FINAL REPORT

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

Test Substance:	Baseline Gasoline Vapor Condensate
Protocol No:	HLS 00-6125
Subcontractor's Sponsor:	Huntingdon Life Sciences Mettlers Road East Millstone, NJ 08875 (732) 873-2550 *Phone* (732) 873-3992 *Fax*
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Sponsor's Representative:	Thomas Gray, M.S., D.A.B.T.
ImmunoTox's Project Number:	ITI 900
Date:	01 April 2005
Principal Investigator:	Kimber L. White, Jr., Ph.D.
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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

1 July 05
Date

Thomas M. Gray, M.S., D.A.B.T.
Sponsor Representative

Date

II. QUALITY ASSURANCE STATEMENT – IMMUNOTOX

Test Substance: Baseline Gasoline Vapor Condensate

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

Protocol Title: Baseline Gasoline 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. 00-6125
Sponsor Study No. 211-B-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 P.I.	Reported to S.D. and Management	Activity
November 28-29, 2000	November 29, 2000	April 3, 2001	Protocol Review (Immunotoxicity Evaluations)
January 10, 2001	January 10, 2001	April 3, 2001	AFC Assay
March 23, 2001	April 3, 2001	April 3, 2001	Data Audit
March 23, 2001	April 3, 2001	April 3, 2001	Draft Report Audit
March 26, 2001	April 3, 2001	April 3, 2001	Review Corrections of Data Notebook
March 26, 2001	April 3, 2001	April 3, 2001	Review Corrections of Draft Report
August 6, 2001	August 7, 2001	August 8, 2001	Review 3rd Draft Audit
August 30, 2001	August 30, 2001	August 30, 2001	Review Changes of 3rd Draft Report
March 31, 2005	March 31, 2005	March 31, 2005	Review Changes of Final Report

Approved and submitted by: _____ 01 April 05
Quality Assurance Manager Date

QUALITY ASSURANCE STATEMENT - HUNTINGDON

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
GLP Protocol Review	13-14 Nov 00	14 Nov 00
Immunization of Immunotoxicity Animals & Dose Positive Control Animals	05 Jan 01	09 Jan 01
Immunotoxicity Necropsy & Training Records	09 Jan 01	09 Jan 01
Subcontractor Final Reports	10, 11 & 13 Jul 01	13 Jul 01
Final In-Life, Pathology Reports & Study Data	31 Jul, 1-3, 6-11 & 13-18 Aug 01	20 Aug 01
Subcontractor In-life Reports & Micronucleus Report	15-17, 27 & 28 Aug 01	28 Aug 01
Sponsor Comments	21-25 Jul & 15 & 22 Aug & 2 Dec 03	2 Dec 03

Fran Jannone

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

26 May 05
Date

III. SIGNATURE OF PRINCIPALS

This report describes the results used to evaluate the relative immunotoxicological potential of the test substance, Baseline Gasoline 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 01 Apr 05

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

Date 01 Apr 05

Approved:

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

Date 1 July 05

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IV. EXECUTIVE SUMMARY

The study was conducted as part of Huntingdon Life Sciences (HLS) Study No. 00-6125 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 Baseline Gasoline 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 Baseline Gasoline 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 ice pack, 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. There were no statistically significant effects observed in either thymus or spleen weight following exposure to Baseline Gasoline Vapor Condensate, when evaluated as either absolute or relative weight (% body weight), as compared to the air control.

Exposure to Baseline Gasoline Vapor Condensate did not result in significant changes 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).

In conclusion, the results of this immunotoxicological evaluation demonstrate that, under the experimental conditions used, exposure to the Baseline Gasoline 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	*Dose*	Comment
Body Weight				
Day 29	No Effect			
Organ Weights				
Spleen	No Effect			
Thymus	No Effect			
Spleen IgM Antibody-Forming Cell Response to Sheep Erythrocytes				
IgM AFC to sRBC	No Effect			

V. INTRODUCTION

The purpose of this study was to provide evaluation of immunological parameters for Huntingdon Life Sciences (HLS) Study No. 00-6125. In this study, the test substance, Baseline 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 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 pack for evaluation the following day. Spleens were received on 10 January 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 Baseline Gasoline Vapor Condensate on the immune response. This assay has been shown to be the most predictive 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 Baseline Gasoline 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 Baseline Gasoline Vapor Condensate.

VI. METHODS OF PROCEDURE

EXPERIMENTAL DESIGN

The immunotoxicological satellite study consisted of a vehicle group, three exposure levels of Baseline Gasoline 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 Baseline Gasoline 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 No. 108H0568, received 21 September 1999, expiration 30 June 2002, white powder, storage 2-8ºC, 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 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 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 were 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. The plaque assay is regarded as the "Gold Standard" for evaluating effects of compounds on humoral immunity. Although the plaque assay is not considered to be an assay for cell-mediated immunity, since the assay utilizes a T-dependent antigen, it does provide limited information on T-helper cells and macrophages. As indicated above, if these cells are adversely affected, then an effect on humoral immunity can be detected with this assay. 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 that 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, 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 effect was observed on terminal body weights of Baseline Gasoline Vapor Condensate-exposed rats as compared to the vehicle controls.

The organ weights of the control and Test Substance-exposed rats are shown in Table 1. No effect was observed, following exposure to Baseline Gasoline Vapor Condensate, on spleen or thymus weight 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 75% 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 72% 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 Baseline Gasoline Vapor Condensate.

