

Summary Report

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

Huntingdon Life Sciences, Inc. Study No. 00-6130

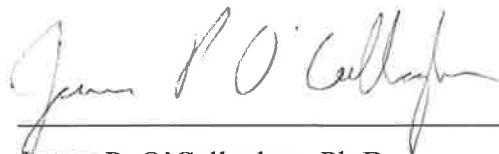
Sponsor Study No 211-DIPE-S

Measurement of Glial Fibrillary Acidic Protein

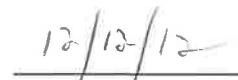
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STATEMENT OF COMPLIANCE

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

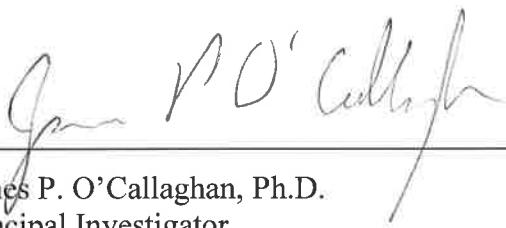

Gary M. Hoffman, B.A., D.A.B.T.
Study Director

Date

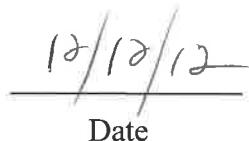
SIGNATURE PAGE

SCIENTIST

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



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



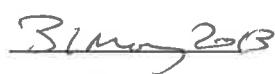
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SCIENTIFIC REVIEW

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 00-6130

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

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

<u>Area Inspected</u>	<u>Date of Inspection</u>	<u>Date Reported to Sponsor</u>
Facility Inspection	2/14/01	2/21/01
Facility Inspection	4/3/01	4/10/01
Draft Final Report Audit	5/3-6/6/04	6/6/04
Second Draft Final Report Review	2/27-29/12	2/29/12

Christine Lehmitte 3/1/12

Christine Sexsmith Date

Sexsmith Consulting Services, LLC

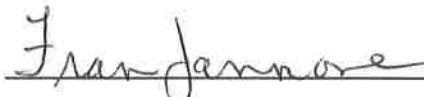
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QUALITY ASSURANCE STATEMENT

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
General Facility Inspection	08 Nov 00	11 Nov 00 ^a
GLP Protocol Review	16 Nov 01	19 Nov 01
Positive Control Dose Preparation and Administration	10 Apr 02	10 Apr 02
Genotoxicity Necropsy	11 Apr 02	11 Apr 02
Subcontractor Report and Study Data	17 Dec 02, 04 Mar 03	04 Mar 03



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



Date

^aGeneral Facility Inspection reported to Testing Facility Management.

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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 DIPE Vapor Condensate did not consistently elevate GFAP levels in any of the nine brain regions examined in either males or females. The data suggest that exposure to Gasoline DIPE 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, 1992). These findings indicate that assays of GFAP represent a sensitive, simple and quantitative approach for evaluation of nervous system damage (O'Callaghan, 1991; Norton et al., 1992).

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

assessment constitutes a portion of these testing activities. Subchronic (13-week) inhalation exposures to gasoline and gasoline plus each of 6 fuel additives have been performed along with a two-generation reproduction toxicity study that includes a neurotoxicity component for gasoline and gasoline plus MTBE vapor condensates only. The purpose of the present study was to use the GFAP assay for assessing the potential neurotoxic effects of Gasoline DIPE 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

17 December 2001 (Date Study Director signed the Protocol)

DATE OF ANIMAL RECEIPT

24 January 2002

EXPOSURE INITIATION

12 February 2002 (Experimental Start Date)

EXPOSURE TERMINATION

14 May 2002

TERMINAL SACRIFICE

15 May 2002

EXPERIMENTAL TERMINATION

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

STUDY COMPLETION

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

I. Basic Protocol 1: GFAP Sandwich ELISA

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

B. **Materials:**

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

02, 2 mm probe)

Pipettes

Hot/Stir Plate

Microplate Reader

96-well Microtiter Plates (Immulon 2, Dynatech)

Pipette tips

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

#Z0334, Lot #096(401))

Non-Fat Dry Milk (Carnation)

Mouse anti-Glial Fibrillary Acidic Protein Antibody (Oncogene Research Products Cat. #IFO3L, Lot #D15158-3)

Phosphate Buffered Saline (e.g. Pierce Cat. #28374)

Alkaline Phosphatase conjugated anti-mouse IgG (Jackson Immuno Research Cat #315-055-003)

Alkaline Phosphatase Substrate Kit (e.g. Bio-Rad Cat. #172-1063)

Triton X-100 (e.g. Bio-Rad Cat. #161-0407)

Sodium Hydroxide (e.g., 0.4N NaOH, Fisher LC 243204)

C. Protocol Steps:

1. Standard Curve Preparation

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

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

2. Sample Preparation

1. Thaw and mix samples: Remove samples from the freezer, thaw at room temperature and vortex or sonify prior to dilution.
2. Prepare dilutions of the samples: Dilute the samples in PBS + 0.5% Triton X-100 to a concentration of approximately 10 μ g total protein/100 μ l. Samples high in GFAP (e.g. cerebellum) may need to be diluted to a concentration of 5 μ g total protein/100 μ l of PBS + 0.5% Triton X-100. Samples low in GFAP (e.g. striatum) may need to be diluted to 20 μ g total protein/100 μ l 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 1.0 μ g total immunoglobulin protein /100 μ l PBS /well. (~25 μ l 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 μ l per well), tapping and blotting between each wash.
5. Block 1 hour with 5% non-fat powdered milk in PBS at 100 μ l 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 μ l per well. Incubate for 1 hour. The template, Table 2, is an example of a typical 96-well microplate layout for GFAP standards and unknowns.
7. Wash 4X with PBS + 0.5% Triton X-100, 200 μ l/well.
8. Incubate for 1 hour in monoclonal anti-GFAP (1:500)(Chemicon) + alkaline phosphatase conjugated anti-mouse IgG (1:3000)(Jackson ImmunoResearch) made up in 5% non-fat dry milk + 0.5% Triton X-100, 100 μ l /well.
9. Wash 4X with PBS + 0.5% Triton X-100, 200 μ l/well.
10. Add P-nitrophenylphosphate substrate (Bio-Rad) in a volume of 100 μ l/well and incubate for 20 minutes.
11. Stop reaction with 0.4N NaOH, 100 μ l/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. Thermolectrics cold plate, Aldrich Chemical Co. or simply an inverted petri dish placed on ice) maintained at approximately 4°C. Rat or mouse brains can be dissected into 10-15 regions, free hand, within approximately 10 minutes. For this study, nine regions were dissected: Striatum, Hippocampus, Cortex, Olfactory Bulb, Thalamus, Hypothalamus, Cerebellum, Pituitary, and Rest of Brain. *This number of regions can be prepared on a cold plate or at room temperature without degradation of GFAP as assessed by immunoblot analysis. All brain*

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

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

III. Support Protocol 2: Assay for Total Protein

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

B. Materials:

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

C. Protocol Steps:

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

a well of the microtiter plate; add 10 μ l 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 μ l aliquot with 190 μ l of 1% SDS. Vortex the dilution tube and add a 10 μ l aliquot into a well of a microtiter plate.
4. Add the protein assay reagent. Add 200 μ l 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 μ l/well and 96 wells per plate (washes take 200 μ l/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(BLOTTTO)**- (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 25 μ l 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 20 μ l of the monoclonal antibody solution stock and 3.3 μ l 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 DIPE Vapor Condensate did not elevate levels in any brain region in either males or females. These data suggest that under the exposure conditions employed, damage-induced gliosis did not occur in the brain regions examined. Toxicant-induced gliosis is highly dose-, region-, and time-dependent. It is possible, therefore, that exposure to higher concentrations, examination of more brain regions, examination of more discrete dissections of a given brain region, or the inclusion of more time points, might have resulted in positive findings. In conclusion, exposure to Gasoline DIPE 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.

