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

HLS Study No.: **00-6127**
Sponsor Study No.: 211-EtOH-S
Date: 12 December 2001

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

Huntingdon Life Sciences, Inc. Study No. 00-6127
Sponsor Study No 211-EtOH-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. — Date
Principal Investigator

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

SIGNATURE PAGE

SCIENTIST

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

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

5/15/09
Date

SCIENTIFIC REVIEW

The following Scientist has reviewed and approved this report.

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

27 Feb 09
Date

Third-Party QA Statement

Study Number HLS 00-6127

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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	4/3/01	4/10/01
Laboratory Inspection (sacrifice inspection)	7/17-19/01	7/30/01
Draft Final Report Audit	2/13-3/19/02	3/21/02

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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	1 & 2 Feb 01	8 Feb 01
Exposure, Monitoring & Equipment Records	17 Apr 01	20 Apr 01
GC Characterization	24 Apr 01	26 Apr 01
Exposure & Monitoring	15 Jun 01	15 Jun 01
GFAP Necropsy	18 Jul 01	23 Jul 01

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26 Jan 10

Date

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Appendix A = Testing Facility Exposure and In-Life Data

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 Ethanol Vapor Condensate resulted in a slight elevation of the levels of GFAP in several regions of the male brains (cortex, thalamus, cerebellum and rest of brain). No regions from the female brains were affected. The data suggest that exposure to Gasoline Ethanol Vapor Condensate under the regimen employed results in a small, sex-dependent gliosis in four of 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 et al., 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, 1998; 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 et al., 1988). 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 along with a two-generation developmental 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 Ethanol 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 April 2001 (Date Study Director signed the Protocol)

DATE OF ANIMAL RECEIPT

5 April 2001

EXPOSURE INITIATION

17 April 2001 (Experimental Start Date)

EXPOSURE TERMINATION

17 July 2001

TERMINAL SACRIFICE

18 July 2001

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)
Non-Fat Dry Milk (Carnation)
Mouse anti-Glial Fibrillary Acidic Protein Antibody (Chemicon Cat. # MAB 3402; formerly Boerhinger Mannheim #814369, Clone GA5)
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 at room temperature 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 at room temperature. 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. Incubate for 1 hour at room temperature 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 at room temperature.

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) 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 on immunohisto-chemistry 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, 1991b; 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-well microplate. 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, as sex-dependent responses to toxic substances are not uncommon, including responses reflected in levels of GFAP. Control levels for GFAP vary markedly according to rat brain region, consistent with known historical levels observed for GFAP across different brain regions (see Fig. 2). The 13-week exposure to Gasoline Ethanol Vapor Condensate caused slight increases in GFAP in several groups of the male subjects (Group III striatum; Group II cortex; group II olfactory bulb; group II & III thalamus; group II, III & IV cerebellum and Group II rest of brain). None of the Gasoline Ethanol Vapor Condensate exposed females showed a significant increase in GFAP in any brain area. While statistically significant, the effects seen in the males were not exposure concentration related; however, the magnitude of the increases reached as much as 150% of control (Group III, striatum). While not statistically significant, the effects seen in the Gasoline Ethanol Vapor Condensate exposed females were increased across almost all groups and brain areas. A trends analysis did not reveal a significant trend associated with exposure to Gasoline Ethanol Vapor Condensate in the females. In aggregate, these data suggest that a small degree of damage-induced gliosis does occur in several brain regions of the male subjects under the exposure conditions employed. Toxicant-induced

gliosis (as reflected by an enhanced expression of GFAP) is highly dose-, region- and time-dependent. Therefore, it is possible that higher dosages, examination of more brain regions or more discrete dissections of the regions examined, or the inclusion of more time points might have resulted in more positive findings. In conclusion, 13-week inhalation exposure to Gasoline Ethanol Vapor Condensate results in a small degree of gliosis in the male subjects, as assessed by analysis of GFAP levels in nine representative areas of the adult rat brain.

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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 ± SEM. Adapted from Martin and O'Callaghan, 1995.

Figure 1

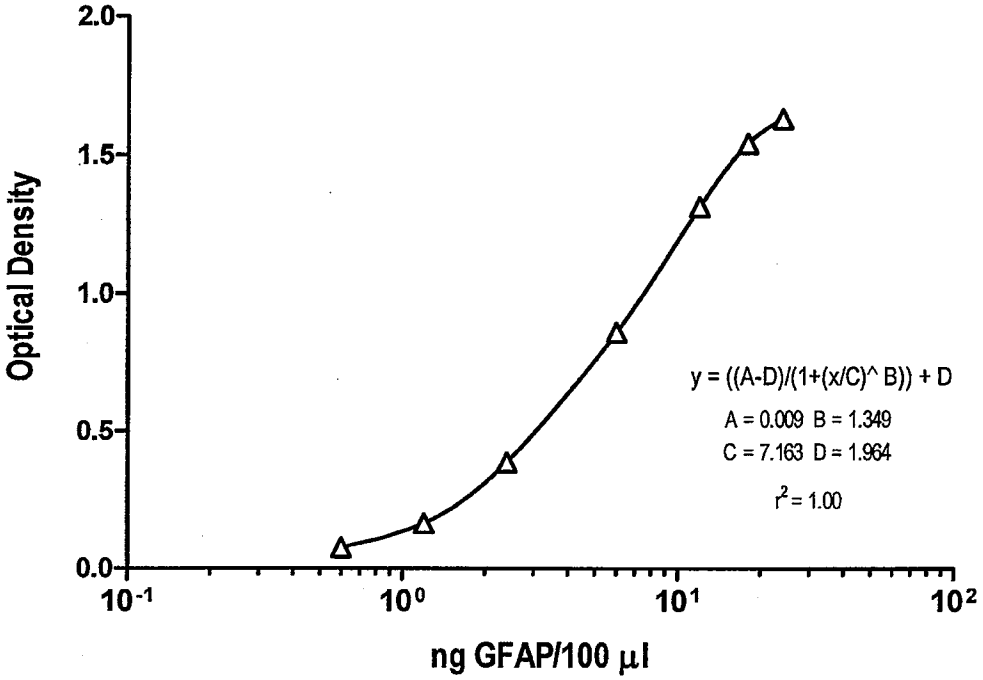
2.0
1.5
1.0
0.5
0.0
Optical Density
10^{-1}
10^{0}
10^{1}
10^{2}
ng GFAP/100 µl
y = ((A-D)/(1+(x/C)^ B)) + D
A = 0.009 B = 1.349
C = 7.163 D = 1.964
r^2 = 1.00

Figure 2

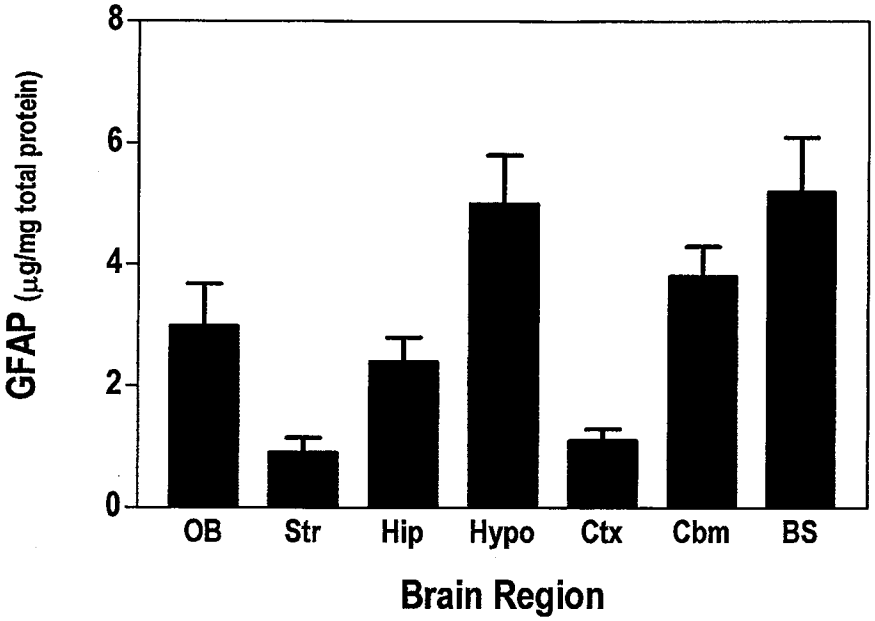
GFAP (µg/mg total protein)
8
6
4
2
0
OB
Str
Hip
Hypo
Ctx
Cbm
BS
Brain Region

TABLE 1: GFAP Standard Curve Preparation

Tube #	µg of total protein/100 µl/well (ng of GFAP)	µl of Hippocampus Std (1034 ng/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 Ethanol 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	0.94 ± 0.10*	1.28 ± 0.18	1.44 ± 0.29$^+$	1.10 ± 0.14
Hippocampus	2.91 ± 0.15	3.46 ± 0.39	3.40 ± 0.31	2.91 ± 0.14
Cortex	1.06 ± 0.07	1.40 ± 0.12$^+$	1.33 ± 0.12	1.18 ± 0.08
Olfactory Bulb	2.28 ± 0.13	3.03 ± 0.36$^+$	2.88 ± 0.15	2.48 ± 0.22
Thalamus	1.53 ± 0.09	2.30 ± 0.18$^+$	1.97 ± 0.30$^+$	1.73 ± 0.14
Hypothalamus	7.52 ± 1.03	8.53 ± 0.27	7.34 ± 0.78	7.44 ± 1.09
Cerebellum	3.49 ± 0.14	4.52 ± 0.25$^+$	4.62 ± 0.36$^+$	4.42 ± 0.32$^+$
Rest of Brain	3.53 ± 0.27	4.54 ± 0.51$^+$	3.92 ± 0.19	3.89 ± 0.32

*Each value represents the mean ± SEM (Standard Error of Mean) for the concentration of GFAP (µg/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 Ethanol 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	0.94 ± 0.12*	1.24 ± 0.19	1.00 ± 0.09	1.04 ± 0.14
Hippocampus	2.76 ± 0.13	3.13 ± 0.13	2.91 ± 0.21	2.90 ± 0.21
Cortex	1.10 ± 0.06	1.26 ± 0.15	1.13 ± 0.08	1.16 ± 0.10
Olfactory Bulb	2.57 ± 0.15	3.15 ± 0.31	2.53 ± 0.26	2.96 ± 0.19
Thalamus	1.54 ± 0.09	1.61 ± 0.14	1.77 ± 0.12	1.57 ± 0.06
Hypothalamus	7.34 ± 0.73	8.21 ± 1.03	9.62 ± 1.05	8.48 ± 0.44
Cerebellum	3.65 ± 0.15	3.93 ± 0.22	4.17 ± 0.34	3.97 ± 0.41
Rest of Brain	3.65 ± 0.13	3.92 ± 0.44	3.92 ± 0.29	3.91 ± 0.31

