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

HLS Study No.: **00-6128**
Sponsor Study No.: 211-TAME-S
Date: 7 January 2011

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

Huntingdon Life Sciences, Inc. Study No. 00-6128
Sponsor Study No 211-TAME-S
Measurement of Glial Fibrillary Acidic Protein

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STATEMENT OF COMPLIANCE

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

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

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Date

Gary M. Hoffman, B.A., D.A.B.T.
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6 Oct 01
Date

SIGNATURE PAGE

SCIENTIST

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

James P. O'Callaghan, Ph.D.
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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

6 Oct 11
Date

Third-Party QA Statement

Study No. HLS 00-6128

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

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

Area Inspected	Date of Inspection	Date Reported to Sponsor
Facility Inspection	4/3/01	4/10/01
Terminal Sacrifice Inspection	9/24/01	10/31/01
Draft Final Report Audit	4/17-6/4/02	6/04/02
Final Report Revew	8/18/09	8/19/09

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QUALITY ASSURANCE STATEMENT

Listed below are the dates that this study was inspected by the Quality Assurance Unit of Huntingdon Life Sciences, East Millstone, New Jersey, and the dates that findings were reported to the Study Director and Management. This report reflects the raw data as far as can be reasonably established.

Type of Inspection	Date(s) of Inspection	Reported to Study Director and Management
GLP Protocol Review	20,24 Apr 01	24 Apr 01
Exposure and Monitoring and Equipment Records	26 Jun 01	26 Jun 01
Exposure and Monitoring	2 Aug 01	2 Aug 01
GFAP Necropsy	26 Sep 01	1 Oct 01
Subcontractor		
Subcontractor Final Report	22-25 Feb 02	26 Feb 02
Final Report Review and Protocol Amendments 1-5	5-7 Jan 09	9 Jan 09
Protocol Amendment 6	11 Aug 11	11 Aug 11

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Summary

Toxicant-induced injury of the adult or developing central nervous system of the rat results in hypertrophy of astrocytes at the site of injury. The hallmark of this response is the enhanced expression of the major intermediate filament protein of astrocytes, GFAP. Exposure to Gasoline TAME Vapor Condensate did not elevate GFAP levels in any of the nine brain regions examined in either males or females. The data suggest that exposure to Gasoline TAME Vapor Condensate under the regimen employed did not consistently elevate the levels of GFAP 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, 1991a; 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, 1991a; Norton et al., 1992). Moreover, the temporal and regional increments in GFAP correspond to the temporal and regional patterns of neuronal damage, as revealed by sensitive silver stains (Balaban et al., 1988). These findings indicate that assays of GFAP represent a sensitive, simple and quantitative approach for evaluation of nervous system damage (O'Callaghan, 1991a; Norton et al., 1992).

As part of the U.S. Environmental Protection Agency's testing requirements under the Clean Air Act, identification and characterization of the potential adverse effects of gasoline and various oxygenate-gasoline blends is to be determined. Neurotoxicity assessment constitutes a portion of these testing activities. Subchronic (13-week) inhalation exposures to gasoline and gasoline plus each of 6 fuel additives have been

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

Materials, Methods/Procedures

STUDY DATES

STUDY INITIATION
30 May 2001 (Date Study Director signed the Protocol)

DATE OF ANIMAL RECEIPT
29 May 2001

EXPOSURE INITIATION
12 June 2001 (Experimental Start Date)

EXPOSURE TERMINATION
9 October 2001

TERMINAL SACRIFICE
10 October 2001

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

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

I. Basic Protocol 1: GFAP Sandwich ELISA

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

B. **Materials**:

Vortex Mixer or Ultrasonic Cell Disruptor (e.g. PGC cat. # 81-6721-02, 2 mm probe)
Pipettes
Hot/Stir Plate
Microplate Reader

96-well Microtiter Plates (Immulon 2, Dynatech)
Pipette tips
Rabbit anti-Glial Fibrillary Acidic Protein Antibody (DAKO, Cat. #Z0334)
Non-Fat Dry Milk (Carnation)
Mouse anti-Glial Fibrillary Acidic Protein Antibody (Chemicon Cat. # MAB 3402; formerly Boerhinger Mannheim #814369, Clone GA5)
Phosphate Buffered Saline (e.g. Pierce Cat. #28374)
Alkaline Phosphatase conjugated anti-mouse IgG (Jackson Immuno Research Cat #315-055-003)
Alkaline Phosphatase Substrate Kit (e.g. Bio-Rad Cat. #172-1063)
Triton X-100 (e.g. Bio-Rad Cat. #161-0407)
Sodium Hydroxide (e.g., 0.4N NaOH, Fisher LC 243204)

C. Protocol Steps:

1. Standard Curve Preparation

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

sample only a single time per tip.

2. Sample Preparation

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

3. GFAP Assay

1. Coat Immulon-2 flat bottom plates with rabbit anti-GFAP. Add 1.0μg total immunoglobulin protein /100 μl PBS /well. (~25μl of anti-GFAP [Dako] in 10 ml of PBS is the quantity needed per plate).
2. Incubate the plate at 37°C for 1 hour. This step may be done at the beginning of the assay or it may be done the night before with storage overnight at 4°C. Perform all other incubation and reagent addition steps at room temperature.
3. Empty the plate into a sink and tap on absorbent paper to remove excess liquid. *This latter procedure is important to eliminate the possibility of any reagent carry-over between steps.*
4. Wash plates 4X with PBS (200 μl per well), tapping and blotting between each wash.
5. Block 1 hour at Room Temperature(Rm Temp) with 5% non-fat powdered milk in PBS at100 μl per well.
6. Empty plate, tap on absorbent paper (upside down) to remove excess liquid, load diluted standard curve and samples in a volume of 100 μl per well. Incubate for 1 hour. The template, Table 2, is an example of a typical 96-well microplate layout for GFAP standards and unknowns.
7. Wash 4X with PBS + 0.5% Triton X-100, 200 μl/well.

Incubate for 1 hour at Rm Temp in monoclonal anti-GFAP (1:500)(Chemicon) + alkaline phosphatase conjugated anti-mouse IgG (1:3000)(Jackson ImmunoResearch) made up in 5% non-fat dry milk + 0.5% Triton X-100, 100 μl /well.

9. Wash 4X with PBS + 0.5% Triton X-100, 200 μl/well.
10. Add P-nitrophenylphosphate substrate (Bio-Rad) in a volume of 100 μl/well and incubate for 20 minutes at room temperature.
11. Stop reaction with 0.4N NaOH, 100 μl/well.
12. "Pop" any bubbles in the plate wells with a needle or pipette tip to insure uniform and accurate readings of standard and sample ODs. Read plate at 405 nm.

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

II. Support Protocol 1: Brain tissue preparation

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

B. Materials:

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

C. Protocol Steps:

1. Sacrifice animals and remove the brain as rapidly as possible.
2. Dissect brain regions. If a number of regions are to be dissected, this process can be aided by keeping the brain firm on a cold plate (e.g. Thermoelectrics cold plate, Aldrich Chemical Co. or simply an inverted petri dish placed on ice) maintained at approximately 4°C. Rat or mouse brains can be dissected into 10-15 regions, free hand, within approximately 10 minutes. For this study, nine regions were dissected: Striatum, Hippocampus, Cortex, Olfactory Bulb, Thalamus, Hypothalamus, Cerebellum, Pituitary, and Rest of Brain. *This number of regions can be prepared on a cold plate or at room temperature without degradation of GFAP as assessed by immunoblot analysis. All brain regions can be stored frozen indefinitely in capped microfuge tubes at this step in the protocol, or you can proceed to the next step.*
3. Weigh and homogenize the dissected brain parts. Tare an appropriately labeled microfuge or other suitable storage tube, or weigh boat for the

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

III. Support Protocol 2: Assay for Total Protein

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

B. Materials:

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

C. Protocol Steps:

1. Prepare total protein standards. Prepare a 1 mg/ml solution of BSA in 1% (w/v) SDS. *Aliquots of this standard can be stored frozen at -70°C for future use. Thaw as needed, but do not re-freeze.*
2. Prepare a total protein standard curve. Prepare dilutions of the BSA standard in 1% SDS as follows: 1.0, 2.5, 5.0, 7.5, and 10 μg/10μl of 1% SDS (no dilution is required for last standard). Vortex each tube and add 10 μl of each standard to a well of the microtiter plate; add 10 μl of 1% SDS to serves as a blank. *Typically, standards are run in duplicate. Detergents act as wetting agents, therefore, more than a single use of a pipette tip with SDS-containing samples*

can lead to carry-over errors. Thus, it is recommended to use only a single pipette tip per sample and to withdraw the sample only a single time per tip.

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

IV. Reagents and Solutions

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

generate a colored reaction product proportional to the amount of antigen (GFAP) present in the samples. Substitution of antibodies from other vendors may not yield suitable results. Make these solutions fresh on the day of use and do not save.

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

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

A. Background Information

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

While GFAP immunohistochemistry has proven useful for revealing patterns of gliosis after brain injury, this approach does not lend itself to quantification or the analysis of large numbers of samples. Small (25-50%), but toxicologically significant increases, also may be difficult to detect by immunohistochemistry. These drawbacks, combined with the need to develop quantitative biomarkers of neurotoxicity (O'Callaghan et al., 1995), and to define quantitative aspects of toxicant- and disease-induced gliosis, has prompted the development and implementation of a number of GFAP assays. These assays have been applied to examine gliosis in specific brain areas already known to be affected by disease or other insult. In addition, they also can be broadly applied in a risk assessment context (U.S. EPA) to screen for potential sites of neural damage resulting from toxic exposures of the CNS. Recently, analysis of GFAP has been used to demonstrate that the degree of cortical gliosis in postmortem brain tissue from victims of Alzheimer's disease correlates with the severity of dementia scores in these individuals prior to death (G. Webster Ross, submitted). Analysis of GFAP in cerebrospinal fluid (CSF) also has been applied to the human condition as an indicator of the severity of traumatic injury to the brain (Rosengren et al., 1994). Finally, analysis of GFAP can be used as an indicator of the presence of brain or spinal cord contamination of meat (Schmidt et al., 1999).

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

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

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

B. Critical Parameters

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

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

C. Troubleshooting (See Table 4)

D. Typical Results

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

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

E. Time Considerations

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

Statistics:

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

Results and Conclusions

The results of the GFAP analysis are presented in Tables 5 and 6. Results are reported according to gender, 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 TAME Vapor Condensate did not elevate GFAP 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. Nevertheless, it is remarkable that a brain-wide survey of GFAP revealed no increases from baseline in any of the examined regions. In conclusion, exposure to Gasoline TAME Vapor Condensate did not result in gliosis in nine representative brain regions (including pituitary samples that were too dilute to detect GFAP; therefore, not of value and not further summarized or discussed) 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

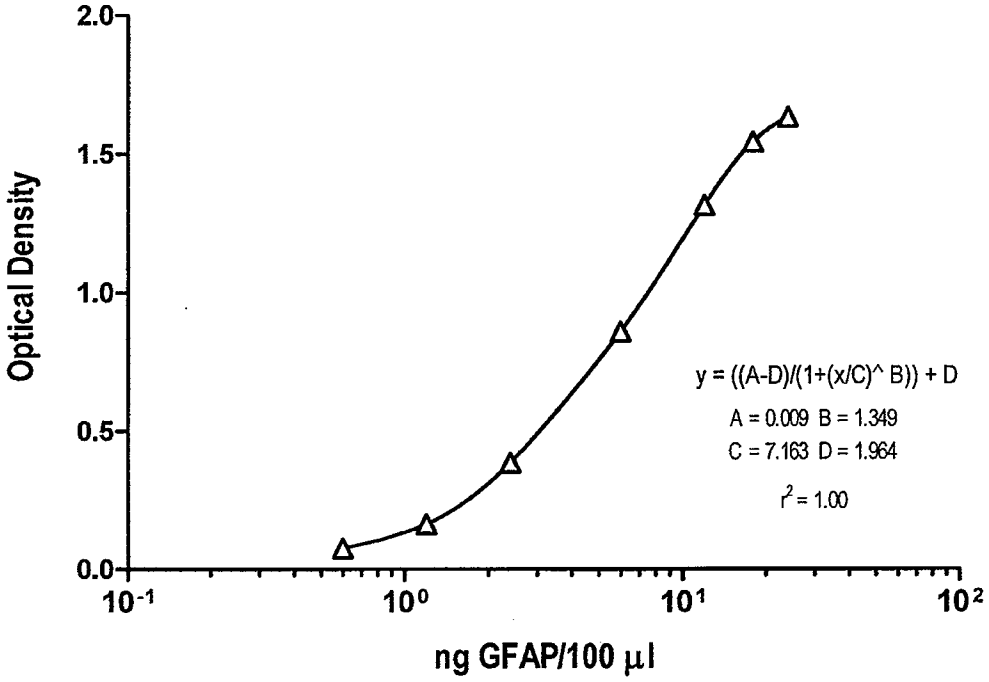
2.0
1.5
1.0
0.5
0.0
Optical Density
y = ((A-D)/(1+(x/C)^ B)) + D
A = 0.009 B = 1.349
C = 7.163 D = 1.964
r2 = 1.00
10-1
100
101
102
ng GFAP/100 µl

