Study Title

SATELLITE PROCEDURE
GASOLINE MTBE VAPOR CONDENSATE
RAT MICRONUCLEUS TEST

TEST GUIDELINES: US EPA Micronucleus Assay 79.64, CFR Vol. 59, No. 122, 27 June 1994.

US EPA (1998) Health Effects Test Guidelines; OPPTS 870.5395 Mammalian Erythrocyte Micronucleus Test.

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STUDY COMPLETED ON: 11 May 2007

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SUBCONTRACTOR'S SPONSOR: Huntingdon Life Sciences
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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The slide evaluation phase of the study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data generated to be valid.

The UK Good Laboratory Practice Regulations 1999 (Statutory Instrument 1999 No. 3106, as amended by Statutory Instrument 2004 No. 994).

OECD Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

EC Commission Directive 1999/11/EC of 8 March 1999 (Official Journal No. L 77/8), as amended by EC Commission Directive 2004/10/EC of 11 February 2004 (Official Journal No. L 50/44).

US EPA 79.60, CFR Vol. 59, No 122, 27 June 1994.

No compliance is claimed for work presented in the Experimental Procedure – In-life phase or Appendix 2 of this report.

C E Mason — 10a May 2007

Christine E. Mason, B.Sc. (Hons.),
Principal Investigator,
Huntingdon Life Sciences Ltd.

Date

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I am claiming compliance for the whole study. This study was performed according to protocol and Standard Operating Procedure with the following exceptions: The identity, strength, purity and composition or other characteristics to define the positive control article has not been determined by the testing facility. The positive control article has been characterized as per the Certificate of Analysis on file with the testing facility. The stability of the positive control article has not been determined by the testing facility. Analyses to determine the uniformity (as applicable) or concentration of the positive control mixture were not performed by the testing facility. The stability of the positive control article mixture has not been determined by the testing facility.

28 Jun 07

Gary M. Hoffman, B.A., D.A.B.T.,
Study Director,
Huntingdon Life Sciences

Date

ERC - QUALITY ASSURANCE STATEMENT

The following inspections and audits have been carried out in relation to the slide evaluation phase for this study:

Study Phase	Date of Inspection	Date of Reporting to Principal Investigator and Test Site Management	Date of Reporting to Study Director, Test Facility Management and Lead QA
Process Based Inspection			
Slide scoring	18 July 2001	18 July 2001	-
Report Audit	24 September 2001 4 – 18 June 2004	24 September 2001 18 June 2004	19 December 2001 18 June 2004

Process Based Inspections: At or about the time this phase of the study was in progress, inspections of routine and repetitive procedures employed on this type of study were carried out. These were conducted and reported to appropriate Company Management as indicated above.

Report Audit: This appendix has been audited by the Quality Assurance Department. These audits were conducted and reported to the Principal Investigator, test site Management, Study Director, test facility Management and lead Quality Assurance Department as indicated above.

Study based inspections were not performed on this phase of the study.

The methods, procedures and observations were found to be accurately described and the reported results of this appendix to reflect the raw data.

N Jolly

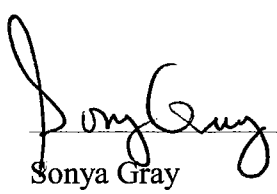
Neal Jolly, B.Sc., M.R.Q.A.,
Group Manager,
Department of Quality Assurance,
Huntingdon Life Sciences Ltd.

3 MAY 2007
Date

PRC - 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	8 – 9 Jan 01	12 Jan 01
Dose Administration of Positive Control Genotoxicity Animals	4 Apr 01	5 Apr 01
Genotoxicity Necropsy	5 Apr 01	5 Apr 01
Subcontractor Micronucleus Report & In-Life Study Data & Report	25 – 26 Oct 01	26 Oct 01
Sponsor's Comments & Report Verification	22 – 24 Aug 05	24 Aug 05



Sonya Gray
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27 jan 07
Date

RESPONSIBLE PERSONNEL AND SCIENTIFIC APPROVAL

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

This satellite micronucleus study was designed to assess the potential induction of micronuclei by Gasoline MTBE Vapor Condensate in bone marrow cells of the rat. Animals were exposed for four weeks (5 days per week) by inhalation administration of the test substance at exposure levels of 2000, 10000 and 20000 mg/m^3.

The test substance and negative control were administered by inhalation. The negative control group received clean air. A positive control group was dosed on one occasion by intraperitoneal injection, with cyclophosphamide at 40 mg/kg bodyweight.

Bone marrow smears were obtained from five male and five female animals in the negative control and each of the test substance groups 24 hours after the 20^{th} exposure and from the positive control group 24 hours after dosing. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes. The proportion of immature erythrocytes was assessed by examination of at least 1000 erythrocytes from each animal. A record of the incidence of micronucleated mature erythrocytes was also kept.

No statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decrease in the proportion of immature erythrocytes were observed in rats treated with Gasoline MTBE Vapor Condensate compared to negative control values.

The positive control substance, Cyclophosphamide, produced large, significant increases in the frequency of micronucleated immature erythrocytes and a decrease in the proportion of immature erythrocytes ($P<0.001$).

It is concluded that Gasoline MTBE Vapor Condensate did not show any evidence, as indicated by micronucleus evaluation, of causing chromosome damage or bone marrow cell toxicity when administered by inhalation exposure in this *in vivo* test procedure.

INTRODUCTION

The purpose of this satellite micronucleus study was to assess the potential of Gasoline MTBE Vapor Condensate to induce mutagenic effects in rats following inhalation administration using an *in vivo* cytogenetic system (Boller and Schmid 1970, MacGregor *et al* 1987, Mavournin *et al* 1990). The inhalation route was selected for use in this test as the most likely route of human exposure.

The procedures used were based on the recommendations of the following guidelines:

- US EPA Micronucleus Assay 79.64, CFR Vol. 59, No. 122, 27 June 1994.
- US EPA (1998) Health Effects Test Guidelines; OPPTS 870.5395 Mammalian Erythrocyte Micronucleus Test.

The bone marrow micronucleus test, originally developed by Matter and Schmid (1971), is a widely employed and internationally accepted short-term assay for identification of genotoxic effects (chromosome damage and aneuploidy) associated with mutagens and carcinogens (Mavournin *et al* 1990). This *in vivo* system allows consideration of various factors including pharmacokinetics, metabolism and DNA repair which cannot be accurately modelled in an *in vitro* system. Young adult rats are chosen for use because of the high rate of cell division in the bone marrow, because of the wealth of background data on this species, and because of their general suitability for toxicological investigations.

In mitotic cells in which chromosomal breakage has been caused by the test substance or its metabolites, acentric fragments of the chromosomes do not separate at the anaphase stage of cell division. After telophase these fragments may not be included in the nuclei of the daughter cells and hence will form single or multiple micronuclei (Howell-Jolly bodies) in the cytoplasm of these cells. Micronuclei are seen in a wide variety of cells, but erythrocytes are chosen for examination since micronuclei are not obscured by the main nucleus and are therefore easily detected in this cell type (Boller and Schmid 1970).

Micronucleated immature erythrocytes appear in the bone marrow approximately 24 hours after induction of chromosome damage. These immature erythrocytes can be differentiated by a variety of staining techniques which rely on their relatively high content of residual RNA. Using the Feulgen method, they stain blue while mature erythrocytes (which contain little RNA) are counterstained orange. An increased incidence of micronucleated immature erythrocytes is indicative of recent exposure to a chromosome-damaging agent. A simultaneous marked increase in the incidence of micronucleated mature erythrocytes is not expected and may be indicative of micronucleus-like artifacts (Schmid 1976).

Substances which interfere with the mitotic spindle apparatus will cause non-disjunction (unequal separation of the chromosomes at anaphase resulting in aneuploidy) or lagging chromosomes at anaphase which may not be incorporated into the daughter nuclei. These lagging chromosomes are not excluded from the erythroblast with the main nucleus and hence also give rise to micronuclei.

Any toxic effects of the test substance on the nucleated cells may lead either to a reduction in cell division or to cell death. These effects in turn lead to a reduction in the number of nucleated cells and immature erythrocytes; to compensate for this, peripheral blood is shunted into the bone marrow (von Ledebur and Schmid 1973). If the proportion of immature erythrocytes is found to be significantly less than the control value, this is taken as being indicative of toxicity. A very large decrease in the proportion would be indicative of a cytostatic or cytotoxic effect.

The slide evaluation phase of the satellite micronucleus study was performed at the Department of Genetic Toxicology, Huntingdon Life Sciences (ERC), Eye, Suffolk, IP23 7PX, England. Subsequently statistical analysis was performed by the Department of Statistics, Huntingdon Life Sciences (HRC), Huntingdon, Cambridgeshire, PE28 4HS, England.

The experimental start and completion dates of the slide evaluation phase of the study were 2 May 2001 and 12 July 2001 respectively.

EXPERIMENTAL PROCEDURE

In-life phase

The in-life phase of the study was carried out at the Princeton Research Centre starting on 8 March 2001 and was completed on 5 April 2001.