Figure 1

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

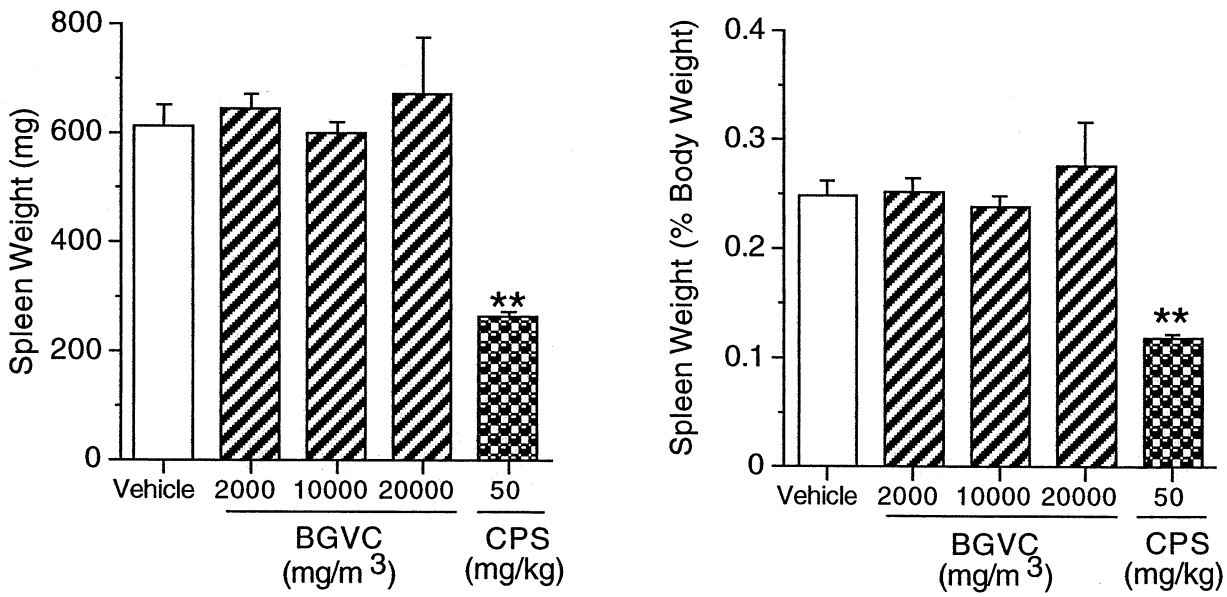
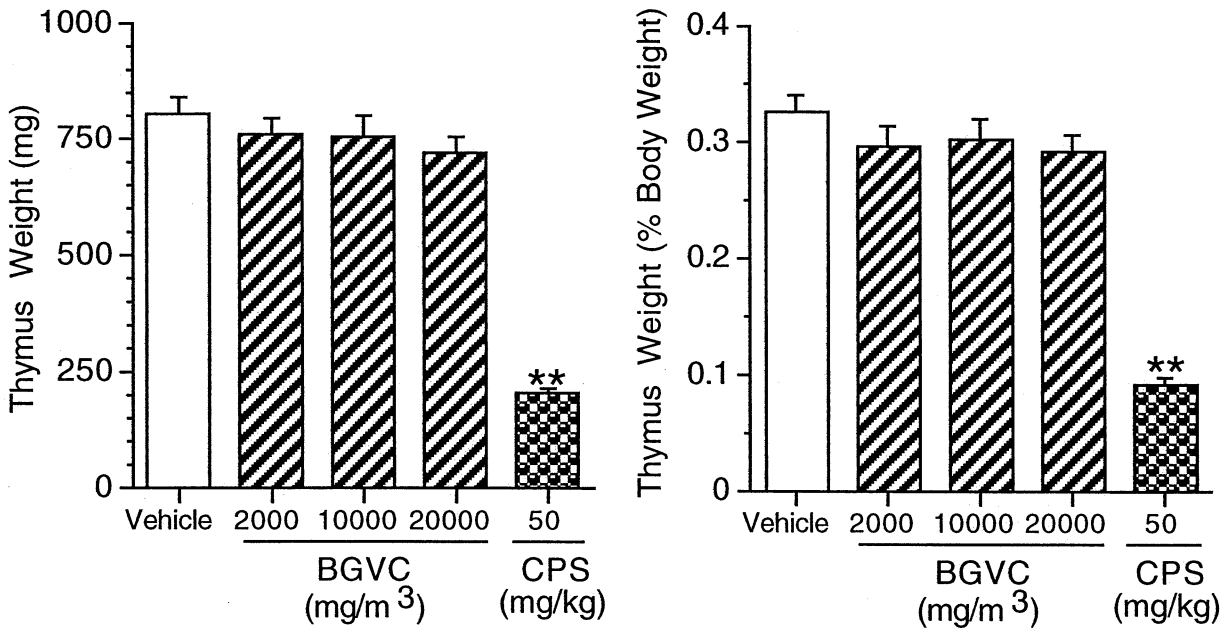


Figure 2

Absolute (mg) and Relative (%) Thymus Weight in Female Sprague Dawley Rats Exposed to Baseline Gasoline Vapor Condensate (BGVC) 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. Animals were sensitized in the morning and also sacrificed in the morning. The results of the AFC response are shown in Table 2 and in Figures 3 and 4. 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 95%.

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.

There was no significant difference in the spleen cell number following exposure to Baseline Gasoline Vapor Condensate (Figure 3). The positive control, cyclophosphamide (CPS), produced an 82% 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, there was no statistically significant difference in the IgM antibody-forming cell response between the Baseline Gasoline Vapor Condensate-exposed animals and the vehicle control group when evaluated either as specific activity (AFC/10^6 spleen cells) or as total spleen activity (AFC/spleen). Furthermore, there was no significant difference in the trend analysis when evaluated by the Jonckheere's Test. This is a very liberal test, often demonstrating statistically significant results when there does not appear visually to be a dose-related trend or dose-related response. The fact that no statistically significant trend was detected with this liberal

test suggests that other statistical analysis for trend would have also shown a non-significant effect.

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. The results of the positive control were consistent with the historical controls for the laboratory. Similarly, the results of the negative control (vehicle) were also consistent with the historical controls for the laboratory.

Figure 3

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

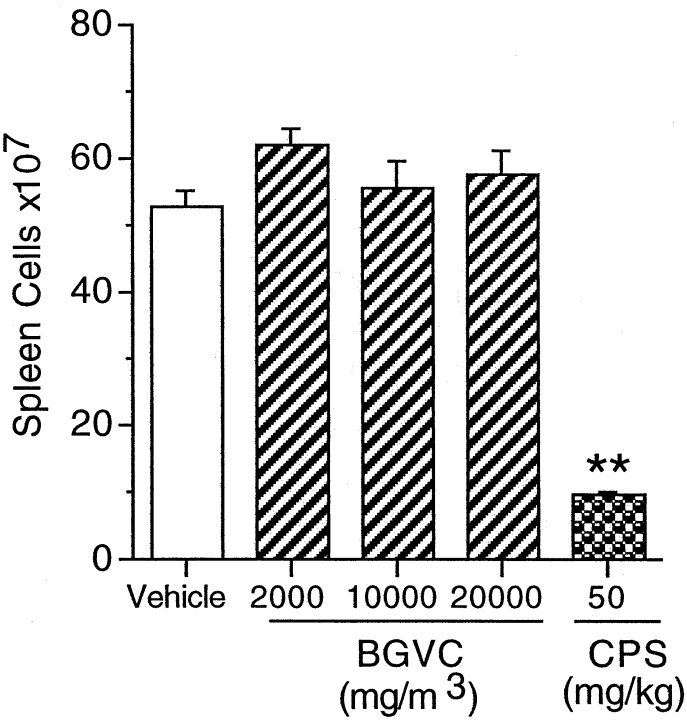
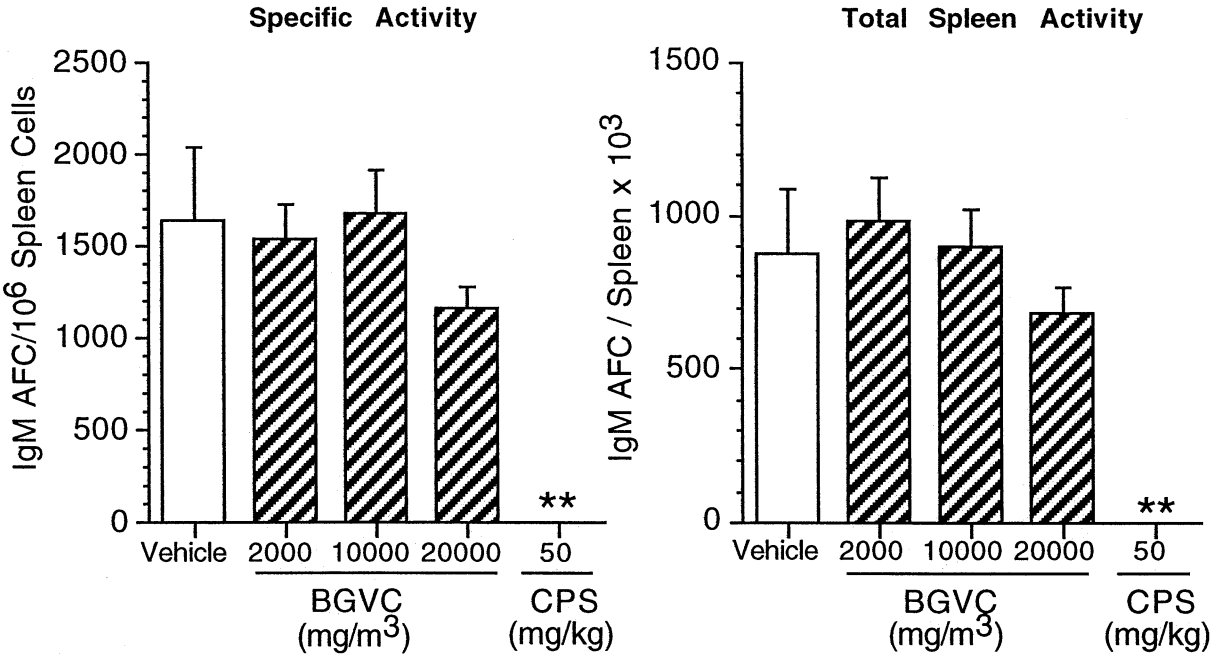


