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

HLS Study No.: **00-6131**
Sponsor Study No.: 211-TBA-S
Date: 14 November 2011

Summary Report
Gasoline TBA Vapor Condensate:
A 13-Week Whole Body Inhalation Toxicity Study in the Rat

Huntingdon Life Sciences, Inc. Study No. 00-6131
Sponsor Study No 211-TBA-S
Measurement of Glial Fibrillary Acidic Protein

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STATEMENT OF COMPLIANCE

This study was conducted in the spirit of compliance with 79.60, CFR Vol. 59, No. 122, 27 June 1994. This study was performed according to protocol and Standard Operating Procedures.

James P. O'Callaghan, Ph.D.
Principal Investigator

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

Summer 2013
Date

SIGNATURE PAGE

SCIENTIST

The following Scientist was responsible for the overall conduct of this study.

James P. O'Callaghan, Ph.D.
Principal Investigator

12/12/12
Date

SCIENTIFIC REVIEW

The following Scientist has reviewed and approved this report.

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

Smarch 2013
Date

Third-Party QA Statement

Study No. HLS 00-6131

Gasoline TBA Vapor Condensate, A 13-Week Whole-Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments (GFAP Portion of Study)

The Sponsor's third-party QA contractor inspected/audited the following aspects of this study for compliance with SOPs and the study protocol:

Area Inspected	Date of Inspection	Date Reported to Sponsor
Facility Inspection	2/14/01	2/21/01
Facility Inspection	4/3/01	4/10/01
Draft Final Report Audit	11/4/04	11/11/04
Second Draft Final Report Review	5/24/12	5/25/12

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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
GLP Protocol Review	9 Apr 02	9 Apr 02
GFAP Necropsy	25 Sep 02	25 Sep 02
Draft GFAP Report and In-Life Study Data	15-19,22 Aug 03 & 2 Dec 03	2 Dec 03

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Quality Assurance Auditor

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Summary

Toxicant-induced injury of the adult or developing central nervous system of the rat results in hypertrophy of astrocytes at the site of injury. The hallmark of this response is the enhanced expression of the major intermediate filament protein of astrocytes, GFAP. A 13-week inhalation exposure to Gasoline TBA Vapor Condensate caused a slight increase in the cortex of the lowest exposure group males and a slight decrease in the hypothalamus of the high exposure group males. Overall, the data suggest that exposure to Gasoline TBA Vapor Condensate under the regimen employed does not result in gliosis in any of the nine brain regions examined.

Introduction

A characteristic feature of chemical-induced damage of the nervous system is selectivity; exposure to different nervous system toxicants results in damage to different brain regions and cell types (Switzer, 1991; Balaban, 1992; O'Callaghan et al., 1995). The differential susceptibility of nervous system cell types to injury often is referred to as "selective vulnerability"(Spencer and Schuamburg, 1980; Baumgarten and Zimmerman, 1992). An implicit assumption underlying this concept is that intrinsic properties of individual neural cell types render them susceptible to damage by specific chemical exposures (Baumgarten and Zimmerman, 1992). Unfortunately, our knowledge of the mechanisms that confer such vulnerability to specific toxic insults is limited. Thus, often there is no *a priori* basis for predicting the cell types affected by toxic exposures of the nervous system. Given the extreme cellular and molecular heterogeneity of the nervous system (McKay and Hockfield, 1982; Sutcliffe, 1988), the fact that targets of chemical-induced neurotoxicity are diverse and unpredictable should not be surprising. This biologically-based situation does, however, make assessment of neurotoxicity difficult because one must face the problem of deciding where to look for damage. Overcoming this obstacle requires a "marker" of neural injury that can be used to localize (i.e. "mark") sites of damage anywhere in the nervous system.

A universal cellular reaction to damage of the central nervous system is hypertrophy of astrocytes. The hallmark of this response, often termed "reactive gliosis," is the enhanced expression of the major intermediate filament protein of astrocytes, glial fibrillary acidic protein (GFAP). Thus, an increase in the brain concentration of GFAP serves as a biochemical indicator of neurotoxicity. To validate the use of GFAP as a biomarker of neurotoxicity, prototype neurotoxicants have been administered to experimental animals and the effects of these agents on the tissue content of GFAP have been determined by immunoassay (O'Callaghan, 1991; Norton et al., 1992). Assays of GFAP were found to reveal dose-, time- and region-dependent patterns of neurotoxicity at toxicant dosages below those that cause light microscopic evidence of cell loss or damage (O'Callaghan, 1988; Norton et al., 1992). Moreover, the temporal and regional increments in GFAP correspond to the temporal and regional patterns of neuronal damage, as revealed by sensitive silver stains (Balaban, 1992). These findings indicate that assays of GFAP represent a sensitive, simple and quantitative approach for evaluation of nervous system damage (O'Callaghan, 1991; Norton et al., 1992).

As part of the U.S. Environmental Protection Agency's testing requirements under the Clean Air Act, identification and characterization of the potential adverse effects of gasoline and various oxygenate-gasoline blends is to be determined. Neurotoxicity

assessment constitutes a portion of these testing activities. Subchronic (13-week) inhalation exposures to gasoline and gasoline plus each of 6 fuel additives have been performed with a two-generation reproduction toxicity study that includes a neurotoxicity component for gasoline and gasoline plus MTBE vapor condensates only. The purpose of the present study was to use the GFAP assay for assessing the potential neurotoxic effects of Gasoline TBA Vapor Condensate. A control (air) and three exposure levels to the test condensate (2,000, 10,000 and 20,000 mg/m^3) were used. Although the EPA Guidelines (CFR 59, No. 122, 79.67, 1994) specify six regions to be analyzed, we expanded our analysis to include an additional three areas of the brain to maximize the potential for detecting enhanced expression of GFAP due to exposure to the test substance.

Materials, Methods/Procedures

STUDY DATES

STUDY INITIATION

6 May 2002 (Date Study Director signed the Protocol)

DATE OF ANIMAL RECEIPT

6 June 2002

EXPOSURE INITIATION

25 June 2002 (Experimental Start Date)

EXPOSURE TERMINATION

24 September 2002

TERMINAL SACRIFICE

25 September 2002

EXPERIMENTAL TERMINATION

Day Month Year (Date Final Report is signed by the Principal Investigator)

STUDY COMPLETION

Day Month Year (Date Final Report is signed by the Study Director)

I. Basic Protocol 1: GFAP Sandwich ELISA

A. **Introduction**: The GFAP sandwich ELISA is suitable for assaying the concentration of GFAP present in homogenates of brain tissue. This method has successfully been applied to analysis of at least the following species: mouse, rat, guinea pig, dog, monkey, man, chicken, pigeon, trout and cod. Because GFAP is evolutionarily conserved, it is likely that this assay can be very broadly applied across many species. The assay does not require preparation of any special materials or reagents; all components are available from commercial sources at modest cost. The 96-well microplate format lends itself to processing large numbers of samples and it makes the assay suitable for automation with a variety of liquid handling systems. It takes a minimum of 5 hours to process a single 96-well plate after preparation of the tissue homogenates (Support Protocol 1).

B. **Materials**:

Vortex Mixer or Ultrasonic Cell Disruptor (e.g. PGC cat. # 81-6721-

02, 2 mm probe)
Pipettes
Hot/Stir Plate
Microplate Reader
96-well Microtiter Plates (Immulon 2, Dynatech)
Pipette tips
Rabbit anti-Glial Fibrillary Acidic Protein Antibody (DAKO, Cat. #Z0334, Lot #096(401))
Non-Fat Dry Milk (Carnation)
Mouse anti-Glial Fibrillary Acidic Protein Antibody (Oncogene Research Products Cat. #IFO3L, Lot #D15158-3)
Phosphate Buffered Saline (e.g. Pierce Cat. #28374)
Alkaline Phosphatase conjugated anti-mouse IgG (Jackson Immuno Research Cat #315-055-003)
Alkaline Phosphatase Substrate Kit (e.g. Bio-Rad Cat. #172-1063)
Triton X-100 (e.g. Bio-Rad Cat. #161-0407)
Sodium Hydroxide (e.g., 0.4N NaOH, Fisher LC 243204)

C. Protocol Steps:

1. Standard Curve Preparation

1. Prepare a GFAP standard: The preferred standard consists of an aliquot of a brain homogenate prepared as described in Support Protocol 1. This standard should be prepared from the same species that was used to prepare the samples to be assayed for GFAP because GFAP immunoreactivity with a given set of immunodetection reagents will differ among different species. A large number of standards can be prepared in advance from a single "pool" of a 1% SDS homogenate prepared as described in Support Protocol 1. This homogenate can be aliquoted and stored frozen at -70°C prior to use. *Thus, the GFAP standard essentially consists of a control sample. This is preferable over using a pure GFAP standard because using control tissue as a standard obviates any influence of the tissue "matrix" on the assay performance.* To express the data in units of GFAP per unit of total protein, aliquots of a 1% SDS homogenate are still to be used as a GFAP standard. This is accomplished by "standardizing the standard" with addition of a known amount of pure GFAP to the 1% SDS homogenate (i.e. an internal standard). Immunoreactivity values generated from standard curves of the GFAP "spiked" homogenate and the homogenate alone then are used to determine the concentration of GFAP in homogenate. For analysis of GFAP in regions of rat brain, we routinely use aliquots of a hippocampal homogenate as a standard. It contains approximately 2.5 μg GFAP per mg of total protein. Other species (e.g. mouse) contain different levels of GFAP in hippocampus.
2. Prepare dilutions of the GFAP standard: Remove a tube of the GFAP standard from the freezer, thaw at room temperature and vortex or sonify prior to dilution. Using a rat hippocampal homogenate as a typical standard (~2.5 μg GFAP/mg total protein), use the total protein value for this homogenate (~ 10 mg/ml) to prepare a standard curve in PBS plus 0.5% Triton X-100. For rat hippocampal homogenate, the protein values for the standard curve should be between approximately 0.25 to 10 μg/100μl/microplate well (i.e. 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, 10μg total protein/100μl). Table 1 shows an example of dilutions needed to prepare a standard curve from a homogenate of rat hippocampus

(hippocampus std.). *Typically, standards are run in duplicate. Detergents act as wetting agents, therefore, more than a single use of a pipette tip with SDS- or Triton X-100-containing samples can lead to carry-over errors. Thus, it is recommended to use only a single pipette tip per sample and to withdraw the sample only a single time per tip.*

2. Sample Preparation

1. Thaw and mix samples: Remove samples from the freezer, thaw at room temperature and vortex or sonify prior to dilution.
2. Prepare dilutions of the samples: Dilute the samples in PBS + 0.5% Triton X-100 to a concentration of approximately 10μg total protein/ 100μl. Samples high in GFAP (e. g. cerebellum) may need to be diluted to a concentration of 5μg total protein/100μl of PBS + 0.5% Triton X-100. Samples low in GFAP (e.g. striatum) may need to be diluted to 20μg total protein/100μl of PBS + 0.5% Triton X-100. These dilution factors are determined empirically. *The best practice is to prepare multiple dilutions of each sample to insure that optical density readings for a given sample fall on the linear portion of the standard curve. Typically, samples (like standards) are run in duplicate. Detergents act as wetting agents, therefore, more than a single use of a pipette tip with SDS- or Triton-X-100-containing samples can lead to carry-over errors. Thus, it is recommended to use only a single pipette tip per sample and to withdraw the sample only a single time per tip.*

3. GFAP Assay

1. Coat Immulon-2 flat bottom plates with rabbit anti-GFAP. Add 1.0μg total immunoglobulin protein /100 μl PBS /well. (~25μl of anti-GFAP [Dako] in 10 ml of PBS is the quantity needed per plate).
2. Incubate the coated plate at 37°C for 1 hour. This step may be done at the beginning of the assay or it may be done the night before with storage overnight at 4°C. Perform all other incubation and reagent addition steps at room temperature.
3. Empty the plate into a sink and tap on absorbent paper to remove excess liquid. *This latter procedure is important to eliminate the possibility of any reagent carry-over between steps.*
4. Wash plates 4X with PBS (200 μl per well), tapping and blotting between each wash.
5. Block 1 hour with 5% non-fat powdered milk in PBS at 100 μl per well.
6. Empty plate, tap on absorbent paper (upside down) to remove excess liquid, load diluted standard curve and samples in a volume of 100 μl per well. Incubate for 1 hour. The template, Table 2, is an example of a typical 96-well microplate layout for GFAP standards and unknowns.
7. Wash 4X with PBS + 0.5% Triton X-100, 200 μl/well.
8. Incubate for 1 hour in monoclonal anti-GFAP (1:500)(Chemicon) + alkaline phosphatase conjugated anti-mouse IgG (1:3000)(Jackson ImmunoResearch) made up in 5% non-fat dry milk + 0.5% Triton X-100, 100 μl /well.
9. Wash 4X with PBS + 0.5% Triton X-100, 200 μl/well.
10. Add P-nitrophenylphosphate substrate (Bio-Rad) in a volume of 100 μl/well and incubate for 20 minutes.
11. Stop reaction with 0.4N NaOH, 100 μl/well.

12. "Pop" any bubbles in the plate wells with a needle or pipette tip to insure uniform and accurate readings of standard and sample ODs. Read plate at 405 nm.
13. Calculate the GFAP concentration in the samples by comparing their optical density (OD) values to those obtained for the linear portion of the GFAP standard curve. Software programs linked to specific plate readers should be programmed to plot OD vs. GFAP values in linear vs. log linear fashion. Typically, we utilize the 4-parameter curve fit equation and generate curves as shown in Fig. 1 and Table 3. Most programs allow for automatic subtraction of blanks and incorporation of dilution factors. Data are expressed as μg GFAP/per mg total protein or, if the absolute amount of GFAP in the standard is not available, data are expressed as GFAP-like immunoreactivity/per mg total protein. Total protein concentration in the samples is estimated from the total protein assay described in Support Protocol 2. Data also can be expressed on the basis of tissue wet weight (μg GFAP/gram wet weight or GFAP immunoreactivity/gram wet weight). Although this approach permits elimination of the total protein assay (Basic Support Protocol 2), we find that the GFAP values obtained are slightly more variable.

II. Support Protocol 1: Brain tissue preparation

A. Introduction: This support protocol describes the procedure for preparing brain tissue for subsequent analysis of GFAP by Sandwich ELISA (Basic Protocol 1). This procedure does not describe or recommend a specific approach for dissecting brain tissue. *It is noted, however, that reliable dissections are essential for obtaining reproducible results with the GFAP ELISA (see commentary).*

B. Materials:

Dissecting Instruments
Balance
Ultrasonic Cell Disruptor (e.g. PGC cat. # 81-6721-02, 2 mm probe)
Pipettes
Hot/Stir Plate
Pipette tips
Microfuge tubes

C. Protocol Steps:

1. Sacrifice animals and remove the brain as rapidly as possible.
2. Dissect brain regions. If a number of regions are to be dissected, this process can be aided by keeping the brain firm on a cold plate (e.g. Thermoelectrics cold plate, Aldrich Chemical Co. or simply an inverted petri dish placed on ice) maintained at approximately 4°C. Rat or mouse brains can be dissected into 10-15 regions, free hand, within approximately 10 minutes. For this study, nine regions were dissected: Striatum, Hippocampus, Cortex, Olfactory Bulb, Thalamus, Hypothalamus, Cerebellum, Pituitary, and Rest of Brain. *This number of regions can be prepared on a cold plate or at room temperature without degradation of GFAP as assessed by immunoblot analysis. All brain*

regions can be stored frozen indefinitely in capped microfuge tubes at this step in the protocol, or you can proceed to the next step.

