ROBUST SUMMARIES OF STUDIES USED TO CHARACTERIZE THE PETROLEUM GASES CATEGORY

Submitted to the USEPA

by

The Petroleum HPV Testing Group

www.petroleumhpv.org

Consortium Registration # 1100997

October 19, 2009

Robust Summaries Of Studies Used To Characterize the Petroleum Hydrocarbon Gases Category
Table of Contents

* within each health effects endpoint, the robust study summaries are presented in CASRN order for the substances as follows:

71-43-2	Benzene
106-97-8	Butane
74-84-0	Ethane
75-28-5	Isobutane (for C1 – C4 hydrocarbon fraction)
78-78-4	Isopentane
106-98-9	1-Butene (for C1 – C4 hydrocarbon fraction)
106-99-0	1,3-Butadiene
107-01-7	2-Butene (for C1 – C4 hydrocarbon fraction)
124-38-9	Carbon dioxide
1333-74-0	Hydrogen
No CASRN	light naphtha (gasoline) stream (for C5 – C6 hydrocarbon fraction
7727-37-9	Nitrogen
109-66-0	Pentane
74-98-6	Propane

Physico-Chemical Data

PHYSICAL-CHEMICAL SIDS MELTING POINT	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
RESULTS	
Melting Indicator :	
Melting Point Input type :	

Melting Point Range : Temperature:

Results Remarks :

Melting point values for major and minor constituents in Petroleum Hydrocarbon Gases cited in referenced literature sources.

Constituent	MP, °C	Reference
C1-C4 Hydrocarbons		
Methane	-164	Lide (1990-1991)
Ethane	-88.6	Lide (1990-1991)
Ethylene	-102.4	Lide (1990-1991)
Propane	-42.1	Lide (1990-1991)
Propylene	-48	O'Neil, 2001
n-butane	-0.5	Lide (1990-1991)
Isobutane	-11.7	Lide (1990-1991)
1,3-butadiene	-4.5	Budavari (1996)
Isobutylene	-6.9	Lide (1998-1999)

C5-C6 Hydrocarbons

C5 and C6 hydrocarbons of various classes (alkanes, alkenes, naphthenes, aromatics) and isomeric structures have melting points that range from -169°C (isopentene) to 6.5°C (cyclohexane) (Lide, 1998-1999; Budavari, 1996). The melting point of benzene is 5.5°C (Budavari, 1996).

Inorganic Gases		
Hydrogen	-252.8	Budavari (1996)
Nitrogen	-196	Budavari (1996)
Carbon dioxide	-78.5	Lide (1994)

STUDY / METHOD	
Key Study Sponsor Indicator :	
Year Study Performed :	
Method / Guideline Followed :	
Method / Guideline and Test Condition Remarks:	
GLP :	
Study Reference :	Lide, D.R. (ed.). 1990-1991. CRC Handbook of Chemistry and Physics. 71st ed. CRC Press Inc., Boca Raton, FL. Lide, D.R. (ed.). 1998-1999. CRC Handbook of Chemistry and Physics. 79th ed. CRC Press Inc., Boca Raton, FL. Lide, D.R., and G.W.A. Milne (eds.). 1994. Handbook of Data on Organic Compounds. Volume I. 3rd ed. CRC Press, Inc. Boca Raton ,FL. p. V3: 2660. O'Neil, M.J. (ed.). 2001. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Merck and Co., Inc. Whitehouse Station, NJ. Budavari, S. (ed.). 1996. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck and Co., Inc., Whitehouse Station, NJ.
RELIABILITY / DATA QUALITY	
Reliability :	2
Reliability Remarks :	This data compendium utilized, to the extent possible, peer reviewed journal articles and reference databases to

	characterize the endpoint values for constituents in Petroleum Hydrocarbon gases.

PHYS CHEM **BOILING POINT**	
Category Chemical:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type:	See Test Plan and Category Analysis
Test Substance Result Type:	
RESULTS	
Boiling Indicator:	
Boiling Point Input type:	Value or Range:

Boiling Point Range:	Temperature: Pressure:
Results Remarks:	Boiling point values for major and minor constituents in Petroleum Hydrocarbon Gases cited in referenced literature sources. **Constituent MP, °C Reference** **C1-C4 Hydrocarbons** Methane -164 Lide (1990-1991) Ethane -88.6 Lide (1990-1991) Ethylene -102.4 Lide (1990-1991) Propane -42.1 Lide (1990-1991) Propylene -48 O'Neil, 2001 n-butane -0.5 Lide (1990-1991) Isobutane -11.7 Lide (1990-1991) 1,3-butadiene -4.5 Budavari (1996) Isobutylene -6.9 Lide (1998-1999) **C5-C6 hydrocarbons** C5 and C6 hydrocarbons of various classes (alkanes, alkenes, naphthenes, aromatics) and isomeric structures have boiling points that range from 20°C (isopentene) to 81°C (cyclohexane) (Lide, 1998-1999; Budavari, 1996). The boiling point of benzene is 80.1°C (Budavari, 1996). **Inorganic Gases** Hydrogen -252.8 Budavari (1996) Nitrogen -196 Budavari (1996) Carbon dioxide -78.5 Lide (1994)

STUDY / METHOD	
Key Study Sponsor Indicator:	
Year Study Performed:	
Method / Guideline Followed:	
Method / Guideline and Test Condition Remarks:	
GLP:	
Study Reference:	Lide, D.R. (ed.). 1990-1991. CRC Handbook of Chemistry and Physics. 71st ed. CRC Press Inc., Boca Raton, FL. Lide, D.R. (ed.). 1998-1999. CRC Handbook of Chemistry and Physics. 79th ed. CRC Press Inc., Boca Raton, FL. Lide, D.R., and G.W.A. Milne (eds.). 1994. Handbook of Data on Organic Compounds. Volume I. 3rd ed. CRC Press, Inc. Boca Raton ,FL. p. V3: 2660. O'Neil, M.J. (ed.). 2001. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. 13th Edition, Merck and Co., Inc. Whitehouse Station, NJ. Budavari, S. (ed.). 1996. The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck and Co., Inc., Whitehouse Station, NJ.
RELIABILITY / DATA QUALITY	
Reliability:	2

Reliability Remarks:	This data compendium utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in Petroleum Hydrocarbon gases.

PHYSICAL-CHEMICAL SIDS VAPOR PRESSURE	
Category Chemical:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type:	See Test Plan and Category Analysis
Test Substance Result Type:	
RESULTS	
Vapor Pressure Input type:	Value or Range?
Vapor Pressure Value : Pressure: **@ Temperature**: 25°C	

Results Remarks:	Vapor Pressure values for major and minor constituents in Petroleum Hydrocarbon Gases cited in referenced literature sources.

Constituent	VP, hPa @25°C	Reference
C1-C4 Hydrocarbons		
Methane	621,282	Daubert and Danner, 1989
Ethane	41,942	Daubert and Danner, 1989
Ethylene	69,461	Daubert and Danner, 1989
Propane	9,533	Daubert and Danner, 1989
Propylene	11,586	Daubert and Danner, 1989
n-butane	2,426	Riddick et al., 1985
Isobutane	3,481	Riddick et al., 1985
1,3-butadiene	2,813	Daubert and Danner, 1989
Isobutylene	3,077	Yaws, 1994

Other hydrocarbons:
C5 and C6 hydrocarbons of various classes (alkanes, alkenes, naphthenes, aromatics) and isomeric structures have vapor pressures that range from 126 hPa (benzene) to 1204 hPa (isopentene) (Daubert and Danner, 1989; Yaws, 1994). The vapor pressure of benzene is 126 hPa (Daubert and Danner, 1989).

Inorganic Gases		
Hydrogen	1,653,198	Ohe (1976)
Nitrogen	1,013[1]	Weast (1984)
Carbon dioxide	64,395	Dauber and Danner (1989)

[1] Value reported for a temperature of -196°C

STUDY / METHOD	
Key Study Sponsor Indicator:	
Year Study Performed:	
Method / Guideline Followed:	
Method / Guideline and Test Condition Remarks:	
GLP:	
Study Reference:	Daubert, T.E., and R.P. Danner. 1989. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Taylor and Francis, Washington, D.C. Yaws, C.L. 1994. Handbook of Vapor Pressure, Vol 1 C1-C4 Compounds. Gulf Pub Co., Houston, TX. Riddick, J.A., W.B. Bunger, and T.K. Sakano. 1985. Techniques of Chemistry 4th ed., Volume II. Organic Solvents. John Wiley and Sons, New York, NY. Weast, R.C. (ed.). 1984 – 1985. Handbook of Chemistry and Physics. 65th ed. CRC Press, Inc. Boca Raton, FL., p. D-219. [Cited in: NLM (U.S. National Library of Medicine), Hazardous Substance Data Bank. Available through the TOXNET Toxicology Data Network, http://toxnet.nlm.nih.gov] Ohe S. 1976. Computer Aided Data Book of Vapor Pressure. Data Book Publ. Co, Tokyo, Japan. [cited in NLM (U.S. National Library of Medicine), Hazardous Substance Data Bank. Available through the TOXNET Toxicology Data Network, http://toxnet.nlm.nih.gov]
RELIABILITY / DATA QUALITY	

Reliability:	2
Reliability Remarks:	This data compendium utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in Petroleum Hydrocarbon gases.

PHYSICAL-CHEMICAL SIDS PARTITION COEFFICIENT	
Category Chemical:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type:	See Test Plan and Category Analysis
Test Substance Result Type:	
RESULTS	
Partition Coefficient Input type:	Value or Range?
Partition Coefficient Range : Log K_{ow}: **@ Temperature**:	

Results Remarks:	Partition coefficient values for major and minor constituents in Hydrocarabon Gases cited in referenced literature sources. **Constituents: Log Kow Reference** **C1-C4 Hydrocarbons** Methane 1.09 Hansch, et al. 1995 Ethane 1.81 Hansch, et al. 1995 Ethylene 1.13 Hansch, et al. 1995 Propane 2.36 Hansch, et al. 1995 Propylene 1.77 Hansch, et al. 1995 n-butane 2.89 SRI International, 2000 Isobutane 2.76 Hansch, et al. 1995 1,3-butadiene 1.99 Hansch, et al. 1995 Isobutylene 2.34 Hansch, et al. 1995 **Other hydrocarbons:** C5 and C6 hydrocarbons of various classes (alkanes, alkenes, naphthenes) and isomeric structures have partition coefficients (Log Kow) that range from 2.13 (benzene) to 3.9 (hexane) (Hansch et al., 1995). **Inorganic Gases** Hydrogen N/A Nitrogen 0.67 Hansch et al. (1995) Carbon dioxide 0.83 US EPA, 2000
STUDY / METHOD	

Key Study Sponsor Indicator:	
Year Study Performed:	
Method/Guideline Followed:	
Method/Guideline and Test Condition Remarks:	
GLP:	
Study Reference:	EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC. Version 3.11. Hansch, C., A. Leo, and D. Hoekman. 1995. Exploring QSAR - Hydrophobic, Electronic, and Steric Constants. American Chemical Society, Washington, DC, p. 3. SRI International. 2000. Directory of Chemical Producers -- United States. SRI Consulting, Menlo Park: CA.
RELIABILITY/DATA QUALITY	
Reliability:	2
Reliability Remarks:	This data compendium utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in Petroleum Hydrocarbon gases.

PHYS CHEM **WATER SOLUBILITY**	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
RESULTS	
Water Solubility Indicator :	
Water Solubility Input type:	Value or Range?

Water Solubility Range : Solubility: @ **Temperature**:	
pH Value :	**Value or Lower Range**: **Upper Range** :
pKa - Protein Kinase:	
pH Value at Saturation :	
Results Remarks :	Water solubility values for major and minor constituents in Petroleum Hydrocarbon Gases cited in referenced literature sources. **Water Solubility** **Constituents:** / **mg/L @25°C** / **Reference** **C1-C4 Hydrocarbons** Methane 22 Yalkowsky and Yan, 2000 Ethane 60.2 McAuliffe, 1966 Ethylene 131 McAuliffe, 1966 Propane 62.4 Yalkowsky and Yan, 2003 Propylene 200 McAuliffe, 1966 n-butane 61.2 McAuliffe, 1966 Isobutane 48.9 McAuliffe, 1966 1,3-butadiene 735[1] McAuliffe, 1966 Isobutylene 263 Yalkowsky and Dannenfelser, 1992

	[1] Value reported for a temperature of 20°C **Other hydrocarbons** C5 and C6 hydrocarbons of various classes (alkanes, alkenes, naphthenes) and isomeric structures have water solubility values that range from 9.5 mg/L (hexane) to 1790 mg/L (benzene) (McAullife, 1966; May et al., 1983). **Inorganic Gases** Hydrogen 1.62 Venable and Fuwa (1922) Nitrogen 18,100 US EPA (2000) Carbon dioxide 1,480 US EPA (2000)
STUDY / METHOD	
Key Study Sponsor Indicator :	
Year Study Performed :	
Method / Guideline Followed :	
Method / Guideline and Test Condition Remarks:	
GLP :	

Study Reference :	Venable C.S., and T. Fuwa. 1922. The solubility of gases in rubber and rubber stock and effect of solubility on penetrability. Ind Eng Chem 14: 139-42. U.S. EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC. Yalkowsky, S.H., and He Yan. 2003. Handbook of Aqueous Solubility Data: An Extensive Compilation of Aqueous Solubility Data for Organic Compounds Extracted from the AQUASOL dATAbASE. CRC Press LLC, Boca Raton, FL. McAuliffe C. 1966. Solubility in water of paraffin, cycloparaffin, olefin, acetylene, cyclo-olefin and aromatic hydrocarbons. J. Phys. Chem. 70(4): 1267-75. Yalkowsky S.H. and R.M. Dannenfelser. 1992. The AQUASOL dATAbASE of Aqueous Solubility. Fifth Ed, Univ Az, College of Pharmacy, Tucson, AZ.
RELIABILITY/DATA QUALITY	
Reliability :	2
Reliability Remarks :	This data compendium utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in Petroleum Hydrocarbon gases.

FATE SIDS PHOTODEGRADATION	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
RESULTS	
Photodegradation Result Description:	

Photodegradation Input type :	
Photodegradation Range :	
Half Life :	
Rate Constant :	
Photo Medium :	
Temperature :	
Sensitizer :	
Sensitizer Concentration and Units :	
Light Source :	
Light Source Spectrum :	
UV/VIS Absorption Spectrum :	
Quantum Yield :	
Breakdown Products Description :	
Results Remarks :	The tendency for constituent substances making up hydrocarbon gases to undergo a gas-phase oxidation reaction with photochemically produced hydroxyl radicals were described below using either

	the AOP subroutine in EPI Suite™ (EPA, 2000) or from published literature data. **Gas Constituent** | **$T_{1/2}$, day** | **Comment and/or Reference** C1-C4 Hydrocarbons | 0.16 to 960 | Values for butadiene and methane, respectively. AOPWIN model in EPI-Suite™ EPA (2000) C5-C6 Hydrocarbons | 0.2 to 5.5 | Values for isopentene and benzene, respectively. AOPWIN model in EPI-Suite™ EPA (2000) Hydrogen | N/A | Non-reactive in troposphere (Boikess and Edelson, 1978) Nitrogen | N/A | Non-reactive in troposphere (Sawyer and McCarty, 1978) Carbon dioxide | N/A | Non-reactive in troposphere (Boikess and Edelson, 1978)
STUDY/METHOD	
Key Study Sponsor Indicator :	
Year Study Performed :	
Method/Guideline Followed :	
Deviations from Method/Guideline :	
Method/Guideline Description :	
Method/Guideline and Test Condition Remarks :	
GLP :	
Study Reference :	Boikess, R.S., and E. Edelson. 1978. Chemical Principles. Harper and Row Publishers, New York, New

	York. 742 p. EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC. Sawyer, C.N., and P.L. McCarty. 1978. Chemistry for Environmental Engineering, 3rd edition. McGraw-Hill Book Company, New York, New York. 532 p.
RELIABILITY/DATA QUALITY	
Reliability :	2
Reliability Remarks :	This data compendium utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in Petroleum Hydrocarbon gases.

FATE SIDS STABILITY IN WATER	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
RESULTS	
Stability in Water Result Description :	Stable
Stability in Water Input type :	

Stability in Water Value : @	
pH Value :	Value or Lower Range: Upper Range :
Hydrolysis Indicator :	
Preliminary Test :	
Stability in Water pH Values	**Half Life** : / **@ pH Value** :
Breakdown Products Description :	
Results Remarks :	Compound types that are known to hydrolyze include alkylhalides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982). The hydrocarbon and non-hydrocarbon constituents in Hydrocarbon Gases do not contain the functional groups or chemical linkages known to undergo hydrolysis reactions. Therefore hydrolysis will not play an important role in the

	environmental fate for the components in Petroleum Hydrocarbon Gas streams.
STUDY/METHOD	
Key Study Sponsor Indicator :	
Year Study Performed :	
Method/Guideline Followed :	
Deviations from Method/Guideline :	
Method/Guideline Description :	
Method/Guideline and Test Condition Remarks :	
GLP :	
Study Reference :	Harris, J.C. 1982. Rate of Hydrolysis. Chapter 7 in: W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt, eds. Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co., NY.
RELIABILITY/DATA QUALITY	
Reliability :	2
Reliability Remarks :	This technical discussion utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in Petroleum Hydrocarbon gases.

FATE SIDS TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS (FUGACITY)	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
Results	
Fugacity/Distribution Result Description:	Multimedia (Fugacity) Modeling

Test Results :	**Hydrocarbon Gas Constituent** — **PERCENT DISTRIBUTION**: **Air** / **Water** / **Soil** / **Sediment** / **Suspended Sediment** / **Biota** C1-C4 alkanes: 100 / <0.1 / <0.1 / <0.1 / <0.1 / <0.1 C5-C6 alkanes: >99.8 / <0.1 / <0.2 / <0.1 / <0.1 / <0.1
Temperature :	
Level of Multi-media Model :	Level 1
Model Input (Water Solubility:)	
Model Input (Vapor Pressure:)	
Model Input (log K_{ow}:)	
Model Input (Melting Point:)	
Henry's Law Constant :	
Model Concentration -- Air :	
Model Concentration -- Water :	
Model Concentration -- Soil :	
Model Concentration -- Sediment :	
Results Remarks :	Equilibrium models can provide information on where a chemical is likely to partition in the environment. These data are

	useful in identifying environmental compartments that could potentially receive a released chemical. A widely used fugacity model is the EQC (Equilibrium Criterion) model (Mackay et al., 1997). In its guidance document for HPV data development, the U.S. EPA states that it accepts Level I fugacity data as an estimate of chemical distribution values. The EQC model is a Level I model that describes the equilibrium distribution of a fixed quantity of conserved (i.e., non-reacting) chemical at steady state within a closed environment with assumed volumes of air, water, soil and sediment. The model assumes the chemical becomes instantaneously distributed to an equilibrium condition using physical-chemical properties to quantify the chemical's behavior. The model does not include degrading reactions, advective processes or inter-media transport between compartments. Results of Level I models are basic partitioning data that allow for comparisons between chemicals and indicate the compartment(s) to which a chemical is likely to partition in the environment. The gases in greatest proportion in the Petroleum Hydrocarbon Gases streams (H_2, N_2, CO_2) typically have very low boiling points. These substances exist as gases at most ambient environmental temperatures. While all of the non-hydrocarbon constituents would be expected to partition to the atmosphere, some have high levels of water solubility and may cause adverse effects on aquatic organisms. Therefore, these substances (hydrogen sulfide, methanethiol, ethanethiol, and ammonia) were assessed for their environmental distribution using the Mackay et al. (1997) EQC model. Hydrocarbon gases (C1 to C4) along with benzene and 1,3-butadiene also were assessed for their potential environmental distribution.
Study / Method	
Key Study Sponsor Indicator :	
Year Study Performed :	
Method/Guideline Followed :	EQC model
Deviations from Method/Guideline :	

Method/Guideline Description :	
Method/Guideline and Test Condition Remarks :	
GLP :	
Study Reference :	Mackay, D., DiGuardo, A. Paterson, S., and Cowan, C. 1997. EQC Model, Version. 1.01, 1997, available from the Environmental Modelling Centre, Trent University, Canada.
Reliability / Data Quality	
Reliability :	2
Reliability Remarks :	This technical discussion utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in hydrocarbon gases.

FATE SIDS BIODEGRADATION	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type :	
Results	
Biodegradability Indicator :	

Concentration Value :	Time in Days :	Biodegradation Value :	Biodegradation Value Range :

			-
			-
			-
			-
			-

Half Life :	
Rate Constant :	
Temperature :	
Incubation Condition :	
Inoculum Type :	
Inoculum Concentration :	
Inoculum Remarks :	

Pre-Exposure Indicator :	
Pre-Exposure Remarks :	
Theoretical Carbon Dioxide :	
Theoretical Oxygen Demand :	
Chemical Oxygen Demand :	
Control Substance Remarks :	
Breakdown Products Description :	
Results Remarks :	Some of the non-hydrocarbon fraction of the Petroleum Hydrocarbon Gases would not be expected to biologically degrade as these substances do not contain the chemical linkages necessary for microbial metabolism. For this reason, hydrogen, nitrogen, and carbon dioxide would not be susceptible to biodegradation. Furthermore, carbon dioxide is the final product in the biological mineralization of organic compounds. Biodegradation of the hydrocarbon components in hydrocarbon gases may occur in soil and water. Gaseous hydrocarbons are widespread in nature and numerous types of microbes have evolved which are capable of oxidizing these substances as their sole energy source (Fuerst and Stephens, 1970; Stephens et al, 1971; O'Brien and Brown, 1967). Vestal (1984) noted that use of gaseous carbon sources for microbial cell growth is common among autotrophic organisms. While volatilization is the predominant behavior for the C1-C4 hydrocarbon gases, biodegradation may occur but would likely not be an important factor in environmental releases. Higher chain length hydrocarbons typical of naphtha streams also are known to inherently biodegrade in the environment (API, 2008).
Study / Method	
Key Study Sponsor Indicator :	

Year Study Performed :	
Method/Guideline Followed :	
Deviations from Method/Guideline :	
Method/Guideline Description :	
Method/Guideline and Test Condition Remarks :	
GLP :	
Study Reference :	Fuerst, R. and S. Stephens. 1970. Studies of Effects of Gases and Gamma Irradiation on *Neurospora crassa*. Dev. Ind. Microbiol. 11:301-310. Stephens, S., C. De Sha, and R. Fuerst. 1971. Phenotypic and Genetic Effects in *Neurospora crassa* Produced by Selected Gases and Gases Mixed with Oxygen. Dev. Ind. Microbiol. 12:346-353. O'Brien, W.E., and L.R. Brown. 1967. The Catabolism of Isobutane and other Alkanes by a Member of the Genus Mycobacterium. Dev. Ind. Microbiol. 9:389-393. Vestal, J.R. 1984. The Metabolism of Gaseous Hydrocarbons by Microorganisms. In, Petroleum Microbiology, R. M. Atlas, ed., MacMillan Publishing Co., New York, NY. API (American Petroleum Institute). 2008. Gasoline Blending Streams Category Assessment Document. American Petroleum Institute, Washington, DC.
Reliability / Data Quality	
Reliability :	2

Reliability Remarks :	This technical discussion utilized, to the extent possible, peer reviewed journal articles and reference databases to characterize the endpoint values for constituents in hydrocarbon gases.

ECOTOXICITY

Acute Toxicity to Aquatic Vertebrates	
Test Substance	
Category Name: Petroleum Hydrocarbon Gases Category	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments :	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type:	
Method	

Year Study Performed :	
Method / Guideline Followed:	
Deviations from Method / Guideline :	
Species:	
GLP:	
Analytical Monitoring :	
Test Type:	
Test Vessel:	
Water Media Type:	
Test Concentrations:	
Nominal and Measured Concentrations:	
Total Exposure Period:	
Vehicle Used:	

Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	
pH Value:	Value or Lower Range : Upper Range :
Test Temperature and Units:	Value or Lower Range : Upper Range :
Photo (Light/Dark):	
Salinity:	
TOC:	
Water Hardness:	Value or Lower Range: Upper Range:

Method/Guideline Test Conditions Remarks:	
Limit Test:	

Test Results

NOEC / LOEC / NOELR / LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:							
LOEC:							
NOELR:							

LC / EC / IC / EL / LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:

Results Remarks:	Petroleum hydrocarbon gases exist in the gaseous phase and are contained in closed systems at the refinery. Inadvertent release of these substances would result in the individual components partitioning to the air. They would not likely enter the aquatic environment unless a direct release to that environment occurred. Even then, the residence time of any dissolved gas would be expected to be short and exposures to aquatic organisms may not persist for a long enough duration to elicit toxic effects. To fulfill the objective of defining the hazard of these gaseous mixtures, a literature review was conducted to identify aquatic hazard data for the predominant constituents in hydrocarbon gases. Some constituents, namely N_2, H_2, and CO_2 were either not known to elicit direct toxic effects to aquatic organisms or no empirical data were found that described their hazard. Upon direct release to aquatic environments, these gases may result in oxygen displacement and thus would act as asphyxiants. Aquatic toxicity data for these substances are tabulated and presented below.

Hydrocarbon Gas Constituent	Range of Toxicity Values (LC50, mg/L)	Reference
C1-C4 Hydrocarbons[1]	6.3 – 137	EPA (2000)
C5-C6 Hydrocarbons[1]	1.0 – 18	EPA (2000)
Benzene[2]	5.3 – 35.7	DeGraeve, et al. (1982); Brooke (1987)

[1] Range of endpoint values for fish as determined using ECOSAR model (EPA, 2000).
[2] Ecotoxicity endpoint values for benzene are empirical data cited in the referenced sources.

Reliability / Data Quality	
Reliability:	2
Reliability Remarks:	The data cited used peer reviewed journal articles and accepted structure activity relationships to characterize the endpoint values for constituents in petroleum hydrocarbon gases.
Key Study Sponsor Indicator:	

Reference

Reference:	DeGraeve, G.M., R.G. Elder, D.C. Woods, and H.L. Bergman. 1982. Effects of naphthalene and benzene on fathead minnows and rainbow trout. Arch. Environ. Contam. Toxicol. 11(4):487-490. Brooke, L. 1987. Acute test comparisons with fathead minnows and acute tests with an Amphipod and a Cladoceran. Center for Lake Superior Environ. Stud., Univ. of Wisconsin-Superior, Superior, WI, 24 p. API (American Petroleum Institute). 2001. Petroleum Gases Test Plant. API, Washington, DC. EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC.

Acute Toxicity to Aquatic Invertebrates	
Test Substance	
Category Name: Petroleum Hydrocarbon Gases Category	
Category Chemical:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance:	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments:	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type:	See Test Plan and Category Analysis
Test Substance Result Type:	

Method	
Year Study Performed:	
Method / Guideline Followed:	
Deviations from Method / Guideline :	
Species:	
GLP:	
Analytical Monitoring :	
Test Type:	
Test Vessel:	
Water Media Type:	
Test Concentrations:	

Nominal and Measured Concentrations:	
Total Exposure Period:	

Vehicle Used:	
Vehicle Name:	
Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	
pH Value:	Value or Lower Range : Upper Range :
Test Temperature and Units:	Value or Lower Range Upper Range :
Photo (Light/Dark):	
Salinity:	
TOC:	

Water Hardness:	Value or Lower Range: Upper Range:

Method / Guideline Test Conditions Remarks:	
Limit Test:	

Test Results

NOEC / LOEC / NOELR / LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:							
LOEC:							
NOELR:							
LOELR:							

LC / EC / IC / EL / LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:

Results Remarks:

Petroleum Hydrocarbon gases exist in the gaseous phase and are contained in closed systems at the refinery. Inadvertent release of these substances would result in the individual components partitioning to the air. They would not likely enter the aquatic environment unless a direct release to that environment occurred. Even then, the residence time of any dissolved gas would be expected to be short and exposures to aquatic organisms may not persist for a long enough duration to elicit toxic effects. To fulfill the objective of defining the hazard of these gaseous mixtures, a literature review was conducted to identify aquatic hazard data for the predominant constituents in hydrocarbon gases. Some constituents, namely N_2, H_2, and CO_2 were either not known to elicit direct toxic effects to aquatic organisms or no empirical data were found that described their hazard. Upon direct release to aquatic environments, these gases may result in oxygen displacement and thus would act as asphyxiants.

Aquatic toxicity data for these substances are tabulated and presented below.

Hydrocarbon Gas Consituent	Range of Toxicity Values (EC50, mg/L)	Reference
C1-C4 Hydrocarbons[1]	7.2 - 138	EPA (2000)
C5-C6 Hydrocarbons[1]	1.3 – 20	EPA (2000)
Benzene[2]	59.6 – 682	MacLean et al. (1989); Eastmond et al. (1984)

[1] Range of endpoint values for fish as determined using ECOSAR model (EPA, 2000).
[2] Ecotoxicity endpoint values for benzene are empirical data cited in the referenced sources.

Reliability / Data Quality

Reliability:	2
Reliability Remarks:	The data cited used peer reviewed journal articles and accepted structure activity relationships to characterize the endpoint values for constituents in petroleum hydrocarbon gases.
Key Study Sponsor Indicator:	
Reference	
Reference:	MacLean, M.M. and K.G. Doe. 1989. The comparative toxicity of crude and refined oils to Daphnia magna and Artemia. Environment Canada, EE-111, Dartmouth, Nova Scotia. 64 p. Eastmond, D.A., G.M. Booth, and M.L. Lee. 1984. Toxicity, accumulation, and elimination of polycyclic aromatic sulfur heterocycles in Daphnia magna. Arch. Environ. Contam. Toxicol. 13(1):105-111. API (American Petroleum Institute). 2001. Petroleum Gases Test Plant. API, Washington, DC. EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S. Environmental Protection Agency, Washington, DC.

Acute Toxicity to Aquatic Plants	
Category Name:	
Category Chemical :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance :	Petroleum Hydrocarbon Gases, multiple CAS numbers
Test Substance Purity/Composition and Other Test Substance Comments :	Major and minor constituents comprising the CAS numbers in the Petroleum Hydrocarbon Gases Category: C1-C4 hydrocarbons representing alkane and alkene structures (methane, ethane, ethylene, propane, propylene, n-butane, isobutane, 1,3-butadiene, isobutylene. C5-C6 hydrocarbons representing alkane, cycloalkane, alkene, cycloalkane, and aromatic structures (n-pentane, isopentane, cyclopentane, isopentene, cyclopentene, hexane, isohexane, cyclohexane, and benzene) Inorganic gases hydrogen, nitrogen, and carbon dioxide (H_2, N_2, CO_2).
Category Chemical Result Type :	See Test Plan and Category Analysis
Test Substance Result Type:	
Method	
Year Study Performed :	

Method / Guideline Followed:	
Deviations from Method / Guideline :	
Species:	
GLP:	
Analytical Monitoring :	
Test Type:	
Test Vessel:	
Water Media Type:	
Test Concentrations:	
Nominal and Measured Concentrations:	
Total Exposure Period:	

Vehicle Used:
Vehicle Name:

Vehicle Amount and Units:	
Alkalinity:	
Dissolved Oxygen:	
pH Value:	Value or Lower Range : Upper Range :
Test Temperature and Units:	Value or Lower Range : Upper Range :
Photo (Light/Dark):	
Salinity:	
TOC:	
Water Hardness:	Value or Lower Range: Upper Range:

Method/Guideline Test Conditions Remarks:	
Limit Test:	

Test Results

NOEC/LOEC/NOELR/LOELR

	Exposure Duration:	Exposure Units:	Value Description:	Value or Lower Range:	Upper Range:	Units:	Basis for Concentration:
NOEC:							
LOEC:							
NOELR:							
LOELR:							

LC/EC/IC/EL/LL Mean Value

Exposure Duration:	Exposure Units:	Type	%:	Value Description:	Mean Value or Lower Mean Value:	Upper Mean Value:	Units:	Basis for Effect:	Basis for Concentration:

Results Remarks:	Petroleum Hydrocarbon gases exist in the gaseous phase and are contained in closed systems at the refinery. Inadvertent release of these substances would result in the individual components partitioning to the air. They would not likely enter the aquatic environment unless a direct release to that environment occurred. Even then, the residence time of any dissolved gas would be expected to be short and exposures to aquatic organisms may not persist for a long enough duration to elicit toxic effects. To fulfill the objective of defining the hazard of these gaseous

mixtures, a literature review was conducted to identify aquatic hazard data for the predominant constituents in hydrocarbon gases. Some constituents, namely N_2, H_2, and CO_2 were either not known to elicit direct toxic effects to aquatic organisms or no empirical data were found that described their hazard. Upon direct release to aquatic environments, these gases may result in oxygen displacement and thus would act as asphyxiants.

Aquatic toxicity data for these substances are tabulated and presented below.

Hydrocarbon Gas Constituent	Range of Toxicity Values (EC50, mg/L)	Reference
C1-C4 Hydrocarbons[1]	4.7 – 82	EPA (2000)
C5-C6 Hydrocarbons[1]	0.9 – 13	EPA (2000)
Benzene[2]	29	Galassi et al. (1988)

[1] Range of endpoint values for fish as determined using ECOSAR model (EPA, 2000).
[2] Ecotoxicity endpoint values for benzene are empirical data cited in the referenced sources.

Reliability / Data Quality

Reliability:	2
Reliability Remarks:	The data cited used peer reviewed journal articles and accepted structure activity relationships to characterize the endpoint values for constituents in petroleum hydrocarbon gases.
Key Study Sponsor Indicator:	

Reference

Reference:

Galassi, S., M. Mingazzini, L. Vigano, D. Cesaeeo, and M.L. Tosato. 1988. Approaches to modeling the toxic responses of aquatic organisms to aromatic hydrocarbons. Ecotoxicol. Environ. Saf. 16(2):158-169.

API (American Petroleum Institute). 2001. Petroleum Gases Test Plant. API, Washington, DC.

EPA (U.S. Environmental Protection Agency). 2000. EPI Suite™, the Estimation Programs Interface (EPI) Suite™. U.S.

	Environmental Protection Agency, Washington, DC.

Human Health Effects Robust Study Summaries

High Production Volume Information System (HPVIS)

Acute Toxicity

Acute Toxicity

Test Substance

Category Chemical (CAS #):	71-43-1
Test Substance (CAS #):	Benzene
Test Substance Purity/Composition and Other Test Substance Comments :	Reagent grade thiophene-free benzene containing no contaminants above a concentration of 0.05% (JT Baker Chemical Co).
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	Acute exposure
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague Dawley
Other Strain:	
Gender:	Females only
Number of Animals per Dose:	10
Concentration:	
Dose:	No data
Year Study Performed :	1974
Method/Guideline Followed:	Other
GLP:	No Data
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 10/sex Exposure time: 4 hours Method approximates OECD Test Guideline, 403 but females only were tested. Groups of 10 female animals; observed for 2 weeks following exposure; animals dying during exposure and those killed at end of study subjected to necropsy. The LC50 value was reported as 13,700 ppm (converts to 44.7 mg/l) with a range of 13,050-14,480 ppm (converts 42.5 – 46.9 mg/l). Death appeared to be caused by a depression of the CNS. These animals had increased lung and liver weights, lung and liver congestion (increase in number of red blood cells and an increased number of vacuolated hepatocytes in the

	liver.

TEST RESULTS

Concentration (LC/LD)

LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
	50	=	13700		ppm (air)
	50	=	13050	14380	ppm (air)

Number of Deaths (Male):	
Number of Deaths (Female):	No data
Number of Deaths (Total):	No data
Results Remarks:	LC50: 13,700 ppm (13050-14380 ppm) Animals which survived the first 24 hours after exposure survived to the end of the 14 day observation period.
Conclusion:	LC50 = 13,700 ppm (13050-14380 ppm)

RELIABILITY/DATA QUALITY

Reliability:	Valid with Restrictions (KS=2)
Reliability Remarks:	Females only used; lack of detail on dose levels
Key Study Sponsor Indicator:	Key

REFERENCE

Reference:	Drew RT, Fouts JR. 1974. The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol Appl Pharmacol 27:183-193.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	74-98-6
Test Substance (CAS #):	Propane
Test Substance Purity/Composition and Other Test Substance Comments :	No data
Category Chemical Result Type :	Measured

Type	:	LC50 ACUTE INHALATION TOXICITY
Value	:	> 800000 ppm
Species	:	rat
Strain	:	
Sex	:	male/female
Number of animals	:	6
Vehicle	:	other: substance administered with air
Doses	:	
Exposure time	:	15 minute(s)
Method	:	other
Year	:	1982
GLP	:	no data
Test substance	:	other TS: Propane
Method	:	Groups of either 6 male or 6 female rats were exposed for 15 minutes in 500 ml whole body inhalation chambers. The animals were observed for effects on the CNS over a 10 minute exposure period.

The EC50 CNS effect concentration (10 mins) was calculated. The concentrations causing death after 15 minutes exposure were recorded and the LC50 (15 mins) was calculated. A range of concentrations was used such that the no effect concentration, the 100% effect concentration and several in-between concentrations were determined. [Details of actual concentrations are not provided].

Result : Propane caused CNS depression. Signs of intoxication were: slight ataxia, loss of righting reflex, loss of movement, narcosis, shallow respiration and death eventually from respiratory depression. Recovery from a non-lethal exposure was rapid and the rats appeared normal within 10 minutes. Where deaths ocurred, they were during exposure, never afterwards.
The calculated EC50 and LC50 values with 95% confidence limits, expressed as concentrations in air (ppm) are as follows:
EC50 (CNS depression, 10 mins.) 280000 (220000-350000)
[=504,996 (396,783-631,245)mg/m3]

LC50 (15 mins.) >800000
[=1,442,847 mg/m3]

Test condition : Propane was passed through a calibrated rotameter and mixed with the required amount of air. As soon as the concentration of propane exceeded 25%, oxygen was mixed with the air to maintain an oxygen concentration of 20%.

Test substance : Propane, purity not specified.

Reliability : (2) valid with restrictions
Study not performed to guidelines and some experimental details lacking.

20.04.2001 Clark, D.G. and Tinson, D.J. (1982) Acute Inhalation Toxicity of some Halogenated and Non-Halogenated Hydrocarbons. Human Toxicol. Vol. 1, pp 239-247.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-28-5
Test Substance (CAS #):	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments :	No data
Category Chemical Result Type :	Measured

Type : LC50 ACUTE INHALATION TOXICITY
Value : = 570000 ppm
Species : rat
Strain :
Sex : male/female
Number of animals : 6
Vehicle : other: substance administered with air
Doses :
Exposure time : 15 minute(s)
Method : other
Year : 1982
GLP : no data
Test substance : other TS: Isobutane
Method : Groups of either 6 male or 6 female rats were exposed for 15 minutes in 500 ml whole body inhalation chambers.
The animals were observed for effects on the CNS over a 10 minute exposure period.

The EC50 CNS effect concentration (10 mins) was calculated.
The concentrations causing death after 15 minutes exposure were recorded and the LC50 (15 mins) was calculated.
A range of concentrations was used such that the no effect concentration, the 100% effect concentration and several in-between concentrations were determined. [Details of actual concentrations are not provided].

Result : Isobutane caused CNS stimulation. Signs of intoxication were: slight tremors of the limbs, marked tremors of the limbs and head, convulsions, narcosis, shallow respiration and death from respiratory depression. Recovery from a non-lethal exposure was rapid and the rats appeared normal within 10 minutes.
Where deaths ocurred, they were during exposure, never afterwards.
The calculated EC50 and LC50 values with 95% confidence limits, expressed as concentrations in air (ppm) are as follows:
EC50 (CNS stimulation 10 mins.) 200000 (160000-230000)
[=475,444 (380,355-546,760)mg/m3]

LC50 (15 mins.) 570000 (480000-650000)
[=1,355,015 (1,141,065-1,545,1920 mg/m3]

Test condition : Isobutane was passed through a calibrated rotameter and mixed with the required amount of air. As soon as the concentration of isobutane exceeded 25%, oxygen was mixed with the air to maintain an oxygen concentration of 20%.

Test substance : Isobutane, purity not specified.

Reliability : (2) valid with restrictions
Study not performed to guidelines and some experimental details lacking.

20.04.2001 Clark, D.G. and Tinson, D.J. (1982) Acute Inhalation Toxicity of some Halogenated and Non-Halogenated Hydrocarbons. Human Toxicol. Vol. 1, pp 239-247.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-28-5
Test Substance (CAS #):	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments :	No data
Category Chemical Result Type :	Measured

Type : LC50 Acute Inhalation Toxicity
Value : = 520400 ppm
Species : mouse
Strain : other: CF-1
Sex : male
Number of animals : 10
Vehicle : other: air
Doses :
Exposure time : 120 minute(s)
Method : other
Year : 1977
GLP : no data
Test substance : other TS: Isobutane
Method : Groups of 10 male mice were exposed by inhalation to isobutane at various concentrations in air for 120 minutes. Oxygen was added (25% of the volume of isobutane) to prevent any death due to hypoxia. An LC50 and 95% fiducial limits

were calculated by a probit method.
No further experimental details are provided.

Result : Signs of central nervous system depression, rapid and shallow respiration, loss of posture and apnea were observed.
Mortality at the various exposure concentrations is as follows.