Figure 2

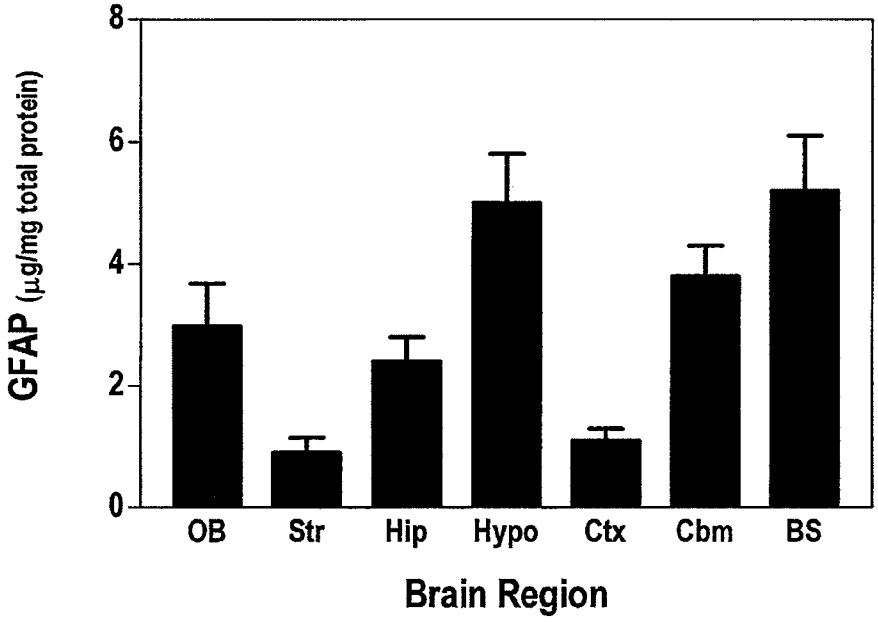
GFAP (μg/mg total protein)
8
6
4
2
0
OB
Str
Hip
Hypo
Ctx
Cbm
BS
Brain Region

TABLE 1: GFAP Standard Curve Preparation

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

Table 2: Microtiter Plate Template

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

Blk= Blank; Std= Standard; ? = Unknowns

Table 3: Sample Values for GFAP Standard Curve

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

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

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

Table 4: Troubleshooting Guide

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

Table 5: Mean GFAP Levels in Specific Regions of Male Rat Brains Following a 13 week Whole -Body Inhalation Exposure to Gasoline TAME 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	1.20 ± 0.07*	1.37 ± 0.09	1.18 ± 0.12	1.05 ± 0.11
Hippocampus	2.77 ± 0.28	3.11 ± 0.34	2.97 ± 0.21	2.74 ± 0.12
Cortex	1.47 ± 0.13	1.65 ± 0.09	1.52 ± 0.12	1.52 ± 0.16
Olfactory Bulb	2.57 ± 0.21	2.49 ± 0.20	2.75 ± 0.16	2.55 ± 0.12
Thalamus	2.41 ± 0.12	2.45 ± 0.25	2.57 ± 0.27	3.04 ± 0.54
Hypothalamus	7.70 ± 0.91	8.50 ± 0.89	7.09 ± 0.91	6.56 ± 0.75
Cerebellum	4.45 ± 0.21	4.16 ± 0.39	3.90 ± 0.45	3.97 ± 0.23
Rest of Brain	4.56 ± 0.20	5.03 ± 0.74	4.93 ± 0.23	4.85 ± 0.10

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

Table 6: Mean GFAP Levels in Specific Regions of Female Rat Brains Following a 13 week Whole -Body Inhalation Exposure to Gasoline TAME 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	1.47 ± 0.16*	1.21 ± 0.14	1.03 ± 0.09	1.18 ± 0.09
Hippocampus	3.30 ± 0.20	3.03 ± 0.30	2.64 ± 0.12	2.63 ± 0.23
Cortex	1.56 ± 0.12	1.88 ± 0.35	1.32 ± 0.10	1.38 ± 0.14
Olfactory Bulb	2.54 ± 0.10	2.76 ± 0.39	2.37 ± 0.16	2.73 ± 0.23
Thalamus	2.38 ± 0.10	2.80 ± 0.45	1.96 ± 0.18	2.31 ± 0.37
Hypothalamus	7.99 ± 0.67	9.67 ± 1.10	7.98 ± 0.46	8.23 ±1.46
Cerebellum	4.58 ± 0.18	4.29 ± 0.45	3.78 ± 0.17	3.87 ± 0.55
Rest of Brain	5.58 ± 0.71	5.40 ± 0.58	4.82 ± 0.23	4.86 ± 0.54

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

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

Group	Animal no.	Sex	Striatum	Hippocampus	Cortex	Olfactory Bulb	Thalamus	Hypothalamus	Cerebellum	Pituitary	Rest of Brain
Group I Air Control 0 mg/m3	1027	Male	1.385•	2.102	1.325	2.244	2.102	6.003	4.019	*	4.481
	1028		1.238	3.678	1.969	3.011	2.173	9.514	5.044	0.054	5.139
	1026		0.966	2.788	1.420	2.962	2.464	5.927	4.832	0.064	4.484
	1030		1.146	2.245	1.359	1.944	2.675	10.249	4.104	0.055	4.743
	1029		1.251	3.037	1.283	2.706	2.613	6.822	4.266	*	3.930
	1530	Female	1.455	3.565	1.529	2.435	2.634	9.763	5.037	*	7.042
	1527		1.065	3.086	1.510	2.348	2.153	6.646	4.189	*	5.080
	1529		1.359	2.837	1.357	2.338	2.193	9.040	4.133	*	3.957
	1526		2.021	3.952	2.006	2.836	2.546	8.201	4.905	*	7.482
	1528		1.460	3.041	1.404	2.744	2.393	6.290	4.630	*	4.359
Group II Test Substance 2,000 mg/m3	2019	Male	1.607	4.396	1.969	2.654	3.302	10.656	5.351	*	7.874
	2017		1.439	2.937	1.483	2.151	2.221	7.335	3.637	0.058	4.068
	2016		1.497	3.049	1.674	3.047	2.174	9.512	4.832	0.055	4.855
	2020		1.145	2.688	1.577	2.677	2.667	5.645	3.634	*	4.592
	2018		1.158	2.457	1.541	1.912	1.872	9.344	3.328	*	3.780
	2519	Female	1.383	2.959	1.386	2.485	2.390	10.062	3.580	*	4.400
	2517		0.947	2.647	1.378	1.952	2.117	8.893	3.846	*	5.442
	2520		1.369	4.108	2.622	3.553	3.713	12.112	5.526	*	7.342
	2516		0.830	2.380	1.175	2.003	1.736	5.883	3.310	*	4.024
	2518		1.516	3.031	2.820	3.809	4.017	11.421	5.179	*	5.770
Group III Test Substance 10,000 mg/m3	3016	Male	1.228	2.773	1.296	2.550	1.704	6.905	2.212	0.063	4.453
	3019		1.227	2.574	1.469	2.486	2.749	5.198	4.745	*	5.600
	3017		1.179	3.407	1.710	2.950	2.954	6.616	3.957	*	5.026
	3020		0.769	2.562	1.247	2.458	2.225	6.184	3.972	*	4.399
	3018		1.508	3.532	1.887	3.301	3.240	10.545	4.588	0.053	5.158
	3516	Female	0.740	2.433	0.945	2.992	1.938	6.848	3.803	*	4.952
	3520		1.084	2.528	1.326	2.264	2.034	6.986	4.308	0.074	5.086
	3518		1.273	2.870	1.405	2.164	2.334	9.156	3.555	*	4.442
	3517		0.916	2.401	1.352	2.127	1.292	8.200	3.295	*	4.163
	3519		1.149	2.983	1.564	2.279	2.184	8.720	3.948	*	5.476
Group IV Test Substance 20,000 mg/m3	4030	Male	0.768	2.692	1.130	2.670	1.644	9.180	4.021	*	4.681
	4028		1.345	2.974	1.576	2.668	2.670	6.143	4.460	*	5.170
	4027		1.258	3.087	2.096	2.856	3.969	6.957	4.426	*	4.712
	4029		1.040	2.508	1.406	2.364	4.597	4.638	3.226	*	4.695
	4026		0.833	2.459	1.382	2.189	2.328	5.883	3.717	0.089	4.999
	4528	Female	1.544	3.158	1.647	3.381	3.559	13.696	5.673	*	6.266
	4527		1.030	3.134	1.268	2.322	1.939	8.695	4.429	*	3.986
	4530		1.173	2.005	1.039	2.119	1.360	5.600	3.307	*	3.306
	4526		1.080	2.287	1.201	2.740	2.139	6.854	3.500	*	5.561
	4529		1.076	2.540	1.762	3.096	2.563	6.278	2.435	*	5.185

• Each value represents the concentration of GFAP (ug/mg Total Protein)
* Pituitary samples too dilute to detect GFAP; therefore, not of value and not further summarized or discussed.

	Animal Exposure and Animal Data Preface	Appendix A

INTRODUCTION: The following is data generated at Huntingdon Life Sciences, East Millstone, NJ. The separately issued main study report should be referenced for details of the procedures used for test atmosphere generation/characterization and animal evaluations.

STUDY DATES:	Date of Animal Receipt:	7 June 2001
	Experimental Initiation Date:	26 June 2001 (in-life)
	Experimental Completion Date:	26 September 2001 (in-life)
	Draft Report Date:	28 February 2002

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

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TABLES

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
0	26-Jun-01	1	0	0	0	0	0	0				25	48
1	27-Jun-01	2	0	0	0	0	0	0				25	50
2	28-Jun-01	3	0	0	0	0	0	0				25	49
3	29-Jun-01	4	0	0	0	0	0	0				25	48
6	2-Jul-01	5	0	0	0	0	0	0	2.809	1.858	3.49E-03	26	48
7	3-Jul-01	6	0	0	0	0	0	0				24	47
8	4-Jul-01	7	0	0	0	0	0	0				24	49
9	5-Jul-01	8	0	0	0	0	0	0				24	52
10	6-Jul-01	9	0	0	0	0	0	0				24	47
13	9-Jul-01	10	0	0	0	0	0	0	1.023	2.369	2.30E-02	24	47
14	10-Jul-01	11	0	0	0	0	0	0				25	46
15	11-Jul-01	12	0	0	0	0	0	0				25	48
16	12-Jul-01	13	0	0	0	0	0	0				25	50
17	13-Jul-01	14	0	0	0	0	0	0	2.799	2.189	3.66E-03	25	52
20	16-Jul-01	15	0	0	0	0	0	0				25	47
21	17-Jul-01	16	0	0	0	0	0	0	3.519	2.858	1.24E-02	24	49
22	18-Jul-01	17	0	0	0	0	0	0				24	50
23	19-Jul-01	18	0	0	0	0	0	0				24	51
24	20-Jul-01	19	0	0	0	0	0	0				24	48
27	23-Jul-01	20	0	0	0	0	0	0				24	48
28	24-Jul-01	21	0	0	0	0	0	0	0.9289	2.087	1.17E-02	25	50
29	25-Jul-01	22	0	0	0	0	0	0				25	53
30	26-Jul-01	23	0	0	0	0	0	0				22	47
31	27-Jul-01	24	0	0	0	0	0	0				25	48
32	28-Jul-01	25	0	0	0	0	0	0				25	47
34	30-Jul-01	26	0	0	0	0	0	0				25	47
35	31-Jul-01	27	0	0	0	0	0	0				24	49

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

Day	Date	Exposure Number	Analytical Chamber Concentration: Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Analytical Chamber Concentration: Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	Particle Size Determinations: GSD	Particle Size Determinations: TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Chamber Environment: Mean Humidity (%)
36	1-Aug-01	28	0	0	0	0	0	0				24	47
37	2-Aug-01	29	0	0	0	0	0	0				24	47
38	3-Aug-01	30	0	0	0	0	0	0	1.281	1.730	2.50E-03	24	47
41	6-Aug-01	31	0	0	0	0	0	0				24	49
42	7-Aug-01	32	0	0	0	0	0	0				25	48
43	8-Aug-01	33	0	0	0	0	0	0				26	50
44	9-Aug-01	34	0	0	0	0	0	0				26	49
45	10-Aug-01	35	0	0	0	0	0	0	0.8679	1.733	6.82E-02	26	51
48	13-Aug-01	36	0	0	0	0	0	0				25	47
49	14-Aug-01	37	0	0	0	0	0	0				24	48
50	15-Aug-01	38	0	0	0	0	0	0				24	48
51	16-Aug-01	39	0	0	0	0	0	0	0.9270	1.631	3.16E-03	24	48
52	17-Aug-01	40	0	0	0	0	0	0				24	50
55	20-Aug-01	41	0	0	0	0	0	0				25	48
56	21-Aug-01	42	0	0	0	0	0	0				25	47
57	22-Aug-01	43	0	0	0	0	0	0				25	48
58	23-Aug-01	44	0	0	0	0	0	0				25	51
59	24-Aug-01	45	0	0	0	0	0	0	1.095	2.289	4.97E-03	25	48
62	27-Aug-01	46	0	0	0	0	0	0				26	46
63	28-Aug-01	47	0	0	0	0	0	0				24	48
64	29-Aug-01	48	0	0	0	0	0	0				24	47
65	30-Aug-01	49	0	0	0	0	0	0				24	48
66	31-Aug-01	50	0	0	0	0	0	0	0.8046	1.439	1.32E-02	24	48
69	3-Sep-01	51	0	0	0	0	0	0				24	49
70	4-Sep-01	52	0	0	0	0	0	0				25	46
71	5-Sep-01	53	0	0	0	0	0	0				25	48
73	7-Sep-01	54	0	0	0	0	0	0	1.991	2.160	2.14E-03	26	49