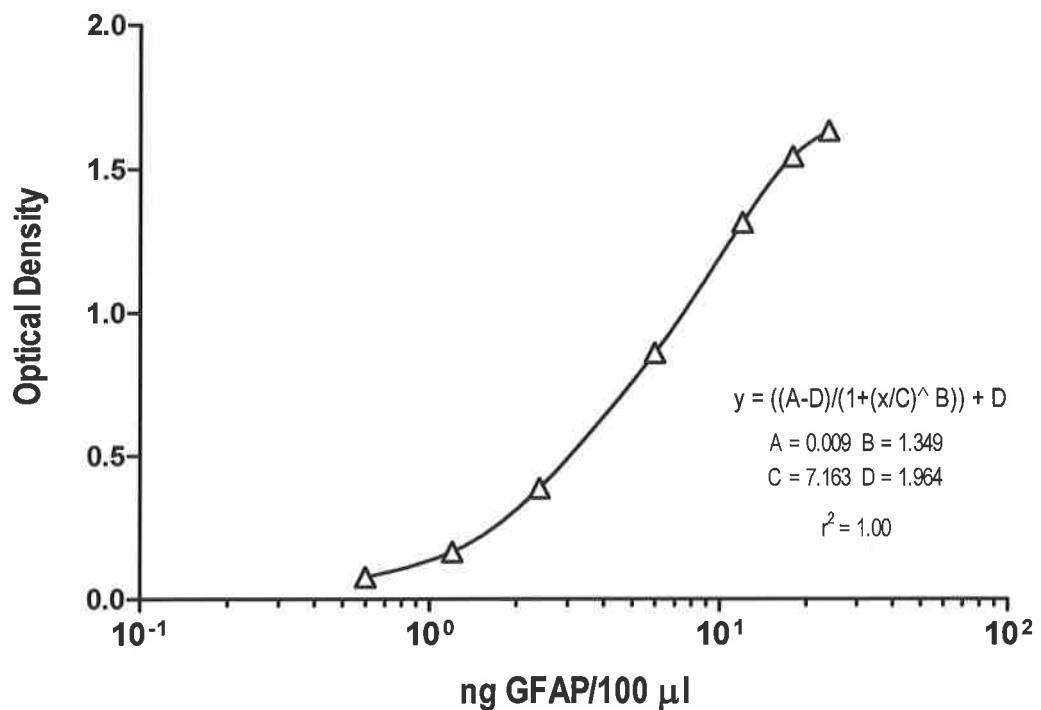
Figure 1

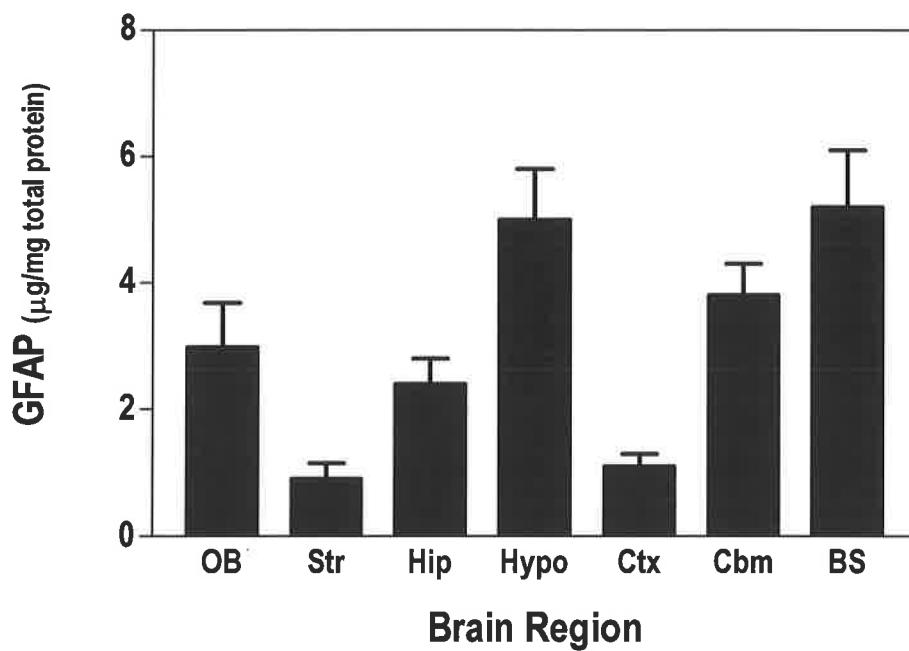
Figure 2

TABLE 1: GFAP Standard Curve Preparation

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

Table 3: Sample Values for GFAP Standard Curve

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

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

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

Table 4: Troubleshooting Guide

Problem	Possible Cause	Solution
No color reaction	Incorrect preparation of color reagent Antibody was not as specified in the protocol One or more antibodies were omitted or used at the wrong dilution	If color reaction has not been terminated, remove reagent, add new color reagent and continue assay Obtain correct antibody and repeat assay Repeat assay with proper reagents used at the correct dilutions
Color reaction abnormally low	Incubator was set at less than 37°C Antibody solution too dilute; incorrect preparation of color reagent	Repeat assay with incubator temperature set at 37°C Repeat assay with correct reagent dilutions
Color reaction abnormally high	P-nitrophenylphosphate substrate kit is too old Color reaction was not terminated	Repeat assay with fresh kit Repeat assay and terminate reaction with 0.4 N NaOH
Standard curve not sigmoid	Incorrect plate template set in the plate reader Incorrect standard dilution	Use correct plate template and re-read plate Repeat assay with correct standard dilution
Samples not on linear portion of curve	Incorrect standard dilution Incorrect sample dilution	Repeat assay with correct dilution of standard 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 Poor pipetting technique Plate washer malfunction Bubbles throughout the plate	Change tips after each use Check precision by weighing Check plate washer for even dispensing and aspiration 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 DIPE 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	1.02 ± 0.11*	1.31 ± 0.17	1.13 ± 0.11	1.15 ± 0.12
Hippocampus	3.31 ± 0.22	3.34 ± 0.15	3.25 ± 0.34	2.96 ± 0.21
Cortex	1.22 ± 0.12	1.27 ± 0.08	1.20 ± 0.17	1.30 ± 0.13
Olfactory Bulb	1.71 ± 0.18	1.99 ± 0.12	1.78 ± 0.05	1.96 ± 0.14
Thalamus	1.98 ± 0.18	1.99 ± 0.16	1.93 ± 0.17	1.91 ± 0.17
Hypothalamus	7.08 ± 1.47	6.68 ± 0.75	6.04 ± 0.38	6.08 ± 0.69
Cerebellum	4.77 ± 0.45	5.52 ± 0.80	4.90 ± 0.61	4.24 ± 0.43
Rest of Brain	4.73 ± 0.20	4.52 ± 0.36	5.31 ± 0.54	4.77 ± 0.34

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

Table 6: Mean GFAP Levels in Specific Regions of Female Rat Brains Following a 13-Week Whole-Body Inhalation Exposure to Gasoline DIPE 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	1.25 ± 0.19*	1.41 ± 0.13	1.23 ± 0.13	0.97 ± 0.13
Hippocampus	3.16 ± 0.13	3.42 ± 0.26	3.17 ± 0.22	2.83 ± 0.16
Cortex	1.10 ± 0.12	1.42 ± 0.10	1.32 ± 0.06	1.15 ± 0.09
Olfactory Bulb	2.00 ± 0.13	2.12 ± 0.24	1.86 ± 0.15	1.75 ± 0.17
Thalamus	1.85 ± 0.11	1.94 ± 0.20	1.84 ± 0.10	1.76 ± 0.18
Hypothalamus	6.28 ± 0.77	8.11 ± 1.29	6.16 ± 0.61	4.73 ± 1.06
Cerebellum	4.19 ± 0.27	4.83 ± 0.23	4.26 ± 0.33	3.96 ± 0.26
Rest of Brain	4.72 ± 0.31	5.04 ± 0.36	4.83 ± 0.27	4.22 ± 0.28

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

**Table 7: Individual GFAP Levels in Specific Regions of Rat Brains
Following a 13 Week Whole-Body Inhalation
Exposure to Gasoline DIPE 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	1079	Male	0.739*	3.420	1.399	2.212	2.398	9.084	5.450	*	4.860
	1080		1.148	3.748	0.892	1.863	1.994	3.556	3.978	*	4.709
	1076		1.187	3.711	1.444	1.733	2.348	8.552	6.185	0.057	5.418
	1077		1.251	3.050	1.374	1.583	1.429	3.620	4.259	0.045	4.362
	1078		0.766	2.595	0.966	1.143	1.751	10.594	3.953	*	4.311
	1580	Female	1.138	3.171	1.079	2.118	1.843	7.724	3.997	*	4.752
	1578		1.596	3.479	0.760	1.986	1.915	5.722	4.137	0.045	5.353
	1577		1.554	2.830	1.186	2.037	1.669	4.269	3.974	*	4.026
	1579		1.418	3.393	1.469	2.330	2.209	8.393	5.232	*	5.451
	1576		0.551	2.904	1.018	1.535	1.614	5.313	3.632	*	4.037
Group II Test Substance 2,000 mg/m ³	2069	Male	0.852	2.807	0.969	1.986	1.541	6.243	4.527	*	3.844
	2068		1.215	3.575	1.307	1.892	1.873	8.475	8.672	*	5.024
	2060		1.913	3.340	1.447	2.296	2.474	8.116	5.337	*	5.701
	2067		1.296	3.293	1.347	1.601	1.817	4.331	4.501	*	4.088
	2066		1.270	3.662	1.301	2.154	2.228	6.233	4.570	*	3.952
	2567	Female	1.453	3.036	1.219	1.938	1.458	12.365	4.243	*	4.691
	2566		0.946	2.973	1.291	1.543	1.542	4.241	4.466	*	4.140
	2569		1.483	4.200	1.603	2.327	2.490	8.167	4.748	*	6.177
	2570		1.431	3.893	1.281	2.927	1.953	7.893	5.139	*	5.526
	2568		1.735	2.998	1.690	1.838	2.254	7.900	5.543	*	4.665
Group III Test Substance 10,000 mg/m ³	3066	Male	1.539	3.777	1.786	1.874	2.443	7.055	4.984	*	6.412
	3068		1.064	2.860	1.120	1.867	1.706	6.380	4.183	*	3.693
	3070		1.129	4.059	0.864	1.651	1.632	5.006	4.924	*	4.781
	3067		1.003	3.432	1.336	1.822	2.219	6.424	7.052	*	6.606
	3069		0.927	2.136	0.911	1.680	1.662	5.313	3.358	*	5.060
	3567	Female	1.158	2.843	1.301	1.763	1.825	4.639	3.588	*	4.577
	3569		1.137	2.559	1.240	1.763	1.466	5.189	3.390	*	4.302
	3568		1.429	3.235	1.551	2.439	1.975	6.022	4.454	*	5.310
	3566		0.853	3.400	1.183	1.700	1.905	6.857	4.944	*	4.338
	3570		1.582	3.808	1.344	1.646	2.046	8.101	4.941	*	5.622
Group IV Test Substance 20,000 mg/m ³	4077	Male	1.040	2.892	1.247	2.100	2.152	6.640	3.839	0.038	4.745
	4079		0.994	2.933	1.204	1.876	1.822	6.714	4.580	0.048	5.207
	4076		1.047	2.439	1.070	1.840	1.603	4.502	3.400	*	3.613
	4078		1.635	3.703	1.819	2.423	2.426	8.040	5.766	0.039	5.610
	4080		1.048	2.842	1.175	1.573	1.527	4.487	3.604	*	4.687
	4576	Female	0.502	2.820	1.090	1.763	1.479	5.424	3.917	*	4.609
	4580		1.197	3.070	1.260	1.667	1.864	5.891	3.931	*	4.623
	4579		1.074	2.592	1.086	1.536	1.626	1.612	3.841	*	3.871
	4578		0.895	2.373	0.907	1.407	1.393	3.122	3.255	*	3.284
	4577		1.196	3.286	1.413	2.389	2.411	7.609	4.874	*	4.705

* 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: 24 January 2002
Experimental Initiation Date: 12 February 2002 (in-life)
Experimental Completion Date: 15 May 2002 (in-life)

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

Note: Animal No. 2070 was sacrificed for humane reasons as a result of accidental injury prior to the Week 13 GFAP evaluations. Therefore, one animal from the main study (Animal No. 2060) was used in its place for the necropsy. All in-life data for Animal No. 2070 is in the following tables/appendices while all in-life data for Animal No. 2060 is located in the main study's tables/appendices. Macroscopic observations for Animal No. 2070 were as follows: skin - moderate thin/absent hair, upper dorsal; GI tract – extreme gaseous distension, entire length; liver – slight tan discoloration, focus, left lobe (0.2 to 0.5 cm); kidney – extreme tan discolored area, right, 1.0 to 1.5 cm).

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Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results							Particle Size Determinations			Chamber Environment				
			Nominal (mg/m ³)	Analytical Chamber Concentration				Mean (mg/m ³)	Individual (mg/m ³)				Mean Temperature (°C)	Humidity (%)			
				Mean (mg/m ³)	Individual (mg/m ³)	Mean (mg/m ³)	Individual (mg/m ³)						Temperature (°C)	Humidity (%)			
0	12-Feb-02	1	0	0	0	0	0	0	0				24	57			
1	13-Feb-02	2	0	0	0	0	0	0	0				25	58			
2	14-Feb-02	3	0	0	0	0	0	0	0				25	58			
3	15-Feb-02	4	0	0	0	0	0	0	0				25	56			
6	18-Feb-02	5	0	0	0	0	0	0	0				25	56			
7	19-Feb-02	6	0	0	0	0	0	0	0	1.209	1.495	1.94E-03	25	56			
8	20-Feb-02	7	0	0	0	0	0	0	0				24	58			
9	21-Feb-02	8	0	0	0	0	0	0	0				24	54			
10	22-Feb-02	9	0	0	0	0	0	0	0				24	55			
13	25-Feb-02	10	0	0	0	0	0	0	0	4.471	2.682	7.37E-03	24	51			
14	26-Feb-02	11	0	0	0	0	0	0	0				26	58			
15	27-Feb-02	12	0	0	0	0	0	0	0				25	53			
16	28-Feb-02	13	0	0	0	0	0	0	0				25	57			
17	01-Mar-02	14	0	0	0	0	0	0	0				25	58			
20	04-Mar-02	15	0	0	0	0	0	0	0	2.303	2.369	3.03E-03	25	54			
21	05-Mar-02	16	0	0	0	0	0	0	0				24	44			
22	06-Mar-02	17	0	0	0	0	0	0	0				24	54			
23	07-Mar-02	18	0	0	0	0	0	0	0				24	59			
24	08-Mar-02	19	0	0	0	0	0	0	0				24	54			
27	11-Mar-02	20	0	0	0	0	0	0	0	1.520	1.876	3.20E-03	24	55			
28	12-Mar-02	21	0	0	0	0	0	0	0				25	52			
29	13-Mar-02	22	0	0	0	0	0	0	0				21	53			
30	14-Mar-02	23	0	0	0	0	0	0	0				25	54			
31	15-Mar-02	24	0	0	0	0	0	0	0				25	53			

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results									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)								Temperature (°C)	Humidity (%)		
32	16-Mar-02	25	0	0	0	0	0	0	0	0.9053	2.137	1.20E-02	27	54		
34	18-Mar-02	26	0	0	0	0	0	0	0				25	53		
35	19-Mar-02	27	0	0	0	0	0	0	0				24	54		
36	20-Mar-02	28	0	0	0	0	0	0	0				24	52		
37	21-Mar-02	29	0	0	0	0	0	0	0				24	53		
38	22-Mar-02	30	0	0	0	0	0	0	0				24	58		
41	25-Mar-02	31	0	0	0	0	0	0	0	2.495	2.130	4.91E-03	24	53		
42	26-Mar-02	32	0	0	0	0	0	0	0				25	51		
43	27-Mar-02	33	0	0	0	0	0	0	0				25	52		
44	28-Mar-02	34	0	0	0	0	0	0	0				25	55		
45	29-Mar-02	35	0	0	0	0	0	0	0	2.357	2.501	7.60E-03	25	54		
48	01-Apr-02	36	0	0	0	0	0	0	0				25	50		
49	02-Apr-02	37	0	0	0	0	0	0	0				24	56		
50	03-Apr-02	38	0	0	0	0	0	0	0				23	52		
51	04-Apr-02	39	0	0	0	0	0	0	0				24	55		
52	05-Apr-02	40	0	0	0	0	0	0	0	2.187	2.049	2.32E-03	23	55		
55	08-Apr-02	41	0	0	0	0	0	0	0				24	51		
56	09-Apr-02	42	0	0	0	0	0	0	0				25	51		
57	10-Apr-02	43	0	0	0	0	0	0	0				25	53		
58	11-Apr-02	44	0	0	0	0	0	0	0				24	52		
59	12-Apr-02	45	0	0	0	0	0	0	0	1.413	1.998	8.01E-03	24	50		
62	15-Apr-02	46	0	0	0	0	0	0	0				25	47		
63	16-Apr-02	47	0	0	0	0	0	0	0				24	54		
64	17-Apr-02	48	0	0	0	0	0	0	0				24	52		

