	225	54.36	564	280.4
4581	GIV	20000 MG/M^3 TBA	F	2405	2088	86.82	653	280.7
4582	GIV	20000 MG/M^3 TBA	F	2343	1773	75.66	651	242.8
4583	GIV	20000 MG/M^3 TBA	F	3180	2007	63.12	582	223.9
4584	GIV	20000 MG/M^3 TBA	F	2224	1197	53.82	504	248.3
4585	GIV	20000 MG/M^3 TBA	F	2193	1359	61.98	534	250.1
4586	GIV	20000 MG/M^3 TBA	F	1964	1593	81.12	644	262.3
4587	GIV	20000 MG/M^3 TBA	F	1146	822	71.70	621	273.3
4588	GIV	20000 MG/M^3 TBA	F	1126	801	71.16	507	257.1
4589	GIV	20000 MG/M^3 TBA	F	1401	1134	80.94	696	243.1
4590	GIV	20000 MG/M^3 TBA	F	4981	4815	96.66	716	260.0
5551	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	80	12	15.00	290	238.6
5552	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	47	9	19.08	394	277.7
5553	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	15.48	317	248.3
5554	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	44	6	13.50	297	225.4
5555	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	12.66	302	226.9
5556	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	43	6	13.86	307	240.3
5557	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	37	6	16.08	281	251.1
5558	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	23	3	13.32	310	239.0
5559	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	12.18	235	230.9
5560	GV	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	13.44	311	255.3

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:	6 June 2002
Experimental Initiation Date:	25 June 2002 (in-life)
Experimental Completion Date:	23 July 2002 (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 TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual							Mean Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				MMAD (μm)	GSD	TMC (mg/m^3)	(°C)	(%)
0	25-Jun-02	1	0	0	0	0	0	0				25	50
1	26-Jun-02	2	0	0	0	0	0	0				26	68
2	27-Jun-02	3	0	0	0	0	0	0				25	52
3	28-Jun-02	4	0	0	0	0	0	0	0.9138	2.460	1.19E-02	25	71
6	1-Jul-02	5	0	0	0	0	0	0				25	51
7	2-Jul-02	6	0	0	0	0	0	0	0.8260	2.332	2.11E-02	24	54
8	3-Jul-02	7	0	0	0	0	0	0				24	53
9	4-Jul-02	8	0	0	0	0	0	0				24	54
10	5-Jul-02	9	0	0	0	0	0	0				24	52
13	8-Jul-02	10	0	0	0	0	0	0				24	52
14	9-Jul-02	11	0	0	0	0	0	0	0.7595	1.840	2.03E-02	25	51
15	10-Jul-02	12	0	0	0	0	0	0				25	52
16	11-Jul-02	13	0	0	0	0	0	0				25	38
17	12-Jul-02	14	0	0	0	0	0	0				25	47
20	15-Jul-02	15	0	0	0	0	0	0				25	50
21	16-Jul-02	16	0	0	0	0	0	0	5.014	2.673	7.41E-03	24	49
22	17-Jul-02	17	0	0	0	0	0	0				24	56
23	18-Jul-02	18	0	0	0	0	0	0				24	52
24	19-Jul-02	19	0	0	0	0	0	0				24	53
27	22-Jul-02	20	0	0	0	0	0	0				24	51
		Mean	**0**		**0**				**1.878**	**2.326**	**1.52E-02**	**24.6**	**52.8**
		S.D.	**0**		**0**				**2.091**	**0.353**	**5.75E-03**	**0.6**	**6.8**