Figure 4

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



VIII. CONCLUSION

Exposure of female Sprague Dawley rats with Baseline Gasoline Vapor Condensate for a period of 5 days per week for 4 weeks did not result in alterations of the humoral immune response as evaluated in the IgM antibody-forming cell response to the T-dependent antigen sheep erythrocytes. There was no statistically significant effect on spleen weight, spleen cell number, or IgM antibody production when evaluated as either specific activity or as total spleen activity. Based on the immunological parameters evaluated, under the experimental conditions of the study, Baseline Gasoline Vapor Condensate did not adversely affect the 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 Baseline Gasoline Vapor Condensate via Inhalation for 5 Days per Week for 4 Weeks

Parameter	Vehicle (10)	Baseline Gasoline Vapor (mg/m^3) 2000 (10)	10000 (10)	20000 (10)	Cyclophosphamide 50 mg/kg (10)	H/NH	Trend Analysis
Body Wgt (g)	247.8 ± 3.6	258.0 ± 5.2	250.0 ± 5.7	245.7 ± 2.7	224.4 ± 5.5**	H	NS
Spleen (mg)	615 ± 38	647 ± 26	600 ± 23	675 ± 102	265 ± 10**	NH	NS
% Body Wgt	0.248 ± 0.014	0.252 ± 0.012	0.239 ± 0.010	0.276 ± 0.041	0.119 ± 0.004**	NH	NS
Thymus (mg)	808 ± 33	761 ± 38	758 ± 44	724 ± 34	205 ± 14**	H	$p \leq 0.05$
% Body Wgt	0.327 ± 0.013	0.297 ± 0.018	0.303 ± 0.018	0.293 ± 0.013	0.093 ± 0.006**	H	$p \leq 0.05$

Female Sprague Dawley rats were administered vehicle control (air only) or Baseline Gasoline Vapor Condensate by inhalation via whole-body exposure for 5 days per week for 4 weeks. The positive control, cyclophosphamide, was admistered 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 pack for next day cell preparation. The rats were necropsied and indicated organ 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 exposure level-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 Baseline Gasoline 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	247.8 ± 3.6 (10)	615 ± 38 (10)	53.18 ± 2.15 (10)	1639 ± 408 (10)	880 ± 209 (10)
Baseline Gasoline Vapor					
2000 mg/m^3	258.0 ± 5.2 (10)	647 ± 26 (10)	62.26 ± 2.35 (10)	1540 ± 194 (10)	980 ± 143 (10)
10000 mg/m^3	250.0 ± 5.7 (10)	600 ± 23 (10)	55.82 ± 4.02 (10)	1687 ± 235 (10)	903 ± 120 (10)
20000 mg/m^3	245.7 ± 2.7 (10)	675 ± 102 (10)	57.96 ± 3.33 (10)	1175 ± 111[a] (9)	685 ± 77[a] (9)
Cyclophosphamide 50 mg/kg	224.4 ± 5.5** (10)	265 ± 10** (10)	9.69 ± 0.41** (10)	3 ± 3** (10)	0 ± 0** (10)
H/NH	H	NH	H	NH	NH
Trend Analysis	NS	NS	NS	NS	NS

Female Sprague Dawley rats were administered vehicle control (air only) or Baseline Gasoline Vapor Condensate by inhalation via whole-body exposure for 5 days per week for 4 weeks. The positive control, cyclophosphamide, was admistered i.p. on 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 pack 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 exposure level-related trends among the vehicle and exposed groups.

[a]Sprague Dawley rats, as a random bred strain, have inherently wide variability in their immune responses. However, it is the opinion of the PI based on his years of experience with this assay that one animal, which had an IgM AFC/10^6 value of 216 and an AFC/spleen of 123, was an outlier and it was scientifically appropriate not to include it in the analysis.

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

Protocol No. HLS Study No. 00-6125
Abbreviated Ttile: Immunological Evaluation of Baseline Gaseline Vapor Condensate

INDIVIDUAL ANIMAL DATA
ORGAN WEIGHTS
BASELINE GASOLINE VAPOR CONDENSATE
00-6125

ANIMAL NO	GROUP	DOSE	SEX	BODY WGT (G)	SPLEEN WGT (MG)	THYMUS WGT (MG)	SPLEEN WGT/% BODY WGT	THYMUS WGT/% BODY WGT
1531	G1	AIR ONLY	F	230.0	605	703	0.260	0.310
1532	G1	AIR ONLY	F	234.0	545	839	0.230	0.360
1533	G1	AIR ONLY	F	246.4	503	821	0.200	0.330
1534	G1	AIR ONLY	F	251.8	721	662	0.290	0.260
1535	G1	AIR ONLY	F	262.6	743	974	0.280	0.370
1536	G1	AIR ONLY	F	245.9	437	673	0.180	0.270
1537	G1	AIR ONLY	F	240.1	521	853	0.220	0.360
1538	G1	AIR ONLY	F	264.9	685	782	0.260	0.300
1539	G1	AIR ONLY	F	246.5	810	867	0.330	0.350
1540	G1	AIR ONLY	F	255.7	584	908	0.230	0.360
2521	G2	2000 MG/M^3 BGVC	F	284.7	686	818	0.240	0.290
2522	G2	2000 MG/M3 BGVC	F	243.1	613	741	0.250	0.300
2523	G2	2000 MG/M3 BGVC	F	273.0	677	780	0.250	0.290
2524	G2	2000 MG/M3 BGVC	F	246.6	540	777	0.220	0.320
2525	G2	2000 MG/M3 BGVC	F	255.9	710	700	0.280	0.270
2526	G2	2000 MG/M3 BGVC	F	256.1	690	494	0.270	0.190
2527	G2	2000 MG/M3 BGVC	F	253.8	541	790	0.210	0.310
2528	G2	2000 MG/M3 BGVC	F	250.2	728	831	0.290	0.330
2529	G2	2000 MG/M3 BGVC	F	281.1	532	720	0.190	0.260
2530	G2	2000 MG/M3 BGVC	F	235.7	753	961	0.320	0.410
3521	G3	10000 MG/M^3 BGVC	F	249.4	484	845	0.190	0.340
3522	G3	10000 MG/M^3 BGVC	F	273.3	641	750	0.230	0.270
3523	G3	10000 MG/M^3 BGVC	F	255.5	610	768	0.240	0.300
3524	G3	10000 MG/M^3 BGVC	F	256.9	596	630	0.230	0.250
3525	G3	10000 MG/M^3 BGVC	F	232.2	535	480	0.230	0.210
3526	G3	10000 MG/M^3 BGVC	F	283.4	607	862	0.210	0.300
3527	G3	10000 MG/M^3 BGVC	F	235.1	605	878	0.260	0.370
3528	G3	10000 MG/M^3 BGVC	F	228.2	529	714	0.230	0.310
3529	G3	10000 MG/M^3 BGVC	F	248.4	728	700	0.290	0.280
3530	G3	10000 MG/M^3 BGVC	F	237.1	668	956	0.280	0.400
4531	G4	20000 MG/M^3 BGVC	F	244.8	535	842	0.220	0.340
4532	G4	20000 MG/M^3 BGVC	F	226.5	562	699	0.250	0.310
4533	G4	20000 MG/M^3 BGVC	F	254.9	487	779	0.190	0.310
4534	G4	20000 MG/M^3 BGVC	F	245.5	553	771	0.230	0.310
4535	G4	20000 MG/M^3 BGVC	F	247.1	1576	529	0.640	0.210
4536	G4	20000 MG/M^3 BGVC	F	243.6	540	631	0.220	0.260
4537	G4	20000 MG/M^3 BGVC	F	253.8	652	841	0.260	0.330
4538	G4	20000 MG/M^3 BGVC	F	255.5	682	829	0.270	0.320
4539	G4	20000 MG/M^3 BGVC	F	241.8	579	687	0.240	0.280
4540	G4	20000 MG/M^3 BGVC	F	243.3	584	628	0.240	0.260
5531	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	200.0	289	170	0.140	0.090
5532	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	219.9	234	174	0.110	0.080
5533	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	230.9	295	203	0.130	0.090
5534	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	222.4	247	222	0.110	0.100
5535	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	218.1	255	314	0.120	0.140
5536	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	251.8	268	223	0.110	0.090
5537	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	244.6	285	169	0.120	0.070
5538	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	234.0	260	201	0.110	0.090
5539	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	197.4	207	168	0.100	0.090
5540	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	224.8	307	204	0.140	0.090