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Mean Humidity (%)
65	18-Apr-02	49	0	0	0	0	0	0	1.767	2.858	5.16E-03	24	52
66	19-Apr-02	50	0	0	0	0	0	0				24	49
69	22-Apr-02	51	0	0	0	0	0	0				24	50
70	23-Apr-02	52	0	0	0	0	0	0				25	54
71	24-Apr-02	53	0	0	0	0	0	0				25	55
72	25-Apr-02	54	0	0	0	0	0	0				25	52
73	26-Apr-02	55	0	0	0	0	0	0	2.130	1.927	4.07E-03	25	54
76	29-Apr-02	56	0	0	0	0	0	0				25	49
77	30-Apr-02	57	0	0	0	0	0	0				24	53
78	01-May-02	58	0	0	0	0	0	0				24	53
79	02-May-02	59	0	0	0	0	0	0				24	51
80	03-May-02	60	0	0	0	0	0	0	1.325	1.605	1.29E-03	24	54
83	06-May-02	61	0	0	0	0	0	0				24	51
84	07-May-02	62	0	0	0	0	0	0				24	52
85	08-May-02	63	0	0	0	0	0	0				25	51
86	09-May-02	64	0	0	0	0	0	0				24	51
87	10-May-02	65	0	0	0	0	0	0	1.594	2.093	2.01E-03	24	50
89	12-May-02	66	0	0	0	0	0	0				25	50
90	13-May-02	67	0	0	0	0	0	0				24	54
91	14-May-02	68	0	0	0	0	0	0				22	53
92	15-May-02	69	0	0	0	0	0	0				22	56
Mean			0		0				1.975	2.132	4.84E-03	24.3	53.3
S.D.			0		0				0.899	0.392	3.13E-03	0.9	2.8

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)
0	12-Feb-02	1	0	0	0	0	0	0				23	58
1	13-Feb-02	2	0	0	0	0	0	0				23	59
2	14-Feb-02	3	0	0	0	0	0	0				24	60
3	15-Feb-02	4	0	0	0	0	0	0				24	58
6	18-Feb-02	5	0	0	0	0	0	0				24	57
7	19-Feb-02	6	0	0	0	0	0	0				24	59
8	20-Feb-02	7	0	0	0	0	0	0				24	55
9	21-Feb-02	8	0	0	0	0	0	0				24	54
10	22-Feb-02	9	0	0	0	0	0	0				24	57
13	25-Feb-02	10	0	0	0	0	0	0	3.222	2.309	7.63E-03	24	54
14	26-Feb-02	11	0	0	0	0	0	0				25	59
15	27-Feb-02	12	0	0	0	0	0	0				24	56
16	28-Feb-02	13	0	0	0	0	0	0				23	69
17	01-Mar-02	14	0	0	0	0	0	0				24	58
20	04-Mar-02	15	0	0	0	0	0	0	2.090	1.807	3.69E-03	24	55
21	05-Mar-02	16	0	0	0	0	0	0				24	45
22	06-Mar-02	17	0	0	0	0	0	0				24	55
23	07-Mar-02	18	0	0	0	0	0	0				25	59
24	08-Mar-02	19	0	0	0	0	0	0				24	55
27	11-Mar-02	20	0	0	0	0	0	0	1.465	1.966	2.72E-03	24	55
28	12-Mar-02	21	0	0	0	0	0	0				23	55
29	13-Mar-02	22	0	0	0	0	0	0				22	56
30	14-Mar-02	23	0	0	0	0	0	0				24	57
31	15-Mar-02	24	0	0	0	0	0	0				23	55

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results									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)							Temperature (°C)	Humidity (%)			
32	16-Mar-02	25	0	0	0	0	0	0	0.9933	2.559	1.41E-02	26	53			
34	18-Mar-02	26	0	0	0	0	0	0				24	51			
35	19-Mar-02	27	0	0	0	0	0	0				24	55			
36	20-Mar-02	28	0	0	0	0	0	0				24	53			
37	21-Mar-02	29	0	0	0	0	0	0				24	53			
38	22-Mar-02	30	0	0	0	0	0	0				24	58			
41	25-Mar-02	31	0	0	0	0	0	0	3.236	2.579	3.65E-03	24	54			
42	26-Mar-02	32	0	0	0	0	0	0				23	55			
43	27-Mar-02	33	0	0	0	0	0	0				24	54			
44	28-Mar-02	34	0	0	0	0	0	0				24	58			
45	29-Mar-02	35	0	0	0	0	0	0	1.240	2.374	4.86E-03	24	56			
48	01-Apr-02	36	0	0	0	0	0	0				24	52			
49	02-Apr-02	37	0	0	0	0	0	0				24	56			
50	03-Apr-02	38	0	0	0	0	0	0				24	53			
51	04-Apr-02	39	0	0	0	0	0	0				24	56			
52	05-Apr-02	40	0	0	0	0	0	0	2.023	1.994	2.81E-03	24	56			
55	08-Apr-02	41	0	0	0	0	0	0				24	52			
56	09-Apr-02	42	0	0	0	0	0	0				24	56			
57	10-Apr-02	43	0	0	0	0	0	0				24	55			
58	11-Apr-02	44	0	0	0	0	0	0				23	55			
59	12-Apr-02	45	0	0	0	0	0	0	1.877	2.932	9.96E-03	23	53			
62	15-Apr-02	46	0	0	0	0	0	0				23	49			
63	16-Apr-02	47	0	0	0	0	0	0				24	55			
64	17-Apr-02	48	0	0	0	0	0	0				24	54			

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Mean Humidity (%)
65	18-Apr-02	49	0	0	0	0	0	0	1.879	2.460	5.66E-03	24	53
66	19-Apr-02	50	0	0	0	0	0	0				24	50
69	22-Apr-02	51	0	0	0	0	0	0				24	51
70	23-Apr-02	52	0	0	0	0	0	0				23	58
71	24-Apr-02	53	0	0	0	0	0	0				24	58
72	25-Apr-02	54	0	0	0	0	0	0				23	55
73	26-Apr-02	55	0	0	0	0	0	0	11.62	2.643	1.06E-02	23	53
76	29-Apr-02	56	0	0	0	0	0	0				24	52
77	30-Apr-02	57	0	0	0	0	0	0				24	54
78	01-May-02	58	0	0	0	0	0	0				24	54
79	02-May-02	59	0	0	0	0	0	0				24	53
80	03-May-02	60	0	0	0	0	0	0	1.503	1.759	1.54E-03	25	55
83	06-May-02	61	0	0	0	0	0	0				25	51
84	07-May-02	62	0	0	0	0	0	0				23	55
85	08-May-02	63	0	0	0	0	0	0				24	54
86	09-May-02	64	0	0	0	0	0	0				23	52
87	10-May-02	65	0	0	0	0	0	0	1.708	1.749	2.43E-03	23	54
89	12-May-02	66	0	0	0	0	0	0				23	53
90	13-May-02	67	0	0	0	0	0	0				23	57
91	14-May-02	68	0	0	0	0	0	0				22	54
Mean			0		0				2.622	2.212	5.49E-03	23.8	55.0
S.D.			0		0				2.790	0.417	3.94E-03	0.7	3.1

Table A

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

00-6130

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)
0	12-Feb-02	1	1940	1985	1990	1860	2020	2070				23	52
1	13-Feb-02	2	1990	1930	1770	1910	2020	2020				24	53
2	14-Feb-02	3	1950	1953	2000	1990	2020	1800				23	52
3	15-Feb-02	4	2150	2085	1900	2170	2150	2120				24	50
6	18-Feb-02	5	2010	1995	1980	2000	1980	2020				24	50
7	19-Feb-02	6	2090	2025	1920	2010	2070	2100				24	52
8	20-Feb-02	7	2060	1985	1920	1980	2020	2020				24	48
9	21-Feb-02	8	2130	1998	2000	1940	2030	2020				24	48
10	22-Feb-02	9	2000	1950	2300	1610	1940	1950				24	49
13	25-Feb-02	10	2090	1995	1920	1900	2050	2110	1.182	2.060	4.38E-03	24	48
14	26-Feb-02	11	1970	1998	2080	1910	2000	2000				25	50
15	27-Feb-02	12	1990	1958	1920	1960	2010	1940				24	48
16	28-Feb-02	13	2030	2010	2050	1980	1990	2020				23	51
17	01-Mar-02	14	2030	2035	2040	2050	2050	2000				23	52
20	04-Mar-02	15	2050	1998	2090	1980	2000	1920	1.832	2.321	2.65E-03	24	50
21	05-Mar-02	16	1900	1865	1940	1730	1800	1990				23	42
22	06-Mar-02	17	2100	2000	2010	1990	2000	2000				23	50
23	07-Mar-02	18	2090	1915	2020	1750	1970	1920				24	55
24	08-Mar-02	19	2150	1983	1900	2030	2020	1980				23	50
27	11-Mar-02	20	2020	1928	2020	1820	1970	1900	1.506	2.053	3.51E-03	24	52
28	12-Mar-02	21	2010	1883	1850	2130	1790	1760				23	50
29	13-Mar-02	22	2010	1988	1950	2020	1980	2000				22	49
30	14-Mar-02	23	2080	1905	1860	1840	1900	2020				23	50
31	15-Mar-02	24	2160	1968	1870	1920	1950	2130				23	50

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Mean Humidity (%)
32	16-Mar-02	25	2190	2068	1900	2050	2100	2220	0.794	1.908	1.19E-02	26	50
34	18-Mar-02	26	2190	2205	2330	2190	2280	2020				23	48
35	19-Mar-02	27	2160	2095	2100	2060	2110	2110				23	50
36	20-Mar-02	28	2130	2040	2020	2030	2070	2040				23	49
37	21-Mar-02	29	2180	2108	2170	2040	2110	2110				23	50
38	22-Mar-02	30	2050	1913	1950	1940	1840	1920				23	54
41	25-Mar-02	31	2090	1918	1900	1900	1830	2040	3.911	2.446	4.72E-03	23	51
42	26-Mar-02	32	2030	1913	2080	1760	1830	1980				24	48
43	27-Mar-02	33	2170	2133	2220	2130	2150	2030				24	48
44	28-Mar-02	34	2110	2090	2130	2030	2020	2180				24	52
45	29-Mar-02	35	2110	2028	2040	2040	2010	2020	0.8422	2.224	4.56E-03	24	50
48	01-Apr-02	36	2090	1843	2120	1630	1830	1790				24	47
49	02-Apr-02	37	2030	1845	1900	1790	1810	1880				23	52
50	03-Apr-02	38	2130	1875	1790	1890	1890	1930				23	48
51	04-Apr-02	39	2060	2093	2240	2130	2010	1990				23	51
52	05-Apr-02	40	2220	2150	1860	2030	2340	2370	1.438	2.053	1.53E-03	23	51
55	08-Apr-02	41	1980	1950	2010	1810	1730	2250				23	48
56	09-Apr-02	42	2040	1938	1980	2030	1720	2020				24	47
57	10-Apr-02	43	2050	1955	2010	1950	1920	1940				24	49
58	11-Apr-02	44	2060	1960	2010	2030	1960	1840				23	49
59	12-Apr-02	45	2060	1820	1870	1890	1730	1790	1.154	2.045	5.62E-03	23	48
62	15-Apr-02	46	2060	1958	2010	2110	1730	1980				23	45
63	16-Apr-02	47	1910	2040	2220	2020	1920	2000				23	51
64	17-Apr-02	48	2000	2080	2240	2020	2010	2050				23	48