Table A GASOLINE TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual							Mean Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				MMAD (μm)	GSD	TMC (mg/m^3)	(°C)	(%)
0	25-Jun-02	1	0	0	0	0	0	0				24	51
1	26-Jun-02	2	0	0	0	0	0	0				25	70
2	27-Jun-02	3	0	0	0	0	0	0				24	54
3	28-Jun-02	4	0	0	0	0	0	0	1.905	2.407	1.36E-02	24	76
6	1-Jul-02	5	0	0	0	0	0	0				24	52
7	2-Jul-02	6	0	0	0	0	0	0	0.8133	3.176	2.72E-02	25	56
8	3-Jul-02	7	0	0	0	0	0	0				25	54
9	4-Jul-02	8	0	0	0	0	0	0				24	55
10	5-Jul-02	9	0	0	0	0	0	0				25	55
13	8-Jul-02	10	0	0	0	0	0	0				24	53
14	9-Jul-02	11	0	0	0	0	0	0	0.8024	2.148	1.92E-02	24	54
15	10-Jul-02	12	0	0	0	0	0	0				24	55
16	11-Jul-02	13	0	0	0	0	0	0				24	39
17	12-Jul-02	14	0	0	0	0	0	0				24	49
20	15-Jul-02	15	0	0	0	0	0	0				24	51
21	16-Jul-02	16	0	0	0	0	0	0	2.076	2.298	4.83E-03	24	51
22	17-Jul-02	17	0	0	0	0	0	0				24	58
23	18-Jul-02	18	0	0	0	0	0	0				24	55
24	19-Jul-02	19	0	0	0	0	0	0				24	54
27	22-Jul-02	20	0	0	0	0	0	0				25	53
		Mean	**0**		**0**				**1.399**	**2.507**	**1.62E-02**	**24.3**	**54.8**
		S.D.	**0**		**0**				**0.686**	**0.458**	**8.16E-03**	**0.4**	**7.4**

Table A GASOLINE TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
0	25-Jun-02	1	1990	1998	2120	2010	1910	1950				24	45
1	26-Jun-02	2	1950	1963	1890	1850	2110	2000				25	63
2	27-Jun-02	3	2070	2063	2180	1960	2030	2080				23	47
3	28-Jun-02	4	2140	2120	2210	1650	2330	2290	0.8293	2.094	7.72E-03	24	61
6	1-Jul-02	5	2070	2063	2280	2020	2010	1940				23	46
7	2-Jul-02	6	2120	2095	2010	2200	2120	2050	0.7460	1.524	1.96E-02	24	49
8	3-Jul-02	7	2010	2050	2280	1970	1970	1980				23	48
9	4-Jul-02	8	2030	2070	1910	2200	2150	2020				24	49
10	5-Jul-02	9	1990	1998	1960	2110	1960	1960				24	48
13	8-Jul-02	10	1930	1963	2000	2030	2000	1820				24	48
14	9-Jul-02	11	1890	1905	2030	1820	1940	1830	0.7361	1.354	1.67E-02	24	47
15	10-Jul-02	12	1920	2058	2380	2200	1990	1660				24	47
16	11-Jul-02	13	2020	2078	2140	2070	2120	1980				24	34
17	12-Jul-02	14	1880	1958	2280	1980	1610	1960				24	42
20	15-Jul-02	15	2010	2033	2240	1960	1990	1940				24	45
21	16-Jul-02	16	2120	1903	1750	1790	2070	2000	1.612	2.152	3.35E-03	23	47
22	17-Jul-02	17	2050	1935	2080	1880	2030	1750				23	53
23	18-Jul-02	18	2090	1890	1750	1690	2000	2120				23	48
24	19-Jul-02	19	2030	1933	2120	1780	1840	1990				23	50
27	22-Jul-02	20	2030	1925	1830	1960	1880	2030				23	48
		Mean	**2017**		**2000**				**0.981**	**1.781**	**1.18E-02**	**23.7**	**48.3**
		S.D.	**75**		**160**				**0.423**	**0.402**	**6.57E-03**	**0.6**	**6.0**