3. Weigh and homogenize the dissected brain parts. Tare an appropriately labeled microfuge or other suitable storage tube, or weigh boat for the large brain areas that don't fit into microfuge tubes, ie; Cerebellum, Cortex, Rest of Brain and Thalamus). Place individual brain regions in the tube or weigh boat, obtain the weight, transfer large brain areas to large glass homogenization tube, and immerse the tissue in 10 volumes of hot (85-95°C) 1% (w/v) SDS. For example, 0.1 grams of tissue would be immersed in 1.0 ml of SDS. While the SDS is still hot, homogenize the tissue by sonification with an ultrasonification microprobe. Large brain areas are sonified in the glass homogenization vessel with the ultrasonification microprobe, then homogenized using a motor-driven Teflon pestle. Samples should be stored frozen (-70°C) at time of sacrifice prior to assay. *Samples prepared and stored in this manner retain their GFAP content for at least 5 years.*

III. Support Protocol 2: Assay for Total Protein

A. **Introduction**: This support protocol describes the procedure for assaying the concentration of total protein in the SDS-homogenates. The procedure described essentially is the bicinchoninic acid (BCA) method described by Smith et al. (1985) which is available in kit form (see materials). To assay total protein concentration of the SDS-homogenates the assay must be compatible with 1% SDS. Use of the BCA assay is not an absolute requirement as other detergent compatible methods are available (e.g. Bio-Rad DC protein assay). Bovine serum albumin is used as the protein standard in the described procedure. Other protein standards can be substituted.

B. Materials:

Microplate Reader
Pipettes
Pipette tips
96-well Microtiter Plates
Microfuge tubes
Incubator
Vortex Mixer
Miscellaneous Laboratory Glassware
BCA Protein Kit (Pierce #23223)
Bovine Serum Albumin (BSA) (Sigma A7888)
Sodium Dodecyl Sulfate (Bio-Rad #161-0302)

C. Protocol Steps:

1. Prepare total protein standards. Prepare a 1 mg/ml solution of BSA in 1% (w/v) SDS. *Aliquots of this standard can be stored frozen at -70°C for future use. Thaw as needed, but do not re-freeze.*
2. Prepare a total protein standard curve. Prepare dilutions of the BSA standard in 1% SDS as follows: 1.0, 2.5, 5.0, 7.5, and 10 μg/10μl of 1% SDS (no dilution is required for last standard). Vortex each tube and add 10 μl of each standard to

a well of the microtiter plate; add 10 µl of 1% SDS to serves as a blank. *Typically, standards are run in duplicate. Detergents act as wetting agents, therefore, more than a single use of a pipette tip with SDS-containing samples can lead to carry-over errors. Thus, it is recommended to use only a single pipette tip per sample and to withdraw the sample only a single time per tip.*

3. Prepare dilutions of the samples. Thaw the samples, vortex and dilute a 10 µl aliquot with 190 µl of 1% SDS. Vortex the dilution tube and add a 10 µl aliquot into a well of a microtiter plate.
4. Add the protein assay reagent. Add 200 µl of the BCA reagent (composed of 50:1 ratio of solution A: solution B of the Pierce BCA reagent) to each standard and sample.
5. Incubate the plate at 37°C for 30 minutes. *Other incubation temperatures are permissible; follow direction provided with the kit.*
6. "Pop" any bubbles in the microtiter plate wells with a needle or pipette tip to insure uniform and accurate readings and read the plate at 562 nm.
7. Calculate the concentration of total protein in the samples from the standard curve. Software programs linked to specific plate readers should be programmed to plot OD vs. total protein in a linear fashion. Most programs allow for automatic subtraction of blanks and incorporation of dilution factors. Because the samples were prepared in 10 volumes of diluent, typically, total protein values are approximately 10 mg/ml.

IV. Reagents and Solutions

1. **Phosphate Buffered Saline (PBS)-** One packet of PBS is mixed thoroughly with 500 ml of deionized water to give a final concentration of: 137 mM NaCl/1.0 mM KCl/2 mM KH_2PO_4/8.0 mM $Na_2HPO4{\cdot}7H_2O$/pH 7.4 (can be stored at 4°C for at least a month). For this and all subsequent reagents and solutions, determine the total volume that needs to be prepared based on the use of 100 µl/well and 96 wells per plate (washes take 200 µl/well).
2. **PBS+0.5% Triton X-100**- 2.5ml of Triton X-100 is added to 500 ml of PBS (can be stored at 4°C for at least a month).
3. **Blocking agent(BLOTTO)- (**PBS + 5% powdered milk or PBS + 0.5% Triton X-100 + 5% powdered milk). Five grams of non-fat powdered milk is added per 100 ml of PBS or per 100 ml of PBS + Triton X-100. Prepare at least 100 ml of each to facilitate dissolving the powdered milk; PBS may be warmed slightly to facilitate this process. Make these solutions up fresh the day of assay and do not save. Also, do not retain the powdered milk for greater than a month or two (room temperature). The dry milk tends to discolor and will not go into solution at shelf times longer than 2 months.
4. **Polyclonal anti-GFAP** (Dako)- Add 25µl of antibody solution /10ml of PBS. The assay is based on the use of this antibody as a "capture" reagent. Substitution of an antibody from another vendor may not yield suitable results. Make this solution fresh on the day of use and do not save.
5. **Monoclonal anti-GFAP** (Chemicon; formerly Boehringer Mannheim) **combined with Alkaline Phosphatase-conjugated anti-mouse IgG** (Jackson ImmunoResearch)- Make up a stock solution of monoclonal anti-GFAP and alkaline phosphatase-conjugated anti-mouse IgG as per the vendors' instructions. Store both stocks at 4°C as per the vendor's instructions. Add 20µl of the monoclonal antibody solution stock and 3.3 µl of the alkaline

phosphatase conjugate stock/10ml powdered milk +0.5% Triton X-100. The assay is based on the use of the monoclonal antibody as a "detection" reagent and the alkaline phosphatase conjugate to bind to the detection antibody and generate a colored reaction product proportional to the amount of antigen (GFAP) present in the samples. Substitution of antibodies from other vendors may not yield suitable results. Make these solutions fresh on the day of use and do not save.

6. **P-nitrophenylphosphate substrate** (BioRad)- Mix 2ml of diethanolamine buffer on a stirrer with 2 p-nitrophenylphosphate tablets and 8 ml of deionized water. Make this solution fresh on the day of use and do not save.

V: General Commentary on GFAP Assays; Specific Commentary on the GFAP ELISA

A. Background Information

It has long been known that damage to the central nervous system results in astrogliosis (gliosis, reactive gliosis, glial activation), a response to brain injury characterized by hypertrophy and, less often, hyperplasia of astrocytes, a sub-type of CNS glia (Eng, 1988; Norenberg, 1994). At the electron microscopic level, astrogliosis is characterized by the accumulation of glial filaments. GFAP was found to be the major protein component of these filaments (Eng, 1988). As such, GFAP serves as a biomarker for filament accumulation and, therefore, of gliosis (Eng, 1988; Norton et al., 1992; O'Callaghan, 1993). With the development of antibodies to GFAP, immunohistochemical analysis of this protein soon documented that gliosis occurs in response to diverse insults of the CNS, including trauma, disease, and toxic exposures (Eng, 1988; Norenberg, 1994; Norton et al., 1992; O'Callaghan, 1993; O'Callaghan et al., 1995). Thus, a large body of evidence now has been accumulated demonstrating the ubiquity of the glial response to all types of CNS damage based onimmunohistochemistry of GFAP. Only recently, however, have methods been introduced to assay levels of GFAP as a means of quantifying gliosis.

While GFAP immunohistochemistry has proven useful for revealing patterns of gliosis after brain injury, this approach does not lend itself to quantification or the analysis of large numbers of samples. Small (25-50%), but toxicologically significant increases, also may be difficult to detect by immunohistochemistry. These drawbacks, combined with the need to develop quantitative biomarkers of neurotoxicity (O'Callaghan et al., 1995), and to define quantitative aspects of toxicant- and disease-induced gliosis, has prompted the development and implementation of a number of GFAP assays. These assays have been applied to examine gliosis in specific brain areas already known to be affected by disease or other insult. In addition, they also can be broadly applied in a risk assessment context (U.S. EPA) to screen for potential sites of neural damage resulting from toxic exposures of the CNS. Recently, analysis of GFAP has been used to demonstrate that the degree of cortical gliosis in postmortem brain tissue from victims of Alzheimer's disease correlates with the severity of dementia scores in these individuals prior to death (G. Webster Ross, submitted). Analysis of GFAP in cerebrospinal fluid (CSF) also has been applied to the human condition as an indicator of the severity of traumatic injury to the brain (Rosengren et al., 1994).

Finally, analysis of GFAP can be used as an indicator of the presence of brain or spinal cord contamination of meat (Schmidt et al., 1999).

Of the number of GFAP assays that have appeared in the literature over the last 15 years, all essentially fall into two categories: 1) solid phase immunoassays where GFAP is immobilized on a solid support matrix and detected by mono- or polyclonal antibodies or 2) liquid-phase assays where GFAP from brain extracts or solubilized brain tissue (or CSF) is "captured" by one antibody and then detected by another antibody raised in a different host species (Butler et al., 1986). The assay described in this study is of the second type and it has a number of advantages over the solid phase assays. Specifically, solid-phase detection and "quantification" of GFAP most commonly involves the time-consuming resolution of a protein mixture by SDS-PAGE, followed by electrophoretic transfer to a solid support membrane. Anti-GFAP antibodies coupled to a variety of detection reagents then can be used for quantification of GFAP bound to the membranes. Unfortunately, this approach has been found to severely underestimate the concentration of GFAP in the resolved mixture of proteins and the effects of treatments known to increase GFAP (O'Callaghan et.al., 1999). Other solid phase assays for GFAP have been developed that do not rely on prior resolution of protein mixtures by SDS-PAGE (Wang et al., 1990; O'Callaghan, 1991b). These assays incorporate manual spotting of brain homogenates on solid supports, with or without the aid of a template. The membranes are then incubated with anti-GFAP polyclonal or monoclonal antibodies, which, in turn, are bound by ^{125}I Protein A. Quantification is achieved by gamma spectrometry or by densitometry of the autoradiographs. These assays give a linear signal over a fairly large range of spotted protein. However, they require large amounts of reagents, including radiolabeled reagents, and they do not have impressive throughput.

The sandwich ELISA for GFAP described in this study or similar ELISAs described previously (Eng et al., 1986; Kretzschmar et al., 1985; O'Callaghan, 1991; Rosengren et al., 1994), have several advantages in comparison to the other methods for assaying GFAP described above. They are easier to perform because they have fewer steps. They are more sensitive. Although they may require a greater number of reagents, ELISAs adapted to the microplate format permit the use of very small volumes, which results in a significant overall cost reduction. The 96-well microplate format also has the advantage of speed and high throughput. From sample application to data collection, all steps can be performed in the plate. Moreover, the microplate-based format permits the entire assay to be automated through the use of robotic liquid handling processors. Finally, radioactivity is not involved, making the assay safer to perform and allowing the user to avoid costly and time-consuming radioactivity disposal procedures. While most of the GFAP sandwich ELISAs described to date are similar and share the advantages afforded by this technique, the assay described in this study may have a few additional advantages. Because it is based on detergent-solubilized homogenates of a given brain area, any treatment effects can be directly related to effects in that brain area, rather than an arbitrarily defined extract or sub-fraction that may contain only a portion of the total GFAP in that area. Using a solubilized homogenate rather than a subfraction of a given brain area also facilitates comparisons of quantitative data on GFAP to immunohistochemical staining of GFAP in that area. Moreover, it also helps rule out inter-laboratory differences associated with assaying GFAP content in

one type of extract in one lab, and another type of extract/fraction in another lab. Finally, the same SDS-denatured homogenate used to assay GFAP can be subjected to multiple assays for additional glial or neuronal proteins, thereby permitting comparisons to be made among multiple markers of neurotoxicity in a single sample. For example, the dopaminergic neurotoxicant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, causes a large increase in GFAP that results from damage to dopaminergic nerve terminals, damage that can be quantified by immunoassay of tyrosine hydroxylase (TH), a marker of dopamine containing nerve terminals in the target region (O'Callaghan et al., 1990). Both markers can be assayed from aliquots of the same tissue sample and, on this basis, we find that larger decreases in TH predict greater increases in GFAP.

B. Critical Parameters

The most critical aspect of the GFAP assay is the absolute requirement for preparation of consistently dissected regions of the brain (see Support Protocol 1). Consistent dissections yield consistent GFAP values with the use of only a few animals per dose or time point (see Anticipated Results). The particular regions to be dissected depend on the questions being addressed. If a target region is known or suspected, dissections can be limited to the region of interest. If the GFAP assay is being applied in a screening context, multiple (10-15) brain regions must be dissected in order to avoid the possibility of diluting localized increases in GFAP. The possibility exists that extremely localized increases in GFAP may fail to be detected with the assay. While GFAP immunohistochemistry is relatively insensitive in comparison to the GFAP assay, and it may not detect small increases in GFAP, it can reveal small "hot spots" of gliosis (e.g. see effects of MK-801) (Fix et al., 1995). Such discrete astrocytic responses could escape quantification with the GFAP assay due to dilution of signal by surrounding tissue. No one approach can be broadly applied to detect all toxicant-induced damage of the CNS. Therefore, it is prudent to use the GFAP ELISA in conjunction with GFAP immunohistochemistry and other sensitive morphological approaches for detection of neural damage, such as silver degeneration stains (Switzer, 2000), Fluoro-Jade (Schmued et al., 2000), and stains that detect activated microglia (Streit et al., 1999).

In terms of the GFAP ELISA itself, the key requirements for optimal performance of the assay include: 1) use of the specified antibodies, 2) addition of the reagents at room temperature, 3) fresh (daily) preparation of all reagents containing antibodies and/or non-fat dry milk and 4) mixing of the standards and samples prior to their dilution or addition to the microplate wells. A troubleshooting guide is provided in Table 4 that covers most problems encountered with the assay.

C. Troubleshooting (see Table 4)

D. Typical Results

Typical GFAP assay values obtained for different regions of the rat brain are presented in Fig. 2 (i.e. historical data). Absolute values for GFAP (μg/mg total protein) may vary depending on the GFAP standard used and the species subjected to evaluation. Region-to-region differences in GFAP values from

untreated animals of a given species, however, should remain stable, if consistent dissections are performed.

E. Time Considerations

The GFAP assay requires approximately 5 hours for one person to process a 96-wellmicroplate. The time required to prepare brain samples depends on the number of brain areas to be dissected and on whether the areas are stored frozen prior to homogenization With practice, 10 brain areas can be prepared (and homogenized) from 50 rats in a day. Two people are required: one to dissect the brains and one (or more) to weigh and homogenize the tissue. The total protein assay requires approximately 1 hour for one person to process a 96-well microplate.

Statistics:

The effect of treatment on GFAP concentration was determined by separate one-way ANOVAs for each of the nine brain areas from males and females utilizing the JMP® statistical package (SAS, 1995). The significance level was set a $P<0.05$ and, to ensure detection of between group treatment effects, The Least Significant-Difference test (Keppel, 1973) was used in *post-hoc* analyses.