All animals in the negative control and test substance groups were exposed for four weeks (5 days per week) by inhalation. The positive control group was dosed with Cyclophosphamide administered by intraperitoneal injection at a volume dosage of 10 ml/kg bodyweight. Cyclophosphamide (CP, CAS # 6055-19-2, lot number 108H0568, received 3 April 2001, expiration June 2001, white powder, storage 2-8°C, purity 99.2%), was obtained from the Sigma Chemical Company (responsible for its characterization), and was dissolved and diluted in sterile distilled water at Huntingdon Life Sciences to stock concentrations of 4.0 mg/mL for use as the positive control for micronucleus study.

The experimental design is shown below:

Group	Treatment	Exposure Level (mg/m^3)	Animal Numbers	
			Male	Female
1	Air control	-	1031 - 1035	1541 – 1545
2	Test Substance	2000	2021 - 2025	2531 – 2535
3	Test Substance	10000	3021 - 3025	3531 – 3535
4	Test Substance	20000	4031 – 4035	4541 – 4545
6	Cyclophosphamide	40 (mg/kg)	6031 – 6035	6541 - 6545

Five males and five females from the negative control, each of the test substance groups and the positive control group were sacrificed 24 hours after the final exposure period by CO_2 asphyxiation. Both femurs were exposed, cut just above the knee and the bone marrow was aspirated into a syringe containing a small volume (about 0.5 mL) of serum. The cells were then flushed into a centrifuge tube of cold serum. The tubes were identified by labels containing the study, group number, and animal number.

The bone marrow cells were pelleted by centrifugation at about 150 x g for about 5 min and the supernatant drawn off, leaving a small amount of serum with the cell pellet. The cells were resuspended by aspiration with a pasteur pipette and a small drop of cells was spread onto a clean glass slide. Four slides were prepared from each animal. The slides were allowed to air dry, fixed by dipping for about 3 minutes in methanol, and aged overnight or longer prior to staining. Slides were labelled with experiment and animal number using a lead pencil.

Two slides from each animal were despatched to Huntingdon Life Sciences (ERC), Eye, Suffolk, IP23 7PX, England for slide staining and analysis. The remaining 2 smears and the cell pellet (refrigerated) were held in reserve at PRC in case of technical problems with the first 2 smears.

Slide evaluation

Due to the presence of mast cell granules in rat bone smears, which appear identical to micronuclei when stained using the Romanowsky methods, a modified Feulgen staining method is employed for the rat micronucleus test in this laboratory. This method specifically stains DNA-containing bodies deep purple while leaving mast cell granules unstained. The method also allows reasonable differentiation of mature and immature erythrocytes and produces permanent preparations.

One slide from each animal was stained as follows, the remaining slide was held in reserve:

1. Hydrolysed in Bouin's fluid at room temperature for 30 hours.
2. Washed three times in purified water (5 minutes per wash).
3. Stained in Schiff's reagent for one hour at room temperature.
4. Washed three times in purified water (5 minutes per wash).
5. Counter-stained for ten minutes in very dilute (approximately 0.06 g/l) aqueous Eosin yellowish.
6. Washed for five minutes in purified water.
7. Stained for 30 minutes in Mayer's Haemalum diluted 9 volumes: 1 volume with aqueous acridine orange solution in purified water (1 mg/ml).
8. Rinsed in purified water.
9. Rinsed in running tap water.
10. Washed for 5 minutes in purified water.
11. Air-dried.
12. Slides were mounted with coverslips using DPX mountant.
13. The mountant was allowed to harden at approximately 37°C.

NB All stains and Bouin's fluid were filtered immediately prior to use to remove particulate material.

The stained smears were examined (under code) by light microscopy to determine the incidence of micronucleated cells per 2000 polychromatic erythrocytes per animal. One smear per animal was examined. The remaining smears were held temporarily in reserve in case of technical problems with the first smear.

Micronuclei are identified by the following criteria:

- Large enough to discern morphological characteristics
- Should possess a generally rounded shape with a clearly defined outline
- Should be deeply stained and similar in colour to the nuclei of other cells - not black
- Should lie in the same focal plane as the cell
- Lack internal structure, *ie* they are pyknotic
- There should be no micronucleus-like debris in the area surrounding the cell

The proportion of immature erythrocytes for each animal was assessed by examination of at least 1000 erythrocytes. A record of the number of micronucleated mature erythrocytes observed during assessment of this proportion was also kept as recommended by Schmid (1976).

Deviation from Protocol

The statistical analysis was performed at Huntingdon Life Sciences Ltd., Huntingdon Cambridgeshire, PE28 4HS, England.

ASSESSMENT OF RESULTS

The results for each treatment group were compared with the results for the negative control group using non-parametric statistics. Non-parametric statistical methods were chosen for analysis of results because:

- They are suited to analysis of data consisting of discrete/integer values with ties such as the incidence of micronucleated immature erythrocytes.
- The methods make few assumptions about the underlying distribution of data and therefore the values do not require transformation to fit a theoretical distribution (where data can be approximately fitted to a normal distribution, the results of non-parametric analysis and classical analysis of variance are very similar).
- 'Outliers' are frequently found in the proportion of immature erythrocytes for both control and treated animals; non-parametric analysis based on rank does not give these values an undue weighting.

For incidences of micronucleated immature erythrocytes, exact one-sided p-values were calculated by permutation (StatXact, CYTEL Software Corporation, Cambridge, Massachussetts). Comparison of several dose levels was made with the negative control using the Linear by Linear Association test for trend. If significance was detected it was repeated in a step-down fashion, Groups 1 to 4 for an increase over control, then groups 1 to 3 etc. (Agresti *et al.* 1990). For the positive control group, this procedure was simplified to a straightforward permutation test (Gibbons 1985).

For assessment of effects on the proportion of immature erythrocytes, equivalent permutation tests based on rank scores were used, *ie* exact versions of Wilcoxon's sum of ranks test and Jonckheere's test for trend.

A positive response is normally indicated by a statistically significant dose-related increase in the incidence of micronucleated immature erythrocytes for the treatment group compared with the negative control group ($P<0.01$); individual and/or group mean values should exceed the laboratory historical control range (Morrison and Ashby 1995).

A negative result is indicated where individual and group mean incidences of micronucleated immature erythrocytes for the group treated with the test substance are not significantly greater than incidences for the negative control group and where these values fall within the historical control range. An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response.

Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant dose-related decrease in the proportion of immature erythrocytes ($P<0.01$).

MAINTENANCE OF RECORDS

All raw data, samples and specimens arising from the performance of this phase of the study will remain the property of the Sponsor.

Types of sample and specimen that are unsuitable, by reason of instability, for long term retention and archiving may be disposed after the periods stated in Huntingdon Life Sciences, Standard Operating Procedures.

All other samples and specimens and all raw data will be retained by Huntingdon Life Sciences PRC in its archive for a period of one year from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and their advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

RESULTS

MICRONUCLEUS TEST

Statistical analysis was performed on the combined sex data and on male and female data separately. Table 1 gives a summary of the results of the micronucleus test and the results of statistical analysis. The results for individual animals are presented in Table 2. Appendix 1 summarises the historical control data for micronucleated immature erythrocyte counts.

Micronucleated immature erythrocyte counts (mie)

The test substance did not cause any statistically significant increases in the number of micronucleated immature erythrocytes.

Individual and group mean values for micronucleated immature erythrocytes observed in animals treated with the positive control, Cyclophosphamide were outside the historical positive control range. However, individual and group mean values were outside the historical negative control range and the increases were statistically significant, indicating a clear positive response [$P<0.001$].

Micronucleated mature erythrocytes (mme)

The test substance did not cause any substantial increases in the incidence of micronucleated mature erythrocytes.

Proportion of immature erythrocytes [% ie/(ie + me)]

The group mean ratio of immature erythrocytes to mature erythrocytes was lower in male animals for all test substance treatment groups and the negative control group compared to female group mean values. Analysis for the separate sexes showed a decrease in the group mean ratio in all treatment groups compared to the negative control value for male animals only. These decreases were not statistically significant and are not considered to be indicative of bone marrow cell toxicity.

The test substance failed to cause any significant decreases in the proportion of immature erythrocytes when the data for both sexes was combined.

Cyclophosphamide caused statistically significant decreases in the proportion in both male and female animals ($P<0.01$).

CONCLUSION

No statistically significant increases in the frequency of micronucleated immature erythrocytes and no substantial decrease in the proportion of immature erythrocytes were observed in rats treated with Gasoline MTBE Vapor Condensate compared to negative control values.

It is concluded that Gasoline MTBE Vapor Condensate did not show any evidence, as indicated by micronuclei evaluation, of causing chromosome damage or bone marrow cell toxicity when administered by inhalation in this *in vivo* test procedure.