Concentration (%)	Mortality (%)
36	0
40	10
50	30
55	50
60	90
65	100

The 120 minute LC50 was determined to be 52.04± 3.26 %v/v (equivalent to 520,400 ppm).

Reliability : (2) valid with restrictions
Full experimental details are not provided; nevertheless, the
data are useful in assessing the acute inhalation toxicity of isobutane.

27.08.2001 Aviado, D.M., Zakhari, S. and Wanatabe, T. (1977) Isobutane, Chapter 6 pp 61-72. in Non-Fluorinated propellants and Solvents for Aerosols. CRC Press, Cleveland, Ohio.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-28-5 (isobutane); 106- 97-8 (butane); 74-98-6 (propane) [**note** – will need 3 separate entries of same RSS for HPVIS – one for each CASRN]
Test Substance (CAS #):	Isobutane; butane; propane
Test Substance Purity/Composition and Other Test Substance Comments :	Mixture of isobutane, butane, and propane
Category Chemical Result Type :	Measured

Type : LC50 acute inhalation toxicity
Value : = 539600 ppm
Species : mouse
Strain : other: CF-1
Sex : male
Number of animals :
Vehicle :
Doses :
Exposure time : 120 minute(s)
Method : other
Year : 1977
GLP : no data
Test substance : other TS: mixture of isobutane, butane and propane
Method : Groups of 10 male mice were exposed by inhalation to the gas mixture at various concentrations in air for 120 minutes. Oxygen was added (25% of the volume of isobutane) to prevent

any death due to hypoxia. An LC50 and 95% fiducial limits were calculated by a probit method.
No further experimental details are provided.

Result : Mortality was reported thus:

Concentration (% v/v)	Mortality (%)
45	0
50	20
55	50
60	60
70	80
75	100

LC50 determined to be 57.42 ±3.46%

The authors reported that the gas mixture exhibited less toxicity than isobutane alone, but did not report any clinical signs. The tendency for the mixture to be less toxic was attributed to the presence of propane which is less toxic than either butane or isobutane.
It was also reported that there was no significant difference
between the LC50 for isobutane alone and the gas mixture.

Test substance : The following gas mixture was tested:

Isobutane (31 psig)	80.4%
Butane (17 psig)	2.5%
Propane (108 psig)	17.1%

Mixture (46 psig)

27.08.2001 Aviado, D.M., Zakhari, S. and Wanatabe, T. (1977) Hydrocarbon mixture: Propane, butane and isobutane, Chapter 7 pp 75-81. in Non-Fluorinated propellants and Solvents for Aerosols. CRC Press, Cleveland, Ohio.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-28-5 (isobutane) and 74-98-6 (propane) [**note** – will need 2 separate entries of same RSS for HPVIS – one for each CASRN]
Test Substance (CAS #):	Isobutane and propane
Test Substance Purity/Composition and Other Test Substance Comments :	isobutane and propane
Category Chemical Result Type :	Measured

Type	**:**	other: EC50 cardiac sensitization to adrenaline following inhalation
Value	**:**	
Species	**:**	dog
Strain	**:**	
Sex	**:**	
Number of animals	**:**	
Vehicle	**:**	
Doses	**:**	
Exposure time	**:**	5 minute(s)
Method	**:**	
Year	**:**	1982
GLP	**:**	no data
Test substance	**:**	other TS: Propane and Isobutane
Method	**:**	Method not described, but reference given to previous publication by authors which does include a description of the method.
Result	**:**	EC50 for cardiac sensitization to adrenaline in dogs after 5 mins. exposure to propane and isobutane are given below.

Values given are the EC50 expressed as concentration in air (ppm) with 95% confidence limits.
Propane 180000 (120000-260000)
[= 324,640 (216,427-468,925) mg/m3]
Isobutane 70000 (47000-106000)
[= 166,405 (111,729-251,9850 mg/m3]

Reliability **:** (4) not assignable
Inadequate description of study.

11.04.2001 Clark, D.G. and Tinson, D.J. (1982) Acute Inhalation Toxicity of some Halogenated and Non-Halogenated Hydrocarbons. Human Toxicol. Vol. 1, pp 239-247.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-28-5 (isobutane); 106- 97-8 (butane); 74-98-6 (propane); 74-84-0 (ethane) [**note** – will need 4 separate entries of same RSS for HPVIS – one for each CASRN]
Test Substance (CAS #):	Isobutane; butane; propane; ethane
Test Substance Purity/Composition and Other Test Substance Comments :	Isobutane, butane, propane, and ethane
Category Chemical Result Type :	Measured

Type	**:**	other: cardiac sensitization to epinephrine (acute inhalation)
Value	**:**	
Species	**:**	dog
Strain	**:**	
Sex	**:**	
Number of animals	**:**	
Vehicle	**:**	other: air
Doses	**:**	
Exposure time	**:**	10 minute(s)
Method	**:**	other: non guideline study
Year	**:**	1948
GLP	**:**	no data
Test substance	**:**	other TS: Ethane, propane, n-Butane, iso-Butane
Method	**:**	Electrocardiograms, Lead II, were recorded from the unanesthetised dogs. Epinephrine hydrochloride solution (1:100000) was injected i.v. at a dose of 0.01 mg/Kg over a 25 to 40 second time interval.

After administration of the epinephrine HCl another ECG was taken.
Each animal was subsequently permitted to breathe a mixture of hydrocarbons in varying concentrations (15 to 90%) mixed with oxygen.
After 10 minutes' inhalation of the hydrocarbon an ECG trace was made following administration of epinephrine HCl as described above.

Remark : This work is an experimental study carried out as part of a programme to examine the usefulness of different materials as anesthetics. Although not a guideline study, it nevertheless demonstrates the potential of some hydrocarbons to sensitise the myocardium to epinephrine.

Result : Sensitization of the myocardium occured at the following incidence:

Hydrocarbon	No. of dogs sensitized/No. exposed
Ethane	2/4
Propane	3/3
n-Butane	2/2
iso-Butane	2/2

The authors reported the results with 12 different hydrocarbons. Only those of relevance to this dossier are summarised here.

Test substance : Ethane, propane, n-Butane, iso-Butane

Reliability : (4) not assignable

27.08.2001

Krantz,J.C., Carr, J. and Vitcha, J.F. (1948) Anesthesia XXXI. A study of cyclic and non cyclic hydrocarbons on cardiac automaticity. J. Pharm. Exp. Therap. Vol 94, pp 315-318

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-28-5 (isobutane); 106- 97-8 (butane); 78-78-4 (isopentane) [**note** – will need 3 separate entries of same RSS for HPVIS – one for each CASRN]
Test Substance (CAS #):	Isobutane; butane; isopentane
Test Substance Purity/Composition and Other Test Substance Comments :	Isobutane, butane, and isopentane
Category Chemical Result Type :	Measured

Type : other: Anesthetic activity (inhalation)
Value :
Species : Mice and dogs
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Exposure time :
Remark : In a cosmetic ingredient review the following information is summarized.

<u>Isobutane</u>
Mice

35% in air for 25 mins	fairly effective as anesthetic
41-52& in air	Lethal in 2-3 minutes

	Dogs	
	45% in air	Relaxation occurs
	55% in air	Lethal
	<u>Isopentane</u>	
	Mice	
	9% in air	Anesthesia in 11 minutes
	11% in air	Anesthesia in 2 minutes
	Dogs	
	12% in air	Not anesthetic
	15-17%	Lethal
	<u>n-Butane</u>	
	Mice	
	13%	Light anesthesia and excitement in 25 minutes
	22%	Anesthesia in 1 minute
	Dogs	
	20-25%	Lethal

Reliability **:** (4) not assignable
Original data were not reviewed and the quality of the data cannot therefore be verified.

20.04.2001 Anon (1982) Final report of the safety assessment of isobutane, isopentane, n-butane and propane. J. Am. College of Toxicol. Vol 1, N0. 4 pp 127-142

Acute Toxicity

Test Substance

Category Chemical (CAS #):	75-28-5
Test Substance (CAS #):	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments :	Isobutane at concentrations of 0 - to 25% was tested in various formulations with propellants and with/without fluorocarbon resins (fabric protection products)
Category Chemical Result Type :	Measured

Type	:	Other – FDA recommended technique for aerosol products
Value	:	
Species	:	rat
Strain	:	no data
Sex	:	male
Number of animals	:	5
Vehicle	:	
Doses	:	
Exposure time	:	
Method	:	other: FDA recommended technique for aerosol products
Year	:	1984
GLP	:	no data
Test substance	:	
Method	:	Five male rats were exposed under static conditions to an aerosol of the test material. In each test, the animals were subjected to 30 seconds of continuous spray and allowed to remain in the sprayed atmosphere for 15 minutes. During the next 15 minutes, the chamber was flushed with room air. The procedure was repeated at 30-minute intervals.

Two series of experiments were carried out. In the first series, each group of animals was subjected to 10 successive exposures within 5 hours.
In the second series, the animals were subjected to only two exposures within one hour.
During the exposures, animals were observed for anesthesia, nasal irritation and other effects (unspecified in report).
At the termination of the exposures, all animals were kept for 2 weeks.
Animals which succumbed were necropsied for gross pathological examinations. Survivors were also sacrificed for examination after 2 weeks.

Remark : This study was of formulations containing isobutane and is of limited value, since it is not possible to attribute any of the effects observed to any single component of the formulations. However, mortality was increased in those formulations containing the highest concentrations of isobutane.

Result : Studies with solvents and propellants alone (10 exposures)

There were no mortalities in these studies. Anesthesia was slight (aerosol containing no isobutane) and moderate in all the other test groups. Respiration was deep and rapid in all groups except the one containing no isobutane and in this group no respiratory distress was observed. Following exposure to the aerosol containing 25% isobutane, the animals lay prostrate but recovered rapidly. In the other isobutane - containing groups the animals were lethargic following exposure. Recovery was rapid in the group containing no isobutane.
There were no visible lesions at necropsy of the animals in this series of tests.

Studies of formulations containing fluorocarbon resin.

Two exposures

There was a 60% mortality during exposure to the

formulations containing 20 and 25% isobutane, whereas no mortalities occured in any other group.
In the 20 and 25% isobutane groups, anesthesia was moderate to deep and respiration was deep and rapid. In the other groups anesthesia was light and respiration was rapid.
Following exposure to all of the formulations, the animals were immediately lethargic but were normal the following day.
Petechial hemorrhages was observed in the lungs of the animals that died.
Necropsy of the surviving animals showed that punctate hemorrhages were present in the lungs of the animals exposed to formulations containing 20 and 25% isobutane. In the other groups there were fewer punctate hemorrhages in the lung.

Ten exposures

Fromulations containing 0, 15, 20 and 25% isobutane were tested. Mortality occurred only during exposure as follows:

% Isobutane	% mortality
0	40
15	0
20	60
25	100

Moderate anesthesia occurred in the groups exposed to formulations containing 0 and 15 % isobutane and recovery was rapid. In the group exposed to 20% isobutane anesthesia was deep and recovery was slow.

Results of the gross necropsies are summarised as follows:

% Isobutane	Gross necropsy	
	Deaths	Survivors
0	Foci of hemorrhage in lung Acute passive	Foci of chronic hemorrhage in lung

		congestion in liver Congestion in kidney	
	15	---	Punctate hemorrhage in lung
	20	Ecchymatic hemorrhage in lung	------
	25	Diffuse congestion in lung	-----

Test substance **:** The following aerosols of the solvents and propellants were tested:

Sample	Constituent	Concentration
1	Chlorothene	75%
	P12 propellant	25%
	Isobutane	0%
2	Chlorothene	75%
	P12 propellant	10%
	Isobutane	15%
3	Chlorothene	75%
	P12 propellant	5%
	Isobutane	20%
4	Chlorothene	75%
	P12 propellant	0%
	Isobutane	25%

Additionally, samples of aerosols of Scotchguard fabric protector formulations were also tested. The formulations were:

Sample	Constituent	Concentration
F1	FCR in Chlorothene	75%

		Propellant P12	25%
		Isobutane	0%
	F2	FCR in NC1815	75%
		Propellant P12	10%
		Isobutane	15%
	F3	FCR in NC1815	75%
		Propellant P12	5%
		Isobutane	20%
	F4	FCR in NC1815	75%
		Propellant P12	0%
		Isobutane	25%

Reliability **:** (2) valid with restrictions

27.08.2001 Dow Chemical Company (undated) Acute inhalation toxicity of aerosols from Scotchgard Fabric Protectors. Report submitted to EPA on November 14, 1984 under Reference No. D-266

Acute Toxicity

Test Substance

Category Chemical (CAS #):	106-97-8
Test Substance (CAS #):	Butane
Test Substance Purity/Composition and Other Test Substance Comments :	No data
Category Chemical Result Type :	Measured

Type	:	LC50 ACUTE INHALATION TOXICITY
Value	:	= 658 mg/l
Species	:	rat
Strain	:	
Sex	:	no data
Number of animals	:	
Vehicle	:	
Doses	:	
Exposure time	:	4 hour(s)
Method	:	other
Year	:	1969
GLP	:	no data
Test substance	:	other TS: Butane
Method	:	Method not described, dose levels, group sizes, observation period not specified. LC50 stated to be estimated by

		Litchfield & Wilcoxon method.
Result	**:**	Study was conducted to determine butane levels in several organs. Butane was found in brain, kidney, liver and perinephric adipose tissue.
Test substance	**:**	Butane, no further specification.
Reliability	**:**	(3) invalid Study not performed to guidelines and some experimental details lacking.
11.04.2001		Shugaev, B.B. (1969) Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health Vol. 18, pp 878-882

Acute Toxicity

Test Substance

Category Chemical (CAS #):	106-97-8
Test Substance (CAS #):	Butane
Test Substance Purity/Composition and Other Test Substance Comments :	No data
Category Chemical Result Type :	Measured

Type	**:**	LC50 Acute inhalation toxicity
Value	**:**	= 680 mg/l
Species	**:**	mouse
Strain	**:**	
Sex	**:**	no data
Number of animals	**:**	
Vehicle	**:**	
Doses	**:**	
Exposure time	**:**	2 hour(s)
Method	**:**	other
Year	**:**	1969
GLP	**:**	no data
Test substance	**:**	other TS: Butane
Method	**:**	No details given of experimental conditions.
Result	**:**	Confidence limits given as 596-775. LC50 value and limits determined by method of Litchfield & Wilcoxon.
Test substance	**:**	Butane, not specified further.
Reliability	**:**	(3) invalid

Study not performed to guidelines and some experimental details lacking.

11.04.2001 Shugaev, B.B. (1969) Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health Vol. 18, pp 878-882

Acute Toxicity

Test Substance	
Category Chemical (CAS #):	106-99-0
Test Substance (CAS #):	1,3-Butadiene
Test Substance Purity/Composition and Other Test Substance Comments :	No data
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	Acute
Species:	Rats
Other Species:	Mice

Mammalian Strain:	No data
Other Strain:	No data
Gender:	No data
Number of Animals per Dose:	No data
Concentration:	No data
Dose:	
Year Study Performed :	1969
Method/Guideline Followed:	No data
GLP:	No data
Method/Guideline and Test Condition Remarks:	Type: LC50 Rats exposed four hours; mice exposed two hours. Age, number, and sex of test animals not specified. Number of groups and exposure concentrations not specified. Dynamic flow exposure system; no description of exposure chambers or conditions.. No post -exposure observation period - mortality study only. Exposure concentrations "controlled" by gas chromatography.

TEST RESULTS

Concentration (LC/LD)

LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
	50	=	129,000		ppm (air)
	50	=	99,126	167,473	ppm (air)

Number of Deaths (Male):	**No data**
Number of Deaths (Female):	**No data**

Number of Deaths (Total):	No data
Results Remarks:	Rat LC50 (4 hour) = 129,000 ppm (99,126-167,473 ppm, p<0.05) Mouse LC50 (2 hour) = 122,000 (113,414-131,037 ppm, p<0.05) No clinical observations or necropsy findings reported. Objective of study was to determine hydrocarbon concentrations in various tissues at lethal exposure concentrations.
Conclusion:	LC50 value reported to be 129,000 ppm (285 mg/L) in rats 122,000 ppm (270 mg/L) in mice.
RELIABILITY / DATA QUALITY	
Reliability:	Unassignable (KS=4)
Reliability Remarks:	Not assignable. Lethality study only; insufficient experimental detail to assess quality.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Shugaev, BB (1969) Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch. Environ. Health 18:878882.

High Production Volume Information System (HPVIS)

Acute Toxicity

Test Substance

Category Chemical (CAS #):	107-07-7
Test Substance (CAS #):	Butene-2
Test Substance Purity/Composition and Other Test Substance Comments :	42.4% cis, 55.3% trans This hydrocarbon is being used to characterize the acute toxicity of the C1-C4 fraction for the refinery gas streams.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation (whole body)
Other Route of Administration:	Not applicable
Type of Exposure:	Acute (limit test)
Species:	Rat:
Other Species:	
Mammalian Strain:	Wistar [Crl:WI(WU)BR]
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	5

Concentration:	
Dose:	10,000 ppm
Year Study Performed :	1992
Method/Guideline Followed:	OECD guideline 403
GLP:	Yes
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 5/sex Exposure time: 4 hours Method: Animals exposed for 4 hours to butane-2 or air and observed for 14 days and examined for gross pathological changes. During exposure, rats were housed individually in wire mesh stainless steel cages within the inhalation chamber (Hazleton Systems Inc, H1000) at a mean temperature of 23.1 degrees C and 49% relative humidity. Chamber concentrations of test article were monitored with a total carbon analyzer (FID) calibrated by passing known atmospheres containing test article over the FID. Rats were exposed for 4 hrs to a test article vapor concentration of 23.1 g/m3 (actual, approx. 10,000 ppm). After exposure, rats were removed from the chambers and returned to their individual living cages for 14 days of observation; the animal room was maintained at 21.5- 230 C with relative humidity of 38-67% and a 12 hr light/dark cycle. Diet and water were available ad lib. Body weight was measured before study initiation and at post-dose days 7 and 14. Rats were observed for clinical signs during exposure, shortly after, and once daily during the observation period. After the observation period, rats were sacrificed, necropsied, and examined for gross pathological changes. Vehicle: Filtered air

TEST RESULTS

Concentration (LC/LD)

LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
	50	>	10,000		ppm (air) [23.1 g/m3]

Number of Deaths (Male):	
Number of Deaths (Female):	
Number of Deaths (Total):	0
Results Remarks:	Restlessness was observed periodically during and after exposure; no clinical signs were seen during the 14 day observation period. Normal growth also occurred during the observation period. No abnormalities were observed at gross necropsy. LC50: >23.1 g/m3 (approximately 10,000 ppm)
Conclusion:	LC50 > 10,000 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline Study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Arts, J.H.E. 1992. Acute (4-hour) inhalation toxicity study of butene-2 in rats. Report No. V92.183/352130. TNO Nutrition and Food Research, Zeist, The Netherlands. [2 butene]

Acute Toxicity

Test Substance

Category Chemical (CAS #):	No CAS number
Test Substance (CAS #):	No CAS Number
Test Substance Purity/Composition and Other Test Substance Comments :	C5-C6 4 light naphtha streams; these hydrocarbons are being used to characterize the acute toxicity of the C5-C6 fraction for the refinery gas streams. API 83-19 CAS# 64741-66-8 (high paraffinic) API 83-20 CAS# 64741-55-5 (high olefinic) API 81-08 CAS# 64741-87-3 (high naphthenic) API 83-05 CAS# 68955-35-1 (high aromatic)
Category Chemical Result Type :	Measured – weight of evidence
Unable to Measure or Estimate Justification :	n/a

METHOD

Route of Administration:	Inhalation
Other Route of Administration:	Oral, dermal (not reported here)
Type of Exposure:	Acute
Species:	rat
Other Species:	rabbit
Mammalian Strain:	Sprague-Dawley
Other Strain:	New Zealand White Rabbit
Gender:	M&F
Number of Animals per Dose:	10 (rats) 4 (rabbits)
Concentration:	Up to 5.3 g/m^3
Dose:	
Year Study Performed :	1980 - 1987
Method/Guideline Followed:	other
GLP:	yes

Method / Guideline and Test Condition Remarks:	*C5 – C6 Light End Naphtha Hydrocarbons* **Light alkylate naphtha** (API 83-19; CAS #64741-66-8; approx 100% paraffinic) is not acutely toxic. A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-19 at a nominal concentration of 5mg/l for 4 hours. This was achieved by total volatilization of the test material and appropriate dilution with air. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically. The mean analytical and nominal exposure concentrations were 5.04 ± 0.74 and 6.31 mg/l respectively. All animals survived the study but exhibited languid behavior and a hunched appearance during the exposure. Female body weights were decreased at day 15 but this was attributed to pre-necropsy fasting. At necropsy there were no remarkable findings and histopathology of the lungs was normal (API, 1987a). **Light catalytic cracked naphtha** (API 83-20; CAS #64741-55-5, approx. 46% olefinic) is not acutely toxic. A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-20 at a nominal concentration of 5mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed and subjected to a gross post-mortem examination. For all animals, including those found dead during the study, the lungs were removed, fixed and examined histologically The mean analytical exposure concentration was measured and found to be 5.28 ±0.55 mgL. Gravimetric samples, collected on glass fiber filters suggested little or no aerosol in the chamber. Most animals exhibited languid behavior and squinted eyes during the second hour of the exposure. Polypnea was observed in all animals when removed from the chamber at the one hour post exposure observation period. Rhinorrhea was exhibited by two animals on day two of the test. All animals appeared normal subsequently and there were no mortalities during the study. With the exception of one animal (female) all animals had body weights that were considered unremarkable. There were no remarkable gross or microscopic findings (API, 1987b). **Sweetened naphtha** (API 81-08, CAS #64741-87-3, approx. 21% naphthenics) is not acutely toxic. A group of 5 male and 5 female rats were exposed by whole body inhalation to API 81-08 at a nominal concentration of 5mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically. The actual chamber concentrations were found to be 5.2 mg/l. No deaths occurred during the study. There were no unusual pharmacotoxic signs or behavior observed in the control animals. There was however, a slight incidence of nasal discharge (2/5 males and 1/5 females) during the exposure period but none during the following 14 day observation period. The body weight gains for the males exposed to API 81-08 was considered normal but the female body weight gains were marginally less than that of the

controls on day 14 post exposure (8.2% compared to 13.8% increase over pre-exposure body weight). No significant macro or microscopic changes were observed that were considered to be treatment related (API, 1987c).

Full range catalytic reformed naphtha (API 83-05, CAS #68955-35-1, approx. 63% aromatics) is not acutely toxic. A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-05 at a nominal concentration of 5mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following methoxyflurane anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically. The exposure chamber TWA concentration was determined to be 5.22 ± 0.14 mg/l. No animal died during the study and no clinical signs of systemic toxicity were observed. There were no significant gross observations at necropsy and no histological changes were observed in the lungs. The 4 hour LC_{50} was therefore greater than 5.22 mg/l (API, 1984).

TEST RESULTS

Concentration (LC/LD)

LC/LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LC	50		> 1063		ppm

Number of Deaths (Male):	
Number of Deaths (Female):	
Number of Deaths (Total):	
Results Remarks:	Results of testing naphtha blending streams for acute toxicity indicate that these materials demonstrate consistently low toxicity by the inhalation [rat LC50 >5g/m^3] exposure route. Weight of the evidence indicates that the C5 – C6 light naphtha hydrocarbon inhalation acute toxicity LC50 is > 5g/m^3 (~1063 ppm). Four hour (rat) LC_{50} > 1063 ppm Full robust study summaries for each study are included separately.Four hour (rat) LC_{50} > 1063 ppm
Conclusion:	Weight of evidence RSS is > 5 g/m3 (~1063 ppm)
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline studies
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	API (American Petroleum Institute) 1984. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 83-05 Full range catalytic reformed naphtha. API Rpt. #31-30681. Washington, DC

	API (American Petroleum Institute) 1987a. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 83-19 Light alkylate naphtha. API Rpt. #34-30636. Washington, DC API (American Petroleum Institute) 1987b. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 83-20 Light catalytic cracked naphtha. API Rpt. #34-32777. Washington, DC API (American Petroleum Institute) 1987c. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 81-08 Sweetened naphtha. API Rpt #33-31827. Washington, DC

High Production Volume Information System (HPVIS)

Acute Toxicity

Test Substance

Category Chemical (CAS #):	64741-66-8
Test Substance (CAS #):	Light Alkylate Naphtha (LAN)

Test Substance Purity/Composition and Other Test Substance Comments :	Sample API 83-19 is a light alkylate naphtha
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation (whole body)
Other Route of Administration:	Not applicable
Type of Exposure:	Acute
Species:	Rat:
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	

Gender:	Both M/F
Number of Animals per Dose:	5
Concentration:	
Dose:	5 mg/l
Year Study Performed :	1987
Method/Guideline Followed:	Other
GLP:	Yes
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 5/sex A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-19 at a nominal concentration of 5mg/l for 4 hours. This was achieved by total volatilization of the test material and appropriate dilution with air. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically. Vehicle: Air
TEST RESULTS	

Concentration (LC/LD)

LC/LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LC	50	>	5		mg/l

Number of Deaths (Male):	
Number of Deaths (Female):	
Number of Deaths (Total):	0
Results Remarks:	The mean analytical and nominal exposure concentrations were 5.04 ± 0.74 and 6.31 mg/l respectively. All animals survived the study but exhibited languid behavior and a hunched appearance during the exposure. Female body weights were decreased at day 15 but this was attributed to pre-necropsy fasting. At necropsy there were no remarkable findings and histopathology of the lungs was normal. LC50: >5 mg/l
Conclusion:	LC50 > 5mg/l

RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline Study
Key Study Sponsor Indicator:	Not a Key Study
REFERENCE	
Reference:	API (American Petroleum Institute) 1987a. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 83-19 Light alkylate naphtha. API Rpt. #34-30636. Washington, DC.

High Production Volume Information System (HPVIS)

Acute Toxicity

Test Substance

Category Chemical (CAS #):	64741-55-5
Test Substance (CAS #):	Light catalytic cracked naphtha (LCCN)
Test Substance Purity/Composition and Other Test Substance Comments :	Sample API 83-20 is a light catalytic cracked naphtha
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation (whole body)
Other Route of Administration:	Not applicable
Type of Exposure:	Acute
Species:	Rat:
Other Species:	

Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	5
Concentration:	
Dose:	5.3 mg/l
Year Study Performed :	1987
Method/Guideline Followed:	Other
GLP:	Yes
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 5/sex A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-20 at a nominal concentration of 5 mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed and subjected to a gross post-mortem examination. For all animals, including those found dead during the study, the lungs were removed, fixed and examined histologically.

	Vehicle: Air

TEST RESULTS

Concentration (LC/LD)

LC/LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LC	50	>	5.3		mg/l

Number of Deaths (Male):	
Number of Deaths (Female):	
Number of Deaths (Total):	0

Results Remarks:	The mean analytical exposure concentration was measured and found to be 5.28 ±0.55 mg/L. Gravimetric samples, collected on glass fiber filters suggested little or no aerosol in the chamber. Most animals exhibited languid behavior and squinted eyes during the second hour of the exposure. Polypnea was observed in all animals when removed from the chamber at the one hour post exposure observation period. Rhinorrhea was exhibited by two animals on day two of the test. All animals appeared normal subsequently and there were no mortalities during the study. With the exception of one animal (female) all animals had body weights that were considered unremarkable. There were no remarkable gross or microscopic findings. LC50: >5.3 mg/l
Conclusion:	LC50 > 5.3 mg/l
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline Study
Key Study Sponsor Indicator:	Not a Key Study
REFERENCE	
Reference:	API (American Petroleum Institute) 1987b. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 83-20 Light catalytic cracked naphtha. API Rpt. #34-32777. Washington, DC.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	64741-87-3
Test Substance (CAS #):	Sweetened naphtha.
Test Substance Purity/Composition and Other Test Substance Comments :	API 81-08 is sweetened naphtha
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Inhalation (whole body)
Other Route of Administration:	Not applicable
Type of Exposure:	Acute
Species:	Rat:
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	5
Concentration:	
Dose:	5.2 mg/l
Year Study Performed :	1986
Method/Guideline Followed:	Other

GLP:	Yes
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 5/sex A group of 5 male and 5 female rats were exposed by whole body inhalation to API 81-08 at a nominal concentration of 5 mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following sodium pentobarbital anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically. Vehicle: Air

TEST RESULTS

Concentration (LC/LD)

LC/LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LC	50	>	5.2		mg/l

Number of Deaths (Male):	

Number of Deaths (Female):	
Number of Deaths (Total):	0
Results Remarks:	The actual chamber concentrations were found to be 5.2 mg/l. No deaths occurred during the study. There were no unusual pharmacotoxic signs or behavior observed in the control animals. There was however, a slight incidence of nasal discharge (2/5 males and 1/5 females) during the exposure period but none during the following 14 day observation period. The body weight gains for the males exposed to API 81-08 was considered normal but the female body weight gains were marginally less than that of the controls on day 14 post exposure (8.2% compared to 13.8% increase over pre-exposure body weight). No significant macro or microscopic changes were observed that were considered to be treatment related. LC50: >5.2 mg/l
Conclusion:	LC50 > 5.2 mg/l
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline Study
Key Study Sponsor Indicator:	Not a Key Study
REFERENCE	
Reference:	API (American Petroleum Institute) 1987c. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 81-08 Sweetened naphtha. API Rpt #33-31827. Washington, DC.

Acute Toxicity

Test Substance

Category Chemical (CAS #):	68955-35-1
Test Substance (CAS #):	Full range catalytic reformed naphtha
Test Substance Purity/Composition and Other Test Substance Comments :	Sample API 83-05 is full range catalytic reformed naphtha
Category Chemical Result Type :	Measured
Unable to Measure or	

Estimate Justification :	
METHOD	
Route of Administration:	Inhalation (whole body)
Other Route of Administration:	Not applicable
Type of Exposure:	Acute
Species:	Rat:
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	5
Concentration:	
Dose:	5.22 mg/l

Year Study Performed :	1984
Method/Guideline Followed:	Other
GLP:	Yes
Method/Guideline and Test Condition Remarks:	Type: LC50 Number of animals: 5/sex A group of 5 male and 5 female rats were exposed by whole body inhalation to API 83-05 at a nominal concentration of 5 mg/l for 4 hours. After the 4 hour exposure the rats were observed twice daily for mortality. The animals were weighed prior to exposure and again on days 7 and 14 post exposure. On day 14 all surviving animals were killed by exsanguination following methoxyflurane anesthesia and were subjected to a full necropsy. For all animals, including those found dead during the study the lungs were removed, fixed and examined histologically. Vehicle: Air

TEST RESULTS

Concentration (LC/LD)

LC/LD	%:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LC	50	>	5.22		mg/l

Number of Deaths (Male):	
Number of Deaths (Female):	
Number of Deaths (Total):	0
Results Remarks:	The exposure chamber TWA concentration was determined to be 5.22 ± 0.14 mg/l. No animal died during the study and no clinical signs of systemic toxicity were observed. There were no significant gross observations at necropsy and no histological changes were observed in the lungs. LC50: >5.22 mg/l
Conclusion:	LC50 > >5.22 mg/l
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline Study
Key Study Sponsor Indicator:	Not a Key Study

REFERENCE	
Reference:	API (American Petroleum Institute) 1984. Acute inhalation toxicity evaluation of a petroleum derived hydrocarbon in rats. API 83-05 Full range catalytic reformed naphtha. API Rpt. #31-30681. Washington, DC.

Skin Irritation

TEST SUBSTANCE

Category Chemical:	74-98-8 (propane); 75-28-5 (isobutane)
Test Substance:	Propane and Isobutane
Test Substance Purity/Composition and Other Test Substance Comments:	Cosmetic formulations containing isobutane alone or propane and isobutane
Category Chemical Result Type:	Measured

Type		SKIN IRRITATION
Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	
Classification	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	
Remark	:	In a Cosmetic ingredients review data are presented (tabulated) on the skin irritation potential of various cosmetics formulations. Erythema and edema scores are provided for each of the formulations tested on the shaved intact skin of rabbits.

15 formulations were tested containing Isobutane at concentrations ranging from 74.25 to 89.55% and the Primary irritation indices for these were similar and ranged from 1.895 to 0.52 respectively

Results are also tabulated for six formulations containing both isobutane and propane (approximately in the proportion of 65 parts isobutane to 12 parts propane) and for these the PIIs ranged from 0.38 to 0.855.

Reliability **:** (4) not assignable

19.04.2001

Anon (1982) Final report of the safety assessment of isobutane, isopentane, n-butane and propane. J. Am. College of Toxicol. Vol 1, N0. 4 pp 127-142

Eye Irritation

TEST SUBSTANCE

Category Chemical:	75-28-5
Test Substance:	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments:	Cosmetic formulations containing isobutane
Category Chemical Result Type:	Measured

type		
Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	
Classification	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	
Remark	:	The following statement is made in a Cosmetic Ingredient Review, published in 1982: " A hair spray containing 22% concentration of isobutane was tested for eye iritation in five rabbits. A 0.1 ml of the undiluted product was sprayed into one eye, and after 4 sec

the eye was irrigated. There was no sign of corneal irritation after 1 h. There was transient iritis and mild conjunctivitis after one hour, but these soon dsiappeared."

Reliability : (4) not assignable
This data does not relate directly to isobutane but is nonetheless useful supporting information.

19.04.2001 Anon (1982) Final report of the safety assessment of isobutane, isopentane, n-butane and propane. J. Am. College of Toxicol. Vol 1, N0. 4 pp 127-142

Eye Irritation

TEST SUBSTANCE

Category Chemical:	106-97-8
Test Substance:	butane
Test Substance Purity/Composition and Other Test Substance Comments:	No data
Category Chemical Result Type:	Measured

Type
Species : rabbit
Concentration : undiluted
Dose :
Exposure time :
Comment :
Number of animals :
Vehicle :
Result :
Classification :
Method :

Year	**:**	
GLP	**:**	
Test substance	**:**	
Remark	**:**	The following statement is made in Grant's Toxicology of the Eye, under the heading Butane: "Butane is an essentially nontoxic petroleum gas which causes no disturbance of the eye, even when injected into the anterior chamber experimentally in rabbits. I found it disappeared spontaneously from the eye in two to four days, causing no disturbance." No other details are provided.
Reliability	**:**	(3) invalid This is an unreliable piece of anecdotal information and should not be used in an evaluation of the eye irritancy potential of butane.
01.03.2000		Grant, W.M. (1974) Toxicology of the eye, 2nd edition. Charles C. Thomas, Springfield, Ill.

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	71-43-2
Test Substance:	Benzene
Test Substance Purity/Composition and Other Test Substance Comments:	Chromatography grade, checked for purity by gas chromatography analysis
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	Subchronic exposure; hematological parameters
Species:	Mouse

Other Species:	Not applicable
Mammalian Strain:	CD-1
Other Strain:	Not applicable
Gender:	Male
Number of Animals per Dose:	11-12 per group
Concentration:	
Dose:	0, 1.1, 9.9, 103, 306, 603, 1276, 2416, 4862 ppm (Experiment 1) 0, 9.6 ppm (Experiment 2) 0, 302 ppm (Experiment 3) Target concentrations of benzene were 1, 10, 100, 300, 600, 1200, 2400 and 4800 ppm.
Year Study Performed:	1981
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	5 days (experiment 1) 10 weeks (experiment 2) [considered 50 days total] 26 weeks (experiment 3)
Frequency of Treatment:	6 hrs/day for 5 days/week
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Control group: Yes, concurrent air exposures. Method: Hematopoietic effects of benzene were evaluated in the mouse following three different exposure regimens: 1) 6 hr/day for 5 days at 0, 1.1, 9.9, 103, 306, 603, 1276, 2416, 4862 ppm benzene, 2) 6 hr/day for 10 weeks [50

days] at 0 and 9.6 ppm benzene, and 3) 6 hr/day for 5 days for 26 weeks at 302 ppm benzene.

Male CD-1 mice (11–12/group) were exposed for 6 hours/day, 5 days/week to concentrations of 0, 1.1, 9.9, 103, 306, 603, 1276, 2416, 4862 ppm benzene in experiment 1. Another group was exposed to 0 or 10 ppm (0 or 32 mg/m^3) benzene for 10 weeks/50 days [giving a total dose equivalent to that delivered over 5 days at 100 ppm benzene] (experiment 2), and another to 10 or 300 ppm (0 or 958 mg/m^3) for 26 weeks (experiment 3).
On the day of the last exposure, samples (pooled from groups of 3–4 mice) were obtained from the peripheral blood, bone marrow, and spleen to evaluate hematologic and hematopoietic cells. Peripheral blood red blood cells and white blood cell counts were determined with a Coulter Counter and 100 cell differentials performed on Wright/Giemsa-stained smears.
Bone marrow cell suspensions were prepared by repeatedly flushing with McCoy's media, and single cell suspensions were prepared and pelleted by centrifugation. Cells were re-suspended in a known volume of fresh media and nucleated cell counts were performed with a Coulter Counter. Splenic cell preparations were similarly prepared with some minor variations in methodology. Smears were prepared following incubation and staining in order to measure a 600-cell differential.

Animals exposed for 50 days or 26 weeks were evaluated against their appropriate, matched air-sham exposed controls. A two-tailed Student's t tesat at $p<0.05$ was selected as the test for significance. Data were evaluated using the Welch approximation. All exposure regimes were performed three to eight times and at each time, the hematological parameters were determined from cells pooled from three or four exposed mice and at least two controls. The calculated degrees of freedom using the Welch approximation were based on the number of experiments.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL		=	10		ppm

Results Remarks:	LOAEL ≤10 ppm (no NOAEL identified) Following the 5-day exposures, granulocytopenia and lymphocytopenia were observed at levels ≥ 100 ppm with no change in white blood cell differential. Red blood cell counts were depressed only at the two highest exposure levels while hematocrits were variably affected and showed no clear dose/response effect. Marrow and splenic cellularities were reduced at all levels ≥100 ppm. Marrow lymphocytes, splenic lymphocytes and marrow granulocytes were reduced in accordance with the reduction in total cellularity, however splenic granulocytes and spleen weights were depressed at almost all exposure levels. Nucleated red blood cells in the marrow and spleen were depressed at almost all levels ≥100 ppm. In mice exposed for 50 days to 10 ppm (32 mg/m^3) [note: a total dose equivalent to that delivered over 5 days at 100 ppm benzene] , no adverse effects were observed with respect to mortality, body weight, or cells in the peripheral blood or bone marrow. Spleen weight, total nucleated cells per spleen, and nucleated red blood cells seen were significantly increased ($p<0.05$) in mice exposed to 10 ppm (32 mg/m^3). Mice exposed to 300 ppm (958 mg/m^3) had the following significant ($p<0.05$) changes: increased mortality rate; decreased numbers of lymphocytes and RBCs in peripheral blood; decreased granulocyte/macrophage progenitor cells in bone marrow; decreased spleen weight and numbers of lymphocytes; multipotential hematopoietic stem cells and committed granulocyte/macrophage progenitor cells in the spleen; and increased incidence of atypical cell morphology in the peripheral blood, bone marrow, and spleen. These studies identify a LOAEL ≤ 10 ppm (32 mg/m^3) for slight hematopoietic effects in mice exposed to benzene for 10 weeks.
Conclusion:	LOAEL ≤ 10 ppm for hematopoetic effects.
RELIABILITY/DATA QUALITY	
Reliability:	Valid With Restrictions (KS= 2)
Reliability Remarks:	Non-guideline developmental toxicity study but in accordance with generally accepted scientific standards and described in sufficient detail.
Key Study Sponsor Indicator:	Key
REFERENCE	

Reference:	Green JD, Snyder CA, LoBue J, BD Goldstein, and RE Albert. 1981. Acute and chronic dose/response effect of benzene inhalation on the peripheral blood, bone marrow, and spleen cell of CD-1 male mice. Toxicol Appl Pharmacol 59:204-214.

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	74-84-0
Test Substance:	Ethane
Test Substance Purity/Composition and Other Test Substance Comments:	Ethane, purity 99%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.

Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 1600, 5000, 16,000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	

Method / Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on ethane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure . The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Developmental Toxicity of this Robust Study Summary submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 160, 1600 and 16000 ppm. Therefore, the high exposure level was established (for safety reasons) at 16000 ppm since it is 50% of the lower explosion limit (3.2% = 32000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 256 230-284 Female: 197 167-217 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 1600, 5000 and 16000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6

hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	1600 ppm	12 males	24 females
Group 3	5000 ppm	12 males	24 females
Group 4	16000 ppm	12 males	24 females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

Viability
Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

Clinical observations
All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response

	to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit

	Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment.

	Functional Observational Battery The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery: **Sensory Observations** startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides

	Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain.

Microscopic pathology
A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded.

F1 Pup Evaluations

Physical Examinations
Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior.

Body Weight
Individual F1 pup weights were recorded on lactation days 1 and 4.

Sexing
Pups were sexed on lactation day 0 and sex verified on lactation day 4.

Macroscopic observations
Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain.

Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation.
F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded.

No protocol deviations occurred during the study.

