

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

Huntingdon Life Sciences, Inc. Study No. 00-6129

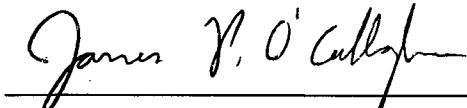
Sponsor Study No 211-ETBE-S

Measurement of Glial Fibrillary Acidic Protein

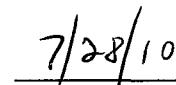
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STATEMENT OF COMPLIANCE

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



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



Date



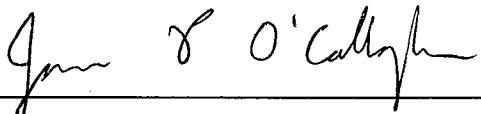
Gary M. Hoffman, B.A., D.A.B.T.
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Date

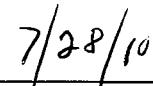
SIGNATURE PAGE

SCIENTIST

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



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



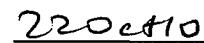
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SCIENTIFIC REVIEW

The following Scientist has reviewed and approved this report.



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



Date

Third-Party QA Statement

Study No. HLS 00-6129

GFAP Levels in Specific Rat Brain Areas Following a 13-Week Whole-Body Inhalation Exposure to Baseline ETBE Vapor Condensate Study

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

<u>Area Inspected</u>	<u>Date of Inspection</u>	<u>Date Reported to Sponsor</u>
Facility Inspection	4/3/01	4/10/01
Terminal Sacrifice Inspection	1/23/02	2/22/02
Draft Final Report Audit	8/11/02-1/13/03	1/13/03
Draft Final Report Review(2cd)	6/25/09	6/25/09

Christine Sexsmith 8/2/10

Christine Sexsmith Date

Sexsmith Consulting Services, LLC

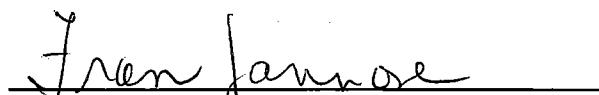
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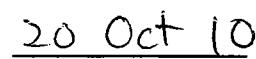
QUALITY ASSURANCE STATEMENT

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	24, 29 Aug 01	29 Aug 01
Exposure, Monitoring and Equipment Records	23 Oct 01	23 Oct 01
GC Characterization	09 Nov 01	09 Nov 01
GFAP Necropsy	23 Jan 02	23 Jan 02
GFAP Report	12, 13 Jun 02	18 Jan 02



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



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 ETBE Vapor Condensate caused small gender- and region-dependent increases and *decreases* in levels of GFAP. The fact that both increases and decreases were seen in only a few brain areas of both genders at the low concentration of the ETBE Vapor Condensate and because, in general, significant effects were seen in brain areas that are difficult to reliably dissect, suggests that the observed changes were not biologically relevant. The data suggest that exposure to Gasoline ETBE Vapor Condensate under the regimen employed does not result in gliosis in any of the nine brain regions examined.

Introduction

A characteristic feature of chemical-induced damage of the nervous system is selectivity; exposure to different nervous system toxicants results in damage to different brain regions and cell types (Switzer, 1991; Balaban 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, 1988; Norton et al., 1992). Moreover, the temporal and regional increments in GFAP correspond to the temporal and regional patterns of neuronal damage, as revealed by sensitive silver stains (Balaban 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 ETBE vapor condensates only. The purpose of the present study was to use the GFAP assay for assessing the potential neurotoxic effects of Gasoline ETBE Vapor Condensate. A control (air) and three exposure levels to the test condensate (2,000, 10,000 and 20,000 mg/m³) 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

28 September 2001 (Date Study Director signed the Protocol)

DATE OF ANIMAL RECEIPT

4 October 2001

EXPOSURE INITIATION

23 October 2001 (Experimental Start Date)

EXPOSURE TERMINATION

22 January 2002

TERMINAL SACRIFICE

23 January 2002

EXPERIMENTAL TERMINATION

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

STUDY COMPLETION

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

I. Basic Protocol 1: GFAP Sandwich ELISA

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

B. **Materials:**

Vortex Mixer or Ultrasonic Cell Disruptor (e.g. PGC cat. # 81-6721-02, 2 mm probe)

Pipettes

Hot/Stir Plate

Microplate Reader

96-well Microtiter Plates (Immulon 2, Dynatech)

Pipette tips

Rabbit anti-Glial Fibrillary Acidic Protein Antibody (DAKO, Cat. #Z0334)

Non-Fat Dry Milk (Carnation)

Mouse anti-Glial Fibrillary Acidic Protein Antibody (Chemicon Cat. # MAB 3402; formerly Boehringer 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 serve 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 KH₂PO₄/8.0 mM Na₂HPO₄·7H₂O/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(BLOTO)**- (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 subtype 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 immunohistochemistry 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 ¹²⁵I 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 ($\mu\text{g}/\text{mg}$ 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 at $P<0.05$ and, to ensure detection of between group treatment effects, The Least Significant-Difference test (Keppel, 1973) was used in *post-hoc* analyses.

Results and Conclusions

The results of the GFAP analysis are presented in Tables 5 and 6. Results are reported according to gender, because sex-dependent responses to toxic substances are not uncommon, including responses reflected in levels of GFAP. Control levels for GFAP varied markedly according to brain region, consistent with known historical levels observed for GFAP across different brain regions (see Fig. 2). The 13-week exposure to Gasoline ETBE Vapor Condensate was associated with slight decreases in GFAP in three of the male groups (Group II and Group IV, cortex and Group II, rest of brain) and slight increases in GFAP in two of the female groups (Group II, striatum and Group II, rest of brain). While statistically significant, the differences seen in males cannot be interpreted as adverse because the neurotoxicological significance of a decrease in GFAP is unknown. Moreover, the decreases were small in magnitude (except rest of brain), not concentration-related and they occurred in brain regions that are difficult to reliably dissect. Likewise, the slight increases observed in the females only occurred at the low concentration of ETBE Vapor Condensate, they were small in magnitude in the case of striatum or they occurred in a brain region (rest of brain) that is difficult to reliably dissect. Thus, it is likely that the

statistically significant differences observed for both males and females reflect normal variations in dissection technique. These data suggest that under the exposure conditions employed, damage-induced gliosis did not occur in the brain regions examined. Toxicant-induced gliosis is highly dose-, region-, and time-dependent. It is possible, therefore, that exposure to higher concentrations, examination of more brain regions, examination of more discrete dissections of a given brain region, or the inclusion of more time points, might have resulted in more positive and biologically significant findings. In conclusion, exposure to Gasoline ETBE Vapor Condensate did not appear to result in gliosis in nine representative brain regions in males or females.

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Figure Legends

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

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

Figure 1

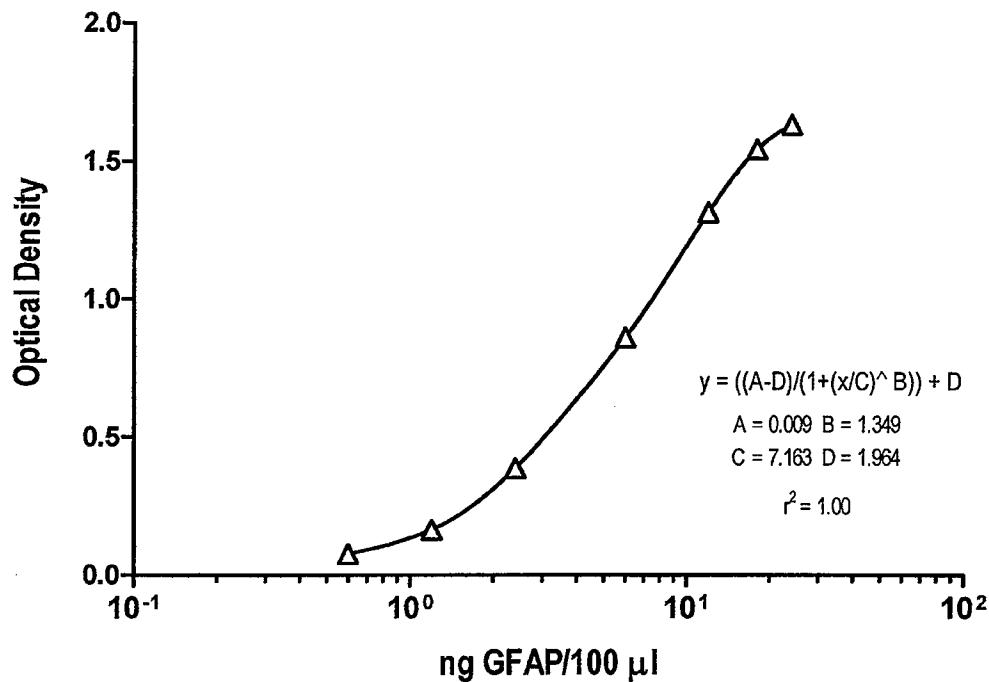


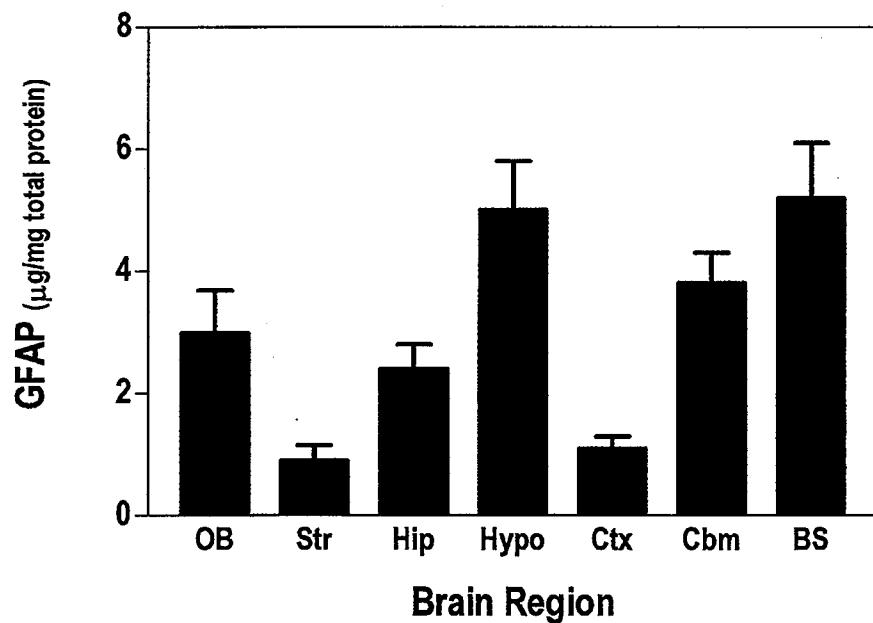
Figure 2

TABLE 1: GFAP Standard Curve Preparation

Tube #	µg of total protein/100 µl/well (ng of GFAP)	µg of GFAP/Campus Std (10.34 ng/ml)	Serum 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

WELL	OD	Mean	Std Dev	CV
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	10 µg (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	5.0 µg (12.50ng)	D1	1.295	1.314	0.027	2.1
		D2	1.334			
STD04	2.5 µg (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 than 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 ETBE Vapor Condensate

Brain Area	Group I Air Control 0 mg/m ³	Group II Test Substance 2,000 mg/m ³	Group III Test Substance 10,000 mg/m ³	Group IV Test Substance 20,000 mg/m ³
Striatum	1.04 ± 0.11*	0.82 ± 0.07	1.01 ± 0.05	0.93 ± 0.07
Hippocampus	2.43 ± 0.15	2.32 ± 0.08	2.26 ± 0.23	2.45 ± 0.09
Cortex	2.15 ± 0.12	1.75 ± 0.11+	1.88 ± 0.11	1.72 ± 0.07+
Olfactory Bulb	2.21 ± 0.15	2.04 ± 0.15	2.22 ± 0.13	2.26 ± 0.16
Thalamus	2.76 ± 0.29	2.18 ± 0.13	2.67 ± 0.12	2.40 ± 0.17
Hypothalamus	7.12 ± 0.95	8.17 ± 1.45	9.49 ± 1.82	6.54 ± 0.93
Cerebellum	4.78 ± 0.32	4.11 ± 0.30	4.28 ± 0.27	4.11 ± 0.36
Rest of Brain	6.46 ± 0.84	4.91 ± 0.28+	5.61 ± 0.32	5.36 ± 0.37