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TABLE 1

Summary of results and statistical analysis – combined sexes

Sampling time	Treatment	Exposure level (mg/m^3)	Proportion of ie † (mean ± SD)	Incidence mie (mean ± SD)	Incidence mme (group mean ± SD) [a]
24 hours	Negative control	-	45±4.8	2.1±1.7	0.4±0.3
	TS	2000	42±5.8	1.4±1.5	0.0±0.0
	TS	10000	43±6.4	0.9±0.6	1.0±0.5
	TS	20000	43±6.8	1.5±1.1	0.0±0.0
	Cyclophosphamide	40 (mg/kg)	26±4.8***	14.9±6.7***	0.0±0.0

TS	Gasoline MTBE Vapor Condensate
ie	Immature erythrocytes
mie	Number of micronucleated cells observed per 2000 immature erythrocytes examined
me	Mature erythrocytes
mme	Number of micronucleated cells observed and calculated per 2000 mature erythrocytes
SD	Standard deviation

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

***	$P < 0.001$	(significant)
otherwise	$P > 0.01$	(not significant)

† Occasional apparent errors of ± 1% may occur due to rounding of values for presentation in the table

[a] Formula for calculation of incidence mme (group mean):

$$\frac{\text{Sum of group incidence mme scored} \times 2000}{\text{Sum of group me scored}}$$

TABLE 1 - continued

Summary of results and statistical analysis – separate sexes

Sampling time	Treatment	Exposure level (mg/m^3)	Proportion of ie † (mean ± SD)	Incidence mie (mean ± SD)	Incidence mme (group mean ± SD) [a]
		MALES			
24 hours	Negative control	-	44±3.2	1.8±1.5	0.7±0.4
	TS	2000	40±6.0	2.0±1.9	0.0±0.0
	TS	10000	38±5.0	1.2±0.4	0.6±0.4
	TS	20000	38±2.6	1.2±0.8	0.0±0.0
	Cyclophosphamide	40 (mg/kg)	25±7.2**	19.2±6.5**	0.0±0.0
		FEMALES			
24 hours	Negative control	-	47±6.0	2.4±2.1	0.0±0.0
	TS	2000	44±5.3	0.8±0.8	0.0±0.0
	TS	10000	48±2.3	0.6±0.5	1.5±0.5
	TS	20000	49±4.2	1.8±1.3	0.0±0.0
	Cyclophosphamide	40 (mg/kg)	26±0.8**	10.6±3.6**	0.0±0.0

TS Gasoline MTBE Vapor Condensate
ie Immature erythrocytes
mie Number of micronucleated cells observed per 2000 immature erythrocytes examined
me Mature erythrocytes
mme Number of micronucleated cells observed and calculated per 2000 mature erythrocytes
SD Standard deviation

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

** $P < 0.01$ (significant)
otherwise $P > 0.01$ (not significant)

† Occasional apparent errors of ± 1% may occur due to rounding of values for presentation in the table

[a] Formula for calculation of incidence mme (group mean):

$$\frac{\text{Sum of group incidence } \textbf{mme} \text{ scored} \times 2000}{\text{Sum of group } \textbf{me} \text{ scored}}$$

TABLE 2

Results for individual animals - 24 hour sampling time

Treatment	Exposure (mg/m^3)	Animal number	ie	me	Proportion of ie	Incidence mie	Incidence mme
Negative control (air)	-	M 1031	452	624	42	0	0
		M 1032	399	619	39	2	1
		M 1033	486	617	44	4	0
		M 1034	519	572	48	2	0
		M 1035	465	553	46	1	0
		F 1541	367	640	36	1	0
		F 1542	488	541	47	4	0
		F 1543	529	491	52	0	0
		F 1544	503	504	50	5	0
		F 1545	499	535	48	2	0
TS	2000	M 2021	508	505	50	0	0
		M 2022	402	705	36	4	0
		M 2023	406	604	40	3	0
		M 2024	400	706	36	0	0
		M 2025	416	733	36	3	0
		F 2531	410	616	40	0	0
		F 2532	411	687	37	0	0
		F 2533	457	551	45	2	0
		F 2534	517	535	49	1	0
		F 2535	491	512	49	1	0
TS	10000	M 3021	389	675	37	1	1
		M 3022	338	736	31	1	0
		M 3023	380	643	37	2	0
		M 3024	440	594	43	1	0
		M 3025	465	592	44	1	0
		F 3531	513	511	50	1	1
		F 3532	512	498	51	1	0
		F 3533	482	532	48	1	1
		F 3534	490	540	48	0	0
		F 3535	450	553	45	0	0
TS	20000	M 4031	417	625	40	2	0
		M 4032	403	709	36	2	0
		M 4033	413	592	41	1	0
		M 4034	396	697	36	1	0
		M 4035	421	778	35	0	0
		F 4541	432	573	43	2	0
		F 4542	523	507	51	1	0
		F 4543	487	549	47	0	0
		F 4544	559	483	54	3	0
		F 4545	522	498	51	3	0

TS Gasoline MTBE Vapor Condensate
ie Immature erythrocytes
mie Number of micronucleated cells observed per 2000 immature erythrocytes
me Total number of mature erythrocytes examined for micronuclei
mme Number of micronucleated mature erythrocytes observed

TABLE 2 - continued

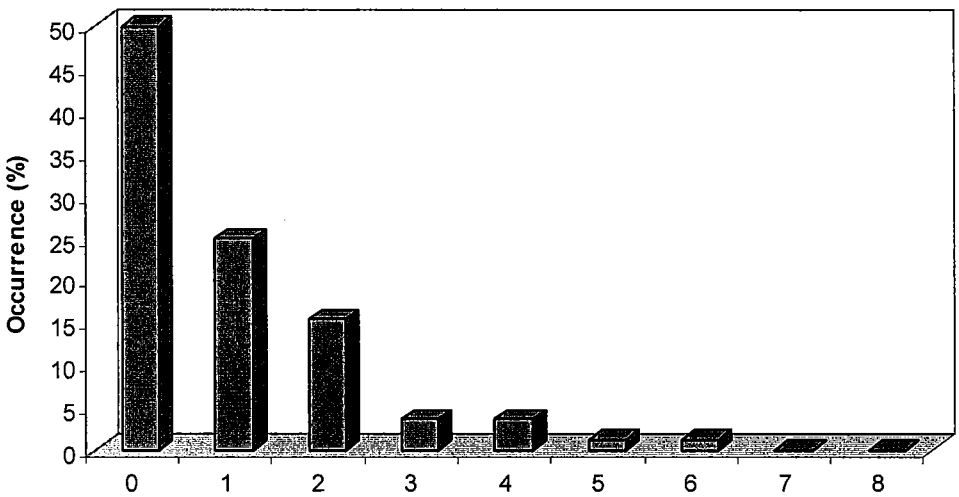
Results for individual animals - 24 hour sampling time

Treatment	Dosage (mg/kg)	Animal number	ie	me	Proportion of ie	Incidence mie	Incidence mme
Cyclophosphamide	40	M 6031	180	876	17	15	0
		M 6032	374	667	36	19	0
		M 6033	305	775	28	29	0
		M 6034	264	925	22	12	0
		M 6035	246	831	23	21	0
		F 6541	287	779	27	15	0
		F 6542	279	791	26	8	0
		F 6543	262	769	25	7	0
		F 6544	259	785	25	14	0
		F 6545	280	806	26	9	0

ie Immature erythrocytes
mie Number of micronucleated cells observed per 2000 immature erythrocytes
me Total number of mature erythrocytes examined for micronuclei
mme Number of micronucleated mature erythrocytes observed

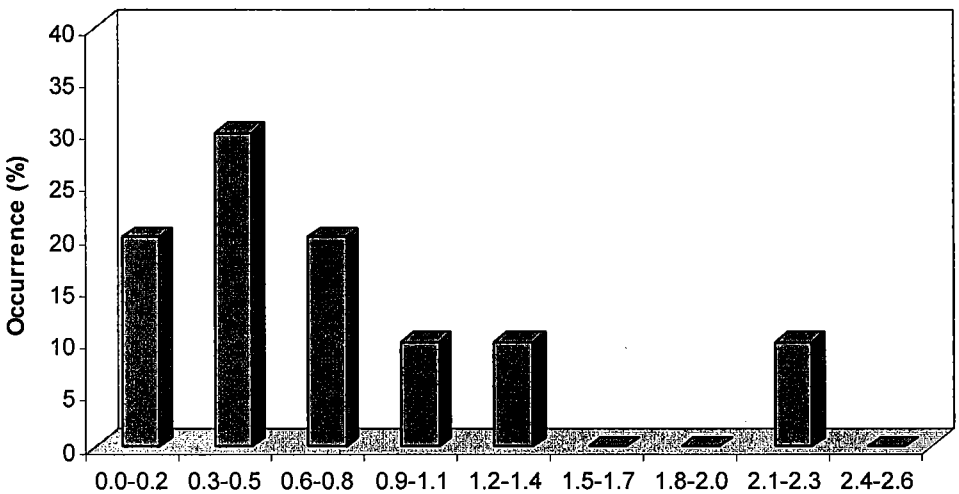
APPENDIX 1

Historical negative control values (1 February 1999 - 31 January 2001)
Frequency of micronucleated immature erythrocytes (individual animals)