Statistical methods:

1. Continuous data
The following parameters were analyzed statistically:
Body weights
Body weight changes
Feed consumption values
Rectal Temperature
Hematology
Coagulation
Clinical Chemistry
Gestation length
Corpora lutea and implantation sites
F1 pup weights (each weighing interval during lactation)
Number of pups (F1 litters) per pregnant female rats
Number of male and females pups
Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit)
Organ weights
Organ weight to body weight ratios
Organ to Brain weight ratios
Grip Strength measurements

Methods of analysis:
Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was

	used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0. 2. Motor Activity Counts The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor. 3. Incidence data The following parameters were analyzed statistically: Mortality rate Mating indices, pregnancy rates, male fertility indices Litter survival indices Gestation indices Incidence of dams with no viable pups Mean pup survival indices (lactation days 0 and 4) Incidence Data Analysis A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.
TEST RESULTS	

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male and female rats (Parental/ systemic)	=	16,000		ppm
NOAEL	Reproductive	=	16,000		ppm
NOAEL	Developmental	=	16,000		ppm

Results Remarks:

Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 1703 ± 23, 4762 ± 124, 15502 ± 194 ppm. The analytically measured levels of airborne test substance were reasonably close to the targeted exposure levels and nominal concentrations. Chamber environmental conditions averaged 23ºC temperature and 49% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers.

The test substance was assayed by gas chromatography versus an analytical standard before and after the study to demonstrate the purity and stability of the test substance. The test substance was determined to be 99.86% ethane before the study and 99.88% ethane after the study demonstrating the purity and the stability of the test substance.

MAIN STUDY ANIMAL OBSERVATIONS
There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes or feed consumption (except the 16000 ppm exposed animals showed marginally lower feed consumption during the first week of exposures but the differences from the air control did not exceed 5% in the test substance exposed animals compared to the air control animals).
There was no apparent exposure-related effect on functional observational battery or motor activity parameters for either sex in this study. There were no exposure-related differences in hematology or coagulation values or clinical chemistry values in test substance exposed animals compared to the air control animals at the terminal interval. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test

	substance exposed animals compared to the air control animals. There were no microscopic findings considered to be related to exposure to ethane. No testiclular (stages of spermatogenesis; testicular interstitial cells) abnormality was evident in any test animal. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red nasal discharge or red/brown staining between the 7th and 28th days of exposure in all groups including the control animals. All animals survived until the termination of the study. These findings were slightly more prevalent among test substance exposed animals. There were no exposure-related differences in body weights or weight changes or feed consumption (except the 16000 ppm exposed animals showed marginally lower feed consumption (6%) during the first week of exposures, consistent with the same marginal trend in the main study animals. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS Almost all mated female animals were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. All but one of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 1600, 5000 or 16,000 ppm of ethane by whole-body inhalation for 4 weeks resulted in no systemic or neurotoxic effects, apart from a marginal feed reduction in high dose animals during the first week of exposure. A no-observed-adverse effect level (NOAEL) of 16,000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 16,000 ppm was concluded for reproductive and developmental endpoints

	in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Ethane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4243

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	74-98-6
Test Substance:	Propane
Test Substance Purity/Composition and Other Test Substance Comments:	Propane, purity 99.5%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured

Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 1200, 4000, 12000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes

Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method / Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on propane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Developmental Toxicity of this Robust Study Summary submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 120, 1200 and 12000 ppm. Therefore, the high exposure level was established (for safety reasons) at 12000 ppm since it is 50% of the lower explosion limit (2.4% = 24000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 269 243-297 Female: 200 180-220 Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

	The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 1200, 4000 and 12000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19. Exposure method: whole body Group 1 0 ppm 12 males 24 females (air control) Group 2 1200 ppm 12 males 24females Group 3 4000 ppm 12 males 24 females Group 4 12000 ppm 12 males 24females The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve. Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure. The following parameters were evaluated: **Viability** Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

	Clinical observations All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus

	Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment

	period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery: **Sensory Observations** startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows:

	Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina

	All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated

	tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. Three protocol deviations occurred during the study, but did not appear to impact the results. **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically: Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval.

	Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0. 2. Motor Activity Counts The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor. 3. Incidence data The following parameters were analyzed statistically: Mortality rate Mating indices, pregnancy rates, male fertility indices Litter survival indices

	Gestation indices Incidence of dams with no viable pups Mean pup survival indices (lactation days 0 and 4) Incidence Data Analysis A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Male rats (Parental)	=	12,000		ppm
NOAEL	Male rats (Parental)	=	4,000		ppm
NOAEL	Female rats (parental)	=	12,000		ppm
NOAEL	Reproductive	=	12,000		ppm
NOAEL	Developmental	=	12,000		ppm

Results Remarks:	Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 1230 ± 34, 3990 ± 156, 12168 ± 415 ppm. Chamber environmental conditions averaged 23º C temperature and 42% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers. Pre-study GC analysis of the test substance showed a purity of 99.91% for propane. This value compared closely to

	the purity of 99.84% for the analytical standard of propane and the 99.5% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.90% for propane. This value compared closely to the purity of 99.80% for the analytical standard of propane and essentially replicated the 99.91% pre-study purity demonstrating stability of the test substance over the period of this study. MAIN STUDY ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red staining between the 22nd and 28th days of exposure in all groups including the control animals. These findings were very slightly more prevalent among test substance exposed animals. There were exposure-related differences in body weights and weight changes in the 12000 ppm exposed male animals as compared to the air control animals. These male animals showed a 25% decrease in weight gain during the 1st week of exposures and this difference persisted for the remainder of the 4 weeks of exposures. Similar differences were not seen at the lower levels for the males or in any of the female groups. There were no meaningful differences in feed consumption in the test substance exposed animals as compared to the air control animals. There was no meaningful effect on functional observational battery parameters or motor activity for either sex in this study. A statistically significant increase in hindlimb grip strength was noted in the 4000 ppm exposed males but there was no exposure level related pattern. There were no meaningful differences in hematology or coagulation values in test substance exposed male animals as compared to the air controls. A statistically significant decrease (up to 21% in the low level) in absolute lymphocytes in the test substance exposed males was seen but not in an exposure-related pattern and there were no accompanying changes in the other hematology parameters for the males, nor were there any similar differences in the females. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures and were within this laboratory's normal range of values. Similarly, the statistically significant increases only in the 12000 ppm exposed females for hemoglobin concentration, hematocrit, erythrocytes and absolute eosinophils were considered in context too small to represent an effect of the exposures. There were no meaningful differences in clinical chemistry values in the test substance exposed animals as compared to the air control animals. A statistically significant increase in sodium concentration in the 12000 ppm exposed females was seen but the absolute difference was less than 1% and there was no similar difference in the males. A statistically significant decrease in chloride concentration in the 1200 ppm exposed females was seen but the absolute difference was only 2% and there was no similar difference at the higher levels in the females or at any level of exposure in the males. In isolation, these minor inter-group differences were not considered to indicate effects of the exposures and were within this laboratory's normal range of values. There were no meaningful inter-group differences in organ weights in the test substance exposed animals as

	compared with air control animals. The 1200 and 12000 ppm exposed males showed decreases in kidney weights (absolute and/or relative to body or brain weight) but no similar differences were seen in the exposed females. The 12000 ppm exposed males also showed decreases in absolute (but not relative) liver weights and similar differences were seen (but only relative to brain weight) in the 4000 and 12000 ppm exposed females. Considering the absence of any correlated pathology findings and clear patterns of treatment, these minor inter-group differences were not considered to indicate effects of the exposures. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations in the test substance exposed animals compared to the air control animals. The testes were examined qualitatively with an awareness of the stages of the spermatogenic cycle to detect any disturbances in spermatogenesis, and with emphasis on the interstitial (Leydig) cells to detect any changes in cellular size or structure. No test article related findings were present. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. For the pregnant females there were no exposure-related differences in body weights or weight changes in the test substance exposed animals as compared to the air control animals. There were no treatment- related changes in feed consumption. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS All mated female animals (except one in the 1200 ppm group – considered incidental) were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. Almost all of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, and the proportion with live litters and/or with stillborn pups when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. Statistically significant decreases in the number of live born pups and converse increases in the number of stillborn pups in the 4000 and 12000 ppm exposed groups were attributable to the single total litter loss in each of these groups very soon after parturition. These losses were

	preceded by severely reduced body weight gain in the last week of gestation for two particular dams. There was no excess of mortality in any of the other litters in these groups and the total litter losses were considered incidental and not related to the exposures. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 1200, 4000 or 12000 ppm of propane by whole-body inhalation for 4 weeks resulted in a reduction of body weight gain in the males resulting from exposures at the12000 ppm dose. Consequently, a no-observed-adverse effect level (NOAEL) of 4000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study (LOAEL – 12,000 ppm). There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 12,000 ppm was concluded for reproductive and developmental endpoints in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Propane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4245.

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	75-28-5
Test Substance:	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments:	Isobutane, purity 99.0%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 900, 3000, 9000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on isobutane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Developmental Toxicity of this Robust Study Summary

	submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 90, 900 and 9000 ppm. Therefore, the high exposure level was established (for safety reasons) at 9000 ppm since it is 50% of the lower explosion limit (1.8% = 18000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 277 249-301 Female: 194 171-216 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 900, 3000 and 9000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19. Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	900 ppm	12 males	24females
Group 3	3000 ppm	12 males	24 females
Group 4	9000 ppm	12 males	24 females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

Viability
Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

Clinical observations
All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded.

Clinical chemistry:
Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed

	to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration

	Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery:

	Sensory Observations startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver

	Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of

	spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. No protocol deviations occurred during the study. **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically:

	Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0.

2. Motor Activity Counts
The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor.

3. Incidence data

The following parameters were analyzed statistically:
Mortality rate
Mating indices, pregnancy rates, male fertility indices
Litter survival indices
Gestation indices
Incidence of dams with no viable pups
Mean pup survival indices (lactation days 0 and 4)

Incidence Data Analysis
A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:

NOAEL	Male and female rats (Parental/ systemic)	=	9,000		ppm
LOAEL	Reproductive	=	9,000		ppm
NOAEL	Reproductive	=	3,000		ppm
NOAEL	Developmental	=	9,000		ppm

Results Remarks:

Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 930.0 ± 27.8, 3122 ± 83 and 9148 ± 201 ppm. Chamber environmental conditions averaged 24°C temperature and 55% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers.

Pre-study GC analysis of the test substance showed a purity of 99.84% for isobutane. This value compared closely to the purity of 99.51% for the analytical standard of Isobutane and the 99.0% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.53% for isobutane. This value compared closely to the purity of 99.50% for the analytical standard of isobutane and the 99.0% pre-study purity demonstrating stability of the test substance over the period of this study.

MAIN STUDY ANIMAL OBSERVATIONS
There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red staining between the 7th and 28th days of exposure in all groups including the control animals. These findings were only slightly more prevalent among test substance exposed animals.
There were no exposure-related differences in body weights and weight changes or feed consumption in the exposed male animals as compared to the air control animals. There was no treatment-related effect on functional observational battery parameters or motor activity for either sex in this study.
There were no meaningful differences in hematology or coagulation values in test substance exposed male animals as compared to the air controls. Statistically significant increases in hemoglobin and mean corpuscular hemoglobin concentration in the 9000 ppm test substance exposed males, decreases in platelets in 3000 and 9000 ppm test substance exposed males and decreases in monocytes in 3000 and 9000 ppm test substance exposed females were seen in exposure-related patterns but there were no accompanying changes in the other hematology parameters, nor

	were there any similar differences in the other sex. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures and were generally within the normal range of these values for a rat. There were no meaningful differences in clinical chemistry values in the test substance exposed animals as compared to the air control animals. Statistically significant increases in sodium concentration in the 9000 ppm test substance exposed males and decreases in phosphorus concentration in all test substance exposed females were seen but the absolute differences were relatively small, were not in an exposure level related pattern and there were no similar differences in the other sex. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures. There were no meaningful inter-group differences in organ weights in the test substance exposed animals as compared with air control animals. The 900 and 3000 ppm exposed males showed an increase in spleen weights (absolute and/or relative to body or brain weight) but no similar difference was seen in the 9000 ppm exposed males or any of the exposed females. In isolation, this minor inter-group difference was not considered to indicate an effect of the exposures. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations in the test substance exposed animals compared to the air control animals. The testes were examined qualitatively with an awareness of the stages of the spermatogenic cycle to detect any disturbances in spermatogenesis, and with emphasis on the interstitial (Leydig) cells to detect any changes in cellular size or structure. No test article related findings were present. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes in the test substance exposed animals as compared to the air control animals. There were no exposure-related differences in feed consumption in the test substance exposed animals as compared to the air control animals. . There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. A statistically significant increase in heart to brain weight ratio in 9000 ppm exposed females was seen but, in the absence of any macroscopic and microscopic abnormalities in the main study animals, this difference was considered to have been incidental. MATING, FERTILITY AND GESTATION PARAMETERS No parental systemic toxicity was observed. In the 9000 ppm group, 25% of the mated females did not become pregnant. Although not statistically significant, the reduction in male and female fertility indices (75%) was considered exposure related since it was below the concurrent control (100%) and the testing facility historical control values (mean 96.4%; range 87.5%-100%). The mating index for male rats treated with the test substance was

	comparable to the air control group. A statistically significant ($p < 0.05$) exposure-related increase in post-implantation loss was also observed for the 9000 ppm group of exposed female rats; mean losses of 0.8± 0.9 and 1.8 ± 0.8 for control and high exposure groups respectively. The data were interpreted as conservatively as possible; and the two reproductive endpoints were attributed to isobutane exposure. All other reproductive endpoints were comparable to controls (number of pairs cohabited, number of pairs mated, mating index, gestation index, mean time to mating, mean gestation length, number of females completing delivery with stillborn pups/all stillborn pups, mean pre-implantation loss, mean pups delivered, live birth index, viability index). Pup endpoints (viabilty to day 4, weight & weight gain, sex ratio) were also comparable to air control pups.
Conclusion:	Exposure of male and female rats to target concentrations of 900, 3000 or 9000 ppm of isobutane by whole-body inhalation for 4 weeks resulted in no general systemic/neurotoxic effects. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. Based on decreased male and female fertility and increased post-implantation loss in the 9000 ppm group, the fertility and reproductive endpoints NOAEL was determined to be 3000 ppm. There were no effects on in offspring survival, body weight and development up to post-natal day 4. A NOAEL of 9000 ppm was concluded for developmental effects.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Isobutane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4244.

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	106-97-8
Test Substance:	Butane
Test Substance Purity/Composition and Other Test Substance Comments:	Butane, purity 99.5%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 900, 3000, 9000 ppm
Year Study Performed:	2004
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on butane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Developmental Toxicity of this Robust Study Summary

submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections.

The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study".

The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 90, 900, and 9000 ppm. Therefore, the high exposure level was established (for safety reasons) at 9000 ppm since it is 50% of the lower explosion limit (1.8% = 18,000 ppm) for the test substance.

The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation.

The weight of animals at initiation of exposures was:

	Mean (g)	Range (g)
Male:	261	241-280
Female:	200	174-229

Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 900, 3000 and 9000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)

	Group 2 900 ppm 12 males 24 females Group 3 3000 ppm 12 males 24 females Group 4 9000 ppm 12 males 24 females The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve. Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure. The following parameters were evaluated: **Viability** Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon). **Clinical observations** All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum.

	The following parameters were evaluated: Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count

	Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery: **Sensory Observations**

	startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi)

	Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during

	macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. Several protocol deviations occurred during the study but were not considered to have compromised the validity or integrity of the study: **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically:

	Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0.

When 75% of the values for a clinical pathology parameter were the same, Fisher's Exact Test (Fisher, 1973) was performed followed by Mantel's test (Mantel, 1963).

2. Motor Activity Counts

The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant (p=0.05), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passed on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the p=0.05 level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor.

3. Incidence Data

The following parameters were analyzed statistically:
Mortality rate
Mating indices, pregnancy rates, male fertility indices
Litter survival indices
Gestation indices
Incidence of dams with no viable pups
Mean pup survival indices (Days 0 and 4)

Incidence Data Analysis
A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male and female rats (Parental/ systemic)	=	9,000		ppm
NOAEL	Reproductive	=	9,000		ppm
NOAEL	Developmental	=	9,000		ppm

Results Remarks:

Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The analytically measured exposure levels of airbone test substance were reasonably close to the targeted exposure levels and nominal concentrations. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 930.6 ± 28.1, 3022 ± 58 and 9157 ± 269 ppm. Chamber environmental conditions averaged 24ºC temperature and 56% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers.

Pre-study GC analysis of the test substance showed a purity of 99.23% for butane. This value compared closely to the purity of 99.96% for the analytical standard of butane and the 99.5% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.98% for butane. This value compared closely to the purity of 99.98% for the analytical standard of butane and the 99.23% pre-study purity demonstrating stability of the test substance over the period of this study.

MAIN STUDY ANIMAL OBSERVATIONS
There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods in-chamber and during the non-exposure periods. There was a low incidence of chromodacryorrhea or transient red nasal discharge among males exposed to 9000 ppm between the 7th and 28th days of exposure. There were no exposure-related differences in body weights or weight changes or feed consumption in the test substance exposed animals compared to the air control animals.
There was no meaningful exposure-related effect on functional observational battery or motor activity parameters for either sex in this study. Minor, but statistically significant decreases in male forelimb grip strength and increases in female forelimb grip strength occurred at 9000 ppm, but were not suggestive of an adverse effect, due to the opposite effect on the same parameter. Intergroup differences of a similar magnitude occurred among hindlimb results for both

	sexes without any apparent exposure-level relationship. There were no apparent exposure-related differences in hematology or coagulation values or clinical chemistry values in test substance exposed animals compared to the air control animals at the terminal interval. A statistically significant decrease in total bilirubin concentration was seen in males at 3000 and 9000 ppm, but not in an exposure-related pattern, and there were no differences in the females. These minor inter-group differences were not indicative of an exposure effect. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. There were no microscopic findings considered to be related to exposure to butane. No testiclular (stages of spermatogenesis; testicular interstitial cells) abnormality was evident in any test animal. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of chromodacryorrhea or transient red nasal discharge among males exposed to 9000 ppm between the 7th and 28th days of exposure. There were no exposure-related differences in body weights or weight changes or feed consumption in the test substance exposed animals compared to the air control animals. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS Almost all mated female animals were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. All but one of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 900, 3000 or 9000 ppm of butane by whole-body inhalation for 4 weeks resulted in no systemic or neuotoxic effects. A no-observed-adverse effect level (NOAEL) of

	9000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for reproductive and developmental endpoints in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Butane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4242.

High Production Volume Information System (HPVIS)

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	106-99-0
Test Substance:	1,3-Butadiene

Test Substance Purity/Composition and Other Test Substance Comments:	Analyzed at 99.2% pure each week (ICI Ltd., Wilton, England)
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	Chronic toxicity study
Species:	Rat
Other Species:	Not applicable
Mammalian Strain:	Sprague-Dawley
Other Strain:	Not applicable
Gender:	Both M/F
Number of Animals per Dose:	110 per sex per dose level
Concentration:	
Dose:	0, 1000, or 8000 ppm
Year Study Performed:	1990

Method / Guideline Followed:	Other
GLP:	No data
Exposure Period:	52 weeks (male and females- interim sacrifice); 105 weeks (females); 111 weeks (males)
Frequency of Treatment:	6 hrs/day 5 days/wk
Post-Exposure Period:	
Method / Guideline and Test Condition Remarks:	Control group: Yes, concurrent air treatment Method: A 2 year inhalation toxicity study using Sprague Dawley rats, were conducted with butadiene vapor. Three groups (110 male/110 female per group) were chamber-exposed to atmospheres of 0, 1000, and 8000 ppm 1,3-butadiene. Control groups were exposed to clean air only. At 52 weeks, 10 males and 10 females from all groups were killed. The remainder were sacrificed when survival was approximately 20-25% (105 weeks for females and 111 weeks for males). The exposure was 6 hrs/day, 5days/wk. All animals were observed twice daily, before and after exposure, and a detailed observation was performed at weekly intervals. Individual body weights were recorded weekly up to week 13, then every 2 weeks to week 52 and monthly thereafter. Clinical chemistries, neuromuscular function and detailed post-mortem examinations were performed at the time of sacrifice. Analysis of the survival data, subcutaneous masses, lesions/tumor incidences was performed using a variety of statistical methods; body weights, laboratory investigations, and organs weights were analyzed by using analysis of variance and Student's t-test.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL		=	8000		ppm
NOAEL		=	1000		ppm

Results Remarks:	LOAEL = 8000 ppm NOAEL = 1000 ppm Clinical signs that appeared to be related to treatment were seen in the second until the fifth month of exposure. Minor, treatment-related clinical signs of toxicity – wet and ruffled fur together with slight limb weakness or incoordination following dosing on the first day of the 5-day schedule – were seen between 2 and 5 months of treatment in animals at 8000 ppm. There were no effects on hematology, blood chemistry, urine analysis, and neuromuscular function that could be associated with treatment with 1,3-butadiene. Changes in clinical condition, suppression of body weight gain, reduced survival and increases in certain organ weights and in both common and uncommon tumor types occurred at 8000 ppm. At 8000 ppm, males had statistically significantly increased kidney, heart, lung and spleen weights, with associated nephrosis of the kidney and focal metaplasia in the lung. At the end of the study, statistically significant increases were seen in liver weight in all exposure groups, but there was no associated pathology.
Conclusion:	LOAEL = 8000 ppm NOAEL = 1000 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Study is comparable to guideline with sufficient level of scientific detail.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Owen PE and JR Glaister. 1990. Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague-Dawley rats. *Environ Health Persp*. **86;** 19-25.

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	107-07-7
Test Substance:	Butene-2
Test Substance Purity/Composition and Other Test Substance Comments:	Butene-2 (cis and trans ≥95%) [UCAR Specialty Gases, The Netherlands. Certificate of analysis provided by the supplier] This hydrocarbon is being used to characterize the repeated dose oxicity of the C1-C4 fraction for the refinery gas streams.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Whole body inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	Subchronic
Species:	Rat

Other Species:	Not applicable
Mammalian Strain:	Wistar (Hsd/Cpd:WU)
Other Strain:	Not applicable
Gender:	Both M/F
Number of Animals per Dose:	12/sex/dose
Concentration:	
Dose:	0, 2500, 5000 ppm
Year Study Performed:	1992
Method/Guideline Followed:	OECD guideline 422 (Combined repeated dose toxicity and reproductive/developmental toxicity test)
GLP:	Yes
Exposure Period:	Males: 39 to 46 days; Females: pre-mating, mating through Gestation day 19
Frequency of Treatment:	6 hr/day, 7 days/wk
Post-Exposure Period:	None
Method/Guideline and Test Condition Remarks:	Post-exposure period: none

	Control group: yes, concurrent no treatment Method: Combined repeated dose toxicity and reproductive/developmental toxicity test. Male and female rats (avg. wt. 299.4 g males, 204.0 g females at study initiation) were assigned to one of three groups by computer randomization based on body weight, and uniquely identified by ear tattoo. During the entire exposure period, animals were housed individually in stainless steel cages within modified multitiered Hazleton 1000 inhalation chambers. Temperature range of 20 to 23 degrees C and relative humidity of 37 to 80% were monitored continuously using thermo-hygrometers with approximately 10 air changes/hour. Lighting in the animal room and Hazleton chamber was 12 hr light/dark cycle. Animals received food and water ad lib except for ½ hr prior to and during exposure. Animals were exposed to a continuous supply of fresh test atmosphere, passed from a cylinder via a pressure reducer, stainless steel tubing and 2 calibrated mass flow controllers and rotameters to the inlet at the top of the inhalation chamber (2.2 m3 capacity), where it was diluted with filtered air-conditioned air to appropriate concentration, directed downward to the animal cages, and eventually exhausted out at the bottom of the chamber. Control rats were exposed to filtered air only. Air flow was monitored by an anemometer and recorded three times/exposure day, providing 11 to 12 air changes/hr. Concentrations of test material were determined with a total carbon analyzer using FID, twice/hr. in each test atmosphere by sampling at locations close to the animal cages. Uniform distribution of butene-2 vapor was verified during preliminary experiments. Nominal concentrations were calculated by mean amount of test material used/hr. divided by mean hourly volume of air passed through the exposure chamber. Top dose level of 5000 ppm was chosen because the estimated body burden was approx. 1000 mg/kg/day, the limit dose for teratology studies in OECD protocol 414. After 2 wks pre-mating exposure, males and females were caged together (1:1) until mating had occurred or one week. Mated females were exposed through day 19 of gestation; males and females that did not mate (1 in control group) were exposed until necropsy at the end of the study. However, data from non-pregnant females was not presented. At terminal necropsy, blood was collected from all parental (F0) animals (males and dams) for hematology and clinical chemistry. Organs were excised and weighed (liver, kidney, thymus, lung, testes, epididymides) and 15 organs/tissues processed for microscopic examination: nose, lungs with trachea and larynx, spleen, heart, brain, seminal vesicles, ovaries (after counting corpora lutea), uterus (after counting implantation sites), any abnormal growths or lesions. All organs in the 5000 ppm and control groups were examined by a pathologist. Clinical findings and pathological changes evaluated Fisher's exact probability test. Body wt and food consumption analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test.
TEST RESULTS	
Concentration (LOAEL / LOAEC / NOAEL / NOAEC)	

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL		=	2500		ppm
LOAEL		=	5000		ppm

Results Remarks:

NOAEL(systemic) = 2500 ppm (based on body wt changes)
LOAEL = 5000 ppm

Mean actual concentration of butene-2 in test atmospheres was 0, 2476 ± 68ppm (5.7 g/m3) and 5009 ± 88 ppm (11.5 g/m3). No mortality or treatment-related clinical signs were observed in parental (F0) animals. Male body wt were comparable in all groups but mean body wt change was statistically significantly
lower in the 1st and 4th wk of exposure for 2500 ppm group and in the 1st wk of exposure for 5000 ppm group. Female rats showed statistically significantly decreased mean body wt compared to controls at 14 days from start of exposure in 2500 ppm group and at 7 and 14 days of exposure in 5000 ppm group. During gestation, all body weights were comparable in treated and control groups; on lactation day 1, body wt of 5000 ppm dams was statistically significantly decreased. Body wt changes in dams were comparable to control throughout the study. Food consumption in males was comparable to controls; food consumption by 5000 ppm females was decreased during the first wk of exposure. No other food consumption differences occurred during the study. In hematology data, the total white blood cell count and number of lymphocytes were increased in male rats in both exposure groups compared to concurrent controls, however there was no dose response, values were within historical control range and concurrent control values were low. No changes were observed in % distribution of white blood cells, any red blood cell parameters, or clotting potential. in males or pregnant females of either exposure group. In clinical chemistry data, plasma calcium concentration was slightly decreased in high-dose males but was
not considered toxicologically significant since there was no accompanying change in inorganic phosphate levels. No other treatment-related differences were observed. Mean absolute organ wt and relative wt were comparable in all groups. No abnormal, treatment-related macroscopic changes (all groups) or pathological
changes (control and 5000 ppm groups) were observed.

Conclusion:	NOAEL(systemic) = 2500 ppm (based on body wt changes) LOAEL = 5000 ppm
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Waalkens-Brendsen, D.H. and Arts, J.H.E. 1992. Combined short term inhalation and reproductive/developmental toxicity screening test with Butene-2 in rats. Proj. #B91-8336 (Study #1410) [2-butene]

Repeated-Dose Toxicity

TEST SUBSTANCE

Category Chemical:	109-66-0 (pentane), 106-97-8 (butane), 75-28-5 (isobutane), 78-78-4 (isopentane) [**note** – will need 4 separate entries of same RSS for HPVIS – one for each CASRN]
Test Substance:	Pentane, Butane, Isobutane, and Isopentane
Test Substance Purity/Composition and Other Test Substance Comments:	Two mixtures were tested as follows: n-butane/n-pentane 50/50 wt.% isobutane/isopentane 50/50 wt.% The mixtures were prepared by gravimetrically filling gas cylinders with 50lb. of each component and then compressing the cylinders to 250 psi. The components were 99% minimum purity.
Category Chemical Result Type:	Measured

Type	:	Repeated dose
Species	:	rat
Sex	:	male/female
Strain	:	Fischer 344
Route of admin.	:	inhalation
Exposure period	:	90 days
Frequency of treatm.	:	6 hours/day, 5 days/week
Post exposure period	:	None
Doses	:	0, 1017 & 4489 ppm
Control group	:	yes, concurrent no treatment
NOAEL	:	= 4489 ppm
Method	:	OECD Guide-line 413 "Subchronic Inhalation Toxicity: 90-day Study"
Year	:	1986
GLP	:	no data
Test substance	:	other TS: n-butane/n-pentane and isobutane/isopentane
Method	:	20 male and 10 female six week old rats were exposed to each concentration, 6 hours each day for 5 days each week. Total

duration of the study was 90 days.
A negative control group of 40 male and 20 female rats were exposed to filtered air under otherwise similar conditions.
The rats were observed daily throughout the study, were weighed weekly and immediately prior to sacrifice.
Necropsies were performed on half the male rats in each treatment group after the 20th exposure and for the remaining animals at the conclusion of the 90 day study.
At necropsy, the presence of lesions and other abnormal conditions was noted and liver and kidney weights determined.
Major tissues, except for kidneys were collected and fixed, but not examined microscopically.
Kidneys were fixed and examined histologically.

Remark : The purpose of this study was to examine the possible nephrotoxic effects of C4 and C5 hydrocarbons present in gasoline. Due to the limited scope of the study, organ weight measurements were restricted to kidney and liver and histopathological examinations also restricted to the kidney.

Result : There were no mortalities in the study.
Possible treatment-related but NOT dose-related effects included transient hunched posture and/or lethargy and intermittent tremor. Statistically significant decreases in body weight occured in both sexes by weeks 3 and 4 when exposed to the n-butane/n-pentane mixture. Recovery occured by the end of the study for males but not for the females.
There were no treatment-related gross lesions observed, nor were there any kidney or liver weight changes following exposure.
Nephrotoxicity was observed after 20 exposures in males at both dose groups of the butane/pentane mixture but this had disappeared by 90 days.
A similar effect was seen in males after 20 exposures to 1000 ppm of the isobutane/isopentane mixture, but again this was not apparent in the rats exposed for 90 days.
The authors concluded that although there had been a slight nephrotoxic response in male rats after 20 exposures, this was transient and was not present after 90 days exposure.

It is concluded, therefore, that the NOAEL (concentration) is
4489 ppm.

Test condition : Inhalation exposures were conducted in Rochester-type 1m3 stainless steel chambers.
Test atmospheres were achieved by flash evaporation of the liquid phase components as they were released from the storage cylinders.
Hydrocarbon concentrations in the chambers were monitored and adjustments made as necessary in order to achieve the desired atmospheres.

Test substance : Two mixtures were tested as follows:
n-butane/n-pentane 50/50 wt.%
isobutane/isopentane 50/50 wt.%
The mixtures were prepared by gravimetrically filling gas cylinders with 50lb. of each component and then compressing the cylinders to 250 psi. The components were 99% minimum purity.

Reliability : (1) valid without restriction

27.08.2001

Aranyi, C., O'Shea, W.J., Halder,C.A., Holdsworth, C.E. and Cockrell, B.Y. (1986). Absence of hydrocarbon-induced nephropathy in rats exposed subchronically to volatile hydrocarbon mixtures pertinent to gasoline. Toxicology and Industrial Health, vol. 2 No. 1, pp 85-98

Repeated-Dose Toxicity

TEST SUBSTANCE	
Category Chemical:	No CAS Number
Test Substance:	Liquid Petroleum Gas
Test Substance Purity/Composition and Other Test Substance Comments:	Liquid Petroleum Gas; 100 % (Chevron Texaco Energy Research & Technology Company, Richmond, CA); lot number 120701-01
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Sub-chronic – 13 weeks (with micronucleus test satellite study)
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague Dawley
Other Strain:	
Gender:	Males and females
Number of Animals per Dose:	15/sex/dose [main study 10/sex/dose; neurotoxicity study 5/sex/dose]
Concentration:	
Dose:	0, 1000, 5000, 10,000 ppm
Year Study Performed:	2005
Method/Guideline Followed:	OECD 413
GLP:	Yes
Exposure Period:	13 weeks
Frequency of Treatment:	6 hr/day; 5 days/week for 13 weeks
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	The study was designed to assess the potential toxicity, including neurotoxicity and genotoxicity parameters, of liquefied petroleum gas (LPG). The exposure levels were based on results of range-find testing which showed no effects at 100 and 1000 and 10000 ppm. The exposure levels were also selected based on establishing (for safety reasons) the high exposure level as no more than 50% of the lower explosion limit (LEL = 2.1% = 21000 ppm) for the test substance. The animals used were approximately 6 weeks old at receipt and approximately 8 weeks old exposure of initiation.

	Note: Females were nulliparous and non-pregnant. The weight of the animals at initiation of exposure was as follows: Mean (grams) Range (grams) Male: 280.0 243 - 308 Female: 209.1 187 - 231 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. Exposure method: whole body Group 1, 0 ppm, 10 males & 10 females Group 2, 1000 ppm, 10 males & 10 females Group 3, 5000 ppm, 10 males & 10 females Group 4, 10000 ppm, 10 males & 10 females VIABILITY CHECKS (IN-CAGE) Animals were observed in their cages twice daily for mortality and signs of severe toxic or pharmacologic effects. Animals in extremely poor health or in a possible moribund condition were identified for further monitoring and possible euthanasia. PHYSICAL EXAMINATIONS In-Chamber: All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Pertinent behavioral changes and all signs of toxicity, including mortality, were recorded. These signs included time of onset, degree and duration. Out-of-Chamber: Each animal (except Neurotoxicity study animals) was removed from its cage and examined twice pretest and once weekly during the study period. Examination included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded as well as evaluations of respiration, palpation for tissue masses, circulatory effects, central nervous system effects, changes in motor activity, and reactivity to handling or sensory stimuli. During treatment period, these evaluation were performed prior to exposures. Neurotoxicity animals were removed from their cages and examined once pretest before selection onto study.

	OPHTHALMOSCOPIC EXAMINATION All animals were examined pretest and at study termination. Eyelids, lacrimal apparatus and conjunctiva were examined grossly; cornea, anterior chamber, lens, vitreous humor, retina and optic disc were examined by indirect opthalmoscopy. The eyes were examined after instillation of a mydriatic (Tropicamide ophthalmic solution 1%). BODY WEIGHT Body weights were recorded for all animals at the time of randomization into test groups, on the day that treatment was initiated and weekly thereafter throughout the study. Terminal, fasted weights were obtained just prior to necropsy. FEED CONSUMPTION Feed consumption was measured (weighed) during the week prior to treatment initiation and weekly throughout the study. Feed was available without restriction 7 days/week except during inhalation exposures and when fasting prior to blood collection. NEUROBEHAVIORAL STUDIES Testing was staggered over 5 sessions and was conducted on non-exposure days at least 16 hours post-exposure. Each session consisted of 10/sex/group. Testing was performed on the main study (5/sex/group) and all neurotoxicity study animals. Noise level was maintained within a level of 55 to 65 decibels by a white noise generator. Temperature, humidity and illumination was measured and recorded to ensure that variations in environmental conditions are minimal during all evaluations. The functional observational battery was performed for all animals before evaluation of motor activity. FUNCTIONAL OBSERVATIONAL BATTERY Method A functional observational battery (Moser, 1989) was performed on all animals. With the exception of pretest, evaluations were performed "blind", i.e., the observer did not know the identity of the animal's exposure group. Time of testing was balanced across treatment groups. The following evaluations were performed as part of the functional observational battery: Home Cage Evaluations: posture, vocalization, palpebral closure and motor movements. Handling Evaluations: ease of removal from cage; reactivity to general stimuli (handling); assessment of signs of autonomic function, i.e., chromodacryorrhea, lacrimation, salivation, altered fur appearance, or red/crusty deposits around eyes. Open Field Evaluations: arousal level and gait; count of urination and defecation; convulsions, tremors, abnormal

	movements or behaviors, locomotion, excessive or repetitive actions; piloerection and exophthalmos. Reflex Assessments: response to visual (approach response) and auditory (finger snap) stimuli; response to a tail pinch; pupillary function,proprioception and pinna reflex. (light touch in ear). Grip Strength: using grip strength meter Hindlimb Extensor Strength: Animals were held in a vertical position facing the observer with a firm grasp around the thorax. The observer placed one finger against the bottom of each hindpaw and pressed towards the animal. Muscular resistance and pressure exerted by the animals were scored. Landing Foot Splay: Each animal was dropped into a pan of sand from a height of one foot. The distance between the marks left by the hindpaws was measured in centimeters. Air Righting Ability: Animals were held upside down and dropped from a height of one foot into a container of bedding. The landing position of each animal was recorded. Body Temperature: Animals were removed from their cages and rectally measure for body temperature with a digital thermometer. Body Weight: Animals were removed from their cages and weighed at pretest and during the 2nd, 4th, 8th and 13th weeks of exposure. MOTOR ACTIVITY Method Using a modified version of Schulze's procedures (Schulze, 1990), the locomotor activity of all animals was monitored using an automated photobeam activity system. Sessions were 60 minutes in length; each session was divided into 12 intervals of 5 minutes. The time of testing was balanced across treatment groups at pretest and during the 2nd, 4th, 8th and 13th weeks of exposure ESTROUS CYCLICITY Daily vaginal smears were taken at approximately the same time each day, and the stage of estrus was determined for each female (main study animals) for three weeks prior to and on the day of termination. Care was taken to ensure that pseudo-pregnancy was not induced. CLINICAL LABORATORY STUDIES Blood was obtained from lightly anesthetized (carbon dioxide/oxygen; 60%/40%) animals via puncture of the orbital sinus (retrobulbar). Rats were fasted overnight prior to blood collection. The main study animals (up to 10/sex/group) were bled at the terminal interval. HEMATOLOGY Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant.

	Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean platelet volume Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Red cell distribution width Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (Henry, 1991) COAGULATION Blood for coagulation studies was collected (approximately 1.0 mL) into tubes containing sodium citrate anticoagulant. Serum samples were analyzed as follows: Prothrombin time Activated partial thromboplastin time CLINICAL CHEMISTRY Blood for clinical chemistry was collected (approximately 1.0 mL) into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Lactate dehydrogenase Sorbitol dehydrogenase Blood urea nitrogen Creatinine

	Glucose Creatine kinase Cholesterol Total protein Albumin Total bilirubin Direct bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transferase Triglycerides Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin ¿ globulin) Indirect bilirubin (calculated value; total bilirubin - direct bilirubin) MACROSCOPIC EXAMINATIONS Complete macroscopic postmortem examinations were performed on all main/neurotoxicity study animals including animals euthanatized in a moribund condition; all abnormal observations were recorded. The necropsy included examination of the external surface and all orifices; the external surfaces of the brain and spinal cord, the organs and tissues of the cranial, thoracic, abdominal and pelvic cavities and neck; and the remainder of the carcass. ORGAN WEIGHTS Organs were taken from all main study animals at the scheduled necropsy, weighed, recorded and organ/body and organ/brain weight ratios calculated. Organs were not weighed for animals euthanatized in a moribund condition during the course of the study. Prior to weighing, all organs were carefully dissected and properly trimmed to remove fat and other contiguous tissue in a uniform manner. Organs were weighed as soon as possible after dissection to avoid drying except the thyroid/parathyroids which were weighed after fixation. Paired organs were weighed together. TISSUES PRESERVED AND EXAMINED HISTOPATHO-LOGICALLY

	The tissues listed below were obtained at the scheduled sacrifice interval and preserved for all main study animals. adrenal gland aorta (thoracic) bone (sternum, left femur) bone marrow (rib) brain (medulla/pons, cerebrum and cerebellum) epididymides esophagus eye heart kidneys large intestine (cecum, colon, and rectum) lacrimal gland larynxc liver lungs (with mainstem bronchi) lymph node (mediastinal and mesenteric) mammary gland muscle (biceps femoris) nasopharyngeal tissued nerve (sciatic) optic nerve ovaries pancreas pituitary prostate salivary gland with submandibular lymph node seminal vesicles skin small intestine (duodenum, jejunum, ileum) spinal cord (cervical, thoracic, lumbar) spleen stomach testes thymic region

	thyroid (with parathyroids) trachea uninary bladder uterus (body/horns with cervix) Zymbal's gland gross lesions NEUROPATHOLOGY SACRIFICE Animals (5/sex/group) were anesthetized with an intraperitoneal injection (~1.0 mL/kg) of 26% sodium pentobarbital and transcardially perfused with phosphate buffered saline followed by 2% glutaraldehyde and 2% paraformaldehyde in the same buffer. After perfusion, the required tissues were dissected out. The carcass was placed in the same fixative as above for approximately 24 hours, followed by 10% NBF prior to dissection of tissues. Postmortem examination was limited to the tissues designated for microscopic evaluation. Measurement of the size (length and width) and weight of the whole brain (cerebrum, cerebellum and pons-medulla) were made. All tissues were then placed in a fresh solution of the same fixative prior to processing. Tissues listed in below were preserved for all designated neurotoxicity study animals (5/sex/group) after 13 weeks of treatment: brain (forebrain, central cerebrum, hippocampus, basal ganglia, midbrain, cerebellum and pons, medulla) eye with optic nerve spinal cord (cervical, thoracic, lumbar, cross and longitudinal sections) sciatic nerve (cross and longitudinal sections) tibial nerve (cross and longitudinal sections) sural nerve (cross and longitudinal sections) trigeminal ganglia dorsal root ganglia (from C3-C6 and L4-L6) dorsal root fibers (from C3-C6 and L4-L6) ventral root fibers (from C3-C6 and L4-L6) lungs and trachea tissues with macroscopic findings The tissues listed above were examined microscopically for all animals as indicated. Tissues with macroscopic lesions were examined in all animals. Any abnormalities not noted during macroscopic postmortem examinations which were seen during histological processing were recorded. Since microscopic findings indicative of an effect of test substance administration were not seen in high-exposure animals, examinations was be made of these tissues/organs for low- and mid-exposure animals.