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
65	18-Apr-02	49	2010	2000	2040	2020	1920	2020	1.072	1.803	3.00E-03	23	49
66	19-Apr-02	50	2140	2108	2030	2130	2180	2090				23	46
69	22-Apr-02	51	1930	1885	1700	1970	2020	1850				23	47
70	23-Apr-02	52	1970	1963	2010	1870	2020	1950				24	50
71	24-Apr-02	53	2060	2045	1950	2390	1880	1960				24	51
72	25-Apr-02	54	1970	1875	1920	1970	1730	1880				24	48
73	26-Apr-02	55	2060	2020	2010	2020	2040	2010	12.34	2.875	9.96E-03	24	49
76	29-Apr-02	56	2060	1985	2030	1970	2020	1920				24	46
77	30-Apr-02	57	1900	1898	1880	1930	1920	1860				23	49
78	01-May-02	58	2090	2163	2290	2210	2110	2040				23	49
79	02-May-02	59	2040	2065	2150	2120	1980	2010				23	47
80	03-May-02	60	2070	2010	2020	1990	2000	2030	1.351	1.783	1.34E-03	24	49
83	06-May-02	61	2060	1958	2150	2000	1850	1830				23	47
84	07-May-02	62	2120	1935	1920	1950	1910	1960				23	50
85	08-May-02	63	2190	1890	1880	2010	1750	1920				23	49
86	09-May-02	64	1990	1808	1880	1790	1830	1730				23	46
87	10-May-02	65	2080	1915	2170	2020	1750	1720	1.631	1.870	2.36E-03	23	48
89	12-May-02	66	2130	1943	2010	1660	1820	2280				23	48
90	13-May-02	67	2230	1945	1920	1950	1970	1940				22	51
91	14-May-02	68	2330	2015	2070	2010	1970	2010				22	51
92	15-May-02	69	2150	2023	2160	1900	2010	2020				22	52
Mean			2068		1982				2.344	2.106	4.51E-03	23.4	49.4
S.D.			83		129				3.103	0.303	3.13E-03	0.7	2.1

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results								Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration				Particle Size Determinations					
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)	
0	12-Feb-02	1	1940	1963	2100	1810	1980	1960			23	54	
1	13-Feb-02	2	1990	1943	2060	1950	1900	1860			23	56	
2	14-Feb-02	3	1950	1983	2020	1920	1880	2110			23	56	
3	15-Feb-02	4	2150	2178	2400	2030	2130	2150			23	55	
6	18-Feb-02	5	2010	2135	2010	2170	2210	2150			24	54	
7	19-Feb-02	6	2090	2063	2180	1990	2000	2080			23	55	
8	20-Feb-02	7	2060	1943	1980	1930	1920	1940			23	51	
9	21-Feb-02	8	2130	2035	2090	2120	1980	1950			23	50	
10	22-Feb-02	9	2000	1903	1910	1710	2000	1990			23	51	
13	25-Feb-02	10	2090	2070	2160	2170	1960	1990	1.378	2.273	5.03E-03	23	49
14	26-Feb-02	11	1970	1938	1730	1920	2000	2100			24	54	
15	27-Feb-02	12	1990	1993	1920	2000	2010	2040			23	52	
16	28-Feb-02	13	2030	1975	2060	2000	2000	1840			23	55	
17	01-Mar-02	14	2030	2050	1920	2150	2210	1920			23	55	
20	04-Mar-02	15	2050	2113	2210	2080	2040	2120	3.380	2.827	3.14E-03	23	52
21	05-Mar-02	16	1900	1918	2000	1860	1860	1950			23	43	
22	06-Mar-02	17	2100	1998	2010	1980	2000	2000			22	52	
23	07-Mar-02	18	2090	2028	2190	1890	2020	2010			23	56	
24	08-Mar-02	19	2150	1958	1910	2020	1950	1950			22	51	
27	11-Mar-02	20	2020	2023	1760	1960	2180	2190	1.174	1.633	2.54E-03	23	53
28	12-Mar-02	21	2010	1963	2080	2210	1850	1710			23	52	
29	13-Mar-02	22	2010	1973	2040	2000	1920	1930			22	52	
30	14-Mar-02	23	2080	1985	1960	1950	1940	2090			23	53	
31	15-Mar-02	24	2160	2020	1920	2020	1980	2160			23	52	

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)
32	16-Mar-02	25	2190	2098	1950	2200	2190	2050	0.8998	2.47	1.47E-02	25	52
34	18-Mar-02	26	2190	2020	2080	2010	1980	2010				23	50
35	19-Mar-02	27	2160	2028	2150	1930	2010	2020				23	52
36	20-Mar-02	28	2130	2040	2110	2060	1980	2010				23	51
37	21-Mar-02	29	2180	2073	2240	2020	2020	2010				23	51
38	22-Mar-02	30	2050	1960	2040	1990	1890	1920				23	55
41	25-Mar-02	31	2090	1968	1980	2050	2080	1760	0.9819	1.762	1.76E-03	23	52
42	26-Mar-02	32	2030	1960	1640	2000	2220	1980				23	52
43	27-Mar-02	33	2170	1978	2030	1980	1950	1950				23	50
44	28-Mar-02	34	2110	1915	2020	1920	1890	1830				23	54
45	29-Mar-02	35	2110	1998	2020	1980	1960	2030	0.8897	1.926	5.04E-03	23	53
48	01-Apr-02	36	2090	2130	1810	2400	2180	2130				23	48
49	02-Apr-02	37	2030	1960	2130	1840	1850	2020				23	54
50	03-Apr-02	38	2130	1970	1900	2020	2040	1920				23	49
51	04-Apr-02	39	2060	2098	2170	2220	2020	1980				23	54
52	05-Apr-02	40	2220	2055	2270	1890	2110	1950	1.403	1.989	1.74E-03	23	54
55	08-Apr-02	41	1980	1858	1790	1790	1730	2120				23	49
56	09-Apr-02	42	2040	1868	1770	1780	2150	1770				23	52
57	10-Apr-02	43	2050	1943	1790	1980	1980	2020				23	52
58	11-Apr-02	44	2060	1985	2100	2020	1960	1860				22	52
59	12-Apr-02	45	2060	1970	2050	2020	1830	1980	1.101	1.755	6.24E-03	22	50
62	15-Apr-02	46	2060	2000	1930	2180	1790	2100				22	47
63	16-Apr-02	47	1910	1903	2140	1840	1790	1840				23	53
64	17-Apr-02	48	2000	1920	2050	1920	1730	1980				23	50

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results								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)						Temperature (°C)	Humidity (%)			
65	18-Apr-02	49	2010	1913	1860	2150	1720	1920			22	52			
66	19-Apr-02	50	2140	2133	2250	2180	2050	2050	0.9187	1.558	2.74E-03	23	48		
69	22-Apr-02	51	1930	2055	2300	1960	2050	1910			23	48			
70	23-Apr-02	52	1970	2043	2240	2040	2020	1870			23	53			
71	24-Apr-02	53	2060	2133	2090	2400	1990	2050			23	55			
72	25-Apr-02	54	1970	2083	2310	2180	1920	1920			22	52			
73	26-Apr-02	55	2060	2168	2280	2130	2180	2080	1.358	1.692	2.33E-03	23	51		
76	29-Apr-02	56	2060	2085	2100	2060	2110	2070			23	49			
77	30-Apr-02	57	1900	1828	2210	1730	1700	1670			23	52			
78	01-May-02	58	2090	2068	2030	2050	2090	2100			23	51			
79	02-May-02	59	2040	1983	2000	1870	2020	2040			23	50			
80	03-May-02	60	2070	2083	2110	2180	2020	2020	1.314	1.631	1.16E-03	23	53		
83	06-May-02	61	2060	2048	1940	2000	2120	2130			23	48			
84	07-May-02	62	2120	1995	2020	2000	1980	1980			22	53			
85	08-May-02	63	2190	1973	2000	2170	1740	1980			23	51			
86	09-May-02	64	1990	2015	1920	1930	2090	2120			22	50			
87	10-May-02	65	2080	1963	2250	2040	1770	1790	1.510	1.720	2.23E-03	22	50		
89	12-May-02	66	2130	2043	2030	1940	1930	2270			22	50			
90	13-May-02	67	2230	2095	2130	2090	2110	2050			22	53			
91	14-May-02	68	2330	2075	2160	2100	2020	2020			22	53			
			Mean	2067			2009		1.377	1.966	4.01E-03	22.9	51.8		
			S.D.	83			134		0.647	0.390	3.54E-03	0.5	2.4		

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)
0	12-Feb-02	1	9470	9685	10000	9580	9580	9580				24	47
1	13-Feb-02	2	9450	9850	9290	10200	9910	10000				24	48
2	14-Feb-02	3	9540	9295	9650	8630	9350	9550				24	47
3	15-Feb-02	4	9890	10400	9910	11500	10200	10000				24	47
6	18-Feb-02	5	9830	10310	10600	10400	9940	10300				24	47
7	19-Feb-02	6	9740	9925	10100	9650	9650	10300				24	50
8	20-Feb-02	7	10800	9680	9910	9190	9420	10200				24	49
9	21-Feb-02	8	9910	10380	9910	10600	10600	10400				24	51
10	22-Feb-02	9	9950	9758	10200	7730	10200	10900				24	50
13	25-Feb-02	10	9840	10480	11400	10200	9910	10400	1.043	2.151	5.74E-03	24	47
14	26-Feb-02	11	9990	9823	9940	10500	9910	8940				25	49
15	27-Feb-02	12	9920	9943	10600	9420	9910	9840				24	47
16	28-Feb-02	13	10100	10650	10700	10300	10700	10900				24	47
17	01-Mar-02	14	10100	10700	11200	10500	10200	10900				24	47
20	04-Mar-02	15	9200	9935	11200	8840	10600	9100	1.708	2.183	2.65E-03	24	46
21	05-Mar-02	16	9890	10200	9580	10600	10500	10100				23	39
22	06-Mar-02	17	9730	10480	10200	10700	10700	10300				23	47
23	07-Mar-02	18	9860	10420	10000	11100	10700	9870				24	51
24	08-Mar-02	19	9960	10240	9650	10100	10800	10400				23	50
27	11-Mar-02	20	9810	10480	10800	10200	10700	10200	2.245	2.622	4.77E-03	24	47
28	12-Mar-02	21	9890	10280	9910	10800	10200	10200				23	47
29	13-Mar-02	22	9700	9968	10900	9580	9810	9580				22	49
30	14-Mar-02	23	10100	10030	8300	10300	11500	10000				24	50
31	15-Mar-02	24	9980	9973	10200	9580	9910	10200				23	50

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results									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)								Temperature (°C)	Humidity (%)	
32	16-Mar-02	25	9530	10260	10800	10600	11900	7720	0.7927	2.452	2.41E-02	26	50		
34	18-Mar-02	26	10000	10000	10000	10100	9910	10000				24	47		
35	19-Mar-02	27	10200	10320	10800	9740	11100	9650				23	50		
36	20-Mar-02	28	10100	10010	9840	10400	9580	10200				23	50		
37	21-Mar-02	29	10300	9970	9580	10000	10300	10000				23	49		
38	22-Mar-02	30	9370	9475	9420	10300	8440	9740				23	50		
41	25-Mar-02	31	9690	9450	9740	9350	9520	9190	1.316	2.451	2.91E-03	23	48		
42	26-Mar-02	32	9940	9773	10700	9190	9910	9290				24	48		
43	27-Mar-02	33	9390	9670	10000	9910	9420	9350				24	48		
44	28-Mar-02	34	9540	9930	8620	10000	10500	10600				24	48		
45	29-Mar-02	35	9670	10010	9580	10400	9870	10200	0.7720	1.889	5.54E-03	24	49		
48	01-Apr-02	36	9830	9790	9840	9740	9580	10000				24	47		
49	02-Apr-02	37	10000	9970	10000	9840	9740	10300				23	50		
50	03-Apr-02	38	10600	9730	9320	10300	9780	9520				23	48		
51	04-Apr-02	39	10000	9745	10200	9520	9740	9520				23	48		
52	05-Apr-02	40	9470	9098	9100	8550	8930	9810	1.490	2.249	1.78E-03	23	49		
55	08-Apr-02	41	10000	10450	10300	10400	10600	10500				23	47		
56	09-Apr-02	42	9880	9828	10600	10000	9290	9420				24	49		
57	10-Apr-02	43	10100	9945	9780	10000	10000	10000				24	49		
58	11-Apr-02	44	9960	10730	10900	10700	10500	10800				24	49		
59	12-Apr-02	45	9840	9475	9260	8260	10600	9780	0.9063	1.662	7.08E-03	24	48		
62	15-Apr-02	46	9950	9743	10000	10500	8170	10300				24	46		
63	16-Apr-02	47	9950	10590	10700	10300	11700	9650				23	52		
64	17-Apr-02	48	9460	10830	11200	10200	11200	10700				23	50		