Results and Conclusions

The results of the GFAP analysis are presented in Tables 5 and 6. Results are reported according to gender, because sex-dependent responses to toxic substances are not uncommon, including responses reflected in levels of GFAP. Control levels for GFAP varied markedly according to brain region, consistent with known levels observed for GFAP across different brain regions (see Fig. 2). The 13-week exposure to Gasoline TBA Vapor Condensate was associated with a slight increase in GFAP in the cortex from group II males and a decrease in GFAP in the hypothalamus of group IV males (see Table 5). These statistically significant changes in GFAP were not likely to be adverse because the increase in GFAP was small in magnitude and not concentration-related and the decrease in GFAP cannot be interpreted as adverse because the neurotoxicological significance of decreases in GFAP are unknown. Given that the decreased levels of GFAP in the hypothalamus occurred at only the high concentration, we cannot rule out the possibility that such an effect represents an adverse effect. Nevertheless, it is noted that the two significant effects on GFAP occurred in brain regions that are difficult to reliably dissect and this may have been a factor in the outcome of the results. None of the values for females were affected in any exposure group. Overall, these data suggest that under the exposure conditions employed, damage-induced gliosis did not occur in the brain regions examined. Toxicant-induced gliosis is highly dose-, region-, and time-dependent. It is possible, therefore, that exposure to higher concentrations, examination of more brain regions, examination of more discrete dissections of a given brain region, or the inclusion of more time points, might have resulted in positive findings. In conclusion, exposure to Gasoline TBA Vapor Condensate did not appear to result in gliosis in nine representative brain regions in males or females.

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Figure Legends

Figure 1. Sample GFAP standard curve. GFAP values in nanograms correspond to levels found in .25 –10 μg total hippocampal homogenate protein. Dilutions of this homogenate were used to construct the GFAP standard curve shown.

Figure 2. Levels of GFAP found in different regions of untreated rat brain. OB, olfactory bulbs; Str, striatum; Hip, hippocampus; Hypo, hypothalamus; Ctx, cortex; Cbm, cerebellum; BS, brain stem. Values are mean ± SE. Adapted from Martin and O'Callaghan, 1995.

Figure 1

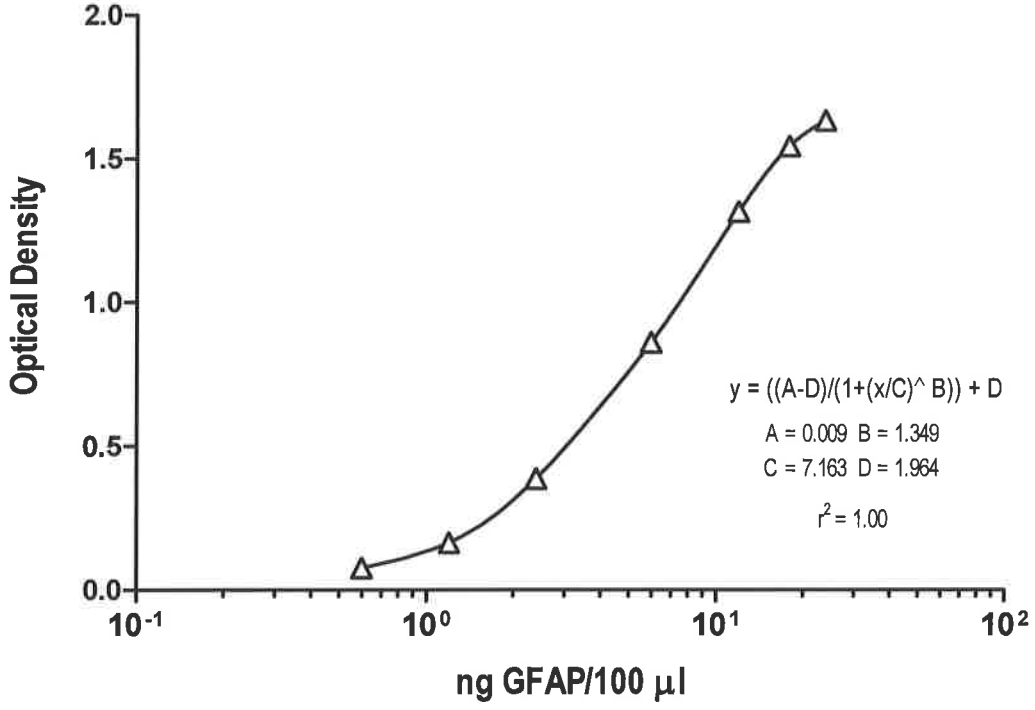
2.0
1.5
1.0
0.5
0.0
Optical Density
y = ((A-D)/(1+(x/C)^ B)) + D
A = 0.009 B = 1.349
C = 7.163 D = 1.964
r2 = 1.00
10-1
100
101
102
ng GFAP/100 µl

Figure 2

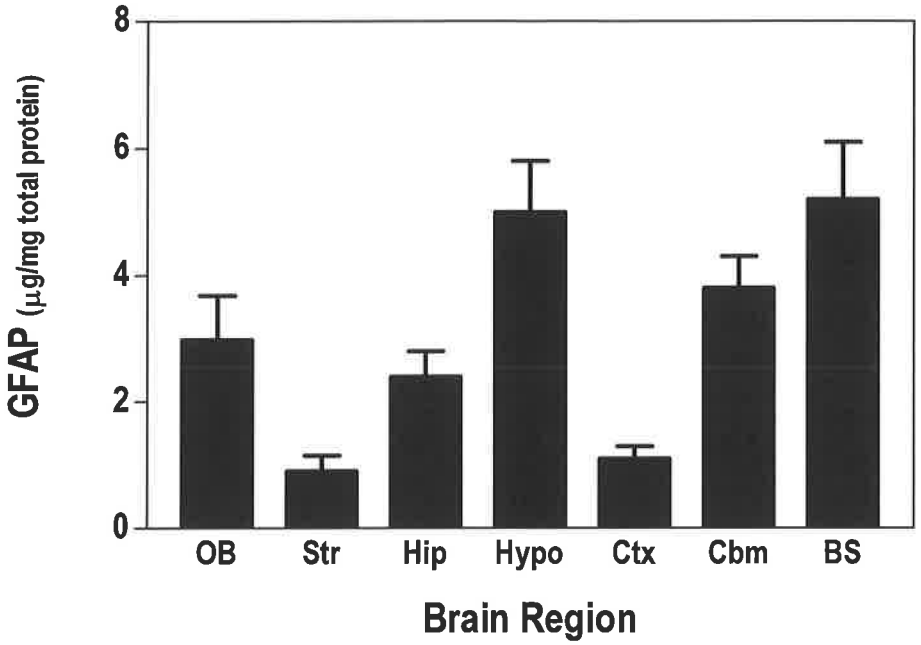
GFAP (μg/mg total protein)
8
6
4
2
0
OB
Str
Hip
Hypo
Ctx
Cbm
BS
Brain Region

TABLE 1: GFAP Standard Curve Preparation

Tube #	µg of total protein/100µl/well (ng of GFAP)	µl of Hippo-campus Std. (10.34 mg/ml)	Serial Dilution	µl of PBS+ 0.5% Triton X-100
1	10 µg (25.00ng)	29 µl		2971 µl
2	7.5 µg (18.75ng)		2063 µl from tube #1	687 µl
3	5.0 µg (12.50ng		1833 µl from tube #2	917 µl
4	2.5 µg (6.25ng)		1000 µl from tube #3	1000 µl
5	1.0 µg (2.50ng)		800 µl from tube #4	1200 µl
6	0.5 µg (1.25ng)		1000 µl from tube #5	1000 µl
7	0.25 µg (0.625ng)		700µl from tube #6	700 µl

Table 2: Microtiter Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blk	Blk	? 1	? 1	? 9	? 9	? 17	? 17	? 25	? 25	? 33	? 33
B	Std 1	Std 1	? 2	? 2	? 10	? 10	? 18	? 18	? 26	? 26	? 34	? 34
C	Std 2	Std 2	? 3	? 3	? 11	? 11	? 19	? 19	? 27	? 27	? 35	? 35
D	Std 3	Std 3	? 4	? 4	? 12	? 12	? 20	? 20	? 28	? 28	? 36	? 36
E	Std 4	Std 4	? 5	? 5	? 13	? 13	? 21	? 21	? 29	? 29	? 37	? 37
F	Std 5	Std 5	? 6	? 6	? 14	? 14	? 22	? 22	? 30	? 30	? 38	? 38
G	Std 6	Std 6	? 7	? 7	? 15	? 15	? 23	? 23	? 31	? 31	? 39	? 39
H	Std 7	Std 7	? 8	? 8	? 16	? 16	? 24	? 24	? 32	? 32	? 40	? 40

Blk= Blank; Std= Standard; ? = Unknowns

Table 3: Sample Values for GFAP Standard Curve

PLATE BLANK	Well	OD	Mean	Std Dev	CV
BL	A1	0.001	0.0	0.001	0.0
	A2	-0.001			

STANDARDS µg total protein (ng GFAP)	Value	Well	OD	Mean	Std Dev	CV
STD01	10µg (25.00ng)	B1	1.694	1.632	0.087	5.4
		B2	1.570			
STD02	7.5 µg (18.75ng)	C1	1.537	1.543	0.008	0.5
		C2	1.549			
STD03	5.0µg (12.50ng)	D1	1.295	1.314	0.027	2.1
		D2	1.334			
STD04	2.5µg (6.25ng)	E1	0.857	0.859	0.002	0.3
		E2	0.861			
STD05	1.0 µg (2.50ng)	F1	0.386	0.386	0.000	0.1
		F2	0.386			
STD06	0.5 µg (1.25ng)	G1	0.175	0.164	0.016	10.0
		G2	0.152			
STD07	0.25 µg (.625ng)	H1	0.072	0.076	0.006	7.6
		H2	0.080			

BL=Blank; STD=Standard; OD=Optical Density; Std Dev=Standard Deviation; CV=Coefficient of Variation

Table 4: Troubleshooting Guide

Problem	Possible Cause	Solution
No color reaction	Incorrect preparation of color reagent	If color reaction has not been terminated, remove reagent, add new color reagent and continue assay
	Antibody was not as specified in the protocol	Obtain correct antibody and repeat assay
	One or more antibodies were omitted or used at the wrong dilution	Repeat assay with proper reagents used at the correct dilutions
Color reaction abnormally low	Incubator was set at less that 37°C	Repeat assay with incubator temperature set at 37°C
	Antibody solution too dilute; incorrect preparation of color reagent	Repeat assay with correct reagent dilutions
Color reaction abnormally high	P-nitrophenylphosphate substrate kit is too old	Repeat assay with fresh kit
	Color reaction was not terminated	Repeat assay and terminate reaction with 0.4 N NaOH
Standard curve not sigmoid	Incorrect plate template set in the plate reader	Use correct plate template and re-read plate
	Incorrect standard dilution	Repeat assay with correct standard dilution
Samples not on linear portion of curve	Incorrect standard dilution	Repeat assay with correct dilution of standard
	Incorrect sample dilution	Run multiple dilutions of samples to obtain OD values from the linear portion of the curve
Duplicates are not similar	Carry over from using same tip	Change tips after each use
	Poor pipetting technique	Check precision by weighing
	Plate washer malfunction	Check plate washer for even dispensing and aspiration
	Bubbles throughout the plate	Pop bubbles and re-read plate
Color reaction obtained for standards and samples, but OD values not as expected	Plate read at incorrect wavelength	Read plate at 405 nm

Table 5: Mean GFAP Levels in Specific Regions of Male Rat Brains Following a 13-Week Whole -Body Inhalation Exposure to Gasoline TBA Vapor Condensate

Brain Area	Group I Air Control 0 mg/m^3	Group II Test Substance 2,000 mg/m^3	Group III Test Substance 10,000 mg/m^3	Group IV Test Substance 20,000 mg/m^3
Striatum	1.22 ± 0.12*	1.25 ± 0.12	1.18 ± 0.07	1.32 ± 0.10
Hippocampus	2.52 ± 0.17	2.76 ± 0.18	3.00 ± 0.30	2.62 ± 0.07
Cortex	1.17 ± 0.07	1.42 ± 0.10+	1.23 ± 0.13	1.27 ± 0.08
Olfactory Bulb	2.12 ± 0.21	2.11 ± 0.18	1.92 ± 0.19	2.28 ± 0.20
Thalamus	2.29 ± 0.11	2.73 ± 0.38	2.18 ± 0.22	2.30 ± 0.15
Hypothalamus	7.08 ± 0.70	7.40 ± 0.96	6.48 ± 0.45	4.97 ± 0.70+
Cerebellum	5.21 ± 0.47	4.66 ± 0.22	4.93 ± 0.44	4.67 ± 0.34
Rest of Brain	4.91 ± 0.33	5.17 ± 0.43	4.45 ± 0.20	4.09 ± 0.16

*Each value represents the mean ± SEM for the concentration of GFAP (mg/mg Total Protein)
n= 5; see Results and Conclusion and Table 7
+Statistically different from Air Control, $P<0.05$

Table 6: Mean GFAP Levels in Specific Regions of Female Rat Brains Following a 13-Week Whole -Body Inhalation Exposure to Gasoline TBA Vapor Condensate

Brain Area	Group I Air Control 0 mg/m^3	Group II Test Substance 2,000 mg/m^3	Group III Test Substance 10,000 mg/m^3	Group IV Test Substance 20,000 mg/m^3
Striatum	1.32 ± 0.20*	1.47 ± 0.12	1.41 ± 0.10	1.05 ± 0.11
Hippocampus	2.72 ± 0.12	2.74 ± 0.07	2.87 ± 0.18	2.32 ± 0.20
Cortex	1.17 ± 0.06	1.24 ± 0.07	1.37 ± 0.07	1.11 ± 0.06
Olfactory Bulb	1.80 ± 0.24	1.91 ± 0.10	2.07 ± 0.17	1.80 ± 0.07
Thalamus	2.16 ± 0.15	2.08 ± 0.07	2.49 ± 0.12	1.96 ± 0.12
Hypothalamus	7.01 ± 0.72	6.27 ± 0.75	6.05 ± 0.47	5.18 ± 0.53
Cerebellum	3.96 ± 0.12	4.42 ± 0.34	4.81 ± 0.22	3.96 ± 0.22
Rest of Brain	4.51 ± 0.32	5.00 ± 0.65	5.18 ± 0.33	3.95 ± 0.12

*Each value represents the mean ± SEM for the concentration of GFAP (mg/mg Total Protein)
n= 5; see Results and Conclusion and Table 7

Table 7: Individual GFAP Levels in Specific Regions of Rat Brains Following a 13 Week Whole-Body Inhalation Exposure to Gasoline TBA Vapor Condensate