*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 ETBE 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	0.88 ± 0.06*	1.12 ± 0.07+	0.99 ± 0.09	1.05 ± 0.12
Hippocampus	2.33 ± 0.16	2.69 ± 0.33	2.36 ± 0.20	2.24 ± 0.10
Cortex	1.77 ± 0.09	1.98 ± 0.13	1.75 ± 0.17	1.76 ± 0.06
Olfactory Bulb	2.04 ± 0.17	2.43 ± 0.21	2.30 ± 0.30	2.33 ± 0.08
Thalamus	1.95 ± 0.15	2.41 ± 0.22	2.37 ± 0.30	2.24 ± 0.17
Hypothalamus	4.89 ± 1.06	7.21 ± 0.69	8.50 ± 1.54	7.50 ± 1.58
Cerebellum	4.02 ± 0.37	4.44 ± 0.18	4.41 ± 0.51	4.25 ± 0.32
Rest of Brain	4.61 ± 0.23	6.17 ± 0.23+	5.18 ± 0.58	5.73 ± 0.43

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

Group	Animal no.	Sex	Striatum	Hippocampus	Cortex	Olfactory Bulb	Thalamus	Hypothalamus	Cerebellum	Pituitary	Rest of Brain
Group I Air Control 0 mg/m ³	1078	Male	0.817*	2.669	1.872	1.968	2.715	8.191	4.695	*	6.077
	1080		1.437	2.769	2.542	2.681	3.666	7.636	5.724	*	9.676
	1077		1.121	2.261	1.966	1.818	2.272	9.690	4.794	*	5.318
	1079		0.988	2.489	2.210	2.352	3.064	5.893	4.978	*	6.214
	1076		0.837	1.943	2.143	2.218	2.064	4.205	3.711	*	5.014
	1578	Female	0.862	2.354	1.963	1.766	2.230	4.121	3.676	*	5.097
	1580		0.818	2.091	1.699	1.924	1.847	8.261	4.197	*	5.222
	1576		0.714	1.982	1.553	1.682	1.418	5.302	3.600	*	4.144
	1579		1.101	2.330	1.665	2.606	2.145	5.093	3.264	*	4.333
	1577		0.890	2.910	1.979	2.239	2.116	1.676	5.382	*	4.243
Group II Test Substance 2,000 mg/m ³	2069	Male	0.987	2.131	1.873	1.634	2.569	6.248	3.900	*	4.941
	2066		0.588	2.227	1.535	1.838	2.184	7.840	3.722	*	5.164
	2067		0.771	2.256	2.103	2.482	2.293	5.189	3.342	*	5.737
	2068		0.840	2.434	1.517	2.007	2.046	13.583	4.675	*	4.724
	2070		0.895	2.562	1.710	2.240	1.802	7.994	4.922	*	4.000
	2567	Female	1.068	2.394	1.687	2.140	1.630	6.668	4.570	*	5.626
	2569		1.134	1.975	1.891	2.381	2.409	7.418	4.316	*	6.490
	2570		1.319	3.930	2.460	3.203	2.918	6.088	4.548	*	6.512
	2566		0.878	2.634	1.968	2.403	2.765	9.805	4.925	*	6.638
	2568		1.216	2.524	1.871	2.030	2.337	6.090	3.862	*	5.579
Group III Test Substance 10,000 mg/m ³	3070	Male	0.900	2.477	1.598	2.453	2.438	5.118	4.522	*	6.634
	3068		0.924	1.817	1.760	1.940	2.456	12.035	3.889	*	5.010
	3066		1.170	2.262	1.783	1.887	2.567	6.200	4.843	*	5.261
	3069		0.971	2.981	2.036	2.343	2.830	14.956	4.741	*	5.036
	3067		1.083	1.763	2.225	2.469	3.057	9.159	3.420	*	6.093
	3567	Female	1.025	2.348	2.127	3.194	2.574	9.168	4.973	*	7.175
	3566		0.711	1.815	1.490	1.422	1.621	5.611	3.802	*	3.895
	3569		1.132	2.942	2.141	2.455	2.690	14.087	6.148	*	5.607
	3570		0.861	2.056	1.668	1.931	1.742	5.970	3.313	*	4.232
	3568		1.212	2.660	1.300	2.475	3.223	7.652	3.827	*	4.991
Group IV Test Substance 20,000 mg/m ³	4080	Male	0.870	2.482	1.626	2.762	2.097	9.045	4.715	*	5.349
	4077		1.137	2.285	1.607	1.952	1.980	6.833	3.244	*	4.438
	4079		1.020	2.645	1.957	2.497	2.917	7.969	5.162	0.073	6.488
	4078		0.914	2.207	1.776	2.019	2.557	4.561	3.891	*	5.817
	4076		0.709	2.638	1.643	2.081	2.444	4.309	3.549	*	4.682
	4576	Female	1.492	2.376	1.761	2.127	2.802	8.446	4.117	*	5.871
	4579		0.995	2.482	1.791	2.444	2.400	7.992	5.461	*	6.997
	4578		0.896	1.909	1.889	2.561	2.119	12.660	3.959	*	5.876
	4580		0.797	2.168	1.551	2.296	1.881	4.393	4.137	0.066	4.284
	4577		1.092	2.265	1.807	2.215	1.991	4.008	3.569	*	5.615

* 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
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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:	4 October 2001
	Experimental Initiation Date:	23 October 2001 (in-life)
	Experimental Completion Date:	23 January 2002 (in-life)
	Draft Report Date:	19 June 2002

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 ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY IN RATS

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IA - 0 (air only) mg/m ³							Particle Size Determinations			Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration				MMAD (μm)	GSD	TMC (mg/m ³)			Mean Temperature (°C)	Humidity (%)
				Mean (mg/m ³)	Individual (mg/m ³)									
0	23-Oct-01	1	0	0	0	0	0						25	53
1	24-Oct-01	2	0	0	0	0	0						25	50
2	25-Oct-01	3	0	0	0	0	0						25	50
3	26-Oct-01	4	0	0	0	0	0						25	54
6	29-Oct-01	5	0	0	0	0	0						25	50
7	30-Oct-01	6	0	0	0	0	0	1.482	1.752	1.72E-03			24	54
8	31-Oct-01	7	0	0	0	0	0						24	53
9	1-Nov-01	8	0	0	0	0	0						24	52
10	2-Nov-01	9	0	0	0	0	0						24	55
13	5-Nov-01	10	0	0	0	0	0						24	52
14	6-Nov-01	11	0	0	0	0	0						25	53
15	7-Nov-01	12	0	0	0	0	0						25	53
16	8-Nov-01	13	0	0	0	0	0						25	52
17	9-Nov-01	14	0	0	0	0	0						25	52
20	12-Nov-01	15	0	0	0	0	0						25	52
21	13-Nov-01	16	0	0	0	0	0						24	53
22	14-Nov-01	17	0	0	0	0	0						24	52
23	15-Nov-01	18	0	0	0	0	0						24	52
24	16-Nov-01	19	0	0	0	0	0						25	49
27	19-Nov-01	20	0	0	0	0	0						24	49
28	20-Nov-01	21	0	0	0	0	0						24	50
29	21-Nov-01	22	0	0	0	0	0						22	56
30	22-Nov-01	23	0	0	0	0	0						25	53
31	23-Nov-01	24	0	0	0	0	0						25	52
32	24-Nov-01	25	0	0	0	0	0						25	53

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IA - 0 (air only) mg/m ³							Chamber Environment			
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean		
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (µm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
34	26-Nov-01	26	0	0	0	0	0	0			25	48	
35	27-Nov-01	27	0	0	0	0	0	0			24	49	
36	28-Nov-01	28	0	0	0	0	0	0			24	50	
37	29-Nov-01	29	0	0	0	0	0	0	1.046	1.964	1.94E-03	24	50
38	30-Nov-01	30	0	0	0	0	0	0			24	56	
41	3-Dec-01	31	0	0	0	0	0	0			24	50	
42	4-Dec-01	32	0	0	0	0	0	0			24	51	
43	5-Dec-01	33	0	0	0	0	0	0			25	51	
44	6-Dec-01	34	0	0	0	0	0	0	0.9233	1.647	2.32E-03	25	52
45	7-Dec-01	35	0	0	0	0	0	0			25	50	
48	10-Dec-01	36	0	0	0	0	0	0			25	50	
49	11-Dec-01	37	0	0	0	0	0	0			23	54	
50	12-Dec-01	38	0	0	0	0	0	0			24	52	
51	13-Dec-01	39	0	0	0	0	0	0	0.7808	1.691	2.30E-03	24	50
52	14-Dec-01	40	0	0	0	0	0	0			24	51	
55	17-Dec-01	41	0	0	0	0	0	0			24	51	
56	18-Dec-01	42	0	0	0	0	0	0			25	53	
57	19-Dec-01	43	0	0	0	0	0	0			25	52	
58	20-Dec-01	44	0	0	0	0	0	0	2.499	3.107	2.29E-03	25	52
59	21-Dec-01	45	0	0	0	0	0	0			25	54	
62	24-Dec-01	46	0	0	0	0	0	0			25	52	
64	26-Dec-01	47	0	0	0	0	0	0			24	53	
65	27-Dec-01	48	0	0	0	0	0	0	0.9215	1.998	1.33E-03	24	54
66	28-Dec-01	49	0	0	0	0	0	0			24	56	
67	29-Dec-01	50	0	0	0	0	0	0			24	55	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IA - 0 (air only) mg/m ³								Particle Size Determinations			Chamber Environment			
			Nominal (mg/m ³)	Analytical Chamber Concentration				MMAD (μm)	GSD	TMC (mg/m ³)	Mean						
				Mean (mg/m ³)	Individual (mg/m ³)	Individua (mg/m ³)	Individua (mg/m ³)				Temperature (°C)	Humidity (%)					
69	31-Dec-01	51	0	0	0	0	0	0			24	54					
70	1-Jan-02	52	0	0	0	0	0	0			25	51					
71	2-Jan-02	53	0	0	0	0	0	0			25	52					
72	3-Jan-02	54	0	0	0	0	0	0			25	50					
73	4-Jan-02	55	0	0	0	0	0	0			25	53					
75	6-Jan-02	56	0	0	0	0	0	0			25	49					
76	7-Jan-02	57	0	0	0	0	0	0			25	48					
77	8-Jan-02	58	0	0	0	0	0	0			24	55					
78	9-Jan-02	59	0	0	0	0	0	0			24	53					
79	10-Jan-02	60	0	0	0	0	0	0			24	49					
83	14-Jan-02	61	0	0	0	0	0	0			24	52					
84	15-Jan-02	62	0	0	0	0	0	0			24	52					
85	16-Jan-02	63	0	0	0	0	0	0			25	49					
86	17-Jan-02	64	0	0	0	0	0	0			24	50					
87	18-Jan-02	65	0	0	0	0	0	0			24	55					
89	20-Jan-02	66	0	0	0	0	0	0			25	52					
90	21-Jan-02	67	0	0	0	0	0	0			24	51					
91	22-Jan-02	68	0	0	0	0	0	0			22	55					
			Mean	0		0			1.228	2.037	2.83E-03	24.4	51.9				
			S.D.	0		0			0.526	0.413	1.67E-03	0.7	2.0				