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results								Particle Size Determinations			Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration				Mean Temperature		Humidity (%)					
				Mean (mg/m ³)	Individual (mg/m ³)			MMAD (μm)	GSD	TMC (mg/m ³)	(°C)				
65	18-Apr-02	49	10100	10090	10100	10500	9910	9840				23	50		
66	19-Apr-02	50	9470	9633	9910	9260	9680	9680	1.618	2.006	6.01E-03	23	46		
69	22-Apr-02	51	9550	10280	10200	11700	9100	10100				23	47		
70	23-Apr-02	52	9780	11100	10700	10800	10900	12000				24	47		
71	24-Apr-02	53	9780	9233	9130	8470	9680	9650				24	47		
72	25-Apr-02	54	9940	9770	9910	10100	9420	9650				24	47		
73	26-Apr-02	55	9680	10090	10100	10100	10900	9260	1.285	1.701	1.84E-03	24	47		
76	29-Apr-02	56	9620	9488	9060	8810	9580	10500				24	45		
77	30-Apr-02	57	9680	9468	9870	9350	10200	8450				23	48		
78	01-May-02	58	10200	10010	9740	10100	10100	10100				24	48		
79	02-May-02	59	9730	9400	9680	9910	9290	8720				23	48		
80	03-May-02	60	10100	10380	10200	10000	11000	10300	1.243	1.646	1.17E-03	24	47		
83	06-May-02	61	10000	9840	10000	9810	10000	9550				24	47		
84	07-May-02	62	9670	9318	10000	9650	9190	8430				23	50		
85	08-May-02	63	9460	9658	9130	8400	10800	10300				24	49		
86	09-May-02	64	9690	9240	8740	9610	8770	9840	4.537	2.571	3.25E-03	23	47		
87	10-May-02	65	9300	9490	9420	9520	8620	10400				23	48		
89	12-May-02	66	9860	9940	9680	10200	9580	10300				23	48		
90	13-May-02	67	9810	9693	10700	9260	9260	9550				23	51		
91	14-May-02	68	9710	9365	8740	9290	10200	9230				22	49		
92	15-May-02	69	9500	10790	9650	10400	11700	11400				22	49		
			Mean	9827			9969		1.534	2.110	5.42E-03	23.6	48.2		
			S.D.	283			704		0.992	0.347	5.91E-03	0.7	1.8		

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment		
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations					
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)	
0	12-Feb-02	1	9470	9930	10200	9740	9580	10200				23	46	
1	13-Feb-02	2	9450	9925	10400	9840	9550	9910				23	46	
2	14-Feb-02	3	9540	10650	10500	10600	10800	10700				24	47	
3	15-Feb-02	4	9890	10270	10900	9160	10400	10600				24	47	
6	18-Feb-02	5	9830	9998	10400	9710	9580	10300				24	46	
7	19-Feb-02	6	9740	10010	10100	9740	9780	10400				24	46	
8	20-Feb-02	7	10800	10150	9780	10000	10000	10800				24	46	
9	21-Feb-02	8	9910	10150	10700	10000	9910	10000				24	47	
10	22-Feb-02	9	9950	10240	11000	8440	11300	10200				24	45	
13	25-Feb-02	10	9840	9480	8460	9580	9480	10400	1.013	2.207	6.20E-03	24	45	
14	26-Feb-02	11	9990	10120	9480	9910	10500	10600				25	49	
15	27-Feb-02	12	9920	10740	9940	10300	11200	11500				24	46	
16	28-Feb-02	13	10100	10650	10800	10800	10700	10300				24	47	
17	01-Mar-02	14	10100	10010	10200	10100	9520	10200				24	47	
20	04-Mar-02	15	9200	9955	9580	9940	10000	10300	1.729	2.339	2.53E-03	24	46	
21	05-Mar-02	16	9890	10140	10900	10300	10100	9260				23	37	
22	06-Mar-02	17	9730	9743	10100	10000	9520	9350				23	44	
23	07-Mar-02	18	9860	10170	9350	10800	10600	9910				24	47	
24	08-Mar-02	19	9960	9590	9100	9480	10200	9580				23	46	
27	11-Mar-02	20	9810	9655	10200	9580	9420	9420	1.443	1.826	3.34E-03	23	44	
28	12-Mar-02	21	9890	9995	10000	10200	9910	9870				24	45	
29	13-Mar-02	22	9700	9168	8140	10400	9100	9030				23	48	
30	14-Mar-02	23	10100	10100	10000	9580	10600	10200				23	49	
31	15-Mar-02	24	9980	9983	10300	9420	9910	10300				23	49	

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)
32	16-Mar-02	25	9530	9325	9580	9520	10200	8000	0.7866	2.33	2.44E-02	26	49
34	18-Mar-02	26	10000	9980	10200	10300	9680	9740				23	46
35	19-Mar-02	27	10200	9548	9350	9100	9740	10000				24	46
36	20-Mar-02	28	10100	9510	9160	9650	9230	10000				24	46
37	21-Mar-02	29	10300	10110	9520	10000	10300	10600				24	46
38	22-Mar-02	30	9370	9188	9650	10000	8550	8550				24	46
41	25-Mar-02	31	9690	9573	9650	9350	10000	9290	4.191	3.203	5.14E-03	24	45
42	26-Mar-02	32	9940	10430	11100	10400	10000	10200				23	46
43	27-Mar-02	33	9390	9818	10200	9840	9580	9650				23	46
44	28-Mar-02	34	9540	9493	9580	9230	9580	9580				23	46
45	29-Mar-02	35	9670	10070	10900	10100	9190	10100	0.7259	1.633	5.49E-03	24	47
48	01-Apr-02	36	9830	10260	10500	10100	9940	10500				24	44
49	02-Apr-02	37	10000	9033	8100	9350	8940	9740				24	47
50	03-Apr-02	38	10600	10630	9910	11500	10400	10700				24	46
51	04-Apr-02	39	10000	10680	11000	10700	10800	10200				24	45
52	05-Apr-02	40	9470	10250	10400	10200	10400	10000	1.767	2.194	3.29E-03	24	45
55	08-Apr-02	41	10000	9763	9910	9810	9910	9420				24	45
56	09-Apr-02	42	9880	10130	10600	9940	10300	9680				24	48
57	10-Apr-02	43	10100	10780	10300	11100	10600	11100				23	46
58	11-Apr-02	44	9960	9203	9520	8360	9350	9580				23	47
59	12-Apr-02	45	9840	9775	10400	8500	10200	10000	0.8941	1.837	8.92E-03	23	46
62	15-Apr-02	46	9950	9888	9780	10700	9230	9840				23	44
63	16-Apr-02	47	9950	10310	10600	9650	11000	10000				24	47
64	17-Apr-02	48	9460	10240	10600	9740	10400	10200				24	46

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
65	18-Apr-02	49	10100	11000	11500	11300	10600	10600	0.8013	1.904	7.49E-03	24	47
66	19-Apr-02	50	9470	10070	9940	9740	10400	10200				24	44
69	22-Apr-02	51	9550	10090	10200	10400	9450	10300				24	44
70	23-Apr-02	52	9780	9758	9130	9480	9320	11100				24	46
71	24-Apr-02	53	9780	10650	10700	10200	11000	10700				24	46
72	25-Apr-02	54	9940	10240	9740	10200	10500	10500				23	47
73	26-Apr-02	55	9680	10530	10100	10400	11200	10400	2.013	2.546	5.59E-03	24	46
76	29-Apr-02	56	9620	9885	9840	9420	9580	10700				24	44
77	30-Apr-02	57	9680	11250	11400	11100	11400	11100				24	45
78	01-May-02	58	10200	11130	10900	11500	11000	11100				24	45
79	02-May-02	59	9730	10480	10800	10100	10600	10400				24	45
80	03-May-02	60	10100	10650	10400	10200	11600	10400	1.661	2.332	3.48E-03	25	44
83	06-May-02	61	10000	11180	11300	11100	11100	11200				25	44
84	07-May-02	62	9670	10350	10600	10700	10400	9710				23	47
85	08-May-02	63	9460	9923	9290	9480	11600	9320				23	47
86	09-May-02	64	9690	10500	10200	10500	10000	11300				23	45
87	10-May-02	65	9300	10180	10400	9420	9910	11000	1.736	1.967	2.83E-03	23	46
89	12-May-02	66	9860	10550	11500	9810	10100	10800				23	46
90	13-May-02	67	9810	9650	10200	10100	8650	9650				22	49
91	14-May-02	68	9710	9218	8710	8580	10000	9580				23	46
Mean			9832		10090				1.547	2.206	6.40E-03	23.7	45.9
S.D.			282		682				0.909	0.402	5.73E-03	0.7	1.7

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Humidity (%)
0	12-Feb-02	1	18400	19680	19400	19200	19700	20400				25	48
1	13-Feb-02	2	17700	19880	20100	18900	19300	21200				25	50
2	14-Feb-02	3	18400	19480	20100	18700	19200	19900				25	50
3	15-Feb-02	4	20500	20430	20300	20300	20700	20400				25	51
6	18-Feb-02	5	20000	20600	19700	22200	20500	20000				25	49
7	19-Feb-02	6	19600	20130	19800	20300	19300	21100				25	50
8	20-Feb-02	7	20000	20650	20900	20900	20700	20100				24	50
9	21-Feb-02	8	19700	20550	20300	20300	20500	21100				24	53
10	22-Feb-02	9	19600	21400	21200	22300	21000	21100				24	51
13	25-Feb-02	10	19600	20480	21600	20400	20400	19500	3.198	3.812	1.15E-02	24	50
14	26-Feb-02	11	19900	20500	20000	20800	20400	20800				26	53
15	27-Feb-02	12	19400	20600	19700	20700	20900	21100				25	50
16	28-Feb-02	13	19100	20030	20700	20600	20700	18100				25	50
17	01-Mar-02	14	18900	20300	20400	20400	20100	20300				25	51
20	04-Mar-02	15	20000	20750	20800	20900	20800	20500	1.192	1.779	1.83E-03	25	49
21	05-Mar-02	16	19600	20950	20900	21300	20800	20800				24	40
22	06-Mar-02	17	18500	20500	21100	20100	20400	20400				24	49
23	07-Mar-02	18	19200	19900	19500	20100	20100	19900				25	52
24	08-Mar-02	19	19000	20050	19800	20500	19900	20000				24	52
27	11-Mar-02	20	18800	19680	18900	18100	21000	20700	1.358	2.17	4.54E-03	25	48
28	12-Mar-02	21	19500	20130	20300	20100	20100	20000				25	49
29	13-Mar-02	22	19300	20950	21200	21300	20900	20400				22	51
30	14-Mar-02	23	19100	19380	18200	19500	20500	19300				25	51
31	15-Mar-02	24	19100	19100	18200	18500	20100	19600				25	52

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Temperature (°C)	Humidity (%)
32	16-Mar-02	25	19400	19980	19600	20500	20100	19700	0.7739	1.949	2.44E-02	28	52
34	18-Mar-02	26	19000	19880	18900	20300	19800	20500				25	48
35	19-Mar-02	27	19300	19250	19500	19000	19500	19000				24	51
36	20-Mar-02	28	19800	19650	18200	20400	19900	20100				24	50
37	21-Mar-02	29	19000	19350	19600	19500	19000	19300				25	50
38	22-Mar-02	30	19500	20280	19900	20300	20400	20500				25	51
41	25-Mar-02	31	19300	19550	19500	19700	20100	18900	2.346	3.191	4.30E-03	25	49
42	26-Mar-02	32	19300	20330	20400	20100	20700	20100				25	49
43	27-Mar-02	33	19600	20250	19800	19700	21000	20500				25	49
44	28-Mar-02	34	18400	19780	19500	19800	20200	19600				25	48
45	29-Mar-02	35	18600	19480	19400	18900	20300	19300	0.8053	2.004	7.67E-03	26	49
48	01-Apr-02	36	18700	20080	20100	19700	20000	20500				26	47
49	02-Apr-02	37	18900	20450	20800	20200	20300	20500				25	50
50	03-Apr-02	38	18800	20300	20100	19800	20400	20900				24	52
51	04-Apr-02	39	18900	19480	19300	19000	20000	19600				24	50
52	05-Apr-02	40	19100	20100	19700	19500	20800	20400	1.220	1.549	1.09E-03	24	50
55	08-Apr-02	41	18800	19830	19900	19500	19300	20600				25	49
56	09-Apr-02	42	17600	19050	18900	18800	19000	19500				25	50
57	10-Apr-02	43	19700	20780	20200	20800	21300	20800				26	51
58	11-Apr-02	44	18400	20330	20500	20500	20100	20200				25	49
59	12-Apr-02	45	19400	20680	20400	21100	21100	20100	1.373	3.011	1.12E-02	25	49
62	15-Apr-02	46	18700	20580	20200	20400	20400	21300				25	48
63	16-Apr-02	47	18900	20900	20800	21000	21000	20800				24	54
64	17-Apr-02	48	18700	20100	20400	20000	20700	19300				24	51
65	18-Apr-02	49	18800	20650	20400	20800	20400	21000				24	51