*Each value represents the mean ± SEM (Standard Error of Mean) for the concentration of GFAP (µg/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 Ethanol 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	1026	Male	0.945*	3.236	1.118	2.007	1.464	6.026	3.191	*	3.460
	1029		1.260	3.183	0.999	2.514	1.774	11.181	3.845	0.049	3.942
	1030		0.783	2.856	1.127	2.666	1.533	5.620	3.166	*	3.629
	1027		1.018	2.872	1.215	2.224	1.623	6.420	3.737	*	4.102
	1028		0.708	2.424	0.820	2.009	1.261	8.375	3.531	*	2.538
	1530	Female	0.803	3.140	1.084	3.015	1.652	6.795	4.151	*	3.538
	1526		0.815	2.321	0.901	2.467	1.254	6.239	3.364	*	3.192
	1528		0.888	2.844	1.169	2.165	1.488	6.242	3.324	*	3.811
	1529		1.408	2.669	1.087	2.427	1.556	7.283	3.615	*	3.819
	1527		0.807	2.817	1.265	2.765	1.771	10.165	3.785	*	3.872
Group II Test Substance 2,000 mg/m3	2018	Male	1.225	4.073	1.398	2.995	2.235	8.548	4.612	0.066	5.000
	2017		1.928	4.114	1.726	3.150	2.864	8.779	4.856	*	6.190
	2020		1.254	3.119	1.362	2.547	2.466	9.234	4.252	*	3.748
	2016		0.817	2.085	0.994	2.139	1.750	7.614	3.735	*	3.236
	2019		1.152	3.921	1.537	4.299	2.172	8.458	5.155	*	4.548
	2519	Female	0.885	3.075	1.098	3.095	1.794	8.730	4.455	*	3.932
	2518		1.194	2.981	0.974	2.453	1.309	7.314	3.891	*	3.783
	2516		1.260	3.441	1.525	3.673	2.051	9.886	4.256	*	5.525
	2520		0.927	2.750	0.998	2.507	1.335	4.695	3.185	*	2.947
	2517		1.919	3.427	1.695	4.040	1.547	10.401	3.887	*	3.414
Group III Test Substance 10,000 mg/m3	3020	Male	0.850	2.434	0.996	2.504	1.281	7.140	3.501	*	3.314
	3017		1.231	3.589	1.272	2.705	1.882	6.465	4.510	*	3.985
	3018		2.538	4.336	1.732	3.197	3.096	9.278	5.467	*	4.395
	3016		1.102	3.477	1.410	3.279	1.862	8.812	5.312	0.072	4.155
	3019		1.485	3.172	1.253	2.719	1.707	4.993	4.303	*	3.727
	3518	Female	0.792	2.619	1.138	2.466	1.668	7.262	3.558	*	4.246
	3517		1.149	3.676	1.354	2.504	1.763	9.804	4.605	*	4.416
	3520		1.252	2.978	1.190	2.385	2.090	13.145	4.475	*	3.883
	3516		0.904	2.798	1.135	3.451	1.970	10.212	4.991	*	4.211
	3519		0.888	2.492	0.839	1.855	1.375	7.690	3.207	*	2.829
Group IV Test Substance 20,000 mg/m3	4029	Male	1.046	2.865	1.277	2.693	2.134	10.280	4.615	0.058	4.653
	4026		0.966	2.965	1.242	2.726	1.601	6.459	3.860	*	3.834
	4028		0.886	2.526	1.113	2.134	1.437	4.546	3.894	*	3.543
	4030		1.605	3.372	1.373	3.038	1.980	9.672	5.584	*	4.528
	4027		0.974	2.822	0.905	1.820	1.502	6.264	4.130	*	2.903
	4527	Female	0.832	2.610	1.272	3.357	1.456	9.998	3.524	*	3.590
	4530		0.656	2.495	0.806	2.237	1.421	7.299	2.826	*	2.919
	4529		1.422	3.686	1.382	3.139	1.614	8.485	5.098	*	4.227
	4526		1.053	2.730	1.272	3.137	1.735	8.100	3.693	*	4.045
	4528		1.234	2.966	1.086	2.944	1.620	8.497	4.684	*	4.763

◆ 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:	5 April 2001
Experimental Initiation Date:	17 April 2001 (in-life)
Experimental Completion Date:	18 July 2001 (in-life)

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

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
0	17-Apr-01	1	0	0	0	0	0	0				24	47
1	18-Apr-01	2	0	0	0	0	0	0				24	37
2	19-Apr-01	3	0	0	0	0	0	0	1.752	2.044	2.68E-03	24	50
3	20-Apr-01	4	0	0	0	0	0	0				24	50
6	23-Apr-01	5	0	0	0	0	0	0				24	49
7	24-Apr-01	6	0	0	0	0	0	0				23	54
8	25-Apr-01	7	0	0	0	0	0	0				24	47
9	26-Apr-01	8	0	0	0	0	0	0	1.399	1.888	2.83E-03	25	46
10	27-Apr-01	9	0	0	0	0	0	0				24	48
13	30-Apr-01	10	0	0	0	0	0	0				24	47
14	1-May-01	11	0	0	0	0	0	0				24	38
15	2-May-01	12	0	0	0	0	0	0				25	44
16	3-May-01	13	0	0	0	0	0	0	0.9981	1.830	3.81E-03	25	44
17	4-May-01	14	0	0	0	0	0	0				23	49
20	7-May-01	15	0	0	0	0	0	0				24	45
21	8-May-01	16	0	0	0	0	0	0				24	46
22	9-May-01	17	0	0	0	0	0	0				24	47
23	10-May-01	18	0	0	0	0	0	0	1.407	1.574	2.26E-03	24	46
24	11-May-01	19	0	0	0	0	0	0				24	45
27	14-May-01	20	0	0	0	0	0	0				24	45
28	15-May-01	21	0	0	0	0	0	0				25	45
29	16-May-01	22	0	0	0	0	0	0				25	41
30	17-May-01	23	0	0	0	0	0	0	1.072	1.890	5.04E-03	22	37
31	18-May-01	24	0	0	0	0	0	0				24	46

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
32	19-May-01	25	0	0	0	0	0	0				24	49
34	21-May-01	26	0	0	0	0	0	0				24	47
35	22-May-01	27	0	0	0	0	0	0				24	50
36	23-May-01	28	0	0	0	0	0	0				24	50
37	24-May-01	29	0	0	0	0	0	0	0.8431	1.531	3.16E-03	24	49
38	25-May-01	30	0	0	0	0	0	0				24	50
41	28-May-01	31	0	0	0	0	0	0				24	52
42	29-May-01	32	0	0	0	0	0	0				25	50
43	30-May-01	33	0	0	0	0	0	0				25	47
44	31-May-01	34	0	0	0	0	0	0	8.759	2.355	2.40E-03	25	47
45	1-Jun-01	35	0	0	0	0	0	0				25	46
48	4-Jun-01	36	0	0	0	0	0	0				25	45
49	5-Jun-01	37	0	0	0	0	0	0				24	48
50	6-Jun-01	38	0	0	0	0	0	0				23	51
51	7-Jun-01	39	0	0	0	0	0	0	2.067	2.172	2.13E-03	24	44
52	8-Jun-01	40	0	0	0	0	0	0				24	45
55	11-Jun-01	41	0	0	0	0	0	0				24	48
56	12-Jun-01	42	0	0	0	0	0	0				26	50
57	13-Jun-01	43	0	0	0	0	0	0				25	50
58	14-Jun-01	44	0	0	0	0	0	0	0.8592	1.676	5.00E-02	25	50
59	15-Jun-01	45	0	0	0	0	0	0				25	50
62	18-Jun-01	46	0	0	0	0	0	0				25	47
63	19-Jun-01	47	0	0	0	0	0	0				24	50
64	20-Jun-01	48	0	0	0	0	0	0				23	50
65	21-Jun-01	49	0	0	0	0	0	0	0.7482	2.632	9.11E-03	24	50

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
66	22-Jun-01	50	0	0	0	0	0	0				23	50
69	25-Jun-01	51	0	0	0	0	0	0				24	48
70	26-Jun-01	52	0	0	0	0	0	0				24	48
71	27-Jun-01	53	0	0	0	0	0	0				24	47
72	28-Jun-01	54	0	0	0	0	0	0				24	48
73	29-Jun-01	55	0	0	0	0	0	0	1.598	2.091	4.07E-03	24	48
76	2-Jul-01	56	0	0	0	0	0	0				25	43
77	3-Jul-01	57	0	0	0	0	0	0				24	46
78	4-Jul-01	58	0	0	0	0	0	0				24	49
79	5-Jul-01	59	0	0	0	0	0	0	1.468	2.634	1.41E-02	24	51
80	6-Jul-01	60	0	0	0	0	0	0				24	44
83	9-Jul-01	61	0	0	0	0	0	0				24	48
84	10-Jul-01	62	0	0	0	0	0	0				24	47
85	11-Jul-01	63	0	0	0	0	0	0				24	48
86	12-Jul-01	64	0	0	0	0	0	0	2.634	1.715	2.39E-03	24	48
87	13-Jul-01	65	0	0	0	0	0	0				24	51
89	15-Jul-01	66	0	0	0	0	0	0				24	48
90	16-Jul-01	67	0	0	0	0	0	0				24	47
91	17-Jul-01	68	0	0	0	0	0	0				22	51
		Mean	0		0				1.970	2.002	8.00E-03	24.1	47.3
		S.D.	0		0				2.109	0.366	1.31E-02	0.7	3.2

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
0	17-Apr-01	1	0	0	0	0	0	0				24	44
1	18-Apr-01	2	0	0	0	0	0	0				24	34
2	19-Apr-01	3	0	0	0	0	0	0	3.223	2.156	4.38E-03	24	46
3	20-Apr-01	4	0	0	0	0	0	0				24	46
6	23-Apr-01	5	0	0	0	0	0	0				23	48
7	24-Apr-01	6	0	0	0	0	0	0				24	53
8	25-Apr-01	7	0	0	0	0	0	0				25	46
9	26-Apr-01	8	0	0	0	0	0	0	1.557	1.896	4.20E-03	25	45
10	27-Apr-01	9	0	0	0	0	0	0				25	45
13	30-Apr-01	10	0	0	0	0	0	0				25	45
14	1-May-01	11	0	0	0	0	0	0				24	37
15	2-May-01	12	0	0	0	0	0	0				24	42
16	3-May-01	13	0	0	0	0	0	0	1.114	2.079	4.27E-03	24	43
17	4-May-01	14	0	0	0	0	0	0				23	48
20	7-May-01	15	0	0	0	0	0	0				24	43
21	8-May-01	16	0	0	0	0	0	0				25	44
22	9-May-01	17	0	0	0	0	0	0				25	44
23	10-May-01	18	0	0	0	0	0	0	1.732	1.802	3.71E-03	25	44
24	11-May-01	19	0	0	0	0	0	0				25	43
27	14-May-01	20	0	0	0	0	0	0				25	43
28	15-May-01	21	0	0	0	0	0	0				25	44
29	16-May-01	22	0	0	0	0	0	0				24	39
30	17-May-01	23	0	0	0	0	0	0	0.9268	1.518	4.14E-03	22	37
31	18-May-01	24	0	0	0	0	0	0				23	44

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
32	19-May-01	25	0	0	0	0	0	0				24	48
34	21-May-01	26	0	0	0	0	0	0				24	47
35	22-May-01	27	0	0	0	0	0	0				25	48
36	23-May-01	28	0	0	0	0	0	0				25	47
37	24-May-01	29	0	0	0	0	0	0	0.8692	1.676	3.48E-03	25	47
38	25-May-01	30	0	0	0	0	0	0				24	47
41	28-May-01	31	0	0	0	0	0	0				24	49
42	29-May-01	32	0	0	0	0	0	0				24	49
43	30-May-01	33	0	0	0	0	0	0				24	44
44	31-May-01	34	0	0	0	0	0	0	1.557	1.915	7.43E-04	24	45
45	1-Jun-01	35	0	0	0	0	0	0				25	44
48	4-Jun-01	36	0	0	0	0	0	0				24	44
49	5-Jun-01	37	0	0	0	0	0	0				25	46
50	6-Jun-01	38	0	0	0	0	0	0				24	49
51	7-Jun-01	39	0	0	0	0	0	0	1.434	1.602	1.49E-03	24	43
52	8-Jun-01	40	0	0	0	0	0	0				25	44
55	11-Jun-01	41	0	0	0	0	0	0				25	46
56	12-Jun-01	42	0	0	0	0	0	0				25	49
57	13-Jun-01	43	0	0	0	0	0	0				25	48
58	14-Jun-01	44	0	0	0	0	0	0	0.8688	1.854	5.01E-02	25	49
59	15-Jun-01	45	0	0	0	0	0	0				24	50
62	18-Jun-01	46	0	0	0	0	0	0				25	46
63	19-Jun-01	47	0	0	0	0	0	0				24	49
64	20-Jun-01	48	0	0	0	0	0	0				24	49
65	21-Jun-01	49	0	0	0	0	0	0	0.7171	1.911	8.62E-03	24	49

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
66	22-Jun-01	50	0	0	0	0	0	0				24	48
69	25-Jun-01	51	0	0	0	0	0	0				25	47
70	26-Jun-01	52	0	0	0	0	0	0				24	47
71	27-Jun-01	53	0	0	0	0	0	0				24	46
72	28-Jun-01	54	0	0	0	0	0	0				24	47
73	29-Jun-01	55	0	0	0	0	0	0	4.660	2.735	6.10E-03	24	47
76	2-Jul-01	56	0	0	0	0	0	0				24	43
77	3-Jul-01	57	0	0	0	0	0	0				25	44
78	4-Jul-01	58	0	0	0	0	0	0				25	47
79	5-Jul-01	59	0	0	0	0	0	0	2.195	3.358	1.67E-02	24	49
80	6-Jul-01	60	0	0	0	0	0	0				25	43
83	9-Jul-01	61	0	0	0	0	0	0				24	46
84	10-Jul-01	62	0	0	0	0	0	0				24	47
85	11-Jul-01	63	0	0	0	0	0	0				23	47
86	12-Jul-01	64	0	0	0	0	0	0	1.872	1.778	1.74E-03	23	47
87	13-Jul-01	65	0	0	0	0	0	0				23	51
89	15-Jul-01	66	0	0	0	0	0	0				23	48
90	16-Jul-01	67	0	0	0	0	0	0				23	47
91	17-Jul-01	68	0	0	0	0	0	0				22	49
		Mean	0		0				1.748	2.022	8.44E-03	24.2	45.8
		S.D.	0		0				1.108	0.503	1.32E-02	0.8	3.2