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IB - 0 (air only) mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
0	23-Oct-01	1	0	0	0	0	0	0			24	56	
1	24-Oct-01	2	0	0	0	0	0	0			24	53	
2	25-Oct-01	3	0	0	0	0	0	0			23	53	
3	26-Oct-01	4	0	0	0	0	0	0			24	57	
6	29-Oct-01	5	0	0	0	0	0	0	5.659	2.488	2.88E-03	24	52
7	30-Oct-01	6	0	0	0	0	0	0			24	53	
8	31-Oct-01	7	0	0	0	0	0	0	0.8546	2.214	5.97E-03	24	54
9	1-Nov-01	8	0	0	0	0	0	0			24	53	
10	2-Nov-01	9	0	0	0	0	0	0			24	55	
13	5-Nov-01	10	0	0	0	0	0	0			24	52	
14	6-Nov-01	11	0	0	0	0	0	0			24	54	
15	7-Nov-01	12	0	0	0	0	0	0			24	56	
16	8-Nov-01	13	0	0	0	0	0	0	1.738	2.383	5.12E-03	24	54
17	9-Nov-01	14	0	0	0	0	0	0			24	53	
20	12-Nov-01	15	0	0	0	0	0	0			24	54	
21	13-Nov-01	16	0	0	0	0	0	0			24	55	
22	14-Nov-01	17	0	0	0	0	0	0			24	53	
23	15-Nov-01	18	0	0	0	0	0	0	0.9337	2.189	6.73E-03	24	54
24	16-Nov-01	19	0	0	0	0	0	0			24	51	
27	19-Nov-01	20	0	0	0	0	0	0			25	50	
28	20-Nov-01	21	0	0	0	0	0	0			23	55	
30	22-Nov-01	22	0	0	0	0	0	0	1.184	2.112	1.56E-03	23	53
31	23-Nov-01	23	0	0	0	0	0	0			24	54	
32	24-Nov-01	24	0	0	0	0	0	0			24	55	
34	26-Nov-01	25	0	0	0	0	0	0			24	50	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IB - 0 (air only) mg/m ³								Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean		
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (µm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
35	27-Nov-01	26	0	0	0	0	0	0			24	51	
36	28-Nov-01	27	0	0	0	0	0	0			24	52	
37	29-Nov-01	28	0	0	0	0	0	0	1.811	2.481	4.57E-03	24	52
38	30-Nov-01	29	0	0	0	0	0	0			24	57	
41	3-Dec-01	30	0	0	0	0	0	0			25	51	
42	4-Dec-01	31	0	0	0	0	0	0			24	53	
43	5-Dec-01	32	0	0	0	0	0	0			24	54	
44	6-Dec-01	33	0	0	0	0	0	0	6.742	3.378	9.03E-03	24	54
45	7-Dec-01	34	0	0	0	0	0	0			24	52	
48	10-Dec-01	35	0	0	0	0	0	0			24	52	
49	11-Dec-01	36	0	0	0	0	0	0			24	54	
50	12-Dec-01	37	0	0	0	0	0	0			24	53	
51	13-Dec-01	38	0	0	0	0	0	0	0.7426	1.477	1.85E-03	24	50
52	14-Dec-01	39	0	0	0	0	0	0			24	52	
55	17-Dec-01	40	0	0	0	0	0	0			25	51	
56	18-Dec-01	41	0	0	0	0	0	0			24	52	
57	19-Dec-01	42	0	0	0	0	0	0			24	55	
58	20-Dec-01	43	0	0	0	0	0	0	2.855	2.088	3.88E-03	23	55
59	21-Dec-01	44	0	0	0	0	0	0			23	58	
62	24-Dec-01	45	0	0	0	0	0	0			24	49	
64	26-Dec-01	46	0	0	0	0	0	0			25	54	
65	27-Dec-01	47	0	0	0	0	0	0	0.9659	1.987	1.29E-03	24	55
66	28-Dec-01	48	0	0	0	0	0	0			24	55	
67	29-Dec-01	49	0	0	0	0	0	0			25	54	
69	31-Dec-01	50	0	0	0	0	0	0			25	54	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IB - 0 (air only) mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
70	1-Jan-02	51	0	0	0	0	0	0				24	54
71	2-Jan-02	52	0	0	0	0	0	0				24	55
72	3-Jan-02	53	0	0	0	0	0	0				24	54
73	4-Jan-02	54	0	0	0	0	0	0				23	57
75	6-Jan-02	55	0	0	0	0	0	0				24	53
76	7-Jan-02	56	0	0	0	0	0	0				24	52
77	8-Jan-02	57	0	0	0	0	0	0				24	55
78	9-Jan-02	58	0	0	0	0	0	0				24	53
79	10-Jan-02	59	0	0	0	0	0	0	1.103	2.244	2.87E-03	24	49
83	14-Jan-02	60	0	0	0	0	0	0				25	51
84	15-Jan-02	61	0	0	0	0	0	0				23	55
85	16-Jan-02	62	0	0	0	0	0	0				23	52
86	17-Jan-02	63	0	0	0	0	0	0				23	52
87	18-Jan-02	64	0	0	0	0	0	0	10.08	3.249	1.05E-02	23	57
89	20-Jan-02	65	0	0	0	0	0	0				24	55
90	21-Jan-02	66	0	0	0	0	0	0				23	55
91	22-Jan-02	67	0	0	0	0	0	0				22	55
Mean			0		0				2.747	2.291	4.40E-03	23.9	53.5
S.D.			0		0				2.919	0.552	3.00E-03	0.6	2.0

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIA - 2,000 mg/m ³								Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean	Temperature (°C)	Humidity (%)
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)			
0	23-Oct-01	1	2090	2038	2280	2160	1960	1750			23	50	
1	24-Oct-01	2	2160	2015	1880	2110	2050	2020			23	47	
2	25-Oct-01	3	2270	2160	2020	1960	2350	2310			23	47	
3	26-Oct-01	4	2090	2023	2010	2060	2060	1960			23	50	
6	29-Oct-01	5	2110	1988	1850	2030	2040	2030	2.045	2.243	2.28E-03	24	46
7	30-Oct-01	6	2030	2020	2170	2050	1900	1960			24	49	
8	31-Oct-01	7	2080	1990	1910	1950	2090	2010			24	50	
9	1-Nov-01	8	2060	1940	1860	1760	2180	1960	0.7630	1.745	3.75E-03	24	48
10	2-Nov-01	9	2120	2010	2040	1980	2000	2020			24	50	
13	5-Nov-01	10	2030	2025	2170	2020	1990	1920			24	48	
14	6-Nov-01	11	2080	1995	1860	1960	2150	2010			24	49	
15	7-Nov-01	12	2100	2063	2060	2090	2040	2060			24	45	
16	8-Nov-01	13	2220	2195	2270	2270	1840	2400	0.8737	1.804	2.31E-03	24	47
17	9-Nov-01	14	2310	2293	2180	2030	2540	2390			24	47	
20	12-Nov-01	15	2140	2048	2060	1960	2140	2030			24	49	
21	13-Nov-01	16	2060	1918	1950	1920	1860	1940			23	50	
22	14-Nov-01	17	2130	2055	2060	1930	2200	2030			24	48	
23	15-Nov-01	18	2120	2043	2230	1970	2110	1860	0.8069	1.867	4.75E-03	24	48
24	16-Nov-01	19	2100	2053	1970	1800	2160	2280			24	47	
27	19-Nov-01	20	1970	1935	1690	1730	1970	2350			24	46	
28	20-Nov-01	21	2140	1733	1470	1760	1730	1970			23	48	
29	21-Nov-01	22	2000	1995	1980	2000	1760	2240			22	51	
30	22-Nov-01	23	2240	1965	1960	1890	1910	2100	1.037	2.383	1.42E-03	23	51
31	23-Nov-01	24	2270	2000	1910	2100	1840	2150			24	49	
32	24-Nov-01	25	2210	2035	2210	2090	1940	1900			24	49	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIA - 2,000 mg/m ³								Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean		
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
34	26-Nov-01	26	2260	1990	1910	1990	2000	2060			24	45	
35	27-Nov-01	27	2250	2045	2090	1840	2300	1950			23	48	
36	28-Nov-01	28	2240	2028	2310	2130	1940	1730			23	47	
37	29-Nov-01	29	2150	2000	1890	1880	1980	2250	1.042	1.662	1.94E-03	23	48
38	30-Nov-01	30	2240	1975	2030	2080	1700	2090			23	54	
41	3-Dec-01	31	2160	2013	2000	2110	1960	1980			23	48	
42	4-Dec-01	32	2160	2000	2050	2030	1920	2000			24	47	
43	5-Dec-01	33	2070	2145	2340	2260	2070	1910			24	48	
44	6-Dec-01	34	2220	1923	2060	1980	1810	1840	0.9014	1.876	2.75E-03	24	48
45	7-Dec-01	35	2080	1968	2080	2040	1680	2070			24	47	
48	10-Dec-01	36	1890	2015	2580	1420	1950	2110			24	47	
49	11-Dec-01	37	2060	2003	1960	1510	2400	1410			23	49	
50	12-Dec-01	38	2360	2220	2380	2000	2480	2020			23	49	
51	13-Dec-01	39	2260	2155	2030	2400	2250	1940	0.9658	2.407	2.51E-03	23	47
52	14-Dec-01	40	2220	2130	2350	2050	2040	2080			23	48	
55	17-Dec-01	41	2130	2050	2350	1840	1920	2090			24	48	
56	18-Dec-01	42	2160	2130	1960	2120	2270	2170			24	47	
57	19-Dec-01	43	2180	2063	2220	2080	1890	2060			24	49	
58	20-Dec-01	44	2180	1993	2090	1870	2010	2000	0.8466	1.508	3.64E-04	23	51
59	21-Dec-01	45	2280	2013	1920	1980	2060	2090			23	51	
62	24-Dec-01	46	2280	2110	1740	2240	2150	2310			24	45	
64	26-Dec-01	47	1990	2070	2400	2110	1980	1790			23	51	
65	27-Dec-01	48	2080	2055	1820	2150	2230	2020	0.8310	1.648	1.51E-03	23	52
66	28-Dec-01	49	2070	2090	2010	1840	1960	2550			23	53	
67	29-Dec-01	50	1920	1955	2050	2030	2120	1620			23	52	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIA - 2,000 mg/m ³								Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean		
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
69	31-Dec-01	51	1950	1943	2230	1760	1760	2020			23	52	
70	1-Jan-02	52	1880	2023	2680	1830	1790	1790			24	48	
71	2-Jan-02	53	1950	2000	1980	1910	2120	1990			24	50	
72	3-Jan-02	54	2090	2100	2110	2150	2030	2110	1.486	2.142	2.16E-03	24	47
73	4-Jan-02	55	1980	2088	2160	2220	2160	1810			24	49	
75	6-Jan-02	56	1880	1948	1870	2240	2200	1480			24	47	
76	7-Jan-02	57	2070	2028	2310	1930	1970	1900			24	45	
77	8-Jan-02	58	1870	1840	2220	1760	1650	1730			23	50	
78	9-Jan-02	59	2020	2015	2060	1970	2060	1970			23	49	
79	10-Jan-02	60	1880	1950	2040	2080	1670	2010	1.779	2.388	4.20E-03	23	45
83	14-Jan-02	61	1910	2073	1990	2320	1920	2060			23	48	
84	15-Jan-02	62	2100	2043	2030	2070	1940	2130			23	51	
85	16-Jan-02	63	2280	2150	1930	2380	2140	2150			23	47	
86	17-Jan-02	64	2190	2078	2010	2230	2090	1980			23	47	
87	18-Jan-02	65	2130	2025	1760	2030	2220	2090	1.222	2.952	6.05E-03	23	51
87	20-Jan-02	66	2160	1965	2040	1910	1820	2090			23	50	
90	21-Jan-02	67	2140	1978	2050	2060	1870	1930			23	48	
91	22-Jan-02	68	2110	1928	1730	2000	2000	1980			22	50	
Mean			2112		2024				1.123	2.048	2.77E-03	23.5	48.6
S.D.			118		190				0.404	0.412	1.54E-03	0.6	2.0