Protocol No. HLS Study No. 00-6125 ITI Study No. ITI 900
Abbreviated Ttile: Immunological Evaluation of Baseline Gaseline Vapor Condensate

INDIVIDUAL ANIMAL DATA
IGM AFC ASSAY
BASELINE GASOLINE VAPOR CONDENSATE
00-6125

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)
1531	G1	AIR ONLY	F	2061	1107	53.70	605	230.0
1532	G1	AIR ONLY	F	958	558	58.26	545	234.0
1533	G1	AIR ONLY	F	752	393	52.26	503	246.4
1534	G1	AIR ONLY	F	967	600	62.04	721	251.8
1535	G1	AIR ONLY	F	1486	855	57.54	743	262.6
1536	G1	AIR ONLY	F	428	174	40.68	437	245.9
1537	G1	AIR ONLY	F	1908	996	52.20	521	240.1
1538	G1	AIR ONLY	F	4745	2340	49.32	685	264.9
1539	G1	AIR ONLY	F	2510	1521	60.60	810	246.5
1540	G1	AIR ONLY	F	571	258	45.18	584	255.7
2521	G2	2000 MG/M^3 BGVC	F	1944	1260	64.80	686	284.7
2522	G2	2000 MG/M^3 BGVC	F	1877	1269	67.62	613	243.1
2523	G2	2000 MG/M^3 BGVC	F	2193	1404	64.02	677	273.0
2524	G2	2000 MG/M^3 BGVC	F	1256	654	52.08	540	246.6
2525	G2	2000 MG/M^3 BGVC	F	768	519	67.56	710	255.9
2526	G2	2000 MG/M^3 BGVC	F	1024	636	62.10	690	256.1
2527	G2	2000 MG/M^3 BGVC	F	538	288	53.58	541	253.8
2528	G2	2000 MG/M^3 BGVC	F	2148	1593	74.16	728	250.2
2529	G2	2000 MG/M^3 BGVC	F	1499	780	52.02	532	281.1
2530	G2	2000 MG/M^3 BGVC	F	2157	1395	64.68	753	235.7
3521	G3	10000 MG/M^3 BGVC	F	1954	972	49.74	484	249.4
3522	G3	10000 MG/M^3 BGVC	F	1540	909	59.04	641	273.3
3523	G3	10000 MG/M^3 BGVC	F	2612	1404	53.76	610	255.5
3524	G3	10000 MG/M^3 BGVC	F	2173	1188	54.66	596	256.9
3525	G3	10000 MG/M^3 BGVC	F	779	405	52.02	535	232.2
3526	G3	10000 MG/M^3 BGVC	F	894	429	48.00	607	283.4
3527	G3	10000 MG/M^3 BGVC	F	1861	825	44.34	605	235.1
3528	G3	10000 MG/M^3 BGVC	F	2633	1098	41.70	529	228.2
3529	G3	10000 MG/M^3 BGVC	F	515	426	82.68	728	248.4
3530	G3	10000 MG/M^3 BGVC	F	1906	1377	72.24	668	237.1
4531	G4	20000 MG/M^3 BGVC	F	946	480	50.76	535	244.8
4532	G4	20000 MG/M3 BGVC	F	1921	990	51.54	562	226.5
4533	G4	20000 MG/M3 BGVC	F	1002	483	48.18	487	254.9
4534	G4	20000 MG/M3 BGVC	F	*	*	56.82	553	245.5
4535	G4	20000 MG/M3 BGVC	F	1186	972	81.96	1576	247.1
4536	G4	20000 MG/M3 BGVC	F	746	393	52.68	540	243.6
4537	G4	20000 MG/M3 BGVC	F	1122	732	65.22	652	253.8
4538	G4	20000 MG/M3 BGVC	F	1407	930	66.12	682	255.5
4539	G4	20000 MG/M3 BGVC	F	1082	621	57.42	579	241.8
4540	G4	20000 MG/M3 BGVC	F	1160	567	48.90	584	243.3
5531	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	11.40	289	200.0
5532	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	9.54	234	219.9
5533	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	28	3	10.86	295	230.9
5534	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	10.02	247	222.4
5535	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	9.90	255	218.1
5536	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	8.34	268	251.8
5537	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	8.28	285	244.6
5538	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	9.66	260	234.0
5539	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	7.62	207	197.4
5540	PC	50 MG/KG CYCLOPHOSPHAMIDE	F	0	0	11.28	307	224.8

* OUTLIER PER PI

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: Experimental Initiation Date: 13 December 2000 (in-life)

Experimental Completion Date: 9 January 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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TABLES