Table A GASOLINE TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

Chamber Monitoring Results
Cumulative Exposure Record
Group IIB - 2,000 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	25-Jun-02	1	1990	2090	2260	2030	2060	2010				23	50
1	26-Jun-02	2	1950	2090	2200	2160	1780	2220				24	68
2	27-Jun-02	3	2070	2038	1930	2080	2150	1990				23	53
3	28-Jun-02	4	2140	2128	2380	2400	1910	1820	0.8183	1.961	8.49E-03	24	71
6	1-Jul-02	5	2070	1965	1730	2010	1940	2180				23	50
7	2-Jul-02	6	2120	2068	2470	2070	1860	1870	0.7358	1.419	2.18E-02	23	51
8	3-Jul-02	7	2010	2020	2030	2010	2050	1990				23	51
9	4-Jul-02	8	2030	2073	1990	2270	2080	1950				23	53
10	5-Jul-02	9	1990	1995	2000	2160	1920	1900				23	52
13	8-Jul-02	10	1930	1895	1810	1940	2030	1800				23	50
14	9-Jul-02	11	1890	1750	1590	1680	1840	1890	14.68	3.869	7.02E-02	23	52
15	10-Jul-02	12	1920	1990	2280	2150	1850	1680				23	52
16	11-Jul-02	13	2020	2033	2110	2030	2050	1940				23	37
17	12-Jul-02	14	1880	1930	2190	1960	1640	1930				23	46
20	15-Jul-02	15	2010	2010	1980	2100	1910	2050				23	49
21	16-Jul-02	16	2120	2058	2140	2060	1990	2040	1.010	2.041	2.96E-03	22	49
22	17-Jul-02	17	2050	1843	1830	1640	1790	2110				22	56
23	18-Jul-02	18	2090	2118	2100	1770	2260	2340				22	51
24	19-Jul-02	19	2030	2095	2500	1810	2080	1990				23	51
27	22-Jul-02	20	2030	1928	1830	1990	1960	1930				23	49
		Mean	**2017**		**2006**				**4.311**	**2.323**	**2.59E-02**	**23.0**	**52.1**
		S.D.	**75**		**187**				**6.914**	**1.067**	**2.65E-02**	**0.5**	**7.0**

Table A GASOLINE TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

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

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)	(%)
0	25-Jun-02	1	9760	10430	9010	11400	10300	11000				24	47
1	26-Jun-02	2	10200	10330	10700	10100	10300	10200				25	64
2	27-Jun-02	3	9440	9878	10500	9040	9670	10300				23	49
3	28-Jun-02	4	9850	9943	9920	10700	10500	8650	0.8742	2.041	8.64E-03	24	61
6	1-Jul-02	5	9970	9763	9580	9070	9800	10600				24	47
7	2-Jul-02	6	10200	10180	10300	9950	10700	9770	0.7422	1.699	2.39E-02	24	48
8	3-Jul-02	7	10100	9665	8890	10000	9070	10700				24	50
9	4-Jul-02	8	10100	10310	11600	10000	9770	9860				24	51
10	5-Jul-02	9	10300	9980	9860	10500	9890	9670				24	49
13	8-Jul-02	10	9970	10070	9890	10300	10200	9890				24	48
14	9-Jul-02	11	10300	9975	9800	10000	10100	10000	0.7415	1.782	1.89E-02	24	48
15	10-Jul-02	12	10300	10300	10600	10600	10000	10000				24	48
16	11-Jul-02	13	10300	10060	9700	10400	10200	9920				24	34
17	12-Jul-02	14	9590	9808	8970	9860	10400	10000				24	42
20	15-Jul-02	15	9840	9778	9670	10000	8940	10500				24	46
21	16-Jul-02	16	9910	10360	10300	9920	10900	10300	1.168	2.319	2.78E-03	23	47
22	17-Jul-02	17	10100	10390	10900	10500	10600	9540				23	53
23	18-Jul-02	18	10100	9895	9800	9480	9800	10500				23	51
24	19-Jul-02	19	9920	10980	10400	10500	11700	11300				23	52
27	22-Jul-02	20	10100	10680	10800	10300	11200	10400				23	49
		Mean	**10020**		**10140**				**0.881**	**1.960**	**1.36E-02**	**23.8**	**49.2**
		S.D.	**239**		**609**				**0.201**	**0.280**	**8.30E-03**	**0.6**	**6.1**