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIB - 2,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean Temperature (°C)	Mean Humidity (%)
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)			
0	23-Oct-01	1	2090	1875	1620	1980	1880	2020				23	54
1	24-Oct-01	2	2160	2043	2060	2060	2010	2040				23	51
2	25-Oct-01	3	2270	2160	2070	1990	2320	2260				23	50
3	26-Oct-01	4	2090	2020	2030	1990	1920	2140				23	53
6	29-Oct-01	5	2110	1995	2160	1980	1880	1960	12.38	3.012	6.67E-03	23	50
7	30-Oct-01	6	2030	2000	2030	1970	2030	1970				23	51
8	31-Oct-01	7	2080	1983	2220	1990	1690	2030				23	53
9	1-Nov-01	8	2090	2030	1990	1820	2280	2030	0.8035	2.139	7.47E-03	23	52
10	2-Nov-01	9	2120	2048	2140	2040	1970	2040				23	53
13	5-Nov-01	10	2030	1983	1890	2020	2010	2010				23	49
14	6-Nov-01	11	2080	2058	1960	1850	2280	2140				23	53
15	7-Nov-01	12	2100	1935	1990	1890	1930	1930				23	52
16	8-Nov-01	13	2220	2088	2190	2300	1740	2120	0.7894	1.578	2.04E-03	24	51
17	9-Nov-01	14	2310	1875	1640	1910	1920	2030				23	51
20	12-Nov-01	15	2140	2013	1940	1890	2020	2200				23	52
21	13-Nov-01	16	2060	1948	1980	1960	2010	1840				22	52
22	14-Nov-01	17	2130	1935	2120	1790	1890	1940				22	50
23	15-Nov-01	18	2120	2143	2160	1980	2170	2260	0.8338	2.476	7.31E-03	23	50
24	16-Nov-01	19	2100	2050	2010	1960	2190	2040				23	48
27	19-Nov-01	20	1970	1958	1730	2030	2200	1870				23	47
28	20-Nov-01	21	2140	1915	1870	1880	2030	1880				22	50
30	22-Nov-01	22	2240	2078	2130	1940	2260	1980	1.176	2.158	1.99E-03	22	51
31	23-Nov-01	23	2270	2103	2150	2100	2110	2050				23	51
32	24-Nov-01	24	2210	1988	1850	1840	2090	2170				23	51
34	26-Nov-01	25	2260	2068	2120	1870	2170	2110				23	47

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIB - 2,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
35	27-Nov-01	26	2250	1938	1610	2070	2090	1980				23	48
36	28-Nov-01	27	2240	2108	1870	2080	2260	2220				23	48
37	29-Nov-01	28	2150	1973	2030	1870	2110	1880	1.046	1.589	1.83E-03	23	50
38	30-Nov-01	29	2240	2120	1840	1760	2340	2540				23	54
41	3-Dec-01	30	2160	2008	2240	1810	2030	1950				23	48
42	4-Dec-01	31	2160	2110	2210	2010	2250	1970				23	50
43	5-Dec-01	32	2070	2110	2150	2020	2100	2170				23	50
44	6-Dec-01	33	2220	1995	2120	2060	1980	1820	0.8575	1.532	2.71E-03	23	52
45	7-Dec-01	34	2080	2238	2290	2350	1670	2640				23	49
48	10-Dec-01	35	1890	2228	2770	1830	2350	1960				23	49
49	11-Dec-01	36	2060	2063	2350	1980	2350	1570				23	50
50	12-Dec-01	37	2360	2378	2770	2570	2100	2070				23	50
51	13-Dec-01	38	2260	2120	2400	1880	2220	1980	3.402	3.001	5.81E-03	23	48
52	14-Dec-01	39	2220	2040	1890	1870	2100	2300				23	49
55	17-Dec-01	40	2130	2115	2300	2010	2230	1920				24	49
56	18-Dec-01	41	2160	1963	2030	1840	1960	2020				23	51
57	19-Dec-01	42	2180	1993	2100	1860	1880	2130				23	51
58	20-Dec-01	43	2180	1973	2120	1910	2010	1850	3.014	2.133	2.46E-03	22	52
59	21-Dec-01	44	2280	1985	1960	1820	2020	2140				22	54
62	24-Dec-01	45	2280	2145	2300	2080	2040	2160				23	47
64	26-Dec-01	46	1990	1978	2210	2000	1880	1820				24	52
65	27-Dec-01	47	2080	1963	1800	2110	1850	2090	0.8323	2.139	2.33E-03	23	55
66	28-Dec-01	48	2070	2035	2300	2030	2080	1730				23	54
67	29-Dec-01	49	1920	1940	2010	2050	1960	1740				23	53
69	31-Dec-01	50	1950	2175	2350	2300	2230	1820				23	53

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIB - 2,000 mg/m ³										Chamber Environment			
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean					
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)				
70	1-Jan-02	51	1880	1900	1450	2110	2000	2040			23	53				
71	2-Jan-02	52	1950	2210	2060	2280	2220	2280			23	53				
72	3-Jan-02	53	2090	2023	1840	1920	2180	2150	2.894	2.310	3.57E-03	23	51			
73	4-Jan-02	54	1980	2063	2280	1860	2090	2020			23	54				
75	6-Jan-02	55	1880	1973	1440	1960	2580	1910			23	51				
76	7-Jan-02	56	2070	2038	2210	1820	2200	1920			23	49				
77	8-Jan-02	57	1870	1825	1970	1600	1910	1820			23	52				
78	9-Jan-02	58	2020	2023	2140	2000	1980	1970			24	51				
79	10-Jan-02	59	1880	1935	2140	1760	1700	2140	8.795	3.240	7.65E-03	23	48			
83	14-Jan-02	60	1910	1968	2170	1920	2000	1780			24	49				
84	15-Jan-02	61	2100	1773	1740	1760	1740	1850			22	53				
85	16-Jan-02	62	2280	2010	2030	1940	1930	2140			22	49				
86	17-Jan-02	63	2190	2018	1830	2030	1900	2310			22	49				
87	18-Jan-02	64	2130	2123	2640	1940	1880	2030	1.677	3.487	7.47E-03	22	53			
89	20-Jan-02	65	2160	2088	2120	2140	2000	2090			23	52				
90	21-Jan-02	66	2140	2005	2090	1810	2060	2060			22	51				
91	22-Jan-02	67	2110	1935	2250	1500	2140	1850			22	52				
Mean			2114		2028				2.962	2.369	4.56E-03	22.9	50.9			
S.D.			118		199				3.586	0.644	2.49E-03	0.5	2.0			

Table A

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

00-6129

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 (mg/m ³)	Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)	
0	23-Oct-01	1	11600	10240	9720	10700	9820	10700				23	50	
1	24-Oct-01	2	11000	9938	9920	10500	9230	10100				24	47	
2	25-Oct-01	3	9890	8895	9430	8040	9130	8980				23	47	
3	26-Oct-01	4	11600	10080	9000	10600	10600	10100				24	46	
6	29-Oct-01	5	11300	10280	10400	10500	10100	10100	2.117	2.220	2.09E-03	24	45	
7	30-Oct-01	6	11000	10180	10100	10100	10400	10100				24	48	
8	31-Oct-01	7	10200	9153	9990	8710	8930	8980				24	49	
9	1-Nov-01	8	11000	10300	9790	10500	10400	10500	0.9487	2.649	5.23E-03	24	48	
10	2-Nov-01	9	11300	10450	10500	9790	10800	10700				24	52	
13	5-Nov-01	10	11000	10380	10700	9530	10800	10500				24	47	
14	6-Nov-01	11	10800	9788	10100	9490	10100	9460				24	47	
15	7-Nov-01	12	10200	10040	10100	10500	9060	10500				24	47	
16	8-Nov-01	13	9970	9823	10000	10800	8900	9590	3.553	2.083	1.22E-02	24	46	
17	9-Nov-01	14	11300	10600	9890	10800	11100	10600				24	45	
20	12-Nov-01	15	10900	10220	10500	10100	10400	9860				24	45	
21	13-Nov-01	16	10500	9645	9890	9130	10100	9460				24	48	
22	14-Nov-01	17	11000	9945	10100	10100	9790	9790				23	49	
23	15-Nov-01	18	10200	9690	9790	9790	9790	9390	0.7951	2.370	1.06E-02	24	49	
24	16-Nov-01	19	10400	9413	8230	9460	10100	9860				24	47	
27	19-Nov-01	20	11500	10520	9790	11000	10500	10800				24	46	
28	20-Nov-01	21	11100	10270	10100	10300	10800	9860				23	48	
29	21-Nov-01	22	10800	10570	11100	10500	9560	11100				22	48	
30	22-Nov-01	23	11400	10770	11000	12000	10800	9290	0.8954	1.722	1.14E-03	23	48	
31	23-Nov-01	24	11200	10040	9790	10700	9460	10200				24	48	
32	24-Nov-01	25	10700	9300	10500	9360	8540	8800				24	50	

Table A

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

00-6129

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 (mg/m ³)	Mean (mg/m ³)	Individual (mg/m ³)				MMAD (µm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Mean Humidity (%)	
34	26-Nov-01	26	10700	9005	8520	10500	9130	7870				24	46	
35	27-Nov-01	27	10700	10420	9060	10500	11800	10300				23	48	
36	28-Nov-01	28	10600	10180	10300	10800	9890	9720				23	48	
37	29-Nov-01	29	11200	10250	9890	10500	10500	10100	1.460	2.555	3.01E-03	23	49	
38	30-Nov-01	30	10400	9750	9090	9490	10800	9620				23	54	
41	3-Dec-01	31	10400	9613	10300	8460	10200	9490				23	47	
42	4-Dec-01	32	11200	10500	10500	10500	10500	10500				24	48	
43	5-Dec-01	33	10700	10200	10500	9790	10000	10500				24	49	
44	6-Dec-01	34	10500	9453	9460	8800	9790	9760	0.9809	1.829	2.97E-03	24	48	
45	7-Dec-01	35	11100	10350	10400	10500	10400	10100				24	47	
48	10-Dec-01	36	10400	10120	10100	10100	10500	9790				24	46	
49	11-Dec-01	37	11200	10120	10100	10900	9390	10100				23	48	
50	12-Dec-01	38	10200	9590	8540	9790	9230	10800				23	50	
51	13-Dec-01	39	10800	10430	11900	11500	8770	9560	0.9910	2.266	3.35E-03	23	48	
52	14-Dec-01	40	11000	10500	10100	10100	11000	10800				23	50	
55	17-Dec-01	41	11000	10450	10800	10100	10800	10100				23	48	
56	18-Dec-01	42	10600	9915	10000	9360	10100	10200				24	48	
57	19-Dec-01	43	10300	10450	10400	10800	10500	10100				24	49	
58	20-Dec-01	44	10800	10050	10100	9720	10500	9890	9.149	2.564	4.51E-03	24	47	
59	21-Dec-01	45	10600	10010	9790	10800	9620	9820				24	48	
62	24-Dec-01	46	10600	10350	10900	10100	10200	10200				24	45	
64	26-Dec-01	47	10500	9883	10200	10100	9130	10100				23	46	
65	27-Dec-01	48	11000	9995	9860	10200	9820	10100	0.7062	1.862	2.74E-03	23	46	
66	28-Dec-01	49	10600	9755	8940	10500	10900	8680				23	47	
67	29-Dec-01	50	10400	10220	9060	11800	10100	9920				23	47	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIIA - 10,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean Temperature	Mean Humidity
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	(°C)	(%)
69	31-Dec-01	51	11000	10350	10000	10100	10500	10800				23	46
70	1-Jan-02	52	11000	10210	10600	9120	11800	9330				24	46
71	2-Jan-02	53	10700	10300	10300	10400	10400	10100				24	45
72	3-Jan-02	54	10800	9065	10000	8550	8840	8870	1.180	2.242	1.75E-03	24	46
73	4-Jan-02	55	10900	9265	8510	9330	9790	9430				24	46
75	6-Jan-02	56	10200	10040	9720	9860	9390	11200				24	45
76	7-Jan-02	57	10700	9575	9790	9790	9000	9720				24	46
77	8-Jan-02	58	11000	10050	10300	10100	9820	9960				23	47
78	9-Jan-02	59	10300	9743	9620	9460	9290	10600				23	47
79	10-Jan-02	60	10900	10440	8150	11200	11800	10600	1.520	3.033	5.37E-03	23	46
83	14-Jan-02	61	10400	10110	10300	10300	9620	10200				23	46
84	15-Jan-02	62	11000	10320	10100	10400	9690	11100				23	49
85	16-Jan-02	63	11100	10250	10300	10100	10500	10100				23	46
86	17-Jan-02	64	11000	10550	10200	10800	10400	10800				23	46
87	18-Jan-02	65	9920	10300	8800	10500	11000	10900	0.7120	1.961	5.98E-03	23	48
89	20-Jan-02	66	9890	10240	11000	9460	10700	9790				23	46
90	21-Jan-02	67	10600	10650	11100	10600	10800	10100				23	48
91	22-Jan-02	68	10200	10700	11500	9390	10500	11400				22	48
Mean			10750		10062				1.924	2.258	4.69E-03	23.5	47.4
S.D.			414		715				2.306	0.376	3.33E-03	0.6	1.7