	Several protocol deviations occurred during the study but were not considered to have compromised the validity or integrity of the study. STATISTICAL ANALYSIS The following parameters were analyzed statistically: · mean body weight values and body weight changes · mean feed consumption values (presented as grams of feed/kg of body weight/day) · mean clinical laboratory values · mean terminal organ weights, organ/body weight ratios and organ/brain weight ratios · sperm analysis · mean motor activity counts · mean FOB data including forelimb and hindlimb grip strength measurements · landing foot splay measurements and body temperature (rectal) · micronucleus counts · sperm analysis METHOD OF ANALYSIS Mean values of all exposure groups was compared to the mean value for the control group at each time interval. Evaluation of equality of group means were made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Bartlett, 1937; Sokal and Rohlf, 1995; Snedecor and Cochran, 1967) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data were analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, were additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953) and if differences were indicated, Shirley's test (Shirley, 1977), Steel's test (Steel, 1959) or Pairwise Comparison with Bonferroni Correction (Games and Howell, 1976) was used to determine which means differ from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. MOTOR ACTIVITY AND FUNCTIONAL OBSERVATIONAL BATTERY (FOB) DATA: All analyses included sex as an independent variable. Since there were not significant effects of sex, separate analyses by sex were not done to explain the nature of the effect. The analyses was performed by Graham Healey,

M.Sc. of Huntingdon Life Sciences, Cambridgeshire, England. The Testing Facility was responsible for the Subcontractor's GLP compliance. After submission of the final report, all of the records of the Subcontractor were shipped to the Testing Facility to be archived.

Motor activity
Repeated measures mixed modelling (using Proc Mixed in SAS) was applied to the motor activity data. The model proposed in the protocol included fixed terms for sex, group and their interaction, random animal, fixed period with AR(1) correlation and (five-minute) interval with unstructured correlation (Galecki 1994). However the input dataset contained over 4000 observations and the program would not run in a reasonable timescale. Hence the model was simplified by removing the interval term and analyzing the mean over the 12 intervals. This is only a minor modification since even in the original model comparisons between sexes, groups and periods would all be based on (weighted) means over the intervals. If the group term in the analysis was significant at the 5% level, then each treatment group was compared with the control using Dunnett's test (Dunnett 1955, 1964). The residuals were checked using the Kolmogorov-Smirnov test (Stephens 1974). If the test was significant at the 1% level, a Blom-transformation (SAS Institute 1999) was considered.

Continuous FOB parameters
Repeated measures mixed modelling (using Proc Mixed in SAS) was applied to the continuous FOB parameters. The model included fixed terms for sex, group and their interaction, random animal, fixed period with AR(1) correlation, the group-by-period interaction, with pre-dose as covariate. The residuals were checked using the Kolmogorov-Smirnov test (Stephens 1974). If the test was significant at the 1% level, a Blom-transformation (SAS Institute 1999) was considered. If the group term in the analysis was significant at the 5% level, then each treatment group was compared with the control using Dunnett's test (Dunnett 1955, 1964). If either of the interactions were strongly significant, then further tests were performed using the SLICE option in Proc Mixed.

Discrete FOB parameters
Repeated measures mixed modelling (using Proc Genmod in SAS), with multinomial distribution and cumulative logit link (Agresti 1989) was used for the discrete FOB parameters. The model included fixed terms for sex, group, random animal, fixed period and pre-dose as covariate. If the dose group effect in the model was statistically significant, each dose group was compared with the control group using pairwise contrasts. Many of the parameters had an insufficient number of non-normal findings to allow this analysis to be carried out.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male and female rats	=	10,000		ppm

Results Remarks:

Pre-study chamber distribution analyses showed that the test substance was evenly distributed within each chamber. Chamber monitoring showed that the chamber oxygen levels were at least 19%. Chamber room monitoring showed that no test substance was present in the room and that the sound and light levels were acceptable.

The mean (± standard deviation) analytical exposure concentrations of LPG were determined to be 0.0 ± 0.0, 1019 + 58, 5009 ± 174 and 9996 ± 261 ppm for the air control and the exposure groups, respectively. Particle sizing results indicated that the atmospheres were essentially gas/vapor only, as expected, since there was no substantial difference between the test substance chambers and the air control chamber.

Analysis of the major components in the neat test substance and the test atmospheres showed a reasonably close comparison between the neat test substance and the vaporized test substance. These data demonstrated that the test animals were exposed, as expected, to all of the major components of the test substance in their reasonably proper proportions. The data were consistent pretest and during the study indicating stability of the test substance and the atmosphere generation techniques.

All animals survived until the scheduled termination of the study except one main study female exposed at the 10000 ppm level which was euthanized on test day 83 because of poor condition and weight loss. From necropsy and histopathological findings the loss of this animal was considered the result of accidental trauma, not related to the exposures.

During the non-exposure periods, all animals were generally unremarkable except for a transient period during the 8th week of exposures where several main study animals in the 10000 ppm exposed group were noted with eye closure. This observation coincided with a transient loss of body weight and decreased feed consumption at this same interval, as discussed below. The cause of these transient differences was not clearly determined but was suspected to have been an otherwise undetected interruption in the water supply to some animal cages. A similar

	pattern was not noted in the neurotoxicity study animals for this same exposure group despite their being housed and exposed together with the main study animals. There were no test substance related differences in absolute body weights or in body weight changes in the test substance exposed animals, compared to the air control animals. However, as noted above, a transient difference (decreased weight or decreased weight gain) was noted during the 8th week of exposures especially in the 10000 ppm exposed group of main study animals. There were no test substance related differences in feed consumption in the test substance exposed animals, compared to the air control animals. However, as noted above, a transient difference (decreased feed consumption) was noted during the 8th week of exposures especially in the 10000 ppm exposed group of Main Study animals. There were no test substance related effects on functional observational battery parameters or on motor activity in the test substance exposed animals, compared to the air control animals. An increase in forelimb grip strength was noted for males only in the 5000 and 10000 ppm exposed groups from Week 4 of exposures at 10000 ppm and at week 13 at 5000 ppm. This minor finding, particularly in the absence of any other apparent functional effects, was considered unlikely to represent an adverse effect of treatment. There were no exposure-related differences in hematology values in test substance exposed animals compared to the air control animals. A few statistically significant decreases in WBC, lymphocyte and monocyte values were noted in all or some test substance exposed groups of female animals but the changes were not in an exposure-level-related pattern and were not similarly seen in the male animals. There were no exposure-related differences in clinical chemistry values in test substance exposed animals compared to the air control animals. A few statistically significant differences in glucose, sodium, potassium and total protein values were noted in all or some test substance exposed groups of animals but were not in a exposure-level-related pattern or the absolute differences were minimal or were only seen in one sex. There were no exposure-related differences s in organ weights and brain measurements in the test substance exposed animals compared to the air control animals. A few statistically significant differences from control animals were noted such as decreased kidney and thymus weights in the 5000 ppm exposed animals. However, these were not in a treatment-related pattern and/or the absolute differences were minimal. The brain weights in perfused Neurotoxicity study animals were lower in general than those in non-perfused main study animals since the perfusion fixatives desiccate the brain tissue.

	Among the various parameters of sperm count, motility and morphology that were examined for control and 10000 ppm exposure groups, there was only a slight increase in the incidence of 'mid-tail blob' (cytoplasmic droplet), as compared with control. This finding can be viewed as indicating relative immaturity of the affected sperm, and the increased incidence (4-12% of the 200 sperm evaluated in each animal) affected 4 of the 10 males examined in the group. The most usual incidence of this finding in control animals of this strain in this laboratory is 0-2% in each animal (similar to the concurrent control group in this study), although occasional control animals have shown 6-16% incidence. There were no correlative findings in the animals showing these higher incidences of cytoplasmic droplet in the 10000 ppm group, in terms of the other sperm parameters, histological findings, or general condition (body weight, etc). It was considered most likely that this difference from control was incidental and not related to treatment.
Conclusion:	A no-observed-adverse effect level (NOAEL) of 10000 ppm was determined for this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Liquified Petroleum Gas: A 13week whole-body inhalation toxicity study in rats with neurotoxicity assessments and in vivo genotoxicity assessements. Conducted for the American Petroleum Institute. Draft report 03-6141.

Repeated-Dose Toxicity

TEST SUBSTANCE

Category Chemical:	No CAS number
Test Substance:	No CAS number
Test Substance Purity/Composition and Other Test Substance Comments:	C5-C6 This hydrocarbon mixture is being used to characterize the repeated dose toxicity of the C5-C6 fraction for the refinery gas streams. Unleaded baseline gasoline API 99-01 Vapor Condensate Test material is a complex mixture of volatile hydrocarbons. The purity of mixture is 100% and stable based on analysis of chamber atmospheres.

Representative Components [98.8%] monitored in Study

Component	Area %
Isobutane	2.70
n-butane	12.78
3-methyl-1-butene	0.41
Isopentane	36.50
n-pentane	9.36
Trans-2-pentene	3.60
2,3-dimethylbutane	1.75
2-methylpentane	7.25

	3-methylpentane: 4.27 n-hexane: 3.62 Methylcyclopentane: 1.87 2,4-dimethylpentane: 1.36 Benzene: 2.75 2-methylhexane: 1.73 2,3-dimethylpentane: 1.52 3-methylhexane: 1.73 Isooctane: 1.92 Toluene: 3.91
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Whole body
Type of Exposure:	
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague Dawley [Crl: CD IGS BR]
Other Strain:	
Gender:	Male and female
Number of Animals per Dose:	10 males/10 females/group and 10 males/10 females in control and in high dose recovery groups
Concentration:	Target: 0, 2000, 10,000, and 20,000mg/m^3 Actual: 0, 2050, 10,153, and 20,324 mg/m^3
Dose:	
Year Study Performed:	2005
Method/Guideline Followed:	EPA OPPTS 870.3465
GLP:	Yes
Exposure Period:	13 weeks, [minimum 65 exposures]
Frequency of Treatment:	6 hours/day, 5 days/week
Post-Exposure Period:	4 weeks

Method / Guideline **and Test Condition Remarks:**	Baseline Gasoline Vapor Condensate was administered via whole-body exposures to Sprague Dawley rats for 13 weeks followed by a 4-week recovery period. The assessment included routine toxicology parameters as well as detailed evaluations of neurotoxicity parameters. The test substance was administered at target concentrations of 2000, 10000 and 20000 mg/m^3 for 6 hours/day, generally 5 days/week for 13 weeks. In addition, an Air Control group received nitrogen-enriched air only while in chamber. Exposure levels were determined using an infra-red spectrophotometer 4 times per chamber per day. The test substance's major components were assayed once per chamber per week. Particle size distribution measurements were also made once per chamber per week using a TSI Aerodynamic Particle Sizer. Viability checks were performed twice daily to check for mortality and signs of severe toxic or pharmacologic effects. Physical observations, body weight and feed consumption measurements were performed on all animals each week. Ophthalmoscopic examinations were conducted pretest and at study termination. Hematology, coagulation and clinical chemistry studies were performed for all animals at 4 weeks and termination of all exposures. Neurobehavioral testing was conducted on non-exposure days at least 16 hours post-exposure on 10 rats/sex/group pretest and at weeks 3, 7 and 12 of exposure according to OPPTS guideline 870.6200 Neurotoxicity Screening Battery. The Functional Observational Battery (FOB) was performed before evaluation of motor activity. FOB included home cage and handling evaluation, open field trials, reflex assessment, grip strength, landing food splay, hindlimb extensor strength and air righting ability. Motor activity was monitored using an automated Photobeam activity system. After 13 weeks of exposures, all animals were sacrificed except recovery animals, which were sacrificed after an additional 4-week recovery period. Selected organs were weighed [brain, heart, liver, lungs, adrenal glands, kidneys, spleen, thymus, ovaries, uterus, testes, seminal vesicles, prostate, epididymides] and organ/body weight and organ/brain weight ratios calculated. Complete macroscopic postmortem examinations were performed on all animals. Histopathological evaluations of 31 tissues were conducted on all Air Control and 20000 mg/m^3 exposed animals at the Terminal interval and at the Recovery interval. Lungs and gross lesions from all animals and kidney tissue from all male rats were examined at the Terminal interval. At terminal sacrifice five rats/sex/group were perfused for neuropathology. Brain size and weight were measured and sections of brain, eye with optic nerve, spinal cord, peripheral nerves and dorsal and ventral root ganglia were examined microscopically. Statistical methods: Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartletts' test was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric method used was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance. If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's, Williams, or Cochran and Cox's modified t-test. The nonparametric method was the Kruskal-Wallis test and if differences were indicated, Shirley's tes, Dunn's test, Steel's test or Pairwise Comparison with Bonferroni Correction were used to determine where means differed from control. Bartlett's test for equality of variance was conducted at

	the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Neurobehavioral statistics: The statistical analysis of the continuous FOB variables was by a mixed model analysis of covariance with a first order autoregressive error structure on the time points. The pretest response was used as the covariate. The residuals from the model were tested for normality by the Shapiro-Wilk test. Those variables that did not exhibit normally distributed residuals at the 0.01 level of significance were transformed by Blom's normalized rank transformation and reanalyzed. The nominal and count data of the FOB were analyzed by a cumulative logit repeated measures analysis. The statistical model partitions the variation of the response variable among the variables sex, dose group, week number and their interactions. Motor activity data were analyzed by a mixed model analysis of covariance with an unstructured error relationship among the five-minute periods, and a first order autoregressive error structure on weeks. The pretest response was used as the covariate. The residuals from the model were tested for normality by the Shapiro-Wilk test. Those variables that did not exhibit normally distributed residuals at the 0.01 level of significance were transformed by Blom's normalized rank transformation and reanalyzed. Satellite groups of animals for genetic toxicity, immunotoxicity and glial fibrillary acidic protein [GFAP] measurement assays were exposed with the subchronic animals. Robust summaries for these studies are provided separately.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Both Sexes	=	20000		mg/m^3
NOAEL	Both Sexes		10000		mg/m^3

Results Remarks:	The mean (± standard deviation) analytical exposure concentrations of Baseline Gasoline Vapor Condensate were determined to be 0 ± 0, 2050 ± 154, 10148 ± 739 and 20324 ± 1183mg/m^3 for the Air Control and the exposure groups, respectively. The analytically measured exposure levels of the airborne test substance were reasonably close to the targeted exposure levels and the nominal exposure levels. Chamber environmental conditions averaged 24^{0}C and 45% relative humidity. Particle sizing results indicated that the atmospheres were essentially vapor only, as expected, since there was no substantial difference between the particulate levels in the test substance chambers and the Air Control chambers. Analysis of the major components in the neat test substance and the test atmospheres showed a close comparison between the neat test substance and the vaporized test substance. This data demonstrated that the test animals were exposed, as expected, to all of the major components of the test substance in their proper proportion. The data was also consistent from week-to-week during the study indicating stability of the test substance and the atmosphere generation techniques. All animals survived throughout the exposures and recovery phases of the study except a 20000 mg/m^3 exposed male which was humanely sacrificed on day 34 of the exposures phase because of an accidental injury, a 10000 mg/m^3 exposed male which was found dead on day 58 of the exposures phase and a 10000 mg/m^3 exposed male which was humanely sacrificed on day 69 of the exposures phase because of swollen limbs. These deaths were considered unrelated to test substance exposure. The test animals were unremarkable in the chambers during the exposure periods. The test animals were also generally unremarkable during the non-exposure periods. However, a slight increase in red nasal discharge was seen in the 20000mg/m^3 animals during the 13 weeks of exposures but not during the 4-week recovery period. There were no toxicologically significant differences in ophthalmoscopic findings in the test animals compared to the Air Control animals. There were no toxicologically significant differences in body weights and feed consumption in the test animals compared to the Air Control animals. Test substance exposure was not associated with any change in counts of the 26 nominal or the 4 continuous FOB measures or with changes in motor activity. There were no toxicologically significant differences in clinical chemistry, hematology and coagulation values in the test substance animals compared to the Air Control animals at the 4th week and terminal intervals. There were no toxicologically significant differences in organ weights and brain measurements in the test animals compared to the Air Control animals at the Terminal and/or Recovery intervals. No gross abnormalities related to test substance exposure were evident on necropsy examination. Some of the male and female rats exposed to 20000 mg/m^3 of Baseline Gasoline Vapor Condensate had eosinophilic material within the nasolacrimal duct lumen at the Terminal sacrifice. This finding was considered to correlate with the increase in red nasal discharge noted previously in this group of test animals. Similar changes were not evident in control animals. Microscopic findings that were considered exposure-related were found also in the kidneys of male animals exposed to all levels of Baseline Gasoline Vapor Condensate following the Terminal sacrifice. These renal histopathologic changes were consistent with hyaline droplet nephropathy, attributable to accumulation of alpha-2 microglobulin

	within renal tubular epithelial cells. This species- and gender-specific change has been well documented in male rats exposed to a variety of hydrocarbon compounds and is not considered relevant to humans (US EPA 1991). The 20000 mg/m^3 exposed males sacrificed following a 4-week recovery period had near complete resolution of the relevant histologic changes. No test substance related histopathologic changes were noted in other protocol-specified tissues including lung, nasoturbinates, and larynx. No neuropathologic microscopic changes attributable to test substance effect were observed in brain, spinal cord, eyes, peripheral nerves, or ganglia among the 20000 mg/m^3 exposed satellite animals.
Conclusion:	Thirteen weeks of exposure of rats to Baseline Gasoline Vapor Condensate resulted in hydrocarbon nephropathy in male animals exposed to all exposure levels of vapor, a species and sex specific syndrome not considered to be relevant to human risk assessment. Exposure also resulted in slight but reversible increases in red nasal discharge in animals exposed to 20000 mg/m^3 of vapor. Therefore, the 10000mg/m^3 exposure level (excluding male rat nephropathy) was considered a no observable adverse effect level. The NOAEL for neurotoxicity =20000mg/m^3
RELIABILITY/DATA QUALITY	
Reliability:	Reliable without Restriction (KS=1)
Reliability Remarks:	HPV Supporting study from Section 211(b) Testing Consortium, Fuels and Fuel Additives Health Effects Testing Regulation, administered by API, Washington DC
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Baseline Gasoline Vapor Condensate: A 13-Week Whole Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments and 4-Week In Vivo Genotoxicity and Immunotoxicity Assessments. 2005. HLS Study No. 00-6125. Huntingdon Life Sciences Laboratories, East Millstone, NJ. US EPA 1991. Alpha 2 microglobulin: Association of chemically induced renal toxicity and neoplasia in male rats. In Risk Assessment Forum, p.85. US Govt Printing Office, Washington DC.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	71-43-2
Test Substance:	Benzene
Test Substance Purity/Composition and Other Test Substance Comments:	No data
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	Bacterial reverse mutation assay
Concentrations:	0, 3, 6, 15, 30, 100, 150, 300, 1000 ppm (exposed by vapor)
Year Study Performed:	1989
Method/Guideline Followed:	Other

GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Not identified.
Method/Guideline and Test Condition Remarks:	Type: Salmonella typhimurium reverse mutation assay Method: The in vitro potential mutagenic activity of benzene was investigated by the Ames test using 4 strains of bacteria Salmonella typhimurium: TA 1535, TA 100, TA 104 and TA 98. This test enables the detection of base-pair substitution and frameshift mutagens. Tests were also conducted on benzene and its following metabolites (additional bacteria strains were added, i.e., TA 102 and TA 97): benzene oxide, phenol, hydroquinone, 4,4'-dihydroxybiphenyl, 2,2'-dihydroxy-biphenyl, quinone, trans-benzene-1,2-dihydrodiol , catechol and 1,2,4-trihydroxybenzene. Duroquinone, and anti-benzene-diol-epoxide, syn-benzene-diol-epoxide and 1,2,3-trihydroxybenzene were also included. S. typhimurium was exposed to benzene vapor in desiccators to allow for longer exposure periods (as opposed to plate incorporation method). Each assay was carried out both in the absence and in the presence of a metabolic activation system, NADPH-fortified, S9 mix derived from Aroclor 1254 induced rat or mouse liver homogenate (17 mg/plate). The test compound, bacteria and S9 fractions or buffer were preincubated for 20 min at 37°C and then added to minimal agar plates. After incubation for 3 days, the colonies were counted. A response was considered to be positive response if the number of colonies was > 2 times the control value. The concentrations were: 0, 3, 6, 15, 30, 100, 150, 300, 1000 ppm (exposed by vapor) Vehicle used: Yes Vehicle Name: Unknown Positive control: none Metabolic Activation: With and Without
TEST RESULTS	
Details for Cytogenetic Assay (if applicable):	**Details for Cytogenetic Assay (if applicable):**

Statistics:	2 times control used as a measure of positive activity

Species:	**Strain:**	**Metabolic Activation:**	**Genotoxic Effect:**	**Conclusion:**
Bacteria	S. typhimurium TA 1535	With Without	Positive Negative	Positive with S9
Bacteria	S. typhimurium TA 104	With Without	Weak + Negative	Weakly positive with S9
Bacteria	S. typhimurium TA 98	With Without	Negative Negative	Negative with and without S9
Bacteria	S. typhimurium TA 100	With Without	Weak + Negative	Weakly positive with S9
Other Species:				
Other Strain:				

Results Remarks:	Benzene was found to be mutagenic in the presence but not the absence of S9. The most responsive strain was TA 1535. A 2-fold increase in the number of mutants above control was observed even at a benzene concentration as low as 10 ppm. However, further increases in the concentration had only a modest effect. The maximal mutant number was about 3-fold the values for the control plates. Similar effects were then seen over a wide concentration range. In this same strain (TA 1535), the metabolites trans-benzene-1,2-dihydrodiol in the presence of S9 and anti-benzene-diol-epoxide and syn-benzene-diol-epoxide in the absence of S9 induced mutations. No other metabolite including catechol to which trans-benzene-1,2-dihydrodiol is converted by cytosolic dihydrodiol dehydrogenase, gave a positive result in strain TA1535. Mutagenic responses, some of them weak, were noted in other strains treated with 1,2,3-trihydroxybenzene, 1,2,4-trihydroxybenzene, catechol, quinine, hydroquinone, syn-benzene-diol-epoxide and anti-benzene-diol-epoxide. Cytotoxic Concentration: not identified
Conclusion Remarks:	Positive in the presence of metabolic activation system.

RELIABILITY / DATA QUALITY

Reliability:	Valid With Restrictions (KS=2)
Reliability Remarks:	Not a guideline test but in accordance with generally accepted scientific standards and described in sufficient detail. No positive control. No data about the test substance.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Glatt H, Padykula R, Berchtold GA, et al. (1989) Multiple activation pathways of benzene leading to products with varying genotoxic characteristics. Environ Health Perspect 82:81-89.

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	71-43-2
Test Substance:	Benzene
Test Substance Purity/Composition and Other Test Substance Comments:	Benzene, Aldrich Chemical Co., Milwaukee, Wis.
Category Chemical Result Type:	Measured

Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	Sister chromatid exchange assay – human peripheral lymphocytes
Concentrations:	16, 78, 391 mg/l
Year Study Performed:	1986
Method/Guideline Followed:	Other
GLP:	No data
Positive, Negative and Solvent Control Substance(s):	No positive control
Method/Guideline and Test Condition Remarks:	Type: Sister chromatid exchange assay – human peripheral lymphocytes Benzene was tested with or without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9) of rats induced with Aroclor 1254. Heparinized whole blood was obtained from healthy adult men. Benzene was dissolved in serum-free culture medium and the metabolic activation system (S9 mix derived from Arachlor-induced rat liver) and incubated in a flask for 2 hours. The flask was agitated to ensure even distribution of active metabolites among the cells. After incubation, the cells were washed, resuspended in the same medium and incubated further. SCEs were analyzed in 35 consecutive second-division cells for each point. 200 metaphase cells were scored to determine the percentage of cells in X1, X2, and X3+ divisions. Vehicle used: Yes Vehicle Name: No Data Metabolic Activation: With
TEST RESULTS	
Details for Cytogenetic Assay (if applicable):	

Statistics:	

Species:	**Strain:**	**Metabolic Activation:**	**Genotoxic Effect:**	**Conclusion:**
Human lymphocytes		With	Positive	Positive
Other Species:				
Other Strain:				

Results Remarks:	An increased number of SCEs were found in cultures treated in the presence of 10% S9 mix. In the absence of S9 and at S9 concentrations of 1 or 90% no increase in the frequency of SCEs was noted. 10% S9 mix is thought to be the optimal concentration for converting benzene into the active metabolites that might be responsible for the induction of SCEs. When the cells were exposed to benzene concentrations of 2 X 10-4, 1X 10-3 and 5 X 10-3M (approximately 16, 78 and 391 mg/l), a dose-related increase in SCEs was seen when the appropriate activation concentration was used. S9 mix at 10-30% converted benzene into active forms that were cytotoxic and delayed cell turnover times. Further examination of this suggested that the metabolites responsible for cell division delay may be different from those which induce SCE. The addition of glutathione to the culture caused a dose-dependent decrease in SCEs in cells exposed to benzene and S9 mix. The addition of glutathione also completely prevented the induction of SCEs by catechol and hydroquinone, two major phenolic metabolites of benzene and potent inducers of SCEs. Genotoxic Effect: Positive No positive control
Conclusion Remarks:	Benzene did induce structural chromosome aberrations in cultured human lymphocytes.

RELIABILITY/DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study
Key Study Sponsor Indicator:	Key

REFERENCE	
Reference:	Morimoto K (1983) Induction of sister chromatid exchanges and cell division delays in human lymphocytes by microsomal activation of benzene. Cancer Res 43:1330-1334.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	106-99-0
Test Substance:	1,3-Butadiene
Test Substance Purity/Composition and Other Test Substance Comments:	No data
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	

METHOD	
Type of Study:	Bacterial reverse mutation assay (Ames *Salmonella* test)
Concentrations:	0, 30, 40, 50, and 60% 1,3-butadiene in air
Year Study Performed:	1990

Method/Guideline Followed:	Other
GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Negative Control Substance Remarks: solvent control
Method/Guideline and Test Condition Remarks:	Type: Salmonella typhimurium reverse mutation assay Method: The in vitro potential mutagenic activity of 1,3-butadiene was investigated by the Ames test using 4 strains of bacteria Salmonella typhimurium: TA97, TA98, TA100, TA1535. This test enables the detection of base-pair substitution and frameshift mutagens. The test substance was tested on two independent assays. Each assay was carried out both in the absence and in the presence of a metabolic activation system (Arochlor 1254-induced and uninduced rat and mouse S9, and human S9) at a level of 0.8 mg/ protein/plate. Concentrations of 1,3-butadiene gas were metered into specially constructed treatment chambers holding the agar plates overlaid with the bacteria and activation system. Actual gas concentrations were determined by gas chromatography before and after the 48 hour exposure period. Different treatment chambers were used for each activation system and for the non-activated treatment. S9 preparations were made according to the procedure of Ames *et al.* (1975).
TEST RESULTS	
Details for Cytogenetic Assay (if applicable):	
Statistics:	2X solvent control used as a measure of positive activity

Species:	Strain:	Metabolic Activation:	Genotoxic Effect:	Conclusion:
Bacteria	S. typhimurium TA 1535	Rat/With - induced	Positive	Barely 2 fold above background at 30 % 1,3-butadiene in air.
Bacteria	S. typhimurium TA	Rat/With -	Positive	Barely 2 fold above

	1535	uninduced		background at 30 % 1,3-butadiene in air.
Bacteria	S. typhimurium TA 1535	Mouse/ With - uninduced	Positive	Barely 2 fold above background at 30 % 1,3-butadiene in air.
Bacteria	S. typhimurium TA 1535	Human/ With	Negative	Negative
Bacteria	S. typhimurium TA 1535	Without	Negative	Negative
Other Species:				
Other Strain: S. typhimurium TA 97, TA 98, TA100		Negative		

Results Remarks:	1,3-Butadiene (BD) induced revertants only in strain TA1535. Mouse S9 showed slightly higher activity than the uninduced rat or human S9 at 30% 1,3-butadiene in air. At concentrations greater than 30%, the number of revertants decreased in the presence of rat or human S9. Results from the human S9-activated treatments did not differ substantially from those of the non-activated treatments. Arochlor 1254-induced rat S9 gave similar results as mouse S9 (uninduced). Since the response was weak, the S9 concentration was increased from 0.8 mg/plate to 4.0 mg/plate. Increasing the concentration of Arochlor 1254-induced rat S9 had no effect on the number of revertants; slightly more revertants were observed using 4.0 than 0.8 mg/plate of uninduced rat S9.
Conclusion Remarks:	*Salmonella typhimurium* reverse gene mutation (Ames) tests of 1,3-butadiene using strains TA1535, TA97, TA98, and TA100 and employing rat, mouse, and human liver S9 metabolic systems were barely 2-fold above background only in strain TA1535 at 30% 1,3-butadiene in air with induced and uninduced rat S9 and mouse S9 (uninduced). In general, 1,3-butadiene was a weak *in vitro* genotoxin

RELIABILITY / DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study
Key Study Sponsor Indicator:	
REFERENCE	
Reference:	Arce GT., DR Vincent, MJ Cunningham, WN Choy and AM Sarrif. 1990. *In vitro* and *in vivo* genotoxicity of 1,3-butadiene and metabolites. Environ. Health Perspect. 86:75-8.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	106-99-0
Test Substance:	1,3-Butadiene
Test Substance Purity/Composition and Other Test Substance Comments:	No data
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	

Type of Study:	Sister chromatid exchange assay – Chinese Hampster Ovary (CHO) cells
Concentrations:	24, 30, 200, 200 uM
Year Study Performed:	1991
Method/Guideline Followed:	Other
GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Solvent control: ethanol Positive control: cyclophosphamide
Method/Guideline and Test Condition Remarks:	Type: Sister chromatid exchange assay – CHO cells. 1.3-Butadiene was tested with or without a metabolic activation system, the S9 mix, prepared from a liver microsomal fraction (S9) of rats induced with Aroclor 1254. Tests were also conducted on 1,3-butadiene and its following metabolites: monoepoxybutene, diepoxybutane. CHO cells were cultured by a method previously described (Hytonen et al, 1983). The test chemicals were added after 24 hr of incubation, and then pulse treated. The duration of the pulse treatment was 4 hr in serum-free bromodeoxyuridine (BudR)-free medium, in the presence or absence of S9 mix. The cultures were rinsed and incubated for the next 24 hr with BudR added. The concentrations of chemicals, used in experiments were as follows: 1,3-butadiene, 25, 50, 100 and 200 uM; monoepoxybutene, 1, 5, 25, 50, 100 and 200 uM; diepoxybutane, 0.1, 1, 50 and 100 uM. Duplicate cultures were set up for each treatment. SCEs were stained and scored from the second-division cells. 60 cells per treatment point were analyzed and the statistical significances were calculated using a one-tailed Student's t-test. Solvent control: ethanol Positive control: cyclophosphamide Metabolic Activation: With and Without
TEST RESULTS	
Details for Cytogenetic Assay (if applicable):	

Statistics:	One-tailed Student's t-test.

Species:	Strain:	Metabolic Activation:	Genotoxic Effect:	Conclusion:
CHO		With	Wk +	Weakly positive with metabolic activation.
CHO		Without	Negative	Negative
Other Species:				
Other Strain:				

Results Remarks:	In the absence of S9 mix no increase of SCEs was observed even at the highest concentration of 1.3-butadiene. In the presence of S9, a slight dose response was observed. Both metabolites of 1.3-butadiene (monoepoxybutene and diepoxybutane) demonstrated a very clear dose-dependent increase in SCEs, both with and without S9 mix. Cytotoxic Concentration: Without S9: >200 uM With S9: >200 uM
Conclusion Remarks:	1,3-Butadiene is weakly positive for inducing SCEs in cultured CHO cells with a metabolic activation system.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Sasiadek M, H Järventaus, M Sorsa. 1991. Sister-chromatid exchanges induced by 1,3-butadiene and its epoxides in CHO cells. *Mutat Res*. 263; 47-50.

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	107-07-7
Test Substance:	Butene-2
Test Substance Purity/Composition and Other Test Substance Comments:	Butene-2 (42.4% cis, 55.3% trans) from Union Carbide Industrial Gases. Certificate of analysis from supplier. This hydrocarbon is being used to characterize the in vitro genotoxicity of the C1-C4 fraction for the refinery gas streams.
Category Chemical Result Type:	Measured
Unable to Measure orEstimate Justification:	
METHOD	
Type of Study:	Bacterial reverse mutation assay
Concentration:	
Concentrations:	0.0, 10, 20, 40, 60, 80%

Year Study Performed:	1992
Method / Guideline Followed:	OECD Guideline #471 (1981), Method B14 of Commission followed Directive 84/449/EEC
GLP:	Yes
Positive, Negative and Solvent Control Substance(s):	Negative Control Substance Remarks: clean dry air Positive control: Without activation system: N-ethyl-N' nitro-N-nitrosoguanidine, 3 µg/plate for TA100, 5 µg/plate for TA1535 9 amino acridine, 80 µg/plate for TA1537; 4-Nitroquinoline-1-oxide, 0.2 µg/plate for TA98; With activation system: 2-aminoanthracene 2 µg/plate for TA1535 benzo(a)pyrene 5 µg/plate for all other strains. Vinyl chloride 50% conc. was gaseous positive control for all strains
Method / Guideline and Test Condition Remarks:	Type: Salmonella typhimurium reverse mutation assay Method: The in vitro potential mutagenic activity of butane-2 was investigated by the Ames test using 4 strains of bacteria Salmonella typhimurium: TA 1535, TA 1537, TA 100, and TA 98. This test enables the detection of base-pair substitution and frameshift mutagens. Activation system: Sprague Dawley male rat liver (S9 fraction). 10% S9 fraction in S9 mix, (0.05 ml S9 fraction/plate) Aroclor 1254 induced; 500mg/kg single ip injection 5 days before sacrifice. A 0.1 ml aliquot of Salmonella, 2.0 ml molten top agar, 0.5 ml S9 mix or 0.5 ml pH 7.4 phosphate buffer were mixed in a test tube and poured on minimal agar plates (3 plates/ conc./± S9 mix). Atmospheres of varying concentrations (0.0, 10, 20, 40, 60, 80%) were generated by mixing Butene-2 with clean dry air, using precalibrated gas flow meters as gas flow indicators. Mixtures passed into 10L stainless steel containers holding Salmonella plates with triple vented lids. Concentrations were selected based on a preliminary range finding test with TA100 ± S9; dose-related reduction in frequency of revertant colonies and reduced growth of background lawn observed at 80, 100%. Containers holding 3 stacks of 8 plates each were flushed with appropriate concentrations of butene-2 for 5 minutes to allow system to equilibrate; containers were incubated at 37 degrees C for 48 hrs and number of revertant colonies counted. Analytical determinations were performed by GC on syringe samples of test atmospheres at representative concentrations.

	Positive control compounds were: -S9, N-ethyl-N' nitro-N-nitrosoguanidine, 3 µg/plate for TA100, 5 µg/plate for TA1535; 9 amino acridine, 80 µg/plate for TA1537; 4-Nitroquinoline-1-oxide, 0.2 µg/plate for TA98; +S9, 2-aminoanthracene 2 µg/plate for TA1535; benzo(a)pyrene 5 µg/plate for all other strains. Vinyl chloride 50% conc. was gaseous positive control for all strains; negative control was clean dry air. The complete experiment was repeated using fresh bacteria cultures, test material and control solutions. Criteria for positive response were induction of dose-related and statistically significant increases in mutation rate in one or more strain of bacteria ± S9 in both experiments at subtoxic doses.

TEST RESULTS

Details for Cytogenetic Assay (if applicable):	
Statistics:	Dunnett's method of linear regression

Species:	Strain:	Metabolic Activation:	Genotoxic Effect:	Conclusion:
Bacteria	S. typhimurium TA 1535	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 1537	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 100	With and Without	Negative	Negative
Bacteria	S. typhimurium TA 98	With and Without	Negative	Negative
Other Species:				
Other Strain:				

Results Remarks:	Toxicity was exhibited in all strains at 80% butene-2. In experiment 2, slight toxicity also occurred at 60%. No significant increases in number of revertant colonies of any strain of bacteria were observed at any dose concentration ± S9. Controls performed appropriately.
Conclusion Remarks:	Butene-2 was not mutagenic in the *Salmonella typhimurium* assay with or without metabolic activation

RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Thompson, PW. 1992. Butene-2: Reverse mutation assay "Ames test" using *Salmonella typhimurium*. Proj. #44/812. SafePharm Laboratories, UK, Derby UK. [2-butene].

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	107-07-7
Test Substance:	Butene-2
Test Substance Purity/Composition and Other Test Substance Comments:	Butene-2 (42.4% cis, 55.3% trans) from Union Carbide Industrial Gases. Certificate of analysis from supplier. This hydrocarbon is being used to characterize the in vitro genotoxicity of the C1-C4 fraction for the refinery gas streams.
Category Chemical Result Type:	Measured

Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	*in vitro* mammalian chromosome aberration test
Concentration:	
Concentrations:	0.0, 10, 20, 40, 50, 60, 80, 100%
Year Study Performed:	1992
Method/Guideline Followed:	OECD Guideline 473 (1981), Method B10 of Commission Directive 84/449/EEC
GLP:	Yes
Positive, Negative and Solvent Control Substance(s):	Negative: yes Positive: yes
Method/Guideline and Test Condition Remarks:	Type: Chromosome aberrations in mammalian cells. Metaphase analysis in primary blood lymphocyte cultures. Metabolic activation system: Sprague Dawley male rat liver (S9 fraction) -20% S9 fraction in S9 mix, (10% v/v S-9 mix/flask) Aroclor 1254 induced; 500 mg/kg single ip injection 5 days before sacrifice. Atmospheres of varying concentrations were generated by mixing butene-2 with clean dry air, using precalibrated gas flow meters as gas flow indicators. Mixtures passed through culture flasks for sufficient time (time not specified) to allow equilibration of the system. Analytical determinations were performed by GC on syringe samples of test atmospheres at representative concentrations. Blood samples were drawn from male rats (Sprague Dawley -CD-1, ages 8-20 wks. from CharlesRiver UK); cells were grown in RPMI medium supplemented with 10% fetal calf serum, 25 mM Hepes and antibiotics, at 37 degrees C in a humidified atmosphere of 5% carbon dioxide in air. Duplicate cultures were incubated for 48 hrs, then transferred to tubes, centrifuged and culture medium drawn off and saved. Cells were resuspended in flasks, in fresh culture medium with or without S9 metabolic activation mix and exposed to appropriate concentrations of butene-2 or control materials. Flasks were sealed and shaken to maximize cell exposure for 4 hrs +S9 or 20 hrs -S9. Cells exposed to butene-2 + S9 were resuspended after 4 hrs in original culture medium; one group was harvested at 20 hrs (16 hr recovery), the other at 30 hrs (26 hr recovery) after initiation of treatment; -S9 cultures were harvested after 20 full hours exposure to butene-2. Positive controls were ethyl methyl sulfonate (500 µg/ml) –S9,

	cyclophosphamide (4.2 µg/ml) +S9; gaseous control was vinyl chloride (50%) in 20 hr group –S9 and 30 hr group +S9. Negative control was clean, dry air.
TEST RESULTS	
Details for Cytogenetic Assay (if applicable):	
Statistics:	Frequency of cells with aberrations (± gaps) and frequency of polyploid cells (duplicate culture data pooled) were compared with concurrent vehicle control using Fisher's Exact Test UKEMS, Statistical Evaluation of Mutagenicity Test Data (1989).

Species:	**Strain:**	**Metabolic Activation:**	**Genotoxic Effect:**	**Conclusion:**
Rat lymphocytes		With and Without	Negative	Negative
Other Species:				
Other Strain:				

Results Remarks:	Butene-2 caused hemolysis in +S9 cultures at concentrations of 50% and above. In –S9 cultures, 80 and 100% concentrations caused cultures to turn dark brown but return to normal red color by cell harvest. Butene-2 induced steep dose-related decreases in mitotic indices ± S9; especially toxic to lymphocytes at 80% in +S9 20 hr harvest group. However, butene-2 did not induce significant dose-related increases in frequency of structural chromosome aberrations or polyploid cells at any concentration level at any harvest period ± S9. Control compounds performed appropriately. Cytotoxic Concentration: Without S9: >80% With S9: >50%
Conclusion Remarks:	Butene-2 produced no significant increases in frequency of chromosome aberrations either in the presence or absence of a liver enzyme metabolizing system. Butene-2 is not clastogenic to rat lymphocytes *in vitro*.
RELIABILITY/DATA QUALITY	

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Wright, NP. 1992. Butene-2: Metaphase analysis in rat lymphocytes *in vitro*. Proj. #44/813. SafePharm Laboratories, UK, Derby UK.