Group	Animal no.	Sex	Striatum	Hippocampus	Cortex	Olfactory Bulb	Thalamus	Hypothalamus	Cerebellum	Pituitary	Rest of Brain
Group I Air Control 0 mg/m3	1076	Male	1.675•	2.954	1.238	2.757	2.378	6.246	6.430	*	5.786
	1078		1.121	2.468	1.185	1.593	2.615	6.352	4.244	*	5.310
	1080		1.085	2.604	1.280	2.376	2.255	7.812	4.798	0.053	4.177
	1077		1.053	2.638	1.242	2.096	2.239	5.527	6.269	*	4.100
	1079		1.158	1.931	0.921	1.777	1.947	9.453	4.321	*	5.184
	1577	Female	1.012	2.653	1.129	1.579	2.099	5.337	3.692	*	4.058
	1579		1.405	2.832	1.146	1.773	2.274	8.462	4.318	*	5.588
	1596		0.986	2.266	1.037	1.440	1.726	8.257	3.681	*	3.673
	1576		2.076	2.889	1.393	2.735	2.658	7.815	4.082	*	4.544
	1578		1.116	2.947	1.158	1.449	2.040	5.181	4.044	*	4.669
Group II Test Substance 2,000 mg/m3	2067	Male	1.009	2.910	1.510	1.685	2.229	7.020	4.392	*	3.925
	2070		1.079	2.633	1.229	1.846	2.280	10.708	4.008	*	5.784
	2069		1.602	3.373	1.776	2.588	4.222	7.805	5.128	*	5.662
	2066		1.509	2.572	1.245	2.469	2.618	6.595	4.625	*	6.110
	2068		1.073	2.330	1.355	1.982	2.313	4.853	5.119	*	4.371
	2569	Female	1.764	2.731	1.253	2.258	2.197	8.602	5.628	*	7.438
	2566		1.480	2.769	1.281	1.772	2.227	5.121	4.487	*	5.052
	2570		1.050	2.610	1.410	1.983	1.892	4.723	3.926	*	3.623
	2568		1.529	2.616	1.000	1.748	1.949	7.483	4.415	0.072	4.425
	2567		1.517	2.966	1.266	1.762	2.126	5.442	3.639	*	4.448
Group III Test Substance 10,000 mg/m3	3067	Male	1.346	3.104	1.354	1.654	2.294	5.280	5.129	0.061	4.141
	3069		0.910	2.637	1.084	1.995	2.033	7.206	4.539	*	4.069
	3068		1.263	3.329	1.399	2.388	2.162	7.470	5.094	*	4.527
	3070		1.215	3.858	1.520	2.192	2.885	6.910	6.286	*	5.172
	3066		1.159	2.076	0.795	1.345	1.546	5.526	3.607	*	4.335
	3566	Female	1.153	2.674	1.309	1.607	2.305	6.444	4.631	*	4.765
	3567		1.628	3.036	1.413	2.418	2.695	4.457	5.054	*	5.964
	3569		1.292	2.462	1.139	1.865	2.209	5.785	5.504	*	4.545
	3570		1.362	2.665	1.446	1.961	2.379	7.284	4.580	*	5.984
	3568		1.635	3.491	1.543	2.472	2.851	6.286	4.259	*	4.655
Group IV Test Substance 20,000 mg/m3	4078	Male	1.630	2.791	1.488	2.505	2.708	7.442	5.193	*	4.657
	4086		1.027	2.662	1.174	2.051	2.274	5.180	4.069	*	3.729
	4076		1.405	2.708	1.406	2.052	2.421	3.850	3.636	*	4.123
	4080		1.230	2.419	1.015	2.966	1.814	3.403	5.059	*	4.093
	4077		1.306	2.502	1.277	1.844	2.296	4.985	5.366	*	3.844
	4596	Female	0.953	2.643	1.249	1.973	1.989	4.357	4.361	*	3.744
	4577		0.866	1.929	0.993	1.646	1.871	4.051	3.643	*	4.279
	4579		1.462	2.582	1.014	1.774	1.647	5.962	4.610	*	3.799
	4578		1.042	2.701	1.265	1.957	2.376	6.836	3.494	*	4.201
	4576		0.907	1.742	1.026	1.667	1.913	4.713	3.680	*	3.741

• Each value represents the concentration of GFAP (ug/mg Total Protein)

* Pituitary samples too dilute to detect GFAP; therefore, not of value and not further summarized or discussed

	Animal Exposure and Animal Data Preface	Appendix A

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: 25 September 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, feed consumption and ophthalmoscopic findings.

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TABLES

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	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	0	0	0	0	0	0	0.8469	1.955	1.87E-02	25	53
29	24-Jul-02	22	0	0	0	0	0	0				22	51
30	25-Jul-02	23	0	0	0	0	0	0				25	47
31	26-Jul-02	24	0	0	0	0	0	0				25	53

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
32	27-Jul-02	25	0	0	0	0	0	0				25	53
34	29-Jul-02	26	0	0	0	0	0	0				25	51
35	30-Jul-02	27	0	0	0	0	0	0				24	51
36	31-Jul-02	28	0	0	0	0	0	0	2.423	2.755	4.95E-03	24	54
37	1-Aug-02	29	0	0	0	0	0	0				24	54
38	2-Aug-02	30	0	0	0	0	0	0				24	55
41	5-Aug-02	31	0	0	0	0	0	0				24	51
42	6-Aug-02	32	0	0	0	0	0	0				25	43
43	7-Aug-02	33	0	0	0	0	0	0				26	47
44	8-Aug-02	34	0	0	0	0	0	0	3.669	2.135	7.68E-03	25	48
45	9-Aug-02	35	0	0	0	0	0	0				25	51
48	12-Aug-02	36	0	0	0	0	0	0				26	50
49	13-Aug-02	37	0	0	0	0	0	0	0.7598	1.887	2.19E-02	25	51
50	14-Aug-02	38	0	0	0	0	0	0				25	52
51	15-Aug-02	39	0	0	0	0	0	0				24	55
52	16-Aug-02	40	0	0	0	0	0	0				24	54
55	19-Aug-02	41	0	0	0	0	0	0				25	51
56	20-Aug-02	42	0	0	0	0	0	0	10.81	3.298	6.53E-03	25	52
57	21-Aug-02	43	0	0	0	0	0	0				25	55
58	22-Aug-02	44	0	0	0	0	0	0				25	55
59	23-Aug-02	45	0	0	0	0	0	0				25	62
62	26-Aug-02	46	0	0	0	0	0	0				24	53
63	27-Aug-02	47	0	0	0	0	0	0	1.075	1.828	2.46E-03	25	53
64	28-Aug-02	48	0	0	0	0	0	0				24	54
65	29-Aug-02	49	0	0	0	0	0	0				24	53

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	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
66	30-Aug-02	50	0	0	0	0	0	0				24	52
69	2-Sep-02	51	0	0	0	0	0	0				24	51
70	3-Sep-02	52	0	0	0	0	0	0	1.132	2.309	4.80E-03	25	53
71	4-Sep-02	53	0	0	0	0	0	0				25	63
72	5-Sep-02	54	0	0	0	0	0	0				25	52
73	6-Sep-02	55	0	0	0	0	0	0				25	44
76	9-Sep-02	56	0	0	0	0	0	0				25	50
77	10-Sep-02	57	0	0	0	0	0	0				24	53
78	11-Sep-02	58	0	0	0	0	0	0	3.109	1.761	4.40E-03	24	51
79	12-Sep-02	59	0	0	0	0	0	0				24	35
80	13-Sep-02	60	0	0	0	0	0	0				25	54
83	16-Sep-02	61	0	0	0	0	0	0				24	61
84	17-Sep-02	62	0	0	0	0	0	0				25	48
85	18-Sep-02	63	0	0	0	0	0	0	0.8813	1.803	3.18E-03	24	55
86	19-Sep-02	64	0	0	0	0	0	0				24	57
87	20-Sep-02	65	0	0	0	0	0	0				24	56
89	22-Sep-02	66	0	0	0	0	0	0				25	51
90	23-Sep-02	67	0	0	0	0	0	0				24	52
91	24-Sep-02	68	0	0	0	0	0	0				24	44
		Mean	**0**		**0**				**2.478**	**2.234**	**1.04E-02**	**24.5**	**52.2**
		S.D.	**0**		**0**				**2.854**	**0.465**	**7.41E-03**	**0.7**	**5.3**

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	0	0	0	0	0	0	2.434	3.100	2.72E-02	23	55
29	24-Jul-02	22	0	0	0	0	0	0				21	53
30	25-Jul-02	23	0	0	0	0	0	0				23	48
31	26-Jul-02	24	0	0	0	0	0	0				23	55

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
32	27-Jul-02	25	0	0	0	0	0	0				24	55
34	29-Jul-02	26	0	0	0	0	0	0				24	53
35	30-Jul-02	27	0	0	0	0	0	0				25	53
36	31-Jul-02	28	0	0	0	0	0	0	3.102	2.358	7.27E-03	25	56
37	1-Aug-02	29	0	0	0	0	0	0				25	56
38	2-Aug-02	30	0	0	0	0	0	0				25	56
41	5-Aug-02	31	0	0	0	0	0	0				25	53
42	6-Aug-02	32	0	0	0	0	0	0				24	45
43	7-Aug-02	33	0	0	0	0	0	0				24	49
44	8-Aug-02	34	0	0	0	0	0	0	2.850	1.972	5.51E-03	24	50
45	9-Aug-02	35	0	0	0	0	0	0				24	52
48	12-Aug-02	36	0	0	0	0	0	0				24	53
49	13-Aug-02	37	0	0	0	0	0	0	0.7383	1.341	2.12E-02	25	54
50	14-Aug-02	38	0	0	0	0	0	0				25	54
51	15-Aug-02	39	0	0	0	0	0	0				25	56
52	16-Aug-02	40	0	0	0	0	0	0				25	54
55	19-Aug-02	41	0	0	0	0	0	0				25	52
56	20-Aug-02	42	0	0	0	0	0	0	3.732	2.486	6.47E-03	24	56
57	21-Aug-02	43	0	0	0	0	0	0				23	56
58	22-Aug-02	44	0	0	0	0	0	0				23	56
59	23-Aug-02	45	0	0	0	0	0	0				23	65
62	26-Aug-02	46	0	0	0	0	0	0				23	57
63	27-Aug-02	47	0	0	0	0	0	0	1.601	2.253	3.51E-03	25	55
64	28-Aug-02	48	0	0	0	0	0	0				24	56

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	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
65	29-Aug-02	49	0	0	0	0	0	0				24	54
66	30-Aug-02	50	0	0	0	0	0	0				25	54
69	2-Sep-02	51	0	0	0	0	0	0				25	53
70	3-Sep-02	52	0	0	0	0	0	0	1.613	2.554	5.32E-03	24	56
71	4-Sep-02	53	0	0	0	0	0	0				24	66
72	5-Sep-02	54	0	0	0	0	0	0				23	56
73	6-Sep-02	55	0	0	0	0	0	0				24	47
76	9-Sep-02	56	0	0	0	0	0	0				24	51
77	10-Sep-02	57	0	0	0	0	0	0				24	54
78	11-Sep-02	58	0	0	0	0	0	0	5.507	2.062	6.18E-03	25	51
79	12-Sep-02	59	0	0	0	0	0	0				25	35
80	13-Sep-02	60	0	0	0	0	0	0				25	53
83	16-Sep-02	61	0	0	0	0	0	0				25	61
84	17-Sep-02	62	0	0	0	0	0	0				23	51
85	18-Sep-02	63	0	0	0	0	0	0	0.8693	2.131	3.13E-03	23	58
86	19-Sep-02	64	0	0	0	0	0	0				23	61
87	20-Sep-02	65	0	0	0	0	0	0				23	57
89	22-Sep-02	66	0	0	0	0	0	0				24	54
90	23-Sep-02	67	0	0	0	0	0	0				23	56
		Mean	**0**		**0**				**2.157**	**2.330**	**1.16E-02**	**24.1**	**54.3**
		S.D.	**0**		**0**				**1.389**	**0.470**	**9.01E-03**	**0.8**	**5.6**

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	2160	2080	2360	1940	1990	2030	0.8157	1.704	1.64E-02	23	50
29	24-Jul-02	22	2070	1923	2010	1800	1880	2000				22	48
30	25-Jul-02	23	2050	2113	2130	2140	2100	2080				23	44
31	26-Jul-02	24	1830	1840	1850	1750	1780	1980				23	50

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
32	27-Jul-02	25	2090	2000	2070	1860	2060	2010				23	50
34	29-Jul-02	26	2070	1935	2120	1860	1860	1900				24	48
35	30-Jul-02	27	1990	1933	1770	1980	1890	2090				23	49
36	31-Jul-02	28	2010	1953	1940	1990	2010	1870	2.312	2.133	4.69E-03	23	51
37	1-Aug-02	29	2150	1975	1930	1960	2030	1980				23	51
38	2-Aug-02	30	2110	2005	2090	1810	1840	2280				23	50
41	5-Aug-02	31	2080	1970	2080	2070	2080	1650				23	48
42	6-Aug-02	32	1950	1963	2010	1990	1980	1870				24	40
43	7-Aug-02	33	2020	2135	2370	2050	2060	2060				24	44
44	8-Aug-02	34	2160	2188	2400	2470	1800	2080	4.144	2.109	9.38E-03	24	44
45	9-Aug-02	35	2020	1953	1770	1870	2120	2050				24	46
48	12-Aug-02	36	2020	1845	2130	1780	1700	1770				24	47
49	13-Aug-02	37	2190	2043	1750	1850	2340	2230	0.7525	1.783	2.35E-02	23	47
50	14-Aug-02	38	2090	2023	1980	2030	2020	2060				23	48
51	15-Aug-02	39	2090	2013	1850	2170	2000	2030				23	50
52	16-Aug-02	40	2140	2070	1890	2030	2020	2340				23	49
55	19-Aug-02	41	2200	2090	2070	2030	2210	2050				23	48
56	20-Aug-02	42	2070	2068	2120	2040	2170	1940	0.8197	1.539	2.61E-03	24	48
57	21-Aug-02	43	2130	2183	2400	2280	2040	2010				24	52
58	22-Aug-02	44	2030	2033	2080	2030	2080	1940				23	50
59	23-Aug-02	45	1950	2065	2220	1990	1990	2060				24	59
62	26-Aug-02	46	1990	2105	2310	2010	2090	2010				23	52
63	27-Aug-02	47	1930	2213	2080	2170	2020	2580	1.124	2.197	3.03E-03	24	48
64	28-Aug-02	48	1950	2020	1970	2090	2060	1960				23	50

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	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
65	29-Aug-02	49	1990	2108	2370	2030	2030	2000				23	50
66	30-Aug-02	50	1510	1840	1940	2030	2000	1390				23	50
69	2-Sep-02	51	1890	2000	2230	1710	2030	2030				23	47
70	3-Sep-02	52	1920	1900	1840	2030	1820	1910	3.784	2.594	8.03E-03	24	49
71	4-Sep-02	53	2080	2070	2090	1910	2280	2000				24	60
72	5-Sep-02	54	1940	2030	2280	1850	1980	2010				24	49
73	6-Sep-02	55	2010	2103	1920	2310	2340	1840				24	40
76	9-Sep-02	56	1980	2028	2070	2090	1970	1980				24	46
77	10-Sep-02	57	1990	1898	1730	1930	1960	1970				23	49
78	11-Sep-02	58	2040	1975	1930	2000	2080	1890	3.475	2.037	5.22E-03	23	47
79	12-Sep-02	59	1990	2113	2280	1980	1960	2230				23	32
80	13-Sep-02	60	1910	2018	2240	1950	1870	2010				24	49
83	16-Sep-02	61	1950	1863	2060	1890	1750	1750				23	57
84	17-Sep-02	62	1980	1880	1940	1840	1820	1920				23	46
85	18-Sep-02	63	2140	2040	2110	2030	2000	2020	0.8395	1.440	3.71E-03	23	51
86	19-Sep-02	64	2140	2013	2010	1960	2020	2060				23	52
87	20-Sep-02	65	2150	2053	2030	2150	2030	2000				23	52
89	22-Sep-02	66	2180	2153	2200	2280	2100	2030				23	48
90	23-Sep-02	67	2070	2000	1970	2000	2000	2030				23	49
91	24-Sep-02	68	1950	2088	2540	2000	1850	1960				24	41
		Mean	**2025**		**2013**				**1.692**	**1.897**	**9.53E-03**	**23.4**	**48.4**
		S.D.	**105**		**163**				**1.290**	**0.368**	**7.10E-03**	**0.6**	**5.0**

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	2160	1888	1530	1930	1980	2110	0.8253	2.100	1.75E-02	22	52
29	24-Jul-02	22	2070	2053	2230	1920	1960	2100				21	49
30	25-Jul-02	23	2050	2155	2210	2170	2160	2080				22	45
31	26-Jul-02	24	1830	1928	1800	1910	1970	2030				22	52