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
76	10-Sep-01	55	0	0	0	0	0	0				26	56
77	11-Sep-01	56	0	0	0	0	0	0				24	49
78	12-Sep-01	57	0	0	0	0	0	0				24	51
79	13-Sep-01	58	0	0	0	0	0	0				24	51
80	14-Sep-01	59	0	0	0	0	0	0	18.34	2.071	1.43E-02	24	50
81	15-Sep-01	60	0	0	0	0	0	0				24	50
83	17-Sep-01	61	0	0	0	0	0	0				24	48
84	18-Sep-01	62	0	0	0	0	0	0				24	52
85	19-Sep-01	63	0	0	0	0	0	0				25	51
86	20-Sep-01	64	0	0	0	0	0	0				24	51
87	21-Sep-01	65	0	0	0	0	0	0	1.645	2.328	2.77E-03	24	54
89	23-Sep-01	66	0	0	0	0	0	0				25	48
90	24-Sep-01	67	0	0	0	0	0	0				24	56
91	25-Sep-01	68	0	0	0	0	0	0				22	50
		Mean	0		0				2.925	2.057	1.27E-02	24.5	49.0
		S.D.	0		0				4.716	0.378	1.78E-02	0.8	2.1

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

Day	Date	Exposure Number	Nominal (mg/m^3)	Analytical Chamber Concentration: Mean (mg/m^3)	Individual (mg/m^3)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m^3)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
0	26-Jun-01	1	0	0	0	0	0	0				24	50
1	27-Jun-01	2	0	0	0	0	0	0				24	52
2	28-Jun-01	3	0	0	0	0	0	0				24	50
3	29-Jun-01	4	0	0	0	0	0	0				24	51
6	2-Jul-01	5	0	0	0	0	0	0	4.591	1.924	1.19E-02	25	50
7	3-Jul-01	6	0	0	0	0	0	0				25	50
8	4-Jul-01	7	0	0	0	0	0	0				24	56
9	5-Jul-01	8	0	0	0	0	0	0				24	57
10	6-Jul-01	9	0	0	0	0	0	0				24	50
13	9-Jul-01	10	0	0	0	0	0	0	0.9107	1.949	1.96E-02	25	50
14	10-Jul-01	11	0	0	0	0	0	0				24	50
15	11-Jul-01	12	0	0	0	0	0	0				24	50
16	12-Jul-01	13	0	0	0	0	0	0				24	51
17	13-Jul-01	14	0	0	0	0	0	0	3.456	1.961	2.88E-03	24	54
20	16-Jul-01	15	0	0	0	0	0	0				24	49
21	17-Jul-01	16	0	0	0	0	0	0	1.980	2.424	1.24E-02	24	51
22	18-Jul-01	17	0	0	0	0	0	0				24	54
23	19-Jul-01	18	0	0	0	0	0	0				24	54
24	20-Jul-01	19	0	0	0	0	0	0				24	50
27	23-Jul-01	20	0	0	0	0	0	0				25	51
28	24-Jul-01	21	0	0	0	0	0	0	0.9115	2.364	1.13E-02	23	52
29	25-Jul-01	22	0	0	0	0	0	0				24	55
30	26-Jul-01	23	0	0	0	0	0	0				21	47
31	27-Jul-01	24	0	0	0	0	0	0				24	50
32	28-Jul-01	25	0	0	0	0	0	0				23	49
34	30-Jul-01	26	0	0	0	0	0	0				24	48
35	31-Jul-01	27	0	0	0	0	0	0				24	51

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
36	1-Aug-01	28	0	0	0	0	0	0				24	51
37	2-Aug-01	29	0	0	0	0	0	0				25	50
38	3-Aug-01	30	0	0	0	0	0	0	1.163	1.719	2.68E-03	25	49
41	6-Aug-01	31	0	0	0	0	0	0				25	51
42	7-Aug-01	32	0	0	0	0	0	0				24	53
43	8-Aug-01	33	0	0	0	0	0	0				24	52
44	9-Aug-01	34	0	0	0	0	0	0				24	51
45	10-Aug-01	35	0	0	0	0	0	0	0.8617	1.638	6.21E-02	25	53
48	13-Aug-01	36	0	0	0	0	0	0				24	49
49	14-Aug-01	37	0	0	0	0	0	0				24	49
50	15-Aug-01	38	0	0	0	0	0	0				25	51
51	16-Aug-01	39	0	0	0	0	0	0	0.9082	1.518	3.36E-03	24	51
52	17-Aug-01	40	0	0	0	0	0	0				24	51
55	20-Aug-01	41	0	0	0	0	0	0				25	51
56	21-Aug-01	42	0	0	0	0	0	0				24	49
57	22-Aug-01	43	0	0	0	0	0	0				24	51
58	23-Aug-01	44	0	0	0	0	0	0				24	51
59	24-Aug-01	45	0	0	0	0	0	0	0.8564	1.629	3.35E-03	24	51
62	27-Aug-01	46	0	0	0	0	0	0				24	49
63	28-Aug-01	47	0	0	0	0	0	0				25	53
64	29-Aug-01	48	0	0	0	0	0	0				25	50
65	30-Aug-01	49	0	0	0	0	0	0				24	50
66	31-Aug-01	50	0	0	0	0	0	0	0.7034	1.798	9.39E-03	25	51
69	3-Sep-01	51	0	0	0	0	0	0				24	52
70	4-Sep-01	52	0	0	0	0	0	0				24	50
71	5-Sep-01	53	0	0	0	0	0	0				23	50
73	7-Sep-01	54	0	0	0	0	0	0	1.341	1.672	1.39E-03	24	52

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
76	10-Sep-01	55	0	0	0	0	0	0				24	59
77	11-Sep-01	56	0	0	0	0	0	0				24	50
78	12-Sep-01	57	0	0	0	0	0	0				25	51
79	13-Sep-01	58	0	0	0	0	0	0				24	52
80	14-Sep-01	59	0	0	0	0	0	0	3.098	2.155	1.53E-03	24	51
81	15-Sep-01	60	0	0	0	0	0	0				25	52
83	17-Sep-01	61	0	0	0	0	0	0				25	49
84	18-Sep-01	62	0	0	0	0	0	0				23	55
85	19-Sep-01	63	0	0	0	0	0	0				23	55
86	20-Sep-01	64	0	0	0	0	0	0				23	54
87	21-Sep-01	65	0	0	0	0	0	0	1.736	2.311	2.63E-03	23	57
89	23-Sep-01	66	0	0	0	0	0	0				23	49
90	24-Sep-01	67	0	0	0	0	0	0				23	58
91	25-Sep-01	68	0	0	0	0	0	0				22	51
		Mean	0		0				1.732	1.928	1.11E-02	24.0	51.4
		S.D.	0		0				1.230	0.303	1.63E-02	0.8	2.4

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
0	26-Jun-01	1	2540	2258	2590	2080	2160	2200				23	45
1	27-Jun-01	2	2520	2113	2010	2220	2010	2210				23	47
2	28-Jun-01	3	2480	2148	2110	2200	2130	2150				23	46
3	29-Jun-01	4	2520	2130	2800	1850	2050	1820				23	45
6	2-Jul-01	5	2450	2135	2080	2180	2090	2190	2.943	1.989	1.98E-03	24	45
7	3-Jul-01	6	2310	2035	1870	1910	2080	2280				24	45
8	4-Jul-01	7	2300	1943	1870	2200	2090	1610				24	47
9	5-Jul-01	8	2420	2088	1990	2300	2250	1810				24	50
10	6-Jul-01	9	2250	2170	1850	2420	2100	2310				24	45
13	9-Jul-01	10	2530	2025	1770	1750	2350	2230	0.9020	1.764	1.89E-02	24	45
14	10-Jul-01	11	2440	2073	2280	1830	2250	1930				23	45
15	11-Jul-01	12	2260	1823	2000	1650	1900	1740				24	44
16	12-Jul-01	13	2310	1925	1840	2040	1910	1910				23	45
17	13-Jul-01	14	2390	2095	2410	1910	1980	2080	2.021	2.043	2.44E-03	24	47
20	16-Jul-01	15	2350	1988	2210	1770	2140	1830				24	44
21	17-Jul-01	16	2270	1913	1820	2090	1890	1850	0.8582	1.737	4.80E-03	23	46
22	18-Jul-01	17	2440	1943	2100	1910	1790	1970				23	47
23	19-Jul-01	18	2760	1985	2210	2000	1950	1780				23	47
24	20-Jul-01	19	2430	2010	1990	1870	2310	1870				23	45
27	23-Jul-01	20	2480	2173	1990	2510	2000	2190				23	45
28	24-Jul-01	21	2450	2100	2060	1850	2220	2270	0.8325	1.603	8.95E-03	23	48
29	25-Jul-01	22	2480	2190	2400	2250	2120	1990				23	50
30	26-Jul-01	23	2750	1973	2010	1860	2040	1980				22	46
31	27-Jul-01	24	2540	2120	2110	1960	2200	2210				23	46
32	28-Jul-01	25	2570	2203	2400	2000	2020	2390				23	45
34	30-Jul-01	26	2470	2020	1920	2000	2060	2100				23	45
35	31-Jul-01	27	2490	1953	1970	1770	2130	1940				22	46

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
36	1-Aug-01	28	2390	1928	1960	1780	1740	2230				22	46
37	2-Aug-01	29	2570	2145	2670	2140	1810	1960				23	46
38	3-Aug-01	30	2330	1905	1940	1750	1970	1960	12.51	3.372	7.50E-03	23	46
41	6-Aug-01	31	2570	1953	2270	2050	1710	1780				23	47
42	7-Aug-01	32	2360	1933	2090	1990	1770	1880				24	45
43	8-Aug-01	33	2310	1873	1780	1920	1770	2020				24	46
44	9-Aug-01	34	2340	2060	2190	2020	2100	1930				24	46
45	10-Aug-01	35	2380	1920	2030	1950	1770	1930	0.8634	1.724	5.97E-02	25	49
48	13-Aug-01	36	2470	1993	1970	2150	1880	1970				24	45
49	14-Aug-01	37	2410	2038	1880	1890	2140	2240				23	45
50	15-Aug-01	38	2420	2045	2350	1890	1850	2090				23	45
51	16-Aug-01	39	2430	1930	2120	1790	1950	1860	0.9240	2.335	5.21E-03	23	45
52	17-Aug-01	40	2480	2070	2200	1960	2150	1970				23	46
55	20-Aug-01	41	2460	1895	1630	1750	1980	2220				23	46
56	21-Aug-01	42	2400	2105	2250	2220	1980	1970				24	44
57	22-Aug-01	43	2440	2100	1850	2250	2150	2150				24	46
58	23-Aug-01	44	2440	2023	2100	1930	2000	2060				24	45
59	24-Aug-01	45	4480	1995	1900	1990	2010	2080	0.9183	1.985	5.49E-03	24	46
62	27-Aug-01	46	2380	1938	2180	1720	1990	1860				24	45
63	28-Aug-01	47	2370	2045	1950	2100	2050	2080				23	46
64	29-Aug-01	48	2540	2303	2320	2400	2250	2240				23	46
65	30-Aug-01	49	2780	2565	2690	2750	2290	2530				23	46
66	31-Aug-01	50	2530	2210	2160	2240	2130	2290	0.6918	1.584	9.83E-03	23	46
69	3-Sep-01	51	2290	2078	2150	2030	2240	1890				23	46
70	4-Sep-01	52	2290	1985	1670	1910	2280	2080				24	44
71	5-Sep-01	53	2330	1945	2080	1900	1820	1980				24	45

[a]Nominal high due to technical problem with the generation system.

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
73	7-Sep-01	54	2370	2050	2030	2030	1980	2160	5.169	2.342	4.49E-03	24	47
76	10-Sep-01	55	2330	2033	1950	1910	2100	2170				24	52
77	11-Sep-01	56	2310	1993	2030	1850	2180	1910				23	46
78	12-Sep-01	57	2320	2013	1980	1950	2030	2090				24	45
79	13-Sep-01	58	2290	2013	2090	1910	2090	1960				23	49
80	14-Sep-01	59	2270	1908	1960	1810	1940	1920	2.354	1.928	1.42E-03	23	48
81	15-Sep-01	60	2350	2110	2130	1960	2120	2230				23	47
83	17-Sep-01	61	2300	1960	2030	1870	1970	1970				24	44
84	18-Sep-01	62	2410	2065	2080	1820	2390	1970				23	49
85	19-Sep-01	63	2390	2023	1890	2030	2040	2130				23	48
86	20-Sep-01	64	2380	1990	2000	1900	2060	2000				23	48
87	21-Sep-01	65	2360	2018	2070	1980	1980	2040	0.8881	2.233	2.02E-03	23	50
89	23-Sep-01	66	2350	2033	2060	2060	1990	2020				23	46
90	24-Sep-01	67	2380	1933	1880	2000	1970	1880				23	51
91	25-Sep-01	68	2450	1893	1910	1540	2060	2060				22	47
		Mean	2451		2037				2.452	2.049	1.02E-02	23.3	46.3
		S.D.	274		192				3.283	0.472	1.56E-02	0.6	1.7