Table A GASOLINE TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
0	25-Jun-02	1	9760	10040	9770	10200	10100	10100				24	45
1	26-Jun-02	2	10200	10140	10600	10000	10200	9770				25	64
2	27-Jun-02	3	9440	9615	9230	9920	9450	9860				24	47
3	28-Jun-02	4	9850	9763	9160	9770	9420	10700	0.8268	1.983	7.73E-03	24	60
6	1-Jul-02	5	9970	10040	10200	10300	10500	9160				24	46
7	2-Jul-02	6	10200	9853	10000	9540	10200	9670	0.7424	1.624	2.36E-02	24	46
8	3-Jul-02	7	10100	9898	9160	10400	9730	10300				24	47
9	4-Jul-02	8	10100	10100	10600	10200	9610	10000				24	48
10	5-Jul-02	9	10300	10070	9390	10700	10300	9890				24	46
13	8-Jul-02	10	9970	9918	9480	10200	10100	9890				24	46
14	9-Jul-02	11	10300	10230	10000	10500	10100	10300	0.7374	1.556	2.03E-02	24	47
15	10-Jul-02	12	10300	10370	10300	10800	10400	9990				24	48
16	11-Jul-02	13	10300	10380	10000	10700	10500	10300				24	33
17	12-Jul-02	14	9590	10250	10000	10800	10200	10000				24	41
20	15-Jul-02	15	9840	9243	8500	8790	10100	9580				24	45
21	16-Jul-02	16	9910	10190	10500	9800	9950	10500	0.8683	1.834	2.96E-03	23	44
22	17-Jul-02	17	10100	9518	9920	9670	9670	8810				23	49
23	18-Jul-02	18	10100	9518	9480	8860	9730	10000				23	47
24	19-Jul-02	19	9920	10060	10000	10200	10600	9420				23	48
27	22-Jul-02	20	10100	10650	10300	10800	10800	10700				23	45
		Mean	**10020**		**9992**				**0.794**	**1.749**	**1.36E-02**	**23.8**	**47.1**
		S.D.	**239**		**509**				**0.064**	**0.196**	**8.55E-03**	**0.5**	**6.2**

Table A GASOLINE TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

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	Individual								Mean Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					MMAD (μm)	GSD	TMC (mg/m^3)	(°C)	(%)
0	25-Jun-02	1	18900	20400	19700	19900	21000	21000					26	49
1	26-Jun-02	2	20600	21000	21400	20800	21100	20700					26	67
2	27-Jun-02	3	19200	19900	20200	20100	19800	19500					26	51
3	28-Jun-02	4	18100	20000	19900	20700	19500	19900		0.8760	2.229	9.70E-03	26	65
6	1-Jul-02	5	19600	20380	19800	20800	21000	19900					25	50
7	2-Jul-02	6	19300	20050	19300	20300	20000	20600		0.7496	1.307	1.50E-02	25	51
8	3-Jul-02	7	19600	19450	19900	20100	18600	19200					25	53
9	4-Jul-02	8	19600	19950	20600	19400	19400	20400					24	56
10	5-Jul-02	9	19400	20080	20100	19400	20700	20100					25	52
13	8-Jul-02	10	19200	19700	18500	20700	19700	19900					25	51
14	9-Jul-02	11	19700	20180	18800	20500	20400	21000		0.7553	2.214	1.34E-02	25	51
15	10-Jul-02	12	19400	20850	20000	21000	21800	20600					26	51
16	11-Jul-02	13	18900	19430	21000	17000	19600	20100					26	37
17	12-Jul-02	14	19100	20680	20400	20600	20700	21000					26	45
20	15-Jul-02	15	18900	19380	18900	19400	19200	20000					26	49
21	16-Jul-02	16	19300	19550	19300	19000	20300	19600		1.008	2.241	3.29E-03	25	49
22	17-Jul-02	17	19200	20100	20200	19900	19500	20800					24	55
23	18-Jul-02	18	19100	19880	19900	19400	20300	19900					24	52
24	19-Jul-02	19	18900	20930	20900	21600	20600	20600					25	50
27	22-Jul-02	20	18600	20980	20400	20300	22000	21200					25	50
		Mean	**19230**		**20141**					**0.8472**	**1.998**	**1.03E-02**	**25.3**	**51.7**
		S.D.	**499**		**802**					**0.1220**	**0.461**	**4.51E-03**	**0.7**	**6.2**