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
32	27-Jul-02	25	2090	2105	2130	2110	2100	2080				22	53
34	29-Jul-02	26	2070	2060	1920	1960	2010	2350				23	50
35	30-Jul-02	27	1990	1935	1820	2000	1890	2030				23	52
36	31-Jul-02	28	2010	1830	1730	1790	2000	1800	3.582	2.537	6.02E-03	23	54
37	1-Aug-02	29	2150	2048	2030	2010	2080	2070				23	55
38	2-Aug-02	30	2110	2123	2400	2130	2140	1820				23	55
41	5-Aug-02	31	2080	2020	1940	1940	1800	2400				23	51
42	6-Aug-02	32	1950	2018	2010	1990	1980	2090				23	43
43	7-Aug-02	33	2020	1970	2050	1950	1900	1980				23	46
44	8-Aug-02	34	2160	2203	1720	2610	2200	2280	2.398	2.155	2.09E-03	23	47
45	9-Aug-02	35	2020	1968	2170	1780	1980	1940				22	50
48	12-Aug-02	36	2020	2088	1870	1980	2220	2280				23	49
49	13-Aug-02	37	2190	2165	2180	1890	2310	2280	0.7703	2.300	1.73E-02	24	50
50	14-Aug-02	38	2090	2008	2050	2010	1960	2010				23	52
51	15-Aug-02	39	2090	1995	2000	1990	2030	1960				23	53
52	16-Aug-02	40	2140	2028	2110	2020	2080	1900				23	53
55	19-Aug-02	41	2200	2073	2050	2090	2180	1970				24	50
56	20-Aug-02	42	2070	2030	1970	1960	1910	2280	0.8346	1.505	2.64E-03	22	54
57	21-Aug-02	43	2130	2025	1980	1950	2090	2080				22	56
58	22-Aug-02	44	2030	2123	2310	2280	2000	1900				22	54
59	23-Aug-02	45	1950	1855	2150	1680	1770	1820				22	64
62	26-Aug-02	46	1990	1993	1970	1960	2010	2030				22	55
63	27-Aug-02	47	1930	1958	1960	2050	2170	1650	2.420	3.024	5.10E-03	24	52
64	28-Aug-02	48	1950	2010	2310	1930	1720	2080				23	55

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
65	29-Aug-02	49	1990	2085	2010	1790	2260	2280				23	53
66	30-Aug-02	50	1510	2008	2310	1810	2260	1650				23	53
69	2-Sep-02	51	1890	1970	1810	1960	2080	2030				23	49
70	3-Sep-02	52	1920	2060	1720	1880	2400	2240	0.8647	1.678	3.13E-03	23	56
71	4-Sep-02	53	2080	1958	2060	1800	2080	1890				23	65
72	5-Sep-02	54	1940	1928	1900	1800	1930	2080				23	54
73	6-Sep-02	55	2010	2030	2080	2140	1960	1940				23	44
76	9-Sep-02	56	1980	1953	1940	1990	1870	2010				23	49
77	10-Sep-02	57	1990	1968	1770	2020	2030	2050				23	52
78	11-Sep-02	58	2040	2135	2260	2150	2140	1990	2.964	1.667	3.22E-03	a	a
79	12-Sep-02	59	1990	2093	2200	2170	2200	1800				24	34
80	13-Sep-02	60	1910	1888	1750	1930	1840	2030				24	53
83	16-Sep-02	61	1950	1993	1870	1890	2120	2090				24	58
84	17-Sep-02	62	1980	1885	1910	1860	1840	1930				22	49
85	18-Sep-02	63	2140	2065	2280	2080	2000	1900	1.148	2.717	5.45E-03	22	54
86	19-Sep-02	64	2140	2020	2040	1980	2030	2030				22	56
87	20-Sep-02	65	2150	2105	2230	2080	2090	2020				22	55
89	22-Sep-02	66	2180	2118	1990	2060	2310	2110				22	50
90	23-Sep-02	67	2070	1920	1750	1740	2400	1790				22	52
		Mean	**2026**		**2014**				**2.542**	**2.229**	**1.28E-02**	**22.8**	**51.9**
		S.D.	**106**		**174**				**3.775**	**0.682**	**1.85E-02**	**0.7**	**5.6**

[a]Gauge fell prior to the 30 minutes exposure reading on Test Day 78. Therefore, the readings could not be recorded for the remainder of the exposure on that day.

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	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	10200	10300	10000	10400	10600	10200	0.7944	1.684	1.69E-02	23	54
29	24-Jul-02	22	10300	10450	10200	10600	10100	10900				22	49
30	25-Jul-02	23	9320	10500	9290	10700	10900	11100				23	44
31	26-Jul-02	24	10100	10490	11200	10700	10100	9950				23	50

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
32	27-Jul-02	25	9960	9908	9130	9480	11100	9920				24	50
34	29-Jul-02	26	10200	10130	10900	9770	9920	9920				24	50
35	30-Jul-02	27	10400	10380	10100	10500	10500	10400				23	50
36	31-Jul-02	28	10300	10780	10300	10900	11800	10100	8.779	2.634	7.41E-03	23	52
37	1-Aug-02	29	10400	10000	10500	10000	9800	9700				23	52
38	2-Aug-02	30	9790	10500	9800	10700	10500	11000				23	52
41	5-Aug-02	31	10000	9818	10100	9610	9160	10400				23	49
42	6-Aug-02	32	9580	10210	8650	10100	11500	10600				24	40
43	7-Aug-02	33	10500	10580	10500	10900	10700	10200				24	44
44	8-Aug-02	34	9910	10200	9920	10900	10100	9860	2.937	2.122	3.72E-03	24	44
45	9-Aug-02	35	9770	10010	10400	9950	10000	9700				24	47
48	12-Aug-02	36	9770	9763	10000	9610	9610	9830				24	47
49	13-Aug-02	37	10000	10190	10700	10100	10000	9950	0.7457	1.589	2.45E-02	23	49
50	14-Aug-02	38	9560	10220	9580	9390	12000	9920				23	51
51	15-Aug-02	39	9660	9463	10000	8700	8650	10500				23	52
52	16-Aug-02	40	9850	10180	10500	10200	9700	10300				23	52
55	19-Aug-02	41	10100	10020	10000	9290	10300	10500				23	48
56	20-Aug-02	42	10600	10440	11200	9770	10200	10600	0.8931	1.546	2.23E-03	24	50
57	21-Aug-02	43	10000	9868	9700	8870	10200	10700				24	51
58	22-Aug-02	44	9820	10070	10200	10600	10100	9390				23	52
59	23-Aug-02	45	10100	10050	10500	9510	10400	9770				24	59
62	26-Aug-02	46	10000	10100	9390	10600	10200	10200				23	52
63	27-Aug-02	47	9830	10520	10100	9890	11200	10900	0.8287	1.699	2.51E-03	23	51
64	28-Aug-02	48	9080	9843	10500	10000	9640	9230				23	51

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
65	29-Aug-02	49	10300	10500	11000	10600	10400	10000				23	50
66	30-Aug-02	50	9600	9718	10000	10000	9260	9610				23	50
69	2-Sep-02	51	9850	9998	10300	9670	10100	9920				23	48
70	3-Sep-02	52	10000	10030	9610	10200	10400	9920	1.870	2.517	5.24E-03	24	51
71	4-Sep-02	53	10300	10260	10200	9920	10400	10500				24	60
72	5-Sep-02	54	10400	10350	10100	10000	10900	10400				24	50
73	6-Sep-02	55	9790	9560	9670	9610	9640	9320				24	40
76	9-Sep-02	56	10100	10470	11400	10900	9860	9700				24	47
77	10-Sep-02	57	10200	9538	9070	9290	9390	10400				23	50
78	11-Sep-02	58	10500	10170	10300	10900	10200	9260	11.81	2.373	4.65E-03	23	47
79	12-Sep-02	59	9910	9805	10400	9450	9670	9700				23	32
80	13-Sep-02	60	9480	10110	10100	10300	9350	10700				24	49
83	16-Sep-02	61	9870	9908	9890	11000	9770	8970				23	59
84	17-Sep-02	62	10400	10040	10000	9640	9730	10800				23	46
85	18-Sep-02	63	9960	10140	9700	10700	10200	9950	0.8990	1.688	2.94E-03	23	51
86	19-Sep-02	64	10000	10150	10700	8690	11000	10200				23	53
87	20-Sep-02	65	9620	10090	9770	10400	9580	10600				23	53
89	22-Sep-02	66	9960	10310	10500	10100	10700	9950				23	49
90	23-Sep-02	67	10100	10240	10000	9450	10800	10700				23	50
91	24-Sep-02	68	10100	10160	9950	10200	10200	10300				24	42
		Mean	**9998**		**10140**				**2.545**	**1.976**	**9.56E-03**	**23.5**	**49.3**
		S.D.	**295**		**587**				**3.550**	**0.379**	**8.39E-03**	**0.6**	**5.1**

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	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	10200	9600	9670	9390	9670	9670	0.7874	1.492	1.72E-02	23	49
29	24-Jul-02	22	10300	9885	9610	9920	9610	10400				22	46
30	25-Jul-02	23	9320	9240	7330	9130	10200	10300				23	42
31	26-Jul-02	24	10100	10010	10200	10100	10100	9640				23	47

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
32	27-Jul-02	25	9960	10500	10700	10100	11000	10200				23	48
34	29-Jul-02	26	10200	10020	9260	11200	9920	9700				23	49
35	30-Jul-02	27	10400	10000	9450	10600	10100	9860				24	47
36	31-Jul-02	28	10300	10090	9540	10100	10400	10300	1.403	1.947	2.32E-03	24	48
37	1-Aug-02	29	10400	10160	10500	10100	10100	9920				24	48
38	2-Aug-02	30	9790	10100	9390	10000	10500	10500				24	48
41	5-Aug-02	31	10000	10240	10100	10700	10900	9260				24	46
42	6-Aug-02	32	9580	10620	9990	11100	11000	10400				24	39
43	7-Aug-02	33	10500	10750	10500	11100	10600	10800				24	42
44	8-Aug-02	34	9910	11150	10900	11800	11300	10600	2.459	1.985	2.80E-03	24	41
45	9-Aug-02	35	9770	10260	10500	9950	10400	10200				23	44
48	12-Aug-02	36	9770	10680	10400	9920	11000	11400				24	45
49	13-Aug-02	37	10000	10280	10300	10300	10200	10300	0.7601	2.395	2.68E-02	24	45
50	14-Aug-02	38	9560	10240	10400	9420	11200	9920				24	46
51	15-Aug-02	39	9660	9605	10300	9200	8420	10500				24	47
52	16-Aug-02	40	9850	10330	10400	10300	10000	10600				24	48
55	19-Aug-02	41	10100	9880	9610	8810	10500	10600				24	45
56	20-Aug-02	42	10600	10380	10600	9800	10600	10500	0.9700	1.968	3.03E-03	24	47
57	21-Aug-02	43	10000	9955	10000	9320	10500	10000				23	48
58	22-Aug-02	44	9820	10200	10300	10900	9730	9860				23	48
59	23-Aug-02	45	10100	10020	10500	10000	9700	9860				23	57
62	26-Aug-02	46	10000	9960	9610	10200	9830	10200				22	51
63	27-Aug-02	47	9830	9715	10200	9230	9130	10300	0.8294	1.716	2.64E-03	24	47
64	28-Aug-02	48	9080	9973	10500	10000	10000	9390				24	47

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
65	29-Aug-02	49	10300	10390	10500	10500	9950	10600				24	46
66	30-Aug-02	50	9600	9490	10000	9640	9160	9160				24	46
69	2-Sep-02	51	9850	9815	10500	9420	9920	9420				24	44
70	3-Sep-02	52	10000	9878	9350	10200	10100	9860	1.726	2.394	4.93E-03	23	50
71	4-Sep-02	53	10300	9910	10000	9580	9860	10200				23	59
72	5-Sep-02	54	10400	10180	10300	9770	10800	9830				23	48
73	6-Sep-02	55	9790	9235	9390	9670	9230	8650				24	38
76	9-Sep-02	56	10100	9655	10400	9860	9390	8970				23	45
77	10-Sep-02	57	10200	10170	9890	9800	10100	10900				24	47
78	11-Sep-02	58	10500	10500	10700	10900	10400	10000	2.221	1.635	1.26E-03	24	45
79	12-Sep-02	59	9910	10170	10700	10000	10300	9670				24	30
80	13-Sep-02	60	9480	10570	10700	10500	9890	11200				25	45
83	16-Sep-02	61	9870	10520	10800	11600	10300	9390				24	53
84	17-Sep-02	62	10400	10080	10500	9670	9260	10900				23	45
85	18-Sep-02	63	9960	9620	9540	10100	9770	9070	0.9092	1.398	1.62E-03	23	49
86	19-Sep-02	64	10000	9635	9920	9010	10000	9610				23	51
87	20-Sep-02	65	9620	10480	10300	10400	10000	11200				23	50
89	22-Sep-02	66	9960	9870	10000	9700	9950	9830				23	47
90	23-Sep-02	67	10100	9633	9390	9480	9830	9830				22	48
		Mean	**9997**		**10050**				**1.172**	**1.841**	**9.01E-03**	**23.6**	**46.8**
		S.D.	**297**		**581**				**0.595**	**0.314**	**9.37E-03**	**0.6**	**5.0**

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	18700	19500	19100	19600	20000	19300	0.8458	2.161	1.82E-02	25	53
29	24-Jul-02	22	18600	17750	17900	18300	16900	17900				22	52
30	25-Jul-02	23	18600	20830	20800	21000	20700	20800				25	47
31	26-Jul-02	24	18900	20500	20600	20400	20500	20500				26	51

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
32	27-Jul-02	25	19100	20650	21300	20700	19800	20800				26	52
34	29-Jul-02	26	18400	19800	20000	18900	20100	20200				26	52
35	30-Jul-02	27	18500	20380	19600	20300	20600	21000				25	51
36	31-Jul-02	28	18900	19500	19500	18900	19800	19800	1.689	2.289	2.75E-03	25	52
37	1-Aug-02	29	19100	19480	19100	19400	19100	20300				25	52
38	2-Aug-02	30	18900	19250	19600	20000	18900	18500				25	53
41	5-Aug-02	31	18800	19530	19300	20000	19100	19700				25	51
42	6-Aug-02	32	19100	19950	18900	20400	20600	19900				26	43
43	7-Aug-02	33	19700	21380	20600	21400	21700	21800				26	45
44	8-Aug-02	34	18800	20530	18000	21300	21600	21200	2.505	2.034	3.68E-03	26	46
45	9-Aug-02	35	18700	19480	19500	19400	19000	20000				26	49
48	12-Aug-02	36	18800	19200	19400	19700	19200	18500				26	49
49	13-Aug-02	37	19000	18500	18200	20000	19400	16400	0.7685	1.346	1.81E-02	25	50
50	14-Aug-02	38	19500	19000	18500	19000	18600	19900				25	53
51	15-Aug-02	39	19400	19430	19200	19600	19200	19700				25	55
52	16-Aug-02	40	19100	20630	20000	21400	20500	20600				25	53
55	19-Aug-02	41	19300	19500	20000	18300	19100	20600				25	50
56	20-Aug-02	42	19200	20250	21600	19700	19700	20000	1.301	2.802	4.37E-03	26	51
57	21-Aug-02	43	18800	20300	20200	20000	20600	20400				26	54
58	22-Aug-02	44	18800	20580	21000	19900	20000	21400				25	53
59	23-Aug-02	45	18100	19830	17300	21000	19900	21100				26	61
62	26-Aug-02	46	19100	20280	19900	20600	20600	20000				24	53
63	27-Aug-02	47	19200	20230	20600	19800	20200	20300	0.8524	1.790	2.71E-03	24	53
64	28-Aug-02	48	18700	19980	19600	20600	19700	20000				24	52