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
0	26-Jun-01	1	2540	1903	1750	1870	2050	1940				23	49
1	27-Jun-01	2	2520	2130	2060	2270	2030	2160				23	50
2	28-Jun-01	3	2480	2143	2540	2080	1940	2010				23	49
3	29-Jun-01	4	2520	2033	2010	2090	2080	1950				23	49
6	2-Jul-01	5	2450	2095	2150	2180	1940	2110	3.662	2.163	2.35E-03	24	48
7	3-Jul-01	6	2310	1938	1700	2040	2010	2000				23	46
8	4-Jul-01	7	2300	2103	2080	1930	1810	2590				23	48
9	5-Jul-01	8	2420	1998	2050	2210	2050	1680				23	51
10	6-Jul-01	9	2250	1858	1680	2090	1910	1750				23	46
13	9-Jul-01	10	2530	1885	1940	1630	2030	1940	0.9443	2.426	2.12E-02	23	47
14	10-Jul-01	11	2440	2028	1900	2040	1980	2190				23	48
15	11-Jul-01	12	2260	1883	1610	2170	2010	1740				23	47
16	12-Jul-01	13	2310	1938	1760	2060	2120	1810				23	47
17	13-Jul-01	14	2390	1885	1610	1890	1980	2060	3.122	2.100	5.53E-03	23	50
20	16-Jul-01	15	2350	2013	1860	2280	1930	1980				23	47
21	17-Jul-01	16	2270	1905	1820	1880	1910	2010	0.8414	1.609	4.19E-03	22	48
22	18-Jul-01	17	2440	1985	1850	1950	2190	1950				22	49
23	19-Jul-01	18	2760	1985	2010	2080	1980	1870				22	48
24	20-Jul-01	19	2430	1963	1860	1860	2080	2050				22	46
27	23-Jul-01	20	2480	2173	2220	2250	2030	2190				23	47
28	24-Jul-01	21	2450	2060	1980	2030	2140	2090	0.8271	1.839	9.12E-03	22	51
29	25-Jul-01	22	2480	1968	1940	1920	1950	2060				22	53
30	26-Jul-01	23	2750	2020	1930	1950	2100	2100				21	48
31	27-Jul-01	24	2540	2093	2050	1930	2250	2140				22	48
32	28-Jul-01	25	2570	2030	1980	1980	1950	2210				22	47
34	30-Jul-01	26	2470	1855	1870	1940	1910	1700				22	47
35	31-Jul-01	27	2490	1985	1940	1940	2140	1920				22	48

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
36	1-Aug-01	28	2390	2070	2050	2120	2170	1940				22	48
37	2-Aug-01	29	2570	2165	1980	2130	2150	2400				23	47
38	3-Aug-01	30	2330	1935	1770	1970	2000	2000	1.044	1.867	3.26E-03	23	47
41	6-Aug-01	31	2570	1970	2150	1910	1890	1930				23	49
42	7-Aug-01	32	2360	2015	1940	2060	2010	2050				23	49
43	8-Aug-01	33	2310	1978	1930	1970	1960	2050				23	49
44	9-Aug-01	34	2340	2148	2260	2150	2120	2060				23	48
45	10-Aug-01	35	2380	2065	1850	1870	2390	2150	0.8638	1.771	5.82E-02	23	51
48	13-Aug-01	36	2470	2030	1910	1640	2400	2170				23	48
49	14-Aug-01	37	2410	2060	2110	2340	1970	1820				23	48
50	15-Aug-01	38	2420	1915	1670	2060	2080	1850				23	49
51	16-Aug-01	39	2430	1938	1780	1980	2050	1940	0.9601	2.240	5.89E-03	23	48
52	17-Aug-01	40	2480	2218	2080	2350	2290	2150				23	49
55	20-Aug-01	41	2460	2085	2250	2090	2100	1900				23	48
56	21-Aug-01	42	2400	2035	2060	1820	2150	2110				23	46
57	22-Aug-01	43	2440	2138	2120	2190	2090	2150				23	47
58	23-Aug-01	44	2440	1945	1890	1790	2080	2020				23	48
59	24-Aug-01	45	4480	1973	1970	1960	2000	1960	0.9272	1.840	5.41E-03	23	48
62	27-Aug-01	46	2380	1918	1860	1910	1820	2080				23	46
63	28-Aug-01	47	2370	2075	2300	2090	1970	1940				24	47
64	29-Aug-01	48	2540	2105	2150	2190	2030	2050				24	48
65	30-Aug-01	49	2780	2270	2370	2110	2380	2220				23	48
66	31-Aug-01	50	2530	2193	2400	2130	2090	2150	0.7421	2.544	1.51E-02	23	48
69	3-Sep-01	51	2290	1978	1980	1960	1850	2120				23	48
70	4-Sep-01	52	2290	2020	2080	2130	2040	1830				23	47
71	5-Sep-01	53	2330	1993	1900	2050	2090	1930				23	48

[a]Nominal high due to technical problem with the generation system.

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
73	7-Sep-01	54	2370	2050	2000	2010	2070	2120	1.334	1.795	1.59E-03	23	50
76	10-Sep-01	55	2330	1935	1910	1840	1940	2050				23	57
77	11-Sep-01	56	2310	2080	2170	2040	2130	1980				23	48
78	12-Sep-01	57	2320	2273	2130	2410	2130	2420				24	47
79	13-Sep-01	58	2290	1993	2160	2070	1780	1960				23	50
80	14-Sep-01	59	2270	2003	2130	2030	1940	1910	2.222	1.866	1.95E-03	24	49
81	15-Sep-01	60	2350	1985	2130	2220	1820	1770				24	48
83	17-Sep-01	61	2300	2023	2050	2120	1950	1970				24	46
84	18-Sep-01	62	2410	1953	1960	1930	1890	2030				22	52
85	19-Sep-01	63	2390	1930	1940	1970	1930	1880				22	51
86	20-Sep-01	64	2380	2063	2000	2050	2150	2050				22	52
87	21-Sep-01	65	2360	1933	2010	1910	1850	1960	0.8731	1.846	2.01E-03	22	54
89	23-Sep-01	66	2350	1908	1850	1890	2010	1880				22	49
90	24-Sep-01	67	2380	1900	2060	1860	1800	1880				22	56
91	25-Sep-01	68	2450	1983	1850	2150	1980	1950				22	50
		Mean	2451		2016				1.413	1.993	1.04E-02	22.8	48.6
		S.D.	274		157				0.964	0.278	1.55E-02	0.6	2.2

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
0	26-Jun-01	1	10400	10560	10100	9950	11300	10900				24	47
1	27-Jun-01	2	10000	10500	9810	10600	10100	11500				24	48
2	28-Jun-01	3	9970	10300	12000	8740	9950	10500				24	48
3	29-Jun-01	4	9170	10560	9140	12000	11000	10100				24	47
6	2-Jul-01	5	9580	9983	8900	9880	9950	11200	5.574	2.285	2.90E-03	25	45
7	3-Jul-01	6	9240	10120	9450	10700	10400	9910				25	47
8	4-Jul-01	7	10100	10690	11700	10900	9660	10500				24	50
9	5-Jul-01	8	9950	9908	9770	10600	9630	9630				24	54
10	6-Jul-01	9	9930	10750	9910	11500	10900	10700				24	47
13	9-Jul-01	10	9870	9815	10900	11100	8600	8660	0.9057	1.859	1.85E-02	24	48
14	10-Jul-01	11	10300	10730	10400	10400	10700	11400				24	47
15	11-Jul-01	12	9660	9438	8540	10900	9370	8940				24	47
16	12-Jul-01	13	10800	11750	11600	12800	11700	10900				24	46
17	13-Jul-01	14	10000	11250	10500	11500	11400	11600	9.339	2.648	4.18E-03	24	48
20	16-Jul-01	15	10200	10190	9810	8530	10900	11500				24	45
21	17-Jul-01	16	10500	11180	9810	11800	11900	11200	0.9086	2.148	5.08E-03	23	50
22	18-Jul-01	17	9930	10340	10600	10600	11400	8760				23	53
23	19-Jul-01	18	9480	11380	11500	10900	11400	11700				23	51
24	20-Jul-01	19	9810	10200	10100	11000	9730	9950				24	47
27	23-Jul-01	20	9900	10330	10400	10300	10100	10500				24	49
28	24-Jul-01	21	10000	9900	8000	10500	10900	10200	0.8701	2.191	1.15E-02	23	52
29	25-Jul-01	22	9900	10520	10400	8570	11100	12000				23	54
30	26-Jul-01	23	10400	10290	10800	9520	9950	10900				22	50
31	27-Jul-01	24	10600	10280	9880	9730	10900	10600				24	47
32	28-Jul-01	25	10600	11030	11500	10500	10100	12000				23	47
34	30-Jul-01	26	10300	10450	11300	10100	10200	10200				23	47
35	31-Jul-01	27	10200	10180	11000	9730	9590	10400				23	48

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Humidity (%)
36	1-Aug-01	28	10200	11100	10100	11400	11200	11700				23	48
37	2-Aug-01	29	9750	10480	12000	10200	9630	10100				23	49
38	3-Aug-01	30	10000	10830	10000	11600	10900	10800	1.228	2.072	4.53E-03	23	48
41	6-Aug-01	31	9710	10500	10800	10300	10600	10300				23	51
42	7-Aug-01	32	9720	10110	10600	11000	10500	8330				24	50
43	8-Aug-01	33	10300	10380	8410	10700	12000	10400				24	51
44	9-Aug-01	34	9930	9495	7460	9130	11400	9990				24	49
45	10-Aug-01	35	10100	10380	10800	10200	10400	10100	0.8637	1.561	6.13E-02	25	52
48	13-Aug-01	36	10200	10350	11100	10100	10600	9590				24	49
49	14-Aug-01	37	9910	10730	10000	10200	12000	10700				23	48
50	15-Aug-01	38	9410	9935	11300	7530	9910	11000				23	48
51	16-Aug-01	39	9760	10110	10700	8620	10600	10500	1.456	2.932	8.24E-03	23	50
52	17-Aug-01	40	10400	11290	9040	11800	12800	11500				23	51
55	20-Aug-01	41	10200	10630	11700	11900	9450	9450				24	50
56	21-Aug-01	42	9130	9303	8990	10600	9140	8480				24	48
57	22-Aug-01	43	9370	10800	11500	11300	9590	10800				24	49
58	23-Aug-01	44	9630	10650	11000	9990	10900	10700				24	49
59	24-Aug-01	45	9960	11000	11300	10600	11400	10700	0.9396	2.104	4.77E-03	25	49
62	27-Aug-01	46	10200	10850	10300	11000	10900	11200				25	47
63	28-Aug-01	47	9710	9763	9000	11100	10000	8950				24	51
64	29-Aug-01	48	9330	9353	7300	8610	10700	10800				24	49
65	30-Aug-01	49	9920	10830	11200	11200	10200	10700				23	48
66	31-Aug-01	50	9930	9903	9590	9910	10200	9910	0.7269	2.066	1.42E-02	24	50
69	3-Sep-01	51	9810	9665	10100	10900	8660	9000				24	48
70	4-Sep-01	52	9720	10350	9520	9880	11600	10400				24	46
71	5-Sep-01	53	9520	10090	10100	9840	10800	9630				24	48
73	7-Sep-01	54	9480	10210	10500	10400	9990	9950	1.526	1.761	2.07E-03	24	49

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
76	10-Sep-01	55	9860	9818	8960	9810	9700	10800				25	57
77	11-Sep-01	56	9880	10950	11400	11000	10700	10700				23	48
78	12-Sep-01	57	9430	9943	8500	11300	10200	9770				24	49
79	13-Sep-01	58	10200	10680	10600	10900	10800	10400				23	51
80	14-Sep-01	59	9310	9535	8450	10300	9730	9660	2.262	1.825	2.80E-03	23	50
81	15-Sep-01	60	9780	9218	8310	10000	9660	8900				24	48
83	17-Sep-01	61	9430	10340	9660	10000	10600	11100				24	46
84	18-Sep-01	62	10200	10110	9520	10400	10400	10100				23	52
85	19-Sep-01	63	9890	10830	10200	10600	11100	11400				24	51
86	20-Sep-01	64	9950	10400	10400	10200	11100	9910				23	52
87	21-Sep-01	65	10100	10680	10400	10900	10600	10800	1.360	2.186	2.29E-03	23	55
89	23-Sep-01	66	10100	11180	10600	11400	11100	11600				23	49
90	24-Sep-01	67	9830	10280	10500	10500	10200	9910				23	54
91	25-Sep-01	68	10000	9758	9340	8790	10600	10300				22	49
		Mean	9913		10372				2.151	2.126	1.10E-02	23.7	49.2
		S.D.	355		932				2.511	0.363	1.60E-02	0.7	2.4

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
0	26-Jun-01	1	10400	11400	11500	11500	11700	10900				24	43
1	27-Jun-01	2	10000	10680	10800	10900	11400	9630				24	46
2	28-Jun-01	3	9970	10140	9660	9910	10100	10900				24	45
3	29-Jun-01	4	9170	9940	10300	10200	9450	9810				24	45
6	2-Jul-01	5	9580	9148	8060	10200	8700	9630	2.528	1.914	1.30E-03	25	44
7	3-Jul-01	6	9240	10310	10800	11100	9590	9730				24	43
8	4-Jul-01	7	10100	10950	11200	10100	11000	11500				24	45
9	5-Jul-01	8	9950	10400	10200	11100	10200	10100				24	48
10	6-Jul-01	9	9930	10220	11700	9990	9590	9590				24	42
13	9-Jul-01	10	9870	10150	9590	9700	10900	10400	0.9015	1.780	1.61E-02	24	44
14	10-Jul-01	11	10300	10850	11000	11600	10300	10500				24	44
15	11-Jul-01	12	9660	9888	9630	10600	9840	9480				23	45
16	12-Jul-01	13	10800	10960	9840	11800	11400	10800				24	44
17	13-Jul-01	14	10000	10540	10600	12000	10800	8760	3.139	2.249	3.16E-03	24	45
20	16-Jul-01	15	10200	10070	8960	9700	10100	11500				24	43
21	17-Jul-01	16	10500	11280	12100	11400	11100	10500	0.8798	1.913	4.37E-03	23	45
22	18-Jul-01	17	9930	10020	10900	9810	10200	9180				23	46
23	19-Jul-01	18	9480	11680	12000	11900	11400	11400				23	46
24	20-Jul-01	19	9810	9598	9480	9100	10000	9810				23	43
27	23-Jul-01	20	9900	10680	10300	11000	10800	10600				24	44
28	24-Jul-01	21	10000	10830	10600	11000	11400	10300	0.8495	2.136	9.36E-03	23	48
29	25-Jul-01	22	9900	9418	10300	8070	9000	10300				23	49
30	26-Jul-01	23	10400	9870	9000	10300	9880	10300				22	46
31	27-Jul-01	24	10600	10370	9590	10400	11000	10500				23	43
32	28-Jul-01	25	10600	10020	11100	9590	8890	10500				23	43
34	30-Jul-01	26	10300	10160	10700	10300	9660	9990				23	43
35	31-Jul-01	27	10200	10700	10600	10800	10500	10900				24	43