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

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
				Mean	Individual								Mean	
			(mg/m^3)	(mg/m^3)	(mg/m^3)					MMAD (μm)	GSD	TMC (mg/m^3)	Temperature (°C)	Humidity (%)
0	13-Dec-00	1	0	0	0	0	0	0					24	50
1	14-Dec-00	2	0	0	0	0	0	0		6.691	2.106	5.91E-03	24	52
2	15-Dec-00	3	0	0	0	0	0	0					23	53
5	18-Dec-00	4	0	0	0	0	0	0					23	49
6	19-Dec-00	5	0	0	0	0	0	0					24	49
7	20-Dec-00	6	0	0	0	0	0	0					23	49
8	21-Dec-00	7	0	0	0	0	0	0		1.547	2.439	5.49E-03	23	50
9	22-Dec-00	8	0	0	0	0	0	0					22	51
10	23-Dec-00	9	0	0	0	0	0	0					23	49
13	26-Dec-00	10	0	0	0	0	0	0					23	46
14	27-Dec-00	11	0	0	0	0	0	0					23	47
15	28-Dec-00	12	0	0	0	0	0	0		4.795	2.430	2.65E-03	24	47
16	29-Dec-00	13	0	0	0	0	0	0					20	45
19	1-Jan-01	14	0	0	0	0	0	0					24	35
20	2-Jan-01	15	0	0	0	0	0	0					24	36
21	3-Jan-01	16	0	0	0	0	0	0					24	34
22	4-Jan-01	17	0	0	0	0	0	0		6.386	2.853	9.53E-03	24	34
23	5-Jan-01	18	0	0	0	0	0	0					24	33
25	7-Jan-01	19	0	0	0	0	0	0					24	34
26	8-Jan-01	20	0	0	0	0	0	0					22	51
		Mean	**0**		**0**					**4.855**	**2.457**	**5.90E-03**	**23.3**	**44.7**
		S.D.	**0**		**0**					**2.357**	**0.306**	**2.82E-03**	**1.0**	**7.2**

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

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(μm)		(mg/m^3)	(°C)	(%)
0	13-Dec-00	1	0	0	0	0	0	0					24	49
1	14-Dec-00	2	0	0	0	0	0	0		4.400	1.871	1.21E-02	24	50
2	15-Dec-00	3	0	0	0	0	0	0					23	51
5	18-Dec-00	4	0	0	0	0	0	0					23	49
6	19-Dec-00	5	0	0	0	0	0	0					24	50
7	20-Dec-00	6	0	0	0	0	0	0					24	50
8	21-Dec-00	7	0	0	0	0	0	0		4.451	2.332	1.17E-02	24	49
9	22-Dec-00	8	0	0	0	0	0	0					24	50
10	23-Dec-00	9	0	0	0	0	0	0					24	48
13	26-Dec-00	10	0	0	0	0	0	0					25	43
14	27-Dec-00	11	0	0	0	0	0	0					24	45
15	28-Dec-00	12	0	0	0	0	0	0		5.507	2.284	5.99E-03	24	44
16	29-Dec-00	13	0	0	0	0	0	0					20	40
19	1-Jan-01	14	0	0	0	0	0	0					24	35
20	2-Jan-01	15	0	0	0	0	0	0					23	34
21	3-Jan-01	16	0	0	0	0	0	0					25	32
22	4-Jan-01	17	0	0	0	0	0	0		3.336	2.996	6.55E-03	25	31
23	5-Jan-01	18	0	0	0	0	0	0					25	33
25	7-Jan-01	19	0	0	0	0	0	0					25	33
26	8-Jan-01	20	0	0	0	0	0	0					23	52
		Mean	**0**		**0**					**4.424**	**2.371**	**9.09E-03**	**23.9**	**43.4**
		S.D.	**0**		**0**					**0.887**	**0.465**	**3.26E-03**	**1.1**	**7.6**

Chamber Monitoring Results
Cumulative Exposure Record
Group IIA - 2000 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	13-Dec-00	1	2440	1955	1650	2030	2210	1930					23	49
1	14-Dec-00	2	2600	2538	3490	2600	1950	2110		4.567	2.014	6.91E-03	23	50
2	15-Dec-00	3	2590	2103	2080	2050	1930	2350					22	61
5	18-Dec-00	4	2460	1905	1860	2000	1860	1900					22	55
6	19-Dec-00	5	2410	1908	1870	1940	2020	1800					22	54
7	20-Dec-00	6	2540	1988	1990	2060	1990	1910					23	51
8	21-Dec-00	7	2680	2065	1920	1920	2190	2230		1.099	2.345	3.48E-03	23	50
9	22-Dec-00	8	2540	1950	1800	1930	2080	1990					23	50
10	23-Dec-00	9	2640	2048	1980	2110	2050	2050					23	50
13	26-Dec-00	10	2640	2218	2340	2080	2160	2290					23	45
14	27-Dec-00	11	2500	2100	2310	2170	2120	1800					23	45
15	28-Dec-00	12	2840	2208	2400	2360	2230	1840		0.9486	1.589	8.27E-04	24	44
16	29-Dec-00	13	2490	2095	2150	2090	2070	2070					20	38
19	1-Jan-01	14	2580	2158	2360	2180	2080	2010					23	35
20	2-Jan-01	15	2490	2103	2170	2110	2110	2020					23	36
21	3-Jan-01	16	2680	2030	2210	1860	1960	2090					24	33
22	4-Jan-01	17	2560	2050	1960	2200	2000	2040		3.091	2.652	7.38E-03	23	33
23	5-Jan-01	18	2530	2065	1930	1960	2330	2040					23	33
25	7-Jan-01	19	2530	1985	1830	2050	2000	2060					23	33
26	8-Jan-01	20	2510	2083	2010	2060	2080	2180					22	51
		Mean	**2563**		**2078**					**2.426**	**2.150**	**4.65E-03**	**22.8**	**44.8**
		S.D.	**100**		**227**					**1.729**	**0.456**	**3.08E-03**	**0.9**	**8.7**

Chamber Monitoring Results
Cumulative Exposure Record
Group IIB - 2000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(μm)		(mg/m^3)	(°C)	(%)
0	13-Dec-00	1	2440	2698	2870	2600	2400	2920					23	53
1	14-Dec-00	2	2600	1975	1700	1950	1990	2260		4.176	2.100	5.92E-03	23	53
2	15-Dec-00	3	2590	2095	2160	2020	2160	2040					22	53
5	18-Dec-00	4	2460	2048	2140	2070	2020	1960					22	51
6	19-Dec-00	5	2410	1920	1830	1950	2050	1850					22	53
7	20-Dec-00	6	2540	1960	2020	2130	1960	1730					22	51
8	21-Dec-00	7	2680	2150	2280	1960	2280	2080		1.141	2.400	3.03E-03	21	55
9	22-Dec-00	8	2540	2000	1840	1990	2130	2040					21	60
10	23-Dec-00	9	2640	2163	2100	2200	2240	2110					22	50
13	26-Dec-00	10	2640	2148	2180	2040	2320	2050					22	46
14	27-Dec-00	11	2500	2058	2110	2290	2040	1790					23	47
15	28-Dec-00	12	2840	2135	2110	1940	2310	2180		6.874	2.344	5.80E-03	23	46
16	29-Dec-00	13	2490	1983	2130	2060	1860	1880					19	51
19	1-Jan-01	14	2580	2098	2110	2080	2170	2030					23	44
20	2-Jan-01	15	2490	2118	2050	2130	2200	2090					23	41
21	3-Jan-01	16	2680	2055	2250	1840	2040	2090					23	39
22	4-Jan-01	17	2560	2068	2020	2230	2040	1980		0.7515	1.603	3.08E-03	23	39
23	5-Jan-01	18	2530	2043	2120	2050	1840	2160					23	38
25	7-Jan-01	19	2530	2048	1780	2110	2140	2160					23	38
26	8-Jan-01	20	2510	2063	2040	2030	2000	2180					22	50
		Mean	**2563**		**2091**					**3.236**	**2.112**	**4.46E-03**	**22.3**	**47.9**
		S.D.	**100**		**199**					**2.868**	**0.363**	**1.62E-03**	**1.0**	**6.3**