Individual mean 0.94

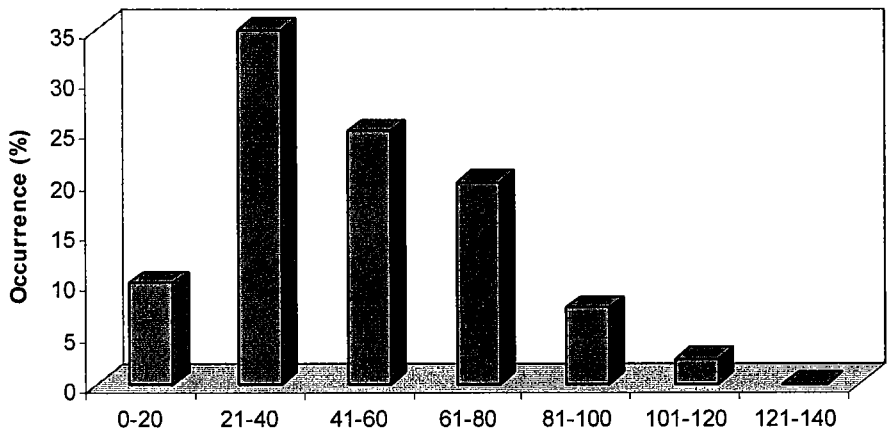
Historical negative control values (1 February 1999 - 31 January 2001)
Frequency of micronucleated immature erythrocytes (Group mean values)



Group mean 0.71

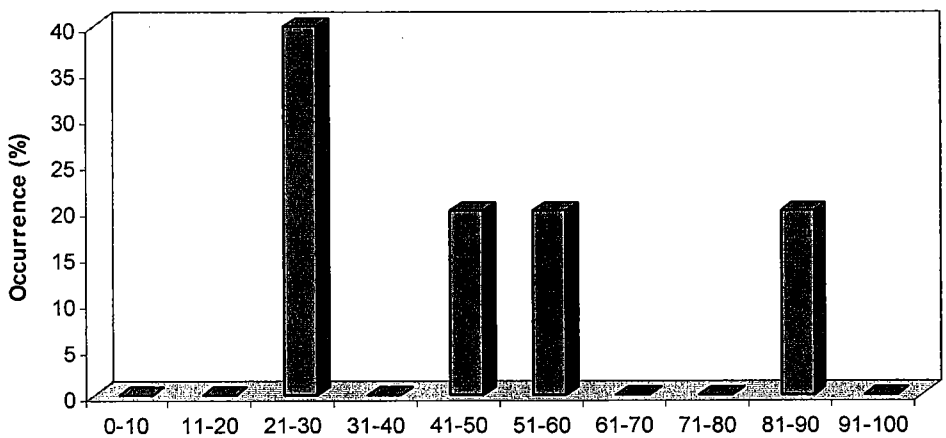
APPENDIX 1 – continued

Historical positive control values (1 February 1999 - 31 January 2001)
Frequency of micronucleated immature erythrocytes (Individual animals)
Cyclophosphamide



Number of micronucleated immature erythrocytes (MIE per 2000 cells)
Individual mean 48.03

Historical positive control values (1 February 1999 - 31 January 2001)
Frequency of micronucleated immature erythrocytes (Group mean values)
Cyclophosphamide



Number of micronucleated immature erythrocytes (MIE per 2000)
Group mean 49.2

APPENDIX 2

ANIMAL EXPOSURE AND OBSERVATIONS DATA

	Animal Exposure and Animal Data Preface	Appendix 2

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:	20 February 2001
Experimental Initiation Date:	8 March 2001 (in-life)
Experimental Completion Date:	5 April 2001 (in-life)

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

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TABLES

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

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

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment	
				Mean	Individual								Mean Temperature	Mean Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					MMAD (µm)	GSD	TMC (mg/m^3)	(°C)	(%)
30	8-Mar-01	23	0	0	0	0	0	0					23	50
31	9-Mar-01	24	0	0	0	0	0	0		1.091	2.189	4.94E-03	25	52
32	11-Mar-01	25	0	0	0	0	0	0					25	52
34	12-Mar-01	26	0	0	0	0	0	0					25	51
35	13-Mar-01	27	0	0	0	0	0	0					24	48
36	14-Mar-01	28	0	0	0	0	0	0					24	49
37	15-Mar-01	29	0	0	0	0	0	0					24	51
38	16-Mar-01	30	0	0	0	0	0	0		1.505	2.305	7.50E-03	24	50
41	19-Mar-01	31	0	0	0	0	0	0					24	53
42	20-Mar-01	32	0	0	0	0	0	0					25	52
43	21-Mar-01	33	0	0	0	0	0	0					25	49
44	22-Mar-01	34	0	0	0	0	0	0					25	48
45	23-Mar-01	35	0	0	0	0	0	0		11.68	2.733	5.62E-03	25	48
48	26-Mar-01	36	0	0	0	0	0	0					25	52
49	27-Mar-01	37	0	0	0	0	0	0					23	56
50	28-Mar-01	38	0	0	0	0	0	0					24	54
51	29-Mar-01	39	0	0	0	0	0	0					24	54
52	30-Mar-01	40	0	0	0	0	0	0		3.050	2.022	2.36E-03	24	48
55	2-Apr-01	41	0	0	0	0	0	0					24	49
56	3-Apr-01	42	0	0	0	0	0	0					25	50
57	4-Apr-01	43	0	0	0	0	0	0					25	53
		Mean	**0**		**0**					**4.332**	**2.312**	**5.11E-03**	**24.4**	**50.9**
		S.D.	**0**		**0**					**4.971**	**0.304**	**2.13E-03**	**0.7**	**2.3**

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

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

Day	Date	Exposure Number	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
			Nominal	Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(µm)		(mg/m^3)	(°C)	(%)
30	8-Mar-01	23	0	0	0	0	0	0				22	55
31	9-Mar-01	24	0	0	0	0	0	0	3.514	2.804	8.02E-03	23	57
32	11-Mar-01	25	0	0	0	0	0	0				24	56
34	12-Mar-01	26	0	0	0	0	0	0				24	56
35	13-Mar-01	27	0	0	0	0	0	0				25	51
36	14-Mar-01	28	0	0	0	0	0	0				25	52
37	15-Mar-01	29	0	0	0	0	0	0				24	52
38	16-Mar-01	30	0	0	0	0	0	0	1.386	2.312	8.77E-03	25	53
41	19-Mar-01	31	0	0	0	0	0	0				25	56
42	20-Mar-01	32	0	0	0	0	0	0				24	56
43	21-Mar-01	33	0	0	0	0	0	0				23	54
44	22-Mar-01	34	0	0	0	0	0	0				24	52
45	23-Mar-01	35	0	0	0	0	0	0	2.302	1.825	2.03E-03	24	51
48	26-Mar-01	36	0	0	0	0	0	0				23	56
49	27-Mar-01	37	0	0	0	0	0	0				24	58
50	28-Mar-01	38	0	0	0	0	0	0				25	57
51	29-Mar-01	39	0	0	0	0	0	0				24	58
52	30-Mar-01	40	0	0	0	0	0	0	6.932	2.434	4.31E-03	24	52
55	2-Apr-01	41	0	0	0	0	0	0				25	51
56	3-Apr-01	42	0	0	0	0	0	0				24	55
57	4-Apr-01	43	0	0	0	0	0	0				24	58
		Mean	**0**		**0**				**3.534**	**2.344**	**5.78E-03**	**24.0**	**54.6**
		S.D.	**0**		**0**				**2.428**	**0.404**	**3.17E-03**	**0.8**	**2.5**

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

Chamber Monitoring Results
Cumulative Exposure Record
Group IIA - 2000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration					Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(µm)		(mg/m^3)	(°C)	(%)
30	8-Mar-01	23	2220	2058	1920	2210	2050	2050				22	58
31	9-Mar-01	24	2140	1960	1770	1830	2020	2220	0.8056	1.998	3.64E-03	23	58
32	11-Mar-01	25	2070	1998	1960	1830	2250	1950				24	57
34	12-Mar-01	26	2080	1900	1910	1840	1890	1960				24	58
35	13-Mar-01	27	2010	1853	1780	1800	1980	1850				23	54
36	14-Mar-01	28	2160	1935	1880	1910	1950	2000				23	56
37	15-Mar-01	29	2070	2050	2070	1970	2310	1850				22	34
38	16-Mar-01	30	2160	2050	1970	1840	2230	2160	0.9256	2.013	6.12E-03	22	33
41	19-Mar-01	31	2080	1993	2080	1920	2020	1950				23	62
42	20-Mar-01	32	2070	2023	1970	2050	2050	2020				24	57
43	21-Mar-01	33	2170	2040	2040	1890	2090	2140				24	54
44	22-Mar-01	34	2100	2023	2000	1990	2010	2090				24	55
45	23-Mar-01	35	2100	2128	2190	2070	2010	2240	1.748	1.808	1.78E-03	24	53
48	26-Mar-01	36	2070	2058	2160	2150	1940	1980				23	60
49	27-Mar-01	37	2090	2005	2000	2130	1970	1920				22	62
50	28-Mar-01	38	2080	1983	2080	2000	1960	1890				23	61
51	29-Mar-01	39	2160	2005	2020	1780	2080	2140				22	59
52	30-Mar-01	40	2120	2005	1800	2310	2020	1890	6.827	2.382	3.16E-03	22	55
55	2-Apr-01	41	2180	2060	1910	1850	2180	2300				23	54
56	3-Apr-01	42	2210	2118	2260	2130	2210	1870				24	56
57	4-Apr-01	43	2140	1950	2030	2230	1910	1630				24	61
		Mean	**2118**		**2009**				**2.577**	**2.050**	**3.68E-03**	**23.1**	**55.1**
		S.D.	**54**		**142**				**2.864**	**0.240**	**1.81E-03**	**0.8**	**7.7**