Table A GASOLINE TBA VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS 00-6131

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	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
0	25-Jun-02	1	18900	20830	20100	20100	21700	21400				25	48
1	26-Jun-02	2	20600	22030	22000	21800	22600	21700				25	67
2	27-Jun-02	3	19200	18680	18300	18000	19400	19000				24	53
3	28-Jun-02	4	18100	19930	18900	20600	20400	19800	0.8428	2.015	8.17E-03	25	68
6	1-Jul-02	5	19600	19750	19800	20100	19500	19600				24	51
7	2-Jul-02	6	19300	19530	18700	19800	19400	20200	0.7643	1.443	2.07E-02	25	50
8	3-Jul-02	7	19600	20100	20400	20600	19900	19500				25	53
9	4-Jul-02	8	19600	19580	19600	19400	19500	19800				25	54
10	5-Jul-02	9	19400	19300	19100	19200	19500	19400				25	52
13	8-Jul-02	10	19200	19830	18900	20600	19200	20600				25	51
14	9-Jul-02	11	19700	19830	19000	20100	20000	20200	0.7572	1.473	1.47E-02	24	51
15	10-Jul-02	12	19400	19200	18300	19500	20100	18900				24	52
16	11-Jul-02	13	18900	20230	19900	20900	20000	20100				24	36
17	12-Jul-02	14	19100	20350	19700	20600	20500	20600				24	45
20	15-Jul-02	15	18900	20080	20000	21000	20300	19000				24	50
21	16-Jul-02	16	19300	20450	20400	20300	20700	20400	1.277	2.081	2.92E-03	25	47
22	17-Jul-02	17	19200	19980	19900	19700	19900	20400				25	54
23	18-Jul-02	18	19100	19930	19900	19000	19400	19400				25	53
24	19-Jul-02	19	18900	19230	19400	18900	19100	19500				25	51
27	22-Jul-02	20	18600	19400	19200	19200	20000	19200				25	51
		Mean	**19230**		**19884**				**0.9103**	**1.753**	**1.16E-02**	**24.7**	**51.9**
		S.D.	**499**		**845**				**0.2475**	**0.342**	**6.70E-03**	**0.5**	**6.7**

TABLE B

GASOLINE TBA 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 TBA 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 0	MEAN	195	189	189	191	192
	S.D.	10.5	15.0	14.8	12.7	9.3
	N	10	10	10	10	10
WEEK 1	MEAN	214	209	211	214	216
	S.D.	10.8	12.9	13.0	12.9	16.0
	N	10	10	10	10	10
WEEK 2	MEAN	234	230	229	232	235
	S.D.	12.8	16.1	16.0	13.7	13.8
	N	10	10	10	10	10
WEEK 3	MEAN	251	244	245	244	250
	S.D.	14.9	18.7	16.9	16.1	17.0
	N	10	10	10	10	10
WEEK 4	MEAN	262	255	253	254	243
	S.D.	16.1	21.1	21.5	16.3	15.7
	N	10	10	10	10	10

No statistically significant differences

TABLE D

GASOLINE TBA 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	19	20	22	22	24
	S.D.	5.1	8.5	6.7	3.7	8.6
	N	10	10	10	10	10
WEEK 0 TO 2	MEAN	39	41	39	40	43
	S.D.	6.8	9.2	8.2	3.9	8.5
	N	10	10	10	10	10
WEEK 0 TO 3	MEAN	56	55	55	53	57
	S.D.	7.2	10.6	9.5	7.4	10.2
	N	10	10	10	10	10
WEEK 0 TO 4	MEAN	67	67	63	63	51**
	S.D.	9.8	11.4	10.8	7.0	9.2
	N	10	10	10	10	10