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	109-66-0 (pentane), 106-97-8 (butane), 74-98-6 (propane), 75-28-5 (isobutane), 78-78-4 (isopentane) [**note** – will need 5 separate entries of same RSS for HPVIS – one for each CASRN]
Test Substance:	Pentane, butane, propane, isobutane, and isopentane
Test Substance Purity/Composition and Other Test Substance Comments:	Six gases of the following composition were tested: Gas A n-Butane 99.7%; iso-Butane 0.3% Gas B iso-Butane 96.3% n-Butane 3.8% propane 0.3% Gas C

	Propane >99.9% iso-Butane trace n-Butane trace Gas D iso-Pentane 97.2% n-Pentane 2.8% Gas E n-Pentane 98.7% cycloPentane 0.6% cis-pentane-2 - Gas F iso-Butane 97.4% n-Butane 2.19% propane 0.4% ethane 0.01%
Category Chemical Result Type:	Measured

Type : Ames test
System of testing : Salmonella typhimurium, reverse mutation assay using strains TA98, TA100, TA1535, TA1537 & TA1538.
Test concentration : Concentrations of ranging between 1 to 50% in air.
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: OECD 471, modified to test gaseous substances
Year : 1980
GLP : no data
Test substance : other TS: 6 gases
Method : Duplicate plates seeded with the respective Salmonella strains (with and without S9 fractions) were placed in desiccators from which air was withdrawn and replaced by the gases under test. Test concentrations were 10, 20, 30, 40 and 50% in air.

The plates were exposed for 6 hours to the gas mixtures in the sealed desiccators, after which time they were removed and incubated at 37°C for an additional 40-45 hours.
The number of histidine revertants were counted and recorded. Negative and positive (methylene chloride) controls were also carried out.
Rat S9 fractions were used for metabolic activation.
The authors do not specify the criteria they used for a positive response.

Result **:** The revertants per plate for each of the test material and controls is shown in the following table. Only the results from the highest non-toxic concentrations are shown.

Material		Salmonella strain				
+/- activation	Conc'n	TA1535	TA1537	TA1538	TA98	TA100
Negative control						
- activation		15	12	10	29	138
+ activation		16	18	30	38	155
Positive control (methylene chloride)						
- activation	2	34	10	16	234	900
+ activation	2	52	12	52	237	1066
Gas A (n-butane)						
- activation	50	24	6	18	22	122
+ activation	50	26	4	37	48	134
Gas B (isobutane)						
- activation	50	23	4	7	26	108
+ activation	50	10	4	16	26	98
Gas C (propane)						
- activation	50	20	18	14	18	114
+ activation	50	18	5	16	21	88
Gas D (isopentane)						
- activation	10	22	3	14	15	124
+ activation	10	10	6	16	22	124

Gas E (n-pentane)						
- activation	10	25	1	8	26	138
+ activation	10	14	6	22	18	116
Gas F (isobutane)						
- activation	40	23	10	10	28	103
+ activation	40	7	6	14	18	91

The positive control (methylene chloride) was mutagenic in strains TA98 and TA100 and was slightly mutagenic in TA1535 Neither n-butane, iso-butane nor propane were toxic or mutagenic at any of the concentrations tested.

Isopentane was toxic at concentrations of 10% and above. Further studies were carried out at 1, 2, 5 and 8% and no mutagenicity was found at these lower concentrations.

n-Pentane was toxic at concentrations of 25 and 50%. Further studies were carried out at 1, 2, 5, 8 and 10% and no mutagenicity was found at these non-toxic concentrations.

Gas F (97.4% Iso butane) was weakly toxic at a concentration of 50% but was not mutagenic at concentrations of 5, 10, 20, 30 or 40%.

In conclusion, none of the hydrocarbons were mutagenic with or without metabolic activation in the Ames Salmonella assay in 5 strains exposed for 6 hours in desiccators.

Test substance **:** Six gases of the following composition were tested:

Gas A

n-Butane	99.7%
iso-Butane	0.3%

Gas B

iso-Butane	96.3%
n-Butane	3.8%
propane	0.3%

Gas C
Propane >99.9%
iso-Butane trace
n-Butane trace

Gas D
iso-Pentane 97.2%
n-Pentane 2.8%

Gas E
n-Pentane 98.7%
cycloPentane 0.6%
cis-pentane-2 -

Gas F
iso-Butane 97.4%
n-Butane 2.19%
propane 0.4%
ethane 0.01%

Reliability : (1) valid without restriction
27.08.2001

Kirwin, C.J and Thomas, W.C. (1980) In vitro microbiological mutagenicity studies of hydrocarbon propellants. J. Soc. Cosmet. Chem. Vol. 31., pp 367-370

TEST SUBSTANCE	
Category Chemical:	No CAS No.
Test Substance:	No CAS No.
Test Substance Purity/Composition and Other Test Substance Comments:	C5-C6 This hydrocarbon mixture is being used to characterize the in vitro genotoxicity of the C5-C6 fraction for the refinery gas streams. Unleaded gasoline (wholly vaporized gasoline)
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	Mouse lymphoma assay Forward mutation assay using cell line L5178Y TK+/-
Concentrations:	0.065, 0.13, 0.26, 0.52, 1.04 ug/ml
Year Study Performed:	1977
Method/Guideline Followed:	Other

GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Solvent control: Acetone Positive control: ethylmethanesulfonate (EMS) – without activation Positive control: dimethylnitrosamine– with activation
Method / Guideline and Test Condition Remarks:	The test material was dissolved in acetone for this assay. The positive control substances were Ethyl methane sulphonate (EMS) and Dimethylnitrosamine (DMN). A cytotoxicity study was carried out prior to the mutagenicity assay. For the mutation assay the lymphoma cells were exposed for 5 hours to test material at concentrations ranging from 0.065 to 1.04 µl/ml for both the activation and non-activation assays. Metabolic activation was accomplished using Araclor-induced rat liver S-9 suspension. After exposure to the test material, the cells were allowed to recover for 3 days and then cultures were selected for cloning and mutant selection. Surviving cell populations were determined by plating diluted aliquots in non-selective growth medium. A mutation index was derived by dividing the number of clones formed in the BUdR-containing selection medium by the number found in the same medium without BUdR. The ratio was then compared to that obtained from other dose levels and from positive and negative controls. A compound is considered mutagenic if: A dose response relationship is observed over 3 of the 4 dose levels employed. The minimum increase at the high level of the dose response curve is at least 2.5 times greater than the solvent control value. The solvent control data are within the normal range of the spontaneous background for the TK locus. Metabolic Activation: With and Without
TEST RESULTS	

Details for Cytogenetic Assay (if applicable):	
Statistics:	

Species:	**Strain:**	**Metabolic Activation:**	**Genotoxic Effect:**	**Conclusion:**
Mouse lymphoma	L5178Y TK+/-	With	Negative	Negative
Mouse lymphoma	L5178Y TK+/-	Without	Negative	Negative
Other Species:				
Other Strain:				

Results Remarks:	Little toxicity was observed with the test material. Positive control values exhibited significant responses over the negative controls, and the negative controls were within the normal range. All results for the test material from the non-activation assay were negative. The results from the activation assay were also considered to be negative. There was an increase in the number of mutants at the 0.52 µl/ml concentration but this appeared to result from a slight increase in the number of viable clones. There was no trend indicating a dose-related response and, therefore, the increases were not believed to be compound related. The results are summarized below. **Dose Rel. Mutant Viable % Rel. Mutant**

(µl/ml)	susp. growth	clones	clones	growth	frequency
Non-activation					
0.065	121.8	76	159	139.3	0.478
0.13	103.7	29	215	160.4	0.1349
0.26	114.6	44	211	174	0.2085
0.52	141.8	66	161	164.3	0.4099
1.04	107.5	58	270	208.9	0.2148
Solvent 100	14	139	100	0.1007	
Negative	129.9	41	140	130.8	0.2929
EMS	58.7	227	67	28.3	3.3881
Activation					
0.065	120.6	66	87	79.5	0.7586
0.13	108.6	46	126	103.7	0.3651
0.26	106	70	130	104.4	0.5385
0.52	112.4	92	108	92	0.8519
1.04	68.9	21	193	100.8	0.1088
Solvent 100	30	132	100	0.2273	
Negative	92.1	41	150	104.7	0.2733
DMN	16.7	91	7	0.9	13

Conclusion Remarks:	Negative in the mouse lymphoma assay with and without a metabolic activation system.

RELIABILITY / DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)

Reliability Remarks:	Comparable to guideline study in protocol and detail
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	American Petroleum Institute. 1977. Mutagenicity evaluation of unleaded gasoline. Study conducted by Litton Bionetics. Inc. API HESD Publication No. 28-30173, March 1977.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vitro*

TEST SUBSTANCE	
Category Chemical:	No CAS No.
Test Substance:	No CAS No.
Test Substance Purity/Composition and Other Test Substance	C5-C6 This hydrocarbon mixture is being used to characterize the in vitro genotoxicity of the C5-C6 fraction for the refinery gas streams.

Comments:	Unleaded gasoline (wholly vaporized gasoline)
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Type of Study:	Bacterial reverse mutation assay /Yeast
Concentrations:	1/8, 1/4, /1/2, and the 50% survival concentration.
Year Study Performed:	1977
Method/Guideline Followed:	Other
GLP:	No data
Positive, Negative and Solvent Control Substance(s):	Not identified.
Method/Guideline and Test Condition Remarks:	Type: Salmonella typhimurium reverse mutation assay/Yeast Method: The in vitro potential mutagenic activity of wholly vaporized gasoline was investigated by the Ames test using 5 strains of bacteria Salmonella typhimurium and one yeast strain. The solubility, toxicity and dose levels for the test material were determined prior to the mutagenicity screening. DMSO was used as solvent.

Based on the preliminary studies the following concentrations of test material were used in the mutagenicity assays:

Test doses	% Concentration	
	Bacteria	Yeast
1/8 50% survival	0.375	0.625
1/4 50% survival	0.75	1.25
1/2 50% survival	1.5	2.5
50% survival	3	5

Plate tests:
For non-activation assays cells in broth were exposed to the test material at the concentrations shown above. The contents of the tubes of broth plus test material were poured over selective agar plates which were then incubated. The test was conducted with and without Araclor-induced rat liver S-9 metabolic activation. Positive control substances (see results section) were also run in the same assay.

The following evaluation criteria were used in this plate test:
Strains TA1535, 1537 and 1538
If the solvent control value is within the normal range a chemical which produces a positive response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

Strains TA98, 100 and D4
If the solvent control value is within the normal range, a chemical which produces a positive response over three concentrations with the highest increase equal to twice the solvent control value for TA100 and two to three times the solvent control value for strains TA98 andD4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

Pattern:
Because TA1535 and TA100 were both derived from the same parental strain (G-46) and because TA1538 and TA98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain responds to a mutagen in non-activation tests it will generally do so in activation tests, but the converse of this is not anticipated. While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

Reproducibility:

	If a chemical produces a response in a single test which cannot be repeated in one or more additional runs, the initial positive test data loses significance. The above criteria are not absolute and other extenuating factors may enter into a final evaluation decision. Suspension tests: Bacteria and yeast cultures were grown in complete broth. The cells were removed, washed and exposed to the test material at the concentrations shown in the results section. For the yeast cells exposure to the test material was for 4 hours whereas for the bacterial cells exposure was for 1 hour. Aliquots of the cells were plated onto the appropriate complete media. After suitable incubation periods, the number of revertant colonies were counted. This assay was also conducted with and without metabolic activation and positive control substances were also included. The following criteria were used in the suspension assay: Surviving population counts A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests show very low (<25%) survival compared to the tissue controls. Data of this type are generally unacceptable and these experiments are repeated at a lower dose level. Total mutant counts: For non mutagens, the ratio of mutant to surviving population should be roughly equivalent for each test point in a given experiment. A mutagenic chemical will produce an altered mutant/surviving population ratio. An attempt is made to keep the surviving population of cells high and to look for positive responses that show increases in both numbers of mutants and mutation frequencies. Dose-response: Dose-related increases in mutants and mutation frequencies are the most convincing data when assessing mutagenic activity. To ensure a proper dose response, dose levels are kept within a relatively low range. Positive control: Metabolic Activation: With and Without
TEST RESULTS	
Details for Cytogenetic Assay	**Details for Cytogenetic Assay**

(if applicable):	**(if applicable):**
Statistics:	

Species:	**Strain:**	**Metabolic Activation:**	**Genotoxic Effect:**	**Conclusion:**
Bacteria	S. typhimurium TA 1535	With Without	Negative Negative	Negative
Bacteria	S. typhimurium TA 1537	With Without	Negative Negative	Negative
Bacteria	S. typhimurium TA 1538	With Without	Negative Negative	Negative
Bacteria	S. typhimurium TA 100	With Without	Negative Negative	Negative
Bacteria	S. typhimurium TA 98	With Without	Negative Negative	Negative
Yeast	D4	With Without	With Without	Negative Negative

Other Species:	
Other Strain:	

Results Remarks:

Plate test
There was no increase in revertants caused by exposure to the test material at any concentration. The results in this assay were negative both with and without metabolic
activation.

Suspension test

The mutation frequencies are summarized in the following table for assays with and without metabolic activation.

Non activation assay

	Salmonella strains					Yeast	
Dose level	TA100	TA1535		TA1537		TA1538	TA98* D4**
-ve control	5.48	3.59	6.15	7.1	41.99	23.69	
+ve control	125.51	185.65	161.54	84.75	100	66.29	
1 (low)	18.18	2.26	12.54	27.78	233.33	9.52	
2	2.9	2.15	8.97	11.76	63.04	36.99	
3	3.1	2.98	7.19	10	9.56	30.02	
4 (high)	4.13	2.66	9.68	3.21	35.74	32.38	

* Assay repeated for negative control and lowest 2 doses.
Results were 54.59 for -ve control
10.84 for lowest dose

14.11 for next highest dose

** Assay repeated at all dose levels

Results were:

-ve control	4.66
+ve control	97.73
dose level 1	1.3
dose level 2	8.33
dose level 4	12.65

Slight increases are observed at the high dose levels with TA100, TA1537 and TA1538. However the responses are not adequate enough to be considered positive. The increases with TA98 could not be reproduced.

With activation

	Salmonella strains					Yeast		
Dose level	TA100	TA1535		TA1537		TA1538	TA98*	D4**
-ve controls*								
A+C	17.08	5.25	6.01	4.8	21.01	52.66		
A-C	17.29	8.77	9.29	8.25	62.02	7.96		
AL1	17.34	7.32	3.99	6.48	45.03	30.06		
+ve control	25.51	89.92	0.22	1253.4	555.35	115.3		
1 (low)	22.97	41.67	100	71.43	100			
2	15.64	7.21	0	300	30.66	27.22		
3	17.26	9.57	20	15.38	83.33	27.03		
4	22.31	7.21	5.43	6.93	60.13	29.04		

* Controls were

A+C	No activation system but including positive control
A-C	Solvent control, no test chemical or activation system
AL1	Liver homogenate control plus solvent

	Scattered increases were found at one or more dose levels (see table above). All apparent positive effects were repeated and were not reproducible indicating problems associated with the initial runs. When the raw data were inspected it was observed that the increases were due to anomalous reductions in viable cell counts. The results of this assay were therefore considered to be negative.
Conclusion Remarks:	Negative
RELIABILITY/DATA QUALITY	
Reliability:	Valid With Restrictions (KS=2)
Reliability Remarks:	Not a guideline test but in accordance with generally accepted scientific standards and described in sufficient detail. Poor quality of initial assay.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	American Petroleum Institute. 1977. Mutagenicity evaluation of unleaded gasoline .Study conducted by Litton Bionetics. Inc. API HESD Publication No. 28-30173, March 1977.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE	
Category Chemical:	74-93-1
Test Substance:	Benzene
Test Substance Purity/Composition and Other Test Substance Comments:	No data
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	Micronucleus assay
Type of Test:	
Route of Administration:	Inhalation
Species:	Mouse and Rat
Strain:	DBA/2 (mouse); Sprague Dawley (rat)

Gender:	Males
Dose:	0, 10, 100, or 1000 ppm (mouse) 0.1, 0.3, 1, 3, 10, or 30 ppm (rat)
Year Study Performed:	1986
Method/Guideline Followed:	Other
GLP:	No data
Duration of Treatment/Expososure Period and Units:	6 Hours
Frequency of Treatment:	Single dose
Positive, Negative and Solvent Control Substance(s):	Negative Control Substance: air
Post-Exposure Period:	18 hours
Number of Animals per Sex per Dose:	5
Method/Guideline and Test Condition Remarks:	Type: Micronucleus assay and SCE The induction of cytogenetic damage after short term inhalation of benzene was studied in rats and mice. Five male mice per treatment group were exposed to benzene vapors by inhalation at 0, 10, 100, or 1,000 ppm. Five male rats per treatment group were exposed to 0.1, 0.3, 1, 3, 10, or 30 ppm benzene for 6 hours. An air-exposed control group of 10-20 male mice/rats were treated similarly and evaluated concurrently with the benzene-treated groups. Exposure chamber atmospheres were analyzed hourly for the top two benzene concentrations and two to three times per hour for the other doses. The animals were killed 18 hours after exposure and peripheral blood lymphocytes and femoral bone marrow samples were taken and slides prepared. The lymphocytes were cultured in the presence of liposaccharides or concanavalin-A to stimulate blastogenesis, and assayed for sister chromatid exchanges (SCEs). 5-Bromo-2'-deoxyuridine was added 24 hours after culture initiation and the cultures harvested at 60 hrs (mice) or 52 hrs (rats) following a 4 hr demecolcine treatment. Two or three slides were prepared per animal for SCE analysis. Slides from five treated and three

	to five concurrent control animals were coded, combined, and randomized prior to analysis. Both parametric (Student t test) and nonparametric (Mann-Whitney U test) statistics were used to analyze the data. Polychromatic erythrocytes (PCEs) in the prepared bone marrow samples (one to four stained slides per animal) were assayed for micronuclei.1000-2000 PCEs were analyzed from each animal. 1000 nuclei and 100 metaphases were scored consecutively for mitotic index and cell cycle kinetics, respectively. A one-tailed Student's t test was used to compare the micronuclei frequencies in the benzene exposed animals with the controls.
TEST RESULTS	
Systemic Toxicity:	
Genotoxic Effect:	Positive
Results Remarks:	MICE: SCE: All levels of benzene induced a statistically significant, dose-related increases in SCE frequency in peripheral lymphocytes. In comparison to the results on micronuclei formation in bone marrow (see below), these effects were relatively weak. Doubling of the spontaneous SCE frequency was achieved only at the highest dose tested (1000 ppm). Micronuclei: All levels of benzene induced a statistically significant, dose-related increases in bone marrow polychromatic erythrocytes containing micronuclei. At 1000 ppm, a 13.4 – fold increase from the control level was observed. Mitotic Index: All levels of benzene induced a statistically significant, dose-related decreases in mitotic index of the lymphocytes. Cell cycle kinetics: Not affected. Leucocyte counts: Not affected. RATS: SCE: At 3, 10, and 30 ppm, benzene caused dose dependent increases in the frequency of SCEs. The 1 ppm dose caused a borderline significant increase in SCE incidence, the level of significance dependent on the type of statistical test chosen. Micronuclei: Doses ranging from 1 to 30 ppm led to significant increases in the frequencies of micronuclei per 1000 polychromatic erythrocytes (frequencies of micronucleated cells were not given) Cell cycle kinetics: Not affected. Leucocyte counts: Not affected. The authors conclude that short term exposures to low concentrations of benzene induce statistically significant cytogenetic effects in lymphocytes and polychromatic erythrocytes in rats and mice

Conclusion:	Positive
RELIABILITY/DATA QUALITY	
Reliability:	Valid With Restrictions (KS=2)
Reliability Remarks:	Not standard test procedure but in accordance with generally accepted scientific standards and described in sufficient detail. No positive control. No data about the test substance.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Erexson, G.L., Wilmer, J.L., Steinhagen, W.H., Kilgerman, A.D. 1986. Induction of Cytogenetic Damage in Rodents after Short-Term Inhalation of Benzene. Environ. Mutagen. 8:29-40.

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE	
Category Chemical:	106-98-9
Test Substance:	Butene-1
Test Substance Purity/Composition and Other Test Substance Comments:	Stability and purity data referred to study sponsor. This hydrocarbon is being used to characterize the in vivo genotoxicity of the C1-C4 fraction for the refinery gas streams.

Category Chemical Result Type:	Measured
METHOD	
Type of Study:	In vivo mouse micronucleus assay
Type of Test:	
Route of Administration:	Whole body inhalation
Species:	Mouse
Strain:	Crl:CDR(IRC)Br Swiss
Gender:	Both M/F
Dose:	Pretest 1000, 9000, 18,000 ppm; full study 1000, 9000, 22,000 ppm
Year Study Performed:	1985
Method/Guideline Followed:	Other
GLP:	Yes
Duration of Treatment/Expososure Period and Units:	2 hours/day for 2 days; one group received 22,000 ppm 2 hrs/day for 1 day
Frequency of Treatment:	2 days; 1 day
Positive, Negative and Solvent Control Substance(s):	Positive Control Substance Remarks: cyclophosphamide Negative Control Substance Remarks: no data
Post-Exposure Period:	

Number of Animals per Sex per Dose:	Pretest: 2 males 2 females/dose group Full study: 10 males, 10 females/group & one group of 15 males, 15 females
Method / Guideline and Test Condition Remarks:	Type: Mammalian Bone Marrow Erythrocyte Micronucleus Test The genotoxic potential of nose-only inhalation exposure of 1-butene to induce micronucleus formation in bone marrow erythrocytes was determined in Swiss-Webster mice. 1-Butene was premixed with ambient air and introduced into inhalation chambers containing groups of mice (10 M,10 F) at concentrations of 0, 1000, 9000,or 22,000 ppm 2 hrs/day for 2 days. One half of each group was killed on day 3 and the remainder on day 4 following exposure. One group (15 M, 15 F) exposed for one day to 22,000 ppm was killed on days 2, 3, 4 after treatment (5/sex/day). Test concentrations were monitored each day by gas chromatography. Positive control mice given cyclophosphamide (75 mg/kg) ip daily for 2 days were killed on day 3. Slides of bone marrow smears were prepared, stained with May-Grunewald/Giemsa stain and examined microscopically. For each mouse, 1000 polychromatic erythrocytes and all mature erythrocytes (normochromatic erythrocytes) were counted. Data collected included group mean body weights for each day, total polychromatic erythrocytes total normochromatic erythrocytes, polychromatic erythrocytes with micronucli, and normochromatic erythrocytes with micronuclei. Values from treated groups for daily mean body weights, group means and std. dev. for polychromatic erythrocytes with micronuclei, (and group mean ratios of polychromatic erythrocytes to normochromatic erythrocytes were calculated and compared with vehicle control values by Student's t-test. Positive response was indicated by statistically significant ($p<0.05$) increases in micronucleated polychromatic erythrocytes at any dose level with a dose related response evident. Results were considered equivocal if only one of these criteria was met.
TEST RESULTS	
Systemic Toxicity:	
Genotoxic Effect:	Negative
Results Remarks:	Mice at all doses were unconscious during exposure to 1-butene but recovered when exposure ended. No other clinical signs were observed and no mortality occurred at any dose level. Inhalation of 1-butene by mice did not induce significant changes in micronucleus formation in polychromatic erythrocytes or NORMs and did not cause significant changes in the ratio of polychromatic erythrocytes /NCE.

	NOAEL = 22,000 ppm
Conclusion:	1-butene given by inhalation 2 hrs/day for 2 days to mice had no effect on the frequency of micronucleated erythrocytes in bone marrow. Under these test conditions, 1-butene does not induce chromosome damage.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Study comparable to guideline study; GLP have been followed and final QA statement is included in the report.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Khan, S.H. Ward, C.O. 1985. Micronucleus test of Gulftene® 4. Unpublished report # 84-2113 by Gulf Life Sciences Center for Gulf Oil Chemicals Co. [1-butene]

High Production Volume Information System (HPVIS)

Genetic Toxicity *in vivo*

TEST SUBSTANCE	
Category Chemical:	106-99-0
Test Substance:	1,3-Butadiene
Test Substance Purity/Composition	No data

and Other Test Substance Comments:	
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	Mammalian micronucleus assay: blood and bone marrow erythrocytes
Type of Test:	
Route of Administration:	Inhalation (gas)
Species:	Rat and Mouse
Strain:	Rat: Wistar Mouse: CB6F1
Gender:	Rat: Male. Mouse: Female
Dose:	0, 50, 200, or 500 ppm.
Year Study Performed:	1994
Method/Guideline Followed:	Other
GLP:	No data
Duration of Treatment/Expososure Period and Units:	6 Hours/day
Frequency of Treatment:	5 days

Positive, Negative and Solvent Control Substance(s):	Positive Control Substance Remarks : none Negative Control Substance Remarks: air
Post-Exposure Period:	6 hours/day for 5 days.
Number of Animals per Sex per Dose:	Rats: 10 per dose (single sex) Mouse: 20 per dose (single sex)
Method / Guideline and Test Condition Remarks:	Type: Micronucleus assay The genotoxic potential of nose-only inhalation exposure of butadiene to induce micronucleus formation in peripheral and bone marrow erythrocytes was determined in rats and mice. Twenty female CB6F1 mice (approximately 25g, 8-10 weeks old) and ten male Wistar rats (300-350g, 10 weeks old) per group were exposed for 5 days, 6 h/day 0, 50, 200, or 500 ppm of 1,3-butadiene by inhalation. An additional high concentration group of mice was exposed to 1300 ppm. Exposure concentrations were monitored by infrared spectroscopy (rats) and gas chromatography (mice). The animals were sacrificed 1 day after the last exposure and smears of blood and bone marrow erythrocytes were prepared and stained.
TEST RESULTS	
Systemic Toxicity:	
Genotoxic Effect:	Negative – rats Positive - mice
Results Remarks:	In the rats, no effects on micronuclei frequencies were observed either in the peripheral blood or bone marrow at all exposure levels. A slight toxic effect in rat bone marrow cells (decreased polychromatic/normochromatic ratio) was observed at the 500 ppm level. This effect was statistically significant at 500 ppm with the Student's t test, 2-tailed. An apparent decrease of the polychromatic/normochromatic ratio in a dose-dependent way was observed , but was not statistically significant with the linear regression test. In the mice, a clear dose-dependent increase in micronuclei frequency was observed in both blood and bone marrow cells at all exposure levels tested. FREQUENCY OF MICRONUCLEATED CELLS IN PERIPHERAL BLOOD ERYTHROCYTES OF RATS

EXPOSED TO 1,3-BUTADIENE

Dose (ppm)	Time (hrs)	# Rats in group	Total number of cells scored	Mean frequency (%) Mean±S.E.
0.0	24	10	9456	2.06±1.13
50	24	10	8607	3.40±1.30*
200	24	10	8500	2.10±1.52
500	24	10	10,000	2.70±1.76

* Statistically different from control ($p < 0.05$) according to Student's t-test (2-tailed)

FREQUENCY OF MICRONUCLEATED CELLS IN BONE MARROW ERYTHROCYTES OF RATS EXPOSED TO 1,3-BUTADIENE

Dose (ppm)	Time (hrs)	# Rats in group	Total number of cells scored	Mean frequency (%) Mean±S.E.
0.0	24	10	10,000	2.40±1.51
50	24	10	10,000	3.10±1.91
200	24	10	10,000	2.40±1.43
500	24	10	10,000	2.20±1.03

FREQUENCY OF MICRONUCLEATED CELLS IN PERIPHERAL BLOOD ERYTHROCYTES OF MICE EXPOSED TO 1,3-BUTADIENE

Dose (ppm)	Time (hrs)	# Rats in group	Total number of cells scored	Mean frequency (%) Mean±S.E.
0.0	24	20	48,000	2.6±1.56
50	24	20	40,000	5.8±2.23*
200	24	20	40,000	15.5±3.75**
500	24	20	40,000	20.2±3.39**
1300	24	20	43,000	23.6±5.0**

* Statistically different from control ($p < 0.05$) according to Student's t-test (2-tailed)

	** Statistically different from control ($p < 0.001$) according to Student's t-test (2-tailed) FREQUENCY OF MICRONUCLEATED CELLS IN BONE MARRO ERYTHROCYTES OF MICE EXPOSED TO 1,3-BUTADIENE

Dose (ppm)	Time (hrs)	# Rats in group	Total number of cells scored	Mean frequency (%) Mean±S.E.
0.0	24	20	408,000	2.8±1.72
50	24	20	40,000	7.5.±1.73**
200	24	20	38,000	15.3±3.82**
500	24	20	40,000	28.5±5.75**
1300	24	20	39,000	29.3±6.3**

	** Statistically different from control ($p < 0.001$) according to Student's t-test (2-tailed) Note: 2000 cells/animal were scored except in three animals where only 1000 cells/animal could be analyzed.
Conclusion:	1,3-butadiene was active in inducing micronuclei in peripheral blood and bone marrow erythrocytes in mice at levels >50 ppm, but not in rats. The genotoxic effects observed in this study parallel the species differences observed in cancer studies. Rat: negative Mouse: positive
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions
Reliability Remarks:	Comparable to guideline study.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Autio, K., Renzi, L., Catalan, J., Albrecht, O.E., and Sorsa, M. 1994. Induction of Micronuclei in Peripheral Blood and Bone Marrow Erythrocytes of Rats and Mice Exposed to 1,3-Butadiene by Inhalation. Mut. Res. 309:315-320.

Genetic Toxicity *in vivo*

TEST SUBSTANCE	
Category Chemical:	No CAS Number
Test Substance:	Liquid Petroleum Gas
Test Substance Purity/Composition and Other Test Substance Comments:	Liquid Petroleum Gas; 100 % (Chevron Texaco Energy Research & Technology Company, Richmond, CA); lot number 120701-01
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	Micronucleus assay; Satellite study to OECD 413 study on LPG
Type of Test:	
Route of Administration:	Inhalation
Species:	Rat
Strain:	Sprague Dawley
Gender:	Males and females

Dose:	0, 1000, 5000, 10,000 ppm
Year Study Performed:	2005
Method/Guideline Followed:	OECD Guide line 474 (satellite to 413 study)
GLP:	Yes
Duration of Treatment/Expososure Period and Units:	13 weeks
Frequency of Treatment:	6 hr/day; 5 days/week
Positive, Negative and Solvent Control Substance(s):	Negative Control Substance: air Positive control: 40 mg/kg cyclophosphamide (i.p. administration)
Post-Exposure Period:	18- 24 hours
Number of Animals per Sex per Dose:	5
Method/Guideline and Test Condition Remarks:	This study was a satellite study to a 13 week study (see Repeat Dose) Sprague Dawley-derived (CD) rats, 5/sex/group, were exposed to liquified petroleum gas by inhalation at exposure levels of 0, 1000, 5000 or 10000 ppm for a 13 week (6 hr/day; 5 days per week) exposure period. A group of non-exposed (5/sex) positive control animals (40 mg/kg cyclophosphamide, injected intraperitoneally with a 4.0 mg/mL solution @ 10 mL/kg, within 24 hours prior to sacrifice) were also dosed. The test animals were sacrificed under carbon dioxide anesthesia. The time between last exposure and tissue harvest was approximately 18 to 24 hours. The right femurs were removed and sampled. Unstained slides (4 per animal - 2 for shipment and 2 for retention) were prepared and shipped to Huntingdon Life Sciences Ltd., Eye Research Centre, Suffolk, UK. Upon receipt, slides were stained (acridine orange) and evaluated using a fluorescent microscope for determination of micronucleus response. The subcontractor was responsible for the QA inspection and audit of the conduct and reporting of the evaluations phase according to their own procedures. The testing facility's quality assurance (QA) department acted as lead QA to ensure that there was adequate quality assurance inspection coverage at the test site throughout the study.

	The exposure levels were based on results of range-find testing (Huntingdon Life Sciences Ltd. Study No. 03-6140) which showed no effects at 100 and 1000 and 10000 ppm. The exposure levels were also selected based on establishing (for safety reasons) the high exposure level as no more than 50% of the lower explosion limit (LEL = 2.1% = 21000 ppm) for the test substance. The animals used were approximately 6 weeks old at receipt and approximately 8 weeks old exposure of initiation. Note: Females were nulliparous and non-pregnant. The weight of the animals at initiation of exposure was: Mean (grams) Range (grams) Male: 280.0 243 - 308 Female: 209.1 187 - 231 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. The following parameters were evaluated as part of the sub-chronic study (see Repeat Dose section): Viability checks (in-cage) Physical examinations Ophthalmoscopic examination Body weight Feed consumption Neurobehavioral studies Functional observational battery Motor activity Estrous cyclicity Clinical laboratory studies Hematology Coagulation Clinical chemistry Macroscopic examinations Organ weights Tissues preserved and examined histopathologically **Statistical analysis:**

	The results obtained for each treatment group was compared with the results obtained for the concurrent air control group using non-parametric statistical methods. As there was no substantial difference (subjective evaluation by Principal Investigator) in response between sexes, results for the two sexes were also combined to facilitate interpretation and maximize the power of statistical analysis. For incidences of micronucleated immature erythrocytes, exact one-sided p-values were calculated by permutation (StatXact, CYTEL Software Corporation, NC, USA). Comparison of several dose levels was made with the concurrent control using the Linear by Linear Association test for trend in a step-down fashion if significance is detected (Agresti et al. 1990); for individual inter-group comparisons (eg the positive control group) this procedure simplifies 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. The following statistical analyses were carried for combined sexes, males and females separately: For the proportion of immature erythrocytes at 24 hours, an asymptotic one-tailed Jonckheere's test for trend (Jonckheere 1954) with "step-down" was used on groups 1 to 4 for a decrease from control. If significant, then the analysis was carried out on groups 1 to 3, then on groups 1 and 2. Also, exact one-tailed Wilcoxon pairwise tests (Wilcoxon 1945), for a decrease from control, were carried out on Group 1 (control) versus Groups 2, 3, 4 and 5. For micronucleated immature erythrocytes at 24 hours, an exact one-tailed Linear-by-Linear association test (Cytel 1995) with "step-down" was used on groups 1 to 4 for an increase from control. Also, exact one-tailed pairwise Permutation tests (Cytel 1995), for an increase from control, were carried out on Group 1 (control) versus Groups 2, 3, 4 and 5. Statistical significance was declared at the 1 % level for all tests. The data were received in a Word document and analysed using SAS 8.2 (SAS Institute 1999) (Jonckheere's and Wilcoxon tests) and StatXact 3 (Cytel 1995) (Linear-by-Linear and Permutation tests).The p-values from the pairwise and trend tests are given in Tables 1, 2 and 3 for combined sexes, males and females respectively. Several protocol deviations occurred during the study but were not considered to have compromised the validity or integrity of the study:
TEST RESULTS	
Systemic Toxicity:	No treatment related effects observed.

Genotoxic Effect:	Negative
Results Remarks:	This study was conducted as a satellite study of the 13-week whole body inhalation toxicity study reported in section 5.4. The genotoxicity study animals for Groups 1-4 were not separate animals but were selected from the main study animals outlined on the prior page. For the genotoxicity evaluation, a separate group (group 5) of animals (5/sex) was used as positive control animals for these evaluations. These positive control animals were given a dose of 40 mg/kg cyclophosphamide (CP) intraperitoneally within 24 hours prior to sacrifice. These positive control animals were not chamber exposed prior to dosing and sacrifice. The CP solutions prepared for dosing were not assayed for purity, uniformity and stability as per GLP's. Exposure method: whole body Group 1, 0 ppm, 10 males & 10 females Group 2, 1000 ppm, 10 males & 10 females Group 3, 5000 ppm, 10 males & 10 females Group 4, 10000 ppm, 10 males & 10 females Pre-study chamber distribution analyses showed that the test substance was evenly distributed within each chamber. Chamber monitoring showed that the chamber oxygen levels were at least 19%. Chamber room monitoring showed that no test substance was present in the room and that the sound and light levels were acceptable. The mean (± standard deviation) analytical exposure concentrations of LPG were determined to be 0.0 ± 0.0, 1019 + 58, 5009 ± 174 and 9996 ± 261 ppm for the air control and the exposure groups, respectively. Particle sizing results indicated that the atmospheres were essentially gas/vapor only, as expected, since there was no substantial difference between the test substance chambers and the air control chamber. Analysis of the major components in the neat test substance and the test atmospheres showed a reasonably close comparison between the neat test substance and the vaporized test substance. These data demonstrated that the test animals were exposed, as expected, to all of the major components of the test substance in their reasonably proper proportions. The data were consistent pretest and during the study indicating stability of the test substance and the atmosphere generation techniques. **Combined sexes**: For the proportion of immature cell and frequency of MIE cells at 24 hours, no statistically significant differences were

	found. **Males** For the proportion of immature cell and frequency of MIE cells at 24 hours, no statistically significant differences were found. **Females** For the proportion of immature cell and frequency of MIE cells at 24 hours, no statistically significant differences were found. The positive control produced significant increases in the frequency of the micronucleated immature erythrocytes and a decrease in the proportion of immature erythrocytes.
Conclusion:	Negative. After 13 weeks of exposures, there were no exposure-related differences in micronucleus incidence in the test substance exposed animals compared to the air control animals.
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Liquified Petroleum Gas: A 13week whole-body inhalation toxicity study in rats with neurotoxicity assessments and in vivo genotoxicity assessements. Conducted for the American Petroleum Institute. Draft report 03-6141.

Genetic Toxicity *in vivo*

TEST SUBSTANCE

Category Chemical:	No CAS number
Test Substance:	No CAS number
Test Substance Purity/Composition and Other Test Substance Comments:	C5-C6 This hydrocarbon mixture is being used to characterize the in vivo genotoxicity of the C5-C6 fraction of the refinery gas streams. Unleaded baseline gasoline API 99-01 Vapor Condensate Test material is a complex mixture of volatile hydrocarbons. The purity of mixture is 100% and stable based on analysis of chamber atmospheres. Representative Components [98.8%] monitored in Study

Component	Area %
Isobutane	2.70
n-butane	12.78
3-methyl-1-butene	0.41
Isopentane	36.50
n-pentane	9.36
Trans-2-pentene	3.60

	2,3-dimethylbutane: 1.75 2-methylpentane: 7.25 3-methylpentane: 4.27 n-hexane: 3.62 Methylcyclopentane: 1.87 2,4-dimethylpentane: 1.36 Benzene: 2.75 2-methylhexane: 1.73 2,3-dimethylpentane: 1.52 3-methylhexane: 1.73 Isooctane: 1.92 Toluene: 3.91
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	In vivo Sister Chromatid Exchange
Type of Test:	
Route of Administration:	Inhalation -Whole body
Species:	Rat
Strain:	Sprague Dawley [Crl: CD IGS BR]
Gender:	M/F

Dose:	Target: 0, 2000, 10,000, and 20,000mg/m^3 Actual: 0, 2050, 10,153, and 20,324 mg/m^3
Year Study Performed:	2005
Method/Guideline Followed:	EPA SCE Assay 79.65, CFR 59, No. 122 [27 June 1994]
GLP:	Yes
Duration of Treatment/Expososure Period and Units:	4 weeks, [20 exposures]
Frequency of Treatment:	6 hours/day, 5 days/week
Positive, Negative and Solvent Control Substance(s):	
Post-Exposure Period:	
Number of Animals per Sex per Dose:	5 males, 5 females/group
Method/Guideline and Test Condition Remarks:	This study was conducted as a satellite study of the 13 week inhalation toxicity study reported in the Repeated Dose section. Baseline Gasoline Vapor Condensate was administered via whole-body exposures to Sprague Dawley rats at target concentrations of 2000, 10000 and 20000 mg/m^3 for 6 hours/day, 5 days/week for 4 weeks. An Air Control group received nitrogen-enriched air only while in chamber. A separate positive control group was treated by intraperitoneal injection with 5mg/kg cyclophosphamide within 24 hours prior to sacrifice. Baseline Gasoline Vapor exposed animals were sacrificed 24

	hours after the 20th exposure. Blood [2-4ml] was collected from the abdominal aorta into sodium heparin tubes and shipped in ice packs on the day of collection to BioReliance, Rockville MD. Within 24 hours of collection, whole blood samples were cultured in supplemented RPMI 1640 culture medium at 37^0C. Approximately 21 hours after initiation, cells were exposed to 5µg/ml bromodeoxyuridine (BrdU). After 68 hours, 0.2µg/ml colcemid was added to each culture flask and incubation continued for 4 more hours. After 72 hours (approximately 51 hours after BrdU exposure) cells were collected, washed, fixed in 0.5ml methanol:acidic acid [3:1] fixative and stored in fixative at least overnight at 2-8^0C. Slides were prepared by removing overnight fixative by centrifugation, resuspending cells in fresh fixative, recentrifuging and aspirating off supernatant leaving 0.1-0.3ml fixative to resuspend the pellet. One or 2 drops of the cell suspension was dropped on a glass slide. Slides were allowed to air dry overnight and were stained using the modified fluorescence-plus Giemsa technique. Slides were coded and evaluated for SCE events without prior knowledge of treatment groups. A minimum of 25 second division metaphases per animal were scored for SCEs. At least 100 consecutive metaphases per animal were scored for the number of cells in first-, second-, or third-division metaphase for each animal as an indicator of toxicity (cell cycle delay). Average generation time [AGT] was estimated as Number of hours in BrdU x 100/[(number of cells in metaphase 1 x 1)+ (number of cells in metaphase 2 x 2) + (number of cells in metaphase 3 x 3)]. At least 1000 cells were scored for mitotic index per animal. Statistical analysis: A regression analysis (trend test) and one-tailed Dunnett's t test for multiple comparisons was performed to compare the average SCE frequency of test exposure levels to the negative control frequency.
TEST RESULTS	
Systemic Toxicity:	
Genotoxic Effect:	Positive
Results Remarks:	Statistically significantly increased SCE frequency ($p \leq 0.05$) was observed at all three dose levels for females and at concentrations of 10000 and 20000 mg/m^3 in males. Regression analysis was also positive ($p \leq 0.05$) for exposure level responses over all three groups for males and females. A dose dependent increase in AGT was observed in test substance and positive control groups. Cyclophosphamide, the positive control, induced increased SCE frequency as expected. No appreciable differences were observed in mitotic index in any test substance exposed group compared to negative controls. Sister chromatid exchanges indicate interaction between test material and DNA, however since no genetic material is unbalanced or lost, these events cannot be considered definitive for clastogenic activity [Reviewer's comment].