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

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

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(μm)		(mg/m^3)	(°C)	(%)
0	17-Apr-01	1	2860	2023	2010	1960	2050	2070					23	45
1	18-Apr-01	2	2840	2170	1980	2160	2230	2310					23	35
2	19-Apr-01	3	2870	2173	2360	2270	1920	2140		2.190	2.590	3.00E-03	24	48
3	20-Apr-01	4	2870	2138	2110	2290	1960	2190					24	47
6	23-Apr-01	5	2840	2078	2010	1920	2310	2070					23	48
7	24-Apr-01	6	3040	2150	2360	1990	1990	2260					23	53
8	25-Apr-01	7	2940	1898	1800	1600	2030	2160					23	47
9	26-Apr-01	8	3050	2118	2040	2150	2030	2250		1.511	2.013	4.08E-03	24	46
10	27-Apr-01	9	3310	2158	2150	1920	2550	2010					24	45
13	30-Apr-01	10	3040	2090	2090	2240	1800	2230					24	46
14	1-May-01	11	2810	2235	2260	2190	2220	2270					24	37
15	2-May-01	12	2830	2105	1920	2070	2080	2350					24	43
16	3-May-01	13	2870	2073	1770	1960	2400	2160		1.088	2.023	5.46E-03	24	44
17	4-May-01	14	2880	2025	1930	2100	2010	2060					23	48
20	7-May-01	15	2840	2130	2170	1800	2390	2200					24	44
21	8-May-01	16	3060	2383	3680	1990	1940	1920					23	47
22	9-May-01	17	2970	2095	2200	1840	2180	2160					23	46
23	10-May-01	18	3010	2090	2080	2020	1860	2400		1.903	1.819	4.36E-03	24	46
24	11-May-01	19	3140	2053	2040	2090	1800	2280					24	45
27	14-May-01	20	3130	2018	1870	2240	1910	2050					24	45
28	15-May-01	21	3080	2043	2120	2070	1970	2010					24	47
29	16-May-01	22	3190	2020	2180	1930	1880	2090					24	41
30	17-May-01	23	3440	1895	1950	1700	1970	1960		0.9306	1.481	4.01E-03	21	39
31	18-May-01	24	3160	2043	2040	2050	2020	2060					23	47

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

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

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(μm)		(mg/m^3)	(°C)	(%)
32	19-May-01	25	3150	1980	2020	2000	1910	1990					23	50
34	21-May-01	26	2900	2045	2030	1940	2190	2020					23	49
35	22-May-01	27	2990	1978	2070	1990	1960	1890					23	50
36	23-May-01	28	2940	2018	2180	1920	1910	2060					23	50
37	24-May-01	29	3010	1978	2050	1740	1930	2190		0.8657	1.945	5.90E-03	23	51
38	25-May-01	30	3160	1948	2020	1860	1720	2190					23	52
41	28-May-01	31	3060	1913	1670	2070	1870	2040					23	52
42	29-May-01	32	3030	2085	2390	1910	2090	1950					24	50
43	30-May-01	33	3000	2065	2150	2200	1920	1990					24	45
44	31-May-01	34	2820	2318	2680	2070	2330	2190		2.020	1.763	1.49E-03	24	46
45	1-Jun-01	35	3000	2043	1870	1900	2050	2350					24	44
48	4-Jun-01	36	2900	2135	2360	1870	2240	2070					24	44
49	5-Jun-01	37	3010	2083	1900	2190	2170	2070					24	47
50	6-Jun-01	38	3030	2083	1930	2070	2260	2070					23	51
51	7-Jun-01	39	3140	2035	1870	1980	2170	2120		2.037	2.179	2.23E-03	23	44
52	8-Jun-01	40	3050	2078	2090	2000	2060	2160					24	46
55	11-Jun-01	41	3240	2180	2060	2180	2180	2300					24	48
56	12-Jun-01	42	2850	2125	1920	2320	2360	1900					25	49
57	13-Jun-01	43	2930	1898	1710	1750	2150	1980					24	48
58	14-Jun-01	44	3200	2023	2090	1960	1960	2080		0.8687	1.992	5.26E-02	24	49
59	15-Jun-01	45	3150	2165	2020	2160	2270	2210					23	51
62	18-Jun-01	46	3140	2025	2070	1790	1980	2260					24	47
63	19-Jun-01	47	2840	2080	2190	2060	2020	2050					23	50
64	20-Jun-01	48	2900	2013	1950	2390	1750	1960					23	50
65	21-Jun-01	49	2850	2050	2060	1940	2030	2170		0.7378	2.246	7.73E-03	23	50

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
66	22-Jun-01	50	2900	2120	2090	2210	2040	2140				23	51
69	25-Jun-01	51	2980	2143	2190	2060	2090	2230				23	48
70	26-Jun-01	52	2330	2040	2080	2070	1950	2060				24	48
71	27-Jun-01	53	2920	2060	2130	2110	1820	2180				24	46
72	28-Jun-01	54	2860	1993	2040	2080	1980	1870				23	48
73	29-Jun-01	55	2830	1960	2130	1820	2020	1870	2.325	2.504	6.42E-03	24	48
76	2-Jul-01	56	2970	1993	2040	1880	2040	2010				24	43
77	3-Jul-01	57	2900	2048	1930	1930	2090	2240				23	46
78	4-Jul-01	58	2930	2100	2020	2000	2040	2340				23	49
79	5-Jul-01	59	2800	2023	2220	2040	1900	1930	0.914	2.538	1.16E-02	23	51
80	6-Jul-01	60	2690	2005	1990	1990	2080	1960				23	44
83	9-Jul-01	61	2880	2100	2400	1820	2190	1990				23	48
84	10-Jul-01	62	2850	2018	2040	1910	1880	2240				23	50
85	11-Jul-01	63	2320	2268	2760	2370	1960	1980				23	49
86	12-Jul-01	64	2790	1985	1700	2150	2050	2040	2.471	1.878	2.31E-03	23	48
87	13-Jul-01	65	2910	1993	2100	1960	1900	2010				23	51
89	15-Jul-01	66	2730	2078	1910	1990	2400	2010				23	48
90	16-Jul-01	67	2510	1908	2400	2010	1610	1610				23	47
91	17-Jul-01	68	3630	2065	2140	2090	2030	2000				22	50
		Mean	2957		2065				1.528	2.075	8.55E-03	23.4	47.1
		S.D.	204		197				0.647	0.328	1.35E-02	0.6	3.3

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
0	17-Apr-01	1	2860	1905	1890	1820	1940	1970				23	45
1	18-Apr-01	2	2840	2080	2050	2010	2160	2100				23	35
2	19-Apr-01	3	2870	2163	2400	2170	1880	2200	1.481	1.915	2.36E-03	23	47
3	20-Apr-01	4	2870	2193	1990	2320	1730	2730				23	47
6	23-Apr-01	5	2840	2160	2200	2010	2360	2070				23	47
7	24-Apr-01	6	3040	2190	2260	2070	2040	2390				23	53
8	25-Apr-01	7	2940	2098	2130	1980	2060	2220				24	47
9	26-Apr-01	8	3050	2163	2220	2240	2140	2050	1.410	1.716	3.50E-03	25	45
10	27-Apr-01	9	3310	2105	1780	2150	2500	1990				24	45
13	30-Apr-01	10	3040	2093	1900	2340	2020	2110				25	46
14	1-May-01	11	2810	2133	2150	1990	2190	2200				24	36
15	2-May-01	12	2830	1970	1920	1920	1900	2140				24	42
16	3-May-01	13	2870	1960	1860	1880	2160	1940	1.304	2.308	6.83E-03	24	43
17	4-May-01	14	2880	1975	1760	2110	2010	2020				22	48
20	7-May-01	15	2840	2168	2150	1930	2210	2380				24	43
21	8-May-01	16	3060	2013	1910	2000	1930	2210				24	46
22	9-May-01	17	2970	2123	2210	2040	2080	2160				24	45
23	10-May-01	18	3010	2200	2180	2130	2090	2400	1.433	1.594	2.88E-03	24	45
24	11-May-01	19	3140	2065	1770	2300	2000	2190				24	45
27	14-May-01	20	3130	1920	1800	1990	1880	2010				24	44
28	15-May-01	21	3080	2048	2270	2120	1890	1910				24	45
29	16-May-01	22	3190	1940	1990	1900	1800	2070				23	40
30	17-May-01	23	3440	1865	1720	1750	2120	1870	3.993	2.642	1.09E-02	22	37
31	18-May-01	24	3160	1975	1700	2210	1980	2010				23	45

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
32	19-May-01	25	3150	2010	2010	2130	1950	1950				23	48
34	21-May-01	26	2900	2100	2210	2220	2030	1940				23	48
35	22-May-01	27	2990	1963	1720	2070	2070	1990				23	49
36	23-May-01	28	2940	2035	2180	1920	1970	2070				23	48
37	24-May-01	29	3010	2005	2190	1890	2140	1800	0.7828	1.492	4.50E-03	23	49
38	25-May-01	30	3160	2025	1840	2210	1790	2260				23	49
41	28-May-01	31	3060	1805	1680	1830	1820	1890				23	51
42	29-May-01	32	3030	2123	2400	2020	2110	1960				24	49
43	30-May-01	33	3000	2155	2190	2040	2150	2240				24	46
44	31-May-01	34	2820	2375	2770	2250	2300	2180	1.726	1.913	1.02E-03	24	46
45	1-Jun-01	35	3000	2043	1890	1890	2070	2320				24	44
48	4-Jun-01	36	2900	2163	2160	1900	2350	2240				24	44
49	5-Jun-01	37	3010	1968	1730	2080	2100	1960				24	46
50	6-Jun-01	38	3030	2010	1710	1820	2330	2180				23	51
51	7-Jun-01	39	3140	2103	1850	1960	2200	2400	1.979	1.925	2.29E-03	24	43
52	8-Jun-01	40	3050	2113	2090	2060	2090	2210				24	44
55	11-Jun-01	41	3240	2128	2200	2130	2170	2010				24	46
56	12-Jun-01	42	2850	2200	2180	2110	2400	2110				25	49
57	13-Jun-01	43	2930	1863	1940	1850	1940	1720				25	48
58	14-Jun-01	44	3200	2213	2320	2090	2110	2330	2.246	2.712	9.40E-02	24	50
59	15-Jun-01	45	3150	2210	2120	2130	2310	2280				24	51
62	18-Jun-01	46	3200	1985	1850	1970	1990	2130				25	46
63	19-Jun-01	47	2840	1913	1890	1940	1910	1910				24	49
64	20-Jun-01	48	2900	2165	2320	1690	2480	2170				24	51
65	21-Jun-01	49	2850	2090	2410	2130	1850	1970	0.7161	2.079	8.89E-03	24	49

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
66	22-Jun-01	50	2900	2090	2110	2140	2050	2060				23	50
69	25-Jun-01	51	2980	2150	2160	1910	2120	2410				24	47
70	26-Jun-01	52	2330	2063	2150	2100	1980	2020				23	47
71	27-Jun-01	53	2920	2050	2090	2180	1820	2110				23	47
72	28-Jun-01	54	2860	2035	2060	2120	1970	1990				23	48
73	29-Jun-01	55	2830	2000	2130	1850	2150	1870	1.122	1.726	3.50E-03	23	47
76	2-Jul-01	56	2970	2063	2280	1960	2020	1990				24	44
77	3-Jul-01	57	2900	2160	2070	2210	2140	2220				24	44
78	4-Jul-01	58	2930	2078	2030	2040	2050	2190				24	47
79	5-Jul-01	59	2800	1990	2020	2040	2020	1880	0.8507	1.809	8.90E-03	24	49
80	6-Jul-01	60	2690	2093	2210	1940	2100	2120				24	43
83	9-Jul-01	61	2880	2188	2400	2070	2400	1880				24	47
84	10-Jul-01	62	2850	1943	1740	1850	1860	2320				22	49
85	11-Jul-01	63	2320	2193	2730	2280	1980	1780				22	49
86	12-Jul-01	64	2790	2038	1750	2180	2140	2080	3.938	2.113	3.04E-03	23	48
87	13-Jul-01	65	2910	2035	2200	2070	1940	1930				23	50
89	15-Jul-01	66	2730	2125	2010	2150	2280	2060				23	47
90	16-Jul-01	67	2510	2038	2570	2240	1730	1610				22	48
91	17-Jul-01	68	3630	2015	2040	2070	2010	1940				22	49
		Mean	2958		2068				1.768	1.996	1.17E-02	23.5	46.4
		S.D.	205		189				1.073	0.373	2.49E-02	0.8	3.3