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIA - 10000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(μm)		(mg/m^3)	(°C)	(%)
0	13-Dec-00	1	9770	9715	9130	10000	9430	10300					23	51
1	14-Dec-00	2	9800	9635	9010	10300	9680	9550		2.365	1.964	1.98E-03	23	50
2	15-Dec-00	3	10800	9870	9920	10000	9460	10100					23	53
5	18-Dec-00	4	10000	10670	11500	11200	10500	9460					23	49
6	19-Dec-00	5	10400	10800	10300	10500	11800	10600					23	50
7	20-Dec-00	6	10600	11280	10900	11800	12000	10400					24	49
8	21-Dec-00	7	9880	10540	10700	12000	9370	10100		12.48	3.100	7.74E-03	23	50
9	22-Dec-00	8	10100	10350	10000	10600	10600	10200					24	50
10	23-Dec-00	9	10700	11020	9160	13800	10800	10300					24	51
13	26-Dec-00	10	9500	7818	5560	7000	9010	9710					23	46
14	27-Dec-00	11	10200	9463	9010	9070	10000	9770					23	47
15	28-Dec-00	12	10200	10060	9220	10500	10400	10100		3.985	2.387	2.75E-03	24	45
16	29-Dec-00	13	10200	10280	9130	11300	10700	10000					20	40
19	1-Jan-01	14	9760	10030	10600	9220	10000	10300					24	38
20	2-Jan-01	15	9860	10280	10300	10500	10300	10000					23	38
21	3-Jan-01	16	10100	10350	10300	10300	9980	10800					24	41
22	4-Jan-01	17	9850	9990	9800	10500	10500	9160		0.9185	2.223	4.59E-03	23	42
23	5-Jan-01	18	10100	10280	10300	10300	10000	10500					23	41
25	7-Jan-01	19	9430	9133	7560	10300	10000	8670					23	40
26	8-Jan-01	20	10300	10070	9010	10700	9770	10800					23	50
		Mean	**10080**		**10080**					**4.937**	**2.419**	**4.27E-03**	**23.2**	**46.1**
		S.D.	**366**		**1066**					**5.182**	**0.487**	**2.56E-03**	**0.9**	**4.9**

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIB - 10000 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	13-Dec-00	1	9770	9923	10100	9860	9430	10300					23	54
1	14-Dec-00	2	9800	10020	9520	9830	9710	11000		2.902	1.869	2.06E-03	23	54
2	15-Dec-00	3	10800	11550	11500	12000	11600	11100					23	54
5	18-Dec-00	4	10000	10050	10600	8580	10100	10900					22	51
6	19-Dec-00	5	10400	10390	9950	10700	10700	10200					23	55
7	20-Dec-00	6	10600	10090	9860	10800	10700	9010					23	51
8	21-Dec-00	7	9880	9385	8520	9220	9400	10400		11.38	3.202	5.39E-03	22	53
9	22-Dec-00	8	10100	9873	9830	10400	9830	9430					22	52
10	23-Dec-00	9	10700	10500	9980	10700	10900	10400					23	52
13	26-Dec-00	10	9500	10070	9520	9160	10200	11400					22	46
14	27-Dec-00	11	10200	10020	9950	9740	11000	10200					23	48
15	28-Dec-00	12	10200	10030	9010	10600	10500	10000		4.375	2.354	4.86E-03	24	47
16	29-Dec-00	13	10200	10280	10400	10900	9710	10100					20	45
19	1-Jan-01	14	9760	9888	7510	11700	10600	9740					23	41
20	2-Jan-01	15	9860	9965	9920	10200	10100	9640					23	40
21	3-Jan-01	16	10100	10030	10100	9680	9950	10400					23	41
22	4-Jan-01	17	9850	9915	9740	9980	9640	10300		0.9483	2.377	4.34E-03	23	42
23	5-Jan-01	18	10100	10420	9370	11000	10500	10800					23	41
25	7-Jan-01	19	9430	9490	10000	9160	9340	9460					23	40
26	8-Jan-01	20	10300	10690	9550	11700	11400	10100					23	49
		Mean	**10080**		**10140**					**4.901**	**2.451**	**4.16E-03**	**22.7**	**47.8**
		S.D.	**366**		**777**					**4.541**	**0.553**	**1.47E-03**	**0.8**	**5.4**

Chamber Monitoring Results
Cumulative Exposure Record
Group IVA - 20000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(μm)		(mg/m^3)	(°C)	(%)
0	13-Dec-00	1	20800	22250	24000	21200	22300	21500					24	52
1	14-Dec-00	2	20400	20180	23800	16000	19700	21200		3.637	2.190	4.62E-03	24	51
2	15-Dec-00	3	20300	20150	20300	20200	19700	20400					24	51
5	18-Dec-00	4	20500	21000	19900	21400	21300	21400					23	50
6	19-Dec-00	5	19900	20030	20300	19600	20500	19700					24	51
7	20-Dec-00	6	20800	21180	21700	21200	21000	20800					24	51
8	21-Dec-00	7	20500	19650	19100	19100	20100	20300		12.61	3.065	6.61E-03	24	52
9	22-Dec-00	8	20500	20200	20800	20000	20200	19800					24	51
10	23-Dec-00	9	21500	21350	22500	21400	20800	20700					24	51
13	26-Dec-00	10	20400	20330	17200	21100	22300	20700					24	47
14	27-Dec-00	11	20500	21950	19300	22900	23300	22300					24	48
15	28-Dec-00	12	20400	21330	21600	21800	20500	21400		3.491	2.309	5.21E-03	24	48
16	29-Dec-00	13	20000	20500	20600	19800	20500	21100					20	53
19	1-Jan-01	14	20800	21730	21600	22000	21800	21500					24	35
20	2-Jan-01	15	20300	20430	20500	19500	21200	20500					24	34
21	3-Jan-01	16	20900	20880	20900	20200	21300	21100					23	37
22	4-Jan-01	17	20400	20350	20100	20400	20600	20300		1.925	3.017	5.64E-03	23	38
23	5-Jan-01	18	20600	20180	20900	20700	20400	18700					23	37
25	7-Jan-01	19	20300	19450	21200	18000	20600	18000					23	38
26	8-Jan-01	20	20600	20230	20200	19700	20600	20400					23	51
		Mean	**20520**		**20660**					**5.416**	**2.645**	**5.52E-03**	**23.5**	**46.3**
		S.D.	**341**		**1272**					**4.858**	**0.460**	**8.38E-04**	**0.9**	**6.8**