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

Chamber Monitoring Results
Cumulative Exposure Record
Group IIB - 2000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration					Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(µm)		(mg/m^3)	(°C)	(%)
30	8-Mar-01	23	2220	2230	1990	2170	2500	2260				21	51
31	9-Mar-01	24	2140	1928	2220	1730	1860	1900	0.8510	2.139	3.77E-03	22	51
32	11-Mar-01	25	2070	2088	2120	2000	2280	1950				23	51
34	12-Mar-01	26	2080	1888	1960	1870	1970	1750				23	52
35	13-Mar-01	27	2010	1870	1960	1740	2120	1660				23	48
36	14-Mar-01	28	2160	2050	2180	1990	2080	1950				23	49
37	15-Mar-01	29	2070	2023	2010	2100	2020	1960				23	50
38	16-Mar-01	30	2160	2158	2230	1970	2220	2210	0.9752	2.318	6.20E-03	23	50
41	19-Mar-01	31	2080	2110	2050	2020	2220	2150				23	53
42	20-Mar-01	32	2070	1943	1660	2110	2010	1990				23	51
43	21-Mar-01	33	2170	2043	1890	2140	2060	2080				22	49
44	22-Mar-01	34	2100	2018	1930	2010	1980	2150				23	47
45	23-Mar-01	35	2100	2045	2110	2230	1840	2000	1.277	1.794	9.58E-04	23	47
48	26-Mar-01	36	2070	1973	1750	1980	2180	1980				22	53
49	27-Mar-01	37	2090	1998	1960	2020	2000	2010				23	55
50	28-Mar-01	38	2080	2008	2020	2020	2010	1980				23	53
51	29-Mar-01	39	2160	1985	2000	1860	1990	2090				23	53
52	30-Mar-01	40	2120	1965	1770	2060	2100	1930	8.151	2.605	2.47E-03	23	49
55	2-Apr-01	41	2180	2048	1890	1770	2280	2250				24	48
56	3-Apr-01	42	2210	2045	2220	1970	1970	2020				23	50
57	4-Apr-01	43	2140	2048	1940	2060	2090	2100				23	54
		Mean	**2118**		**2022**				**2.814**	**2.214**	**3.35E-03**	**22.8**	**50.7**
		S.D.	**54**		**150**				**3.563**	**0.339**	**2.22E-03**	**0.6**	**2.3**

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIA - 10000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
30	8-Mar-01	23	10800	9853	9080	9730	10500	10100					22	48
31	9-Mar-01	24	11500	10900	11700	10000	11100	10800		0.8529	1.953	3.85E-03	24	49
32	11-Mar-01	25	10700	10030	11200	9310	10200	9410					24	47
34	12-Mar-01	26	10700	10430	10700	10500	11000	9520					24	47
35	13-Mar-01	27	10800	10400	10400	10400	10700	10100					23	47
36	14-Mar-01	28	10800	10070	9580	10000	10300	10400					23	46
37	15-Mar-01	29	10600	10060	10000	11400	9170	9650					23	46
38	16-Mar-01	30	11200	9990	9750	9710	10100	10400		0.9905	2.570	6.44E-03	24	47
41	19-Mar-01	31	10800	10080	10000	10000	10200	10100					23	48
42	20-Mar-01	32	10800	10150	9680	10100	10300	10500					25	47
43	21-Mar-01	33	10600	10380	10700	10200	10500	10100					24	46
44	22-Mar-01	34	10900	10450	10700	10500	10200	10400					24	46
45	23-Mar-01	35	11000	10430	10600	10600	10400	10100		1.628	1.760	1.46E-03	25	43
48	26-Mar-01	36	10700	10600	10400	10700	10800	10500					24	45
49	27-Mar-01	37	10700	10350	10200	10400	10500	10300					23	44
50	28-Mar-01	38	10900	10350	9680	10800	10200	10700					24	45
51	29-Mar-01	39	10800	10350	10400	10000	10800	10200					23	46
52	30-Mar-01	40	10000	10100	10900	10700	9720	9080		2.622	2.029	1.72E-03	23	46
55	2-Apr-01	41	11100	9753	8810	10100	9700	10400					24	46
56	3-Apr-01	42	11300	9790	9480	9640	9540	10500					25	45
57	4-Apr-01	43	11300	9800	10000	9280	9820	10100					25	46
		Mean	**10857**		**10204**					**1.523**	**2.078**	**3.37E-03**	**23.8**	**46.2**
		S.D.	**316**		**534**					**0.807**	**0.347**	**2.31E-03**	**0.8**	**1.4**

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

Chamber Monitoring Results
Cumulative Exposure Record
Group IIIB - 10000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
30	8-Mar-01	23	10800	8950	8170	8680	9700	9250					22	44
31	9-Mar-01	24	11500	10200	10500	10100	10200	10000		4.347	3.673	6.80E-03	23	44
32	11-Mar-01	25	10700	9895	9970	9640	10300	9670					23	43
34	12-Mar-01	26	10700	10250	10300	10100	10400	10200					24	43
35	13-Mar-01	27	10800	10350	10200	10400	10700	10100					24	42
36	14-Mar-01	28	10800	10270	10300	9970	10500	10300					24	40
37	15-Mar-01	29	10600	10310	11300	9720	9530	10700					24	43
38	16-Mar-01	30	11200	9863	9690	9630	10200	9930		1.264	2.447	7.44E-03	24	43
41	19-Mar-01	31	10800	10280	10600	10100	9910	10500					24	43
42	20-Mar-01	32	10800	10230	10000	9630	10900	10400					24	43
43	21-Mar-01	33	10600	10530	11200	10100	10500	10300					23	42
44	22-Mar-01	34	10900	9775	9750	9470	9580	10300					24	42
45	23-Mar-01	35	11000	10380	9800	10200	10500	11000		1.447	1.627	1.14E-03	24	39
48	26-Mar-01	36	10700	10080	10200	10200	10300	9620					23	42
49	27-Mar-01	37	10700	10050	9910	10600	9970	9710					24	39
50	28-Mar-01	38	10900	10150	9880	9870	10900	9950					24	41
51	29-Mar-01	39	10800	9905	9140	9890	10600	9990					24	42
52	30-Mar-01	40	10000	10560	10900	11000	10800	9540		1.530	1.670	1.18E-03	24	43
55	2-Apr-01	41	11100	10070	9950	9440	10500	10400					24	43
56	3-Apr-01	42	11300	10140	9810	9960	9800	11000					24	43
57	4-Apr-01	43	11300	9738	9360	9800	9790	10000					24	42
		Mean	**10857**		**10094**					**2.147**	**2.354**	**4.14E-03**	**23.7**	**42.2**
		S.D.	**316**		**526**					**1.471**	**0.957**	**3.45E-03**	**0.6**	**1.4**

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

Chamber Monitoring Results
Cumulative Exposure Record
Group IVA - 20000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration						Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual					MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)					(µm)		(mg/m^3)	(°C)	(%)
30	8-Mar-01	23	23300	20680	19400	21600	21500	20200					24	46
31	9-Mar-01	24	25400	21830	22800	22300	22000	20200		0.8242	2.202	4.64E-03	26	49
32	11-Mar-01	25	24400	21080	19700	20400	22800	21400					25	48
34	12-Mar-01	26	24000	21830	26400	20000	21300	19600					26	46
35	13-Mar-01	27	24100	20030	20900	22000	17100	20100					25	47
36	14-Mar-01	28	25100	21080	20400	21100	21300	21500					25	46
37	15-Mar-01	29	22000	21150	21000	23300	20000	20300					25	47
38	16-Mar-01	30	21700	20580	21400	20600	20300	20000		1.213	2.681	9.47E-03	25	45
41	19-Mar-01	31	22200	20850	20700	20000	20900	21800					25	46
42	20-Mar-01	32	22400	20300	18800	20300	21300	20800					26	46
43	21-Mar-01	33	21600	20450	19800	20700	21100	20200					26	47
44	22-Mar-01	34	22000	20850	21300	20800	20500	20800					26	46
45	23-Mar-01	35	22000	20900	19000	21600	21300	21700		2.536	2.616	2.08E-03	26	44
48	26-Mar-01	36	22200	20530	19200	20300	20900	21700					25	47
49	27-Mar-01	37	21900	20680	20600	21400	20400	20300					25	44
50	28-Mar-01	38	21400	20530	21000	21100	19900	20100					25	45
51	29-Mar-01	39	21900	20080	20000	20300	19200	20800					25	47
52	30-Mar-01	40	21900	20280	19900	20700	20200	20300		1.999	2.246	1.54E-03	25	46
55	2-Apr-01	41	21700	20230	19500	21100	20100	20200					25	48
56	3-Apr-01	42	21600	20780	20600	21500	20000	21000					26	46
57	4-Apr-01	43	21500	20300	20500	20200	20500	20000					26	47
		Mean	**22586**		**20712**					**1.643**	**2.436**	**4.43E-03**	**25.3**	**46.3**
		S.D.	**1249**		**1120**					**0.770**	**0.247**	**3.62E-03**	**0.6**	**1.2**