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIIB - 10,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
0	23-Oct-01	1	11600	10780	11200	10700	10100	11100				24	47
1	24-Oct-01	2	11000	10080	10800	10500	9560	9460				24	47
2	25-Oct-01	3	9890	9985	10100	8850	9890	11100				24	46
3	26-Oct-01	4	11600	10150	9790	10100	10600	10100				24	46
6	29-Oct-01	5	11300	10030	10100	9960	9960	10100	5.488	2.816	3.57E-03	24	44
7	30-Oct-01	6	11000	10530	11000	10100	10800	10200				24	44
8	31-Oct-01	7	10200	9230	10300	8910	8950	8760				24	46
9	1-Nov-01	8	11000	10020	10500	9990	9860	9720	0.7852	1.929	3.90E-03	24	47
10	2-Nov-01	9	11300	10400	10500	10400	10500	10200				24	48
13	5-Nov-01	10	11000	10120	10100	9460	10500	10400				24	44
14	6-Nov-01	11	10800	9728	10100	9020	10000	9790				24	46
15	7-Nov-01	12	10200	9790	9990	10100	8970	10100				24	47
16	8-Nov-01	13	9970	9483	10000	10200	8110	9620	0.8095	2.085	4.71E-03	24	46
17	9-Nov-01	14	11300	10330	9530	10800	10300	10700				24	45
20	12-Nov-01	15	10900	10030	9620	10300	10200	10000				24	44
21	13-Nov-01	16	10500	9293	10100	8350	9030	9690				23	46
22	14-Nov-01	17	11000	10370	10100	11100	9990	10300				23	46
23	15-Nov-01	18	10200	9795	9720	10000	10000	9460	0.7347	2.097	1.04E-02	23	45
24	16-Nov-01	19	10400	9755	9460	9460	10000	10100				24	44
27	19-Nov-01	20	11500	10160	9920	10500	10100	10100				23	45
28	20-Nov-01	21	11100	9700	9260	9720	10100	9720				23	45
30	22-Nov-01	22	11400	9833	9790	9050	9890	10600	5.383	3.304	4.59E-03	23	45
31	23-Nov-01	23	11200	10230	10100	11100	9620	10100				23	46
32	24-Nov-01	24	10700	10410	8930	10500	10800	11400				23	48
34	26-Nov-01	25	10700	9455	7670	9790	9860	10500				23	45

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIIB - 10,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
35	27-Nov-01	26	10700	9840	10300	9330	10400	9330			24	45	
36	28-Nov-01	27	10600	10470	11000	11100	10300	9460			24	46	
37	29-Nov-01	28	11200	10260	11100	10600	9890	9460	1.035	1.778	2.97E-03	24	45
38	30-Nov-01	29	10400	9853	9960	10100	9960	9390			24	50	
41	3-Dec-01	30	10400	10090	10500	9960	10100	9790			24	45	
42	4-Dec-01	31	11200	10500	10900	10100	10500	10500			23	46	
43	5-Dec-01	32	10700	10630	11000	9820	10900	10800			23	47	
44	6-Dec-01	33	10500	9828	10500	9330	9790	9690	0.8592	1.606	2.51E-03	24	46
45	7-Dec-01	34	11100	10430	10200	10100	10600	10800			23	45	
48	10-Dec-01	35	10400	9603	8690	9460	9460	10800			23	45	
49	11-Dec-01	36	11200	10850	11800	11300	9790	10500			24	45	
50	12-Dec-01	37	10200	10190	8960	10800	9790	11200			24	46	
51	13-Dec-01	38	10800	9518	9960	9560	8760	9790	0.7420	2.021	5.17E-03	24	45
52	14-Dec-01	39	11000	10230	10100	10200	10500	10100			24	45	
55	17-Dec-01	40	11000	9960	9290	9960	10700	9890			24	44	
56	18-Dec-01	41	10600	9848	9990	8900	10000	10500			23	45	
57	19-Dec-01	42	10300	10160	9790	10300	9460	11100			24	46	
58	20-Dec-01	43	10800	10340	10900	9820	10800	9820	13.62	2.510	6.39E-03	23	45
59	21-Dec-01	44	10600	10300	10100	10900	10100	10100			23	45	
62	24-Dec-01	45	10600	10150	10700	10100	9890	9890			23	44	
64	26-Dec-01	46	10500	10550	10700	10500	10500	10500			24	44	
65	27-Dec-01	47	11000	10310	9620	10800	10300	10500	0.7724	2.193	3.67E-03	24	43
66	28-Dec-01	48	10600	10200	9590	11100	10000	10100			24	44	
67	29-Dec-01	49	10400	10190	8970	11100	10600	10100			24	44	
69	31-Dec-01	50	11000	10730	10800	10300	10800	11000			24	43	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IIIB - 10,000 mg/m ³								Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean		
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
70	1-Jan-02	51	11000	9758	10300	8730	11400	8600			23	45	
71	2-Jan-02	52	10700	9678	9790	9360	9460	10100	2.837	2.479	3.08E-03	23	44
72	3-Jan-02	53	10800	10140	11200	9460	9790	10100				23	45
73	4-Jan-02	54	10900	9915	8260	10100	10900	10400				23	45
75	6-Jan-02	55	10200	10140	10100	9490	9760	11200				23	44
76	7-Jan-02	56	10700	10020	10300	10200	10100	9490				23	45
77	8-Jan-02	57	11000	10730	11500	9920	10900	10600				24	44
78	9-Jan-02	58	10300	10040	10400	9430	9720	10600				24	44
79	10-Jan-02	59	10900	10460	10300	9330	11500	10700	7.657	3.667	8.94E-03	24	44
83	14-Jan-02	60	10400	10400	11000	9790	10600	10200				24	42
84	15-Jan-02	61	11000	9920	9560	10200	9620	10300				23	47
85	16-Jan-02	62	11100	9755	9460	8760	10500	10300				23	45
86	17-Jan-02	63	11000	10170	9790	10100	10500	10300				23	45
87	18-Jan-02	64	9920	9775	9920	10200	8480	10500	0.7065	2.252	7.40E-03	23	46
89	20-Jan-02	65	9890	9518	10400	8980	9230	9460				23	46
90	21-Jan-02	66	10600	9895	9790	10200	10100	9490				22	46
91	22-Jan-02	67	10200	9410	9070	8580	9690	10300				22	46
Mean			10749		10065				3.187	2.364	5.18E-03	23.5	45.3
S.D.			417		661				3.921	0.594	2.44E-03	0.6	1.3

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IVA - 20,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean Temperature	Humidity
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	(°C)	(%)
0	23-Oct-01	1	19100	19000	21000	18100	18400	18500				25	52
1	24-Oct-01	2	19900	19980	19300	19700	21500	19400				25	52
2	25-Oct-01	3	19600	20250	22300	19000	19700	20000				25	50
3	26-Oct-01	4	20300	19530	19600	19600	19600	19300				25	49
6	29-Oct-01	5	19600	20250	18500	20400	22300	19800	1.051	1.750	7.17E-04	25	48
7	30-Oct-01	6	20500	20030	19700	20900	20100	19400				25	50
8	31-Oct-01	7	19900	19630	19400	19800	19300	20000				24	50
9	1-Nov-01	8	20200	19580	19600	20100	19100	19500	0.8223	1.803	4.14E-03	25	49
10	2-Nov-01	9	19200	19800	19700	19700	20100	19700				24	54
13	5-Nov-01	10	20200	20250	20400	19400	19800	21400				24	50
14	6-Nov-01	11	19600	19650	20100	19400	19500	19600				26	48
15	7-Nov-01	12	20200	19480	19400	19400	19700	19400				25	51
16	8-Nov-01	13	20700	19680	17100	21400	21400	18800	0.9253	1.866	2.15E-03	25	49
17	9-Nov-01	14	20200	19330	19700	16000	21200	20400				26	47
20	12-Nov-01	15	20400	19630	18900	21400	19300	18900				25	47
21	13-Nov-01	16	19900	19900	18500	20300	20000	20800				24	49
22	14-Nov-01	17	19700	19630	20200	19000	19400	19900				24	51
23	15-Nov-01	18	19100	18880	18000	18800	19300	19400	0.8504	2.121	4.80E-03	24	51
24	16-Nov-01	19	20000	20680	20000	21600	20100	21000				25	48
27	19-Nov-01	20	19400	19050	19500	19400	19700	17600				25	48
28	20-Nov-01	21	19700	19600	18900	21200	19400	18900				25	48
29	21-Nov-01	22	19500	21030	19400	20900	23000	20800				23	50
30	22-Nov-01	23	20900	20230	20100	19000	21500	20300	0.9666	2.254	1.43E-03	25	50
31	23-Nov-01	24	20400	20480	19700	20900	21100	20200				25	50
32	24-Nov-01	25	20200	20330	20500	20200	20100	20500				25	51

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IVA - 20,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
34	26-Nov-01	26	19800	20080	20200	19700	20500	19900				26	48
35	27-Nov-01	27	19700	20380	19400	20800	20200	21100				24	48
36	28-Nov-01	28	19200	19750	19500	18800	20500	20200				24	49
37	29-Nov-01	29	19900	20150	20600	19900	19900	20200	0.9850	1.432	1.66E-03	24	50
38	30-Nov-01	30	19000	19480	18800	19700	19700	19700				24	55
41	3-Dec-01	31	18900	19400	19200	19800	18000	20600				25	48
42	4-Dec-01	32	19600	20250	18900	21200	21200	19700				26	49
43	5-Dec-01	33	20600	20430	20400	19900	20600	20800				26	49
44	6-Dec-01	34	19400	19980	21300	17700	20000	20900	0.9092	1.905	2.69E-03	26	50
45	7-Dec-01	35	20000	19900	20000	21200	19000	19400				26	47
48	10-Dec-01	36	20800	19550	18900	19400	18900	21000				26	47
49	11-Dec-01	37	18700	19730	19700	18300	21200	19700				24	48
50	12-Dec-01	38	20300	20630	20700	20900	20500	20400				25	49
51	13-Dec-01	39	20000	19880	20000	20400	19500	19600	0.7134	1.424	2.95E-03	24	48
52	14-Dec-01	40	19400	19150	18100	20100	19900	18500				24	50
55	17-Dec-01	41	18000	18880	18800	20800	19000	16900				25	48
56	18-Dec-01	42	20100	20030	19400	20100	20100	20500				26	47
57	19-Dec-01	43	19200	19880	19000	20000	20800	19700				26	48
58	20-Dec-01	44	21000	20700	20900	20900	21200	19800	2.022	1.982	7.33E-04	25	48
59	21-Dec-01	45	20700	20080	19900	21300	19400	19700				25	49
62	24-Dec-01	46	19100	19900	20500	19500	20200	19400				26	46
64	26-Dec-01	47	20400	20330	20900	20100	20200	20100				25	48
65	27-Dec-01	48	18900	19430	19600	19400	19700	19000	0.9029	2.190	2.16E-03	24	48
66	28-Dec-01	49	19400	20050	20700	20000	19800	19700				24	49
68	30-Dec-01	50	19400	19730	20100	19700	19700	19400				24	49