Chamber Monitoring Results
Cumulative Exposure Record
Group IVB - 20000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(μm)		(mg/m^3)	(°C)	(%)
0	13-Dec-00	1	20800	21900	23800	21000	21100	21700					24	55
1	14-Dec-00	2	20400	23280	19100	27600	24000	22400		4.319	2.227	2.60E-03	24	53
2	15-Dec-00	3	20300	20630	20200	21100	19800	21400					24	54
5	18-Dec-00	4	20500	20550	18600	21200	21100	21300					24	51
6	19-Dec-00	5	19900	18530	16800	19700	18400	19200					24	52
7	20-Dec-00	6	20800	20080	20800	20000	20400	19100					24	51
8	21-Dec-00	7	20500	19250	19100	18700	19200	20000		2.347	2.173	4.09E-03	24	50
9	22-Dec-00	8	20500	20200	20800	19900	20200	19900					24	50
10	23-Dec-00	9	21500	21130	22800	21400	20100	20200					28	50
13	26-Dec-00	10	20400	20200	24600	17000	18800	20400					24	46
14	27-Dec-00	11	20500	19480	21700	19100	18400	18700					23	48
15	28-Dec-00	12	20400	21400	21900	21900	20900	20900		3.037	2.262	4.26E-03	24	48
16	29-Dec-00	13	20000	19180	18100	18400	19800	20400					20	42
19	1-Jan-01	14	20800	20100	21100	19400	20100	19800					24	32
20	2-Jan-01	15	20300	19780	19900	19000	20000	20200					24	31
21	3-Jan-01	16	20900	20350	20600	19200	21200	20400					25	35
22	4-Jan-01	17	20400	19700	19900	19800	19800	19300		1.177	2.527	5.06E-03	24	37
23	5-Jan-01	18	20600	20250	21000	20200	20300	19500					24	35
25	7-Jan-01	19	20300	20050	18400	20900	19400	21500					24	35
26	8-Jan-01	20	20600	20430	20100	19300	21700	20600					24	49
		Mean	**20520**		**20330**					**2.720**	**2.297**	**4.00E-03**	**24.0**	**45.2**
		S.D.	**341**		**1599**					**1.314**	**0.157**	**1.03E-03**	**1.3**	**8.0**

TABLE B

BGVC: 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 WEEKLY CLINICAL OBSERVATIONS

	GROUP#	WEEK OF STUDY -1	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

BGVC: 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)

GROUP:		I	II	III	IV	V
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000	POSITIVE CONTROL
WEEK -1	MEAN	118	119	119	118	118
	S.D.	8.0	8.3	7.5	7.9	8.2
	N	10	10	10	10	10
WEEK 0	MEAN	161	163	163	163	162
	S.D.	7.5	10.1	9.8	11.1	10.3
	N	10	10	10	10	10
WEEK 1	MEAN	185	189	186	186	188
	S.D.	8.4	12.8	12.0	8.8	15.1
	N	10	10	10	10	10
WEEK 2	MEAN	214	218	214	209	211
	S.D.	8.4	13.4	13.0	11.3	18.7
	N	10	10	10	10	10
WEEK 3	MEAN	236	240	237	233	233
	S.D.	8.8	15.1	16.0	10.7	17.5
	N	10	10	10	10	10

No statistically significant differences

TABLE D

BGVC: 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)

	GROUP:	I	II	III	IV	V
	EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000	POSITIVE CONTROL
WEEK 0 TO 1	MEAN	24	25	23	23	26
	S.D.	3.4	5.4	4.0	7.8	6.9
	N	10	10	10	10	10
WEEK 0 TO 2	MEAN	53	55	51	46	49
	S.D.	5.3	7.0	6.0	8.7	10.3
	N	10	10	10	10	10
WEEK 0 TO 3	MEAN	75	77	74	71	71
	S.D.	7.7	10.6	10.9	9.6	9.8
	N	10	10	10	10	10

No statistically significant differences

Huntingdon Life Sciences 00-6125I
Immunotoxicity Sub-Group

TABLE E

BGVC: 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)

	GROUP:	I	II	III	IV	V
	EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000	POSITIVE CONTROL
WEEK 0	MEAN	138	137	138	140	135
	S.D.	8.7	4.2	5.9	5.3	3.5
	N	9	10	10	10	10
WEEK 1	MEAN	103	100	103	106	106
	S.D.	6.1	5.7	4.6	5.6	4.8
	N	10	10	10	10	8
WEEK 2	MEAN	96	96	97	96	95
	S.D.	5.4	5.0	3.9	5.3	3.3
	N	10	10	10	10	9
WEEK 3	MEAN	89	91	94	91	91
	S.D.	6.6	4.0	4.9	4.1	3.2
	N	9	10	10	10	9

No statistically significant differences

TABLE F

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

INDIVIDUAL WEEKLY CLINICAL OBSERVATIONS

FEMALES GROUP I 0 mg/m3

ANIMAL#	OBSERVATIONS	WEEK OF STUDY	- 1
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

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

INDIVIDUAL WEEKLY CLINICAL OBSERVATIONS

FEMALES GROUP II 2,000 mg/m3

ANIMAL#	OBSERVATIONS	WEEK OF STUDY	- 1
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

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

INDIVIDUAL WEEKLY CLINICAL OBSERVATIONS

FEMALES GROUP III 10,000 mg/m3

ANIMAL#	OBSERVATIONS	WEEK OF STUDY	- 1
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

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

INDIVIDUAL WEEKLY CLINICAL OBSERVATIONS

FEMALES GROUP IV 20,000 mg/m3

ANIMAL#	OBSERVATIONS	WEEK OF STUDY - 1
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

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

INDIVIDUAL WEEKLY CLINICAL OBSERVATIONS

FEMALES GROUP V POSITIVE CONTROL

ANIMAL#	OBSERVATIONS	WEEK OF STUDY	- 1
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

BGVC: 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
1531	108	152	176	198	215
1532	114	151	178	202	231
1533	130	168	190	216	239
1534	125	170	192	215	244
1535	121	168	192	225	241
1536	129	168	196	222	230
1537	107	152	173	213	235
1538	120	166	189	217	244
1539	113	158	176	213	240
1540	119	160	189	219	241
MEAN	118	161	185	214	236
S.D.	8.0	7.5	8.4	8.4	8.8
N	10	10	10	10	10

TABLE G

BGVC: 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
2521	127	176	206	239	269
2522	118	164	184	207	219
2523	130	170	202	237	258
2524	109	145	173	204	228
2525	120	168	190	221	237
2526	123	166	185	216	236
2527	118	162	193	211	239
2528	112	155	179	209	233
2529	126	175	204	232	253
2530	105	151	169	206	230
MEAN	119	163	189	218	240
S.D.	8.3	10.1	12.8	13.4	15.1
N	10	10	10	10	10

TABLE G

BGVC: 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
3521	121	160	183	208	235
3522	124	176	203	230	256
3523	123	164	195	224	252
3524	128	171	194	215	231
3525	112	152	174	199	216
3526	129	178	200	238	267
3527	115	156	174	209	234
3528	107	154	173	201	222
3529	119	168	192	212	227
3530	110	152	174	202	231
MEAN	119	163	186	214	237
S.D.	7.5	9.8	12.0	13.0	16.0
N	10	10	10	10	10

TABLE G

BGVC: 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
4531	121	170	181	215	235
4532	106	140	167	187	209
4533	113	150	187	207	236
4534	119	165	186	212	230
4535	111	168	186	220	246
4536	127	174	200	224	245
4537	121	161	193	199	238
4538	126	169	190	219	240
4539	110	157	180	208	231
4540	129	175	188	201	225
MEAN	118	163	186	209	233
S.D.	7.9	11.1	8.8	11.3	10.7
N	10	10	10	10	10

TABLE G

BGVC: 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
5531	110	157	175	187	212
5532	112	158	189	202	229
5533	115	161	184	209	232
5534	120	159	187	212	229
5535	118	157	185	208	226
5536	126	181	207	234	248
5537	126	169	209	233	258
5538	122	162	188	217	237
5539	104	143	159	180	203
5540	131	173	202	233	255
MEAN	118	162	188	211	233
S.D.	8.2	10.3	15.1	18.7	17.5
N	10	10	10	10	10

TABLE H

BGVC: 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
1531	23	46	63
1532	27	50	80
1533	22	48	71
1534	22	46	74
1535	24	56	73
1536	28	54	61
1537	21	61	83
1538	24	52	79
1539	18	55	82
1540	29	60	82
MEAN	24	53	75
S.D.	3.4	5.3	7.7
N	10	10	10