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration					Particle Size Determinations				
				Mean (mg/m ³)	Individual (mg/m ³)				MMAD (μm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)	Mean Humidity (%)
66	19-Apr-02	50	18400	20430	20500	21100	19700	20400	0.9267	1.578	2.63E-03	24	48
69	22-Apr-02	51	18600	20330	19800	20500	20100	20900				24	48
70	23-Apr-02	52	18900	21200	21200	21100	21200	21300				25	48
71	24-Apr-02	53	18700	20480	19800	20300	20600	21200				25	49
72	25-Apr-02	54	19000	20630	20500	21700	20100	20200				25	49
73	26-Apr-02	55	18800	21080	21200	20100	22200	20800	1.413	1.826	1.91E-03	25	49
76	29-Apr-02	56	19000	19830	19300	20300	19600	20100				26	47
77	30-Apr-02	57	19000	20800	20600	21200	20200	21200				24	49
78	01-May-02	58	18900	20350	20400	20200	20100	20700				25	50
79	02-May-02	59	18800	20230	20600	20300	20300	19700				24	50
80	03-May-02	60	18800	20100	20900	19700	19900	19900	3.580	2.863	6.05E-03	25	49
83	06-May-02	61	19000	20930	20300	20900	21600	20900				25	48
84	07-May-02	62	19100	20100	20700	19000	20000	20700				25	52
85	08-May-02	63	19100	20130	20700	20000	20400	19400				25	51
86	09-May-02	64	18600	18550	17200	19100	18900	19000				25	48
87	10-May-02	65	18900	19180	18900	18700	19800	19300	1.597	1.681	1.85E-03	24	49
89	12-May-02	66	19100	18830	18300	18000	19300	19700				25	49
90	13-May-02	67	19500	19580	19000	19300	19600	20400				24	52
91	14-May-02	68	19100	19450	20000	19800	19300	18700				23	49
92	15-May-02	69	18700	19900	20000	20100	19500	20000				23	51
Mean			19070		20130				1.659	2.311	6.46E-03	24.7	49.7
S.D.			513		775				0.878	0.719	6.38E-03	0.8	1.9

Table A

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

Day	Date	Exposure Number	Chamber Monitoring Results									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	12-Feb-02	1	18400	20000	21700	20100	18800	19400				24	49
1	13-Feb-02	2	17700	19200	18200	19200	19300	20100				24	50
2	14-Feb-02	3	18400	19900	19800	21100	17900	20800				24	51
3	15-Feb-02	4	20500	22680	23300	22600	22600	22200				24	52
6	18-Feb-02	5	20000	21680	22500	20900	22500	20800				24	50
7	19-Feb-02	6	19600	21300	19200	22000	21400	22600				25	49
8	20-Feb-02	7	20000	20400	20000	20700	20200	20700				25	50
9	21-Feb-02	8	19700	20030	19600	19600	20100	20800				25	52
10	22-Feb-02	9	19600	20530	20400	21400	20200	20100				25	53
13	25-Feb-02	10	19600	21200	20500	21300	21500	21500	1.041	2.111	7.73E-03	25	50
14	26-Feb-02	11	19900	21380	21700	22600	21700	19500				25	53
15	27-Feb-02	12	19400	20530	20900	19900	20500	20800				24	51
16	28-Feb-02	13	19100	20450	20700	20500	20200	20400				24	51
17	01-Mar-02	14	18900	20900	21300	21700	20200	20400				24	50
20	04-Mar-02	15	20000	21100	20700	21200	21100	21400	1.147	1.782	1.69E-03	24	50
21	05-Mar-02	16	19600	21450	21400	21700	22000	20700				25	40
22	06-Mar-02	17	18500	19950	20000	19700	20000	20100				25	48
23	07-Mar-02	18	19200	21000	21700	20700	20900	20700				25	51
24	08-Mar-02	19	19000	19650	19400	19900	19500	19800				25	51
27	11-Mar-02	20	18800	19350	19300	17400	20700	20000	1.182	2.056	4.70E-03	25	47
28	12-Mar-02	21	19500	20350	20700	20500	20000	20200				24	48
29	13-Mar-02	22	19300	19400	19400	19500	19200	19500				23	51
30	14-Mar-02	23	19100	21080	20700	20700	21200	21700				24	51
31	15-Mar-02	24	19100	20430	20800	19600	20600	20700				24	51

Table A

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

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Day	Date	Exposure Number	Chamber Monitoring Results										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 (%)	
32	16-Mar-02	25	19400	20680	20300	21200	20500	20700	0.7578	1.842	2.31E-02	26	52	
34	18-Mar-02	26	19000	20980	20700	21300	20300	21600				24	49	
35	19-Mar-02	27	19300	20580	20700	20400	20700	20500				25	50	
36	20-Mar-02	28	19800	20800	20500	20800	20800	21100				25	49	
37	21-Mar-02	29	19000	20730	20300	20600	21100	20900				25	49	
38	22-Mar-02	30	19500	20750	20700	20700	21100	20500				25	50	
41	25-Mar-02	31	19300	21030	21000	21200	20700	21200	0.9018	2.195	3.14E-03	25	48	
42	26-Mar-02	32	19300	19850	20000	20000	19900	19500				24	51	
43	27-Mar-02	33	19600	20380	20000	20300	20800	20400				24	50	
44	28-Mar-02	34	18400	19930	18900	20400	20400	20000				24	50	
45	29-Mar-02	35	18600	19650	19400	20800	19900	18500	0.7779	2.114	9.04E-03	24	50	
48	01-Apr-02	36	18700	18930	18500	18900	19200	19100				24	48	
49	02-Apr-02	37	18900	18830	17900	19000	19100	19300				25	49	
50	03-Apr-02	38	18800	19250	19700	18900	18900	19500				25	50	
51	04-Apr-02	39	18900	19900	19600	19000	20700	20300				25	49	
52	05-Apr-02	40	19100	19700	19600	20600	19300	19300	1.517	2.038	2.38E-03	25	48	
55	08-Apr-02	41	18800	19880	19600	19300	20000	20600				25	48	
56	09-Apr-02	42	17600	18230	18300	18200	18100	18300				25	52	
57	10-Apr-02	43	19700	20350	20100	20800	20800	19700				24	50	
58	11-Apr-02	44	18400	20150	20500	20100	20000	20000				24	49	
59	12-Apr-02	45	19400	20200	19800	20800	20200	20000	1.301	2.253	9.39E-03	24	49	
62	15-Apr-02	46	18700	19650	19600	19200	19800	20000				24	48	
63	16-Apr-02	47	18900	20450	20100	20800	20800	20100				25	53	
64	17-Apr-02	48	18700	19300	18100	19200	20200	19700				25	51	

Table A

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

Chamber Monitoring Results Cumulative Exposure Record Group IVB - 20,000 mg/m ³														
Day	Date	Exposure Number								Particle Size Determinations			Chamber Environment	
			Nominal (mg/m ³)	Analytical Chamber Concentration		Mean (mg/m ³)		Individual (mg/m ³)			MMAD (µm)	GSD	TMC (mg/m ³)	Mean Temperature (°C)
65	18-Apr-02	49	18800	20080	20400	20400	19700	19800	0.7490	1.593	5.03E-03	25	51	
66	19-Apr-02	50	18400	18850	18500	19400	18300	19200				25	48	
69	22-Apr-02	51	18600	19780	19000	20000	19400	20700				25	48	
70	23-Apr-02	52	18900	19850	20500	19900	20500	18500				24	51	
71	24-Apr-02	53	18700	19430	19200	19800	19000	19700				24	50	
72	25-Apr-02	54	19000	19480	19400	20400	19600	18500				24	50	
73	26-Apr-02	55	18800	19930	20800	19200	19200	20500	1.357	1.997	3.45E-03	24	49	
76	29-Apr-02	56	19000	19300	18800	19400	19300	19700				24	48	
77	30-Apr-02	57	19000	19030	18400	19200	18400	20100				25	49	
78	01-May-02	58	18900	19900	20200	20100	19300	20000				25	49	
79	02-May-02	59	18800	18800	17900	19200	19400	18700				25	49	
80	03-May-02	60	18800	19200	19000	19500	19300	19000	6.591	3.086	3.95E-03	26	49	
83	06-May-02	61	19000	19750	19500	20400	19700	19400				26	49	
84	07-May-02	62	19100	19680	20400	18600	19700	20000				24	52	
85	08-May-02	63	19100	20330	20900	20200	20700	19500				24	51	
86	09-May-02	64	18600	20000	20200	20100	19700	20000				24	49	
87	10-May-02	65	18900	20000	20000	20100	19900	20000	1.699	2.025	2.97E-03	24	49	
89	12-May-02	66	19100	20350	20000	20000	20800	20600				24	49	
90	13-May-02	67	19500	20100	20000	20000	19800	20600				24	54	
91	14-May-02	68	19100	18880	20000	19000	18300	18200				23	49	
Mean			19070		20100				1.550	2.078	6.20E-03	24.5	49.8	
S.D.			515		967				1.542	0.351	5.64E-03	0.6	1.9	

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE B

GASOLINE DIPE 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 92 TOTAL			
# OF ANIMALS EXAMINED	1	5	5	
	2	5	5	
	3	5	5	
	4	5	5	
 NORMAL				
WITHIN NORMAL LIMITS	1	5	0	5
	2	5	0	5
	3	5	0	5
	4	5	0	5
 DEAD				
HUMANE SACRIFICE	1	0	0	0
	2	0	1	1
	3	0	0	0
	4	0	0	0
TERMINAL SACRIFICE	1	0	5	5
	2	0	4	4
	3	0	5	5
	4	0	5	5

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE B

GASOLINE DIPE 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

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

NORMAL

WITHIN NORMAL LIMITS	1	5	0	5
1	5	0	5	
2	5	0	5	
3	5	0	5	
4	5	0	5	

DEAD

TERMINAL SACRIFICE	1	0	5	5
1	0	5	5	
2	0	5	5	
3	0	5	5	
4	0	5	5	

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

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February 2, 2002

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

Re: study A-12 00-6130F

Ophthalmoscopic examination of study A-12 00-6130F pretest rats was performed January 31, 2002. Rats with ocular abnormalities were identified and should be withdrawn from inclusion in the study if convenient.



Lionel F. Rubin, V. M. D.

Table C
LIONEL F. RUBIN, V.M.D.
1116 Saint Andrews Road
Bryn Mawr, PA 19010
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May 11, 2002

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

Re: study A-12 00-6130F

Ophthalmoscopic examination of study A-12 00-6130F rats was performed May 10, 2002 (terminal examination). I have reviewed the findings of the type and incidence of ocular abnormalities. There is no indication of dose or compound related ocular disease. In my opinion, none of the ocular abnormalities is attributable to the administration of the test compound.



Lionel F. Rubin, V. M. D.