Conclusion:	Baseline Gasoline Vapor Condensate administered by inhalation for 4 weeks induced sister chromatid exchanges in rat peripheral lymphocytes in both male and female rats in this *in vivo* study.
RELIABILITY/DATA QUALITY	
Reliability:	Reliable without restriction (KS=1)
Reliability Remarks:	HPV Supporting study from Section 211(b) Testing Consortium, Fuels and Fuel Additives Health Effects Testing Regulation, administered by API, Washington DC.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Baseline Gasoline Vapor Condensate: A 13-Week Whole Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments and 4-Week In Vivo Genotoxicity and Immunotoxicity Assessments. HLS Study No. 00-6125, Vol IV, Appendix Y: *In vivo-In vitro* Rat Peripheral Lymphocyte Sister Chromatid Exchange Assay, R. Gudi, Principal Investigator, BioReliance study designation AA40NU.130.BTL. 2005. Huntingdon Life Sciences Laboratories, East Millstone, NJ and BioReliance Laboratories, Rockville, MD.

Genetic Toxicity *in vivo*

TEST SUBSTANCE

Category Chemical:	No CAS number
Test Substance:	No CAS number
Test Substance Purity/Composition and Other Test Substance Comments:	C5-C6 This hydrocarbon mixture is being used to characterize the in vivo genotoxicity of the C5-C6 fraction of the refinery gas streams. Unleaded baseline gasoline API 99-01 Vapor Condensate Test material is a complex mixture of volatile hydrocarbons. The purity of mixture is 100% and stable based on analysis of chamber atmospheres. Representative Components [98.8%] monitored in Study

Component	Area %
Isobutane	2.70
n-butane	12.78
3-methyl-1-butene	0.41
Isopentane	36.50
n-pentane	9.36
Trans-2-pentene	3.60

	2,3-dimethylbutane: 1.75 2-methylpentane: 7.25 3-methylpentane: 4.27 n-hexane: 3.62 Methylcyclopentane: 1.87 2,4-dimethylpentane: 1.36 Benzene: 2.75 2-methylhexane: 1.73 2,3-dimethylpentane: 1.52 3-methylhexane: 1.73 Isooctane: 1.92 Toluene: 3.91
Category Chemical Result Type:	Measured
METHOD	
Type of Study:	In vivo micronucleus assay
Type of Test:	

Route of Administration:	Inhalation - Whole body
Species:	Rat
Strain:	Sprague Dawley [Crl: CD IGS BR]
Gender:	Male and female
Dose:	Target: 0, 2000, 10,000, and 20,000mg/m^3 Actual: 0, 2050, 10,153, and 20,324 mg/m^3
Year Study Performed:	2005
Method/Guideline Followed:	EPA OPPTS 870.5395 [1998]
GLP:	Yes
Duration of Treatment/Expososure Period and Units:	4 weeks, [20 exposures]
Frequency of Treatment:	6 hours/day, 5 days/week
Positive, Negative and Solvent Control Substance(s):	
Post-Exposure Period:	None
Number of Animals per Sex per Dose:	5 males, 5 females/group

Method / Guideline and Test Condition Remarks:	Type: Micronucleus assay This study was conducted as a satellite study of the 13 week inhalation toxicity study reported in the Repeated Dose section. Baseline Gasoline Vapor Condensate was administered via whole-body exposures to Sprague Dawley rats at target concentrations of 2000, 10000 and 20000 mg/m^3 for 6 hours/day, 5 days/week for 4 weeks. An Air Control group received nitrogen-enriched air only while in chamber. A separate positive control group was treated by intraperitoneal injection with 40mg/kg cyclophosphamide within 24 hours prior to sacrifice. Baseline Gasoline Vapor-exposed animals were sacrificed 24 hours after the 20th exposure. Bone marrow was extracted from femurs and the fixed, unstained slides were prepared and shipped via overnight delivery to Huntingdon's Eye Research Center, Suffolk UK where the slides were stained by the modified Feulgen method. One smear from each animal was examined for the presence of micronuclei in 2000 immature erythrocytes. Slides were coded and evaluated without knowledge of treatment groups. The proportion of immature erythrocytes was assessed by examination of at least 1000 mature and immature erythrocytes from each animal. The incidence of micronucleated mature erythrocytes was also recorded. Statistical methods: The results for each treatment group were compared with the results for the concurrent negative control group using non-parametric statistics. Since there was no substantial difference in response between sexes results for the two sexes are combined to facilitate interpretation and maximise the power of statistical analysis. For incidences of micronucleated immature erythrocytes, exact one-sided p-values are calculated by permutation (StatXact, CYTEL Software Corporation, Cambridge, Mass.). Comparison of several dose levels is made with the concurrent control using the Linear by Linear Association test for trend, in a step-down fashion if significance is detected; for individual intergroup comparisons (i.e. the positive control group) this procedure simplifies to a straightforward permutation test. For assessment of effects on the proportion of immature erythrocytes, equivalent permutation tests based on rank scores are used, i.e. exact versions of Wilcoxon's sum of ranks test and Jonckheere's test for trend. Positive Control Substance Remarks: Cyclophosphamide
TEST RESULTS	
Systemic Toxicity:	
Genotoxic Effect:	Negative

Results Remarks:	The test substance did not cause any statistically significant increases in the number of micronucleated immature erythrocytes [P>0.01]. As expected, the positive control cyclophosphamide caused large, highly significant increases in the frequency of micronucleated immature erythrocytes [P<0.001]. The test substance did not cause any substantial increases in the incidence of micronucleated mature erythrocytes, did not induce differential cytotoxicity and did not cause any significant decreases in the proportion of immature erythrocytes [P>0.01]. Cyclophosphamide caused statistically significant decreases in the proportion of immature erythrocytes [P<0.001].
Conclusion:	Baseline Gasoline Vapor Condensate administered by inhalation for 4 weeks did not induce cytogenetic damage expressed as increases in micronucleated immature erythrocytes, nor bone marrow cell toxicity in this *in vivo* test procedure
RELIABILITY/DATA QUALITY	
Reliability:	Reliable without restriction (KS=1)
Reliability Remarks:	HPV Supporting study from Section 211(b) Testing Consortium, Fuels and Fuel Additives Health Effects Testing Regulation, administered by API, Washington DC.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Baseline Gasoline Vapor Condensate: A 13-Week Whole Body Inhalation Toxicity Study in Rats with Neurotoxicity Assessments and 4-Week In Vivo Genotoxicity and Immunotoxicity Assessments. HLS Study No. 00-6125: Micronucleus Assay, Vol IV, Appendix X. 2005. Huntingdon Life Sciences Laboratories, East Millstone, NJ and Huntingdon Eye Research Center, Suffolk UK.

High Production Volume Information System (HPVIS)

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	71-43-2
Test Substance:	Benzene
Test Substance Purity/Composition and Other Test Substance Comments:	Commercial benzene, >99.9% pure, from API, Washington, DC
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Subchronic inhalation toxicity, 13 weeks
Species:	Mouse

Other Species:	Rats
Mammalian Strain:	CD-1
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	80 (40 per sex)
Concentration:	
Dose:	0, 1, 10, 30, 300 ppm
Year Study Performed :	1985
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** 6 hrs/day
Frequency of Treatment:	5 days/week , 13 weeks.
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	Type: Subchronic inhalation toxicity Control group: yes, filtered air Method: This study investigated the systemic effects of a 13 week benzene (whole chamber, vapor) exposure by inhalation. Male and female CD-1 mice (40 mice/sex/dose) were exposed (0, 1, 10, 30, or 300 ppm benzene; 6h/day,

	5 days/week) for 13 weeks. Criteria used to evaluate exposure related effects included behavior, body weights, organ weights, clinical pathology, gross pathology, and histopathology. All animals were observed twice daily, before and after exposure and on nonexposure days, for mortality and moribundity throughout the study. At weekly intervals animals were observed for signs of toxicity, weighed and individual body weights recorded. On study days 7, 14, 28, 56, and 91, blood samples were taken from randomly selected 20 mice/sex/group for full range hematological and clinical chemistry examinations. Blood was collected for clinical pathology analyses from an additional 30 mice one day prior to the start of the study. For interim sacrifice on days 7, 14, 28, 56, and for terminal sacrifice on day 91, 20 mice/sex/group were randomly selected and killed. Complete necropsies were performed on all these animals and on animals found dead or sacrificed in a moribund condition during the study. With respect to reproductive organs, absolute testes weight and testes/terminal body weight ratios were determined for each animal that was necropsied at each interval. In addition, the following tissues from each animal necropsied at each sacrifice interval was taken and fixed: testes or ovaries, prostate or uterus, and mammary gland. Sections from the control and high-level groups at each sacrifice period were subject to histopathological examinations. The testes and ovaries of all animals at all exposure levels at the 91-day terminal sacrifice were examined microscopically.
Pre-Mating Exposure / Males :	Not applicable
Pre-Mating Exposure / Females:	Not applicable

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Males	=	300		ppm
NOAEL	Males	=	30		ppm
LOAEL	Females	=	300		ppm
NOAEL	Males	=	30		ppm

Results:	No consistent exposure-related apparent trends in clinical observations or mean body weight data in either species (no

	data provided). At 300 ppm, changes in blood composition occurred, consisting of decreases in red blood cell counts, white blood cell counts, platelets, hemoglobin, myeloid/erythroid ratios and hematocrit. Also femoral myeloid hypoplasia, extramedullary hematopoiesis in the spleen and thymic atrophy were noted. With respect to reproductive organs a statistically significant and exposure-time related decrease in absolute mean testes weights at sacrifices on days 28, 56, and 91, as well as in relative mean testes at sacrifices on days 59 and 91 occurred at the 300 ppm level (data not provided). Histomorphologic changes in reproductive organs were also reported at 300 ppm in male mice at the 91 day-interval (7 mice with minimal to moderately severe bilateral atrophy/degeneration, 6 mice with moderate to moderaely severe decrease in spermatozoa, 9 mice with minimal to moderate increase in abnormal sperm forms) but not in those sacrificed at the earlier intervals. At the 300 ppm dose level, four female mice showed bilateral ovarian cysts. The severity of gonadal lesions was greater in the males. Similar lesions were reported to be observed in both sexes also at lower dose levels (data not provided), which by the authors themselves were considered of doubtful biological significance, and it is assumed that these levels did not represent any significant changes from the controls. Other histopathological changes observed at the high-dose level included the thymus, femoral marrow, spleen, mesenteric lymph nodes, mandibular lymph nodes and the liver, the severity increasing with time. In a few instances, the lesions seen at the high-dose level were detected at the lower levels but these were not reported in detail. Male mice were reported to be much more susceptible than the females.
Results Remarks:	Lacks detail on testes weight data and numerical incidences on histomorphological changes. Also, no data on the performance of the control animals are given during the study.
Conclusion:	LOAEL = 300 ppm (males, females) NOAEL = 30 ppm (males, females)
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Non-guideline reproductive toxicity study but in accordance with generally accepted scientific standards for a subchronic study.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Ward, CO, RA Kuna, NK Snyder, RD Alsaker, WB Coate, PH Craig (1985) Subchronic inhalation toxicity of

	benzene in rats and mice. Americ. Journ. of Industrial Medicine 7: 457-473.

High Production Volume Information System (HPVIS)

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	74-84-0
Test Substance:	Ethane
Test Substance Purity/Composition and Other Test Substance Comments:	Ethane, purity 99%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.

Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 1600, 5000, 16,000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	

Method / Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on ethane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Repeated-Dose Toxicity and Developmental Toxicity of this Robust Study Summary submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 160, 1600 and 16000 ppm. Therefore, the high exposure level was established (for safety reasons) at 16000 ppm since it is 50% of the lower explosion limit (3.2% = 32000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 256 230-284 Female: 197 167-217 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 1600, 5000 and 16000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6

hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	1600 ppm	12 males	24 females
Group 3	5000 ppm	12 males	24 females
Group 4	16000 ppm	12 males	24 females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

Viability
Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

Clinical observations
All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response

	to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit

	Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment.

	Functional Observational Battery The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery: **Sensory Observations** startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides

	Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain.

Microscopic pathology
A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded.

F1 Pup Evaluations

Physical Examinations
Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior.

Body Weight
Individual F1 pup weights were recorded on lactation days 1 and 4.

Sexing
Pups were sexed on lactation day 0 and sex verified on lactation day 4.

Macroscopic observations
Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain.

Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation.
F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded.

No protocol deviations occurred during the study.

Statistical methods:

1. Continuous data
The following parameters were analyzed statistically:
Body weights
Body weight changes
Feed consumption values
Rectal Temperature
Hematology
Coagulation
Clinical Chemistry
Gestation length
Corpora lutea and implantation sites
F1 pup weights (each weighing interval during lactation)
Number of pups (F1 litters) per pregnant female rats
Number of male and females pups
Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit)
Organ weights
Organ weight to body weight ratios
Organ to Brain weight ratios
Grip Strength measurements

Methods of analysis:
Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was

	used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0. 2. Motor Activity Counts The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor. 3. Incidence data The following parameters were analyzed statistically: Mortality rate Mating indices, pregnancy rates, male fertility indices Litter survival indices Gestation indices Incidence of dams with no viable pups Mean pup survival indices (lactation days 0 and 4) Incidence Data Analysis A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.
TEST RESULTS	

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male and female rats (Parental/ systemic)	=	16,000		ppm
NOAEL	Reproductive	=	16,000		ppm
NOAEL	Developmental	=	16,000		ppm

Results Remarks:

Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 1703 ± 23, 4762 ± 124, 15502 ± 194 ppm. The analytically measured levels of airborne test substance were reasonably close to the targeted exposure levels and nominal concentrations. Chamber environmental conditions averaged 23ºC temperature and 49% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers.

The test substance was assayed by gas chromatography versus an analytical standard before and after the study to demonstrate the purity and stability of the test substance. The test substance was determined to be 99.86% ethane before the study and 99.88% ethane after the study demonstrating the purity and the stability of the test substance.

MAIN STUDY ANIMAL OBSERVATIONS
There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes or feed consumption (except the 16000 ppm exposed animals showed marginally lower feed consumption during the first week of exposures but the differences from the air control did not exceed 5% in the test substance exposed animals compared to the air control animals).
There was no apparent exposure-related effect on functional observational battery or motor activity parameters for either sex in this study. There were no exposure-related differences in hematology or coagulation values or clinical chemistry values in test substance exposed animals compared to the air control animals at the terminal interval. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test

	substance exposed animals compared to the air control animals. There were no microscopic findings considered to be related to exposure to ethane. No testiclular (stages of spermatogenesis; testicular interstitial cells) abnormality was evident in any test animal. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red nasal discharge or red/brown staining between the 7th and 28th days of exposure in all groups including the control animals. All animals survived until the termination of the study. These findings were slightly more prevalent among test substance exposed animals. There were no exposure-related differences in body weights or weight changes or feed consumption (except the 16000 ppm exposed animals showed marginally lower feed consumption (6%) during the first week of exposures, consistent with the same marginal trend in the main study animals. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS Almost all mated female animals were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. All but one of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 1600, 5000 or 16,000 ppm of ethane by whole-body inhalation for 4 weeks resulted in no systemic or neurotoxic effects, apart from a marginal feed reduction in high dose animals during the first week of exposure. A no-observed-adverse effect level (NOAEL) of 16,000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 16,000 ppm was concluded for reproductive and developmental endpoints

	in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Ethane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4243

High Production Volume Information System (HPVIS)

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	74-98-6
Test Substance:	Propane
Test Substance Purity/Composition and Other Test Substance Comments:	Propane, purity 99.5%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.

Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 1200, 4000, 12000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650

GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on propane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Repeated-Dose Toxicity and Developmental Toxicity of this Robust Study Summary submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 120, 1200 and 12000 ppm. Therefore, the high exposure level was established (for safety reasons) at 12000 ppm since it is 50% of the lower explosion limit (2.4% = 24000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 269 243-297 Female: 200 180-220

Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 1200, 4000 and 12000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	1200 ppm	12 males	24females
Group 3	4000 ppm	12 males	24 females
Group 4	12000 ppm	12 males	24females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

Viability

	Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon). **Clinical observations** All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium

	Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights.

Feed consumption
Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment.

Functional Observational Battery
The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery:

Sensory Observations
startle response to auditory stimuli
tail pinch response

Neuromuscular Observations
grip strength - hindlimb and forelimb

Physiological Observations
rectal temperature

Motor activity
Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups.

Clinical pathology (termination)
Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized

	(CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve

	Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations**

	Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. Three protocol deviations occurred during the study, but did not appear to impact the results. **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically: Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements

Methods of analysis:
Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels.

Exceptions
Statistical evaluations were not performed when the standard deviation for the control group was 0.

2. Motor Activity Counts
The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor.

3. Incidence data

The following parameters were analyzed statistically:

	Mortality rate Mating indices, pregnancy rates, male fertility indices Litter survival indices Gestation indices Incidence of dams with no viable pups Mean pup survival indices (lactation days 0 and 4) Incidence Data Analysis A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Male rats (Parental)	=	12,000		ppm
NOAEL	Male rats (Parental)	=	4,000		ppm
NOAEL	Female rats (parental)	=	12,000		ppm
NOAEL	Reproductive	=	12,000		ppm
NOAEL	Developmental	=	12,000		ppm

Results Remarks:	Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 1230 ± 34, 3990 ± 156, 12168 ± 415 ppm. Chamber environmental conditions averaged 23º C temperature and 42% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance

	chambers and the air control chambers. Pre-study GC analysis of the test substance showed a purity of 99.91% for propane. This value compared closely to the purity of 99.84% for the analytical standard of propane and the 99.5% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.90% for propane. This value compared closely to the purity of 99.80% for the analytical standard of propane and essentially replicated the 99.91% pre-study purity demonstrating stability of the test substance over the period of this study. MAIN STUDY ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red staining between the 22nd and 28th days of exposure in all groups including the control animals. These findings were very slightly more prevalent among test substance exposed animals. There were exposure-related differences in body weights and weight changes in the 12000 ppm exposed male animals as compared to the air control animals. These male animals showed a 25% decrease in weight gain during the 1st week of exposures and this difference persisted for the remainder of the 4 weeks of exposures. Similar differences were not seen at the lower levels for the males or in any of the female groups. There were no meaningful differences in feed consumption in the test substance exposed animals as compared to the air control animals. There was no meaningful effect on functional observational battery parameters or motor activity for either sex in this study. A statistically significant increase in hindlimb grip strength was noted in the 4000 ppm exposed males but there was no exposure level related pattern. There were no meaningful differences in hematology or coagulation values in test substance exposed male animals as compared to the air controls. A statistically significant decrease (up to 21% in the low level) in absolute lymphocytes in the test substance exposed males was seen but not in an exposure-related pattern and there were no accompanying changes in the other hematology parameters for the males, nor were there any similar differences in the females. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures and were within this laboratory's normal range of values. Similarly, the statistically significant increases only in the 12000 ppm exposed females for hemoglobin concentration, hematocrit, erythrocytes and absolute eosinophils were considered in context too small to represent an effect of the exposures. There were no meaningful differences in clinical chemistry values in the test substance exposed animals as compared to the air control animals. A statistically significant increase in sodium concentration in the 12000 ppm exposed females was seen but the absolute difference was less than 1% and there was no similar difference in the males. A statistically significant decrease in chloride concentration in the 1200 ppm exposed females was seen but the absolute difference was only 2% and there was no similar difference at the higher levels in the females or at any level of

	exposure in the males. In isolation, these minor inter-group differences were not considered to indicate effects of the exposures and were within this laboratory's normal range of values. There were no meaningful inter-group differences in organ weights in the test substance exposed animals as compared with air control animals. The 1200 and 12000 ppm exposed males showed decreases in kidney weights (absolute and/or relative to body or brain weight) but no similar differences were seen in the exposed females. The 12000 ppm exposed males also showed decreases in absolute (but not relative) liver weights and similar differences were seen (but only relative to brain weight) in the 4000 and 12000 ppm exposed females. Considering the absence of any correlated pathology findings and clear patterns of treatment, these minor inter-group differences were not considered to indicate effects of the exposures. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations in the test substance exposed animals compared to the air control animals. The testes were examined qualitatively with an awareness of the stages of the spermatogenic cycle to detect any disturbances in spermatogenesis, and with emphasis on the interstitial (Leydig) cells to detect any changes in cellular size or structure. No test article related findings were present. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. For the pregnant females there were no exposure-related differences in body weights or weight changes in the test substance exposed animals as compared to the air control animals. There were no treatment- related changes in feed consumption. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS All mated female animals (except one in the 1200 ppm group – considered incidental) were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. Almost all of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, and the proportion with live litters and/or with stillborn pups when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live

	pups/litter, when compared to the air control group. Statistically significant decreases in the number of live born pups and converse increases in the number of stillborn pups in the 4000 and 12000 ppm exposed groups were attributable to the single total litter loss in each of these groups very soon after parturition. These losses were preceded by severely reduced body weight gain in the last week of gestation for two particular dams. There was no excess of mortality in any of the other litters in these groups and the total litter losses were considered incidental and not related to the exposures. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 1200, 4000 or 12000 ppm of propane by whole-body inhalation for 4 weeks resulted in a reduction of body weight gain in the males resulting from exposures at the12000 ppm dose. Consequently, a no-observed-adverse effect level (NOAEL) of 4000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study (LOAEL – 12,000 ppm). There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 12,000 ppm was concluded for reproductive and developmental endpoints in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Propane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4245.

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	75-28-5
Test Substance:	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments:	Isobutane, purity 99.0%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 900, 3000, 9000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on isobutane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Repeated-Dose Toxicity and Developmental Toxicity of this Robust Study Summary

submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections.

The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study".

The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 90, 900 and 9000 ppm. Therefore, the high exposure level was established (for safety reasons) at 9000 ppm since it is 50% of the lower explosion limit (1.8% = 18000 ppm) for the test substance.

The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation.

The weight of animals at initiation of exposures was:

	Mean (g)	Range (g)
Male:	277	249-301
Female:	194	171-216

Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 900, 3000 and 9000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	900 ppm	12 males	24females
Group 3	3000 ppm	12 males	24 females
Group 4	9000 ppm	12 males	24 females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

Viability
Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

Clinical observations
All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded.

Clinical chemistry:
Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed

	to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration

	Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery:

	Sensory Observations startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver

	Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of

spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded.

F1 Pup Evaluations

Physical Examinations
Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior.

Body Weight
Individual F1 pup weights were recorded on lactation days 1 and 4.

Sexing
Pups were sexed on lactation day 0 and sex verified on lactation day 4.

Macroscopic observations
Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain.

Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation.
F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded.

No protocol deviations occurred during the study.

Statistical methods:

1. Continuous data
The following parameters were analyzed statistically:

	Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0.

2. Motor Activity Counts
The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor.

3. Incidence data

The following parameters were analyzed statistically:
Mortality rate
Mating indices, pregnancy rates, male fertility indices
Litter survival indices
Gestation indices
Incidence of dams with no viable pups
Mean pup survival indices (lactation days 0 and 4)

Incidence Data Analysis
A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:

NOAEL	Male and female rats (Parental/ systemic)	=	9,000		ppm
LOAEL	Reproductive	=	9,000		ppm
NOAEL	Reproductive	=	3,000		ppm
NOAEL	Developmental	=	9,000		ppm

Results Remarks:

Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 930.0 ± 27.8, 3122 ± 83 and 9148 ± 201 ppm. Chamber environmental conditions averaged 24°C temperature and 55% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers.

Pre-study GC analysis of the test substance showed a purity of 99.84% for isobutane. This value compared closely to the purity of 99.51% for the analytical standard of Isobutane and the 99.0% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.53% for isobutane. This value compared closely to the purity of 99.50% for the analytical standard of isobutane and the 99.0% pre-study purity demonstrating stability of the test substance over the period of this study.

MAIN STUDY ANIMAL OBSERVATIONS
There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red staining between the 7th and 28th days of exposure in all groups including the control animals. These findings were only slightly more prevalent among test substance exposed animals.
There were no exposure-related differences in body weights and weight changes or feed consumption in the exposed male animals as compared to the air control animals. There was no treatment-related effect on functional observational battery parameters or motor activity for either sex in this study.
There were no meaningful differences in hematology or coagulation values in test substance exposed male animals as compared to the air controls. Statistically significant increases in hemoglobin and mean corpuscular hemoglobin concentration in the 9000 ppm test substance exposed males, decreases in platelets in 3000 and 9000 ppm test substance exposed males and decreases in monocytes in 3000 and 9000 ppm test substance exposed females were seen in exposure-related patterns but there were no accompanying changes in the other hematology parameters, nor

	were there any similar differences in the other sex. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures and were generally within the normal range of these values for a rat. There were no meaningful differences in clinical chemistry values in the test substance exposed animals as compared to the air control animals. Statistically significant increases in sodium concentration in the 9000 ppm test substance exposed males and decreases in phosphorus concentration in all test substance exposed females were seen but the absolute differences were relatively small, were not in an exposure level related pattern and there were no similar differences in the other sex. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures. There were no meaningful inter-group differences in organ weights in the test substance exposed animals as compared with air control animals. The 900 and 3000 ppm exposed males showed an increase in spleen weights (absolute and/or relative to body or brain weight) but no similar difference was seen in the 9000 ppm exposed males or any of the exposed females. In isolation, this minor inter-group difference was not considered to indicate an effect of the exposures. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations in the test substance exposed animals compared to the air control animals. The testes were examined qualitatively with an awareness of the stages of the spermatogenic cycle to detect any disturbances in spermatogenesis, and with emphasis on the interstitial (Leydig) cells to detect any changes in cellular size or structure. No test article related findings were present. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes in the test substance exposed animals as compared to the air control animals. There were no exposure-related differences in feed consumption in the test substance exposed animals as compared to the air control animals. . There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. A statistically significant increase in heart to brain weight ratio in 9000 ppm exposed females was seen but, in the absence of any macroscopic and microscopic abnormalities in the main study animals, this difference was considered to have been incidental. MATING, FERTILITY AND GESTATION PARAMETERS No parental systemic toxicity was observed. In the 9000 ppm group, 25% of the mated females did not become pregnant. Although not statistically significant, the reduction in male and female fertility indices (75%) was considered exposure related since it was below the concurrent control (100%) and the testing facility historical control values (mean 96.4%; range 87.5%-100%). The mating index for male rats treated with the test substance was

	comparable to the air control group. A statistically significant ($p < 0.05$) exposure-related increase in post-implantation loss was also observed for the 9000 ppm group of exposed female rats; mean losses of 0.8± 0.9 and 1.8 ± 0.8 for control and high exposure groups respectively. The data were interpreted as conservatively as possible; and the two reproductive endpoints were attributed to isobutane exposure. All other reproductive endpoints were comparable to controls (number of pairs cohabited, number of pairs mated, mating index, gestation index, mean time to mating, mean gestation length, number of females completing delivery with stillborn pups/all stillborn pups, mean pre-implantation loss, mean pups delivered, live birth index, viability index). Pup endpoints (viabilty to day 4, weight & weight gain, sex ratio) were also comparable to air control pups.
Conclusion:	Exposure of male and female rats to target concentrations of 900, 3000 or 9000 ppm of isobutane by whole-body inhalation for 4 weeks resulted in no general systemic/neurotoxic effects. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. Based on decreased male and female fertility and increased post-implantation loss in the 9000 ppm group, the fertility and reproductive endpoints NOAEL was determined to be 3000 ppm. There were no effects on in offspring survival, body weight and development up to post-natal day 4. A NOAEL of 9000 ppm was concluded for developmental effects.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Isobutane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4244.

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	106-97-8
Test Substance:	Butane
Test Substance Purity/Composition and Other Test Substance Comments:	Butane, purity 99.5%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 900, 3000, 9000 ppm
Year Study Performed:	2004
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on butane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Repeated-Dose Toxicity and Developmental Toxicity of this Robust Study Summary

	submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 90, 900, and 9000 ppm. Therefore, the high exposure level was established (for safety reasons) at 9000 ppm since it is 50% of the lower explosion limit (1.8% = 18,000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 261 241-280 Female: 200 174-229 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 900, 3000 and 9000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19. Exposure method: whole body Group 1 0 ppm 12 males 24 females (air control)

	Group 2 900 ppm 12 males 24 females Group 3 3000 ppm 12 males 24 females Group 4 9000 ppm 12 males 24 females The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve. Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure. The following parameters were evaluated: **Viability** Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon). **Clinical observations** All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum.

	The following parameters were evaluated: Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count

	Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery: **Sensory Observations**

	startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi)

	Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during

	macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. Several protocol deviations occurred during the study but were not considered to have compromised the validity or integrity of the study: **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically:

	Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0.

When 75% of the values for a clinical pathology parameter were the same, Fisher's Exact Test (Fisher, 1973) was performed followed by Mantel's test (Mantel, 1963).

2. Motor Activity Counts

The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant (p=0.05), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passed on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the p=0.05 level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor.

3. Incidence Data

The following parameters were analyzed statistically:
Mortality rate
Mating indices, pregnancy rates, male fertility indices
Litter survival indices
Gestation indices
Incidence of dams with no viable pups
Mean pup survival indices (Days 0 and 4)

Incidence Data Analysis
A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male and female rats (Parental/ systemic)	=	9,000		ppm
NOAEL	Reproductive	=	9,000		ppm
NOAEL	Developmental	=	9,000		ppm

Results Remarks:

Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The analytically measured exposure levels of airbone test substance were reasonably close to the targeted exposure levels and nominal concentrations. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 930.6 ± 28.1, 3022 ± 58 and 9157 ± 269 ppm. Chamber environmental conditions averaged 24ºC temperature and 56% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers.

Pre-study GC analysis of the test substance showed a purity of 99.23% for butane. This value compared closely to the purity of 99.96% for the analytical standard of butane and the 99.5% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.98% for butane. This value compared closely to the purity of 99.98% for the analytical standard of butane and the 99.23% pre-study purity demonstrating stability of the test substance over the period of this study.

MAIN STUDY ANIMAL OBSERVATIONS
There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods in-chamber and during the non-exposure periods. There was a low incidence of chromodacryorrhea or transient red nasal discharge among males exposed to 9000 ppm between the 7th and 28th days of exposure. There were no exposure-related differences in body weights or weight changes or feed consumption in the test substance exposed animals compared to the air control animals.
There was no meaningful exposure-related effect on functional observational battery or motor activity parameters for either sex in this study. Minor, but statistically significant decreases in male forelimb grip strength and increases in female forelimb grip strength occurred at 9000 ppm, but were not suggestive of an adverse effect, due to the opposite effect on the same parameter. Intergroup differences of a similar magnitude occurred among hindlimb results for both

	sexes without any apparent exposure-level relationship. There were no apparent exposure-related differences in hematology or coagulation values or clinical chemistry values in test substance exposed animals compared to the air control animals at the terminal interval. A statistically significant decrease in total bilirubin concentration was seen in males at 3000 and 9000 ppm, but not in an exposure-related pattern, and there were no differences in the females. These minor inter-group differences were not indicative of an exposure effect. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. There were no microscopic findings considered to be related to exposure to butane. No testiclular (stages of spermatogenesis; testicular interstitial cells) abnormality was evident in any test animal. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of chromodacryorrhea or transient red nasal discharge among males exposed to 9000 ppm between the 7th and 28th days of exposure. There were no exposure-related differences in body weights or weight changes or feed consumption in the test substance exposed animals compared to the air control animals. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY and gestation parameterS Almost all mated female animals were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. All but one of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 900, 3000 or 9000 ppm of butane by whole-body

	inhalation for 4 weeks resulted in no systemic or neuotoxic effects. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for reproductive and developmental endpoints in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Butane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4242.

High Production Volume Information System (HPVIS)

Reproductive Toxicity

TEST SUBSTANCE	
Category Chemical:	106-99-0
Test Substance:	1,3-Butadiene

Test Substance Purity/Composition and Other Test Substance Comments:	Purity 99.88%
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Reproductive/developmental toxicity screen
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	24 (12/sex)
Concentration:	
Dose:	0, 300, 1500, 6000 ppm
Year Study Performed :	2003

Method / Guideline Followed:	OECD 421
GLP:	Yes
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** 6 hrs/day
Frequency of Treatment:	14 days prior to breeding, continuing throughout gestation day 21, and resumed following lactation day 5 until weaning.
Post-Exposure Period:	
Method / Guideline and Test Condition Remarks:	Type: OECD 421 Premating exposure period: Male: 2 weeks Female: 2 weeks Duration of test: 2 weeks prior to breeding, 2 wk mating period. Females - gestation day 0-19, postnatal days 5-18. Males exposed for 70 consecutive days Control group: yes, clean, unfiltered air. Method: This study was conducted to provide information on the potential adverse effects of 1,3-butadiene on male and female reproduction within the scope of a screening study. Three groups of 12 male and 12 female Sprague-Dawley rats were exposed to 0, 300, 1,500, and 6,000 ppm 1,3-butadiene via whole-body inhalation exposure 6 h/day for 14 days prior to the breeding period and continuing throughout the gestation and lactation periods. A control group was exposed to clean, filtered air on a comparable regimen. For F_0 dams, the daily inhalation exposures were suspended on gestation day 21 through lactation day 4, to avoid any confounding effects of exposure on nesting or nursing behavior. Exposures were resumed for these dams on lactation day 5. The F_1 generation pups were exposed to ,3-butadiene *in utero* and through nursing during lactation until weaning. Beginning on postnatal day 21, one male and one female from each litter were exposed for seven consecutive days to the same concentration of 1,3-butadiene concentration as its dam. Beginning on postnatal day 28, one previously unexposed male and one previously unexposed female per litter were exposed for seven consecutive days to the same 1,3-butadiene concentration as its dam.

	Assessments of gonadal function, mating behavior, conception, gestation, parturition, lactation of the F_0 generation, and the development of F_1 offspring from conception through weaning and post-weaning exposure were included in this study.
Pre-Mating Exposure / Males :	14 days
Pre-Mating Exposure / Females:	14 days

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Parental (F0)/Offspring (F1)	=	300		ppm
LOAEL	Parental (F0)/Offspring (F1)	=	1500		ppm
NOAEL	Reproductive	=	6000		ppm

Results:	No adverse treatment-related effects on any parameter measured in either the F_0 or F_1 animals at the exposure level of 300 ppm. At 1,500 and 6,000 ppm , effects consisted of persistent reductions in body weight parameters in F_0 and F_1 males and females and transient reductions in food consumption (week 0-1) for F_0 males and females. Adverse effects noted only at the high dose of 6,000 ppm consisted of clinical observations indicative of chromodacryorrhea, chromorhinorrhea, and salivation in F_0 males and females as well as infrequent

	occurrences of dried red material in the perioral and perinasal regions of four exposed F_1 pups (three males and one female). Based on the results of this study, an exposure level of 300 ppm was considered to be the NOAEL in rats for F_0 parental systemic toxicity and for systemic toxicity for F_1 animals following post-weaning 6-h daily exposures (postnatal day 21-27 or postnatal day 28-34). The NOAEL for effects on gonadal function, mating behavior, conception, gestation, parturition, lactation of the F_0 generation, and the development of F_1 offspring from conception through weaning was considered to be 6,000 ppm. Reduction in body weight parameters was determined to be the most sensitive endpoint in male and female rats with a NOAEL of 300 ppm. Developmental effects were not observed.
Results Remarks:	
Conclusion:	NOAEL (reproductive effects) = 6000 ppm in the presence of maternal toxicity
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	WIL Research Laboratories. 2003. An inhalation reproduction/developmental toxicity screening study of 1,3-butadiene in rats (unpublished report). (WIL-186024). WIL Research Laboratories, Inc., Ashland, OH, USA.