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IVA - 20,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
69	31-Dec-01	51	20200	20380	20500	20100	20800	20100				24	49
70	1-Jan-02	52	20300	19730	18800	19400	20700	20000				25	45
71	2-Jan-02	53	20300	20450	20100	19400	20400	21900				25	47
72	3-Jan-02	54	19900	19880	19400	19300	20800	20000				25	47
73	4-Jan-02	55	18100	20280	19300	21200	21100	19500				25	47
75	6-Jan-02	56	20700	19900	19800	19800	20000	20000				26	46
76	7-Jan-02	57	18900	19680	21200	18600	20100	18800				25	47
77	8-Jan-02	58	19200	20580	20200	19800	21500	20800				24	48
78	9-Jan-02	59	19900	20880	20200	20800	21700	20800				24	48
79	10-Jan-02	60	19700	21000	20700	20300	19700	23300	0.7752	1.684	3.25E-03	24	47
83	14-Jan-02	61	18400	20880	19900	21100	20700	21800				25	47
84	15-Jan-02	62	20300	21080	21800	22100	20600	19800				25	49
85	16-Jan-02	63	16800	19030	17200	20000	18800	20100				25	48
86	17-Jan-02	64	19200	19550	19400	21200	19000	18600				25	47
87	18-Jan-02	65	18400	19500	19700	20600	20200	17500	0.7750	2.044	4.73E-03	25	50
89	20-Jan-02	66	19600	19750	19700	20800	17700	20800				25	47
90	21-Jan-02	67	19900	20480	20500	20100	21000	20300				25	49
91	22-Jan-02	68	18300	20180	19700	20800	20800	19400				23	49
Mean			19676		19950				1.421	1.926	2.91E-03	24.8	48.7
S.D.			770		983				1.643	0.324	1.72E-03	0.8	1.8

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IVB - 20,000 mg/m ³								Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations			Mean		
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)	
0	23-Oct-01	1	19100	20630	17700	20500	22700	21600			24	51	
1	24-Oct-01	2	19900	19150	20800	18800	18200	18800			24	51	
2	25-Oct-01	3	19600	18750	17200	18800	18900	20100			24	51	
3	26-Oct-01	4	20300	19330	18500	20100	19600	19100			24	50	
6	29-Oct-01	5	19600	18930	20400	17500	19500	18300	8.942	3.141	2.73E-03	24	49
7	30-Oct-01	6	20500	20030	19600	20800	20400	19300			25	49	
8	31-Oct-01	7	19900	19500	19100	19800	19200	19900			25	49	
9	1-Nov-01	8	20200	19850	19700	20100	19700	19900	0.7851	2.273	5.82E-03	25	48
10	2-Nov-01	9	19200	19750	19700	20200	20200	18900			25	53	
13	5-Nov-01	10	20200	20750	20800	20800	21200	20200			25	48	
14	6-Nov-01	11	19600	19080	17300	19900	19900	19200			24	48	
15	7-Nov-01	12	20200	19880	19900	19400	20000	20200			24	51	
16	8-Nov-01	13	20700	21280	18400	22000	23900	20800	0.8337	1.690	2.09E-03	24	49
17	9-Nov-01	14	20200	20050	20800	16000	22000	21400			24	49	
20	12-Nov-01	15	20400	20300	19500	20400	20800	20500			24	49	
21	13-Nov-01	16	19900	19750	19700	20100	19500	19700			25	49	
22	14-Nov-01	17	19700	19600	18200	19700	19900	20600			25	49	
23	15-Nov-01	18	19100	20480	20100	20200	20800	20800	0.9512	3.722	1.36E-02	25	49
24	16-Nov-01	19	20000	19080	20500	17400	19000	19400			25	47	
27	19-Nov-01	20	19400	18930	17200	20100	20100	18300			25	48	
28	20-Nov-01	21	19700	19950	18800	21600	20200	19200			24	48	
30	22-Nov-01	22	20900	20150	20500	18900	20200	21000	0.7119	1.795	2.93E-03	24	48
31	23-Nov-01	23	20400	21150	20600	21200	21600	21200			24	49	
32	24-Nov-01	24	20200	19900	19400	20400	19600	20200			24	52	
34	26-Nov-01	25	19800	20280	19800	21200	20200	19900			24	48	

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IVB - 20,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
35	27-Nov-01	26	19700	19450	19200	19500	18600	20500				25	48
36	28-Nov-01	27	19200	20130	18600	21600	20600	19700				25	49
37	29-Nov-01	28	19900	19600	18600	20500	19900	19400	1.201	2.588	3.51E-03	25	49
38	30-Nov-01	29	19000	19130	19100	19400	19400	18600				25	54
41	3-Dec-01	30	18900	19600	19200	19800	18800	20600				25	48
42	4-Dec-01	31	19600	20400	19400	20500	21200	20500				24	49
43	5-Dec-01	32	20600	20780	20500	21200	20700	20700				24	49
44	6-Dec-01	33	19400	19800	21200	17300	19900	20800	1.784	3.162	7.82E-03	24	50
45	7-Dec-01	34	20000	19800	19400	19700	20100	20000				24	48
48	10-Dec-01	35	20800	19780	19700	19700	19400	20300				24	48
49	11-Dec-01	36	18700	19330	18600	17700	21300	19700				26	47
50	12-Dec-01	37	20300	19630	18500	20500	19400	20100				25	48
51	13-Dec-01	38	20000	19130	19300	19600	18900	18700	0.7145	1.806	6.92E-03	25	48
52	14-Dec-01	39	19400	19730	18600	19700	19800	20800				25	48
55	17-Dec-01	40	18000	19280	18800	18800	20100	19400				25	48
56	18-Dec-01	41	20100	19850	19700	19700	19400	20600				24	49
57	19-Dec-01	42	19200	20000	19200	20700	19700	20400				24	49
58	20-Dec-01	43	21000	20500	19700	21000	20800	20500	5.752	2.569	2.14E-03	24	49
59	21-Dec-01	44	20700	19930	19700	21200	19100	19700				24	50
62	24-Dec-01	45	19100	18880	19100	18000	20000	18400				24	48
64	26-Dec-01	46	20400	20530	20900	20100	20500	20600				25	47
65	27-Dec-01	47	18900	19680	19400	20100	20600	18600	1.097	2.323	1.54E-03	25	47
66	28-Dec-01	48	19400	19730	20500	19000	20000	19400				25	48
67	29-Dec-01	49	19400	19930	19200	20800	20200	19500				25	48

Table A

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

00-6129

Day	Date	Exposure Number	Chamber Monitoring Results Cumulative Exposure Record Group IVB - 20,000 mg/m ³									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations			Mean	
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
69	31-Dec-01	50	20200	20280	20500	20100	20000	20500				25	47
70	1-Jan-02	51	20300	19280	17000	19100	20000	21000				24	49
71	2-Jan-02	52	20300	20100	19000	19400	20400	21600				24	47
72	3-Jan-02	53	19900	20280	19700	19600	21600	20200	2.353	2.512	3.02E-03	24	50
73	4-Jan-02	54	18100	19530	19900	19400	19400	19400				24	49
75	6-Jan-02	55	20700	19950	18400	19600	19900	21900				24	47
76	7-Jan-02	56	18900	20230	21300	19700	20500	19400				24	48
77	8-Jan-02	57	19200	19650	19300	19300	19900	20100				25	48
78	9-Jan-02	58	19900	20450	21600	20100	20800	19300				25	47
79	10-Jan-02	59	19700	19900	19400	20100	18300	22200	2.188	3.245	6.14E-03	25	48
83	14-Jan-02	60	18400	19850	19900	20400	19400	19700				25	46
84	15-Jan-02	61	20300	20430	21500	21600	19700	18900				24	49
85	16-Jan-02	62	16800	19500	19200	21100	17900	19800				24	49
86	17-Jan-02	63	19200	20900	20100	22700	21100	19700				24	48
87	18-Jan-02	64	18400	19950	19400	21200	20900	18300	0.7771	2.092	4.75E-03	24	49
89	20-Jan-02	65	19600	19780	20200	20600	16900	21400				24	49
90	21-Jan-02	66	19900	21150	21300	21400	20800	21100				24	49
91	22-Jan-02	67	18300	21250	21900	21500	21900	19700				23	49
Mean			19679		19903				2.161	2.532	4.85E-03	24.4	48.7
S.D.			775		1085				2.455	0.631	3.31E-03	0.6	1.4

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TABLE B

GASOLINE ETBE 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

DAY OF STUDY
GROUP# -11 TOTAL

# OF ANIMALS EXAMINED	1	5
	2	5
	3	5
	4	5

NORMAL

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

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TABLE B

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

Table C - Week -1
LIONEL F. RUBIN, V.M.D.
1116 Saint Andrews Road
Bryn Mawr, PA 19010
(610) 520 9430

October 10, 2001

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

Re: study A-12 00-6129F

Ophthalmoscopic examination of study A-12 00-6129F rats was performed October 10, 2001 (pretest examination). No ocular abnormalities were identified. All rats are suitable for inclusion in the study.



Lionel F. Rubin, V. M. D.

Table C - Week 13
JAMES M. CLINTON, V.M.D.
Animal Eye Clinic at South Jersey Animal Hospital
204 Route 541
Medford, New Jersey 08055
Telephone: (609) 654-0304
Fax: (609) 714-1479

Huntingdon Life Sciences
Study: 00-6129

Examination Date:
17 January 2002
Interval: Week 13

Ophthalmoscopic Examination Summary

Both eyes of all of the rats selected for ocular examination were examined by focal illumination and indirect ophthalmoscopy. Mydriasis was produced with 1% tropicamide and the eyes examined in subdued light. The dose levels and group identifications were not disclosed to me prior to my examinations.

With just one exception, a unilateral lesion was identified, as follows:

<u>Rat</u>	<u>Observation</u>
4570	Focal retinopathy

Comment

I would expect this lesion to be demonstrable histologically if the fixed section includes the optic disc. The lesion typifies one of the commonest posterior segment changes identified in well managed rat colonies. It is not likely to be caused by exposure to an ocular toxicant.

James M. Clinton

James M. Clinton, V.M.D.