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

DAY -12

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

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE C

GASOLINE DIPE VAPOR CONDENSATE: A 13-WEEK WHOLE-BODY
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SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

DAY -12

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

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE C

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

DAY 87

	DOSE GROUP: DOSE LEVEL(MG/M ³):	1 0	2 2000	3 10000	4 20000	
MALES	total number examined	5	4	5	5	
RETINA	N	1	0	0	0	
FOCAL RETINOPATHY	N %	1 20.0	0 0.0	0 0.0	0 0.0	

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

DAY 87

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

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE D

GASOLINE DIPE 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	225	224	223
		S.D.	7.1	8.3	9.8
		N	5	5	5
WEEK 0		MEAN	308	307	306
		S.D.	18.0	18.0	16.3
		N	5	5	5
WEEK 1		MEAN	345	344	348
		S.D.	18.7	24.1	21.0
		N	5	5	5
WEEK 2		MEAN	377	373	385
		S.D.	22.7	30.3	24.1
		N	5	5	5
WEEK 3		MEAN	406	399	414
		S.D.	22.2	36.2	26.7
		N	5	5	5
WEEK 4		MEAN	430	422	441
		S.D.	26.8	43.3	28.5
		N	5	5	5
WEEK 5		MEAN	455	446	463
		S.D.	30.5	45.7	29.1
		N	5	5	5
WEEK 6		MEAN	473	458	489
		S.D.	32.2	46.1	31.5
		N	5	5	5
WEEK 7		MEAN	494	457	517
		S.D.	35.0	102.0	30.6
		N	5	5	5

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE D

GASOLINE DIPE 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	505	463	527
		S.D.	37.0	119.5	32.7
		N	5	5	5
WEEK	9	MEAN	524	480	545
		S.D.	39.5	127.0	35.8
		N	5	5	5
WEEK	10	MEAN	535	485	562
		S.D.	43.9	146.9	35.3
		N	5	5	5
WEEK	11	MEAN	549	562	577
		S.D.	44.4	28.1	34.8
		N	5	4	5
WEEK	12	MEAN	564	579	593
		S.D.	44.4	26.2	36.8
		N	5	4	5
WEEK	13	MEAN	567	582	595
		S.D.	50.6	26.9	41.9
		N	5	4	5

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE D

GASOLINE DIPE 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/M ³):	1 0	2 2000	3 10000	4 20000
WEEK -1	MEAN	176	174	172	176
	S.D.	7.0	6.5	9.7	9.2
	N	5	5	5	5
WEEK 0	MEAN	218	215	217	216
	S.D.	9.0	17.4	13.1	16.6
	N	5	5	5	5
WEEK 1	MEAN	240	235	231	234
	S.D.	14.2	19.7	17.4	13.7
	N	5	5	5	5
WEEK 2	MEAN	261	249	242	246
	S.D.	16.3	22.0	17.1	13.3
	N	5	5	5	5
WEEK 3	MEAN	271	261	247	250
	S.D.	17.3	27.1	13.8	8.5
	N	5	5	5	5
WEEK 4	MEAN	283	271	262	259
	S.D.	25.5	34.4	16.5	11.8
	N	5	5	5	5
WEEK 5	MEAN	293	281	268	267
	S.D.	27.0	33.5	20.3	16.0
	N	5	5	5	5
WEEK 6	MEAN	295	282	272	270
	S.D.	25.8	31.6	18.6	13.1
	N	5	5	5	5
WEEK 7	MEAN	306	289	276	275
	S.D.	27.5	35.2	16.9	13.7
	N	5	5	5	5

No statistically significant differences

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211-DIPE-S

TABLE D

GASOLINE DIPE 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/M ³):	1 0	2 2000	3 10000	4 20000
WEEK	8	MEAN	312	293	283	279
		S.D.	38.4	36.9	17.5	15.1
		N	5	5	5	5
WEEK	9	MEAN	317	302	292	287
		S.D.	36.9	33.9	20.6	19.0
		N	5	5	5	5
WEEK	10	MEAN	321	304	292	292
		S.D.	35.1	31.3	16.0	19.0
		N	5	5	5	5
WEEK	11	MEAN	324	309	298	296
		S.D.	35.6	32.6	19.4	13.1
		N	5	5	5	5
WEEK	12	MEAN	329	314	305	299
		S.D.	39.1	38.9	21.5	19.6
		N	5	5	5	5
WEEK	13	MEAN	325	312	304	299
		S.D.	35.2	34.9	17.9	19.9
		N	5	5	5	5

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE E

GASOLINE DIPE 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	4 20000
WEEK	0	TO	1	MEAN	37	37
				S.D.	5.2	6.3
				N	5	5
WEEK	0	TO	2	MEAN	70	66
				S.D.	6.4	13.5
				N	5	5
WEEK	0	TO	3	MEAN	98	92
				S.D.	8.2	19.3
				N	5	5
WEEK	0	TO	4	MEAN	122	115
				S.D.	10.8	26.8
				N	5	5
WEEK	0	TO	5	MEAN	147	139
				S.D.	16.4	29.5
				N	5	5
WEEK	0	TO	6	MEAN	166	151
				S.D.	18.5	29.5
				N	5	5

No statistically significant differences

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211-DIPE-S

TABLE E

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

MALES

MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

		DOSE GROUP:	1	2	3	4
		DOSE LEVEL (MG/M3):	0	2000	10000	20000
WEEK	0 TO 7	MEAN	187	150	211	182
		S.D.	24.5	86.2	20.6	17.8
		N	5	5	5	5
WEEK	0 TO 8	MEAN	197	156	221	198
		S.D.	24.7	103.5	23.4	17.2
		N	5	5	5	5
WEEK	0 TO 9	MEAN	216	173	239	213
		S.D.	30.6	111.1	25.4	21.5
		N	5	5	5	5
WEEK	0 TO 10	MEAN	227	178	256	230
		S.D.	34.8	131.0	27.6	22.8
		N	5	5	5	5
WEEK	0 TO 11	MEAN	241	248	271	242
		S.D.	37.1	20.4	27.6	25.4
		N	5	4	5	5
WEEK	0 TO 12	MEAN	256	265	287	254
		S.D.	38.5	18.3	30.0	26.4
		N	5	4	5	5

No statistically significant differences

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TABLE E

GASOLINE DIPE 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	4 20000
WEEK	0 TO 13	MEAN	259	268	289
		S.D.	42.5	17.5	36.3
		N	5	4	5
					256
					25.9

No statistically significant differences

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TABLE E

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

FEMALES

MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

			DOSE GROUP:	1	2	3	4
			DOSE LEVEL (MG/M3):	0	2000	10000	20000
WEEK	0	TO	1	MEAN	21	20	14
				S.D.	7.4	3.3	5.8
				N	5	5	5
WEEK	0	TO	2	MEAN	43	34	25**
				S.D.	9.8	4.6	7.2
				N	5	5	5
WEEK	0	TO	3	MEAN	53	46	30**
				S.D.	10.4	12.5	5.7
				N	5	5	5
WEEK	0	TO	4	MEAN	64	55	46
				S.D.	18.7	20.0	6.2
				N	5	5	5
WEEK	0	TO	5	MEAN	75	66	51*
				S.D.	20.1	17.5	10.3
				N	5	5	5
WEEK	0	TO	6	MEAN	77	67	55
				S.D.	18.5	15.8	8.9
				N	5	5	5

Statistical key: * = p<0.05 ** = p<0.01

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TABLE E

GASOLINE DIPE 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	7	MEAN S.D. N	87 20.4 5	74 19.9 5	59 5.8 5
WEEK	0	TO	8	MEAN S.D. N	94 31.6 5	78 20.9 5	66 7.4 5
WEEK	0	TO	9	MEAN S.D. N	99 29.6 5	86 18.3 5	75 11.4 5
WEEK	0	TO	10	MEAN S.D. N	102 28.1 5	89 14.7 5	75 9.8 5
WEEK	0	TO	11	MEAN S.D. N	105 28.2 5	93 17.4 5	81 10.7 5
WEEK	0	TO	12	MEAN S.D. N	111 31.7 5	99 23.3 5	88 13.1 5

Statistical key: * = p<0.05

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TABLE E

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

FEMALES

MEAN BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

	DOSE GROUP:	1	2	3	4
	DOSE LEVEL (MG/M3):	0	2000	10000	20000
WEEK	0 TO 13	MEAN	106	97	87
		S.D.	27.6	18.8	12.6
		N	5	5	5

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE F

GASOLINE DIPE 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/M3) :	1 0	2 2000	3 10000
WEEK	0	MEAN	96	97	94
		S.D.	6.0	2.6	4.9
		N	5	5	5
WEEK	1	MEAN	81	81	80
		S.D.	4.6	2.5	2.9
		N	5	5	5
WEEK	2	MEAN	75	73	73
		S.D.	3.4	1.9	2.8
		N	5	5	5
WEEK	3	MEAN	69	70	68
		S.D.	3.5	3.3	2.4
		N	5	5	5
WEEK	4	MEAN	65	63	65
		S.D.	2.7	2.4	0.8
		N	5	5	5
WEEK	5	MEAN	62	62	63
		S.D.	2.8	3.6	1.7
		N	5	5	5
WEEK	6	MEAN	59	56	58
		S.D.	2.8	2.3	1.4
		N	5	5	5
WEEK	7	MEAN	56	47	55
		S.D.	3.4	21.3	1.4
		N	5	5	5
WEEK	8	MEAN	52	45	50
		S.D.	2.0	15.3	1.5
		N	5	5	5

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE F

GASOLINE DIPE 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/M3) :	1 0	2 2000	3 10000	4 20000
WEEK 9	MEAN	53	48	51	55
	S.D.	3.4	12.5	2.6	3.7
	N	5	5	5	5
WEEK 10	MEAN	53	54	49	55
	S.D.	3.9	7.2	2.5	3.6
	N	5	5	5	5
WEEK 11	MEAN	50	49	47	52
	S.D.	3.1	2.8	2.5	3.2
	N	5	4	5	5
WEEK 12	MEAN	48	48	45	50
	S.D.	1.8	2.3	2.6	3.1
	N	5	4	5	5
WEEK 13	MEAN	46	44	42	46
	S.D.	1.8	1.1	2.4	3.3
	N	5	4	5	5

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE F

GASOLINE DIPE 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: DOSE LEVEL (MG/M ³):	1 0	2 2000	3 10000
WEEK	0	MEAN	98	100	98
		S.D.	4.8	8.3	7.2
		N	5	5	5
WEEK	1	MEAN	87	86	82
		S.D.	4.8	2.9	2.3
		N	5	5	5
WEEK	2	MEAN	83	81	77
		S.D.	3.2	4.9	2.9
		N	5	5	5
WEEK	3	MEAN	77	76	74
		S.D.	2.6	6.8	2.8
		N	5	5	5
WEEK	4	MEAN	72	73	72
		S.D.	3.9	7.0	5.1
		N	5	5	5
WEEK	5	MEAN	71	69	70
		S.D.	3.0	1.9	4.4
		N	5	5	5
WEEK	6	MEAN	63	66	70*
		S.D.	2.6	4.8	2.3
		N	5	5	5
WEEK	7	MEAN	63	64	67
		S.D.	2.9	4.9	3.1
		N	5	5	5
WEEK	8	MEAN	58	61	63
		S.D.	4.1	2.1	5.6
		N	5	5	5

Statistical key: * = p<0.05

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211-DIPE-S

TABLE F

GASOLINE DIPE 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: DOSE LEVEL (MG/M3):	1 0	2 2000	3 10000	4 20000
WEEK 9	MEAN	60	64	65	65
	S.D.	2.3	3.3	4.1	3.9
	N	5	5	5	5
WEEK 10	MEAN	60	62	64	66
	S.D.	2.0	2.6	3.1	6.8
	N	5	5	5	5
WEEK 11	MEAN	58	59	63	64
	S.D.	2.4	3.7	4.3	4.7
	N	5	5	5	5
WEEK 12	MEAN	59	60	62	63
	S.D.	1.9	3.4	4.5	2.4
	N	5	5	5	5
WEEK 13	MEAN	55	56	56	58
	S.D.	1.4	3.1	4.6	2.9
	N	5	5	5	5

No statistically significant differences

Huntingdon Life Sciences 00-6130F
211-DIPE-S

TABLE G

GASOLINE DIPE 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 9
		STUDY	1 2
1076	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1077	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1078	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1079	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1080	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P

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

Huntingdon Life Sciences 00-6130F
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TABLE G

GASOLINE DIPE 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 9
		STUDY	1 2
2066	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
2067	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
2068	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
2069	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
2070	WITHIN NORMAL LIMITS	P	

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

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TABLE G

GASOLINE DIPE 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	1 9
		STUDY	1 2
3066	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
3067	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
3068	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
3069	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
3070	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P

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

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP 4 20000 MG/M³

ANIMAL#	OBSERVATIONS	DAY OF	1 9
		STUDY	1 2
4076	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
4077	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
4078	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
4079	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
4080	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P 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 9
		STUDY	1 2
1576	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1577	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1578	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1579	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P
1580	WITHIN NORMAL LIMITS TERMINAL SACRIFICE		P P

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

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP 2 2000 MG/M³

ANIMAL#	OBSERVATIONS	DAY OF	1 9
		STUDY	1 2
2566	WITHIN NORMAL LIMITS TERMINAL SACRIFICE	P	P
2567	WITHIN NORMAL LIMITS TERMINAL SACRIFICE	P	P
2568	WITHIN NORMAL LIMITS TERMINAL SACRIFICE	P	P
2569	WITHIN NORMAL LIMITS TERMINAL SACRIFICE	P	P
2570	WITHIN NORMAL LIMITS TERMINAL SACRIFICE	P	P

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

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP 3 10000 MG/M³

ANIMAL#	OBSERVATIONS	DAY OF	1 9
		STUDY	1 2
3566	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
3567	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
3568	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
3569	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	
3570	WITHIN NORMAL LIMITS	P	
	TERMINAL SACRIFICE	P	

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

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INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP 4 20000 MG/M³