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
0	17-Apr-01	1	12800	10470	10600	9980	10200	11100				24	46
1	18-Apr-01	2	12500	9993	8750	10700	9720	10800				24	35
2	19-Apr-01	3	12300	9908	10300	9130	10100	10100	1.824	2.273	2.73E-03	24	47
3	20-Apr-01	4	12300	9700	9130	9820	9850	10000				24	46
6	23-Apr-01	5	12100	10200	9980	10100	10700	10000				23	47
7	24-Apr-01	6	12500	9765	9520	9520	10100	9920				23	53
8	25-Apr-01	7	12500	10250	10500	10000	10300	10200				24	49
9	26-Apr-01	8	12700	10430	10500	10300	10200	10700	1.404	1.569	2.61E-03	24	48
10	27-Apr-01	9	12200	10900	10400	11800	10500	10900				24	47
13	30-Apr-01	10	12000	9745	10400	8730	10200	9650				25	47
14	1-May-01	11	11800	10600	11700	10500	9790	10400				25	37
15	2-May-01	12	11500	10710	11500	9820	11100	10400				25	42
16	3-May-01	13	11300	10450	11200	10500	10000	10100	1.077	1.863	4.56E-03	25	43
17	4-May-01	14	11700	9748	9460	10400	9030	10100				23	47
20	7-May-01	15	11900	10360	10900	9950	10200	10400				25	44
21	8-May-01	16	11900	10100	11200	9690	9390	10100				24	45
22	9-May-01	17	11900	9793	10100	10100	9320	9650				24	45
23	10-May-01	18	11700	10190	9850	10500	10000	10400	1.516	1.720	2.88E-03	24	46
24	11-May-01	19	12400	10070	9880	10200	10000	10200				24	45
27	14-May-01	20	12600	10070	9490	9790	10600	10400				24	45
28	15-May-01	21	12400	10080	10700	9650	10200	9750				25	47
29	16-May-01	22	12500	10410	11000	11300	9980	9360				24	41
30	17-May-01	23	11800	10280	10100	10500	10200	10300	0.9372	1.717	4.77E-03	22	37
31	18-May-01	24	12100	9728	9220	11200	8770	9720				24	45

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
32	19-May-01	25	12200	10070	9420	9850	10200	10800				24	49
34	21-May-01	26	11900	9858	10600	9520	9520	9790				24	48
35	22-May-01	27	12500	10170	9920	9950	10600	10200				24	50
36	23-May-01	28	12600	10160	9950	9690	10400	10600				24	49
37	24-May-01	29	12400	10230	9720	10200	10300	10700	0.7874	1.582	5.47E-03	24	50
38	25-May-01	30	12600	10210	9820	10200	10400	10400				24	49
41	28-May-01	31	12600	9310	7950	9950	9490	9850				24	51
42	29-May-01	32	11900	10380	10300	10500	10200	10500				24	49
43	30-May-01	33	12200	10780	10900	10400	10600	11200				24	46
44	31-May-01	34	10400	11120	13000	11200	10500	9790	2.164	1.913	1.58E-03	24	45
45	1-Jun-01	35	11800	9905	8490	11500	9130	10500				24	45
48	4-Jun-01	36	12000	10060	10100	10300	9620	10200				24	44
49	5-Jun-01	37	12300	10040	9790	10300	10100	9950				25	47
50	6-Jun-01	38	12100	9930	9690	9950	10200	9880				24	49
51	7-Jun-01	39	12300	10350	10600	10300	10200	10300	1.591	1.735	2.40E-03	24	43
52	8-Jun-01	40	12400	10180	10400	10000	10100	10200				24	45
55	11-Jun-01	41	12500	10450	9690	10600	10400	11100				24	47
56	12-Jun-01	42	11700	10550	11300	9790	10100	11000				25	48
57	13-Jun-01	43	12000	9808	9320	9790	9620	10500				25	48
58	14-Jun-01	44	12500	9908	10700	9720	9690	9520	0.8862	1.735	4.44E-02	24	50
59	15-Jun-01	45	12200	10180	10200	10500	10000	10000				24	50
62	18-Jun-01	46	12300	10070	10600	10200	9360	10100				25	45
63	19-Jun-01	47	12800	10510	9520	11000	10800	10700				23	50
64	20-Jun-01	48	12500	9325	8960	9790	9290	9260				23	50
65	21-Jun-01	49	12500	9678	9460	9590	9260	10400	0.7336	2.039	7.52E-03	23	50

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
66	22-Jun-01	50	12600	10230	10500	10200	10100	10100				23	50
69	25-Jun-01	51	12000	10230	10100	9320	11300	10200				24	47
70	26-Jun-01	52	12000	10600	10900	10900	10300	10300				24	46
71	27-Jun-01	53	11800	10550	10700	9880	11100	10500				24	47
72	28-Jun-01	54	12500	10810	12200	9950	10700	10400				24	47
73	29-Jun-01	55	11800	10270	10600	9980	10300	10200	1.153	1.818	3.58E-03	24	46
76	2-Jul-01	56	12000	10420	11500	10100	9590	10500				25	43
77	3-Jul-01	57	11900	10180	10500	10300	9720	10200				24	44
78	4-Jul-01	58	12400	10550	10400	10500	10500	10800				24	48
79	5-Jul-01	59	11700	11280	13500	11200	10400	10000	0.9041	2.472	1.13E-02	24	50
80	6-Jul-01	60	11700	10470	11400	10100	10400	9980				24	44
83	9-Jul-01	61	11900	10220	10300	10900	9490	10200				24	48
84	10-Jul-01	62	11600	10850	12000	10200	10300	10900				24	47
85	11-Jul-01	63	9640	11900	14800	10800	12000	9980				24	47
86	12-Jul-01	64	11300	9888	8700	9650	10700	10500	1.979	1.741	1.66E-03	24	47
87	13-Jul-01	65	12000	10020	9590	10100	10300	10100				24	49
89	15-Jul-01	66	11500	10450	10100	10200	10600	10900				24	47
90	16-Jul-01	67	10250	9463	11400	9850	8430	8170				23	47
91	17-Jul-01	68	11300	10480	10100	10500	10600	10700				23	48
		Mean	12040		10230				1.304	1.860	7.34E-03	24.0	46.5
		S.D.	566		731				0.478	0.263	1.15E-02	0.6	3.2

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(μm)		(mg/m^3)	(°C)	(%)
0	17-Apr-01	1	12800	10210	10200	9820	10200	10600				24	45
1	18-Apr-01	2	12500	11910	14700	11900	11600	9455				23	35
2	19-Apr-01	3	12300	10500	10500	9790	10900	10800	8.854	2.709	6.29E-03	24	47
3	20-Apr-01	4	12300	10520	11300	10200	9880	10700				24	47
6	23-Apr-01	5	12100	10430	10500	10000	10800	10400				23	47
7	24-Apr-01	6	12500	10480	10400	10500	10300	10700				24	53
8	25-Apr-01	7	12500	10430	10200	10600	10300	10600				24	49
9	26-Apr-01	8	12700	10700	10700	10200	9980	11900	1.384	1.639	2.98E-03	25	47
10	27-Apr-01	9	12200	9528	9690	8020	10300	10100				25	46
13	30-Apr-01	10	12000	10340	9650	11200	10800	9720				25	46
14	1-May-01	11	11800	10360	11300	10000	9820	10300				24	36
15	2-May-01	12	11500	10900	10900	10200	11700	10800				24	42
16	3-May-01	13	11300	10480	10200	10800	10600	10300	1.352	2.089	5.55E-03	24	43
17	4-May-01	14	11700	9828	8600	9490	11400	9820				23	48
20	7-May-01	15	11900	9945	9790	9790	10000	10200				24	44
21	8-May-01	16	11900	9858	10800	9390	9390	9850				24	44
22	9-May-01	17	11900	10420	10400	10800	10500	9980				25	45
23	10-May-01	18	11700	10260	9650	10600	10300	10500	1.565	1.713	3.34E-03	25	45
24	11-May-01	19	12400	10320	9690	10600	10200	10800				25	44
27	14-May-01	20	12600	10320	10900	10900	9820	9650				25	44
28	15-May-01	21	12400	10230	9320	10600	10700	10300				25	46
29	16-May-01	22	12500	9935	9650	9390	10400	10300				24	40
30	17-May-01	23	11800	10190	10500	11500	9320	9420	1.285	2.412	6.16E-03	22	37
31	18-May-01	24	12100	10200	10300	10000	10500	10000				24	45

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
32	19-May-01	25	12200	10230	10600	10400	9820	10100				23	49
34	21-May-01	26	11900	10210	10900	9850	10100	10000				24	48
35	22-May-01	27	12500	10480	10200	11000	10300	10400				24	49
36	23-May-01	28	12600	10400	10100	10100	10800	10600				24	49
37	24-May-01	29	12400	10900	10000	10800	11800	11000	0.7889	1.540	5.63E-03	24	50
38	25-May-01	30	12600	10090	10300	9850	10200	10000				24	49
41	28-May-01	31	12600	9680	9825	9590	9490	9820				24	50
42	29-May-01	32	11900	10110	10100	10400	9820	10100				25	49
43	30-May-01	33	12200	10020	9490	10100	10400	10100				24	46
44	31-May-01	34	10400	10600	12800	10600	10100	8900	2.518	2.423	2.13E-03	25	46
45	1-Jun-01	35	11800	9710	8050	10700	9490	10600				25	45
48	4-Jun-01	36	12000	10380	10500	10700	9620	10700				25	44
49	5-Jun-01	37	12300	10630	10600	10700	10800	10400				24	46
50	6-Jun-01	38	12100	10040	9590	9850	10600	10100				24	49
51	7-Jun-01	39	12300	10220	10200	10700	10000	9980	2.076	1.926	2.14E-03	24	42
52	8-Jun-01	40	12400	10240	10200	9950	10400	10400				24	43
55	11-Jun-01	41	12500	10510	9130	10800	10600	11500				24	47
56	12-Jun-01	42	11700	10700	11800	10100	10300	10600				26	49
57	13-Jun-01	43	12000	10250	10200	10100	10600	10100				25	49
58	14-Jun-01	44	12500	9993	10200	10100	9820	9850	0.8732	1.657	4.46E-02	25	50
59	15-Jun-01	45	12200	10080	9790	9920	10100	10500				24	51
62	18-Jun-01	46	12300	10360	9850	10100	10400	11100				25	46
63	19-Jun-01	47	12800	10290	11500	9790	9880	10000				24	48
64	20-Jun-01	48	12500	11350	11900	11800	10900	10800				25	49
65	21-Jun-01	49	12500	11050	11000	11400	11300	10500	0.7145	1.760	6.86E-03	24	49

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
66	22-Jun-01	50	12600	10400	10300	10500	10400	10400				24	49
69	25-Jun-01	51	12000	10350	11100	9980	9130	11200				24	47
70	26-Jun-01	52	12000	10500	10700	10900	10200	10200				24	47
71	27-Jun-01	53	11800	10010	10100	9130	10400	10400				24	47
72	28-Jun-01	54	12500	11650	11900	12800	11100	10800				24	47
73	29-Jun-01	55	11800	10860	9920	11200	10900	11400	2.152	2.267	5.39E-03	24	46
76	2-Jul-01	56	12000	10630	10600	11100	10700	10100				25	43
77	3-Jul-01	57	11900	10230	10600	10000	10000	10300				25	43
78	4-Jul-01	58	12400	10780	10100	10900	10700	11400				24	47
79	5-Jul-01	59	11700	11300	14200	10400	10200	10400	0.8582	1.924	1.03E-02	25	49
80	6-Jul-01	60	11700	10600	11200	10500	10500	10200				25	42
83	9-Jul-01	61	11900	10420	9880	10300	10300	11200				25	46
84	10-Jul-01	62	11600	9848	9490	9520	9980	10400				23	48
85	11-Jul-01	63	9640	11480	12800	10400	12000	10700				23	47
86	12-Jul-01	64	11300	10130	9750	9650	10300	10800	2.141	1.804	1.88E-03	24	47
87	13-Jul-01	65	12000	10490	9550	10400	10900	11100				23	49
89	15-Jul-01	66	11500	10680	10300	10400	10800	11200				24	46
90	16-Jul-01	67	10250	10080	12700	11000	8510	8100				23	46
91	17-Jul-01	68	11300	10140	9650	10300	10400	10200				23	48
		Mean	12040		10400				2.043	1.989	7.94E-03	24.2	46.2
		S.D.	566		781				2.130	0.363	1.13E-02	0.7	3.3