24 Jan 2002

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TABLE C

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

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TABLE C

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

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TABLE C

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

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TABLE C

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

TABLE D

GASOLINE ETBE 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: DOSE LEVEL (MG/M3):	1 0	2 2000	3 10000
WEEK -1		MEAN	215	216	216
		S.D.	4.2	6.8	9.4
		N	5	5	5
WEEK 0		MEAN	303	300	310
		S.D.	11.9	16.8	11.1
		N	5	5	5
WEEK 1		MEAN	338	331	350
		S.D.	14.6	21.6	17.6
		N	5	5	5
WEEK 2		MEAN	372	368	388
		S.D.	16.6	28.8	20.7
		N	5	5	5
WEEK 3		MEAN	401	397	418
		S.D.	21.0	28.3	27.3
		N	5	5	5
WEEK 4		MEAN	427	417	446
		S.D.	23.9	27.2	27.3
		N	5	5	5
WEEK 5		MEAN	450	440	471
		S.D.	25.6	29.4	32.9
		N	5	5	5
WEEK 6		MEAN	470	461	482
		S.D.	28.0	34.6	54.0
		N	5	5	5
WEEK 7		MEAN	493	474	508
		S.D.	32.8	36.8	48.1
		N	5	5	5

No statistically significant differences

TABLE D

GASOLINE ETBE 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: DOSE LEVEL (MG/M ³) :	1 0	2 2000	3 10000
WEEK 8		MEAN	502	485	522
		S.D.	36.8	34.1	50.3
		N	5	5	5
WEEK 9		MEAN	511	495	536
		S.D.	30.6	30.0	50.1
		N	5	5	5
WEEK 10		MEAN	538	514	554
		S.D.	43.4	36.8	52.9
		N	5	5	5
WEEK 11		MEAN	550	525	566
		S.D.	44.6	30.3	50.9
		N	5	5	5
WEEK 12		MEAN	563	537	581
		S.D.	46.9	30.7	55.5
		N	5	5	5
WEEK 13		MEAN	576	548	586
		S.D.	48.4	34.5	62.0
		N	5	5	5

No statistically significant differences

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TABLE D

GASOLINE ETBE 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: DOSE LEVEL(MG/M3) :	1 0	2 2000	3 10000	4 20000
WEEK -1		MEAN	176	175	176	176
		S.D.	5.3	5.0	6.6	8.0
		N	5	5	5	5
WEEK 0		MEAN	220	217	210	224
		S.D.	10.0	14.0	12.2	14.0
		N	5	5	5	5
WEEK 1		MEAN	244	238	233	239
		S.D.	16.9	14.6	19.6	19.6
		N	5	5	5	5
WEEK 2		MEAN	257	255	247	250
		S.D.	16.3	14.6	18.3	18.0
		N	5	5	5	5
WEEK 3		MEAN	267	262	255	259
		S.D.	17.0	14.7	20.3	18.1
		N	5	5	5	5
WEEK 4		MEAN	276	269	261	269
		S.D.	16.0	17.6	23.9	13.7
		N	5	5	5	5
WEEK 5		MEAN	280	285	273	276
		S.D.	19.9	18.0	26.3	9.0
		N	5	5	5	5
WEEK 6		MEAN	285	287	282	283
		S.D.	21.3	18.5	27.4	15.6
		N	5	5	5	5
WEEK 7		MEAN	292	289	284	287
		S.D.	21.6	11.6	27.4	16.9
		N	5	5	5	5

No statistically significant differences

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TABLE D

GASOLINE ETBE 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: DOSE LEVEL(MG/M3):	1 0	2 2000	3 10000
WEEK	8	MEAN	291	293	281
		S.D.	22.0	18.2	32.8
		N	5	5	5
WEEK	9	MEAN	296	299	293
		S.D.	27.5	19.6	28.0
		N	5	5	5
WEEK	10	MEAN	300	306	294
		S.D.	26.4	16.4	25.0
		N	5	5	5
WEEK	11	MEAN	306	305	300
		S.D.	23.2	21.2	31.3
		N	5	5	5
WEEK	12	MEAN	308	307	300
		S.D.	27.1	21.1	32.4
		N	5	5	5
WEEK	13	MEAN	307	317	308
		S.D.	32.5	18.6	28.2
		N	5	5	5

No statistically significant differences

TABLE E

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

MALES			MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)					
			DOSE GROUP: DOSE LEVEL(MG/M ³):	1 0	2 2000	3 10000		
WEEK	0	TO	1	MEAN S.D. N	35 3.3 5	31 7.4 5	40 7.0 5	33 3.4 5
WEEK	0	TO	2	MEAN S.D. N	69 6.1 5	68 13.6 5	78 10.9 5	68 4.8 5
WEEK	0	TO	3	MEAN S.D. N	98 11.2 5	97 13.1 5	108 17.2 5	95 8.7 5
WEEK	0	TO	4	MEAN S.D. N	124 15.6 5	117 12.9 5	136 18.0 5	116 14.3 5
WEEK	0	TO	5	MEAN S.D. N	147 17.8 5	140 14.0 5	160 24.7 5	137 13.7 5
WEEK	0	TO	6	MEAN S.D. N	167 20.8 5	161 18.8 5	172 46.7 5	152 16.7 5

No statistically significant differences

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TABLE E

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

MALES				MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)			
				DOSE GROUP: DOSE LEVEL(MG/M3) :	1 0	2 2000	3 10000
WEEK	0	TO	7	MEAN	191	174	198
				S.D.	25.1	21.4	40.4
				N	5	5	5
WEEK	0	TO	8	MEAN	199	185	211
				S.D.	29.5	19.0	43.0
				N	5	5	5
WEEK	0	TO	9	MEAN	208	195	226
				S.D.	29.3	16.0	42.7
				N	5	5	5
WEEK	0	TO	10	MEAN	235	214	244
				S.D.	35.5	21.7	45.3
				N	5	5	5
WEEK	0	TO	11	MEAN	247	225	256
				S.D.	36.5	17.5	43.4
				N	5	5	5
WEEK	0	TO	12	MEAN	260	237	270
				S.D.	39.3	16.4	47.9
				N	5	5	5

No statistically significant differences

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TABLE E

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

MALES		MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)				
		DOSE GROUP:	1	2	3	4
		DOSE LEVEL (MG/M ³) :	0	2000	10000	20000
WEEK	0 TO 13	MEAN	273	248	276	255
		S.D.	40.9	20.2	54.3	15.6
		N	5	5	5	5

No statistically significant differences

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TABLE E

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

FEMALES			MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)			
			DOSE GROUP: DOSE LEVEL(MG/M ³):	1 0	2 2000	3 10000
WEEK	0	TO	1	MEAN	23	21
				S.D.	11.7	9.8
				N	5	5
WEEK	0	TO	2	MEAN	37	38
				S.D.	9.1	9.7
				N	5	5
WEEK	0	TO	3	MEAN	47	45
				S.D.	10.8	13.7
				N	5	5
WEEK	0	TO	4	MEAN	56	53
				S.D.	10.5	13.4
				N	5	5
WEEK	0	TO	5	MEAN	60	69
				S.D.	12.8	14.1
				N	5	5
WEEK	0	TO	6	MEAN	65	70
				S.D.	13.2	18.9
				N	5	5

NO statistically significant differences

TABLE E

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

FEMALES				MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)			
				DOSE GROUP: DOSE LEVEL(MG/M3) :	1 0	2 2000	3 10000
WEEK	0	TO	7	MEAN	71	73	74
				S.D.	14.7	12.4	19.1
				N	5	5	5
WEEK	0	TO	8	MEAN	71	77	71
				S.D.	15.0	15.2	22.9
				N	5	5	5
WEEK	0	TO	9	MEAN	76	82	83
				S.D.	20.0	18.3	19.2
				N	5	5	5
WEEK	0	TO	10	MEAN	80	89	84
				S.D.	19.2	16.4	17.6
				N	5	5	5
WEEK	0	TO	11	MEAN	85	88	90
				S.D.	16.3	22.8	22.5
				N	5	5	5
WEEK	0	TO	12	MEAN	87	90	90
				S.D.	19.3	21.3	22.5
				N	5	5	5

No statistically significant differences

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TABLE E

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

FEMALES		MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)				
		DOSE GROUP: DOSE LEVEL(MG/M3) :	1 0	2 2000	3 10000	4 20000
WEEK	0 TO 13	MEAN	86	100	98	88
		S.D.	24.6	20.0	18.9	8.0
		N	5	5	5	5

No statistically significant differences

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TABLE F

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

		MEAN FEED CONSUMPTION VALUES (GRAMS/KG/DAY)			
		DOSE GROUP: DOSE LEVEL (MG/M3):	1 0	2 2000	3 10000
WEEK	0	MEAN	99	101	100
		S.D.	4.3	2.1	3.3
		N	5	5	5
WEEK	1	MEAN	80	81	82
		S.D.	3.2	2.6	2.3
		N	5	5	5
WEEK	2	MEAN	74	75	73
		S.D.	2.6	2.3	5.0
		N	5	5	5
WEEK	3	MEAN	69	69	68
		S.D.	1.6	1.7	3.4
		N	5	5	5
WEEK	4	MEAN	65	62	63
		S.D.	1.6	3.0	3.9
		N	4	5	5
WEEK	5	MEAN	63	61	60
		S.D.	4.1	1.5	3.6
		N	5	5	5
WEEK	6	MEAN	58	57	54
		S.D.	3.7	2.2	5.1
		N	5	5	5
WEEK	7	MEAN	56	55	54
		S.D.	2.6	2.1	3.5
		N	5	5	5
WEEK	8	MEAN	54	52	51
		S.D.	3.1	2.2	1.7
		N	5	5	5

No statistically significant differences

TABLE F

GASOLINE ETBE 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: DOSE LEVEL(MG/M ³):	1 0	2 2000	3 10000
WEEK 9		MEAN	57	56	53
		S.D.	4.5	2.4	2.7
		N	5	5	5
WEEK 10		MEAN	54	53	49
		S.D.	4.6	2.1	2.7
		N	5	5	5
WEEK 11		MEAN	52	51	49
		S.D.	2.1	3.1	2.2
		N	5	5	5
WEEK 12		MEAN	52	51	47
		S.D.	4.7	2.9	1.9
		N	5	5	5
WEEK 13		MEAN	49	49	45*
		S.D.	2.9	1.1	1.5
		N	5	5	5

Statistical key: * = p<0.05

TABLE F

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

		MEAN FEED CONSUMPTION VALUES (GRAMS/KG/DAY)			
		DOSE GROUP: DOSE LEVEL(MG/M3):	1 0	2 2000	3 10000
WEEK	0	MEAN	103	104	95
		S.D.	6.0	2.9	7.1
		N	5	5	4
WEEK	1	MEAN	91	90	87
		S.D.	5.6	7.3	9.4
		N	5	5	4
WEEK	2	MEAN	83	87	81
		S.D.	6.2	5.0	5.9
		N	5	5	5
WEEK	3	MEAN	78	80	77
		S.D.	5.4	5.9	6.5
		N	5	5	5
WEEK	4	MEAN	74	77	72
		S.D.	3.5	6.1	2.2
		N	5	5	4
WEEK	5	MEAN	74	80	73
		S.D.	4.0	9.8	7.7
		N	5	5	5
WEEK	6	MEAN	70	70	74
		S.D.	2.9	5.9	12.3
		N	5	4	5
WEEK	7	MEAN	67	69	67
		S.D.	4.7	3.9	4.6
		N	5	5	5
WEEK	8	MEAN	61	68	56
		S.D.	3.5	5.6	15.9
		N	5	5	5

No statistically significant differences

TABLE F

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

FEMALES

		MEAN FEED CONSUMPTION VALUES (GRAMS/KG/DAY)				
		DOSE GROUP:	1	2	3	4
		DOSE LEVEL (MG/M ³):	0	2000	10000	20000
WEEK	9	MEAN	65	67	74	68
		S.D.	1.9	4.3	15.5	3.0
		N	5	4	5	5
WEEK	10	MEAN	65	64	63	63
		S.D.	4.0	4.9	7.0	2.7
		N	5	5	5	5
WEEK	11	MEAN	63	64	65	65
		S.D.	2.7	4.7	6.3	5.3
		N	5	5	5	5
WEEK	12	MEAN	63	61	66	65
		S.D.	2.6	4.6	7.3	2.6
		N	5	4	5	5
WEEK	13	MEAN	60	62	62	62
		S.D.	3.9	5.1	6.3	3.3
		N	5	5	5	5

No statistically significant differences

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TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP 1 0 MG/M³

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

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

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TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP 2 2000 MG/M³

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

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

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP 3 10000 MG/M³

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

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

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GASOLINE ETBE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTSMALES GROUP 4 20000 MG/M³