Table A Gasoline MTBE Vapor Condensate: A 13-Week Whole-Body Inhalation Toxicity Study in Rats

Chamber Monitoring Results
Cumulative Exposure Record
Group IVB - 20000 mg/m^3

Day	Date	Exposure Number	Nominal	Analytical Chamber Concentration					Particle Size Determinations			Chamber Environment Mean	
				Mean	Individual				MMAD	GSD	TMC	Temperature	Humidity
			(mg/m^3)	(mg/m^3)	(mg/m^3)				(µm)		(mg/m^3)	(°C)	(%)
30	8-Mar-01	23	23300	22050	23300	21800	22700	20800				23	47
31	9-Mar-01	24	25400	20350	20500	19900	19300	21700	0.7992	2.043	5.14E-03	25	50
32	11-Mar-01	25	24400	20180	18500	20800	21200	20200				25	49
34	12-Mar-01	26	24000	19450	19400	17900	18900	21600				25	48
35	13-Mar-01	27	24100	18530	19300	18000	16800	20000				26	47
36	14-Mar-01	28	25100	19280	23600	15100	17500	20900				26	47
37	15-Mar-01	29	22000	20600	24200	20800	18400	19000				26	48
38	16-Mar-01	30	21700	20330	20900	20300	19800	20300	0.8359	1.868	7.41E-03	26	47
41	19-Mar-01	31	22200	20350	19500	19900	21500	20500				26	47
42	20-Mar-01	32	22400	19250	18100	19200	19200	20500				25	50
43	21-Mar-01	33	21600	20800	21600	21700	20200	19700				25	48
44	22-Mar-01	34	22000	19530	18600	20100	19400	20000				24	48
45	23-Mar-01	35	22000	19530	18500	19200	20400	20000	2.364	2.249	1.93E-03	25	45
48	26-Mar-01	36	22200	20650	19700	20800	22300	19800				24	47
49	27-Mar-01	37	21900	20450	19900	21500	19900	20500				25	46
50	28-Mar-01	38	21400	20380	20200	21100	20200	20000				25	51
51	29-Mar-01	39	21900	19900	19700	18800	20600	20500				25	48
52	30-Mar-01	40	21900	20130	20000	20500	20700	19300	1.793	1.810	1.63E-03	25	48
55	2-Apr-01	41	21700	21030	21300	21100	20100	21600				26	49
56	3-Apr-01	42	21600	20250	20100	21200	20000	19700				25	49
57	4-Apr-01	43	21500	20800	20900	19900	21000	21400				25	47
		Mean	**22586**		**20185**				**1.448**	**1.993**	**4.03E-03**	**25.1**	**47.9**
		S.D.	**1249**		**1390**				**0.765**	**0.198**	**2.76E-03**	**0.8**	**1.4**

TABLE B

G MTBE VC: 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 -9	TOTAL
# OF ANIMALS EXAMINED	1	5	
	2	5	
	3	5	
	4	5	
	6	5	
NORMAL			
WITHIN NORMAL LIMITS	1	5	5
	2	5	5
	3	5	5
	4	5	5
	6	5	5

TABLE B

G MTBE VC: 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 -9	TOTAL
# OF ANIMALS EXAMINED	1	5	
	2	5	
	3	5	
	4	5	
	6	5	
NORMAL			
WITHIN NORMAL LIMITS	1	5	5
	2	5	5
	3	5	5
	4	5	5
	6	5	5

TABLE C

G MTBE VC: 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 LEVEL:		I	II	III	IV	VI
EXPOSURE LEVEL (mg/m3):		0	2,000	10,000	20,000	MICRO+CONTROL
WEEK -1	MEAN	147	147	146	146	148
	S.D.	5.2	5.8	5.3	6.5	5.8
	N	5	5	5	5	5
WEEK 0	MEAN	214	221	216	217	216
	S.D.	6.9	11.4	9.7	8.2	11.8
	N	5	5	5	5	5
WEEK 1	MEAN	265	274	272	268	272
	S.D.	12.0	16.3	12.0	13.7	15.9
	N	5	5	5	5	5
WEEK 2	MEAN	317	332	327	322	321
	S.D.	14.7	19.5	15.1	13.8	18.3
	N	5	5	5	5	5
WEEK 3	MEAN	363	377	377	363	361
	S.D.	15.6	21.7	21.8	21.3	23.9
	N	5	5	5	5	5
WEEK 4	MEAN	403	417	417	398	396
	S.D.	17.2	23.7	26.3	28.2	31.8
	N	5	5	5	5	5

No statistically significant differences

TABLE C

G MTBE VC: 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 LEVEL:	I	II	III	IV	VI
		EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000	MICRO+CONTROL
WEEK	-1	MEAN	125	126	125	126	127
		S.D.	6.7	5.2	9.1	9.0	5.3
		N	5	5	5	5	5
WEEK	0	MEAN	162	166	166	163	169
		S.D.	10.1	8.4	11.2	12.1	6.7
		N	5	5	5	5	5
WEEK	1	MEAN	184	190	194	183	197
		S.D.	13.3	3.9	7.4	11.7	9.3
		N	5	5	5	5	5
WEEK	2	MEAN	211	218	223	207	225
		S.D.	13.2	8.1	14.0	10.7	18.5
		N	5	5	5	5	5
WEEK	3	MEAN	227	239	246	222	241
		S.D.	19.7	6.1	19.1	16.8	15.9
		N	5	5	5	5	5
WEEK	4	MEAN	245	249	255	239	253
		S.D.	21.2	9.5	17.8	16.2	14.5
		N	5	5	5	5	5

No statistically significant differences

TABLE D

G MTBE VC: 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 LEVEL:	I	II	III	IV	VI
	EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000	MICRO+CONTROL
WEEK 0 TO 1	MEAN	51	52	55	50	56
	S.D.	9.8	7.5	6.7	6.6	4.5
	N	5	5	5	5	5
WEEK 0 TO 2	MEAN	103	111	111	104	105
	S.D.	13.9	11.0	14.2	8.0	7.6
	N	5	5	5	5	5
WEEK 0 TO 3	MEAN	149	156	161	145	145
	S.D.	15.1	15.4	20.8	17.1	13.2
	N	5	5	5	5	5
WEEK 0 TO 4	MEAN	189	196	200	180	181
	S.D.	16.2	15.1	26.6	24.1	20.9
	N	5	5	5	5	5

No statistically significant differences

TABLE D

G MTBE VC: 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 LEVEL:	I	II	III	IV	VI
	EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000	MICRO+CONTROL
WEEK 0 TO 1	MEAN	22	24	27	21	28
	S.D.	4.1	5.3	9.3	3.6	4.9
	N	5	5	5	5	5
WEEK 0 TO 2	MEAN	49	52	56	44	56
	S.D.	5.3	7.5	13.2	8.2	13.2
	N	5	5	5	5	5
WEEK 0 TO 3	MEAN	66	74	79	59	72
	S.D.	10.2	6.7	16.2	6.7	9.8
	N	5	5	5	5	5
WEEK 0 TO 4	MEAN	83	83	88	77	85
	S.D.	11.6	10.9	13.2	8.1	8.6
	N	5	5	5	5	5

No statistically significant differences

TABLE E

G MTBE VC: 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 LEVEL:	I	II	III	IV	VI
	EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000	MICRO+CONTROL
WEEK 0	MEAN	130	135	134	136	132
	S.D.	3.7	5.9	4.5	5.8	4.9
	N	5	5	5	5	5
WEEK 1	MEAN	105	110	107	108	112
	S.D.	4.6	3.2	5.2	4.2	4.1
	N	5	5	5	5	5
WEEK 2	MEAN	93	97	94	92	97
	S.D.	3.0	3.6	5.4	3.2	4.3
	N	5	5	5	5	4
WEEK 3	MEAN	84	86	84	81	85
	S.D.	3.2	2.4	2.9	4.1	3.9
	N	5	5	5	5	4
WEEK 4	MEAN	76	80	75	75	78
	S.D.	2.2	3.4	4.4	4.5	3.7
	N	5	5	5	5	4