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
0	17-Apr-01	1	24300	20200	20200	19500	20500	20600				24	46
1	18-Apr-01	2	25100	20580	18400	21500	20200	22200				24	36
2	19-Apr-01	3	25500	20480	22400	19000	19600	20900	1.272	2.164	3.02E-03	25	48
3	20-Apr-01	4	25800	19780	20000	19500	19600	20000				25	49
6	23-Apr-01	5	26200	20750	20300	21200	22200	19300				24	49
7	24-Apr-01	6	26500	19600	19700	19900	18700	20100				23	55
8	25-Apr-01	7	26800	20150	20200	19600	20700	20100				24	49
9	26-Apr-01	8	26700	20430	20400	20500	20200	20600	1.628	1.807	3.52E-03	24	50
10	27-Apr-01	9	25800	20900	20900	20800	21600	20300				24	48
13	30-Apr-01	10	26400	21030	21600	21000	21100	20400				24	48
14	1-May-01	11	24600	20130	20900	19000	19700	20900				25	37
15	2-May-01	12	23600	19980	20200	19400	19900	20400				25	45
16	3-May-01	13	24500	19580	20200	18100	19300	20700	4.508	2.831	8.30E-03	25	46
17	4-May-01	14	25300	19830	18500	20000	20700	20100				23	52
20	7-May-01	15	25300	20380	19600	20300	21600	20000				25	46
21	8-May-01	16	25000	19930	20100	20400	19100	20100				24	46
22	9-May-01	17	25700	20700	19900	20900	21300	20700				24	46
23	10-May-01	18	26600	20200	19600	20300	20600	20300	1.321	1.568	2.30E-03	24	47
24	11-May-01	19	27300	20230	18700	20300	20100	21800				24	47
27	14-May-01	20	26600	20450	19900	20500	20500	20900				24	45
28	15-May-01	21	26600	20030	19300	19500	21400	19900				25	48
29	16-May-01	22	26300	19550	19900	18700	19600	20000				25	44
30	17-May-01	23	26400	20800	21200	21100	21000	19800	0.9327	1.603	4.37E-03	22	38
31	18-May-01	24	26700	20030	20200	19300	19100	21500				24	47

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
32	19-May-01	25	26600	20250	20500	20600	19700	20200				24	51
34	21-May-01	26	26000	20900	20900	21400	20600	20700				24	49
35	22-May-01	27	25900	20400	19300	20600	21800	19900				24	52
36	23-May-01	28	26100	21300	22700	20600	20600	21300				24	49
37	24-May-01	29	25700	20350	21400	19600	20400	20000	4.411	2.361	2.14E-02	24	51
38	25-May-01	30	26500	21030	21400	21300	20800	20600				24	50
41	28-May-01	31	25600	18430	17500	18100	19000	19100				24	53
42	29-May-01	32	26800	20630	21200	21000	20400	19900				25	51
43	30-May-01	33	26600	20000	20100	19800	20000	20100				25	52
44	31-May-01	34	22900	21830	25100	21000	20300	20900	1.555	1.642	1.49E-03	25	48
45	1-Jun-01	35	23900	18430	17000	18100	19000	19600				25	46
48	4-Jun-01	36	26200	20650	20100	20800	20800	20900				25	46
49	5-Jun-01	37	26700	21150	20100	21600	22300	20600				24	49
50	6-Jun-01	38	27000	20780	19200	20600	21900	21400				23	54
51	7-Jun-01	39	25600	20980	21400	20900	20500	21100	1.392	1.555	1.10E-03	24	45
52	8-Jun-01	40	25300	21300	22400	22000	20400	20400				24	45
55	11-Jun-01	41	24900	20400	21100	20100	19600	20800				24	50
56	12-Jun-01	42	23600	20980	22700	19800	21300	20100				26	51
57	13-Jun-01	43	24600	20550	20200	21100	20800	20100				25	51
58	14-Jun-01	44	25000	20150	20000	19600	20200	20800	0.8922	1.727	4.61E-02	25	54
59	15-Jun-01	45	25200	20780	20800	21600	20100	20600				25	53
62	18-Jun-01	46	25000	19380	17400	19900	20500	19700				25	48
63	19-Jun-01	47	28500	18400	16400	19000	19100	19100				23	50
64	20-Jun-01	48	25300	20250	17400	21000	21100	21500				23	51
65	21-Jun-01	49	26400	20480	20400	20000	20500	21000	0.7091	1.733	8.27E-03	23	51

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
66	22-Jun-01	50	24800	20800	23000	20100	20200	19900				23	51
69	25-Jun-01	51	24400	20650	20000	21500	20800	20300				24	48
70	26-Jun-01	52	24400	20580	21600	19300	21600	19800				24	48
71	27-Jun-01	53	24500	20800	21500	20800	20500	20400				24	49
72	28-Jun-01	54	24800	20630	21300	20600	20300	20300				24	48
73	29-Jun-01	55	25100	20580	20400	20500	19900	21500	2.434	2.110	6.60E-03	24	48
76	2-Jul-01	56	24300	20300	20900	20200	20300	19800				25	44
77	3-Jul-01	57	24000	20630	20100	21200	21200	20000				24	46
78	4-Jul-01	58	24400	20280	19200	20700	20500	20700				23	51
79	5-Jul-01	59	24300	21050	20900	21300	21300	20700	0.9100	1.928	9.78E-03	24	51
80	6-Jul-01	60	23600	20580	20600	20700	20700	20300				24	45
83	9-Jul-01	61	24400	20050	19500	21000	20000	19700				24	49
84	10-Jul-01	62	24300	20430	19900	21100	20700	20000				24	49
85	11-Jul-01	63	20000	21650	25200	21900	16800	22700				24	48
86	12-Jul-01	64	23600	19830	18000	20500	20500	20300	2.151	1.950	2.42E-03	24	48
87	13-Jul-01	65	24400	20680	20900	20500	20900	20400				24	51
89	15-Jul-01	66	23800	20080	20800	19400	20400	19700				24	48
90	16-Jul-01	67	22600	21050	25200	22300	16600	20100				24	48
91	17-Jul-01	68	23700	20400	19700	20100	20900	20900				22	49
		Mean	25250		20390				1.855	1.921	9.13E-03	24.1	48.3
		S.D.	1338		1122				1.257	0.370	1.24E-02	0.7	3.5

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
0	17-Apr-01	1	24300	18450	17500	17600	19000	19700				24	49
1	18-Apr-01	2	25100	19130	17200	20200	18400	20700				24	37
2	19-Apr-01	3	25500	19500	20000	18400	19300	20300	0.9562	1.715	2.50E-03	24	51
3	20-Apr-01	4	25800	19830	19800	20200	19700	19600				24	51
6	23-Apr-01	5	26200	20850	19700	20600	22400	20700				24	53
7	24-Apr-01	6	26500	19350	19600	19500	18700	19600				24	58
8	25-Apr-01	7	26800	20880	21300	20000	21000	21200				25	53
9	26-Apr-01	8	26700	20480	20500	21000	20300	20100	2.933	2.023	9.27E-03	25	51
10	27-Apr-01	9	25800	20200	20200	19900	21000	19700				25	50
13	30-Apr-01	10	26400	20000	20200	19300	19300	21200				25	51
14	1-May-01	11	24600	21150	22300	21000	20600	20700				25	40
15	2-May-01	12	23600	20050	18000	20000	20100	22100				25	47
16	3-May-01	13	24500	20680	20500	18600	21800	21800	1.187	1.849	3.58E-03	24	47
17	4-May-01	14	25300	19600	18400	19600	20300	20100				23	53
20	7-May-01	15	25300	19880	19100	20000	20800	19600				24	49
21	8-May-01	16	25000	19550	19100	20000	19300	19800				25	49
22	9-May-01	17	25700	20130	19500	19600	20900	20500				25	49
23	10-May-01	18	26600	20430	18800	20900	20900	21100	1.459	1.870	2.45E-03	25	51
24	11-May-01	19	27300	21180	20300	21100	21000	22300				25	49
27	14-May-01	20	26600	20400	19600	20700	20800	20500				26	47
28	15-May-01	21	26600	19380	18500	18700	20200	20100				25	50
29	16-May-01	22	26300	19980	20200	19400	20000	20300				25	45
30	17-May-01	23	26400	19800	20500	19700	19600	19400	1.204	1.917	4.39E-03	22	40
31	18-May-01	24	26700	20800	21500	20900	20300	20500				24	50

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
32	19-May-01	25	26600	20830	22000	21000	19700	20600				24	55
34	21-May-01	26	26000	20180	20600	20500	19600	20000				24	53
35	22-May-01	27	25900	20830	20300	21300	21600	20100				25	56
36	23-May-01	28	26100	20500	21600	19600	20800	20000				25	54
37	24-May-01	29	25700	20230	20800	19600	20600	19900	0.7816	1.437	4.78E-03	25	54
38	25-May-01	30	26500	20400	20900	20800	20100	19800				25	54
41	28-May-01	31	25600	19430	19000	19100	19800	19800				25	54
42	29-May-01	32	26800	20380	21400	20300	19400	20400				25	55
43	30-May-01	33	26600	20680	21100	20700	20100	20800				24	47
44	31-May-01	34	22900	21650	23000	21800	20800	21000	1.786	2.141	1.16E-03	25	48
45	1-Jun-01	35	23900	19230	18700	18100	19800	20300				25	49
48	4-Jun-01	36	26200	20450	20300	20600	20500	20400				25	48
49	5-Jun-01	37	26700	20750	19700	21000	21300	21000				25	53
50	6-Jun-01	38	27000	21400	20300	21600	21900	21800				25	56
51	7-Jun-01	39	25600	20430	20400	20300	20000	21000	1.618	1.703	1.63E-03	25	48
52	8-Jun-01	40	25300	20430	19800	21200	20100	20600				25	49
55	11-Jun-01	41	24900	21580	21400	20100	22600	22200				25	53
56	12-Jun-01	42	23600	21230	23200	21300	20100	20500				26	54
57	13-Jun-01	43	24600	20380	20000	21000	20600	19900				25	54
58	14-Jun-01	44	25000	19750	19200	19300	20000	20500	0.9184	2.066	4.89E-02	25	57
59	15-Jun-01	45	25200	19950	19700	20900	19100	20100				24	56
62	18-Jun-01	46	25000	20550	21800	19900	20400	20100				25	51
63	19-Jun-01	47	28500	18700	17800	16300	20200	20500				25	53
64	20-Jun-01	48	25300	19980	18200	19500	21000	21200				25	53
65	21-Jun-01	49	26400	19650	19900	19100	19900	19700	0.7025	1.677	9.87E-03	25	52

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

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Mean Temperature	Mean Humidity
			(mg/m³)	(mg/m³)	(mg/m³)				(μm)		(mg/m³)	(°C)	(%)
66	22-Jun-01	50	24800	20350	19900	20300	20700	20500				25	55
69	25-Jun-01	51	24400	20130	19500	20600	20200	20200				26	49
70	26-Jun-01	52	24400	20300	20400	19000	21700	20100				24	53
71	27-Jun-01	53	24500	20830	20800	20500	21000	21000				24	52
72	28-Jun-01	54	24800	20530	21100	20400	20300	20300				24	53
73	29-Jun-01	55	25100	20650	20900	20400	20000	21300	0.9784	1.959	5.71E-03	24	52
76	2-Jul-01	56	24300	20000	19600	19900	19600	20900				25	48
77	3-Jul-01	57	24000	20650	19800	21600	21100	20100				26	48
78	4-Jul-01	58	24400	20400	19400	20700	20900	20600				25	53
79	5-Jul-01	59	24300	21180	21500	21300	21000	20900	0.8715	1.709	9.12E-03	25	53
80	6-Jul-01	60	23600	21000	20200	20900	21200	21700				25	46
83	9-Jul-01	61	24400	20580	20600	21600	20300	19800				25	51
84	10-Jul-01	62	24300	20750	20400	21200	21100	20300				24	51
85	11-Jul-01	63	20000	21530	24100	22400	17000	22600				24	51
86	12-Jul-01	64	23600	20150	18300	20800	21200	20300	2.056	1.685	1.55E-03	24	51
87	13-Jul-01	65	24400	21350	21300	21000	21800	21300				24	53
89	15-Jul-01	66	23800	20730	20900	20000	21100	20900				24	50
90	16-Jul-01	67	22600	21500	25200	21900	17900	21000				24	51
91	17-Jul-01	68	23700	21000	19600	20700	21900	21800				23	51
		Mean	25250		20360				1.342	1.827	8.07E-03	24.6	50.8
		S.D.	1338		1080				0.631	0.195	1.26E-02	0.7	3.8

TABLE B

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

MALES SUMMARY OF CLINICAL OBSERVATIONS

	GROUP#	DAY OF STUDY -8	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 ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

FEMALES — SUMMARY OF CLINICAL OBSERVATIONS

	GROUP#	DAY OF STUDY -8	TOTAL
# OF ANIMALS EXAMINED	1	5	
	2	5	
	3	5	
	4	5	
NORMAL			
WITHIN NORMAL LIMITS	1	5	5
	2	5	5
	3	5	5
	4	5	5

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1116 Saint Andrews Road
Bryn Mawr, PA 19010
(610) 520 9430

April 9, 2001

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

Re: study A-12 00-6127F

Ophthalmoscopic examination of study A-12 00-6127F pretest rats was performed April 9, 2001. Rats with ocular abnormalities were identified and should be withdrawn from inclusion in the study if convenient.