TABLE H

BGVC: 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
2521	30	63	93
2522	20	43	55
2523	32	67	88
2524	28	59	83
2525	22	52	69
2526	19	50	71
2527	31	48	76
2528	24	54	78
2529	29	57	78
2530	18	55	79
MEAN	25	55	77
S.D.	5.4	7.0	10.6
N	10	10	10

TABLE H

BGVC: 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
3521	23	48	75
3522	28	55	80
3523	32	60	88
3524	23	44	60
3525	22	47	64
3526	22	59	89
3527	19	53	78
3528	18	46	68
3529	23	44	58
3530	22	50	79
MEAN	23	51	74
S.D.	4.0	6.0	10.9
N	10	10	10

TABLE H

BGVC: 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
4531	12	45	65
4532	26	47	69
4533	37	57	86
4534	21	47	65
4535	19	52	78
4536	26	50	71
4537	31	37	77
4538	21	50	71
4539	23	52	75
4540	13	27	50
MEAN	23	46	71
S.D.	7.8	8.7	9.6
N	10	10	10

TABLE H

BGVC: 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
5531	18	30	55
5532	31	43	71
5533	23	48	70
5534	28	53	70
5535	29	52	69
5536	26	53	67
5537	40	64	89
5538	26	55	75
5539	15	37	59
5540	28	60	81
MEAN	26	49	71
S.D.	6.9	10.3	9.8
N	10	10	10

Huntingdon Life Sciences 00-6125I
Immunotoxicity Sub-Group

TABLE I

BGVC: 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
1531	140	101	86	82
1532	141	111	97	97
1533	127	112	91	82
1534	SF	100	100	96
1535	140	103	99	94
1536	131	101	92	85
1537	152	109	105	94
1538	137	100	95	93
1539	125	91	93	82
1540	147	104	97	SF
MEAN	138	103	96	89
S.D.	8.7	6.1	5.4	6.6
N	9	10	10	9

SF=Spilled Feeder

TABLE I

BGVC: 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
2521	137	95	90	87
2522	140	93	90	91
2523	129	99	98	88
2524	139	105	101	94
2525	142	99	98	89
2526	130	91	90	86
2527	139	109	100	100
2528	136	100	97	91
2529	139	99	94	91
2530	141	106	104	91
MEAN	137	100	96	91
S.D.	4.2	5.7	5.0	4.0
N	10	10	10	10

TABLE I

BGVC: 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
3521	131	95	92	87
3522	136	100	94	91
3523	144	110	100	99
3524	138	105	94	91
3525	134	102	96	100
3526	131	98	94	88
3527	138	103	100	91
3528	149	108	103	100
3529	144	106	93	96
3530	140	106	101	96
MEAN	138	103	97	94
S.D.	5.9	4.6	3.9	4.9
N	10	10	10	10

TABLE I

BGVC: 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
4531	135	96	97	87
4532	144	109	97	92
4533	147	113	100	96
4534	138	102	94	87
4535	150	112	105	94
4536	137	104	91	86
4537	137	109	91	96
4538	140	108	101	93
4539	136	103	91	85
4540	133	100	90	90
MEAN	140	106	96	91
S.D.	5.3	5.6	5.3	4.1
N	10	10	10	10

TABLE I

BGVC: 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
5531	138	101	89	94
5532	139	115	95	92
5533	132	103	95	93
5534	131	103	94	90
5535	138	103	97	88
5536	137	106	96	84
5537	136	110	93	90
5538	137	SF	SF	SF
5539	129	SF	100	93
5540	137	105	98	93
MEAN	135	106	95	91
S.D.	3.5	4.8	3.3	3.2
N	10	8	9	9

SF=Spilled Feeder

Huntingdon Life Sciences 00-6125I
Immunotoxicity Sub-Group

TABLE J

BGVC: 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	9-JAN-01	4	27
1532	TERMINAL SACRIFICE	9-JAN-01	4	27
1533	TERMINAL SACRIFICE	9-JAN-01	4	27
1534	TERMINAL SACRIFICE	9-JAN-01	4	27
1535	TERMINAL SACRIFICE	9-JAN-01	4	27
1536	TERMINAL SACRIFICE	9-JAN-01	4	27
1537	TERMINAL SACRIFICE	9-JAN-01	4	27
1538	TERMINAL SACRIFICE	9-JAN-01	4	27
1539	TERMINAL SACRIFICE	9-JAN-01	4	27
1540	TERMINAL SACRIFICE	9-JAN-01	4	27

TABLE J

BGVC: 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	9-JAN-01	4	27
2522	TERMINAL SACRIFICE	9-JAN-01	4	27
2523	TERMINAL SACRIFICE	9-JAN-01	4	27
2524	TERMINAL SACRIFICE	9-JAN-01	4	27
2525	TERMINAL SACRIFICE	9-JAN-01	4	27
2526	TERMINAL SACRIFICE	9-JAN-01	4	27
2527	TERMINAL SACRIFICE	9-JAN-01	4	27
2528	TERMINAL SACRIFICE	9-JAN-01	4	27
2529	TERMINAL SACRIFICE	9-JAN-01	4	27
2530	TERMINAL SACRIFICE	9-JAN-01	4	27

TABLE J

BGVC: 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	9-JAN-01	4	27
3522	TERMINAL SACRIFICE	9-JAN-01	4	27
3523	TERMINAL SACRIFICE	9-JAN-01	4	27
3524	TERMINAL SACRIFICE	9-JAN-01	4	27
3525	TERMINAL SACRIFICE	9-JAN-01	4	27
3526	TERMINAL SACRIFICE	9-JAN-01	4	27
3527	TERMINAL SACRIFICE	9-JAN-01	4	27
3528	TERMINAL SACRIFICE	9-JAN-01	4	27
3529	TERMINAL SACRIFICE	9-JAN-01	4	27
3530	TERMINAL SACRIFICE	9-JAN-01	4	27

TABLE J

BGVC: 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	9-JAN-01	4	27
4532	TERMINAL SACRIFICE	9-JAN-01	4	27
4533	TERMINAL SACRIFICE	9-JAN-01	4	27
4534	TERMINAL SACRIFICE	9-JAN-01	4	27
4535	TERMINAL SACRIFICE	9-JAN-01	4	27
4536	TERMINAL SACRIFICE	9-JAN-01	4	27
4537	TERMINAL SACRIFICE	9-JAN-01	4	27
4538	TERMINAL SACRIFICE	9-JAN-01	4	27
4539	TERMINAL SACRIFICE	9-JAN-01	4	27
4540	TERMINAL SACRIFICE	9-JAN-01	4	27

TABLE J

BGVC: 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	9-JAN-01	4	27
5532	TERMINAL SACRIFICE	9-JAN-01	4	27
5533	TERMINAL SACRIFICE	9-JAN-01	4	27
5534	TERMINAL SACRIFICE	9-JAN-01	4	27
5535	TERMINAL SACRIFICE	9-JAN-01	4	27
5536	TERMINAL SACRIFICE	9-JAN-01	4	27
5537	TERMINAL SACRIFICE	9-JAN-01	4	27
5538	TERMINAL SACRIFICE	9-JAN-01	4	27
5539	TERMINAL SACRIFICE	9-JAN-01	4	27
5540	TERMINAL SACRIFICE	9-JAN-01	4	27