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
65	29-Aug-02	49	19400	20080	20000	19100	19400	21800				24	52
66	30-Aug-02	50	19900	20030	21400	20000	19600	19100				24	51
69	2-Sep-02	51	19700	20280	19400	21000	21000	19700				24	50
70	3-Sep-02	52	19700	20330	19400	20700	20800	20400	0.8903	1.814	3.33E-03	26	52
71	4-Sep-02	53	18900	20050	19700	20800	18900	20800				26	61
72	5-Sep-02	54	19800	20850	20700	20700	20400	21600				26	51
73	6-Sep-02	55	19200	20580	21000	21400	19800	20100				26	41
76	9-Sep-02	56	18700	20430	21100	21600	19500	19500				26	48
77	10-Sep-02	57	19100	20400	19800	20800	20300	20700				24	53
78	11-Sep-02	58	19800	20600	20300	20500	21300	20300	4.819	2.008	4.25E-03	24	52
79	12-Sep-02	59	18800	19600	20000	20000	18500	19900				25	33
80	13-Sep-02	60	19100	20180	19500	20000	19200	22000				25	50
83	16-Sep-02	61	19100	20150	19700	20500	20400	20000				25	60
84	17-Sep-02	62	19500	20250	20000	19900	21000	20100				25	46
85	18-Sep-02	63	19000	20480	20400	21600	19900	20000	0.9657	1.410	1.90E-03	25	53
86	19-Sep-02	64	19000	19850	18600	20200	20000	20600				25	54
87	20-Sep-02	65	19800	19500	19700	20100	19000	19200				25	54
89	22-Sep-02	66	18200	19830	19400	20400	19600	19900				25	50
90	23-Sep-02	67	19000	20680	21000	21500	21600	18600				25	51
91	24-Sep-02	68	19700	20200	18800	19100	22500	20400				24	43
		Mean	**19120**		**20050**				**1.387**	**1.973**	**7.74E-03**	**25.1**	**51.1**
		S.D.	**452**		**914**				**1.145**	**0.432**	**6.25E-03**	**0.8**	**5.2**

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	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
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
28	23-Jul-02	21	18700	20080	20600	20500	19600	19600	0.8255	1.505	1.37E-02	24	53
29	24-Jul-02	22	18600	19400	18700	19400	19900	19600				22	51
30	25-Jul-02	23	18600	20150	20100	20400	20300	19800				24	48

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
31	26-Jul-02	24	18900	19550	19000	19000	20400	19800				24	52
32	27-Jul-02	25	19100	19650	19300	19600	19500	20200				24	54
34	29-Jul-02	26	18400	20080	19900	20200	20100	20100				24	52
35	30-Jul-02	27	18500	19400	18700	18600	19700	20600				26	51
36	31-Jul-02	28	18900	20250	20200	20400	20400	20000	1.032	1.790	1.88E-03	26	51
37	1-Aug-02	29	19100	20180	20300	19800	19900	20700				25	53
38	2-Aug-02	30	18900	19600	19500	19500	18900	20500				26	52
41	5-Aug-02	31	18800	19880	20000	19900	19600	20000				26	51
42	6-Aug-02	32	19100	19880	20000	20500	19300	19700				25	43
43	7-Aug-02	33	19700	20650	20600	20300	20600	21100				25	47
44	8-Aug-02	34	18800	19330	17400	19500	20300	20100	1.793	1.769	2.30E-03	24	46
45	9-Aug-02	35	18700	20300	20700	20300	19600	20600				24	48
48	12-Aug-02	36	18800	20050	19900	20200	20200	19900				25	50
49	13-Aug-02	37	19000	19750	20100	20200	19800	18900	0.7982	2.481	2.51E-02	26	50
50	14-Aug-02	38	19500	19980	19600	20200	19500	20600				25	51
51	15-Aug-02	39	19400	20580	20700	20300	20100	21200				25	55
52	16-Aug-02	40	19100	20650	19700	21000	21000	20900				25	53
55	19-Aug-02	41	19300	20330	19700	20300	20000	21300				26	50
56	20-Aug-02	42	19200	20030	21300	19700	20200	18900	0.9155	1.711	3.08E-03	25	52
57	21-Aug-02	43	18800	20500	20200	20600	20600	20600				24	55
58	22-Aug-02	44	18800	20830	21900	21000	21000	19400				24	54
59	23-Aug-02	45	18100	18730	16800	19100	18800	20200				24	62
62	26-Aug-02	46	19100	19000	18800	19500	18900	18800				23	55

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (μm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
63	27-Aug-02	47	19200	19480	20000	18600	19400	19900	0.8423	1.656	2.57E-03	25	52
64	28-Aug-02	48	18700	19400	19800	19300	19200	19300				25	52
65	29-Aug-02	49	19400	19650	18700	19100	19400	21400				25	51
66	30-Aug-02	50	19900	20400	20100	21000	20400	20100				25	51
69	2-Sep-02	51	19700	20800	19800	21400	21400	20600				25	49
70	3-Sep-02	52	19700	20830	20500	21900	20600	20300	2.275	2.699	6.20E-03	24	54
71	4-Sep-02	53	18900	19680	19400	20500	19000	19800				24	63
72	5-Sep-02	54	19800	20800	21700	20000	20200	21300				24	52
73	6-Sep-02	55	19200	20530	20900	21400	19900	19900				24	42
76	9-Sep-02	56	18700	20350	19700	19700	21500	20500				24	50
77	10-Sep-02	57	19100	20250	19600	20400	20400	20600				26	51
78	11-Sep-02	58	19800	20750	20600	20600	20800	21000	3.337	1.960	2.26E-03	25	49
79	12-Sep-02	59	18800	20400	21000	19900	19900	20800				25	33
80	13-Sep-02	60	19100	19980	20300	19600	18400	21600				26	49
83	16-Sep-02	61	19100	20880	21800	21000	20600	20100				26	58
84	17-Sep-02	62	19500	20850	20700	19900	21900	20900				24	47
85	18-Sep-02	63	19000	20580	21000	21600	20000	19700	0.8639	1.446	1.07E-03	24	54
86	19-Sep-02	64	19000	21000	20000	21800	21200	21000				24	56
87	20-Sep-02	65	19800	20250	20000	21100	19900	20000				23	56
89	22-Sep-02	66	18200	20800	21400	20700	20100	21000				24	51
90	23-Sep-02	67	19000	20230	19200	19800	20500	21400				24	52
		Mean	**19110**		**20060**				**1.256**	**1.848**	**8.05E-03**	**24.6**	**51.5**
		S.D.	**450**		**831**				**0.775**	**0.395**	**7.98E-03**	**0.8**	**5.3**

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

MALES

SUMMARY OF CLINICAL OBSERVATIONS

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

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	5	
	2	5	
	3	5	
	4	5	
NORMAL			
WITHIN NORMAL LIMITS	1	5	5
	2	5	5
	3	5	5
	4	5	5

Table C - Pretest

LIONEL F. RUBIN, V.M.D.
1116 Saint Andrews Road
Bryn Mawr, PA 19010
(610) 520 9430

June 14, 2002

Huntingdon Life Sciences, Inc.
Mettlers Road, Box 2360
East Millstone, NJ 08875-2360

Re: study A-12 00-6131F

Ophthalmoscopic examination of study A-12 00-6131F pretest rats was performed June 13, 2002. No abnormality was seen. All rats are suitable for inclusion in the study.

Lionel F. Rubin, V. M. D.

LIONEL F. RUBIN, V.M.D.
1116 Saint Andrews Road
Bryn Mawr, PA 19010
(610) 520 9430

September 18, 2002

Huntingdon Life Sciences, Inc.
Mettlers Road, Box 2360
East Millstone, NJ 08875-2360

Re: study A-12 00-6131F

Ophthalmoscopic examination of study A-12 00-6131F rats was performed September 17, 2002 (terminal examination). I have reviewed the findings of the type and incidence of ocular abnormalities. There is no indication of dose or compound related ocular disease. In my opinion, none of the ocular abnormalities is attributable to the administration of the test compound.

Lionel F. Rubin, V. M. D.

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

DAY -12

	DOSE GROUP:	1	2	3	4
	DOSE LEVEL (MG/M3):	0	2000	10000	20000
MALES	total number examined	5	5	5	5
	NO ABNORMALITIES DETECTED				

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

DAY -12

	DOSE GROUP:	1	2	3	4
	DOSE LEVEL (MG/M3):	0	2000	10000	20000
FEMALES	total number examined	5	5	5	5
	NO ABNORMALITIES DETECTED				

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

DAY 84

	DOSE GROUP:	1	2	3	4
	DOSE LEVEL (MG/M3):	0	2000	10000	20000
MALES	total number examined	5	5	5	5
CONJUNCTIVA	N	1	0	2	0
CONJUNCTIVITIS	N	1	0	2	0
	%	20.0	0.0	40.0	0.0

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

DAY 84

	DOSE GROUP:	1	2	3	4
	DOSE LEVEL (MG/M3):	0	2000	10000	20000
FEMALES	total number examined	5	5	5	5
	NO ABNORMALITIES DETECTED				

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

MALES

MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK -1	MEAN	247	248	247	251
	S.D.	7.1	9.5	10.3	12.4
	N	5	5	5	5
WEEK 0	MEAN	336	336	324	337
	S.D.	16.4	12.6	18.7	18.2
	N	5	5	5	5
WEEK 1	MEAN	372	372	352	377
	S.D.	20.1	18.1	23.7	26.4
	N	5	5	5	5
WEEK 2	MEAN	409	409	386	414
	S.D.	25.8	22.9	15.6	34.0
	N	5	5	5	5
WEEK 3	MEAN	442	443	420	445
	S.D.	35.2	28.6	13.7	36.0
	N	5	5	5	5
WEEK 4	MEAN	468	462	444	469
	S.D.	42.1	26.3	17.1	37.7
	N	5	5	5	5
WEEK 5	MEAN	491	488	459	486
	S.D.	46.2	31.7	20.1	36.3
	N	5	5	5	5
WEEK 6	MEAN	509	509	487	511
	S.D.	54.9	33.3	28.1	39.1
	N	5	5	5	5
WEEK 7	MEAN	534	531	507	534
	S.D.	56.9	41.1	30.4	42.8
	N	5	5	5	5

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

MALES

MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 8	MEAN	549	542	519	548
	S.D.	63.8	45.8	27.6	44.4
	N	5	5	5	5
WEEK 9	MEAN	562	554	529	560
	S.D.	63.9	49.3	29.5	46.5
	N	5	5	5	5
WEEK 10	MEAN	578	571	543	576
	S.D.	67.7	55.1	32.1	46.9
	N	5	5	5	5
WEEK 11	MEAN	593	586	557	594
	S.D.	76.0	53.0	31.4	46.9
	N	5	5	5	5
WEEK 12	MEAN	607	598	565	605
	S.D.	81.9	55.7	34.4	47.2
	N	5	5	5	5
WEEK 13	MEAN	616	605	573	607
	S.D.	78.7	53.9	31.8	46.2
	N	5	5	5	5

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 WEIGHTS (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK -1	MEAN	175	180	179	181
	S.D.	8.9	10.1	10.9	8.9
	N	5	5	5	5
WEEK 0	MEAN	208	217	212	217
	S.D.	11.0	14.5	20.4	11.7
	N	5	5	5	5
WEEK 1	MEAN	224	232	224	227
	S.D.	11.8	17.4	13.7	15.1
	N	5	5	5	5
WEEK 2	MEAN	238	245	235	239
	S.D.	15.9	14.5	23.0	15.8
	N	5	5	5	5
WEEK 3	MEAN	255	258	247	246
	S.D.	21.8	16.7	22.3	19.0
	N	5	5	5	5
WEEK 4	MEAN	256	261	254	256
	S.D.	15.5	17.0	24.2	16.4
	N	5	5	5	5
WEEK 5	MEAN	261	268	257	255
	S.D.	16.1	19.5	25.0	21.2
	N	5	5	5	5
WEEK 6	MEAN	262	273	267	261
	S.D.	15.4	18.9	28.1	23.5
	N	5	5	5	5
WEEK 7	MEAN	268	279	269	263
	S.D.	19.8	19.2	31.0	23.2
	N	5	5	5	5

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 WEIGHTS (GRAMS)

	DOSE GROUP:	1	2	3	4
	DOSE LEVEL (MG/M3):	0	2000	10000	20000
WEEK 8	MEAN	275	276	270	269
	S.D.	16.8	16.2	33.6	23.8
	N	5	5	5	5
WEEK 9	MEAN	275	282	272	272
	S.D.	20.2	22.3	34.1	26.3
	N	5	5	5	5
WEEK 10	MEAN	283	289	282	272
	S.D.	19.6	19.2	33.3	25.7
	N	5	5	5	5
WEEK 11	MEAN	278	292	281	276
	S.D.	19.5	19.5	29.9	27.4
	N	5	5	5	5
WEEK 12	MEAN	290	295	289	282
	S.D.	19.4	17.7	33.9	22.7
	N	5	5	5	5
WEEK 13	MEAN	291	297	290	281
	S.D.	19.4	20.4	35.5	22.5
	N	5	5	5	5

No statistically significant differences

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

MALES — MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0 TO 1	MEAN	36	36	28	40
	S.D.	4.6	7.2	6.1	10.1
	N	5	5	5	5
WEEK 0 TO 2	MEAN	73	73	63	77
	S.D.	11.5	13.0	5.1	17.6
	N	5	5	5	5
WEEK 0 TO 3	MEAN	105	107	97	108
	S.D.	20.7	18.4	14.1	20.1
	N	5	5	5	5
WEEK 0 TO 4	MEAN	132	126	120	132
	S.D.	27.3	17.8	18.1	20.9
	N	5	5	5	5
WEEK 0 TO 5	MEAN	155	152	136	149
	S.D.	32.3	23.2	23.4	20.7
	N	5	5	5	5
WEEK 0 TO 6	MEAN	173	173	163	174
	S.D.	39.1	26.8	28.2	24.4
	N	5	5	5	5

No statistically significant differences

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

MALES — MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0 TO 7	MEAN	198	195	184	196
	S.D.	41.5	33.5	33.2	28.2
	N	5	5	5	5
WEEK 0 TO 8	MEAN	213	206	196	211
	S.D.	48.3	38.6	30.9	28.8
	N	5	5	5	5
WEEK 0 TO 9	MEAN	225	218	205	223
	S.D.	48.1	41.5	33.2	31.6
	N	5	5	5	5
WEEK 0 TO 10	MEAN	242	235	219	239
	S.D.	51.9	48.4	35.0	31.6
	N	5	5	5	5
WEEK 0 TO 11	MEAN	257	250	233	256
	S.D.	60.0	46.3	33.9	32.2
	N	5	5	5	5
WEEK 0 TO 12	MEAN	271	262	241	268
	S.D.	65.9	48.9	34.5	33.3
	N	5	5	5	5

No statistically significant differences

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

MALES — MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0 TO 13	MEAN	280	269	249	269
	S.D.	62.8	47.3	30.5	31.6
	N	5	5	5	5

No statistically significant differences

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 BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0 TO 1	MEAN	16	15	13	10
	S.D.	4.6	6.9	7.5	4.7
	N	5	5	5	5
WEEK 0 TO 2	MEAN	31	27	23	21
	S.D.	7.2	8.5	6.1	4.6
	N	5	5	5	5
WEEK 0 TO 3	MEAN	47	41	35	28*
	S.D.	13.4	5.1	7.7	10.0
	N	5	5	5	5
WEEK 0 TO 4	MEAN	49	44	42	39
	S.D.	8.3	5.3	8.0	6.3
	N	5	5	5	5
WEEK 0 TO 5	MEAN	54	50	45	37
	S.D.	7.8	8.0	8.4	11.2
	N	5	5	5	5
WEEK 0 TO 6	MEAN	55	56	55	44
	S.D.	7.6	9.9	9.1	12.3
	N	5	5	5	5