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
36	1-Aug-01	28	10200	10480	11500	11200	9520	9700				23	45
37	2-Aug-01	29	9750	10090	11000	8850	9700	10800				24	43
38	3-Aug-01	30	10000	9630	9550	10200	9630	9140	0.9181	1.528	3.26E-03	24	43
41	6-Aug-01	31	9710	9265	8930	9590	9520	9020				24	45
42	7-Aug-01	32	9720	9240	8970	9810	9550	8630				24	46
43	8-Aug-01	33	10300	9843	10700	8940	8830	10900				24	46
44	9-Aug-01	34	9930	10800	12500	10500	10300	9910				24	45
45	10-Aug-01	35	10100	9865	9550	9450	10800	9660	0.8640	1.582	5.60E-02	24	49
48	13-Aug-01	36	10200	9798	9110	10100	10100	9880				24	45
49	14-Aug-01	37	9910	9865	10200	10300	9480	9480				24	45
50	15-Aug-01	38	9410	9565	9370	9630	9630	9630				24	45
51	16-Aug-01	39	9760	10680	10600	10100	11100	10900	0.9497	2.008	5.76E-03	24	45
52	17-Aug-01	40	10400	11780	11700	11800	12200	11400				24	48
55	20-Aug-01	41	10200	10120	9410	9080	10700	11300				25	45
56	21-Aug-01	42	9130	9758	11200	9630	9150	9050				24	45
57	22-Aug-01	43	9370	9945	11000	10600	9100	9080				24	45
58	23-Aug-01	44	9630	9913	10300	9410	9950	9990				24	47
59	24-Aug-01	45	9960	9838	8820	9730	10300	10500	0.8748	1.664	4.06E-03	24	46
62	27-Aug-01	46	10200	10580	9910	10300	11100	11000				24	44
63	28-Aug-01	47	9710	11400	12000	12000	10200	11400				25	46
64	29-Aug-01	48	9330	10500	11500	10900	10100	9480				24	45
65	30-Aug-01	49	9920	10800	9910	11900	10600	10800				24	44
66	31-Aug-01	50	9930	11100	12000	11400	10700	10300	0.7253	2.106	1.47E-02	24	46
69	3-Sep-01	51	9810	10360	12000	11000	9050	9370				24	44
70	4-Sep-01	52	9720	10850	10400	11200	10800	11000				23	45
71	5-Sep-01	53	9520	10190	9770	9990	10600	10400				23	47
73	7-Sep-01	54	9480	9623	9840	8510	9840	10300	1.863	2.230	2.08E-03	23	46

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
76	10-Sep-01	55	9860	9923	9490	10300	9950	9950				24	54
77	11-Sep-01	56	9880	10440	11500	10400	10200	9660				24	45
78	12-Sep-01	57	9430	9755	8580	10500	9840	10100				25	43
79	13-Sep-01	58	10200	10530	10300	9810	11100	10900				24	46
80	14-Sep-01	59	9310	9620	9520	8790	9170	11000	2.542	1.842	2.48E-03	24	46
81	15-Sep-01	60	9780	11230	12000	10900	11100	10900				24	43
83	17-Sep-01	61	9430	10180	10600	10200	10200	9730				24	42
84	18-Sep-01	62	10200	10330	10300	10200	10300	10500				23	48
85	19-Sep-01	63	9890	10020	11000	9130	10100	9840				23	48
86	20-Sep-01	64	9950	10480	9410	10900	11400	10200				23	49
87	21-Sep-01	65	10100	10070	9550	10300	10500	9910	1.470	2.569	2.48E-03	23	51
89	23-Sep-01	66	10100	10200	9880	10300	10300	10300				23	46
90	24-Sep-01	67	9830	10460	11700	10600	9910	9630				23	53
91	25-Sep-01	68	10000	9345	8540	8840	10000	10000				23	47
		Mean	9913		10274				1.423	1.963	9.62E-03	23.7	45.5
		S.D.	355		854				0.821	0.296	1.47E-02	0.6	2.3

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
0	26-Jun-01	1	17600	20150	20000	20600	20900	19100				25	49
1	27-Jun-01	2	19100	21100	19200	22900	19900	22400				25	51
2	28-Jun-01	3	20100	20680	21400	19400	21300	20600				25	50
3	29-Jun-01	4	18900	20950	21500	21500	19600	21200				25	50
6	2-Jul-01	5	18000	20980	21400	20000	19700	22800	2.988	2.234	2.03E-03	26	49
7	3-Jul-01	6	19800	20400	20200	18500	22700	20200				25	48
8	4-Jul-01	7	18900	20380	20300	20400	19700	21100				25	52
9	5-Jul-01	8	19800	20850	21500	20800	19800	21300				25	54
10	6-Jul-01	9	19200	19650	18600	19100	19600	21300				25	49
13	9-Jul-01	10	19000	19880	20300	21200	18700	19300	0.9082	1.748	1.72E-02	25	50
14	10-Jul-01	11	18600	19630	19400	20000	19600	19500				26	50
15	11-Jul-01	12	19100	20780	17600	22500	23000	20000				26	49
16	12-Jul-01	13	18800	19600	18600	20000	19700	20100				26	49
17	13-Jul-01	14	17400	20100	18700	21700	20100	19900	1.920	1.859	1.96E-03	26	50
20	16-Jul-01	15	19000	20200	20300	20700	20600	19200				26	49
21	17-Jul-01	16	18200	19650	19100	19300	20000	20200	0.8551	1.671	4.38E-03	24	51
22	18-Jul-01	17	18800	21380	21600	20400	22000	21500				24	53
23	19-Jul-01	18	18900	21200	22000	22900	21000	18900				24	51
24	20-Jul-01	19	18700	19650	20300	20800	18400	19100				24	46
27	23-Jul-01	20	17800	20580	21300	20500	20700	19800				25	51
28	24-Jul-01	21	18800	20550	20700	21500	20600	19400	0.8367	1.441	8.55E-03	25	52
29	25-Jul-01	22	19400	21480	22600	21100	21700	20500				25	55
30	26-Jul-01	23	18800	19250	18700	19300	19900	19100				23	51
31	27-Jul-01	24	19800	19200	18700	20200	19700	18200				25	48
32	28-Jul-01	25	19600	19300	19100	19700	18700	19700				25	47
34	30-Jul-01	26	19100	19530	19200	19400	21400	18100				25	48
35	31-Jul-01	27	18600	19980	19100	20800	19700	20300				24	47

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
36	1-Aug-01	28	19300	20400	20500	21300	20400	19400				25	48
37	2-Aug-01	29	18200	20150	20000	21100	19700	19800				25	48
38	3-Aug-01	30	19300	20630	19900	20600	21300	20700	4.537	2.957	8.77E-03	25	47
41	6-Aug-01	31	17900	19730	19400	20100	19700	19700				25	50
42	7-Aug-01	32	18500	20030	21000	18800	20900	19400				26	49
43	8-Aug-01	33	17900	18900	21100	16400	19000	19100				26	53
44	9-Aug-01	34	19400	20250	20600	21800	20200	18400				26	50
45	10-Aug-01	35	19200	20930	21300	19400	20200	22800	0.8520	1.431	5.01E-02	27	52
48	13-Aug-01	36	18500	19830	21400	18500	19800	19600				26	50
49	14-Aug-01	37	17700	19500	17900	21200	19300	19600				25	48
50	15-Aug-01	38	18000	18150	17200	18300	19500	17600				25	51
51	16-Aug-01	39	18000	19580	16100	19800	21200	21200	0.9696	2.431	5.60E-03	25	51
52	17-Aug-01	40	19100	20430	20100	20600	20700	20300				25	52
55	20-Aug-01	41	18800	20180	19700	20600	20300	20100				25	53
56	21-Aug-01	42	18500	18850	22400	18500	17400	17100				25	49
57	22-Aug-01	43	18500	18530	17100	17800	20100	19100				26	50
58	23-Aug-01	44	20000	20650	21700	19600	20900	20400				25	56
59	24-Aug-01	45	18500	19630	19700	20300	19400	19100	0.9608	2.041	5.93E-03	26	50
62	27-Aug-01	46	19200	19280	18200	19300	20500	19100				26	50
63	28-Aug-01	47	19300	20630	18700	21800	21100	20900				25	52
64	29-Aug-01	48	19600	19980	20900	17200	22000	19800				25	51
65	30-Aug-01	49	19700	20580	20300	20300	20500	21200				25	49
66	31-Aug-01	50	19800	20100	20000	19800	20000	20600	0.6992	1.653	1.13E-02	24	52
69	3-Sep-01	51	19200	20130	20800	17900	21600	20200				25	50
70	4-Sep-01	52	19600	20680	19200	21000	21400	21100				25	48
71	5-Sep-01	53	19700	19780	18100	18800	21100	21100				25	50
73	7-Sep-01	54	18900	20230	22000	19400	20300	19200	1.764	2.188	2.50E-03	25	50

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
76	10-Sep-01	55	19500	20330	20500	19700	20800	20300				26	59
77	11-Sep-01	56	18800	19930	19200	20100	19800	20600				24	51
78	12-Sep-01	57	19000	20800	20300	20500	21200	21200				25	50
79	13-Sep-01	58	19400	19630	22100	20100	17100	19200				24	52
80	14-Sep-01	59	20100	19900	19400	20300	19100	20800	1.979	1.681	1.62E-03	24	51
81	15-Sep-01	60	18700	20050	20100	19900	19900	20300				25	49
83	17-Sep-01	61	17700	18880	17100	19400	18400	20600				25	47
84	18-Sep-01	62	19100	19730	18500	21000	21600	17800				25	51
85	19-Sep-01	63	19100	18680	17400	19100	19400	18800				25	50
86	20-Sep-01	64	20100	19380	19500	18400	20000	19600				25	54
87	21-Sep-01	65	19000	19400	18700	19600	19700	19600	1.058	2.511	2.70E-03	25	56
89	23-Sep-01	66	19900	20200	19800	19400	20900	20700				25	49
90	24-Sep-01	67	18800	20050	19200	20300	20400	20300				25	56
91	25-Sep-01	68	19200	20180	19400	19700	20700	20900				23	52
		Mean	**18949**		**20026**				**1.564**	**1.988**	**9.43E-03**	**25.0**	**50.5**
		S.D.	**661**		**1219**				**1.114**	**0.457**	**1.30E-02**	**0.7**	**2.4**

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
0	26-Jun-01	1	17600	19380	19000	19100	19400	20000				24	49
1	27-Jun-01	2	19100	20630	19400	19200	22300	21600				24	51
2	28-Jun-01	3	20100	20830	22100	20600	20600	20000				25	51
3	29-Jun-01	4	18900	18530	18100	17100	19000	19900				24	51
6	2-Jul-01	5	18000	19530	18700	19700	19400	20300	3.133	2.363	2.02E-03	25	50
7	3-Jul-01	6	19800	21030	20400	21700	20100	21900				26	48
8	4-Jul-01	7	18900	20380	19500	20700	20000	21300				25	54
9	5-Jul-01	8	19800	21180	21600	22300	20400	20400				25	55
10	6-Jul-01	9	19200	21080	20700	21100	22200	20300				25	50
13	9-Jul-01	10	19000	19550	19400	18800	20200	19800	0.8877	1.583	1.61E-02	25	52
14	10-Jul-01	11	18600	20030	19600	20200	20100	20200				24	49
15	11-Jul-01	12	19100	20180	21500	19300	18600	21300				24	50
16	12-Jul-01	13	18800	20630	21400	21000	20800	19300				24	55
17	13-Jul-01	14	17400	19430	18300	20600	19400	19400	0.9560	2.242	7.88E-03	24	52
20	16-Jul-01	15	19000	20430	21000	20800	20500	19400				25	50
21	17-Jul-01	16	18200	19800	21400	18500	18900	20400	0.8593	1.735	4.90E-03	25	50
22	18-Jul-01	17	18800	19980	21100	19800	19600	19400				25	53
23	19-Jul-01	18	18900	19950	20800	21400	19100	18500				25	51
24	20-Jul-01	19	18700	20000	19600	21000	20000	19400				25	46
27	23-Jul-01	20	17800	21330	20600	21400	22200	21100				25	51
28	24-Jul-01	21	18800	20050	20500	21000	19600	19100	0.8624	1.890	8.63E-03	24	55
29	25-Jul-01	22	19400	20950	22200	20200	20800	20600				24	55
30	26-Jul-01	23	18800	19850	18700	20100	20600	20000				22	50
31	27-Jul-01	24	19800	19930	20200	20500	19500	19500				24	49
32	28-Jul-01	25	19600	21550	21100	21500	23200	20400				24	47
34	30-Jul-01	26	19100	21130	21400	21200	20600	21300				24	49
35	31-Jul-01	27	18600	21300	21700	21400	20500	21600				25	48