INDIVIDUAL CLINICAL OBSERVATIONS

ANIMAL#	OBSERVATIONS	DAY OF	1
		STUDY	1
4076	WITHIN NORMAL LIMITS		P
4077	WITHIN NORMAL LIMITS		P
4078	WITHIN NORMAL LIMITS		P
4079	WITHIN NORMAL LIMITS		P
4080	WITHIN NORMAL LIMITS		P

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

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP 1 0 MG/M3

ANIMAL#	OBSERVATIONS	DAY OF	1
		STUDY	1
1576	WITHIN NORMAL LIMITS	P	
1577	WITHIN NORMAL LIMITS	P	
1578	WITHIN NORMAL LIMITS	P	
1579	WITHIN NORMAL LIMITS	P	
1580	WITHIN NORMAL LIMITS	P	

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

FEMALES GROUP 2 2000 MG/M³

INDIVIDUAL CLINICAL OBSERVATIONS

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

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP 3 10000 MG/M³

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

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

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP 4 20000 MG/M³

ANIMAL#	OBSERVATIONS	DAY OF STUDY
4576	WITHIN NORMAL LIMITS	P
4577	WITHIN NORMAL LIMITS	P
4578	WITHIN NORMAL LIMITS	P
4579	WITHIN NORMAL LIMITS	P
4580	WITHIN NORMAL LIMITS	P

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

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

MALES	GROUP 1	0 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	WEEK -1
ANIMAL#	PART OF EYE	OBSERVATION		
1076		NO VISIBLE LESIONS		
1077		NO VISIBLE LESIONS		
1078		NO VISIBLE LESIONS		
1079		NO VISIBLE LESIONS		
1080		NO VISIBLE LESIONS		

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

MALES	GROUP 2	2000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS		WEEK -1
			ANIMAL#	PART OF EYE	
			2066		NO VISIBLE LESIONS
			2067		NO VISIBLE LESIONS
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	3069	NO VISIBLE LESIONS		
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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

MALES	GROUP 4	20000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	WEEK -1
ANIMAL#	PART OF EYE	OBSERVATION		
4076		NO VISIBLE LESIONS		
4077		NO VISIBLE LESIONS		
4078		NO VISIBLE LESIONS		
4079		NO VISIBLE LESIONS		
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ANIMAL#	PART OF EYE	OBSERVATION		
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ANIMAL#	PART OF EYE	OBSERVATION		
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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

MALES	GROUP 4	20000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	WEEK 13
ANIMAL#	PART OF EYE	OBSERVATION		
4076		NO VISIBLE LESIONS		
4077		NO VISIBLE LESIONS		
4078		NO VISIBLE LESIONS		
4079		NO VISIBLE LESIONS		
4080		NO VISIBLE LESIONS		

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
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			ANIMAL#	PART OF EYE	OBSERVATION	
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			2569		NO VISIBLE LESIONS	
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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

FEMALES	GROUP 3	10000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	WEEK 13
ANIMAL#	PART OF EYE	OBSERVATION		
3566		NO VISIBLE LESIONS		
3567		NO VISIBLE LESIONS		
3568		NO VISIBLE LESIONS		
3569		NO VISIBLE LESIONS		
3570		NO VISIBLE LESIONS		

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

FEMALES	GROUP 4	20000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	WEEK 13
ANIMAL#	PART OF EYE	OBSERVATION		
4576		NO VISIBLE LESIONS		
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4578		NO VISIBLE LESIONS		
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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

MALES GROUP 2 2000 MG/M3

INDIVIDUAL BODY WEIGHTS (GRAMS)

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

MALES GROUP 3 10000 MG/M3

INDIVIDUAL BODY WEIGHTS (GRAMS)

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

MNL EG GROUP 4 20000 MG/M3

INDIVIDUAL BODY WEIGHTS (GRAMS)

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INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP 1 0 MG/M3

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

FEMALES GROUP 2 2000 MG/M³

INDIVIDUAL BODY WEIGHTS (GRAMS)

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INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP 3 10000 MG/M3

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

FEMALES GROUP 4 20000 MG/M³

INDIVIDUAL BODY WEIGHTS (GRAMS)

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 1 0 MG/M3

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 2 2000 MG/M3

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 3 10000 MG/M3

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

MALES GROUP 4 20000 MG/M3

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INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
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INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 2 2000 MG/M3

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 3 10000 MG/M3

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GASOLINE ETBE VAPOR CONDENSATE: A 13 WEEK WHOLE-BODY
INHALATION TOXICITY STUDY IN RATS WITH NEUROTOXICITY ASSESSMENTS
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INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 4 20000 MG/M3

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GASOLINE ETBE 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	GROUP 1	0 MG/M3	INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)													
			WEEK OF STUDY													
ANIMAL#			0	1	2	3	4	5	6	7	8	9	10	11	12	13
1076			96	77	72	68	63	61	56	54	51	53	50	52	50	48
1077			104	82	75	68	64	60	55	54	52	57	55	52	50	47
1078			104	85	78	71	SF	69	64	60	59	64	61	55	60	54
1079			97	80	73	69	67	65	61	56	55	54	54	51	52	48
1080			96	78	73	68	64	59	56	55	52	55	49	50	48	48
MEAN			99	80	74	69	65	63	58	56	54	57	54	52	52	49
S.D.			4.3	3.2	2.6	1.6	1.6	4.1	3.7	2.6	3.1	4.5	4.6	2.1	4.7	2.9
N			5	5	5	5	4	5	5	5	5	5	5	5	5	5

SF=Spilled Feeder

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP 2 2000 MG/M3

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MALES GROUP 3 10000 MG/M3

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

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GASOLINE ETBE 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	GROUP 4	INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)													
		WEEK OF STUDY													
ANIMAL#		0	1	2	3	4	5	6	7	8	9	10	11	12	13
4076		95	77	78	72	65	61	61	58	58	58	55	54	56	50
4077		95	77	68	66	59	59	55	54	49	52	50	50	50	48
4078		98	82	75	69	64	64	59	60	62	61	57	59	59	55
4079	SF	83	77	71	65	64	61	58	62	57	56	55	54	52	
4080		99	82	75	70	63	61	59	55	49	53	51	52	51	49
MEAN		97	80	75	69	63	62	59	57	56	56	54	54	54	51
S.D.		2.0	3.1	3.8	2.4	2.7	2.4	2.6	2.0	6.5	3.9	2.9	3.4	3.4	2.9
N		4	5	5	5	5	5	5	5	5	5	5	5	5	5

SF=Spilled Feeder

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP 1 0 MG/M3

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

		INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)													
FEMALES	GROUP 2	2000 MG/M ³													
ANIMAL#		WEEK OF STUDY													
		0	1	2	3	4	5	6	7	8	9	10	11	12	13
2566		100	83	86	73	71	76	69	70	64	64	65	58	56	67
2567		106	90	90	81	80	79	74	74	75	SF	70	69	SF	66
2568		107	84	79	74	70	69	62	63	61	62	57	60	58	54
2569		103	94	93	86	85	95	SF	70	71	71	67	67	63	65
2570		106	100	88	84	79	79	75	70	69	69	61	66	66	60
MEAN		104	90	87	80	77	80	70	69	68	67	64	64	61	62
S.D.		2.9	7.3	5.0	5.9	6.1	9.8	5.9	3.9	5.6	4.3	4.9	4.7	4.6	5.1
N		5	5	5	5	5	5	4	5	5	4	5	5	4	5

SF=Spilled Feeder

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GASOLINE ETBE 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)													
		WEEK OF STUDY													
ANIMAL#		0	1	2	3	4	5	6	7	8	9	10	11	12	13
3566	SF	SF	89	87	SF	82	96	73	29	101	74	65	78	70	
3567	95	89	76	71	72	75	69	68	67	73	64	75	68	67	
3568	104	97	82	75	73	78	69	65	65	69	60	64	63	59	
3569	93	85	82	77	74	67	70	65	63	67	62	61	63	62	
3570	87	75	74	72	69	63	66	61	58	62	55	59	59	54	
MEAN	95	87	81	77	72	73	74	67	56	74	63	65	66	62	
S.D.	7.1	9.4	5.9	6.5	2.2	7.7	12.3	4.6	15.9	15.5	7.0	6.3	7.3	6.3	
N	4	4	5	5	4	5	5	5	5	5	5	5	5	5	

SF=Spilled Feeder

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GASOLINE ETBE 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 GROUP 4 20000 MG/M3

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

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

ANIMAL TERMINATION HISTORY

MALES GROUP 1 0 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1076	TERMINAL SACRIFICE	23-JAN-02	13	92
1077	TERMINAL SACRIFICE	23-JAN-02	13	92
1078	TERMINAL SACRIFICE	23-JAN-02	13	92
1079	TERMINAL SACRIFICE	23-JAN-02	13	92
1080	TERMINAL SACRIFICE	23-JAN-02	13	92

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

ANIMAL TERMINATION HISTORY

MALES GROUP 2 2000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2066	TERMINAL SACRIFICE	23-JAN-02	13	92
2067	TERMINAL SACRIFICE	23-JAN-02	13	92
2068	TERMINAL SACRIFICE	23-JAN-02	13	92
2069	TERMINAL SACRIFICE	23-JAN-02	13	92
2070	TERMINAL SACRIFICE	23-JAN-02	13	92

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

ANIMAL TERMINATION HISTORY

MALES GROUP 3 10000 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF	STUDY
			STUDY	DAY
3066	TERMINAL SACRIFICE	23-JAN-02	13	92
3067	TERMINAL SACRIFICE	23-JAN-02	13	92
3068	TERMINAL SACRIFICE	23-JAN-02	13	92
3069	TERMINAL SACRIFICE	23-JAN-02	13	92
3070	TERMINAL SACRIFICE	23-JAN-02	13	92

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

ANIMAL TERMINATION HISTORY

MALES GROUP 4 20000 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4076	TERMINAL SACRIFICE	23-JAN-02	13	92
4077	TERMINAL SACRIFICE	23-JAN-02	13	92
4078	TERMINAL SACRIFICE	23-JAN-02	13	92
4079	TERMINAL SACRIFICE	23-JAN-02	13	92
4080	TERMINAL SACRIFICE	23-JAN-02	13	92

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP 1 0 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1576	TERMINAL SACRIFICE	23-JAN-02	13	92
1577	TERMINAL SACRIFICE	23-JAN-02	13	92
1578	TERMINAL SACRIFICE	23-JAN-02	13	92
1579	TERMINAL SACRIFICE	23-JAN-02	13	92
1580	TERMINAL SACRIFICE	23-JAN-02	13	92

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

ANIMAL TERMINATION HISTORY

FEMALES GROUP 2 2000 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2566	TERMINAL SACRIFICE	23-JAN-02	13	92
2567	TERMINAL SACRIFICE	23-JAN-02	13	92
2568	TERMINAL SACRIFICE	23-JAN-02	13	92
2569	TERMINAL SACRIFICE	23-JAN-02	13	92
2570	TERMINAL SACRIFICE	23-JAN-02	13	92

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ANIMAL TERMINATION HISTORY

FEMALES GROUP 3 10000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3566	TERMINAL SACRIFICE	23-JAN-02	13	92
3567	TERMINAL SACRIFICE	23-JAN-02	13	92
3568	TERMINAL SACRIFICE	23-JAN-02	13	92
3569	TERMINAL SACRIFICE	23-JAN-02	13	92
3570	TERMINAL SACRIFICE	23-JAN-02	13	92

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

ANIMAL TERMINATION HISTORY

FEMALES GROUP 4 20000 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4576	TERMINAL SACRIFICE	23-JAN-02	13	92
4577	TERMINAL SACRIFICE	23-JAN-02	13	92
4578	TERMINAL SACRIFICE	23-JAN-02	13	92
4579	TERMINAL SACRIFICE	23-JAN-02	13	92
4580	TERMINAL SACRIFICE	23-JAN-02	13	92