Statistical key: * = $p<0.05$

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 BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0 TO 7	MEAN	60	61	57	45
	S.D.	11.9	9.4	13.7	13.6
	N	5	5	5	5
WEEK 0 TO 8	MEAN	68	59	58	51
	S.D.	8.4	7.2	15.5	13.2
	N	5	5	5	5
WEEK 0 TO 9	MEAN	68	65	60	55
	S.D.	11.6	11.4	15.7	15.4
	N	5	5	5	5
WEEK 0 TO 10	MEAN	76	72	70	55
	S.D.	12.5	9.8	15.3	14.8
	N	5	5	5	5
WEEK 0 TO 11	MEAN	71	75	69	59
	S.D.	11.5	12.7	12.7	16.9
	N	5	5	5	5
WEEK 0 TO 12	MEAN	82	78	77	65
	S.D.	13.1	13.9	17.6	11.4
	N	5	5	5	5

No statistically significant differences

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 BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0 TO 13	MEAN	83	80	78	63
	S.D.	12.9	12.2	18.2	11.3
	N	5	5	5	5

No statistically significant differences

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

MALES MEAN FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0	MEAN	95	96	89	98
	S.D.	6.0	8.2	9.1	6.2
	N	5	5	5	5
WEEK 1	MEAN	77	80	76	78
	S.D.	5.2	3.8	1.7	4.4
	N	5	5	5	5
WEEK 2	MEAN	72	73	68	72
	S.D.	4.2	2.4	5.8	3.6
	N	5	5	5	5
WEEK 3	MEAN	68	68	69	67
	S.D.	4.9	3.5	4.8	3.6
	N	5	5	5	5
WEEK 4	MEAN	60	61	63	63
	S.D.	3.4	0.8	2.3	2.9
	N	5	5	5	5
WEEK 5	MEAN	60	61	61	61
	S.D.	1.5	1.7	3.9	3.6
	N	5	5	5	5
WEEK 6	MEAN	57	57	58	58
	S.D.	6.7	2.3	1.4	3.3
	N	5	5	5	5
WEEK 7	MEAN	57	56	56	57
	S.D.	3.6	1.7	2.0	2.6
	N	5	5	5	5
WEEK 8	MEAN	53	52	52	55
	S.D.	2.4	1.7	2.5	2.0
	N	5	5	5	5

No statistically significant differences

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

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

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 9	MEAN	53	52	51	55
	S.D.	3.5	1.5	3.7	0.9
	N	5	5	5	5
WEEK 10	MEAN	54	51	49*	54
	S.D.	3.1	2.0	2.4	2.1
	N	5	5	5	5
WEEK 11	MEAN	52	49	48	51
	S.D.	3.7	1.4	2.9	3.7
	N	5	5	5	5
WEEK 12	MEAN	50	48	47	50
	S.D.	3.0	1.4	3.2	2.8
	N	5	5	5	5
WEEK 13	MEAN	49	46	45	47
	S.D.	5.3	2.0	3.3	2.0
	N	5	5	5	5

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

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

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 0	MEAN	99	102	94	110
	S.D.	4.4	6.7	4.5	14.9
	N	5	5	5	5
WEEK 1	MEAN	86	90	83	87
	S.D.	4.1	8.9	11.4	11.5
	N	5	5	5	5
WEEK 2	MEAN	82	83	80	81
	S.D.	2.8	4.2	3.5	8.0
	N	5	5	5	4
WEEK 3	MEAN	79	79	78	78
	S.D.	5.2	4.1	4.2	7.3
	N	5	5	5	5
WEEK 4	MEAN	67	72	72	79
	S.D.	5.6	5.8	2.7	14.8
	N	5	5	4	5
WEEK 5	MEAN	70	72	73	70
	S.D.	4.1	3.7	3.0	3.6
	N	5	5	5	5
WEEK 6	MEAN	65	69	76**	71
	S.D.	4.7	2.6	7.0	3.8
	N	4	5	5	5
WEEK 7	MEAN	68	68	69	69
	S.D.	4.6	1.7	3.7	5.9
	N	5	5	4	5
WEEK 8	MEAN	65	64	65	67
	S.D.	7.0	2.1	2.8	3.9
	N	5	5	4	5

Statistical key: ** = p<0.01

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

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

DOSE GROUP:		1	2	3	4
DOSE LEVEL (MG/M3):		0	2000	10000	20000
WEEK 9	MEAN	64	64	71	69
	S.D.	7.3	4.2	6.4	4.4
	N	5	3	5	5
WEEK 10	MEAN	67	64	70	67
	S.D.	4.8	4.2	6.8	5.2
	N	5	5	4	5
WEEK 11	MEAN	65	63	71	67
	S.D.	4.8	3.1	9.6	5.0
	N	5	5	5	5
WEEK 12	MEAN	63	63	67	65
	S.D.	5.7	4.0	5.1	7.1
	N	5	5	5	5
WEEK 13	MEAN	61	60	64	65
	S.D.	6.1	6.2	10.8	9.6
	N	5	5	5	5

No statistically significant differences

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 CLINICAL OBSERVATIONS

MALES GROUP 1 0 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
1076	WITHIN NORMAL LIMITS		P
1077	WITHIN NORMAL LIMITS		P
1078	WITHIN NORMAL LIMITS		P
1079	WITHIN NORMAL LIMITS		P
1080	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 CLINICAL OBSERVATIONS

MALES GROUP 2 2000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
2066	WITHIN NORMAL LIMITS		P
2067	WITHIN NORMAL LIMITS		P
2068	WITHIN NORMAL LIMITS		P
2069	WITHIN NORMAL LIMITS		P
2070	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 CLINICAL OBSERVATIONS

MALES GROUP 3 10000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	1 1
3066	WITHIN NORMAL LIMITS		P
3067	WITHIN NORMAL LIMITS		P
3068	WITHIN NORMAL LIMITS		P
3069	WITHIN NORMAL LIMITS		P
3070	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 CLINICAL OBSERVATIONS

MALES GROUP 4 20000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	1 1
4076	WITHIN NORMAL LIMITS		P
4077	WITHIN NORMAL LIMITS		P
4078	WITHIN NORMAL LIMITS		P
4086	WITHIN NORMAL LIMITS		P
4080	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 CLINICAL OBSERVATIONS

FEMALES GROUP 1 0 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
1576	WITHIN NORMAL LIMITS		P
1577	WITHIN NORMAL LIMITS		P
1578	WITHIN NORMAL LIMITS		P
1579	WITHIN NORMAL LIMITS		P
1596	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 CLINICAL OBSERVATIONS

FEMALES GROUP 2 2000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	1 1
2566	WITHIN NORMAL LIMITS		P
2567	WITHIN NORMAL LIMITS		P
2568	WITHIN NORMAL LIMITS		P
2569	WITHIN NORMAL LIMITS		P
2570	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 CLINICAL OBSERVATIONS

FEMALES GROUP 3 10000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
3566	WITHIN NORMAL LIMITS		P
3567	WITHIN NORMAL LIMITS		P
3568	WITHIN NORMAL LIMITS		P
3569	WITHIN NORMAL LIMITS		P
3570	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 CLINICAL OBSERVATIONS

FEMALES GROUP 4 20000 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
4576	WITHIN NORMAL LIMITS		P
4577	WITHIN NORMAL LIMITS		P
4578	WITHIN NORMAL LIMITS		P
4579	WITHIN NORMAL LIMITS		P
4596	WITHIN NORMAL LIMITS		P

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

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 1 0 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
1076		NO VISIBLE LESIONS
1077		NO VISIBLE LESIONS
1078		NO VISIBLE LESIONS
1079		NO VISIBLE LESIONS
1080		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 2 2000 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
2066		NO VISIBLE LESIONS
2067		NO VISIBLE LESIONS
2068		NO VISIBLE LESIONS
2069		NO VISIBLE LESIONS
2070		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 3 10000 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
3066		NO VISIBLE LESIONS
3067		NO VISIBLE LESIONS
3068		NO VISIBLE LESIONS
3069		NO VISIBLE LESIONS
3070		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 4 20000 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
4076		NO VISIBLE LESIONS
4077		NO VISIBLE LESIONS
4078		NO VISIBLE LESIONS
4086		NO VISIBLE LESIONS
4080		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 1 0 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
1576		NO VISIBLE LESIONS
1577		NO VISIBLE LESIONS
1578		NO VISIBLE LESIONS
1579		NO VISIBLE LESIONS
1596		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 2 2000 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
2566		NO VISIBLE LESIONS
2567		NO VISIBLE LESIONS
2568		NO VISIBLE LESIONS
2569		NO VISIBLE LESIONS
2570		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 3 10000 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
3566		NO VISIBLE LESIONS
3567		NO VISIBLE LESIONS
3568		NO VISIBLE LESIONS
3569		NO VISIBLE LESIONS
3570		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 4 20000 MG/M3 DAY -12

ANIMAL#	PART OF EYE	OBSERVATION
4576		NO VISIBLE LESIONS
4577		NO VISIBLE LESIONS
4578		NO VISIBLE LESIONS
4579		NO VISIBLE LESIONS
4596		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 1 0 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
1076		NO VISIBLE LESIONS
1077	CONJUNCTIVA	CONJUNCTIVITIS; RIGHT
1078		NO VISIBLE LESIONS
1079		NO VISIBLE LESIONS
1080		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 2 2000 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
2066		NO VISIBLE LESIONS
2067		NO VISIBLE LESIONS
2068		NO VISIBLE LESIONS
2069		NO VISIBLE LESIONS
2070		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 3 10000 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
3066		NO VISIBLE LESIONS
3067		NO VISIBLE LESIONS
3068	CONJUNCTIVA	CONJUNCTIVITIS; LEFT
3069		NO VISIBLE LESIONS
3070	CONJUNCTIVA	CONJUNCTIVITIS; LEFT

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 OPHTHALMOLOGY OBSERVATIONS

MALES GROUP 4 20000 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
4076		NO VISIBLE LESIONS
4077		NO VISIBLE LESIONS
4078		NO VISIBLE LESIONS
4086		NO VISIBLE LESIONS
4080		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 1 0 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
1576		NO VISIBLE LESIONS
1577		NO VISIBLE LESIONS
1578		NO VISIBLE LESIONS
1579		NO VISIBLE LESIONS
1596		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 2 2000 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
2566		NO VISIBLE LESIONS
2567		NO VISIBLE LESIONS
2568		NO VISIBLE LESIONS
2569		NO VISIBLE LESIONS
2570		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 3 10000 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
3566		NO VISIBLE LESIONS
3567		NO VISIBLE LESIONS
3568		NO VISIBLE LESIONS
3569		NO VISIBLE LESIONS
3570		NO VISIBLE LESIONS

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 OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP 4 20000 MG/M3 DAY 84

ANIMAL#	PART OF EYE	OBSERVATION
4576		NO VISIBLE LESIONS
4577		NO VISIBLE LESIONS
4578		NO VISIBLE LESIONS
4579		NO VISIBLE LESIONS
4596		NO VISIBLE LESIONS

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 BODY WEIGHTS (GRAMS)

MALES GROUP 1 0 MG/M3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1076	248	318	349	379	397	414	435	453	473	479	492	503	517	522	526
1077	239	323	358	399	432	456	483	473	503	516	524	538	538	549	574
1078	255	340	371	404	436	466	482	502	520	539	550	572	590	605	615
1079	253	359	400	449	494	531	564	595	622	649	659	682	711	733	738
1080	241	343	382	415	449	474	491	524	551	561	582	594	610	627	628
MEAN	247	336	372	409	442	468	491	509	534	549	562	578	593	607	616
S.D.	7.1	16.4	20.1	25.8	35.2	42.1	46.2	54.9	56.9	63.8	63.9	67.7	76.0	81.9	78.7
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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 BODY WEIGHTS (GRAMS)

MALES GROUP 2 2000 MG/M3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2066	233	323	352	387	418	441	472	495	514	524	536	561	577	593	599
2067	253	342	388	421	453	466	488	513	540	547	556	574	582	595	597
2068	257	344	376	405	439	454	480	486	505	512	528	533	552	564	573
2069	244	350	390	442	487	506	542	565	598	620	637	663	676	690	696
2070	251	322	354	391	419	445	460	485	497	508	512	524	542	546	558
MEAN	248	336	372	409	443	462	488	509	531	542	554	571	586	598	605
S.D.	9.5	12.6	18.1	22.9	28.6	26.3	31.7	33.3	41.1	45.8	49.3	55.1	53.0	55.7	53.9
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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 BODY WEIGHTS (GRAMS)

MALES GROUP 3 10000 MG/M3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3066	257	328	353	387	414	425	437	454	474	487	492	505	520	533	546
3067	230	314	343	377	407	437	443	467	483	498	505	516	529	527	535
3068	253	352	386	412	443	471	484	524	540	549	557	575	589	604	611
3069	250	324	357	383	416	438	460	486	501	518	532	543	560	567	578
3070	246	301	321	372	423	448	474	504	537	545	557	573	585	593	593
MEAN	247	324	352	386	420	444	459	487	507	519	529	543	557	565	573
S.D.	10.3	18.7	23.7	15.6	13.7	17.1	20.1	28.1	30.4	27.6	29.5	32.1	31.4	34.4	31.8
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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 BODY WEIGHTS (GRAMS)

MALES GROUP 4 20000 MG/M3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4076	251	316	341	366	395	416	434	454	471	480	488	503	520	530	534
4077	260	357	395	436	465	495	505	526	549	574	582	601	613	623	626
4078	233	326	367	403	434	456	474	498	524	541	557	571	587	609	603
4086	265	356	409	455	492	514	532	561	589	599	614	630	648	660	661
4080	247	332	372	411	439	464	487	516	537	549	560	577	600	604	609
MEAN	251	337	377	414	445	469	486	511	534	548	560	576	594	605	607
S.D.	12.4	18.2	26.4	34.0	36.0	37.7	36.3	39.1	42.8	44.4	46.5	46.9	46.9	47.2	46.2
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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 BODY WEIGHTS (GRAMS)

FEMALES GROUP 1 0 MG/M3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1576	174	220	236	260	290	278	286	283	299	301	305	317	311	322	323
1577	184	204	226	240	249	247	258	260	258	273	275	277	272	287	293
1578	174	201	210	229	235	240	247	244	266	260	256	273	266	283	278
1579	181	219	234	244	261	267	267	272	271	282	282	283	281	286	288
1596	161	195	214	218	239	250	248	253	245	261	258	267	262	271	273
MEAN	175	208	224	238	255	256	261	262	268	275	275	283	278	290	291
S.D.	8.9	11.0	11.8	15.9	21.8	15.5	16.1	15.4	19.8	16.8	20.2	19.6	19.5	19.4	19.4
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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 BODY WEIGHTS (GRAMS)

FEMALES GROUP 2 2000 MG/M3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2566	192	241	254	258	281	286	292	294	301	297	309	310	308	307	317
2567	177	211	220	232	247	252	257	256	265	268	266	276	275	284	281
2568	183	217	240	255	264	269	279	287	293	288	299	302	311	319	316
2569	180	217	239	251	262	257	269	277	280	271	283	296	297	292	300
2570	165	201	209	226	237	243	242	251	255	257	255	264	269	275	272
MEAN	180	217	232	245	258	261	268	273	279	276	282	289	292	295	297
S.D.	10.1	14.5	17.4	14.5	16.7	17.0	19.5	18.9	19.2	16.2	22.3	19.2	19.5	17.7	20.4
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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 BODY WEIGHTS (GRAMS)