Lionel F. Rubin, V. M. D.

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

July 10, 2001

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

Re: study A-12 00-6127F

Ophthalmoscopic examination of study A-12 00-6127F rats was performed July 10, 2001 (week 13 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 ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK -1

DOSE GROUP:	I	II	III	IV
EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000
MALES total number examined	5	5	5	5
NO ABNORMALITIES DETECTED				

TABLE C

GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
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SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK -1

DOSE GROUP:	I	II	III	IV
EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000
FEMALES total number examined	5	5	5	5
NO ABNORMALITIES DETECTED				

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK 13

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
MALES total number examined		5	5	5	5
CONJUNCTIVA	N	0	0	2	0
CONJUNCTIVITIS	N	0	0	2	0
	%	0.0	0.0	40.0	0.0

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK 13

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
FEMALES	total number examined	5	5	5	5
CONJUNCTIVA	N	0	2	0	0
CONJUNCTIVITIS	N	0	2	0	0
	%	0.0	40.0	0.0	0.0

TABLE D

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

MALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK -1	MEAN	227	228	228	227
	S.D.	3.4	6.8	3.3	2.8
	N	5	5	5	5
WEEK 0	MEAN	297	292	296	294
	S.D.	8.4	19.6	3.5	7.7
	N	5	5	5	5
WEEK 1	MEAN	343	330	330	322
	S.D.	16.5	26.7	8.7	5.4
	N	5	5	5	5
WEEK 2	MEAN	372	356	358	347
	S.D.	25.9	39.8	10.4	4.9
	N	5	5	5	5
WEEK 3	MEAN	402	375	381	374
	S.D.	34.9	47.0	16.3	11.8
	N	5	5	5	5
WEEK 4	MEAN	426	395	400	397
	S.D.	37.6	55.3	16.7	11.2
	N	5	5	5	5
WEEK 5	MEAN	448	417	421	423
	S.D.	46.2	60.4	17.8	14.8
	N	5	5	5	5
WEEK 6	MEAN	461	435	437	439
	S.D.	44.0	61.1	21.0	19.4
	N	5	5	5	5
WEEK 7	MEAN	476	447	441	458
	S.D.	46.5	61.0	50.1	24.5
	N	5	5	5	5

No statistically significant differences

TABLE D

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

MALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 8	MEAN	496	458	458	470
	S.D.	52.1	65.1	29.6	23.8
	N	5	5	5	5
WEEK 9	MEAN	511	472	476	484
	S.D.	56.1	66.9	24.7	26.8
	N	5	5	5	5
WEEK 10	MEAN	526	484	494	500
	S.D.	58.5	70.1	21.9	27.4
	N	5	5	5	5
WEEK 11	MEAN	537	496	494	512
	S.D.	64.2	71.6	21.0	24.6
	N	5	5	5	5
WEEK 12	MEAN	544	504	506	523
	S.D.	64.2	73.2	30.0	29.9
	N	5	5	5	5
WEEK 13	MEAN	557	509	516	531
	S.D.	71.4	72.7	22.0	36.4
	N	5	5	5	5

No statistically significant differences

TABLE D

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

FEMALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK -1	MEAN	181	181	181	181
	S.D.	4.3	5.4	6.8	5.7
	N	5	5	5	5
WEEK 0	MEAN	217	212	216	209
	S.D.	9.9	5.6	9.9	5.2
	N	5	5	5	5
WEEK 1	MEAN	236	228	231	227
	S.D.	15.6	11.8	8.8	10.2
	N	5	5	5	5
WEEK 2	MEAN	249	238	245	235
	S.D.	11.9	14.4	12.8	9.4
	N	5	5	5	5
WEEK 3	MEAN	264	246	259	248
	S.D.	11.2	18.9	19.5	14.6
	N	5	5	5	5
WEEK 4	MEAN	276	255	269	256
	S.D.	16.6	16.0	12.5	8.9
	N	5	5	5	5
WEEK 5	MEAN	286	269	274	264
	S.D.	23.6	20.3	9.7	9.6
	N	5	5	5	5
WEEK 6	MEAN	291	271	283	267
	S.D.	19.0	21.8	14.8	12.5
	N	5	5	5	5
WEEK 7	MEAN	303	275	292	273
	S.D.	21.4	25.4	16.1	15.1
	N	5	5	5	5

No statistically significant differences

TABLE D

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

FEMALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 8	MEAN	307	281	294	277
	S.D.	26.2	23.5	10.0	11.2
	N	5	5	5	5
WEEK 9	MEAN	309	279	293	281
	S.D.	29.1	19.0	7.3	10.4
	N	5	5	5	5
WEEK 10	MEAN	318	290	302	283
	S.D.	28.8	21.6	12.8	12.8
	N	5	5	5	5
WEEK 11	MEAN	318	294	307	283
	S.D.	26.0	26.0	10.5	12.1
	N	5	5	5	5
WEEK 12	MEAN	321	301	311	292
	S.D.	25.9	24.6	9.8	13.5
	N	5	5	5	5
WEEK 13	MEAN	324	304	307	294
	S.D.	31.8	24.4	6.7	13.3
	N	5	5	5	5

No statistically significant differences

TABLE E

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

MALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 1	MEAN	46	37	35	28*
	S.D.	12.0	10.8	6.4	3.1
	N	5	5	5	5
WEEK 0 TO 2	MEAN	75	64	63	53
	S.D.	21.7	22.9	10.5	6.4
	N	5	5	5	5
WEEK 0 TO 3	MEAN	105	83	85	80
	S.D.	30.1	29.5	16.5	8.6
	N	5	5	5	5
WEEK 0 TO 4	MEAN	128	103	105	103
	S.D.	33.5	37.5	17.7	8.0
	N	5	5	5	5
WEEK 0 TO 5	MEAN	151	125	125	129
	S.D.	42.8	42.3	19.0	11.2
	N	5	5	5	5
WEEK 0 TO 6	MEAN	164	143	142	145
	S.D.	40.9	43.7	22.5	16.3
	N	5	5	5	5

Statistical key: * = p<0.05

TABLE E

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

MALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 7	MEAN	179	155	145	164
	S.D.	43.6	44.0	51.0	20.2
	N	5	5	5	5
WEEK 0 TO 8	MEAN	199	166	162	176
	S.D.	48.9	47.7	30.7	19.2
	N	5	5	5	5
WEEK 0 TO 9	MEAN	214	179	181	190
	S.D.	52.9	49.2	25.7	22.4
	N	5	5	5	5
WEEK 0 TO 10	MEAN	229	192	199	206
	S.D.	54.2	51.8	22.5	23.8
	N	5	5	5	5
WEEK 0 TO 11	MEAN	240	203	198	218
	S.D.	60.5	52.8	22.7	19.7
	N	5	5	5	5
WEEK 0 TO 12	MEAN	247	212	210	229
	S.D.	60.5	54.8	30.5	25.1
	N	5	5	5	5

No statistically significant differences

Huntingdon Life Sciences 00-6127F
GFAP Sub-Group

TABLE E

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

MALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 13	MEAN	260	217	221	237
	S.D.	67.1	54.3	22.9	30.8
	N	5	5	5	5

No statistically significant differences

TABLE E

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

FEMALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:			I	II	III	IV
EXPOSURE LEVEL (mg/m3):			0	2,000	10,000	20,000
WEEK 0 TO 1	MEAN		19	16	15	19
	S.D.		8.1	6.5	2.0	6.8
	N		5	5	5	5
WEEK 0 TO 2	MEAN		32	26	29	26
	S.D.		8.8	9.0	5.5	8.8
	N		5	5	5	5
WEEK 0 TO 3	MEAN		46	34	43	40
	S.D.		5.9	13.9	12.9	10.9
	N		5	5	5	5
WEEK 0 TO 4	MEAN		59	43*	53	47
	S.D.		8.6	10.8	5.9	3.8
	N		5	5	5	5
WEEK 0 TO 5	MEAN		69	57	58	55
	S.D.		15.4	15.2	7.3	5.8
	N		5	5	5	5
WEEK 0 TO 6	MEAN		74	59	66	59
	S.D.		10.3	17.0	8.5	10.0
	N		5	5	5	5

Statistical key: * = p<0.05

TABLE E

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

FEMALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 7	MEAN	86	63	76	64
	S.D.	12.0	20.6	12.4	11.9
	N	5	5	5	5
WEEK 0 TO 8	MEAN	90	68	78	68
	S.D.	16.9	18.7	6.7	8.3
	N	5	5	5	5
WEEK 0 TO 9	MEAN	92	67	77	73
	S.D.	19.8	16.0	5.7	8.4
	N	5	5	5	5
WEEK 0 TO 10	MEAN	101	78	86	75
	S.D.	19.1	17.0	8.3	12.0
	N	5	5	5	5
WEEK 0 TO 11	MEAN	101	82	91	74
	S.D.	16.1	21.9	8.1	9.5
	N	5	5	5	5
WEEK 0 TO 12	MEAN	104	89	95	84
	S.D.	16.4	20.5	7.0	10.8
	N	5	5	5	5

No statistically significant differences

TABLE E

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

FEMALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 13	MEAN	106	91	91	86
	S.D.	22.2	20.0	6.1	9.8
	N	5	5	5	5

No statistically significant differences

TABLE F

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

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

DOSE GROUP:			I	II	III	IV
EXPOSURE LEVEL (mg/m3):			0	2,000	10,000	20,000
WEEK	0	MEAN	93	91	89	91
		S.D.	3.3	5.6	3.6	4.7
		N	5	5	5	5
WEEK	1	MEAN	84	81	81	79
		S.D.	2.5	5.3	2.6	3.4
		N	5	5	5	5
WEEK	2	MEAN	74	72	70	70
		S.D.	3.1	5.1	3.5	1.7
		N	5	5	5	5
WEEK	3	MEAN	69	67	66	69
		S.D.	2.8	3.6	1.5	6.2
		N	5	5	5	5
WEEK	4	MEAN	63	62	61	61
		S.D.	2.9	2.5	1.0	1.6
		N	5	5	5	4
WEEK	5	MEAN	59	61	58	60
		S.D.	2.6	1.8	1.3	2.1
		N	5	5	5	5
WEEK	6	MEAN	55	59	58	58
		S.D.	2.8	2.1	2.8	1.9
		N	5	5	5	5
WEEK	7	MEAN	54	56	50	56
		S.D.	2.0	2.6	17.8	3.7
		N	5	5	5	5
WEEK	8	MEAN	53	51	56	53
		S.D.	1.8	2.2	3.6	2.3
		N	5	5	5	5

No statistically significant differences

TABLE F

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

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

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 9	MEAN	51	52	54	51
	S.D.	1.6	0.9	4.2	1.8
	N	5	5	5	5
WEEK 10	MEAN	51	52	52	53
	S.D.	1.4	1.9	4.2	1.3
	N	5	5	5	5
WEEK 11	MEAN	49	51	48	51
	S.D.	1.3	2.1	3.3	2.4
	N	5	5	5	5
WEEK 12	MEAN	48	49	48	49
	S.D.	1.4	4.1	2.1	1.4
	N	5	5	5	4
WEEK 13	MEAN	47	48	50	48
	S.D.	1.5	3.0	4.6	1.6
	N	5	5	5	5

No statistically significant differences

TABLE F

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

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

DOSE GROUP:			I	II	III	IV
EXPOSURE LEVEL (mg/m3):			0	2,000	10,000	20,000
WEEK	0	MEAN	92	93	88	86
		S.D.	5.0	6.1	2.7	1.8
		N	5	5	5	5
WEEK	1	MEAN	88	85	81	82
		S.D.	6.3	6.0	2.6	4.4
		N	5	4	5	5
WEEK	2	MEAN	83	81	78	77
		S.D.	5.8	5.3	3.1	1.9
		N	5	5	5	4
WEEK	3	MEAN	80	80	76	76
		S.D.	12.5	4.3	2.5	3.4
		N	5	5	5	4
WEEK	4	MEAN	71	75	67	66
		S.D.	7.0	5.2	2.9	4.7
		N	5	5	5	5
WEEK	5	MEAN	69	73	71	65
		S.D.	3.6	6.1	3.3	4.0
		N	5	5	5	5
WEEK	6	MEAN	68	67	67	64
		S.D.	3.0	3.5	5.1	4.6
		N	5	5	5	5
WEEK	7	MEAN	68	67	62**	64
		S.D.	3.2	2.4	2.2	2.7
		N	5	5	5	5
WEEK	8	MEAN	63	62	57	61
		S.D.	6.0	2.9	2.5	5.0
		N	5	5	5	4