Statistical key: ** = p<0.01

TABLE E

GASOLINE TBA 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	116	121	117	117	117
	S.D.	4.2	11.1	4.9	5.2	18.6
	N	10	10	10	9	10
WEEK 1	MEAN	92	96	95	93	99*
	S.D.	4.0	5.7	6.1	3.0	5.4
	N	10	10	10	8	10
WEEK 2	MEAN	87	90	90	87	91
	S.D.	2.2	4.8	7.1	4.1	4.7
	N	10	9	9	9	7
WEEK 3	MEAN	82	85	85	82	84
	S.D.	3.7	4.8	6.7	3.7	4.3
	N	10	10	10	10	7

Statistical key: * = p<0.05

TABLE F

GASOLINE TBA 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 TBA 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 TBA 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 TBA 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 TBA 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 TBA 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	155	209	228	240	268	277
1582	141	188	200	222	234	230
1583	139	193	219	236	259	269
1584	147	197	212	236	251	266
1585	147	205	226	247	260	273
1586	128	178	203	219	235	250
1587	140	183	206	225	236	252
1588	143	198	210	241	252	267
1589	150	208	230	255	278	285
1590	137	192	208	216	241	253
MEAN	143	195	214	234	251	262
S.D.	7.7	10.5	10.8	12.8	14.9	16.1
N	10	10	10	10	10	10

TABLE G

GASOLINE TBA 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	147	202	223	244	263	285
2572	136	183	203	217	225	233
2573	142	200	229	248	250	270
2574	148	194	202	220	239	241
2575	141	183	212	232	248	258
2576	149	182	195	223	242	249
2577	125	172	193	208	214	233
2578	139	185	209	222	237	253
2579	157	218	225	261	281	293
2580	139	168	198	228	241	241
MEAN	142	189	209	230	244	255
S.D.	8.6	15.0	12.9	16.1	18.7	21.1
N	10	10	10	10	10	10

TABLE G

GASOLINE TBA 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	149	195	216	232	245	256
3572	131	165	196	215	226	233
3573	141	191	205	212	233	242
3574	138	189	207	225	240	249
3575	152	198	223	250	271	270
3576	146	206	229	246	258	276
3577	143	171	196	212	227	226
3578	129	173	194	210	226	222
3579	149	209	218	248	257	271
3580	140	196	226	237	264	280
MEAN	142	189	211	229	245	253
S.D.	7.8	14.8	13.0	16.0	16.9	21.5
N	10	10	10	10	10	10

TABLE G

GASOLINE TBA 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	144	207	229	249	260	281
4582	130	179	208	215	229	243
4583	132	168	189	207	211	224
4584	140	193	209	230	244	248
4585	143	186	213	235	249	250
4586	151	203	225	241	259	262
4587	149	210	233	250	260	273
4588	153	192	216	236	253	257
4589	142	189	210	227	231	243
4590	139	186	205	228	248	260
MEAN	142	191	214	232	244	254
S.D.	7.5	12.7	12.9	13.7	16.1	16.3
N	10	10	10	10	10	10

Huntingdon Life Sciences 00-6131I
Immunotoxicity Sub-Group

TABLE G

GASOLINE TBA 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	148	198	216	234	252	239
5552	158	211	248	265	284	278
5553	138	193	219	237	247	248
5554	150	190	206	214	235	225
5555	130	177	198	220	229	227
5556	139	188	206	231	229	240
5557	145	194	221	237	265	251
5558	142	195	213	233	255	239
5559	132	182	199	233	244	231
5560	144	198	237	246	257	255
MEAN	143	192	216	235	250	243
S.D.	8.3	9.3	16.0	13.8	17.0	15.7
N	10	10	10	10	10	10