Reproductive Toxicity

TEST SUBSTANCE

Category Chemical:	No CAS number
Test Substance:	No CAS number
Test Substance Purity/Composition and Other Test Substance Comments:	C5-C6 This hydrocarbon mixture is being used to characterize the reproductive toxicity of the C5-C6 fraction of the refinery gas streams. Unleaded baseline gasoline API 99-01 Vapor Condensate Test material is a complex mixture of volatile hydrocarbons. The purity of mixture is 100% and stable based on analysis of chamber atmospheres. Representative Components [98.44%] monitored in Study

Component	Area %
Isobutane	2.14
n-butane	10.89
3-methyl-1-butene	0.41
Isopentane	35.13
n-pentane	10.44
Trans-2-pentene	2.71
2,3-dimethylbutane	2.26

	2-methylpentane: 7.82 3-methylpentane: 4.62 n-hexane: 4.14 Methylcyclopentane: 2.05 2,4-dimethylpentane: 1.42 Benzene: 2.89 2-methylhexane: 1.71 2,3-dimethylpentane: 1.74 3-methylhexane: 1.93 Isooctane: 2.15 Toluene: 4.03
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation -Whole body
Other Route of Administration:	
Type of Exposure:	2- Generation Reproduction Study
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague Dawley [Crl: CD IGS BR]
Other Strain:	
Gender:	Male and female
Number of Animals per Dose:	26 males, 26 females/group
Concentration:	
Dose:	Target: 0, 2000, 10,000, and 20,000mg/m^3 Actual: 0, 2014, 10,139, and 20,004 mg/m^3
Year Study Performed :	2006
Method/Guideline Followed:	EPA OPPTS 870.3800 None
GLP:	Yes
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :**

	P0 and F1: 10 weeks before mating, 2 weeks during mating, 3weeks gestation, 4 weeks lactation prior to weaning.
Frequency of Treatment:	6 hrs/day ,7 days/week
Post-Exposure Period:	
Method / Guideline and Test Condition Remarks:	Baseline Gasoline Vapor Condensate was administered via whole-body exposures to Sprague Dawley rats over 2 generations at target concentrations of 2000, 10000 and 20000 mg/m^3 for 6 hours/day, 7 days/week. In addition, an Air Control group received nitrogen-enriched air only while in chamber. Exposure levels were determined using an infra-red spectrophotometer 4 times per chamber per day. The test substance's major components were assayed once per chamber per week. Particle size distribution measurements were also made once per chamber per week using a TSI Aerodynamic Particle Sizer. Viability checks were performed twice daily to check for mortality and signs of severe toxic or pharmacologic effects. Physical observations and body weights were collected twice pretest (P0 generation) and at least weekly during the study (P0 and F1). Feed consumption was measured beginning the week prior to treatment initiation (P0 generation) and at least weekly during the study (P0 and F1). For P0 and F1 dams, body weight and food consumption were measured on Gestation Days [GD] 0, 7, 14, 20 and on Lactation Days [LD] 1,4,7,14,21 and 28. After approximately 16 weeks of exposure, all parental male animals (P0 and F1) were sacrificed and all parental females (P0 and F1) were sacrificed on their respective LD28. Females that failed to mate were sacrificed 25 days after the end of the mating period and females with confirmed mating but without delivery were sacrificed on presumed GD25. Selected organs [adrenals, brain, heart, liver, lungs, kidneys, spleen, thymus, ovaries, uterus testes, seminal vesicles, prostate, epididymides] were weighed and organ/body weight and organ/brain weight ratios calculated. Macroscopic examinations were performed on all parental rats and histological evaluations of the tissue samples from the weighed organs of 10 randomly selected rats in the Control and 20000mg/m^3 groups were performed. Reproductive organs from all male and bred female rats in control and high dose groups were evaluated. Sperm evaluations included motility, testicular homogenization-resistant sperm and cauda epididymal sperm count, sperm morphology in the cauda epididymis. Ovary histopathology included evaluation of the primordial follicle population, number of growing follicles and corpora lutea. <u>Mating</u>: Vaginal smears were taken daily for each female beginning three weeks prior to cohabitation for P0 and F1 rats and continuing until there was evidence of mating or until the 14-day mating period was ended. Following 10 weeks premating exposure, one male and one female from the same group were mated overnight until evidence of mating was observed or 14 days had elapsed. Animals were not paired during the daily exposure period. During mating of F1 generation, male and female

	littermates were never paired together. At weaning of each F1 litter on Lactation day 28, one pup/sex/litter was chosen at random to continue with exposure to BGVC as the F1 parental generation. When less than 26 litters were available in a group, additional pups from other litters within the group were selected at random to make up 26 mating pairs/group. Parturition and Lactation: On Day 18 of gestation exposure was ended and each female was transferred to a plastic shoebox with bedding material and observed for evidence of parturition. The day on which parturition was observed was Day 0 of Lactation. These females were not exposed from GD19 [P0 and F1 dams] until exposure was resumed on LD5 to weaning at LD28. Pups (F1 and F2 generations) were observed as soon as possible after delivery for sex, number of live and dead pups and pup abnormalities. Pup dead at delivery were identified as stillborn or liveborn found dead based on lung floatation evaluation. Thereafter litters were observed twice daily. On LD 4, F1 litters with more than 10 pups were randomly culled to 10 pups with sex distribution equalized if possible. Pups were examined and weighed on LD1 (delivery day), 4 (preculled), 7, 14, 21 and 28. At weaning one pup/sex/group was selected for mating to produce the F2 generation. F1 pups [5/sex/group/assessment] not selected for F1 mating were evaluated for standard Tier 2 neuropathology [40 CFR79.66] or for GFAP assessments [40 CFR79.67] on postpartum day 28 [Results of GFAP study are reported in separate Neurotoxicity Robust Summary]. The remaining pups were sacrificed. Three pups/sex/litter in each group (F1 and F2) were selected from macroscopic examination and selected organs [brain, spleen, thymus] were weighed from one pup /sex/litter. Statistical methods: For continuous data [Body weights, Body weight change, Feed consumption, Organ weight data, Gestation length, Pup body weights, Number of pups (live, dead, total), Mean age-to-criteria for vaginal opening and preputial separation], mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made with standard one-way analysis of variance (ANOVA) using the F ratio followed by Dunnett's if needed. Sperm and ovary analysis: The following parameters were analysed statistically: Mean sperm count (testicular sperm count and caudal epididymal sperm count) and motility data and numbers of primordial and growing follicles by ovary and total. If a significant difference occurred ($p<0.05$) between groups using the nonparametric Kruskal-Wallis test, the Wilcoxon (Mann-Whitney U) test was used for pair-wise comparisons of each treated group to the vehicle control group. Incidence data [Mortality, Mating Indices, Pregnancy rates, Male fertility Indices, Live birth indices, and Pup viability indices (Days 0-4) and lactation indices (Days 4-28)] were analyzed using the Chi-square test (2 x n). If Chi-square analysis was not significant, no additional analyses were performed. If Chi-square is significant, a Fisher Exact Test with Bonferroni correction was performed to identify differences between the groups.
Pre-Mating Exposure / Males :	

Pre-Mating Exposure / Females:	

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Repro.	≥	20000		mg/m^3
LOAEL	Systemic for P0 females, F1 males	=	20000		mg/m^3
NOAEL	Systemic for P0 females, F1 males	=	10000		mg/m^3

Results:	
Results Remarks:	Exposure conditions: The analytically measured exposure levels of the airborne test substance were reasonably close to the targeted exposure levels. Chamber environmental conditions averaged 24ºC and 43% relative humidity. Particle sizing results indicated that the atmospheres were essentially vapor only. Analysis of the major components in the neat test substance and the test atmospheres showed a reasonably close comparison between the neat test substance and the vaporized test substance. This data demonstrated that the test animals were exposed, as expected, to all of the major components of the test substance in their reasonably proper proportion. The data was consistent from week-to-week during the study indicating stability of the test substance and the atmosphere generation

	techniques. Parental data (P0 and F1 generations): There was no effect of treatment on survival. The test animals were generally unremarkable in-chamber during the exposure periods and during the non-exposure periods (afternoon evaluations) during the premating period in both sexes, the mating/postmating period in the male rats, and the gestation and lactation periods in the female rats. There were exposure-related differences in body weights or weight changes in the test substance exposed animals compared to the Air Control animals. These differences were decreases in weight gain in the P0 female rats in the 20000mg/m³ group during the latter 3 weeks of the premating period and in the F1 male rats in the 20000 mg/m³ group during the initial 8 weeks of the premating period. There were no exposure-related differences in feed consumption in the test substance exposed animals compared to the Air Control animals. There were no exposure-related differences in estrous cycle data (as measured by cycle length and number of estrous cycles) in the test substance exposed animals compared to the Air Control animals. Mating indices for the male rats treated with the test substance were comparable to the Air Control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the Air Control group. The pregnancy rates for the Air Control, 2000, 10000 and 20000 mg/m³ groups were 96.0%, 96.2%, 92.3% and 100%, respectively, for the P0 animals and 100%, 100%, 91.7% and 100%, respectively, for the F1 animals. Treatment with the test substance also resulted in no statistically significant differences in most other reproductive parameters including the percent of females completing delivery and the duration of gestation, when compared to the Air Control group. There were no exposure-related differences in body weights or weight changes in the test substance exposed animals compared to the Air Control animals during the gestation and lactation periods. There were no exposure-related differences in feed consumption during the gestation and lactation periods in the test substance exposed animals compared to the Air Control animals. Treatment with the test substance resulted in no statistically significant differences in all parturition parameters including the total number of pups delivered, the number of pups dying, the viability (4 day survival) and lactation (28 day survival) indices, the number of implantation sites per litter, the sex ratio and the number of live pups/litter, when compared to the Air Control group. There were no exposure-related temporal differences in males showing preputial separation and females showing vaginal opening in the F1 pups weaned from test substance exposed animals compared to the F1 pups weaned from Air Control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the test substance exposed animals compared to the Air Control animals. Exposure-related effects on organ weights included statistically significant increases in kidney weights (absolute and relative to body and brain weight) at the 2 higher exposure levels in the P0 and F1 males and at the highest exposure level in the P0 females. These differences for the males (but not the females) were consistent with the microscopic findings discussed below. The percent sperm motility, caudal epididymal and homogenization-resistant testicular sperm counts, sperm morphology, and primordial and growing follicle counts, as individual ovaries and total per animal, were not affected by treatment with test substance at an exposure level of 20,000 mg/m³. Microscopic findings that were considered exposure-related were found only in the kidneys of male animals exposed to 20,000 mg/m³ of test substance and are consistent with hyaline droplet nephropathy, attributable to attributable to

	accumulation of alpha-2 microglobulin within renal tubular epithelial cells. This species- and gender-specific change has been well documented in male rats exposed to a variety of hydrocarbon compounds and is not considered relevant to humans. No test substance related microscopic changes were noted in male and female reproductive organs or other protocol-specified tissues in this study. Pup data (F1 & F2 generations): There were no exposure-related differences in body weights and weight changes in the pups from test substance exposed animals compared to the pups from Air Control animals. The pups were unremarkable during the lactation period. There were no exposure-related differences in macroscopic postmortem evaluations and organ weights in the pups from test substance exposed animals compared to the pups from Air Control animals. No adverse neuropathological findings were observed.
Conclusion:	Exposure of rats to 2000, 10000 and 20000mg/m³ of vapor of test substance resulted in decreased body weight gains in the P0 females and F1 males prior to mating in the 20000 mg/m³ exposed group. Increases in kidney weights in parental male animals exposed to the 2 higher exposure levels of vapor were consistent with hydrocarbon nephropathy seen in these animals, a finding has been generally accepted not to be relevant to human risk assessment (US EPA, 1991). There was no effect at any of the exposure levels on reproductive performance in the study, including mating, fertility, parturition, lactation, offspring survival and development or maturation, in either the P0 or F1 generations. There was no evidence of any neuropathology in F1 pups as a result of the exposures [GFAP results reported in separate Robust summary]. The NOAEL for systemic toxicity [excluding kidney effects in male rats] is 10000mg/m³. The NOAEL for neuropathology in F1 animals is >20,000mg/m³ The Reproductive NOAEL is ≥20,000mg/m³ (6521 ppm).
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	HPV Supporting study from Section 211(b) Testing Consortium, Fuels and Fuel Additives Health Effects Testing Regulation, administered by API, Washington DC
Key Study Sponsor Indicator:	Key
REFERENCE	

Reference:	Baseline Gasoline Vapor Condensate: A Two-Generation Whole Body Inhalation Reproductive Study in Rats. 2006. HLS Study No. 00-4207. Huntingdon Life Sciences Laboratories, East Millstone, NJ. US EPA 1991. Alpha 2 microglobulin: Association of chemically induced renal toxicity and neoplasia in male rats. In Risk Assessment Forum, p.85. US Govt Printing Office, Washington DC.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	71-43-2
Test Substance:	Benzene
Test Substance Purity/Composition and Other Test Substance Comments:	Glass distilled benzene of chromatographic quality (Burdick and Jackson Laboratories, Inc., Muskegon, MI).
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Developmental toxicity study
Species:	Mice
Other Species:	
Mammalian Strain:	Crl: CFW(SW)Br, Charles River Laboratories
Other Strain:	
Gender:	Females only
Number of Animals per Dose:	5-10
Concentration:	

Dose:	0, 5, 10, 20 ppm
Year Study Performed :	1988
Method/Guideline Followed:	Other
GLP:	No data
Exposure Period:	Gestation days 6-15
Frequency of Treatment:	Daily; 6 hrs/day
Post-Exposure Period:	Examinations on gestation day 16; 2 days post-partum; 6 weeks post partum
Method/Guideline and Test Condition Remarks:	Duration of test: Female mice exposed for gestation days 6-15 Control group: yes, filtered conditioned air. Method: This study investigated the effects of inhalation exposure to benzene *in utero* on fetuses. In three experiments, female Swiss-Webster (Crl: CFW(SW)Br) mice (5-10 mice/concentration level) were exposed (0, 5, 10, or 20 ppm benzene, 6h/day, gestation days 6-15. Experiment 1: Five benzene-exposed and five air-exposed pregnant mice were sacrificed on the 16^{th} day of gestation, their uteri removed, and the number of live, dead, and resorbed fetuses recorded. Two male and two female fetuses were then randomly selected, weighed, and examined for any external gross morphological malformation. Peripheral blood samples were taken for red and white cell counts and for hemoglobin analysis. Livers were removed for enumeration of recognizable cells in the hematopoietic differentiating, proliferating pool (DPP). Experiment 2: Five benzene-exposed and five air-exposed pregnant females were allowed to proceed through normal parturition. Two male and two female neonates were then randomly selected at 2 days of age and subjected to the same protocol as that described above with 16-day old fetuses. Experiment 3: Five benzene-exposed and five air-exposed pregnant dams were allowed to proceed through normal parturition. At 6 weeks of age, one male and one female were randomly selected from each litter. Peripheral blood samples were obtained from tail veins for red and white cell counts and for hemoglobin analysis. These animals were then sacrificed and their spleen and femurs removed for enumeration of recognizable cells in the DPP. Peripheral and organ blood cell counts were determined, each benzene exposed animal having its own age-matched air control. Differences in the cell counts were evaluated by the Student *t* test using the litter as the experimental unit. Differences greater than the two-tailed, $p < 0.05$, Student *t* value were considered significant. Differential counts of peripheral blood cells and DPP cells were evaluated against age-matched control slides. These data were evaluated first using a two-way analysis of variance with treatment and gender as covariates. *F* ratios were significant for differences in the responses of the genders in only 3 of the 42 two-way analyses of variance performed. In contrast, *F* ratios were significant for the responses due to benzene treatment in 15 of the 42 two way

	analyses ofvariance performed. For this reason total litter responses (male and female) vs treatment were assessed by one-way analysis of variance followed by Dunnett's tests. Ratios of hemoglobin A major to hemoglobin A minor were also analyzed by one-way analyses of variance followed by Dunnett's tests. Differences between treatments were considered significant when they were greater than the two-tailed, $p < 0.05$ Dunnett's critical value.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Female (Maternal)	=	20		ppm
LOAEL	Offspring	=	20		ppm
NOAEL	Offspring	=	10		ppm

Results Remarks:	In the dams, no hematological investigations were performed. Also, no data on any gestational parameters were provided from the three experiments, since investigating any embryo-/fetotoxic or teratogenic properties of benzene was not the focus of the study. However, there was no evidence of maternal toxicity among dams exposed to any concentration of benzene tested as determined by maternal morbidity, mortality, or weight loss during the exposures. There was no evidence of non-hematopoietic toxicity among any of the fetal or neonatal progeny exposed *in utero* to any concentration of benzene studied. Litter sizes, male/female ratios, and body weights, as well as the numbers of dead, resorbed, or malformed fetuses, were all within control limits. There were < 5 litters/age group per treatment. Peripheral blood cell indices (red blood cell count, mean corpuscular hemoglobin, nucleated cells/mm3 and ratio of HbA major to HbA minor)- no significant differences between benzene-exposed and air-exposed progeny across the different stages of development. Peripheral blood cell differentials (numbers of blasts, dividing/nondividing granulocytes, early/late and primitive nucleated red cells and lymphocytes determined from a total of 100 cells)-

	16-day fetuses: no significant differences between benzene-exposed and air-exposed groups. 2-day neonates: benzene-exposed groups showed significantly less counts of erythroid precursor cells (early nucleated cells), and the 20 ppm group also exhibited depressed numbers of late nucleated red cells and elevated numbers of granulocytic precursor cells (nondividing granulocytes). 6-week old offspring: no significant differences between benzene-exposed and air-exposed groups. Hemopoietic organs cell differentials (numbers of blasts, dividing/nondividing granulocytes, early/late and primitive nucleated red cells and lymphocytes determined from a total of 500 cells in fetal and neonatal liver, respectively in femural bone marrow and spleen of 6-week old offspring): 16-day fetuses-no significant differences between benzene-exposed and air-exposed groups. 2-day neonates: The 20 ppm level group showed significanly lower counts of late nucleated red cells and decreased counts of early nucleated red cells, whereas the numbers of blasts, dividing/nondividing granulocytes and lymphocytes were elevated. 6-week old offspring: the 20 ppm level group showed a slightly higher numbers of blasts, dividing/nondividing granulocytes and lymphocytes in comparison to their age-matched controls. Note: The lower numbers of erythroid precursor cells (early nucleated cells) observed in the 2-day neonates apparently did not negatively affect the circulating red cells in these animals as indicated from their normal cell counts in peripheral blood. Also, no changes in the HBA major/HbA minor ratios, indicative for disturbances of normal maturation of erythroid precursor cells, were determined in this group of animals.
Conclusion:	*in utero* exposures to concentrations of benzene as low as 20 ppm can induce persistent enhanced production of recognizable granulopoietic elements in the hematopoietic systems of offspring LOAEL = 20 ppm NOAEL= 10 ppm
RELIABILITY / DATA QUALITY	
Reliability:	Valid With Restrictions (KS-2)
Reliability Remarks:	Non-guideline developmental toxicity study but in accordance with generally accepted scientific standards and described in sufficient detail.
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Keller, KA., Snyder, CA. 1988. Mice exposed in utero to 20 ppm benzene exhibit altered numbers of recognizable hematopoietic cells up to seven weeks after exposure. Fundam. Appl. Toxicol. 10: 224-232.

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	74-84-0
Test Substance:	Ethane
Test Substance Purity/Composition and Other Test Substance Comments:	Ethane, purity 99%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	

Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 1600, 5000, 16,000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on ethane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Repeated-Dose Toxicity of this Robust Study Summary

	submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 160, 1600 and 16000 ppm. Therefore, the high exposure level was established (for safety reasons) at 16000 ppm since it is 50% of the lower explosion limit (3.2% = 32000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 256 230-284 Female: 197 167-217 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 1600, 5000 and 16000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19. Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	1600 ppm	12 males	24 females
Group 3	5000 ppm	12 males	24 females
Group 4	16000 ppm	12 males	24 females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

Viability
Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

Clinical observations
All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded.

Clinical chemistry:
Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed

	to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration

	Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery:

	Sensory Observations startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver

	Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of

	spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. No protocol deviations occurred during the study. **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically:

	Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0.

2. Motor Activity Counts
The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor.

3. Incidence data

The following parameters were analyzed statistically:
Mortality rate
Mating indices, pregnancy rates, male fertility indices
Litter survival indices
Gestation indices
Incidence of dams with no viable pups
Mean pup survival indices (lactation days 0 and 4)

Incidence Data Analysis
A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:

NOAEL	Male and female rats (Parental/ systemic)	=	16,000		ppm
NOAEL	Reproductive	=	16,000		ppm
NOAEL	Developmental	=	16,000		ppm

Results Remarks:

Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 1703 ± 23, 4762 ± 124, 15502 ± 194 ppm. The analytically measured levels of airborne test substance were reasonably close to the targeted exposure levels and nominal concentrations. Chamber environmental conditions averaged 23ºC temperature and 49% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers.

The test substance was assayed by gas chromatography versus an analytical standard before and after the study to demonstrate the purity and stability of the test substance. The test substance was determined to be 99.86% ethane before the study and 99.88% ethane after the study demonstrating the purity and the stability of the test substance.

MAIN STUDY ANIMAL OBSERVATIONS
There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes or feed consumption (except the 16000 ppm exposed animals showed marginally lower feed consumption during the first week of exposures but the differences from the air control did not exceed 5% in the test substance exposed animals compared to the air control animals).
There was no apparent exposure-related effect on functional observational battery or motor activity parameters for either sex in this study. There were no exposure-related differences in hematology or coagulation values or clinical chemistry values in test substance exposed animals compared to the air control animals at the terminal interval. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. There were no microscopic findings considered to be related to exposure to ethane. No testiclular (stages of spermatogenesis; testicular interstitial cells) abnormality was evident in any test animal.

SATELLITE FEMALE ANIMAL OBSERVATIONS

	There was no effect of treatment on survival. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red nasal discharge or red/brown staining between the 7th and 28th days of exposure in all groups including the control animals. All animals survived until the termination of the study. These findings were slightly more prevalent among test substance exposed animals. There were no exposure-related differences in body weights or weight changes or feed consumption (except the 16000 ppm exposed animals showed marginally lower feed consumption (6%) during the first week of exposures, consistent with the same marginal trend in the main study animals. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS Almost all mated female animals were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. All but one of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 1600, 5000 or 16,000 ppm of ethane by whole-body inhalation for 4 weeks resulted in no systemic or neurotoxic effects, apart from a marginal feed reduction in high dose animals during the first week of exposure. A no-observed-adverse effect level (NOAEL) of 16,000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 16,000 ppm was concluded for reproductive and developmental endpoints in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1

Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Ethane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4243

	High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	74-98-6
Test Substance:	Propane
Test Substance Purity/Composition and Other Test Substance Comments:	Propane, purity 99.5%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	

Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 1200, 4000, 12000 ppm
Year Study Performed:	2003
Method/Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen:

	Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method / Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on propane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Repeated-Dose Toxicity of this Robust Study Summary submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 120, 1200 and 12000 ppm. Therefore, the high exposure level was established (for safety reasons) at 12000 ppm since it is 50% of the lower explosion limit (2.4% = 24000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation. The weight of animals at initiation of exposures was: Mean (g) Range (g) Male: 269 243-297 Female: 200 180-220 Individual weights of animals placed on test were within ±20% of the mean weight for each sex. The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 1200, 4000 and 12000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times

per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	1200 ppm	12 males	24females
Group 3	4000 ppm	12 males	24 females
Group 4	12000 ppm	12 males	24females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

Viability
Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon).

Clinical observations
All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior

	to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other

	Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when

	main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery: **Sensory Observations** startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature **Motor activity** Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant.

	Organ weights A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations**

	Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during

	lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. Three protocol deviations occurred during the study, but did not appear to impact the results. **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically: Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were

equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels.

Exceptions
Statistical evaluations were not performed when the standard deviation for the control group was 0.

2. Motor Activity Counts
The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the $p<=0.05$ level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor.

3. Incidence data

The following parameters were analyzed statistically:
Mortality rate
Mating indices, pregnancy rates, male fertility indices
Litter survival indices
Gestation indices
Incidence of dams with no viable pups
Mean pup survival indices (lactation days 0 and 4)

	Incidence Data Analysis A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Male rats (Parental)	=	12,000		ppm
NOAEL	Male rats (Parental)	=	4,000		ppm
NOAEL	Female rats (parental)	=	12,000		ppm
NOAEL	Reproductive	=	12,000		ppm
NOAEL	Developmental	=	12,000		ppm

Results Remarks:	Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 1230 ± 34, 3990 ± 156, 12168 ± 415 ppm. Chamber environmental conditions averaged 23º C temperature and 42% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers. Pre-study GC analysis of the test substance showed a purity of 99.91% for propane. This value compared closely to the purity of 99.84% for the analytical standard of propane and the 99.5% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.90% for propane. This value compared closely to the purity of 99.80% for the analytical standard of propane and essentially replicated the 99.91%

	pre-study purity demonstrating stability of the test substance over the period of this study. MAIN STUDY ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red staining between the 22nd and 28th days of exposure in all groups including the control animals. These findings were very slightly more prevalent among test substance exposed animals. There were exposure-related differences in body weights and weight changes in the 12000 ppm exposed male animals as compared to the air control animals. These male animals showed a 25% decrease in weight gain during the 1st week of exposures and this difference persisted for the remainder of the 4 weeks of exposures. Similar differences were not seen at the lower levels for the males or in any of the female groups. There were no meaningful differences in feed consumption in the test substance exposed animals as compared to the air control animals. There was no meaningful effect on functional observational battery parameters or motor activity for either sex in this study. A statistically significant increase in hindlimb grip strength was noted in the 4000 ppm exposed males but there was no exposure level related pattern. There were no meaningful differences in hematology or coagulation values in test substance exposed male animals as compared to the air controls. A statistically significant decrease (up to 21% in the low level) in absolute lymphocytes in the test substance exposed males was seen but not in an exposure-related pattern and there were no accompanying changes in the other hematology parameters for the males, nor were there any similar differences in the females. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures and were within this laboratory's normal range of values. Similarly, the statistically significant increases only in the 12000 ppm exposed females for hemoglobin concentration, hematocrit, erythrocytes and absolute eosinophils were considered in context too small to represent an effect of the exposures. There were no meaningful differences in clinical chemistry values in the test substance exposed animals as compared to the air control animals. A statistically significant increase in sodium concentration in the 12000 ppm exposed females was seen but the absolute difference was less than 1% and there was no similar difference in the males. A statistically significant decrease in chloride concentration in the 1200 ppm exposed females was seen but the absolute difference was only 2% and there was no similar difference at the higher levels in the females or at any level of exposure in the males. In isolation, these minor inter-group differences were not considered to indicate effects of the exposures and were within this laboratory's normal range of values. There were no meaningful inter-group differences in organ weights in the test substance exposed animals as compared with air control animals. The 1200 and 12000 ppm exposed males showed decreases in kidney weights (absolute and/or relative to body or brain weight) but no similar differences were seen in the exposed females. The 12000 ppm exposed males also showed decreases in absolute (but not relative) liver weights and similar differences

	were seen (but only relative to brain weight) in the 4000 and 12000 ppm exposed females. Considering the absence of any correlated pathology findings and clear patterns of treatment, these minor inter-group differences were not considered to indicate effects of the exposures. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations in the test substance exposed animals compared to the air control animals. The testes were examined qualitatively with an awareness of the stages of the spermatogenic cycle to detect any disturbances in spermatogenesis, and with emphasis on the interstitial (Leydig) cells to detect any changes in cellular size or structure. No test article related findings were present. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. For the pregnant females there were no exposure-related differences in body weights or weight changes in the test substance exposed animals as compared to the air control animals. There were no treatment- related changes in feed consumption. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS All mated female animals (except one in the 1200 ppm group – considered incidental) were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. Almost all of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, and the proportion with live litters and/or with stillborn pups when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. Statistically significant decreases in the number of live born pups and converse increases in the number of stillborn pups in the 4000 and 12000 ppm exposed groups were attributable to the single total litter loss in each of these groups very soon after parturition. These losses were preceded by severely reduced body weight gain in the last week of gestation for two particular dams. There was no excess of mortality in any of the other litters in these groups and the total litter losses were considered incidental and not related to the exposures. There were no exposure-related differences in body weights or weight gains in the pups

	feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 1200, 4000 or 12000 ppm of propane by whole-body inhalation for 4 weeks resulted in a reduction of body weight gain in the males resulting from exposures at the12000 ppm dose. Consequently, a no-observed-adverse effect level (NOAEL) of 4000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study (LOAEL – 12,000 ppm). There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 12,000 ppm was concluded for reproductive and developmental endpoints in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Propane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4245.

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	75-28-5
Test Substance:	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments:	Isobutane, purity 99.0%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)

Concentration:	
Dose:	0, 900, 3000, 9000 ppm
Year Study Performed:	2003
Method / Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method / Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on isobutane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Repeated-Dose Toxicity of this Robust Study Summary submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 90, 900 and 9000 ppm. Therefore, the high exposure level was established (for safety reasons) at 9000 ppm since it is 50% of the lower explosion limit (1.8% = 18000 ppm) for the test substance.

The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation.

The weight of animals at initiation of exposures was:

	Mean (g)	Range (g)
Male:	277	249-301
Female:	194	171-216

Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 900, 3000 and 9000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	900 ppm	12 males	24females
Group 3	3000 ppm	12 males	24 females
Group 4	9000 ppm	12 males	24 females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

	Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure. The following parameters were evaluated: **Viability** Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon). **Clinical observations** All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose

	Cholesterol Total protein Triglycerides Albumin Total bilirubin Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on

	the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights. **Feed consumption** Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment. **Functional Observational Battery** The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery: **Sensory Observations** startle response to auditory stimuli tail pinch response **Neuromuscular Observations** grip strength - hindlimb and forelimb **Physiological Observations** rectal temperature

	Motor activity Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups. **Clinical pathology (termination**) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum)

	Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior.

	Body Weight Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing** Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. No protocol deviations occurred during the study. **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically: Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites

	F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit) Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0. 2. Motor Activity Counts The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant ($p<=0.05$), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passes on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment

	group effects were deemed significant at the p<=0.05 level. Plots, tables, listings and analyses were generated using SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor. 3. Incidence data The following parameters were analyzed statistically: Mortality rate Mating indices, pregnancy rates, male fertility indices Litter survival indices Gestation indices Incidence of dams with no viable pups Mean pup survival indices (lactation days 0 and 4) Incidence Data Analysis A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male and female rats (Parental/ systemic)	=	9,000		ppm
LOAEL	Reproductive	=	9,000		ppm
NOAEL	Reproductive	=	3,000		ppm
NOAEL	Developmental	=	9,000		ppm

Results Remarks:	Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The mean

	(± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 930.0 ± 27.8, 3122 ± 83 and 9148 ± 201 ppm. Chamber environmental conditions averaged 24°C temperature and 55% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers. Pre-study GC analysis of the test substance showed a purity of 99.84% for isobutane. This value compared closely to the purity of 99.51% for the analytical standard of Isobutane and the 99.0% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.53% for isobutane. This value compared closely to the purity of 99.50% for the analytical standard of isobutane and the 99.0% pre-study purity demonstrating stability of the test substance over the period of this study. MAIN STUDY ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of transient red staining between the 7th and 28th days of exposure in all groups including the control animals. These findings were only slightly more prevalent among test substance exposed animals. There were no exposure-related differences in body weights and weight changes or feed consumption in the exposed male animals as compared to the air control animals. There was no treatment-related effect on functional observational battery parameters or motor activity for either sex in this study. There were no meaningful differences in hematology or coagulation values in test substance exposed male animals as compared to the air controls. Statistically significant increases in hemoglobin and mean corpuscular hemoglobin concentration in the 9000 ppm test substance exposed males, decreases in platelets in 3000 and 9000 ppm test substance exposed males and decreases in monocytes in 3000 and 9000 ppm test substance exposed females were seen in exposure-related patterns but there were no accompanying changes in the other hematology parameters, nor were there any similar differences in the other sex. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures and were generally within the normal range of these values for a rat. There were no meaningful differences in clinical chemistry values in the test substance exposed animals as compared to the air control animals. Statistically significant increases in sodium concentration in the 9000 ppm test substance exposed males and decreases in phosphorus concentration in all test substance exposed females were seen but the absolute differences were relatively small, were not in an exposure level related pattern and there were no similar differences in the other sex. In isolation, these minor inter-group differences were not considered to indicate an effect of the exposures. There were no meaningful inter-group differences in organ weights in the test substance exposed animals as compared with air control animals. The 900 and 3000 ppm exposed males showed an increase in spleen weights

	(absolute and/or relative to body or brain weight) but no similar difference was seen in the 9000 ppm exposed males or any of the exposed females. In isolation, this minor inter-group difference was not considered to indicate an effect of the exposures. There were no exposure-related differences in macroscopic or microscopic postmortem evaluations in the test substance exposed animals compared to the air control animals. The testes were examined qualitatively with an awareness of the stages of the spermatogenic cycle to detect any disturbances in spermatogenesis, and with emphasis on the interstitial (Leydig) cells to detect any changes in cellular size or structure. No test article related findings were present. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There were no exposure-related differences in body weights or weight changes in the test substance exposed animals as compared to the air control animals. There were no exposure-related differences in feed consumption in the test substance exposed animals as compared to the air control animals. . There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. A statistically significant increase in heart to brain weight ratio in 9000 ppm exposed females was seen but, in the absence of any macroscopic and microscopic abnormalities in the main study animals, this difference was considered to have been incidental. MATING, FERTILITY AND GESTATION PARAMETERS No parental systemic toxicity was observed. In the 9000 ppm group, 25% of the mated females did not become pregnant. Although not statistically significant, the reduction in male and female fertility indices (75%) was considered exposure related since it was below the concurrent control (100%) and the testing facility historical control values (mean 96.4%; range 87.5%-100%). The mating index for male rats treated with the test substance was comparable to the air control group. A statistically significant ($p < 0.05$) exposure-related increase in post-implantation loss was also observed for the 9000 ppm group of exposed female rats; mean losses of 0.8 ± 0.9 and 1.8 ± 0.8 for control and high exposure groups respectively. The data were interpreted as conservatively as possible; and the two reproductive endpoints were attributed to isobutane exposure. All other reproductive endpoints were comparable to controls (number of pairs cohabited, number of pairs mated, mating index, gestation index, mean time to mating, mean gestation length, number of females completing delivery with stillborn pups/all stillborn pups, mean pre-implantation loss, mean pups delivered, live birth index, viability index). Pup endpoints (viabilty to day 4, weight & weight gain, sex ratio) were also comparable to air control pups.

Conclusion:	Exposure of male and female rats to target concentrations of 900, 3000 or 9000 ppm of isobutane by whole-body inhalation for 4 weeks resulted in no general systemic/neurotoxic effects. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. Based on decreased male and female fertility and increased post-implantation loss in the 9000 ppm group, the fertility and reproductive endpoints NOAEL was determined to be 3000 ppm. There were no effects on in offspring survival, body weight and development up to post-natal day 4. A NOAEL of 9000 ppm was concluded for developmental effects.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Isobutane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4244.

DEVELOPMENTAL TOXICITY / TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	106-97-8
Test Substance:	Butane

Test Substance Purity/Composition and Other Test Substance Comments:	Butane, purity 99.5%. (MG Industries, Malvern, Pennsylvania); assayed by gas chromatography by testing laboratory.
Category Chemical Result Type:	Measured
Unable to Measure or Estimate Justification:	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	Not applicable
Type of Exposure:	4-week subchronic toxicity study combined with reproduction/developmental toxicity screening and neurotoxicity screening study.
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	Males -12/dose (used for main study and repro/dev screen) Females – 24/dose (12 /dose for main study; 12/dose for repro/dev screen)
Concentration:	
Dose:	0, 900, 3000, 9000 ppm

Year Study Performed:	2004
Method / Guideline Followed:	OECD 422/EPA OPPTS 879.3650
GLP:	Yes
Exposure Period:	Main study: 28 days (males and females) Repro/dev screen: Males – 2 weeks prior to mating and post-mating until euthanized for a minimum exposure of 28 days Females - 2 weeks prior to mating, during mating period and gestation days 0-19
Frequency of Treatment:	6 hrs/day 7 days/wk
Post-Exposure Period:	
Method / Guideline and Test Condition Remarks:	An OECD Test No. 422 Combined Repeated Dose Toxicity Study with Reproduction/Developmental Toxicity Screening Test was conducted on butane. This study assessed the repeated-dose, reproductive, and developmental toxicity potential of this material when administered by whole-body inhalation exposure. The subchronic portion of the study includes neurotoxicity assessment for both behavioral and motor activity toxicity potential. This same study is also described in the Reproductive Toxicity and Repeated-Dose Toxicity of this Robust Study Summary submission for Petroleum Hydrocarbon Gases Category; the same RSS is presented in all three (repeated-dose, reproductive toxicity, and developmental toxicity) human health endpoint sections. The subchronic portion of the study is referred to as the "main study"; the reproductive/developmental portion is referred to as the "satellite study". The exposure levels were based on results of a 2-week range-finding study which showed no toxicity at exposure levels of 90, 900, and 9000 ppm. Therefore, the high exposure level was established (for safety reasons) at 9000 ppm since it is 50% of the lower explosion limit (1.8% = 18,000 ppm) for the test substance. The animals used in the study were approximately 6 weeks old at receipt and approximately 8 weeks old at exposure initiation.

The weight of animals at initiation of exposures was:

	Mean (g)	Range (g)
Male:	261	241-280
Female:	200	174-229

Individual weights of animals placed on test were within ±20% of the mean weight for each sex.

The test substance was administered as a gas to Sprague Dawley CD rats (12/sex/main study group and 12 females/satellite group) at target concentrations of 900, 3000 and 9000 ppm for 6 hours/day, 7 days/week for 2 weeks prior to mating initiation. Main study male rats were exposed during the mating and post-mating periods until euthanized for a minimum exposure of 28 days. Main study female rats (12/group) were exposed once daily (6 hours/day), seven days/week for 4 weeks (28 days), and then evaluated for subchronic study parameters per guideline. Satellite female rats (12/group) for the reproduction study were exposed once daily (6 hours/day), 7 days/week for at least two weeks prior to mating initiation. Satellite female rats continued to be treated once daily (6 hours/day) during mating. Once mated, satellite female rats were treated once daily (6 hours/day) during gestation (Days 0-19). Satellite female rats without evidence of mating continued treatment for 19 days (6 hours/day) following completion of the mating period and then held for an additional 7 days. For satellite female rats without evidence of mating that appeared to be pregnant, exposure was terminated on the estimated gestation day 19.

Exposure method: whole body

Group 1	0 ppm	12 males	24 females (air control)
Group 2	900 ppm	12 males	24 females
Group 3	3000 ppm	12 males	24 females
Group 4	9000 ppm	12 males	24 females

The test substance was administered as a gas in the breathing air of the animal. It was delivered from a single cylinder, through a regulator and backpressure gauge to each of three chambers via a flow meter and regulator valve.

Determination of the exposure levels were made using an ambient air analyzer equipped with a strip chart recorder. The test atmosphere was drawn from the sampling portal through the air analyzer and measurements were recorded at least 4 times during each exposure.

The following parameters were evaluated:

	Viability Observations for mortality and general condition were made at least twice daily (once in the morning and once in the afternoon). **Clinical observations** All animals were observed as a group at least once during each exposure. This was routinely performed near the middle of each exposure. Each animal was removed from its cage and a detailed physical observation performed prior to randomization. Main study male rats were observed once weekly beginning during the pre-mating period and throughout the study. Main study female rats were observed weekly after randomization and continuing throughout the study. Satellite female rats were observed weekly during the pre-mating period and on gestation days 0, 7, 14, 20 and lactation days 0 (except for females whose parturition was not complete on lactation day 0), 1 and 4. Satellite female rats without evidence of mating continued to be observed weekly during the mating and post-mating period until euthanized. Examinations during non-exposure periods included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, stereotypy (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backward) were recorded. **Clinical chemistry:** Blood for clinical chemistry studies (approximately 1.0 mL) was collected into tubes with no anticoagulant, allowed to clot, and centrifuged to obtain serum. The following parameters were evaluated: Blood samples were analyzed as follows: Aspartate aminotransferase Alanine aminotransferase Alkaline phosphatase Blood urea nitrogen Creatinine Glucose Cholesterol Total protein Triglycerides Albumin Total bilirubin

	Sodium Potassium Chloride Calcium Inorganic phosphorus Gamma-glutamyl transpeptidase Other Globulin (calculated value; total protein - albumin) Albumin/globulin ratio (calculated value; albumin -globulin) **Hematology:** Blood for hematology studies was collected (approximately 0.25 mL) into tubes containing EDTA anticoagulant. Blood samples were analyzed as follows: Hemoglobin concentration Hematocrit Erythrocyte count Platelet count Mean corpuscular volume Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Total leukocyte count Reticulocyte count Differential leukocyte count Other Erythrocyte and platelet morphology (from peripheral blood smear) (Henry, 1991) **Body weights** Body weights of the main study male and female rats were recorded at the time of randomization into test groups, on the first day of treatment and weekly thereafter throughout the study. Satellite female rats for the reproduction study were weighed at the time of randomization into test groups, on the first day of treatment and twice weekly until evidence of copulation was observed. Mated satellite female rats were weighed on gestation days 0, 7, 14 and 20 and Satellite female rats that delivered litters were weighed on lactation days 1 and 4. Satellite female rats without evidence of mating were weighed twice weekly during the mating and post-mating period. Terminal body weights

for main study animals were recorded after fasting. Satellite females were not fasted prior to recording of terminal body weights.

Feed consumption
Feed consumption for the main study male rats was recorded pretest and weekly during the pre-mating treatment period. Feed consumption for the main study female rats was recorded pretest and weekly during the treatment period. Feed consumption for satellite female rats was recorded pretest and weekly during the pre-mating period, and if not mated, during the post-mating period. Feed consumption was not recorded during the mating period when main study male rats were co-housed with satellite female rats. For pregnant or confirmed mated satellite female rats, feed consumption was recorded on gestation days 0-7, 7-14 and 14-20 and on lactation days 1-4. Feed was available without restriction, except during exposures and at terminal fasting for the main study animals. Animals were presented with full feeders of known weight. After 7 days (pre-mating), 6 or 7 days (gestation) or 3 days (lactation), the feeders were reweighed and the resulting weight subtracted from the initial feeder weight to obtain the grams of feed consumed per animal over the 7, 6 or 3-day period. Feed consumption was measured (weighed) weekly, beginning one week prior to treatment.

Functional Observational Battery
The time of testing was balanced across treatment groups. All observations during the treatment period were performed blind, i.e., the observer was unaware of the animals' treatment. The following evaluations were performed as part of the functional observational battery:

Sensory Observations
startle response to auditory stimuli
tail pinch response

Neuromuscular Observations
grip strength - hindlimb and forelimb

Physiological Observations
rectal temperature

Motor activity
Activity was monitored using an automated Photobeam Activity System. Sessions were 60 minutes in length; each session was divided into 12 five-minute intervals. The time of testing was balanced across treatment groups.