Statistical key: ** = p<0.01

TABLE F

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

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

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 9	MEAN	61	58	57	57
	S.D.	3.6	6.3	3.1	4.1
	N	5	5	5	5
WEEK 10	MEAN	61	63	60	59
	S.D.	5.3	5.4	4.3	3.7
	N	5	4	5	5
WEEK 11	MEAN	61	63	58	61
	S.D.	5.0	4.5	3.8	3.3
	N	5	5	5	5
WEEK 12	MEAN	62	63	56	61
	S.D.	6.0	4.7	3.7	2.5
	N	5	4	5	5
WEEK 13	MEAN	58	57	55	57
	S.D.	4.3	3.3	4.3	3.1
	N	5	5	5	5

No statistically significant differences

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP I 0 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
1026	WITHIN NORMAL LIMITS	P
1027	WITHIN NORMAL LIMITS	P
1028	WITHIN NORMAL LIMITS	P
1029	WITHIN NORMAL LIMITS	P
1030	WITHIN NORMAL LIMITS	P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP II 2,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
2016	WITHIN NORMAL LIMITS	P
2017	WITHIN NORMAL LIMITS	P
2018	WITHIN NORMAL LIMITS	P
2019	WITHIN NORMAL LIMITS	P
2020	WITHIN NORMAL LIMITS	P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP III 10,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
3016	WITHIN NORMAL LIMITS	P
3017	WITHIN NORMAL LIMITS	P
3018	WITHIN NORMAL LIMITS	P
3019	WITHIN NORMAL LIMITS	P
3020	WITHIN NORMAL LIMITS	P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP IV 20,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
4026	WITHIN NORMAL LIMITS	P
4027	WITHIN NORMAL LIMITS	P
4028	WITHIN NORMAL LIMITS	P
4029	WITHIN NORMAL LIMITS	P
4030	WITHIN NORMAL LIMITS	P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP I 0 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
1526	WITHIN NORMAL LIMITS	P
1527	WITHIN NORMAL LIMITS	P
1528	WITHIN NORMAL LIMITS	P
1529	WITHIN NORMAL LIMITS	P
1530	WITHIN NORMAL LIMITS	P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP II 2,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
2516	WITHIN NORMAL LIMITS	P
2517	WITHIN NORMAL LIMITS	P
2518	WITHIN NORMAL LIMITS	P
2519	WITHIN NORMAL LIMITS	P
2520	WITHIN NORMAL LIMITS	P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP III 10,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
3516	WITHIN NORMAL LIMITS	P
3517	WITHIN NORMAL LIMITS	P
3518	WITHIN NORMAL LIMITS	P
3519	WITHIN NORMAL LIMITS	P
3520	WITHIN NORMAL LIMITS	P

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

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INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP IV 20,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 8
4526	WITHIN NORMAL LIMITS	P
4527	WITHIN NORMAL LIMITS	P
4528	WITHIN NORMAL LIMITS	P
4529	WITHIN NORMAL LIMITS	P
4530	WITHIN NORMAL LIMITS	P

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

TABLE H

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP I 0 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
1026		NO VISIBLE LESIONS
1027		NO VISIBLE LESIONS
1028		NO VISIBLE LESIONS
1029		NO VISIBLE LESIONS
1030		NO VISIBLE LESIONS

TABLE H

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP II 2,000 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
2016		NO VISIBLE LESIONS
2017		NO VISIBLE LESIONS
2018		NO VISIBLE LESIONS
2019		NO VISIBLE LESIONS
2020		NO VISIBLE LESIONS

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP III 10,000 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
3016		NO VISIBLE LESIONS
3017		NO VISIBLE LESIONS
3018		NO VISIBLE LESIONS
3019		NO VISIBLE LESIONS
3020		NO VISIBLE LESIONS

TABLE H

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP IV 20,000 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
4026		NO VISIBLE LESIONS
4027		NO VISIBLE LESIONS
4028		NO VISIBLE LESIONS
4029		NO VISIBLE LESIONS
4030		NO VISIBLE LESIONS

TABLE H

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INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP I 0 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
1526		NO VISIBLE LESIONS
1527		NO VISIBLE LESIONS
1528		NO VISIBLE LESIONS
1529		NO VISIBLE LESIONS
1530		NO VISIBLE LESIONS

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP II 2,000 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
2516		NO VISIBLE LESIONS
2517		NO VISIBLE LESIONS
2518		NO VISIBLE LESIONS
2519		NO VISIBLE LESIONS
2520		NO VISIBLE LESIONS

TABLE H

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP III 10,000 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
3516		NO VISIBLE LESIONS
3517		NO VISIBLE LESIONS
3518		NO VISIBLE LESIONS
3519		NO VISIBLE LESIONS
3520		NO VISIBLE LESIONS

TABLE H

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP IV 20,000 mg/m3 WEEK -1

ANIMAL#	PART OF EYE	OBSERVATION
4526		NO VISIBLE LESIONS
4527		NO VISIBLE LESIONS
4528		NO VISIBLE LESIONS
4529		NO VISIBLE LESIONS
4530		NO VISIBLE LESIONS

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP I 0 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
1026		NO VISIBLE LESIONS
1027		NO VISIBLE LESIONS
1028		NO VISIBLE LESIONS
1029		NO VISIBLE LESIONS
1030		NO VISIBLE LESIONS

TABLE H

GASOLINE ETHANOL VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP II 2,000 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
2016		NO VISIBLE LESIONS
2017		NO VISIBLE LESIONS
2018		NO VISIBLE LESIONS
2019		NO VISIBLE LESIONS
2020		NO VISIBLE LESIONS

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP III 10,000 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
3016		NO VISIBLE LESIONS
3017	CONJUNCTIVA	CONJUNCTIVITIS; LEFT
3018		NO VISIBLE LESIONS
3019		NO VISIBLE LESIONS
3020	CONJUNCTIVA	CONJUNCTIVITIS; BILATERAL

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP IV 20,000 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
4026		NO VISIBLE LESIONS
4027		NO VISIBLE LESIONS
4028		NO VISIBLE LESIONS
4029		NO VISIBLE LESIONS
4030		NO VISIBLE LESIONS

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP I 0 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
1526		NO VISIBLE LESIONS
1527		NO VISIBLE LESIONS
1528		NO VISIBLE LESIONS
1529		NO VISIBLE LESIONS
1530		NO VISIBLE LESIONS

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP II 2,000 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
2516	CONJUNCTIVA	CONJUNCTIVITIS; LEFT
2517	CONJUNCTIVA	CONJUNCTIVITIS; RIGHT
2518		NO VISIBLE LESIONS
2519		NO VISIBLE LESIONS
2520		NO VISIBLE LESIONS

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP III 10,000 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
3516		NO VISIBLE LESIONS
3517		NO VISIBLE LESIONS
3518		NO VISIBLE LESIONS
3519		NO VISIBLE LESIONS
3520		NO VISIBLE LESIONS

TABLE H

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP IV 20,000 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
4526		NO VISIBLE LESIONS
4527		NO VISIBLE LESIONS
4528		NO VISIBLE LESIONS
4529		NO VISIBLE LESIONS
4530		NO VISIBLE LESIONS

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP I 0 mg/m3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1026	232	300	351	387	419	456	481	494	513	535	549	573	589	599	617
1027	226	286	339	371	400	425	456	469	487	506	523	522	541	549	552
1028	229	308	367	406	449	468	502	511	525	554	577	597	610	615	641
1029	223	293	323	338	356	376	392	413	421	435	447	462	466	473	481
1030	227	300	336	360	386	404	411	419	434	450	460	477	479	486	495
MEAN	227	297	343	372	402	426	448	461	476	496	511	526	537	544	557
S.D.	3.4	8.4	16.5	25.9	34.9	37.6	46.2	44.0	46.5	52.1	56.1	58.5	64.2	64.2	71.4
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP II 2,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2016	237	320	362	406	430	457	488	502	513	530	546	571	590	595	600
2017	226	278	307	319	336	347	371	389	399	401	421	434	444	449	453
2018	218	270	302	319	324	333	346	364	380	387	393	402	414	420	429
2019	227	296	326	348	371	397	413	430	436	454	467	476	487	496	499
2020	230	298	352	389	416	442	466	492	506	518	531	537	542	559	565
MEAN	228	292	330	356	375	395	417	435	447	458	472	484	496	504	509
S.D.	6.8	19.6	26.7	39.8	47.0	55.3	60.4	61.1	61.0	65.1	66.9	70.1	71.6	73.2	72.7
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP III 10,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3016	226	293	317	344	361	382	399	415	426	440	456	473	490	494	502
3017	233	296	334	367	396	416	435	458	487	492	508	522	493	528	536
3018	227	292	327	360	381	408	435	462	480	483	495	511	524	529	538
3019	229	301	337	352	368	383	404	424	449	453	469	493	497	520	520
3020	224	297	336	369	397	413	430	428	363	422	452	474	465	458	487
MEAN	228	296	330	358	381	400	421	437	441	458	476	494	494	506	516
S.D.	3.3	3.5	8.7	10.4	16.3	16.7	17.8	21.0	50.1	29.6	24.7	21.9	21.0	30.0	22.0
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4026	228	300	328	351	387	413	438	455	480	496	510	528	539	556	574
4027	224	298	326	352	378	395	419	427	446	462	468	478	501	509	526
4028	227	289	317	349	356	383	402	417	429	440	454	472	484	488	485
4029	225	300	325	343	380	403	436	463	486	493	514	530	536	552	562
4030	231	283	316	341	371	392	418	436	449	461	473	492	499	510	511
MEAN	227	294	322	347	374	397	423	439	458	470	484	500	512	523	531
S.D.	2.8	7.7	5.4	4.9	11.8	11.2	14.8	19.4	24.5	23.8	26.8	27.4	24.6	29.9	36.4
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP I 0 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1526	186	230	256	262	269	300	313	315	327	330	341	349	348	358	362
1527	174	208	232	247	257	266	270	275	290	284	286	290	293	298	297
1528	181	214	233	254	262	276	292	294	294	302	309	313	308	316	323
1529	180	209	215	230	251	256	254	269	280	281	275	291	298	298	289
1530	183	226	246	251	280	283	300	303	326	339	335	347	344	336	348
MEAN	181	217	236	249	264	276	286	291	303	307	309	318	318	321	324
S.D.	4.3	9.9	15.6	11.9	11.2	16.6	23.6	19.0	21.4	26.2	29.1	28.8	26.0	25.9	31.8
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP II 2,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2516	189	218	242	255	270	271	293	301	307	306	276	315	329	334	339
2517	181	213	231	242	255	264	280	279	288	294	298	299	302	310	306
2518	176	204	210	218	220	230	241	241	238	243	248	256	257	266	270
2519	176	209	223	230	238	248	257	265	269	277	286	292	294	303	304
2520	184	216	233	246	249	262	274	271	274	283	287	290	285	293	300
MEAN	181	212	228	238	246	255	269	271	275	281	279	290	294	301	304
S.D.	5.4	5.6	11.8	14.4	18.9	16.0	20.3	21.8	25.4	23.5	19.0	21.6	26.0	24.6	24.4
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP III 10,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3516	176	210	227	239	254	267	278	281	292	296	294	300	307	311	304
3517	185	223	235	246	252	268	272	280	282	293	296	299	301	307	305
3518	173	205	219	231	236	254	259	263	271	279	281	285	295	299	298
3519	190	229	243	265	288	289	286	304	312	307	300	318	322	326	316
3520	181	214	230	247	264	267	276	286	302	297	296	311	312	314	311
MEAN	181	216	231	245	259	269	274	283	292	294	293	302	307	311	307
S.D.	6.8	9.9	8.8	12.8	19.5	12.5	9.7	14.8	16.1	10.0	7.3	12.8	10.5	9.8	6.7
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