TABLE H

GASOLINE TBA 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	31	59	68
1582	13	35	47	42
1583	26	43	66	76
1584	14	39	54	68
1585	21	42	54	68
1586	25	41	57	72
1587	24	42	53	69
1588	12	43	53	68
1589	22	47	70	77
1590	16	24	49	61
MEAN	19	39	56	67
S.D.	5.1	6.8	7.2	9.8
N	10	10	10	10

TABLE H

GASOLINE TBA 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	21	42	61	83
2572	20	34	43	50
2573	29	48	50	70
2574	8	26	45	47
2575	29	49	65	75
2576	13	41	60	67
2577	21	36	42	61
2578	24	36	51	68
2579	7	43	63	75
2580	30	60	73	73
MEAN	20	41	55	67
S.D.	8.5	9.2	10.6	11.4
N	10	10	10	10

TABLE H

GASOLINE TBA 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	21	37	50	62
3572	31	50	61	68
3573	14	21	42	51
3574	18	36	50	60
3575	24	51	73	72
3576	23	40	52	70
3577	25	41	56	55
3578	21	37	53	48
3579	9	40	48	63
3580	30	41	68	84
MEAN	22	39	55	63
S.D.	6.7	8.2	9.5	10.8
N	10	10	10	10

TABLE H

GASOLINE TBA 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	22	42	53	74
4582	29	36	50	64
4583	21	38	43	56
4584	16	37	51	56
4585	26	48	63	64
4586	22	38	55	59
4587	23	40	50	63
4588	24	45	61	65
4589	21	38	43	55
4590	19	42	62	74
MEAN	22	40	53	63
S.D.	3.7	3.9	7.4	7.0
N	10	10	10	10

TABLE H

GASOLINE TBA 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	17	35	54	40
5552	37	54	74	67
5553	26	44	54	55
5554	17	25	45	36
5555	21	43	52	50
5556	19	44	42	53
5557	28	44	71	58
5558	18	39	61	45
5559	17	51	62	49
5560	40	49	60	58
MEAN	24	43	57	51
S.D.	8.6	8.5	10.2	9.2
N	10	10	10	10

Huntingdon Life Sciences 00-6131I
Immunotoxicity Sub-Group

TABLE I

GASOLINE TBA 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
1581	112	89	87	87
1582	116	91	87	77
1583	119	97	90	85
1584	110	87	86	79
1585	118	91	86	80
1586	121	96	91	85
1587	119	99	88	81
1588	114	91	86	78
1589	112	89	83	79
1590	123	89	88	85
MEAN	116	92	87	82
S.D.	4.2	4.0	2.2	3.7
N	10	10	10	10

TABLE I

GASOLINE TBA 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
2571	135	101	99	94
2572	121	96	86	80
2573	127	97	88	81
2574	117	94	92	84
2575	124	103	SF	92
2576	107	95	92	82
2577	142	98	88	81
2578	110	90	82	83
2579	116	85	88	86
2580	114	103	93	85
MEAN	121	96	90	85
S.D.	11.1	5.7	4.8	4.8
N	10	10	9	10

SF=Spilled Feeder

TABLE I

GASOLINE TBA 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
3571	108	90	82	80
3572	116	102	93	85
3573	115	85	91	85
3574	121	93	SF	86
3575	118	97	88	84
3576	124	105	106	102
3577	110	97	82	77
3578	120	96	89	83
3579	115	89	89	82
3580	120	93	88	86
MEAN	117	95	90	85
S.D.	4.9	6.1	7.1	6.7
N	10	10	9	10

SF=Spilled Feeder

TABLE I

GASOLINE TBA 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
4581	121	93	88	83
4582	119	96	83	76
4583	122	SF	96	87
4584	115	87	83	79
4585	113	94	87	80
4586	112	96	89	83
4587	125	93	83	79
4588	SF	SF	SF	79
4589	110	90	86	87
4590	113	93	89	86
MEAN	117	93	87	82
S.D.	5.2	3.0	4.1	3.7
N	9	8	9	10