ANIMAL#	OBSERVATIONS	DAY OF	1 9
		STUDY	1 2
4576	WITHIN NORMAL LIMITS		P
	TERMINAL SACRIFICE		P
4577	WITHIN NORMAL LIMITS		P
	TERMINAL SACRIFICE		P
4578	WITHIN NORMAL LIMITS		P
	TERMINAL SACRIFICE		P
4579	WITHIN NORMAL LIMITS		P
	TERMINAL SACRIFICE		P
4580	WITHIN NORMAL LIMITS		P
	TERMINAL SACRIFICE		P

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

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GASOLINE DIPE 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 OPHTHALMOLOGY OBSERVATIONS	DAY -12
ANIMAL#	PART OF EYE	OBSERVATION		
1076		NO VISIBLE LESIONS		
1077		NO VISIBLE LESIONS		
1078		NO VISIBLE LESIONS		
1079		NO VISIBLE LESIONS		
1080		NO VISIBLE LESIONS		

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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	DAY -12
ANIMAL#	PART OF EYE	OBSERVATION		
2066		NO VISIBLE LESIONS		
2067		NO VISIBLE LESIONS		
2068		NO VISIBLE LESIONS		
2069		NO VISIBLE LESIONS		
2070		NO VISIBLE LESIONS		

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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 OPHTHALMOLOGY OBSERVATIONS	DAY -12
ANIMAL#	PART OF EYE	OBSERVATION		
3066		NO VISIBLE LESIONS		
3067		NO VISIBLE LESIONS		
3068		NO VISIBLE LESIONS		
3069		NO VISIBLE LESIONS		
3070		NO VISIBLE LESIONS		

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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	DAY -12
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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FEMALES	GROUP 1	0 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY -12
ANIMAL#	PART OF EYE	OBSERVATION		
1576		NO VISIBLE LESIONS		
1577		NO VISIBLE LESIONS		
1578		NO VISIBLE LESIONS		
1579		NO VISIBLE LESIONS		
1580		NO VISIBLE LESIONS		

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FEMALES	GROUP 2	2000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY -12
ANIMAL#	PART OF EYE	OBSERVATION		
2566		NO VISIBLE LESIONS		
2567		NO VISIBLE LESIONS		
2568		NO VISIBLE LESIONS		
2569		NO VISIBLE LESIONS		
2570		NO VISIBLE LESIONS		

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FEMALES	GROUP 3	10000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY -12
ANIMAL#	PART OF EYE	OBSERVATION		
3566		NO VISIBLE LESIONS		
3567		NO VISIBLE LESIONS		
3568		NO VISIBLE LESIONS		
3569		NO VISIBLE LESIONS		
3570		NO VISIBLE LESIONS		

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FEMALES	GROUP 4	20000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY -12
ANIMAL#	PART OF EYE	OBSERVATION		
4576		NO VISIBLE LESIONS		
4577		NO VISIBLE LESIONS		
4578		NO VISIBLE LESIONS		
4579		NO VISIBLE LESIONS		
4580		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	1	0 MG/M ³	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY	87
ANIMAL#	PART OF EYE			OBSERVATION		
1076				NO VISIBLE LESIONS		
1077	RETINA			FOCAL RETINOPATHY; LEFT		
1078				NO VISIBLE LESIONS		
1079				NO VISIBLE LESIONS		
1080				NO VISIBLE LESIONS		

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MALES	GROUP 2	2000 MG/M ₃	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY 87
ANIMAL#	PART OF EYE	OBSERVATION		
2066		NO VISIBLE LESIONS		
2067		NO VISIBLE LESIONS		
2068		NO VISIBLE LESIONS		
2069		NO VISIBLE LESIONS		

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MALES	GROUP 3	10000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY 87
ANIMAL#	PART OF EYE	OBSERVATION		
3066		NO VISIBLE LESIONS		
3067		NO VISIBLE LESIONS		
3068		NO VISIBLE LESIONS		
3069		NO VISIBLE LESIONS		
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MALES	GROUP 4	20000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY 87
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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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

FEMALES	GROUP 1	0 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY 87
ANIMAL#	PART OF EYE	OBSERVATION		
1576		NO VISIBLE LESIONS		
1577		NO VISIBLE LESIONS		
1578		NO VISIBLE LESIONS		
1579		NO VISIBLE LESIONS		
1580		NO VISIBLE LESIONS		

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FEMALES	GROUP 2	2000 MG/M ³	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY 87
ANIMAL#	PART OF EYE	OBSERVATION		
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2567		NO VISIBLE LESIONS		
2568		NO VISIBLE LESIONS		
2569		NO VISIBLE LESIONS		
2570		NO VISIBLE LESIONS		

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FEMALES	GROUP 3	10000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY 87
ANIMAL#	PART OF EYE	OBSERVATION		
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3567		NO VISIBLE LESIONS		
3568		NO VISIBLE LESIONS		
3569		NO VISIBLE LESIONS		
3570		NO VISIBLE LESIONS		

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FEMALES	GROUP 4	20000 MG/M3	INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS	DAY	87
ANIMAL#	PART OF EYE	OBSERVATION			
4576		NO VISIBLE LESIONS			
4577		NO VISIBLE LESIONS			
4578		NO VISIBLE LESIONS			
4579		NO VISIBLE LESIONS			
4580		NO VISIBLE LESIONS			

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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 2	INDIVIDUAL BODY WEIGHTS (GRAMS)														
		WEEK OF STUDY														
ANIMAL#		-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2066	232	324	367	394	422	443	471	488	516	530	548	562	574	585	600	
2067	223	302	338	367	392	409	436	447	472	484	505	513	521	543	543	
2068	232	323	364	399	432	465	483	492	513	534	553	570	584	606	601	
2069	221	306	346	383	411	439	470	480	508	514	538	554	570	582	583	
2070	212	280	307	324	340	353	371	382	278	252	255	225				
MEAN	224	307	344	373	399	422	446	458	457	463	480	485	562	579	582	
S.D.	8.3	18.0	24.1	30.3	36.2	43.3	45.7	46.1	102.0	119.5	127.0	146.9	28.1	26.2	26.9	
N	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	

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INDIVIDUAL BODY WEIGHTS (GRAMS)

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INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP 4 20000 MG/M3

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INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP 1 0 MG/M3

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AND 4-WEEK IN VIVO GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP 2 2000 MG/M3

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP 3 10000 MG/M3

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP 4 20000 MG/M3

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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/M ³	INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)												
			WEEK OF STUDY												
ANIMAL#			0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
2066			43	70	98	119	147	164	192	206	224	238	250	261	276
2067			36	66	90	108	135	145	170	183	203	211	219	241	242
2068			42	76	109	142	160	169	191	211	230	247	261	283	278
2069			40	77	105	133	164	174	202	207	232	248	264	276	277
2070			27	44	60	73	91	102	-2	-28	-25	-55			
MEAN			37	66	92	115	139	151	150	156	173	178	248	265	268
S.D.			6.3	13.5	19.3	26.8	29.5	29.5	86.2	103.5	111.1	131.0	20.4	18.3	17.5
N			5	5	5	5	5	5	5	5	5	5	4	4	4

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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 4 20000 MG/M3

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 1 0 MG/M3

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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)

FEMALES GROUP 2 2000 MG/M3

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GASOLINE DIPE 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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TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE FROM BASELINE (GRAMS)

FEMALES GROUP 4 20000 MG/M3

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TABLE K

GASOLINE DIPE 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)

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TABLE K

GASOLINE DIPE 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 2	2000 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
2066			100	84	71	66	60	59	55	57	49	52	48	47	45	44
2067			96	82	74	74	66	67	58	61	54	56	53	54	51	46
2068			96	78	73	67	61	59	55	52	52	51	52	49	48	43
2069			100	81	75	69	63	61	54	55	52	53	49	49	47	44
2070			95	78	70	73	66	65	59	9	18	26	66			
MEAN			97	81	73	70	63	62	56	47	45	48	54	49	48	44
S.D.			2.6	2.5	1.9	3.3	2.4	3.6	2.3	21.3	15.3	12.5	7.2	2.8	2.3	1.1
N			5	5	5	5	5	5	5	5	5	5	5	4	4	4

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TABLE K

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

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INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP 4 20000 MG/M3

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INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP 1 0 MG/M3

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FEMALES GROUP 2 2000 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	INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)												
		20000 MG/M ³												
ANIMAL#	WEEK OF STUDY													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4576	90	79	73	70	64	64	66	63	60	62	60	60	59	61
4577	SF	97	87	79	75	74	69	67	68	70	67	65	64	61
4578	103	81	80	85	75	73	71	71	64	69	77	71	63	56
4579	SF	84	81	79	69	70	74	69	67	63	66	64	65	55
4580	96	79	72	70	66	65	66	67	63	62	61	59	63	56
MEAN	96	84	79	77	70	69	69	67	64	65	66	64	63	58
S.D.	6.3	7.6	6.2	6.5	5.0	4.5	3.3	3.2	3.4	3.9	6.8	4.7	2.4	2.9
N	3	5	5	5	5	5	5	5	5	5	5	5	5	5

SF=Spilled Feeder

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TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP 1 0 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF	STUDY
			STUDY	DAY
1076	TERMINAL SACRIFICE	15-MAY-02	13	92
1077	TERMINAL SACRIFICE	15-MAY-02	13	92
1078	TERMINAL SACRIFICE	15-MAY-02	13	92
1079	TERMINAL SACRIFICE	15-MAY-02	13	92
1080	TERMINAL SACRIFICE	15-MAY-02	13	92

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ANIMAL TERMINATION HISTORY

MALES GROUP 2 2000 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2066	TERMINAL SACRIFICE	15-MAY-02	13	92
2067	TERMINAL SACRIFICE	15-MAY-02	13	92
2068	TERMINAL SACRIFICE	15-MAY-02	13	92
2069	TERMINAL SACRIFICE	15-MAY-02	13	92
2070	HUMANE SACRIFICE	29-APR-02	10	76

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ANIMAL TERMINATION HISTORY

MALES GROUP 3 10000 MG/M3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF	STUDY
			STUDY	DAY
3066	TERMINAL SACRIFICE	15-MAY-02	13	92
3067	TERMINAL SACRIFICE	15-MAY-02	13	92
3068	TERMINAL SACRIFICE	15-MAY-02	13	92
3069	TERMINAL SACRIFICE	15-MAY-02	13	92
3070	TERMINAL SACRIFICE	15-MAY-02	13	92

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ANIMAL TERMINATION HISTORY

MALES GROUP 4 20000 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4076	TERMINAL SACRIFICE	15-MAY-02	13	92
4077	TERMINAL SACRIFICE	15-MAY-02	13	92
4078	TERMINAL SACRIFICE	15-MAY-02	13	92
4079	TERMINAL SACRIFICE	15-MAY-02	13	92
4080	TERMINAL SACRIFICE	15-MAY-02	13	92

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ANIMAL TERMINATION HISTORY

FEMALES GROUP 1 0 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1576	TERMINAL SACRIFICE	15-MAY-02	13	92
1577	TERMINAL SACRIFICE	15-MAY-02	13	92
1578	TERMINAL SACRIFICE	15-MAY-02	13	92
1579	TERMINAL SACRIFICE	15-MAY-02	13	92
1580	TERMINAL SACRIFICE	15-MAY-02	13	92

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ANIMAL TERMINATION HISTORY

FEMALES GROUP 2 2000 MG/M³

ANIMAL#	TYPE OF DEATH	DATE OF	WEEK OF	STUDY
		DEATH	STUDY	DAY
2566	TERMINAL SACRIFICE	15-MAY-02	13	92
2567	TERMINAL SACRIFICE	15-MAY-02	13	92
2568	TERMINAL SACRIFICE	15-MAY-02	13	92
2569	TERMINAL SACRIFICE	15-MAY-02	13	92
2570	TERMINAL SACRIFICE	15-MAY-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	15-MAY-02	13	92
3567	TERMINAL SACRIFICE	15-MAY-02	13	92
3568	TERMINAL SACRIFICE	15-MAY-02	13	92
3569	TERMINAL SACRIFICE	15-MAY-02	13	92
3570	TERMINAL SACRIFICE	15-MAY-02	13	92

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ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4576	TERMINAL SACRIFICE	15-MAY-02	13	92
4577	TERMINAL SACRIFICE	15-MAY-02	13	92
4578	TERMINAL SACRIFICE	15-MAY-02	13	92
4579	TERMINAL SACRIFICE	15-MAY-02	13	92
4580	TERMINAL SACRIFICE	15-MAY-02	13	92