No statistically significant differences

TABLE E

G MTBE VC: 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 LEVEL:	I	II	III	IV	VI
	EXPOSURE LEVEL (mg/m3):	0	2,000	10,000	20,000	MICRO+CONTROL
WEEK 0	MEAN	127	127	131	126	129
	S.D.	4.0	1.6	6.2	3.4	3.8
	N	5	5	5	4	5
WEEK 1	MEAN	103	108	103	102	109
	S.D.	2.3	4.0	5.3	3.4	3.1
	N	5	5	5	5	5
WEEK 2	MEAN	98	104	97	96	100
	S.D.	6.6	3.5	1.7	5.4	4.5
	N	5	5	4	5	5
WEEK 3	MEAN	90	96*	88	90	90
	S.D.	4.5	1.5	1.6	2.3	1.6
	N	5	5	5	5	3
WEEK 4	MEAN	88	91	81	83	85
	S.D.	3.7	5.2	5.0	6.0	2.5
	N	5	5	4	4	5

Statistical key: * = p<0.05

TABLE F

G MTBE VC: 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 - 9
1031	WITHIN NORMAL LIMITS	P
1032	WITHIN NORMAL LIMITS	P
1033	WITHIN NORMAL LIMITS	P
1034	WITHIN NORMAL LIMITS	P
1035	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 - 9
2021	WITHIN NORMAL LIMITS	P
2022	WITHIN NORMAL LIMITS	P
2023	WITHIN NORMAL LIMITS	P
2024	WITHIN NORMAL LIMITS	P
2025	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 - 9
3021	WITHIN NORMAL LIMITS	P
3022	WITHIN NORMAL LIMITS	P
3023	WITHIN NORMAL LIMITS	P
3024	WITHIN NORMAL LIMITS	P
3025	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 - 9
4031	WITHIN NORMAL LIMITS	P
4032	WITHIN NORMAL LIMITS	P
4033	WITHIN NORMAL LIMITS	P
4034	WITHIN NORMAL LIMITS	P
4035	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 VI MICRO+CONTROL

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 9
6031	WITHIN NORMAL LIMITS	P
6032	WITHIN NORMAL LIMITS	P
6033	WITHIN NORMAL LIMITS	P
6034	WITHIN NORMAL LIMITS	P
6035	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 - 9
1541	WITHIN NORMAL LIMITS	P
1542	WITHIN NORMAL LIMITS	P
1543	WITHIN NORMAL LIMITS	P
1544	WITHIN NORMAL LIMITS	P
1545	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 - 9
2531	WITHIN NORMAL LIMITS	P
2532	WITHIN NORMAL LIMITS	P
2533	WITHIN NORMAL LIMITS	P
2534	WITHIN NORMAL LIMITS	P
2535	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 - 9
3531	WITHIN NORMAL LIMITS	P
3532	WITHIN NORMAL LIMITS	P
3533	WITHIN NORMAL LIMITS	P
3534	WITHIN NORMAL LIMITS	P
3535	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 - 9
4541	WITHIN NORMAL LIMITS	P
4542	WITHIN NORMAL LIMITS	P
4543	WITHIN NORMAL LIMITS	P
4544	WITHIN NORMAL LIMITS	P
4545	WITHIN NORMAL LIMITS	P

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

TABLE F

G MTBE VC: 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 VI MICRO+CONTROL

ANIMAL#	OBSERVATIONS	DAY OF STUDY - 9
6541	WITHIN NORMAL LIMITS	P
6542	WITHIN NORMAL LIMITS	P
6543	WITHIN NORMAL LIMITS	P
6544	WITHIN NORMAL LIMITS	P
6545	WITHIN NORMAL LIMITS	P

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

TABLE G

G MTBE VC: 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

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
1031	145	213	276	336	382	424
1032	147	211	271	323	373	410
1033	139	206	245	295	342	377
1034	152	224	271	316	365	401
1035	151	217	264	317	354	404
MEAN	147	214	265	317	363	403
S.D.	5.2	6.9	12.0	14.7	15.6	17.2
N	5	5	5	5	5	5

TABLE G

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP II 2,000 mg/m3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
2021	154	226	281	348	396	433
2022	145	220	282	340	384	423
2023	143	219	261	315	349	392
2024	140	205	253	307	359	395
2025	151	236	292	350	395	445
MEAN	147	221	274	332	377	417
S.D.	5.8	11.4	16.3	19.5	21.7	23.7
N	5	5	5	5	5	5

TABLE G

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

MALES GROUP III 10,000 mg/m3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
3021	153	223	275	325	365	403
3022	144	215	261	307	355	385
3023	139	200	258	323	372	419
3024	148	222	281	334	383	422
3025	145	222	285	348	411	456
MEAN	146	216	272	327	377	417
S.D.	5.3	9.7	12.0	15.1	21.8	26.3
N	5	5	5	5	5	5

TABLE G

G MTBE VC: 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
4031	138	206	252	309	351	386
4032	155	218	270	313	340	369
4033	145	227	289	344	397	445
4034	148	222	268	324	364	392
4035	143	214	260	318	363	397
MEAN	146	217	268	322	363	398
S.D.	6.5	8.2	13.7	13.8	21.3	28.2
N	5	5	5	5	5	5

TABLE G

G MTBE VC: 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 VI MICRO+CONTROL

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
6031	143	213	271	325	368	405
6032	156	236	299	350	398	447
6033	142	207	262	305	337	366
6034	146	213	268	318	353	382
6035	150	209	260	307	346	381
MEAN	148	216	272	321	361	396
S.D.	5.8	11.8	15.9	18.3	23.9	31.8
N	5	5	5	5	5	5

TABLE G

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP I 0 mg/m3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
1541	115	148	166	194	202	221
1542	125	159	176	207	217	231
1543	126	161	186	214	232	246
1544	129	167	191	209	232	253
1545	132	176	200	230	255	276
MEAN	125	162	184	211	227	245
S.D.	6.7	10.1	13.3	13.2	19.7	21.2
N	5	5	5	5	5	5

TABLE G

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHTS (GRAMS)

FEMALES GROUP II 2,000 mg/m3

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
2531	133	177	193	225	246	246
2532	125	156	186	217	238	249
2533	119	162	187	218	239	239
2534	124	164	188	205	230	245
2535	129	171	195	225	244	264
MEAN	126	166	190	218	239	249
S.D.	5.2	8.4	3.9	8.1	6.1	9.5
N	5	5	5	5	5	5

TABLE G

G MTBE VC: 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
3531	134	175	199	230	256	267
3532	111	149	193	224	245	249
3533	131	169	190	221	238	251
3534	125	162	184	200	219	230
3535	124	177	203	238	270	277
MEAN	125	166	194	223	246	255
S.D.	9.1	11.2	7.4	14.0	19.1	17.8
N	5	5	5	5	5	5

TABLE G

G MTBE VC: 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
4541	126	161	179	211	214	238
4542	129	170	191	218	237	256
4543	112	146	172	198	205	224
4544	124	159	175	194	211	222
4545	137	178	200	214	243	256
MEAN	126	163	183	207	222	239
S.D.	9.0	12.1	11.7	10.7	16.8	16.2
N	5	5	5	5	5	5

TABLE G

G MTBE VC: 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 VI MICRO+CONTROL

	WEEK OF STUDY					
ANIMAL#	-1	0	1	2	3	4
6541	127	172	194	242	246	255
6542	134	170	204	238	252	263
6543	124	166	196	218	236	252
6544	129	177	207	231	255	266
6545	120	159	184	196	216	229
MEAN	127	169	197	225	241	253
S.D.	5.3	6.7	9.3	18.5	15.9	14.5
N	5	5	5	5	5	5

TABLE H

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP I 0 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
1031	62	122	168	211
1032	60	112	162	199
1033	39	89	136	171
1034	47	92	141	177
1035	47	100	137	187
MEAN	51	103	149	189
S.D.	9.8	13.9	15.1	16.2
N	5	5	5	5

TABLE H

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP II 2,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
2021	54	122	170	207
2022	62	120	164	203
2023	43	97	130	173
2024	47	102	154	189
2025	56	114	159	209
MEAN	52	111	156	196
S.D.	7.5	11.0	15.4	15.1
N	5	5	5	5

TABLE H

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP III 10,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
3021	52	102	142	180
3022	46	92	140	170
3023	58	123	172	219
3024	59	112	161	200
3025	63	126	189	234
MEAN	55	111	161	200
S.D.	6.7	14.2	20.8	26.6
N	5	5	5	5

TABLE H

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

MALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
4031	46	103	145	181
4032	52	95	122	151
4033	61	117	170	218
4034	46	102	142	171
4035	46	104	149	182
MEAN	50	104	145	180
S.D.	6.6	8.0	17.1	24.1
N	5	5	5	5

TABLE H

G MTBE VC: 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 VI MICRO+CONTROL

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
6031	58	112	155	192
6032	63	114	162	211
6033	55	98	130	159
6034	55	105	140	169
6035	51	98	138	172
MEAN	56	105	145	181
S.D.	4.5	7.6	13.2	20.9
N	5	5	5	5

TABLE H

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP I 0 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
1541	18	46	54	72
1542	17	49	59	72
1543	26	54	72	85
1544	24	42	65	86
1545	24	55	79	100
MEAN	22	49	66	83
S.D.	4.1	5.3	10.2	11.6
N	5	5	5	5

TABLE H

G MTBE VC: 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
2531	16	48	68	68
2532	30	61	82	94
2533	26	56	78	77
2534	24	42	66	81
2535	24	54	73	93
MEAN	24	52	74	83
S.D.	5.3	7.5	6.7	10.9
N	5	5	5	5