SF=Spilled Feeder

TABLE I

GASOLINE TBA 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
5551	152	88	88	79
5552	77	101	86	84
5553	118	98	89	84
5554	112	98	93	90
5555	118	100	SF	SF
5556	116	96	101	87
5557	130	109	SF	SF
5558	119	97	SF	SF
5559	112	96	91	78
5560	119	104	90	88
MEAN	117	99	91	84
S.D.	18.6	5.4	4.7	4.3
N	10	10	7	7

SF=Spilled Feeder

TABLE J

GASOLINE TBA 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	23-JUL-02	4	28
1582	TERMINAL SACRIFICE	23-JUL-02	4	28
1583	TERMINAL SACRIFICE	23-JUL-02	4	28
1584	TERMINAL SACRIFICE	23-JUL-02	4	28
1585	TERMINAL SACRIFICE	23-JUL-02	4	28
1586	TERMINAL SACRIFICE	23-JUL-02	4	28
1587	TERMINAL SACRIFICE	23-JUL-02	4	28
1588	TERMINAL SACRIFICE	23-JUL-02	4	28
1589	TERMINAL SACRIFICE	23-JUL-02	4	28
1590	TERMINAL SACRIFICE	23-JUL-02	4	28

TABLE J

GASOLINE TBA 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	23-JUL-02	4	28
2572	TERMINAL SACRIFICE	23-JUL-02	4	28
2573	TERMINAL SACRIFICE	23-JUL-02	4	28
2574	TERMINAL SACRIFICE	23-JUL-02	4	28
2575	TERMINAL SACRIFICE	23-JUL-02	4	28
2576	TERMINAL SACRIFICE	23-JUL-02	4	28
2577	TERMINAL SACRIFICE	23-JUL-02	4	28
2578	TERMINAL SACRIFICE	23-JUL-02	4	28
2579	TERMINAL SACRIFICE	23-JUL-02	4	28
2580	TERMINAL SACRIFICE	23-JUL-02	4	28

TABLE J

GASOLINE TBA 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	23-JUL-02	4	28
3572	TERMINAL SACRIFICE	23-JUL-02	4	28
3573	TERMINAL SACRIFICE	23-JUL-02	4	28
3574	TERMINAL SACRIFICE	23-JUL-02	4	28
3575	TERMINAL SACRIFICE	23-JUL-02	4	28
3576	TERMINAL SACRIFICE	23-JUL-02	4	28
3577	TERMINAL SACRIFICE	23-JUL-02	4	28
3578	TERMINAL SACRIFICE	23-JUL-02	4	28
3579	TERMINAL SACRIFICE	23-JUL-02	4	28
3580	TERMINAL SACRIFICE	23-JUL-02	4	28

TABLE J

GASOLINE TBA 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	23-JUL-02	4	28
4582	TERMINAL SACRIFICE	23-JUL-02	4	28
4583	TERMINAL SACRIFICE	23-JUL-02	4	28
4584	TERMINAL SACRIFICE	23-JUL-02	4	28
4585	TERMINAL SACRIFICE	23-JUL-02	4	28
4586	TERMINAL SACRIFICE	23-JUL-02	4	28
4587	TERMINAL SACRIFICE	23-JUL-02	4	28
4588	TERMINAL SACRIFICE	23-JUL-02	4	28
4589	TERMINAL SACRIFICE	23-JUL-02	4	28
4590	TERMINAL SACRIFICE	23-JUL-02	4	28

TABLE J

GASOLINE TBA 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	23-JUL-02	4	28
5552	TERMINAL SACRIFICE	23-JUL-02	4	28
5553	TERMINAL SACRIFICE	23-JUL-02	4	28
5554	TERMINAL SACRIFICE	23-JUL-02	4	28
5555	TERMINAL SACRIFICE	23-JUL-02	4	28
5556	TERMINAL SACRIFICE	23-JUL-02	4	28
5557	TERMINAL SACRIFICE	23-JUL-02	4	28
5558	TERMINAL SACRIFICE	23-JUL-02	4	28
5559	TERMINAL SACRIFICE	23-JUL-02	4	28
5560	TERMINAL SACRIFICE	23-JUL-02	4	28