FEMALES GROUP 3 10000 MG/M3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3566	162	183	204	203	218	227	220	229	228	225	223	238	239	247	241
3567	185	221	232	241	244	254	256	271	264	267	272	279	278	282	283
3568	178	204	223	235	245	241	257	261	262	259	262	276	274	273	287
3569	191	238	241	267	281	292	290	308	314	317	317	331	321	336	340
3570	181	213	221	229	248	255	261	265	276	281	286	288	293	308	299
MEAN	179	212	224	235	247	254	257	267	269	270	272	282	281	289	290
S.D.	10.9	20.4	13.7	23.0	22.3	24.2	25.0	28.1	31.0	33.6	34.1	33.3	29.9	33.9	35.5
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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 BODY WEIGHTS (GRAMS)

FEMALES GROUP 4 20000 MG/M3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4576	175	211	223	228	246	255	257	249	260	262	265	256	269	271	270
4577	171	200	203	217	215	230	218	228	227	233	231	238	236	249	248
4578	187	228	242	253	265	274	272	287	288	298	296	301	307	306	303
4579	180	224	230	245	243	261	261	266	259	271	276	276	275	287	286
4596	193	224	238	250	258	262	265	277	278	280	292	292	295	296	298
MEAN	181	217	227	239	246	256	255	261	263	269	272	272	276	282	281
S.D.	8.9	11.7	15.1	15.8	19.0	16.4	21.2	23.5	23.2	23.8	26.3	25.7	27.4	22.7	22.5
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 1 0 MG/M3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
1076	31	61	79	95	117	135	155	161	174	185	199	204	208
1077	35	76	110	134	160	151	181	194	201	216	216	227	252
1078	32	64	97	127	142	163	180	200	211	233	251	266	276
1079	42	90	136	172	205	236	264	291	301	324	353	374	379
1080	39	73	107	131	149	181	209	218	240	252	267	285	286
MEAN	36	73	105	132	155	173	198	213	225	242	257	271	280
S.D.	4.6	11.5	20.7	27.3	32.3	39.1	41.5	48.3	48.1	51.9	60.0	65.9	62.8
N	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 2 2000 MG/M3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
2066	29	64	95	118	149	172	191	202	213	239	254	271	276
2067	46	79	112	125	146	171	199	206	214	232	241	254	256
2068	32	61	95	110	136	142	161	169	184	189	208	220	229
2069	41	93	138	157	193	215	249	270	288	313	326	341	347
2070	32	69	97	122	138	163	174	186	189	202	220	224	236
MEAN	36	73	107	126	152	173	195	206	218	235	250	262	269
S.D.	7.2	13.0	18.4	17.8	23.2	26.8	33.5	38.6	41.5	48.4	46.3	48.9	47.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 3 10000 MG/M3

	WEEK OF STUDY												
ANIMAL#	0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
3066	25	59	86	97	109	126	146	159	164	177	192	205	218
3067	30	64	94	124	129	153	170	184	191	202	216	213	222
3068	35	61	92	119	132	172	189	198	206	224	237	252	260
3069	33	59	92	114	136	162	177	194	208	219	236	243	254
3070	20	71	122	147	173	203	236	244	255	272	284	292	292
MEAN	28	63	97	120	136	163	184	196	205	219	233	241	249
S.D.	6.1	5.1	14.1	18.1	23.4	28.2	33.2	30.9	33.2	35.0	33.9	34.5	30.5
N	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 4 20000 MG/M3

	WEEK OF STUDY												
ANIMAL#	0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
4076	25	50	79	100	118	138	155	164	172	187	204	214	218
4077	39	80	108	138	148	169	192	217	225	244	257	266	269
4078	41	77	108	130	148	172	198	215	231	245	261	283	277
4086	54	99	136	159	176	205	233	243	258	274	292	304	305
4080	40	79	107	132	155	184	205	217	228	245	269	273	277
MEAN	40	77	108	132	149	174	196	211	223	239	256	268	269
S.D.	10.1	17.6	20.1	20.9	20.7	24.4	28.2	28.8	31.6	31.6	32.2	33.3	31.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 1 0 MG/M3

	WEEK OF STUDY												
ANIMAL#	0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
1576	17	40	70	58	66	63	79	81	86	97	91	103	103
1577	22	36	45	43	54	56	53	69	71	72	67	82	89
1578	9	28	34	39	46	43	65	59	55	72	65	81	77
1579	16	26	43	48	49	54	53	63	63	64	63	67	70
1596	19	23	45	56	53	58	51	67	63	72	67	76	78
MEAN	16	31	47	49	54	55	60	68	68	76	71	82	83
S.D.	4.6	7.2	13.4	8.3	7.8	7.6	11.9	8.4	11.6	12.5	11.5	13.1	12.9
N	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 2 2000 MG/M3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
2566	13	18	40	46	51	54	60	56	69	69	68	66	76
2567	9	21	36	41	46	45	53	56	55	64	63	73	70
2568	23	38	47	52	62	70	76	71	82	85	94	102	99
2569	22	34	45	40	52	60	63	54	66	79	80	75	83
2570	8	25	36	41	40	50	53	56	54	62	67	74	70
MEAN	15	27	41	44	50	56	61	59	65	72	75	78	80
S.D.	6.9	8.5	5.1	5.3	8.0	9.9	9.4	7.2	11.4	9.8	12.7	13.9	12.2
N	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 3 10000 MG/M3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
3566	22	20	35	45	37	46	45	42	40	55	56	64	58
3567	11	20	23	33	35	50	43	46	51	57	57	61	62
3568	19	31	40	37	53	57	57	54	58	72	69	69	83
3569	3	29	43	54	52	70	76	79	78	93	83	97	102
3570	8	17	35	42	49	53	63	68	73	76	80	95	86
MEAN	13	23	35	42	45	55	57	58	60	70	69	77	78
S.D.	7.5	6.1	7.7	8.0	8.4	9.1	13.7	15.5	15.7	15.3	12.7	17.6	18.2
N	5	5	5	5	5	5	5	5	5	5	5	5	5

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 4 20000 MG/M3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
4576	12	17	35	44	46	38	49	50	54	44	58	60	58
4577	3	17	16	30	18	28	27	34	31	38	36	49	48
4578	14	26	37	46	44	59	60	70	69	73	79	79	76
4579	6	21	20	38	38	43	35	47	52	52	51	64	62
4596	13	26	34	38	40	53	54	56	68	67	71	72	74
MEAN	10	21	28	39	37	44	45	51	55	55	59	65	63
S.D.	4.7	4.6	10.0	6.3	11.2	12.3	13.6	13.2	15.4	14.8	16.9	11.4	11.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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)

MALES GROUP 1 0 MG/M3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
1076	87	70	67	60	58	61	58	56	50	53	52	51	48	48
1077	92	73	69	65	56	60	46	53	52	48	51	48	48	50
1078	96	79	78	73	64	61	62	61	56	57	59	58	55	57
1079	99	79	74	70	61	58	57	54	52	52	52	50	47	45
1080	102	83	74	69	63	61	62	60	54	56	54	52	50	44
MEAN	95	77	72	68	60	60	57	57	53	53	54	52	50	49
S.D.	6.0	5.2	4.2	4.9	3.4	1.5	6.7	3.6	2.4	3.5	3.1	3.7	3.0	5.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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)

MALES GROUP 2 2000 MG/M3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
2066	97	77	70	67	61	63	59	57	55	53	53	50	49	46
2067	102	84	76	75	60	62	59	58	52	53	53	50	49	46
2068	93	75	70	68	61	60	54	56	53	53	52	50	49	47
2069	103	83	74	67	62	59	55	54	51	50	50	47	45	43
2070	83	79	72	66	60	61	59	54	51	50	49	48	48	48
MEAN	96	80	73	68	61	61	57	56	52	52	51	49	48	46
S.D.	8.2	3.8	2.4	3.5	0.8	1.7	2.3	1.7	1.7	1.5	2.0	1.4	1.4	2.0
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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)

MALES GROUP 3 10000 MG/M3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
3066	90	77	76	74	63	62	58	57	54	53	52	51	50	49
3067	94	74	68	65	61	58	56	54	51	51	48	47	45	43
3068	95	74	61	63	61	57	58	56	51	51	48	49	47	44
3069	95	77	72	72	66	66	59	58	55	55	52	51	50	48
3070	74	77	65	71	64	63	56	54	49	45	47	44	43	42
MEAN	89	76	68	69	63	61	58	56	52	51	49	48	47	45
S.D.	9.1	1.7	5.8	4.8	2.3	3.9	1.4	2.0	2.5	3.7	2.4	2.9	3.2	3.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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)

MALES GROUP 4 20000 MG/M3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
4076	92	76	77	70	68	67	59	58	56	56	55	55	53	49
4077	92	73	68	61	61	58	55	54	55	54	54	48	48	45
4078	105	83	72	68	62	60	56	57	54	55	52	49	48	47
4086	104	83	74	70	62	61	63	61	57	56	56	55	53	48
4080	100	76	70	66	62	58	56	55	52	54	51	48	48	45
MEAN	98	78	72	67	63	61	58	57	55	55	54	51	50	47
S.D.	6.2	4.4	3.6	3.6	2.9	3.6	3.3	2.6	2.0	0.9	2.1	3.7	2.8	2.0
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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 1 0 MG/M3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
1576	96	84	80	76	59	66	59	62	58	57	65	59	57	56
1577	93	82	77	73	64	67	64	66	61	59	61	63	60	57
1578	100	87	85	77	69	75	68	71	62	61	70	65	63	58
1579	102	87	83	85	71	70	70	67	67	68	67	65	65	64
1596	103	93	82	84	73	74	SF	73	76	75	74	72	72	70
MEAN	99	86	82	79	67	70	65	68	65	64	67	65	63	61
S.D.	4.4	4.1	2.8	5.2	5.6	4.1	4.7	4.6	7.0	7.3	4.8	4.8	5.7	6.1
N	5	5	5	5	5	5	4	5	5	5	5	5	5	5

SF=Spilled Feeder

TABLE K

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 2 2000 MG/M3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
2566	91	77	78	73	63	68	65	65	63	64	59	58	58	57
2567	106	87	81	77	68	70	67	68	63	68	63	64	59	65
2568	105	98	84	79	78	72	69	69	67	SF	64	65	66	50
2569	108	98	85	84	75	78	72	68	66	SF	70	66	67	64
2570	101	94	89	82	74	74	70	69	63	60	66	64	65	62
MEAN	102	90	83	79	72	72	69	68	64	64	64	63	63	60
S.D.	6.7	8.9	4.2	4.1	5.8	3.7	2.6	1.7	2.1	4.2	4.2	3.1	4.0	6.2
N	5	5	5	5	5	5	5	5	5	3	5	5	5	5

SF=Spilled Feeder

TABLE K

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 3 10000 MG/M3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3566	92	82	77	81	73	74	74	71	66	80	79	73	71	69
3567	101	102	86	81	74	74	75	69	69	73	72	84	68	68
3568	92	79	81	77	SF	76	73	SF	SF	73	SF	73	73	77
3569	95	71	78	71	68	68	89	63	62	66	64	59	60	50
3570	90	80	80	79	73	74	71	71	64	64	66	65	64	56
MEAN	94	83	80	78	72	73	76	69	65	71	70	71	67	64
S.D.	4.5	11.4	3.5	4.2	2.7	3.0	7.0	3.7	2.8	6.4	6.8	9.6	5.1	10.8
N	5	5	5	5	4	5	5	4	4	5	4	5	5	5

SF=Spilled Feeder

TABLE K

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 4 20000 MG/M3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
4576	97	79	81	81	73	71	70	73	67	69	64	69	61	65
4577	132	98	SF	82	103	70	75	75	73	74	75	72	74	81
4578	112	77	75	71	66	66	70	61	62	62	63	61	57	57
4579	115	101	92	86	82	75	75	70	68	70	71	71	71	65
4596	95	79	76	69	69	67	66	65	65	70	64	63	63	58
MEAN	110	87	81	78	79	70	71	69	67	69	67	67	65	65
S.D.	14.9	11.5	8.0	7.3	14.8	3.6	3.8	5.9	3.9	4.4	5.2	5.0	7.1	9.6
N	5	5	4	5	5	5	5	5	5	5	5	5	5	5

SF=Spilled Feeder

TABLE L

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

MALES GROUP 1 0 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1076	TERMINAL SACRIFICE	25-SEP-02	13	92
1077	TERMINAL SACRIFICE	25-SEP-02	13	92
1078	TERMINAL SACRIFICE	25-SEP-02	13	92
1079	TERMINAL SACRIFICE	25-SEP-02	13	92
1080	TERMINAL SACRIFICE	25-SEP-02	13	92

TABLE L

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

MALES GROUP 2 2000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2066	TERMINAL SACRIFICE	25-SEP-02	13	92
2067	TERMINAL SACRIFICE	25-SEP-02	13	92
2068	TERMINAL SACRIFICE	25-SEP-02	13	92
2069	TERMINAL SACRIFICE	25-SEP-02	13	92
2070	TERMINAL SACRIFICE	25-SEP-02	13	92

TABLE L

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

MALES GROUP 3 10000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3066	TERMINAL SACRIFICE	25-SEP-02	13	92
3067	TERMINAL SACRIFICE	25-SEP-02	13	92
3068	TERMINAL SACRIFICE	25-SEP-02	13	92
3069	TERMINAL SACRIFICE	25-SEP-02	13	92
3070	TERMINAL SACRIFICE	25-SEP-02	13	92

TABLE L

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

MALES GROUP 4 20000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4076	TERMINAL SACRIFICE	25-SEP-02	13	92
4077	TERMINAL SACRIFICE	25-SEP-02	13	92
4078	TERMINAL SACRIFICE	25-SEP-02	13	92
4086	TERMINAL SACRIFICE	25-SEP-02	13	92
4080	TERMINAL SACRIFICE	25-SEP-02	13	92

TABLE L

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 1 0 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1576	TERMINAL SACRIFICE	25-SEP-02	13	92
1577	TERMINAL SACRIFICE	25-SEP-02	13	92
1578	TERMINAL SACRIFICE	25-SEP-02	13	92
1579	TERMINAL SACRIFICE	25-SEP-02	13	92
1596	TERMINAL SACRIFICE	25-SEP-02	13	92

TABLE L

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 2 2000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2566	TERMINAL SACRIFICE	25-SEP-02	13	92
2567	TERMINAL SACRIFICE	25-SEP-02	13	92
2568	TERMINAL SACRIFICE	25-SEP-02	13	92
2569	TERMINAL SACRIFICE	25-SEP-02	13	92
2570	TERMINAL SACRIFICE	25-SEP-02	13	92

TABLE L

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 3 10000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3566	TERMINAL SACRIFICE	25-SEP-02	13	92
3567	TERMINAL SACRIFICE	25-SEP-02	13	92
3568	TERMINAL SACRIFICE	25-SEP-02	13	92
3569	TERMINAL SACRIFICE	25-SEP-02	13	92
3570	TERMINAL SACRIFICE	25-SEP-02	13	92

TABLE L

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 4 20000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4576	TERMINAL SACRIFICE	25-SEP-02	13	92
4577	TERMINAL SACRIFICE	25-SEP-02	13	92
4578	TERMINAL SACRIFICE	25-SEP-02	13	92
4579	TERMINAL SACRIFICE	25-SEP-02	13	92
4596	TERMINAL SACRIFICE	25-SEP-02	13	92