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment Mean Temperature (°C)	Humidity (%)
36	1-Aug-01	28	19300	20230	19200	20600	21300	19800				24	50
37	2-Aug-01	29	18200	19850	21200	19200	19600	19400				25	48
38	3-Aug-01	30	19300	20750	21200	21200	20600	20000	0.9733	1.736	3.11E-03	26	48
41	6-Aug-01	31	17900	20680	20600	20300	20900	20900				26	50
42	7-Aug-01	32	18500	19750	19900	19400	20400	19300				25	50
43	8-Aug-01	33	17900	19830	18300	21400	19700	19900				25	52
44	9-Aug-01	34	19400	20330	19500	20900	20900	20000				25	50
45	10-Aug-01	35	19200	20700	20200	21100	21600	19900	0.8423	1.453	4.13E-02	25	54
48	13-Aug-01	36	18500	19430	18300	20700	19100	19600				25	52
49	14-Aug-01	37	17700	19380	19400	18700	19900	19500				26	47
50	15-Aug-01	38	18000	20280	19800	19900	20300	21100				25	50
51	16-Aug-01	39	18000	19250	19800	18600	19800	18800	0.9267	1.785	4.15E-03	25	50
52	17-Aug-01	40	19100	19900	19500	20100	20100	19900				25	50
55	20-Aug-01	41	18800	20030	19700	19400	20500	20500				26	51
56	21-Aug-01	42	18500	21230	20400	21300	21900	21300				25	49
57	22-Aug-01	43	18500	20250	21600	19400	20700	19300				25	49
58	23-Aug-01	44	20000	20000	17600	21100	21400	19900				24	50
59	24-Aug-01	45	18500	20050	19400	20800	20200	19800	0.8972	1.956	5.41E-03	25	51
62	27-Aug-01	46	19200	19730	19800	20000	18400	20700				25	50
63	28-Aug-01	47	19300	19950	20200	20500	19600	19500				26	50
64	29-Aug-01	48	19600	20600	18100	22100	21600	20600				26	51
65	30-Aug-01	49	19700	20150	18600	19900	20500	21600				25	48
66	31-Aug-01	50	19800	21280	21500	21500	21300	20800	0.6975	1.513	1.00E-02	25	51
69	3-Sep-01	51	19200	20630	21900	20600	19700	20300				25	49
70	4-Sep-01	52	19600	20800	20400	21600	20200	21000				24	50
71	5-Sep-01	53	19700	19980	19800	21300	19400	19400				24	50
73	7-Sep-01	54	18900	19800	20900	18500	20100	19700	1.557	1.927	1.98E-03	24	51

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

Day	Date	Exposure Number	Nominal (mg/m³)	Analytical Chamber Concentration: Mean (mg/m³)	Individual (mg/m³)				Particle Size Determinations: MMAD (µm)	GSD	TMC (mg/m³)	Chamber Environment: Mean Temperature (°C)	Mean Humidity (%)
76	10-Sep-01	55	19500	19980	20300	19900	19700	20000				25	60
77	11-Sep-01	56	18800	20300	19900	20500	20500	20300				25	48
78	12-Sep-01	57	19000	20580	20300	20700	20800	20500				26	48
79	13-Sep-01	58	19400	21550	19900	21600	22900	21800				25	51
80	14-Sep-01	59	20100	21180	21900	21300	21400	20100	2.203	1.826	1.73E-03	25	50
81	15-Sep-01	60	18700	20030	20600	20700	19400	19400				26	47
83	17-Sep-01	61	17700	20050	19100	19900	19100	22100				26	47
84	18-Sep-01	62	19100	20130	21000	19400	18700	21400				24	50
85	19-Sep-01	63	19100	20350	20600	19900	20600	20300				24	50
86	20-Sep-01	64	20100	20300	20500	19000	21000	20700				24	53
87	21-Sep-01	65	19000	19630	20000	19100	20000	19400	10.03	3.370	4.19E-03	24	56
89	23-Sep-01	66	19900	19930	19300	19600	20600	20200				24	48
90	24-Sep-01	67	18800	19800	19400	20000	20100	19700				24	57
91	25-Sep-01	68	19200	19100	19000	18300	19800	19300				23	49
		Mean	18949		20238				1.910	1.952	8.57E-03	24.7	50.5
		S.D.	661		984				2.538	0.499	1.06E-02	0.8	2.5

TABLE B

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

MALES — SUMMARY OF CLINICAL OBSERVATIONS

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

TABLE B

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

FEMALES SUMMARY OF CLINICAL OBSERVATIONS

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

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK -1

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

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK -1

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

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK 13

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

TABLE C

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

SUMMARY OF OPHTHALMOLOGY OBSERVATIONS

WEEK 13

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
FEMALES total number examined		5	5	5	5
RETINA	N	0	0	1	0
FOCAL RETINOPATHY	N	0	0	1	0
	%	0.0	0.0	20.0	0.0

LIONEL F. RUBIN, V.M.D.
2020 Walnut St.
Philadelphia PA 19103
(215) 557-0237

21 September 2011

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

Re: study 00-6128F

Ophthalmoscopic examination of study 00-6128F pretest rats was performed 14 June 2001 (day -1). No abnormality was seen.

21 Sept 2011

Lionel F. Rubin, V. M. D.

LIONEL F. RUBIN, V.M.D.
2020 Walnut St.
Philadelphia PA 19103
(215) 557-0237

21 September 2011

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

Re: study 00-6128F

Ophthalmoscopic examination of study 00-6128F rats was performed 20 June 2001 (week 13, terminal examination). Rat 3520 (group III) had focal retinopathy in the right eye, probably secondary to infectious disease. No other abnormality was seen. There is no indication of substance-related ocular disease.

21 Sept 2011

Lionel F. Rubin, V. M. D.

TABLE D

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

MALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK -1	MEAN	219	220	220	220
	S.D.	7.7	9.2	10.8	7.9
	N	5	5	5	5
WEEK 0	MEAN	307	316	312	312
	S.D.	12.2	11.5	14.3	15.4
	N	5	5	5	5
WEEK 1	MEAN	345	353	346	346
	S.D.	18.4	11.4	17.7	20.8
	N	5	5	5	5
WEEK 2	MEAN	378	386	356	375
	S.D.	22.2	13.6	10.2	19.7
	N	5	5	5	5
WEEK 3	MEAN	410	420	413	401
	S.D.	25.4	14.1	19.4	20.1
	N	5	5	5	5
WEEK 4	MEAN	431	446	436	416
	S.D.	25.6	14.1	21.9	21.2
	N	5	5	5	5
WEEK 5	MEAN	455	465	455	436
	S.D.	31.8	18.3	23.4	23.7
	N	5	5	5	5
WEEK 6	MEAN	478	488	475	458
	S.D.	30.0	17.0	27.0	20.9
	N	5	5	5	5
WEEK 7	MEAN	494	508	493	471
	S.D.	33.4	20.0	29.0	22.3
	N	5	5	5	5

No statistically significant differences

TABLE D

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

MALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 8	MEAN	504	520	510	490
	S.D.	36.3	18.9	31.5	25.2
	N	5	5	5	5
WEEK 9	MEAN	519	534	522	506
	S.D.	42.1	20.2	33.4	23.5
	N	5	5	5	5
WEEK 10	MEAN	531	545	529	514
	S.D.	45.4	23.4	37.5	24.3
	N	5	5	5	5
WEEK 11	MEAN	545	556	543	530
	S.D.	46.4	23.3	35.1	31.0
	N	5	5	5	5
WEEK 12	MEAN	553	560	543	532
	S.D.	44.3	25.2	38.0	27.0
	N	5	5	5	5
WEEK 13	MEAN	566	571	552	545
	S.D.	48.6	31.1	38.6	32.5
	N	5	5	5	5

No statistically significant differences

TABLE D

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

FEMALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK -1	MEAN	175	176	177	176
	S.D.	9.3	9.0	9.9	8.8
	N	5	5	5	5
WEEK 0	MEAN	213	215	225	218
	S.D.	9.9	17.3	17.9	6.0
	N	5	5	5	5
WEEK 1	MEAN	229	235	241	233
	S.D.	11.2	15.6	17.7	9.2
	N	5	5	5	5
WEEK 2	MEAN	241	251	253	245
	S.D.	15.5	15.4	24.9	11.1
	N	5	5	5	5
WEEK 3	MEAN	242	262	260	255
	S.D.	26.2	18.1	29.3	14.3
	N	5	5	5	5
WEEK 4	MEAN	259	270	268	266
	S.D.	18.4	22.8	26.2	13.7
	N	5	5	5	5
WEEK 5	MEAN	264	277	280	271
	S.D.	16.0	20.3	28.8	9.2
	N	5	5	5	5
WEEK 6	MEAN	274	286	287	275
	S.D.	20.5	19.8	32.0	14.8
	N	5	5	5	5
WEEK 7	MEAN	275	292	286	275
	S.D.	20.5	18.2	39.6	16.6
	N	5	5	5	5

No statistically significant differences

TABLE D

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

FEMALES — MEAN BODY WEIGHTS (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 8	MEAN	280	294	299	280
	S.D.	20.0	21.6	47.8	14.5
	N	5	5	5	5
WEEK 9	MEAN	287	305	297	287
	S.D.	24.8	22.0	41.4	13.8
	N	5	5	5	5
WEEK 10	MEAN	287	308	301	286
	S.D.	21.8	21.0	39.9	10.1
	N	5	5	5	5
WEEK 11	MEAN	290	309	304	289
	S.D.	26.8	21.8	40.5	16.8
	N	5	5	5	5
WEEK 12	MEAN	294	312	306	289
	S.D.	23.2	24.4	39.2	14.2
	N	5	5	5	5
WEEK 13	MEAN	302	317	309	298
	S.D.	25.6	24.6	32.8	11.6
	N	5	5	5	5

No statistically significant differences

TABLE E

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

MALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 1	MEAN	39	37	35	33
	S.D.	7.2	3.2	4.9	7.6
	N	5	5	5	5
WEEK 0 TO 2	MEAN	71	70	45	62
	S.D.	11.4	3.4	23.1	7.5
	N	5	5	5	5
WEEK 0 TO 3	MEAN	103	104	101	88
	S.D.	13.9	4.7	8.4	11.8
	N	5	5	5	5
WEEK 0 TO 4	MEAN	124	130	125	103*
	S.D.	14.1	5.9	11.5	11.6
	N	5	5	5	5
WEEK 0 TO 5	MEAN	148	149	143	124
	S.D.	20.2	11.4	12.4	14.7
	N	5	5	5	5
WEEK 0 TO 6	MEAN	171	172	164	146*
	S.D.	18.2	10.8	16.2	14.0
	N	5	5	5	5

Statistical key: * = $p<0.05$

TABLE E

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

MALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 7	MEAN	187	192	181	159
	S.D.	21.2	13.0	17.5	18.5
	N	5	5	5	5
WEEK 0 TO 8	MEAN	197	204	199	178
	S.D.	24.1	13.7	19.8	15.7
	N	5	5	5	5
WEEK 0 TO 9	MEAN	212	218	211	193
	S.D.	29.9	15.4	21.7	15.3
	N	5	5	5	5
WEEK 0 TO 10	MEAN	224	229	217	202
	S.D.	33.5	19.4	26.4	16.7
	N	5	5	5	5
WEEK 0 TO 11	MEAN	238	241	232	217
	S.D.	34.3	18.8	24.1	20.1
	N	5	5	5	5
WEEK 0 TO 12	MEAN	246	244	231	219
	S.D.	32.6	22.4	27.4	18.2
	N	5	5	5	5

No statistically significant differences

TABLE E

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

MALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 13	MEAN	259	255	241	233
	S.D.	36.9	28.3	27.7	19.9
	N	5	5	5	5

No statistically significant differences

TABLE E

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

FEMALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 1	MEAN	16	20	16	15
	S.D.	4.7	7.0	8.9	8.5
	N	5	5	5	5
WEEK 0 TO 2	MEAN	28	36	28	28
	S.D.	9.5	8.7	10.0	8.2
	N	5	5	5	5
WEEK 0 TO 3	MEAN	29	46	35	37
	S.D.	30.0	12.6	15.0	9.8
	N	5	5	5	5
WEEK 0 TO 4	MEAN	46	55	43	48
	S.D.	16.8	8.5	8.9	9.3
	N	5	5	5	5
WEEK 0 TO 5	MEAN	51	62	55	54
	S.D.	8.8	9.8	12.5	8.4
	N	5	5	5	5
WEEK 0 TO 6	MEAN	61	71	62	57
	S.D.	13.0	14.2	16.7	10.7
	N	5	5	5	5

No statistically significant differences

TABLE E

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

FEMALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 7	MEAN	62	77	61	58
	S.D.	12.5	14.8	25.3	11.7
	N	5	5	5	5
WEEK 0 TO 8	MEAN	68	79	74	62
	S.D.	12.6	10.8	32.3	10.3
	N	5	5	5	5
WEEK 0 TO 9	MEAN	74	90	72	70
	S.D.	16.4	12.2	25.9	10.5
	N	5	5	5	5
WEEK 0 TO 10	MEAN	75	93	76	68
	S.D.	13.5	12.1	25.7	5.0
	N	5	5	5	5
WEEK 0 TO 11	MEAN	77	94	79	72
	S.D.	18.9	11.4	25.4	11.2
	N	5	5	5	5
WEEK 0 TO 12	MEAN	81	97	81	71
	S.D.	15.3	12.7	23.3	10.7
	N	5	5	5	5