TABLE H

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP III 10,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
3531	24	55	80	92
3532	44	75	96	99
3533	20	52	69	82
3534	22	39	58	69
3535	26	61	94	100
MEAN	27	56	79	88
S.D.	9.3	13.2	16.2	13.2
N	5	5	5	5

TABLE H

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL BODY WEIGHT CHANGE (GRAMS)

FEMALES GROUP IV 20,000 mg/m3

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
4541	19	50	53	78
4542	21	48	67	85
4543	26	52	59	79
4544	16	34	52	63
4545	22	36	65	78
MEAN	21	44	59	77
S.D.	3.6	8.2	6.7	8.1
N	5	5	5	5

TABLE H

G MTBE VC: 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 VI MICRO+CONTROL

	WEEK OF STUDY			
ANIMAL#	0-1	0-2	0-3	0-4
6541	22	70	75	83
6542	34	68	82	93
6543	30	52	70	86
6544	31	55	78	90
6545	25	38	57	70
MEAN	28	56	72	85
S.D.	4.9	13.2	9.8	8.6
N	5	5	5	5

TABLE I

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP I 0 mg/m3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
1031	129	107	96	84	76
1032	135	111	96	88	79
1033	128	103	94	83	76
1034	132	102	92	84	73
1035	125	100	89	79	76
MEAN	130	105	93	84	76
S.D.	3.7	4.6	3.0	3.2	2.2
N	5	5	5	5	5

TABLE I

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

MALES GROUP II 2,000 mg/m3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
2021	129	107	94	83	74
2022	136	111	94	87	79
2023	133	105	96	84	81
2024	134	113	103	89	84
2025	145	112	96	87	80
MEAN	135	110	97	86	80
S.D.	5.9	3.2	3.6	2.4	3.4
N	5	5	5	5	5

TABLE I

G MTBE VC: 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
3021	138	108	93	85	81
3022	128	99	87	82	69
3023	133	113	101	88	78
3024	130	107	94	82	74
3025	138	110	96	82	72
MEAN	134	107	94	84	75
S.D.	4.5	5.2	5.4	2.9	4.4
N	5	5	5	5	5

TABLE I

G MTBE VC: 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
4031	135	110	92	83	79
4032	128	102	87	76	69
4033	143	113	92	84	79
4034	140	109	96	85	78
4035	134	105	91	78	71
MEAN	136	108	92	81	75
S.D.	5.8	4.2	3.2	4.1	4.5
N	5	5	5	5	5

TABLE I

G MTBE VC: 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 VI MICRO+CONTROL

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
6031	135	113	100	90	82
6032	134	109	91	81	75
6033	133	115	99	87	81
6034	137	115	SF	SF	SF
6035	124	106	96	83	75
MEAN	132	112	97	85	78
S.D.	4.9	4.1	4.3	3.9	3.7
N	5	5	4	4	4

SF=Spilled Feeder

TABLE I

G MTBE VC: 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
1541	131	104	107	92	90
1542	122	100	98	90	91
1543	124	103	92	84	82
1544	131	106	101	97	89
1545	126	101	91	89	86
MEAN	127	103	98	90	88
S.D.	4.0	2.3	6.6	4.5	3.7
N	5	5	5	5	5

TABLE I

G MTBE VC: A 13-WEEK WHOLE-BODY INHALATION TOXICITY STUDY
IN RATS WITH NEUROTOXICITY ASSESSMENTS AND 4-WEEK IN VIVO
GENOTOXICITY AND IMMUNOTOXICITY ASSESSMENTS

INDIVIDUAL FEED CONSUMPTION VALUES (GRAMS/KG/DAY)

FEMALES GROUP II 2,000 mg/m3

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
2531	125	109	108	98	94
2532	128	112	104	95	85
2533	129	110	105	98	98
2534	128	106	103	95	87
2535	126	102	99	94	92
MEAN	127	108	104	96	91
S.D.	1.6	4.0	3.5	1.5	5.2
N	5	5	5	5	5

TABLE I

G MTBE VC: 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
3531	128	97	CF	87	CF
3532	139	111	99	89	82
3533	124	100	99	86	84
3534	126	102	95	90	85
3535	136	107	97	89	74
MEAN	131	103	97	88	81
S.D.	6.2	5.3	1.7	1.6	5.0
N	5	5	4	5	4

CF=Contaminated Feeder

TABLE I

G MTBE VC: 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
4541	SF	103	104	88	SF
4542	128	103	98	92	85
4543	128	107	97	93	91
4544	121	101	89	88	79
4545	128	98	94	90	77
MEAN	126	102	96	90	83
S.D.	3.4	3.4	5.4	2.3	6.0
N	4	5	5	5	4

SF=Spilled Feeder

TABLE I

G MTBE VC: 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 VI MICRO+CONTROL

	WEEK OF STUDY				
ANIMAL#	0	1	2	3	4
6541	131	104	106	SF	84
6542	125	111	103	89	87
6543	134	111	95	SF	86
6544	128	107	96	89	81
6545	125	111	98	91	87
MEAN	129	109	100	90	85
S.D.	3.8	3.1	4.5	1.6	2.5
N	5	5	5	3	5

SF=Spilled Feeder

TABLE J

G MTBE VC: 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
1031	TERMINAL SACRIFICE	5-APR-01	4	28
1032	TERMINAL SACRIFICE	5-APR-01	4	28
1033	TERMINAL SACRIFICE	5-APR-01	4	28
1034	TERMINAL SACRIFICE	5-APR-01	4	28
1035	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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
2021	TERMINAL SACRIFICE	5-APR-01	4	28
2022	TERMINAL SACRIFICE	5-APR-01	4	28
2023	TERMINAL SACRIFICE	5-APR-01	4	28
2024	TERMINAL SACRIFICE	5-APR-01	4	28
2025	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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
3021	TERMINAL SACRIFICE	5-APR-01	4	28
3022	TERMINAL SACRIFICE	5-APR-01	4	28
3023	TERMINAL SACRIFICE	5-APR-01	4	28
3024	TERMINAL SACRIFICE	5-APR-01	4	28
3025	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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
4031	TERMINAL SACRIFICE	5-APR-01	4	28
4032	TERMINAL SACRIFICE	5-APR-01	4	28
4033	TERMINAL SACRIFICE	5-APR-01	4	28
4034	TERMINAL SACRIFICE	5-APR-01	4	28
4035	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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 VI MICRO+CONTROL

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
6031	TERMINAL SACRIFICE	5-APR-01	4	28
6032	TERMINAL SACRIFICE	5-APR-01	4	28
6033	TERMINAL SACRIFICE	5-APR-01	4	28
6034	TERMINAL SACRIFICE	5-APR-01	4	28
6035	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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
1541	TERMINAL SACRIFICE	5-APR-01	4	28
1542	TERMINAL SACRIFICE	5-APR-01	4	28
1543	TERMINAL SACRIFICE	5-APR-01	4	28
1544	TERMINAL SACRIFICE	5-APR-01	4	28
1545	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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
2531	TERMINAL SACRIFICE	5-APR-01	4	28
2532	TERMINAL SACRIFICE	5-APR-01	4	28
2533	TERMINAL SACRIFICE	5-APR-01	4	28
2534	TERMINAL SACRIFICE	5-APR-01	4	28
2535	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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
3531	TERMINAL SACRIFICE	5-APR-01	4	28
3532	TERMINAL SACRIFICE	5-APR-01	4	28
3533	TERMINAL SACRIFICE	5-APR-01	4	28
3534	TERMINAL SACRIFICE	5-APR-01	4	28
3535	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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
4541	TERMINAL SACRIFICE	5-APR-01	4	28
4542	TERMINAL SACRIFICE	5-APR-01	4	28
4543	TERMINAL SACRIFICE	5-APR-01	4	28
4544	TERMINAL SACRIFICE	5-APR-01	4	28
4545	TERMINAL SACRIFICE	5-APR-01	4	28

TABLE J

G MTBE VC: 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 VI MICRO+CONTROL

ANIMAL#	TYPE OF DEATH	DATE OF DEATH	WEEK OF STUDY	STUDY DAY
6541	TERMINAL SACRIFICE	5-APR-01	4	28
6542	TERMINAL SACRIFICE	5-APR-01	4	28
6543	TERMINAL SACRIFICE	5-APR-01	4	28
6544	TERMINAL SACRIFICE	5-APR-01	4	28
6545	TERMINAL SACRIFICE	5-APR-01	4	28

FINAL REPORT

Study Title

In Vivo-In Vitro Rat Peripheral Lymphocyte Sister Chromatid Exchange Assay

Test Substance

Gasoline MTBE Vapor Condensate

Author

Ramadevi Gudi, Ph.D.

Study Completion Date

21 June 2007

Performing Laboratory

BioReliance
9630 Medical Center Drive
Rockville, Maryland 20850

Laboratory Study Number

AA40NV.130.BTL

Subcontractor's Sponsor Project Number

00-6126

Subcontractor's Sponsor

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