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TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4526	181	211	241	249	264	261	275	284	290	291	298	302	293	309	312
4527	180	210	222	226	241	257	261	262	265	271	279	280	278	286	293
4528	184	206	225	238	250	252	262	271	277	278	281	290	291	297	293
4529	173	201	215	227	228	243	251	250	251	262	269	268	264	273	275
4530	188	215	235	235	259	267	271	270	280	282	281	277	288	296	298
MEAN	181	209	227	235	248	256	264	267	273	277	281	283	283	292	294
S.D.	5.7	5.2	10.2	9.4	14.6	8.9	9.6	12.5	15.1	11.2	10.4	12.8	12.1	13.5	13.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP I 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
1026	50	87	118	156	180	194	213	235	249	272	289	299	317
1027	53	85	114	139	170	183	201	220	237	236	255	263	266
1028	59	98	141	160	194	203	217	246	269	289	302	307	333
1029	31	45	63	84	100	120	129	142	154	170	173	181	188
1030	36	60	86	104	111	119	134	150	160	177	179	186	195
MEAN	46	75	105	128	151	164	179	199	214	229	240	247	260
S.D.	12.0	21.7	30.1	33.5	42.8	40.9	43.6	48.9	52.9	54.2	60.5	60.5	67.1
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP II 2,000 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
2016	42	86	110	137	168	182	193	210	226	251	270	275	280
2017	28	41	58	69	92	111	121	123	142	156	166	171	175
2018	33	50	55	63	77	94	110	118	124	133	145	151	159
2019	30	52	75	101	117	134	140	158	171	180	191	200	203
2020	55	91	119	145	169	194	209	220	234	239	245	261	267
MEAN	37	64	83	103	125	143	155	166	179	192	203	212	217
S.D.	10.8	22.9	29.5	37.5	42.3	43.7	44.0	47.7	49.2	51.8	52.8	54.8	54.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP III 10,000 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
3016	24	51	68	89	106	123	133	147	163	180	197	201	210
3017	39	71	100	120	140	162	191	197	213	227	197	232	240
3018	35	68	89	116	144	170	189	191	203	219	233	237	247
3019	37	52	67	82	104	124	148	152	168	192	197	219	219
3020	40	72	101	116	133	131	66	125	155	177	169	162	190
MEAN	35	63	85	105	125	142	145	162	181	199	198	210	221
S.D.	6.4	10.5	16.5	17.7	19.0	22.5	51.0	30.7	25.7	22.5	22.7	30.5	22.9
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP IV 20,000 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
4026	28	51	87	113	138	155	180	196	210	228	239	256	274
4027	28	54	80	97	121	129	148	164	170	180	203	211	228
4028	28	60	67	94	113	127	139	151	165	183	195	199	196
4029	25	43	80	103	136	163	186	193	214	230	236	252	262
4030	33	58	88	109	135	153	166	178	190	209	216	227	228
MEAN	28	53	80	103	129	145	164	176	190	206	218	229	237
S.D.	3.1	6.4	8.6	8.0	11.2	16.3	20.2	19.2	22.4	23.8	19.7	25.1	30.8
N	5	5	5	5	5	5	5	5	5	5	5	5	5

Huntingdon Life Sciences 00-6127F
GFAP Sub-Group

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP I 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
1526	27	32	39	70	84	85	97	100	112	119	118	129	133
1527	24	39	49	59	62	67	82	77	78	82	85	90	89
1528	19	41	48	63	79	80	81	88	96	100	95	103	109
1529	6	20	42	47	45	60	71	71	66	82	89	89	79
1530	20	25	54	57	74	76	99	113	109	121	118	110	122
MEAN	19	32	46	59	69	74	86	90	92	101	101	104	106
S.D.	8.1	8.8	5.9	8.6	15.4	10.3	12.0	16.9	19.8	19.1	16.1	16.4	22.2
N	5	5	5	5	5	5	5	5	5	5	5	5	5

Huntingdon Life Sciences 00-6127F
GFAP Sub-Group

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP II 2,000 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
2516	24	38	52	54	76	83	89	88	59	98	111	116	121
2517	17	29	42	51	67	66	75	81	84	86	89	97	93
2518	6	14	16	26	37	37	34	39	44	52	53	62	66
2519	14	21	29	39	48	56	59	68	77	83	85	94	94
2520	17	29	33	45	57	55	58	66	71	74	69	77	83
MEAN	16	26	34	43	57	59	63	68	67	78	82	89	91
S.D.	6.5	9.0	13.9	10.8	15.2	17.0	20.6	18.7	16.0	17.0	21.9	20.5	20.0
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP III 10,000 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
3516	18	29	44	57	69	71	83	87	85	90	97	102	94
3517	12	22	29	45	49	57	59	69	73	76	78	84	82
3518	15	26	31	49	55	58	67	75	76	80	90	94	94
3519	14	36	59	60	57	75	83	78	71	89	93	97	87
3520	15	33	50	53	62	72	87	82	81	97	98	100	97
MEAN	15	29	43	53	58	66	76	78	77	86	91	95	91
S.D.	2.0	5.5	12.9	5.9	7.3	8.5	12.4	6.7	5.7	8.3	8.1	7.0	6.1
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP IV 20,000 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
4526	29	38	53	50	64	73	79	80	87	90	82	98	100
4527	12	16	31	47	51	52	55	61	69	70	68	76	83
4528	19	32	44	47	57	66	72	72	75	84	86	91	88
4529	14	26	26	42	49	48	50	60	67	67	62	72	73
4530	20	21	45	52	57	55	66	67	66	62	73	82	84
MEAN	19	26	40	47	55	59	64	68	73	75	74	84	86
S.D.	6.8	8.8	10.9	3.8	5.8	10.0	11.9	8.3	8.4	12.0	9.5	10.8	9.8
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP I 0 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1026	94	82	77	72	66	61	56	55	54	51	52	50	49	46
1027	87	83	74	68	64	61	56	56	54	51	50	51	47	46
1028	93	87	77	72	62	61	54	51	52	52	52	49	48	46
1029	94	86	69	66	62	59	58	55	55	52	53	50	49	49
1030	95	82	74	69	58	55	51	54	50	49	50	47	45	45
MEAN	93	84	74	69	63	59	55	54	53	51	51	49	48	47
S.D.	3.3	2.5	3.1	2.8	2.9	2.6	2.8	2.0	1.8	1.6	1.4	1.3	1.4	1.5
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP II 2,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2016	98	84	77	68	64	62	56	53	52	52	54	51	48	47
2017	84	73	65	65	59	59	59	55	49	51	49	49	43	44
2018	89	79	70	62	61	60	62	60	50	51	52	53	50	49
2019	94	82	74	71	66	63	59	57	55	53	54	54	53	52
2020	93	87	76	69	62	61	59	56	52	51	52	50	52	49
MEAN	91	81	72	67	62	61	59	56	51	52	52	51	49	48
S.D.	5.6	5.3	5.1	3.6	2.5	1.8	2.1	2.6	2.2	0.9	1.9	2.1	4.1	3.0
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP III 10,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3016	90	80	70	65	60	59	55	56	56	54	52	50	49	48
3017	83	78	66	65	61	59	61	59	55	50	46	48	46	50
3018	90	83	75	68	62	59	59	55	52	50	49	50	49	48
3019	93	84	69	68	60	58	60	62	56	56	53	51	50	48
3020	89	82	72	65	59	56	55	19	62	59	58	43	45	58
MEAN	89	81	70	66	61	58	58	50	56	54	52	48	48	50
S.D.	3.6	2.6	3.5	1.5	1.0	1.3	2.8	17.8	3.6	4.2	4.2	3.3	2.1	4.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4026	92	76	68	71	63	62	59	60	55	51	53	50	48	49
4027	92	78	69	68	61	60	56	57	53	49	51	51	IW	48
4028	93	82	73	63	60	60	57	51	56	53	52	51	49	47
4029	94	83	69	79	SF	63	61	58	54	53	55	55	51	50
4030	82	76	70	65	61	58	57	52	50	49	52	48	47	46
MEAN	91	79	70	69	61	60	58	56	53	51	53	51	49	48
S.D.	4.7	3.4	1.7	6.2	1.6	2.1	1.9	3.7	2.3	1.8	1.3	2.4	1.4	1.6
N	5	5	5	5	4	5	5	5	5	5	5	5	4	5

IW=Invalid Weight: Feed left greater than feed fed weight SF=Spilled Feeder

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP I 0 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1526	92	86	77	92	72	66	63	67	59	56	56	59	59	54
1527	85	88	81	60	71	68	67	68	58	60	64	62	60	58
1528	97	99	92	87	83	76	71	73	69	64	66	70	72	65
1529	88	83	81	75	67	68	69	64	58	60	65	58	56	55
1530	96	84	83	84	64	69	70	69	69	65	56	58	62	60
MEAN	92	88	83	80	71	69	68	68	63	61	61	61	62	58
S.D.	5.0	6.3	5.8	12.5	7.0	3.6	3.0	3.2	6.0	3.6	5.3	5.0	6.0	4.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP II 2,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2516	99	SF	82	78	75	78	66	66	64	49	SF	67	SF	58
2517	99	93	88	86	78	77	71	71	59	63	66	64	63	59
2518	94	85	83	83	81	76	70	68	66	64	69	68	69	61
2519	88	81	78	80	68	66	65	66	60	58	58	60	59	53
2520	86	79	74	75	71	66	62	65	62	56	59	58	59	55
MEAN	93	85	81	80	75	73	67	67	62	58	63	63	63	57
S.D.	6.1	6.0	5.3	4.3	5.2	6.1	3.5	2.4	2.9	6.3	5.4	4.5	4.7	3.3
N	5	4	5	5	5	5	5	5	5	5	4	5	4	5

SF=Spilled Feeder

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP III 10,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3516	84	77	76	71	64	66	61	59	54	53	54	52	52	50
3517	92	79	75	76	71	70	65	63	59	56	59	60	56	52
3518	88	82	76	77	69	70	75	64	58	61	62	62	52	56
3519	87	83	81	77	64	72	67	62	55	56	63	58	61	60
3520	88	83	82	77	68	75	67	64	60	60	65	57	58	59
MEAN	88	81	78	76	67	71	67	62	57	57	60	58	56	55
S.D.	2.7	2.6	3.1	2.5	2.9	3.3	5.1	2.2	2.5	3.1	4.3	3.8	3.7	4.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4526	84	83	75	73	65	66	62	61	60	53	57	56	57	52
4527	88	81	79	79	70	66	63	65	SF	58	58	61	62	57
4528	88	89	SF	SF	73	72	71	68	66	62	65	64	64	61
4529	86	80	78	73	64	62	59	65	65	58	57	58	61	55
4530	85	78	75	79	61	62	65	63	55	53	58	64	62	58
MEAN	86	82	77	76	66	65	64	64	61	57	59	61	61	57
S.D.	1.8	4.4	1.9	3.4	4.7	4.0	4.6	2.7	5.0	4.1	3.7	3.3	2.5	3.1
N	5	5	4	4	5	5	5	5	4	5	5	5	5	5

SF=Spilled Feeder

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP I 0 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1026	TERMINAL SACRIFICE	18-JUL-01	13	92
1027	TERMINAL SACRIFICE	18-JUL-01	13	92
1028	TERMINAL SACRIFICE	18-JUL-01	13	92
1029	TERMINAL SACRIFICE	18-JUL-01	13	92
1030	TERMINAL SACRIFICE	18-JUL-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP II 2,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2016	TERMINAL SACRIFICE	18-JUL-01	13	92
2017	TERMINAL SACRIFICE	18-JUL-01	13	92
2018	TERMINAL SACRIFICE	18-JUL-01	13	92
2019	TERMINAL SACRIFICE	18-JUL-01	13	92
2020	TERMINAL SACRIFICE	18-JUL-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP III 10,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3016	TERMINAL SACRIFICE	18-JUL-01	13	92
3017	TERMINAL SACRIFICE	18-JUL-01	13	92
3018	TERMINAL SACRIFICE	18-JUL-01	13	92
3019	TERMINAL SACRIFICE	18-JUL-01	13	92
3020	TERMINAL SACRIFICE	18-JUL-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP IV 20,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4026	TERMINAL SACRIFICE	18-JUL-01	13	92
4027	TERMINAL SACRIFICE	18-JUL-01	13	92
4028	TERMINAL SACRIFICE	18-JUL-01	13	92
4029	TERMINAL SACRIFICE	18-JUL-01	13	92
4030	TERMINAL SACRIFICE	18-JUL-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP I 0 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1526	TERMINAL SACRIFICE	18-JUL-01	13	92
1527	TERMINAL SACRIFICE	18-JUL-01	13	92
1528	TERMINAL SACRIFICE	18-JUL-01	13	92
1529	TERMINAL SACRIFICE	18-JUL-01	13	92
1530	TERMINAL SACRIFICE	18-JUL-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP II 2,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2516	TERMINAL SACRIFICE	18-JUL-01	13	92
2517	TERMINAL SACRIFICE	18-JUL-01	13	92
2518	TERMINAL SACRIFICE	18-JUL-01	13	92
2519	TERMINAL SACRIFICE	18-JUL-01	13	92
2520	TERMINAL SACRIFICE	18-JUL-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP III 10,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3516	TERMINAL SACRIFICE	18-JUL-01	13	92
3517	TERMINAL SACRIFICE	18-JUL-01	13	92
3518	TERMINAL SACRIFICE	18-JUL-01	13	92
3519	TERMINAL SACRIFICE	18-JUL-01	13	92
3520	TERMINAL SACRIFICE	18-JUL-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP IV 20,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4526	TERMINAL SACRIFICE	18-JUL-01	13	92
4527	TERMINAL SACRIFICE	18-JUL-01	13	92
4528	TERMINAL SACRIFICE	18-JUL-01	13	92
4529	TERMINAL SACRIFICE	18-JUL-01	13	92
4530	TERMINAL SACRIFICE	18-JUL-01	13	92