No statistically significant differences

TABLE E

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

FEMALES — MEAN BODY WEIGHT CHANGE (GRAMS)

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0 TO 13	MEAN	89	102	84	80
	S.D.	18.2	12.6	17.6	6.6
	N	5	5	5	5

No statistically significant differences

TABLE F

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

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

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 0	MEAN	95	94	91	95
	S.D.	2.7	3.7	2.1	3.7
	N	5	5	4	5
WEEK 1	MEAN	80	80	76	79
	S.D.	4.0	3.9	1.7	2.8
	N	5	5	5	5
WEEK 2	MEAN	74	72	71	71
	S.D.	4.4	3.3	2.0	1.4
	N	5	5	5	5
WEEK 3	MEAN	69	68	68	66
	S.D.	4.0	2.8	1.9	1.9
	N	5	5	5	5
WEEK 4	MEAN	63	64	61	62
	S.D.	4.8	2.8	2.4	1.9
	N	5	5	5	5
WEEK 5	MEAN	64	61	60	63
	S.D.	4.1	2.8	3.9	1.3
	N	5	5	5	5
WEEK 6	MEAN	62	59	60	63
	S.D.	3.7	2.8	2.5	2.2
	N	5	5	5	5
WEEK 7	MEAN	56	56	55	58
	S.D.	1.8	1.4	2.0	2.4
	N	4	5	5	5
WEEK 8	MEAN	54	51	51	55
	S.D.	3.7	2.1	2.4	1.7
	N	5	5	5	5

No statistically significant differences

TABLE F

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

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

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 9	MEAN	54	51	52	54
	S.D.	3.2	2.0	2.7	1.4
	N	5	5	5	5
WEEK 10	MEAN	54	51*	50*	54
	S.D.	1.8	2.0	2.6	1.6
	N	5	5	5	5
WEEK 11	MEAN	53	47	49	53
	S.D.	1.6	6.5	2.9	1.9
	N	5	5	5	5
WEEK 12	MEAN	51	46	45*	49
	S.D.	2.6	2.5	2.7	4.2
	N	5	5	5	5
WEEK 13	MEAN	52	47*	48	51
	S.D.	2.5	1.0	3.2	2.0
	N	5	5	5	5

Statistical key: * = $p<0.05$

TABLE F

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

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

DOSE GROUP:			I	II	III	IV
EXPOSURE LEVEL (mg/m3):			0	2,000	10,000	20,000
WEEK	0	MEAN	87	94	94	90
		S.D.	3.5	7.9	8.7	2.4
		N	5	5	5	5
WEEK	1	MEAN	83	86	80	82
		S.D.	3.1	5.5	8.1	6.4
		N	5	5	5	5
WEEK	2	MEAN	81	82	74	79
		S.D.	2.5	4.9	5.5	4.4
		N	5	5	5	5
WEEK	3	MEAN	66	78	71	78
		S.D.	25.8	5.5	2.0	6.6
		N	5	5	5	5
WEEK	4	MEAN	78	74	69	72
		S.D.	10.5	5.2	4.4	4.4
		N	5	5	5	5
WEEK	5	MEAN	73	75	66	69
		S.D.	4.5	4.9	4.2	6.3
		N	5	5	5	4
WEEK	6	MEAN	71	72	66*	68
		S.D.	1.8	3.4	4.2	2.2
		N	5	5	5	5
WEEK	7	MEAN	65	71*	63	65
		S.D.	4.4	2.3	1.1	4.2
		N	5	5	5	5
WEEK	8	MEAN	61	65	65	64
		S.D.	2.0	4.1	4.8	2.7
		N	5	5	5	5

Statistical key: * = $p<0.05$

TABLE F

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

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

DOSE GROUP:		I	II	III	IV
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000
WEEK 9	MEAN	61	67*	62	63
	S.D.	2.7	2.8	2.0	3.6
	N	5	5	5	5
WEEK 10	MEAN	63	66	62	63
	S.D.	3.7	4.2	6.0	2.4
	N	5	5	5	5
WEEK 11	MEAN	62	64	61	64
	S.D.	3.2	6.5	5.5	3.8
	N	5	5	5	5
WEEK 12	MEAN	60	63	61	61
	S.D.	2.5	6.3	5.1	5.6
	N	5	5	5	5
WEEK 13	MEAN	58	60	59	62
	S.D.	3.4	3.4	4.2	3.4
	N	5	5	5	5

Statistical key: * = $p<0.05$

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP I 0 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
1026	WITHIN NORMAL LIMITS		P
1027	WITHIN NORMAL LIMITS		P
1028	WITHIN NORMAL LIMITS		P
1029	WITHIN NORMAL LIMITS		P
1030	WITHIN NORMAL LIMITS		P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP II 2,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
2016	WITHIN NORMAL LIMITS		P
2017	WITHIN NORMAL LIMITS		P
2018	WITHIN NORMAL LIMITS		P
2019	WITHIN NORMAL LIMITS		P
2020	WITHIN NORMAL LIMITS		P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP III 10,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	1 1
3016	WITHIN NORMAL LIMITS		P
3017	WITHIN NORMAL LIMITS		P
3018	WITHIN NORMAL LIMITS		P
3019	WITHIN NORMAL LIMITS		P
3020	WITHIN NORMAL LIMITS		P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

MALES GROUP IV 20,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
4026	WITHIN NORMAL LIMITS		P
4027	WITHIN NORMAL LIMITS		P
4028	WITHIN NORMAL LIMITS		P
4029	WITHIN NORMAL LIMITS		P
4030	WITHIN NORMAL LIMITS		P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP I 0 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
1526	WITHIN NORMAL LIMITS		P
1527	WITHIN NORMAL LIMITS		P
1528	WITHIN NORMAL LIMITS		P
1529	WITHIN NORMAL LIMITS		P
1530	WITHIN NORMAL LIMITS		P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP II 2,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
2516	WITHIN NORMAL LIMITS		P
2517	WITHIN NORMAL LIMITS		P
2518	WITHIN NORMAL LIMITS		P
2519	WITHIN NORMAL LIMITS		P
2520	WITHIN NORMAL LIMITS		P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP III 10,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
3516	WITHIN NORMAL LIMITS		P
3517	WITHIN NORMAL LIMITS		P
3518	WITHIN NORMAL LIMITS		P
3519	WITHIN NORMAL LIMITS		P
3520	WITHIN NORMAL LIMITS		P

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

TABLE G

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

INDIVIDUAL CLINICAL OBSERVATIONS

FEMALES GROUP IV 20,000 mg/m3

ANIMAL#	OBSERVATIONS	DAY OF STUDY	- 1 1
4526	WITHIN NORMAL LIMITS		P
4527	WITHIN NORMAL LIMITS		P
4528	WITHIN NORMAL LIMITS		P
4529	WITHIN NORMAL LIMITS		P
4530	WITHIN NORMAL LIMITS		P

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP I 0 mg/m3 WEEK -1

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

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

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

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

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

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

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP I 0 mg/m3 WEEK -1

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

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

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

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

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

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

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP I 0 mg/m3 WEEK 13

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP II 2,000 mg/m3 WEEK 13

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP III 10,000 mg/m3 WEEK 13

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

MALES GROUP IV 20,000 mg/m3 WEEK 13

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP I 0 mg/m3 WEEK 13

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP II 2,000 mg/m3 WEEK 13

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

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP III 10,000 mg/m3 WEEK 13

ANIMAL#	PART OF EYE	OBSERVATION
3516		NO VISIBLE LESIONS
3517		NO VISIBLE LESIONS
3518		NO VISIBLE LESIONS
3519		NO VISIBLE LESIONS
3520	RETINA	FOCAL RETINOPATHY; RIGHT

TABLE H

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

INDIVIDUAL OPHTHALMOLOGY OBSERVATIONS

FEMALES GROUP IV 20,000 mg/m3 WEEK 13

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

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP I 0 mg/m3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1026	223	307	339	369	404	431	461	483	492	501	521	534	553	566	573
1027	214	302	336	365	397	414	435	459	478	490	507	524	535	545	562
1028	221	307	350	389	422	443	468	488	500	504	515	519	541	553	567
1029	209	292	327	355	381	400	414	440	454	462	467	476	484	488	494
1030	229	325	375	411	447	466	496	519	545	561	584	602	614	611	631
MEAN	219	307	345	378	410	431	455	478	494	504	519	531	545	553	566
S.D.	7.7	12.2	18.4	22.2	25.4	25.6	31.8	30.0	33.4	36.3	42.1	45.4	46.4	44.3	48.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP II 2,000 mg/m3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2016	221	320	360	390	426	454	487	505	522	528	548	564	570	574	589
2017	211	305	340	371	404	428	441	463	476	488	499	504	515	515	517
2018	210	303	344	372	406	433	452	478	500	520	537	550	562	573	592
2019	230	330	368	400	432	455	474	494	518	530	547	553	568	567	586
2020	226	321	354	397	432	458	472	500	523	535	538	552	567	572	572
MEAN	220	316	353	386	420	446	465	488	508	520	534	545	556	560	571
S.D.	9.2	11.5	11.4	13.6	14.1	14.1	18.3	17.0	20.0	18.9	20.2	23.4	23.3	25.2	31.1
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP III 10,000 mg/m3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3016	211	297	335	355	404	430	448	471	485	501	512	523	537	542	547
3017	235	331	371	345	435	461	487	510	531	547	560	570	582	590	596
3018	220	314	348	358	420	437	461	481	495	516	530	532	550	541	544
3019	226	318	352	350	421	449	456	479	501	525	538	549	559	556	579
3020	209	298	325	372	385	403	422	435	450	463	471	470	488	485	495
MEAN	220	312	346	356	413	436	455	475	493	510	522	529	543	543	552
S.D.	10.8	14.3	17.7	10.2	19.4	21.9	23.4	27.0	29.0	31.5	33.4	37.5	35.1	38.0	38.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4026	222	328	373	401	425	442	466	482	492	519	535	547	572	563	586
4027	232	324	359	387	416	429	446	466	482	504	514	514	536	540	562
4028	217	316	345	374	397	416	442	466	477	498	513	524	541	544	553
4029	218	303	328	356	375	388	403	427	434	454	472	482	492	493	506
4030	210	291	323	356	391	403	423	449	472	477	495	502	507	519	518
MEAN	220	312	346	375	401	416	436	458	471	490	506	514	530	532	545
S.D.	7.9	15.4	20.8	19.7	20.1	21.2	23.7	20.9	22.3	25.2	23.5	24.3	31.0	27.0	32.5
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP I 0 mg/m3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1526	184	227	240	250	206	250	276	287	292	293	306	307	307	311	316
1527	166	201	213	218	224	237	240	242	243	250	254	257	252	261	268
1528	179	212	236	252	259	268	261	277	274	284	284	281	293	289	310
1529	165	207	222	231	253	254	261	269	272	274	273	283	277	288	284
1530	182	218	235	252	269	285	281	295	295	302	316	310	321	321	332
MEAN	175	213	229	241	242	259	264	274	275	280	287	287	290	294	302
S.D.	9.3	9.9	11.2	15.5	26.2	18.4	16.0	20.5	20.5	20.0	24.8	21.8	26.8	23.2	25.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP II 2,000 mg/m3

ANIMAL#	WEEK OF STUDY -1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
2516	169	191	211	225	232	233	244	253	261	258	266	270	270	269	276
2517	165	207	239	258	275	272	286	303	309	302	312	316	316	315	328
2518	185	224	240	256	271	282	284	290	297	307	317	318	322	323	326
2519	184	217	233	253	256	269	276	284	295	290	313	317	313	326	317
2520	179	237	253	265	274	294	298	300	299	311	317	319	322	328	340
MEAN	176	215	235	251	262	270	277	286	292	294	305	308	309	312	317
S.D.	9.0	17.3	15.6	15.4	18.1	22.8	20.3	19.8	18.2	21.6	22.0	21.0	21.8	24.4	24.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP III 10,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3516	181	234	245	261	269	281	285	290	291	298	302	296	306	311	311
3517	174	201	224	232	240	239	250	259	261	260	264	273	274	272	282
3518	163	221	246	240	241	258	266	269	258	275	282	281	284	293	300
3519	176	219	223	240	244	257	275	275	267	280	272	284	281	283	288
3520	190	249	266	294	308	307	326	340	353	380	367	371	373	371	364
MEAN	177	225	241	253	260	268	280	287	286	299	297	301	304	306	309
S.D.	9.9	17.9	17.7	24.9	29.3	26.2	28.8	32.0	39.6	47.8	41.4	39.9	40.5	39.2	32.8
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE I

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

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY														
ANIMAL#	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4526	165	209	222	233	234	250	265	260	253	264	276	272	263	277	280
4527	185	223	242	261	274	286	287	296	298	302	310	295	308	313	312
4528	170	217	226	236	252	267	269	262	270	281	277	280	289	281	299
4529	184	215	243	249	257	257	270	274	274	269	284	287	287	284	294
4530	178	224	231	247	259	268	266	282	281	282	289	297	300	291	302
MEAN	176	218	233	245	255	266	271	275	275	280	287	286	289	289	298
S.D.	8.8	6.0	9.2	11.1	14.3	13.7	9.2	14.8	16.6	14.5	13.8	10.1	16.8	14.2	11.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP I 0 mg/m3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
1026	32	62	97	124	154	176	185	194	214	227	246	259	266
1027	34	63	95	112	133	158	176	188	205	222	233	243	261
1028	43	81	114	135	160	180	193	196	208	211	233	245	260
1029	36	63	89	108	122	148	163	170	175	185	192	196	202
1030	50	85	122	141	171	194	219	236	258	277	288	286	305
MEAN	39	71	103	124	148	171	187	197	212	224	238	246	259
S.D.	7.2	11.4	13.9	14.1	20.2	18.2	21.2	24.1	29.9	33.5	34.3	32.6	36.9
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP II 2,000 mg/m3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
2016	40	70	106	134	167	185	202	208	228	244	250	254	269
2017	35	66	99	123	136	158	171	183	194	199	210	210	212
2018	41	69	103	130	149	175	197	217	234	247	259	270	289
2019	38	70	102	125	144	164	188	200	217	223	238	237	256
2020	33	75	111	137	151	178	202	213	217	231	245	250	251
MEAN	37	70	104	130	149	172	192	204	218	229	241	244	255
S.D.	3.2	3.4	4.7	5.9	11.4	10.8	13.0	13.7	15.4	19.4	18.8	22.4	28.3
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP III 10,000 mg/m3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
3016	38	58	107	133	150	173	188	204	215	225	240	245	250
3017	40	14	104	130	156	179	199	216	229	239	251	259	265
3018	35	45	106	123	147	168	182	203	216	218	236	227	231
3019	35	32	103	132	139	162	184	208	221	231	242	239	262
3020	27	74	87	105	124	137	152	165	173	172	190	187	197
MEAN	35	45	101	125	143	164	181	199	211	217	232	231	241
S.D.	4.9	23.1	8.4	11.5	12.4	16.2	17.5	19.8	21.7	26.4	24.1	27.4	27.7
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP IV 20,000 mg/m3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
4026	45	73	97	114	138	154	164	191	207	219	244	235	258
4027	35	63	91	105	121	142	158	180	190	190	212	216	238
4028	29	58	82	100	127	150	161	183	197	209	225	229	237
4029	25	53	72	85	100	123	130	151	169	179	189	189	203
4030	33	65	100	112	133	159	182	186	205	212	217	228	228
MEAN	33	62	88	103	124	146	159	178	193	202	217	219	233
S.D.	7.6	7.5	11.8	11.6	14.7	14.0	18.5	15.7	15.3	16.7	20.1	18.2	19.9
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP I 0 mg/m3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
1526	13	23	-21	24	49	61	65	66	80	80	81	85	89
1527	12	17	23	36	39	41	42	49	53	56	51	60	67
1528	24	41	47	56	50	65	62	72	72	69	81	77	98
1529	16	25	46	47	55	62	65	67	66	76	70	81	77
1530	17	34	50	67	63	77	76	84	97	92	103	102	114
MEAN	16	28	29	46	51	61	62	68	74	75	77	81	89
S.D.	4.7	9.5	30.0	16.8	8.8	13.0	12.5	12.6	16.4	13.5	18.9	15.3	18.2
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP II 2,000 mg/m3

	WEEK OF STUDY												
ANIMAL#	0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
2516	20	34	42	42	53	63	70	67	75	79	80	79	85
2517	32	51	68	65	79	96	102	95	105	109	109	108	120
2518	16	32	47	58	60	66	73	83	93	94	98	99	102
2519	16	36	38	52	59	66	78	73	96	99	96	109	100
2520	17	28	38	57	61	63	63	75	80	82	85	91	104
MEAN	20	36	46	55	62	71	77	79	90	93	94	97	102
S.D.	7.0	8.7	12.6	8.5	9.8	14.2	14.8	10.8	12.2	12.1	11.4	12.7	12.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP III 10,000 mg/m3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
3516	11	27	36	47	52	56	57	64	68	62	73	78	78
3517	23	31	39	38	48	58	59	58	63	72	72	71	80
3518	25	19	20	37	45	48	38	54	62	60	64	72	80
3519	3	21	24	38	55	55	47	60	52	65	62	63	68
3520	16	44	59	58	77	91	104	131	118	122	123	122	114
MEAN	16	28	35	43	55	62	61	74	72	76	79	81	84
S.D.	8.9	10.0	15.0	8.9	12.5	16.7	25.3	32.3	25.9	25.7	25.4	23.3	17.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE J

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

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP IV 20,000 mg/m3

ANIMAL#	WEEK OF STUDY 0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13
4526	13	24	25	41	56	51	43	55	67	63	54	67	71
4527	19	38	51	64	65	73	75	79	88	72	85	90	89
4528	9	19	35	49	52	44	53	63	60	62	72	64	82
4529	28	34	42	42	55	59	59	54	69	72	72	69	79
4530	7	23	35	44	41	58	57	58	65	72	75	66	78
MEAN	15	28	37	48	54	57	58	62	70	68	72	71	80
S.D.	8.5	8.2	9.8	9.3	8.4	10.7	11.7	10.3	10.5	5.0	11.2	10.7	6.6
N	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP I 0 mg/m3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
1026	93	77	69	67	66	64	62	55	54	55	55	54	51	52
1027	93	75	71	64	59	58	58	55	51	52	52	50	47	47
1028	97	79	73	68	58	63	61	58	52	51	54	54	53	52
1029	95	82	73	71	66	64	63	58	54	54	54	53	52	53
1030	99	85	81	75	68	69	68	SF	61	59	56	53	50	54
MEAN	95	80	74	69	63	64	62	56	54	54	54	53	51	52
S.D.	2.7	4.0	4.4	4.0	4.8	4.1	3.7	1.8	3.7	3.2	1.8	1.6	2.6	2.5
N	5	5	5	5	5	5	5	4	5	5	5	5	5	5

SF=Spilled Feeder

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP II 2,000 mg/m3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
2016	93	80	72	66	61	61	56	56	49	50	51	52	43	47
2017	99	83	76	69	65	64	61	56	52	53	50	36	44	46
2018	95	84	72	71	67	62	62	58	53	54	52	51	49	48
2019	89	75	68	65	61	57	56	54	49	49	47	46	46	47
2020	95	79	74	71	65	59	60	56	52	52	52	49	48	48
MEAN	94	80	72	68	64	61	59	56	51	51	51	47	46	47
S.D.	3.7	3.9	3.3	2.8	2.8	2.8	2.8	1.4	2.1	2.0	2.0	6.5	2.5	1.0
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP III 10,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3016	92	75	69	65	58	56	56	51	48	48	47	47	43	45
3017	90	77	74	68	61	63	62	55	51	50	50	48	45	47
3018	89	74	72	69	59	59	59	54	51	52	49	48	42	46
3019	94	78	73	69	60	56	62	55	52	54	52	49	46	47
3020	SF	77	70	70	64	65	62	57	54	55	54	54	49	53
MEAN	91	76	71	68	61	60	60	55	51	52	50	49	45	48
S.D.	2.1	1.7	2.0	1.9	2.4	3.9	2.5	2.0	2.4	2.7	2.6	2.9	2.7	3.2
N	4	5	5	5	5	5	5	5	5	5	5	5	5	5

SF=Spilled Feeder

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4026	101	83	70	65	59	61	60	55	54	52	54	52	43	51
4027	92	79	72	69	64	62	65	58	56	54	53	56	53	55
4028	95	78	70	64	61	64	62	57	54	53	52	52	48	51
4029	93	77	70	65	64	64	65	57	57	56	55	55	52	50
4030	93	76	73	67	61	62	61	62	56	54	55	51	51	50
MEAN	95	79	71	66	62	63	63	58	55	54	54	53	49	51
S.D.	3.7	2.8	1.4	1.9	1.9	1.3	2.2	2.4	1.7	1.4	1.6	1.9	4.2	2.0
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP I 0 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
1526	81	81	77	21	96	80	71	63	59	61	58	59	57	52
1527	89	79	79	82	76	72	74	71	64	61	65	63	63	61
1528	88	85	83	73	75	69	70	66	62	64	68	67	62	60
1529	87	82	80	76	68	69	70	59	59	57	63	59	60	57
1530	90	86	84	79	76	73	71	65	60	63	60	63	58	60
MEAN	87	83	81	66	78	73	71	65	61	61	63	62	60	58
S.D.	3.5	3.1	2.5	25.8	10.5	4.5	1.8	4.4	2.0	2.7	3.7	3.2	2.5	3.4
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP II 2,000 mg/m3

ANIMAL#	WEEK OF STUDY 0	1	2	3	4	5	6	7	8	9	10	11	12	13
2516	88	83	75	71	68	72	67	67	62	64	61	57	57	57
2517	101	96	87	83	73	77	73	70	64	68	63	60	63	60
2518	92	83	80	77	71	72	75	70	65	64	65	61	57	56
2519	85	84	85	76	79	82	75	72	62	71	69	67	65	60
2520	103	84	84	84	79	70	71	73	72	67	71	74	72	65
MEAN	94	86	82	78	74	75	72	71	65	67	66	64	63	60
S.D.	7.9	5.5	4.9	5.5	5.2	4.9	3.4	2.3	4.1	2.8	4.2	6.5	6.3	3.4
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP III 10,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
3516	87	75	73	69	66	62	61	62	58	59	59	59	56	57
3517	91	84	70	70	67	67	63	62	63	64	60	59	59	58
3518	109	93	67	74	73	65	65	64	65	62	65	65	69	58
3519	92	73	80	70	74	73	72	64	69	61	71	68	61	66
3520	91	77	78	72	64	65	67	62	70	63	56	54	57	55
MEAN	94	80	74	71	69	66	66	63	65	62	62	61	61	59
S.D.	8.7	8.1	5.5	2.0	4.4	4.2	4.2	1.1	4.8	2.0	6.0	5.5	5.1	4.2
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5

TABLE K

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

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY													
ANIMAL#	0	1	2	3	4	5	6	7	8	9	10	11	12	13
4526	93	77	79	80	74	73	65	65	68	60	62	61	66	59
4527	88	84	80	82	74	64	68	70	63	64	62	67	65	61
4528	91	87	85	85	76	SF	71	69	65	62	66	68	55	65
4529	90	89	79	72	73	76	69	64	65	69	65	65	65	66
4530	87	74	73	69	65	64	68	59	61	60	61	59	55	60
MEAN	90	82	79	78	72	69	68	65	64	63	63	64	61	62
S.D.	2.4	6.4	4.4	6.6	4.4	6.3	2.2	4.2	2.7	3.6	2.4	3.8	5.6	3.4
N	5	5	5	5	5	4	5	5	5	5	5	5	5	5

SF=Spilled Feeder

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP I 0 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1026	TERMINAL SACRIFICE	26-SEP-01	13	92
1027	TERMINAL SACRIFICE	26-SEP-01	13	92
1028	TERMINAL SACRIFICE	26-SEP-01	13	92
1029	TERMINAL SACRIFICE	26-SEP-01	13	92
1030	TERMINAL SACRIFICE	26-SEP-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP II 2,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2016	TERMINAL SACRIFICE	26-SEP-01	13	92
2017	TERMINAL SACRIFICE	26-SEP-01	13	92
2018	TERMINAL SACRIFICE	26-SEP-01	13	92
2019	TERMINAL SACRIFICE	26-SEP-01	13	92
2020	TERMINAL SACRIFICE	26-SEP-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP III 10,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3016	TERMINAL SACRIFICE	26-SEP-01	13	92
3017	TERMINAL SACRIFICE	26-SEP-01	13	92
3018	TERMINAL SACRIFICE	26-SEP-01	13	92
3019	TERMINAL SACRIFICE	26-SEP-01	13	92
3020	TERMINAL SACRIFICE	26-SEP-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

MALES GROUP IV 20,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4026	TERMINAL SACRIFICE	26-SEP-01	13	92
4027	TERMINAL SACRIFICE	26-SEP-01	13	92
4028	TERMINAL SACRIFICE	26-SEP-01	13	92
4029	TERMINAL SACRIFICE	26-SEP-01	13	92
4030	TERMINAL SACRIFICE	26-SEP-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP I 0 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
1526	TERMINAL SACRIFICE	26-SEP-01	13	92
1527	TERMINAL SACRIFICE	26-SEP-01	13	92
1528	TERMINAL SACRIFICE	26-SEP-01	13	92
1529	TERMINAL SACRIFICE	26-SEP-01	13	92
1530	TERMINAL SACRIFICE	26-SEP-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP II 2,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
2516	TERMINAL SACRIFICE	26-SEP-01	13	92
2517	TERMINAL SACRIFICE	26-SEP-01	13	92
2518	TERMINAL SACRIFICE	26-SEP-01	13	92
2519	TERMINAL SACRIFICE	26-SEP-01	13	92
2520	TERMINAL SACRIFICE	26-SEP-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP III 10,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
3516	TERMINAL SACRIFICE	26-SEP-01	13	92
3517	TERMINAL SACRIFICE	26-SEP-01	13	92
3518	TERMINAL SACRIFICE	26-SEP-01	13	92
3519	TERMINAL SACRIFICE	26-SEP-01	13	92
3520	TERMINAL SACRIFICE	26-SEP-01	13	92

TABLE L

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

ANIMAL TERMINATION HISTORY

FEMALES GROUP IV 20,000 mg/m3

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
4526	TERMINAL SACRIFICE	26-SEP-01	13	92
4527	TERMINAL SACRIFICE	26-SEP-01	13	92
4528	TERMINAL SACRIFICE	26-SEP-01	13	92
4529	TERMINAL SACRIFICE	26-SEP-01	13	92
4530	TERMINAL SACRIFICE	26-SEP-01	13	92