	Clinical pathology (termination) Blood samples for hematology, coagulation and clinical chemistry studies were obtained from lightly anesthetized (CO2/O2) main study animals (12/sex/group) via puncture of the orbital sinus (retrobulbar) at study termination. Animals were fasted overnight prior to blood collection. Blood was collected and studies performed as follows: Blood for coagulation studies was collected (approximately 0.75 mL) into tubes containing sodium citrate anticoagulant. **Organ weights** A wide range of organs (30) were taken at the scheduled necropsy, recorded and organ/body and organ/brain weight ratios calculated. The following organs were taken: Adrenal glands Bone (sternum/femur) Bone marrow (rib) Brain (medulla/pons, cerebrum and cerebellum) Epididymides Heart Kidneys Large intestine (cecum, colon and rectum) Larynx Liver Lungs (with mainstem bronchi) Lymph node (mesenteric) Lymph node (mediastinal) Mammary glands (with adjacent skin) Nasopharynx Ovaries (with oviducts) Prostate Seminal vesicles Small intestine (duodenum, ileum and jejunum) Spinal cord (cervical, thoracic and lumbar) Spleen Stomach Testes Thymus

	Thyroid with parathyroids Tibial nerve Trachea Urinary bladder Uterus with vagina All macroscopic lesions and tissue masses **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study male rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. **Microscopic pathology** A wide range of organs (30) were obtained at necropsy for all main study male and female rats as well as all satellite females. Slides of the indicated tissues were prepared and examined microscopically for control and high exposure main study animals. During the microscopic examination of the testes, special emphasis was placed on the stages of spermatogenesis and the histopathology of interstitial testicular cell structure. Any abnormalities not noted during macroscopic postmortem examinations, which were seen during histological processing were recorded. **F1 Pup Evaluations** **Physical Examinations** Each F1 pup was given a gross examination on lactation days 0 and 4. Pups were also observed for any abnormal behavior. **Body Weight** Individual F1 pup weights were recorded on lactation days 1 and 4. **Sexing**

	Pups were sexed on lactation day 0 and sex verified on lactation day 4. **Macroscopic observations** Macroscopic postmortem examinations were performed on all main study and satellite female rats. Postmortem examinations included examination of external surface, all orifices, cranial cavity, nasal cavity (external examination), neck and its associated tissues and organs, thoracic, abdominal and pelvic cavities and their associated tissues and organs, and external surfaces of the brain. Macroscopic postmortem examinations (internal and external) were performed on F1 pups found dead during lactation. F1 pups found dead at birth were identified (lung floatation test) as stillborn or alive but found dead. Macroscopic postmortem examinations (external only) were performed on all F1 pups on lactation day 4 for pups surviving to that interval. Unusual observations, including gross abnormalities and the absence of milk in the stomach, were noted and then the carcasses discarded. Several protocol deviations occurred during the study but were not considered to have compromised the validity or integrity of the study: **Statistical methods**: 1. Continuous data The following parameters were analyzed statistically: Body weights Body weight changes Feed consumption values Rectal Temperature Hematology Coagulation Clinical Chemistry Gestation length Corpora lutea and implantation sites F1 pup weights (each weighing interval during lactation) Number of pups (F1 litters) per pregnant female rats Number of male and females pups Pup weight distinguished by sex and as a composite for both sexes (litter as experimental unit)

	Organ weights Organ weight to body weight ratios Organ to Brain weight ratios Grip Strength measurements Methods of analysis: Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method, followed by a multiple comparison test if needed. Bartlett's test (Snedecor and Cochran, 1967; Bartlett, 1937; Sokal and Rohlf, 1995) was performed to determine if groups had equal variances. For all parameters except organ weights, if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. Organ weight data was analyzed only by parametric methods. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, additional tests were used to determine which means were significantly different from the control: Dunnett's (Dunlap et al., 1981; Dunnett, 1955, 1964), Williams (Williams, 1971, 1972), or Cochran and Cox's modified t-test (Cochran and Cox, 1959). The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953; Siegel, 1956) and if differences were indicated, Shirley's test (Shirley, 1977) was used to determine which means differed from control. Bartlett's test for equality of variance was conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Exceptions Statistical evaluations were not performed when the standard deviation for the control group was 0. When 75% of the values for a clinical pathology parameter were the same, Fisher's Exact Test (Fisher, 1973) was performed followed by Mantel's test (Mantel, 1963). 2. Motor Activity Counts The data was analyzed using split-plot repeated measures ANOVA with model terms for group, animal within group, interval and group by interval interaction. If the group x interval interaction was statistically significant (p=0.05), indicating non-parallelism in the behavioral profile between groups, a separate one-way ANOVA for group effects was performed at each interval. If the response data passed on the parallel hypothesis, an ANOVA (using summed responses over intervals) was used to test for the overall treatment effect, which constitutes the level hypothesis. If any significant overall treatment group effect was found by any of the above ANOVAs, Dunnett's t-test was used to find groups that differed from control. Analyses were performed for sexes separately and combined. Treatment group effects were deemed significant at the p=0.05 level. Plots, tables, listings and analyses were generated using

	SAS(R) version 8.2 for WINDOWS. Analyses were conducted by CATO Research, 200 Westpark Corporate Center, 4364 South Alston Avenue, Durham, NC 27713-2280. The Testing Facility was responsible for the GLP compliance of this subcontractor. 3. Incidence Data The following parameters were analyzed statistically: Mortality rate Mating indices, pregnancy rates, male fertility indices Litter survival indices Gestation indices Incidence of dams with no viable pups Mean pup survival indices (Days 0 and 4) Incidence Data Analysis A Fisher Exact Test with Bonferonni correction was performed to identify differences between the control and treatment groups (Siegel, 1956). All statistical tests were conducted at the 5% and 1%, two-sided risk levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Male and female rats (Parental/ systemic)	=	9,000		ppm
NOAEL	Reproductive	=	9,000		ppm
NOAEL	Developmental	=	9,000		ppm

Results Remarks:	Chamber distribution analyses showed that the test substance was evenly distributed within each chamber. The analytically measured exposure levels of airbone test substance were reasonably close to the targeted exposure levels

	and nominal concentrations. The mean (± standard deviation) analytical (IR) concentrations for the control and the respective exposure groups were as follows: 0 ± 0, 930.6 ± 28.1, 3022 ± 58 and 9157 ± 269 ppm. Chamber environmental conditions averaged 24ºC temperature and 56% relative humidity. Mean particle size distribution measurements for the exposures indicated that the atmospheres were gas only, as expected, since there was no substantial difference between the test substance chambers and the air control chambers. Pre-study GC analysis of the test substance showed a purity of 99.23% for butane. This value compared closely to the purity of 99.96% for the analytical standard of butane and the 99.5% purity as guaranteed by the supplier of the test substance. Post-study GC analysis of the test substance showed a purity of 99.98% for butane. This value compared closely to the purity of 99.98% for the analytical standard of butane and the 99.23% pre-study purity demonstrating stability of the test substance over the period of this study. MAIN STUDY ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test animals were generally unremarkable during the exposure periods in-chamber and during the non-exposure periods. There was a low incidence of chromodacryorrhea or transient red nasal discharge among males exposed to 9000 ppm between the 7th and 28th days of exposure. There were no exposure-related differences in body weights or weight changes or feed consumption in the test substance exposed animals compared to the air control animals. There was no meaningful exposure-related effect on functional observational battery or motor activity parameters for either sex in this study. Minor, but statistically significant decreases in male forelimb grip strength and increases in female forelimb grip strength occurred at 9000 ppm, but were not suggestive of an adverse effect, due to the opposite effect on the same parameter. Intergroup differences of a similar magnitude occurred among hindlimb results for both sexes without any apparent exposure-level relationship. There were no apparent exposure-related differences in hematology or coagulation values or clinical chemistry values in test substance exposed animals compared to the air control animals at the terminal interval. A statistically significant decrease in total bilirubin concentration was seen in males at 3000 and 9000 ppm, but not in an exposure-related pattern, and there were no differences in the females. These minor inter-group differences were not indicative of an exposure effect. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. There were no microscopic findings considered to be related to exposure to butane. No testiclular (stages of spermatogenesis; testicular interstitial cells) abnormality was evident in any test animal. SATELLITE FEMALE ANIMAL OBSERVATIONS There was no effect of treatment on survival. All animals survived until the termination of the study. The test

	animals were generally unremarkable during the exposure periods (in-chamber) and during the non-exposure periods. There was a low incidence of chromodacryorrhea or transient red nasal discharge among males exposed to 9000 ppm between the 7th and 28th days of exposure. There were no exposure-related differences in body weights or weight changes or feed consumption in the test substance exposed animals compared to the air control animals. There were no exposure-related differences in macroscopic postmortem evaluations or organ weights in the test substance exposed animals compared to the air control animals. MATING, FERTILITY AND GESTATION PARAMETERS Almost all mated female animals were found pregnant and delivered live pups. Mating indices for the male rats treated with the test substance were comparable to the air control group. Mating, fertility and gestation indices for the female rats treated with the test substance were comparable to the air control group. All but one of the females in each group mated at the first opportunity. There were also no treatment-related differences in the other reproductive parameters up to the time of parturition including the percent of females completing delivery and the duration of gestation, when compared to the air control group. There were no exposure-related differences in any of the parturition parameters including pre-implantation loss, post-implantation loss, the total number of pups delivered, the number of pups dying, the viability (4 day survival) index, the pup sex ratio and the number of live pups/litter, when compared to the air control group. There were no exposure-related differences in body weights or weight gains in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals. There were no exposure-related differences in macroscopic postmortem evaluations in the pups feeding from test substance exposed animals compared to the pups feeding from air control animals.
Conclusion:	Exposure of male and female rats to target concentrations of 900, 3000 or 9000 ppm of butane by whole-body inhalation for 4 weeks resulted in no systemic or neuotoxic effects. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for general systemic/neurotoxic (parental) endpoints in this study. There were no effects on fertility or reproductive performance, including offspring survival and weight development up to post-natal day 4. A no-observed-adverse effect level (NOAEL) of 9000 ppm was concluded for reproductive and developmental endpoints in this study.
RELIABILITY/DATA QUALITY	
Reliability:	Valid Without Restrictions; KS=1
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key

REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2008. Butane: Combined repeated exposure toxicity with reproduction/ developmental toxicity and neurotoxicity screening in rats via whole-body inhalation exposures. Conducted for the American Petroleum Institute. Draft report 03-4242.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY/TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	106-99-0
Test Substance:	1,3-Butadiene
Test Substance Purity/Composition and Other Test Substance Comments:	Purity 99.88%
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation (gas)
Other Route of Administration:	
Type of Exposure:	Developmental toxicity (teratogenicity) study
Species:	Rat
Other Species:	
Mammalian Strain:	Sprague-Dawley

Other Strain:	
Gender:	Females only
Number of Animals per Dose:	24-28 pregnant females per group
Concentration:	
Dose:	0, 40, 200, or 1000 ppm.
Year Study Performed :	1987
Method/Guideline Followed:	OECD 414
GLP:	Yes
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** 6 hrs/day
Frequency of Treatment:	Days 6-15 of gestation
Post-Exposure Period:	Females sacrificed on gestation day 20.
Method/Guideline and Test Condition Remarks:	Control group: yes, air-exposed only Method: This study investigated the effects of perinatal exposure by inhalation to 1,3-butadiene on pregnancy outcomes and fetal developmental effects. Female rats were mated to unexposed males and exposed from days 6-15 of gestation to 0, 40, 200, or 1000 ppm of the test substance. Analytical chamber concentrations were measured by on-line gas chromatography. Body weights were recorded on gestation days 0, 6, 11, 16, and 20. Maternal animals were observed daily for mortality, morbidity, and signs of toxicity and examined for gross tissue abnormalities at necropsy (day 20). The uterus and placenta was removed and weighed; the number of implantation sites, resorptions, live and dead fetuses were recorded. Live fetuses were weighed and subjected to external, visceral, and skeletal examinations. Approximately 50% of the fetal heads were sectioned and examined. Analysis of variance for body weights, number of resorptions, implants, live, dead or affected fetuses per litter. Significant differences among the groups were also analyzed by Duncan's multiple range test or arcsin transformation of the response proportion. Binary-response variables between groups were compared using chi-square or Fisher's exact test.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Female (Maternal)	=	1000 ppm		ppm
NOAEL	Female (Maternal)	=	200 ppm		ppm
NOAEL	Offspring (F1)	=	1000 ppm		ppm

Results Remarks:	The only toxicity observed was decreased body weight gains in the dams at 1000 ppm. The percentage of pregnant animals and number of litters with live fetuses were unaffected by treatment. There were no significant differences among the groups for number of live fetuses per litter, percent resorptions or malformations per litter, placental or fetal body weights, or sex ratio.
Conclusion:	There was no evidence of teratagenicity or adverse reproductive effects in any of the exposed groups. NOAEL for developmental effects = 1000 ppm

RELIABILITY / DATA QUALITY

Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key

REFERENCE

Reference:	Morrissey R, Schwetz B, Hackett P, Sikov M, Hardin B, McClanahan B, Decker J and Mast T .1990. Overview of reproductive and developmental toxicity studies of 1,3-butadiene in rodents. Environ. Health Perspect. **86**, 79-84.

TEST SUBSTANCE	
Category Chemical:	107-07-7
Test Substance:	Butene-2
Test Substance Purity/Composition and Other Test Substance Comments:	Butene-2 (cis and trans ≥95%), mol. wt 56.1, from UCAR Specialty Gases, The Netherlands. Certificate of analysis provided by the supplier This hydrocarbon has been used to characterize the developmental toxicity of the C1-C4 fraction of the refinery gas streams.
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	
METHOD	
Route of Administration:	Inhalation (whole body)
Other Route of Administration:	
Type of Exposure:	Reproductive/developmental toxicity screen
Species:	Rat
Other Species:	
Mammalian Strain:	Wistar (Hsd/Cpd:WU) from Charles River, Sulzfeld, F.R.G.; 13 wks old at study initiation
Other Strain:	
Gender:	Both M/F
Number of Animals per Dose:	24 (12/sex/dose level)
Concentration:	
Dose:	0, 2500, 5000 ppm
Year Study Performed :	2003
Method/Guideline Followed:	OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening

	Test.
GLP:	Yes
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** 6 hrs/day
Frequency of Treatment:	7 days/week
Post-Exposure Period:	None
Method / Guideline and Test Condition Remarks:	Control: 12 M, 12 F; filtered air-conditioned air, 6 hr/day, 7 days/wk Premating exposure period: Male-2 weeks Female- 2 weeks Method: The reproductive toxicity of 2-butene was assessed in an OECD 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test. Male and female rats (avg. wt. 299.4 g males, 204.0 g females at study initiation) were assigned to one of three groups by computer randomization based on body weight, and uniquely identified by ear tattoo. During the entire exposure period, animals were housed individually in stainless steel cages within modified multitiered inhalation chambers. Animals were exposed to a continuous supply of fresh test atmosphere, passed from a cylinder via a pressure reducer, stainless steel tubing and 2 calibrated mass flow controllers and rotameters to the inlet at the top of the inhalation chamber where it was diluted with filtered air-conditioned air to appropriate concentration, directed downward to the animal cages, and eventually exhausted out at the bottom of the chamber. Control rats were exposed to filtered air only. Air flow was monitored by an anemometer and recorded three times/exposure day, providing 11 to 12 air changes/hr. Concentrations of test material were determined with a total carbon analyzer using FID, twice/hr in each test atmosphere by sampling at locations close to the animal cages. Uniform distribution of butene-2 vapor was verified during preliminary experiments. Nominal concentrations were calculated by mean amount of test material used/hr divided by mean hourly volume of air passed through the exposure chamber. Top dose level of 5000 ppm was chosen because the estimated body burden was approx. 1000 mg/kg/day, the limit dose for teratology studies in OECD protocol 414. After 2 wks pre-mating exposure, males and females were caged together (1:1) until mating had occurred or for 1 wk. Mating was verified by a vaginal plug or sperm in a vaginal smear = Gestation day (GD) 0. Pregnant females were exposed through GD19; after which they were removed from the inhalation chambers and housed individually in the animal room, allowed to litter normally and to rear pups to day 4 of lactation, when both

	dams and pups were killed. Males, and females that did not mate (1 in control group), were housed individually in chambers and exposed until necropsy at the end of the study. Each rat was observed twice a day for reaction to treatment, ill health or mortality. Body wt of males were recorded weekly; body wt of all females were recorded weekly during pre-mating, mated females on GD0, 7, 14, 21, and on lactation days 1, 4. Food consumption was measured weekly for all rats pre-mating and for males after the mating period ended until study termination; for pregnant females, food consumption was recorded weekly during gestation and days 1 to 4 of lactation. Total litter size and number of pups of each sex, number of stillbirths, grossly malformed pups, if any, and pup body wt were recorded on day 1 and 4 postpartum. Necropsies were performed on stillborns and pups dying during lactation. Macroscopic examinations were performed on these pups and all pups killed on day 4 post-partum, and any abnormalities were recorded. Blood was collected from all parental (F0) animals (males and dams) at terminal necropsy for hematology and clinical chemistry analyses in the subchronic portion of this study. All F0 males and dams were examined macroscopically. Organs were excised and weighed, and tissues processed for microscopic examination. Pregnancies were verified by counting of implantation sites at necropsy; corpora lutea were counted in ovaries prior to fixation. Fisher's exact probability test for parametric data; Kruskal-Wallis analysis of variance followed by Mann-Whitney U-test for nonparametric data. Analysis of variance followed by Dunnet's multiple comparison tests for body weights and food consumption.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Parental (systemic)	=	5000		ppm
NOAEL	Parental (systemic)	=	2500		ppm
NOAEL	Developmental	=	5000		ppm

Results Remarks:	NOAEL(developmental) = 5000 ppm Mean actual concentration of butene-2 in test atmospheres was 0, 2476 ± 68 ppm (5.7 g/m3) and 5009 ± 88 ppm

	(11.5 g/m3). No mortality or treatment-related clinical signs were observed in parental (F0) animals. Male body wt were comparable in all groups but mean body wt change was statistically significantly lower in the 1st and 4th wk of exposure for 2500 ppm group and in the 1st wk of exposure for 5000 ppm group. Female rats showed statistically significantly decreased mean body wt compared to controls at 14 days from start of exposure in 2500 ppm group andat 7 and 14 days of exposure in 5000 ppm group. During gestation, all body weights were comparable in treated and control groups; on lactation day 1, body wt of 5000 ppm dams was statistically significantly decreased. Body wt changes in dams were comparable to control throughout the study. Food consumption in males was comparable to controls; food consumption by 5000 ppm females was decreased during the first wk of exposure. No other food consumption differences occurred during the study. Mating was successful in 11/12 females in the control group and all females 12/12 in each treated group; precoital times were comparable. Female fecundity index was 73% (8/12), 75% (9/12), 83% (10/12) in control, 2500 ppm and 5000 ppm groups, respectively. Duration of pregnancy was comparable in all groups. One high dose female delivered 1 stillborn pup and 12 live pups; all other dams in all groups delivered live pups. Gestation and live birth indices were approx. 100% in all groups. No treatment-related increase in pre-implantation loss occurred. Post-implantation loss was slightly increased in 5000 ppm group but was within historical control limits and the number of implantation sites in the control group was low. Total number of live births in exposed groups was slightly higher than controls. In the control and 2500 ppm groups, one pup died between days 1 and 4 of lactation, viability index was 97 to 100%; sex ratio of pups was similar in all groups. Mean body weight of pups was slightly but not statistically significantly lower in 2500 and 5000 ppm groups, which might be explained by the higher number of pups in these groups compared to controls. No treatment related effects were noted in pups during lactation or at necropsy. Parental Systemic NOAEL = 2500 ppm Reproductive NOAEL = 5000 ppm Developmental NOAEL = 5000 ppm
Conclusion:	Developmental NOAEL = 5000 ppm
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Waalkens-Brendsen, DH and JHE Arts. 1992. Combined short term inhalation and reproductive/developmental

	toxicity screening test with Butene-2 in rats. Proj. #B91-8336 (Study #1410)

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY / TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	No CAS Number
Test Substance:	Liquid Petroleum Gas
Test Substance Purity / Composition and Other Test Substance Comments:	Liquid Petroleum Gas; 100 % (Chevron Texaco Energy Research & Technology Company, Richmond, CA); lot number 120701-01
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD

Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Prenatal developmental toxicity study

Species:	Rat
Other Species:	
Mammalian Strain:	Sprague Dawley
Other Strain:	
Gender:	Female
Number of Animals per Dose:	24/dose
Concentration:	
Dose:	0, 1000, 5000, 10000 ppm
Year Study Performed :	2005
Method/Guideline Followed:	OECD 414 (Prenatal Developmental Toxicity Study)
GLP:	Yes
Exposure Period:	Gestation Days 6-19
Frequency of Treatment:	6 hr/day; 7 days/wk
Post-Exposure Period:	
Method/Guideline and Test Condition Remarks:	This study was designed to assess the potential maternal and/or developmental toxicity of liquified petroleum gas (LPG) in the pregnant rat when administered by whole-body inhalation exposure. The study design permitted detection of gross maternal organ changes and effects on the developing conceptus, including death, structural abnormalities or altered growth. The exposure levels were based on results of range-find testing which showed no effects at 100 and 1000 and 10000 ppm. The exposure levels were also selected based on establishing (for safety reasons) the high exposure level as no more than 50% of the lower explosion limit (LEL = 2.1% = 21000 ppm) for the test substance.

	Pregnant female Sprague-Dawley CD® rats (24/sex/group) were exposed daily for six hours per day to 0 (air control), 1000, 5000 or 10000 ppm LPG for 7 days per week for gestation days 6 to 19. Exposure levels were determined using an infrared spectrophotometer (IR) 4 times per chamber per day. At the end of the treatment period, all animals were euthanized and necropsied. The following parameters were evaluated: viability, clinical observations, body weights, feed consumption, necropsy observations, pre- and post-implantation losses, fetal weights and the incidences of fetal abnormalities and variants including state of skeletal ossification. The animals were 70 to 84 days in age and weighing 225 to 250 grams (non-GLP weights) on gestation day (gestation day) 0. Animals were received on gestation day 0, 1 or 2. Acclimation was 4 to 6 days at the testing facility prior to commencement of exposure, as dictated by the mating and delivery schedule for the animals. Exposure method: whole body Group 1, 0 ppm, 24 females Group 2, 1000 ppm, 24 females Group 3, 5000 ppm, 24 females Group 4, 10000 ppm, 24 females **Viability examination (cage-side)** Observations for mortality and general condition were made at least twice daily (nominally once in the morning and once in the afternoon). **Detailed physical examinations** Out-of-Chamber: Each animal on study was examined daily from receipt through to terminal euthanasia on gestation day 20. Examination included observations of general condition, skin and fur, eyes, nose, oral cavity, abdomen and external genitalia, occurrence of secretions and excretions, and autonomic activity (e.g., lacrimation, piloerection, pupil size, unusual respiratory pattern). Changes in gait, posture and response to handling as well as the presence of repetitive circling or bizarre behavior (e.g., self-mutilation, walking backward) were recorded as well as evaluations were performed prior to exposures. Each rat was observed at least once a day throughout gestation until sacrifice for signs of pathosis, abortion premature delivery and/or death. All unusual findings were noted. In-Chamber: All animals were observed as a group at least once during each exposure. This was routinely

performed near the middle of each exposure. Pertinent behavioral changes and any signs of toxicity were recorded.

Body weight
Body weights were recorded on gestation day 3, 6, 9, 12, 15, 18 and 20 (day of scheduled sacrifice). Weight gain was calculated for intervals as found appropriate, including gestation day 6 to 15, gestation day 15 to 20 and gestation day 6 to 20.

Feed consumption
Feed consumption was recorded on gestation day 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18 and 18 to 20. Feed consumption was calculated for these same intervals and for gestation day 6 to 20.
Calculation
Feed consumption (g/kg/day) =

$$\frac{\text{grams of feed consumed}}{\text{body weight (kg)}} \div \text{2 to 3 days}$$

Method of Euthanasia
All dams were euthanized on gestation day 20 with an overdose of carbon dioxide inhalation. All live fetuses were euthanized by an intraperitoneal overdose of sodium pentobarbital.

Macroscopic postmortem examinations
A necropsy was performed for all the dams in the study. Thoracic and abdominal organs were examined, and the uterus and ovaries were examined grossly for evidence of pathosis. Macroscopic lesions or tissues with significant findings were preserved in 10% neutral buffered formalin (NBF).
The following examinations of the reproductive system were made:
The intact uteri (ovaries attached) were removed from the abdominal cavity. The gravid uteri, including the cervix, were weighed. Corpora lutea were counted and the number per ovary recorded.

The number and location of the following were recorded for each uterine horn:
live fetuses
late embryo-fetal deaths (recognizable dead fetus undergoing degeneration, regardless of size)
early embryonic deaths (evidence of implantation but no recognizable fetus), or ammonium sulphide positive (see below)
Each placenta was examined macroscopically. The maternal carcass, uterus, ovaries and placentas were then discarded. Females that did not have visible implantations had their apparent non-pregnant status confirmed by means of staining the uterus with ammonium sulphide (Salewski, 1964).

	Fetal Evaluations External examinations: All live gestation day 20 fetuses were weighed and individually identified. Each live fetus was given a macroscopic external examination for defects (including observation of the palate). Soft tissue (visceral) examinations: Approximately one-half of the fetuses in each litter (alternating fetuses within the litter, nominally) were placed in Modified Davidson's fixative for preservation and decalcification. These fetuses were subject to soft tissue examination by gross dissection of the torso and a razor blade sectioning technique for the head derived from that of Wilson (1965). All malformations and variations were recorded. During the dissection process, the sex of each fetus was confirmed by internal inspection of the gonads. Following complete dissection of the fetuses, all carcasses and sections were preserved in 10% neutral buffered formalin. Skeletal Examinations: Approximately one-half of the fetuses in each litter (alternating fetuses within the litter, nominally) were eviscerated, placed in 70% isopropyl alcohol for preservation and processed for staining of the skeleton using Alizarin Red S. Subsequently, these fetuses were evaluated for skeletal malformations and ossification variations. These skeletons were then stored in 100% glycerin with a mold inhibitor. During the dissection process, the sex of each fetus was confirmed by internal inspection of the gonads. One protocol deviation occurred during the study, but was not considered to affect the integrity of the study. **Statistical analysis** Analysis was performed by the Statistics Department of Huntingdon Life Sciences Ltd, Woolley Road, Alconbury, Huntingdon Cambridgeshire, PE28 4HS, England (Graham Healey, principal investigator). The testing facility was responsible for the GLP compliance of this work and the associated archiving of the raw data and original final sub-report. Continuous Data: The following mean measures were analyzed as described below: maternal body weight values and body weight changes during gestation maternal feed consumption values (presented as grams of feed/kg of body weight/day) gravid uterine weights corpora lutea implantation data pre-implantation loss

	early embryonic deaths (evidence of implantation but no recognizabl fetus) live fetuses late embryo-fetal deaths (recognizable dead fetus undergoing degeneration, regardless of size) total embryo-fetal deaths and as % of implant sites mean percent female fetuses Mean values of all exposure groups were compared to the mean value for the control group at each time interval. Evaluation of equality of group means was made by the appropriate statistical method (either parametric or non-parametric), followed by a multiple comparison test if needed. Bartlett's test (Bartlett, 1937; Sokal and Rohlf, 1995; Snedecor and Cochran, 1967) was performed to determine if groups have equal variances. For all parameters if the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric method was the standard one-way analysis of variance (ANOVA) using the F ratio to assess significance (Armitage, 1971; Dunlap and Duffy, 1975). If significant differences among the means were indicated, Dunnett's test (Dunlap et al., 1981; Dunnett, 1955, 1964) was used to determine which means were significantly different from the control. The nonparametric method was the Kruskal-Wallis test (Kruskal and Wallis, 1952, 1953), and if differences were indicated, Steel's test (Steel, 1959) was used to determine which means differed from control. Bartlett's test for equality of variance were conducted at the 1% significance level; all other statistical tests were conducted at the 5% and 1% significance levels. Incidence Data: premature deliveries total pregnancy loss (no live fetuses) maternal necropsy findings external fetal defects skeletal malformations and variations soft tissue malformations and variations These data was analyzed based on a Generalized Estimating Equation (GEE) application of the linearized model (Ryan, 1992). For litter endpoints, the model used the litter as the basis for analysis and considered correlation among littermates by incorporating an estimated constant correlation and the litter size as a covariate. If the dose group effect in the model was statistically significant, the dose group least squares means were tested pairwise vs. the control group using t-tests associated with least squares means. The least squares means allow comparisons that account for differences in litter size. Statistical significance of differences from control was recognized at the 5% or 1%, two-sided levels. T he fetal body weights (by sex and as a composite for both sexes) were analyzed by a mixed model analysis of

	variance that provides an accurate statistical model of the biology. The analysis used the litter as the basis for analysis and effectively used the litter size as a covariate. The model considered dose group, litter size and fetal sex as explanatory variables. If the dose group effect in the model was statistically significant, the dose group least squares means were tested pairwise vs. the control group using t-tests associated with least squares means. The least squares means allow comparisons that account for differences in litter size and sex. The mathematical model was based on a paper by Chen et. al. (1996). Statistical significance of differences from control was recognized at the 5% or 1%, two-sided levels.

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Maternal	=	10,000		ppm
NOAEL	Developmental	=	10,000		ppm

Results Remarks:	Pre-study chamber distribution analyses showed that the test substance was evenly distributed within each chamber. Chamber monitoring showed that the chamber oxygen levels were at least 19%. Chamber room monitoring showed that no test substance was present in the room and that the sound and light levels were acceptable. The mean (± standard deviation) analytical exposure concentrations of LPG were determined to be 0±0, 1013 ± 60, 5079 ± 217 and 10426 ± 527 ppm for the air control and the exposure groups, respectively. Particle sizing

	results indicated that the atmospheres were essentially gas/vapor only, as expected, since there was no substantial difference in particle concentration between the test substance chambers and the air control chamber. Analysis of the major components in the neat test substance and the test atmospheres showed an acceptably close comparison between the neat test substance and the vaporized test substance. The data were consistent pretest and during the study indicating stability of the test substance and the atmosphere generation techniques. **Mortality and pregnancy** All animals survived until the scheduled termination of the study and pregnancy rates were 96 to 100% across the groups. **Clinical observations** During the exposure and non-exposure periods, all animals were generally unremarkable. **Body weights** There were no test substance related differences in absolute body weights or in body weight changes in the test substance exposed animals, compared to the air control animals. **Feed consumption** There were no test substance related differences in feed consumption in the test substance exposed animals, compared to the air control animals. **Female necropsy data and pregnancy outcome** There were no test substance related effects on gross necropsy observations, pregnancy outcome, in terms of corpora lutea numbers, pre- or post-implantation loss, early or late resorptions, or litter size and gravid uterine weight. All values were normal for this strain of animals. **Fetal body weights** There were no test substance related differences in fetal body weights among the test substance exposed animals, compared to the air control animals. **Fetal observations** There were low, sporadic incidences of fetal abnormalities and variants across all the groups. There was no indication of any association with exposure to the test substance. Similarly, there was no evidence of an effect on the state of ossification of the fetal skeleton.
Conclusion:	Exposure of pregnant female rats to target concentrations of 1000, 5000 or 10000 ppm of LPG by whole-body inhalation for gestation days 6 to 19 resulted in no exposure-related effects. . Therefore, a no observed adverse effect level (NOAEL) for maternal toxicity and developmental toxicity of 10000 ppm was determined for this

	study.
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Comparable to guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	HLS (Huntington Life Sciences), 2009. Liquefied Petroleum Gas: Embryo-Fetal Toxicity Study in Rats by Inhalation Exposure. Conducted for the American Petroleum Institute. Draft report 03-4253.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY / TERATOGENICITY

TEST SUBSTANCE	
Category Chemical:	No CAS No.
Test Substance:	No CAS No.

Test Substance Purity/Composition and Other Test Substance Comments:	C5-C6 This hydrocarbon mixture is being used to characterize the developmental toxicity of the C5-C6 fraction of the refinery gas streams. Unleaded baseline gasoline API 99-01 Vapor Condensate Test material is a complex mixture of volatile hydrocarbons. The purity of mixture is 100% and stable based on analysis of chamber atmospheres.

Representative Components monitored in Study

Component	Area % range for the three exposure levels
Isobutane	2.0 – 2.9
n-butane	11 – 15
Isopentane	33 - 39
n-pentane	10 - 14
Trans-2-pentene	2.5 – 3.5
2-methyl-2-butene	0.17 – 3.9
2,3,-dimethylbutane	1.5 – 1.9
2-methylpentane	6.8 – 7.9
3-methylpentane	3.9 - 4.5
n-hexane	3.2 – 4.0
methylcyclopentane	1.6 – 1.8
2,4-dimethylpentane	1.1 – 1.4
Benzene	2.2 – 3.4
2-methylhexane	1.1 – 1.5
2,3-dimethylpentane	1.1 – 1.5
3-methylhexane	1.4 – 1.7
Isooctane	1.5 – 1.8
Toluene	2.7 – 4.0

Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	

METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Vapor
Species:	Mice
Other Species:	
Mammalian Strain:	Crl:CD-1®(ICR)BR
Other Strain:	
Gender:	female
Number of Animals per Dose:	25
Concentration:	
Dose:	Target: 0, 2000, 10000, 20000 mg/m^3 Analytical: 0, 2086, 10625, 20903
Year Study Performed :	2008
Method/Guideline Followed:	EPA OPPTS 870.3600
GLP:	yes
Exposure Period:	**Value or Lower Exposure Duration :**

	Upper Exposure Duration : 6 hrs/day
Frequency of Treatment:	Gestation Day 5 - 17
Post-Exposure Period:	none
Method / Guideline and Test Condition Remarks:	A developmental toxicity study in rats of Baseline Gasoline Vapor Condensate (BGVC), a 20% light fraction of whole unleaded gasoline was performed according to OPPTS 870.3600, 870.3700 and OECD 414 guidelines. This test material was a representative evaporative emission tested under the USEPA 211(b) Fuels and Fuel Additives Health Effects Testing Program (1994b). BGVC was administered to 25 confirmed-mated female Crl:CD-1®(ICR)BR mice/exposure group at target concentrations of 0, 2000, 10,000, and 20,000 mg/m^3 (mean analytical concentrations 0, 2086, 10625 and 20,903 mg/m^3; 0, 680, 3463, and 6814 ppm) in air. The animals were exposed daily for six hours from Gestation Day 5 through Gestation Day 17. The Sponsor selected the exposure levels based upon safety considerations and previously conducted mammalian toxicity studies. The highest exposure level was one-half the lower explosive limit. The concentration of the test atmosphere in each chamber and the chamber room was determined approximately hourly during each exposure by on-line gas chromatography. The chamber concentrations were measured in the breathing zone of the rats. Additionally, a sorbent tube sample of the test atmosphere was collected once during each week of the study. These samples were analyzed by the detailed capillary/GC method used for the initial characterization analysis of the liquid test substance. This analysis was done to determine component proportions of the test material atmosphere compared to the liquid test material. Chamber Homogeneity was evaluated during the validation of the exposure system for this study. Distribution samples were drawn from twelve different points within the chamber at each exposure level. A particle size determination of the aerosol portion of the test atmosphere was conducted three times during the chamber trials from the 20,000 mg/m^3 concentration. The samples were taken using a multistage cascade impactor. Preweighed glass fiber filters were used to collect aerosol on each stage, which are associated with specific cutoff diameters for aerodynamic particle size in microns. Since minimal aerosol was present, no further calculations were performed. Clinical observations were made daily during gestation. Body weight and food consumption measurements were made on GD 0, 5, 8, 11, 14, 17, and 18. On GD 18, animals were sacrificed by CO_2 asphyxiation followed by exsanguination. and cesarean sections (C-sections) were performed. . The reproductive organs and the abdominal and thoracic cavities were examined grossly. Evaluations of dams during cesarean section were conducted without knowledge of treatment group in order to minimize bias. Uterine weights with ovaries attached were recorded. Uterine contents were examined, and the numbers of live, dead and resorbed fetuses were recorded. Corpora lutea were also

	counted. All fetuses were weighed, sexed externally, and examined externally for gross malformations. Apparent non-gravid uteri were placed in 10% ammonium sulfide solution for confirmation of non-pregnancy status. The fetuses were placed in a refrigerator to slow down and eventually terminate vital signs after the external examination and weighing. The viscera of approximately one-half of the fetuses of each litter were examined by fresh dissection. After these fetuses were examined, they were decapitated. The heads were preserved in Bouin's solution for at least two weeks, rinsed, and subsequently stored in 70% ethanol. The fetal heads were sectioned and examined with a dissecting microscope for the presence of abnormalities. The remaining fetuses judged to be alive at the C-section were eviscerated, processed for skeletal staining, stained for bone and cartilage, and examined for the presence of skeletal malformations and variations. Statistical Analysis: Statistical evaluation of equality of means was done by an appropriate one way analysis of variance and a test for ordered response in the dose groups. First, Bartlett's Test was performed to determine if the dose groups had equal variance (Snedecor and Cochran, 1989). If the variances were equivalent, the hypothesis that there was no difference in response between the groups was tested using a standard one-way analysis of variance (Snedecor and Cochran, 1989). If the variances were equal, the testing was done using parametric methods, otherwise nonparametric techniques were used. Continuous data will be tested for statistical significance as follows: Where applicable, percentages were calculated and transformed by Cochran's transformation, followed by the arc sine transformation (Snedecor and Cochran, 1989). The raw percentages and the transformed percentages both were tested for statistical significance. For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used (Snedecor and Cochran, 1989). If significant differences among the means were indicated, Dunnett's Test was used to determine which treatment groups differed significantly from control (Dunnett, 1964). In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model. For the nonparametric procedures, the test of equality of means was performed using the Kruskal-Wallis Test (Hollander and Wolfe, 1973). If significant differences among the means were indicated, Dunn's Summed Rank Test was used to determine which treatment groups differed significantly from the control (Hollander and Wolfe, 1973). In addition to the Kruskal-Wallis Test, Jonckheere's Test for monotonic trend in the dose response was performed. Bartlett's Test for equal variance was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of The following data was not included in the statistical analyses: • Gestation body weight and body weight change data for females that were not pregnant • Gestation food consumption for females that were not pregnant Means and standard deviations were calculated for animal, exposure and chamber environmental data. The coefficient of variation also was calculated when considered relevant for the exposure data.

Fetal body weight was analyzed by a mixed model analysis of variance that provided an accurate statistical model of the biology. The analysis used the litter as the basis for analysis and effectively used the litter size as a covariate. The model considered dose group, litter size, and fetal sex as explanatory variables. If the overall effect of dose, or the dose by sex effect, was statistically significant the dose groups means were tested pairwise vs. the control group using least squares means. The least squares means allowed comparisons that accounted for differences in litter size and sex. The mathematical model was based on a paper by Chen, et al (1996). The analysis was run using SAS with code suggested in Little, et al (1997).
The analysis of anomalies (malformations or variations) was based on a Generalized Estimating Equation (GEE) application of the linearized model, Ryan (1992). The model used the litter as the basis for analysis and considered correlation among littermates by incorporating an estimated constant correlation and the litter size as a covariate. If the overall effect of dose, or the dose by sex effect, was statistically significant the dose groups were tested pairwise vs. the control group using least squares means. The least squares means allowed comparisons that accounted for differences in litter size. Three categories of anomalies were tested, and within each category specific anomalies also were tested. In addition to the category specific anomalies a series of combined analyses were performed within each category as applicable:

Combined Malformations and Variations for All Fetuses
Combined Malformations and Variations for Alive Fetuses
Malformations for All Fetuses
Malformations for Alive Fetuses
Variations for All Fetuses
Variations for Alive Fetuses

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
LOAEL	Maternal (dams)	=	20000		mg/m^3
NOAEL	Maternal (dams)	=	10000		mg/m^3

LOAEL	Fetal (F1)	≥	10000		mg/m^3
NOAEL	Fetal (F1)	≥	2000		mg/m^3

Results Remarks:

All dams were free of clinical or postmortem findings attributable to treatment with BGVC. One control, one 10,000 mg/m3, and two 20,000 mg/m3 dams were determined at the scheduled terminal sacrifice to be not pregnant. Additionally, one control and one 20,000 mg/m3 dam delivered their litters on Day 18, prior to their scheduled sacrifice.

Maternal toxicity was evident as statistically significant decreases in mean gestation body weight and mean gestation body weight change in the 20,000 mg/m3 target concentration group. The only clinical sign observed was emaciation, noted in a single dam at 20,000 mg/m3 on GD 11; since this finding was not seen in other dams at this target concentration, this is unlikely to be related to exposure to the test substance.

Statistically significant reduced fetal body weights, compared with the control fetal weights, were noted in the 10,000 and 20,000 mg/m3 target concentration groups. The reduction of these fetal weights occurred in the absence of statistically significant reductions in maternal body weight and body weight change in the 10,000 mg/m3 target concentration group. There were no statistically significant differences detected in the incidence of other fetal observations.

The uterine implantation data revealed a statistically significant decrease in the number of live fetuses in the 20,000 mg/m3 target concentration group and also a statistically significant increase in the transformed resorptions to implantation ratio in this group. This difference is not considered to be exposure-related for two reasons. First, the mean number of corpora lutea(CL) per litter at 20,000 mg/m3 was nearly two CL less than the control group. The number of corpora lutea was determined prior to initiation of exposure to the test material, and hence cannot be due to exposure. The difference in the mean number of corpora lutea per litter alone is insufficient to explain the cascading differences in mean litter implantation number and live fetuses per litter. The reduced number of live fetuses primarily was a function of a reduction in the number of implantations prior to commencement of exposure.

	Additionally the litter of one dam in this group was completely resorbed. The dam lost 26% of her body weight on GD 8-11. Although there is no apparent explanation for this animal's weight loss, weight loss during gestation in mice due to food restriction is associated with increased resorptions (Chapin et al., 1993). When the uterine implantation data for this litter was removed from the statistical analyses as an outlier, there was no statistical significance in the transformed resorptions to implantation ratio. This dam also was noted as emaciated on GD 11 and its body weight data indicates that resorption of the litter probably occurred between GD 5 and GD 8. The NOAELSs for developmental and maternal toxicity were considered to be 2000 (680 ppm) and 10,000 mg/m3 (3,463 ppm) target concentrations, respectively.
Conclusion:	Based upon reduced fetal body weights in the absence of reduced maternal body weights, BGVC was determined to be a developmental toxicant in CD-1 mice. The NOAEL for developmental toxicity was 2,086 mg/m^3 (680 ppm); the LOAEL for developmental toxicity was 10,625 mg/m^3 3,463 ppm). Based upon reduced gestation body weight and mean gestation body weight change, the Maternal NOAEL was 10,625 mg/m3 (3,463 ppm); the maternal LOAEL was 20,903 mg/m3 (6,814 ppm).
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Key
REFERENCE	
Reference:	Whole-Body Inhalation Developmental Toxicity Study in Mice with Baseline Gasoline Vapor Condensate (MRD-00-695). Laboratory (EMBSI) study number 169534. ExxonMobil Biomedical Sciences, Inc., Annadale, NJ. Study conducted for the American Petroleum Institute 211(b) Research Group in compliance of the Clean Air Act 211(b) testing requirements. Other references cited in study summary: Dunnett, C., New Tables for Multiple Comparisons with a Control, Biometrics 20, 1964, pp. 482-491. Hollander, M. and Wolfe, D.A. Nonparametric Statistical Methods, John Wiley and Sons, New York, 1973.

	Little, Milliken, Stroup, and Wolfinger, "SAS System for Mixed Models", SAS Institute, Cary, NC, 1997, section 5.6.2, pg 203. Ryan, L., "The use of generalized estimating equations for risk assessment in developmental toxicity", Risk Analysis, 12(3), pg 439-447, 1992. Snedecor, G.W., and Cochran, W.G., Statistical Methods, 8th ed., Iowa State University Press, Ames, Iowa, 1989.

High Production Volume Information System (HPVIS)

DEVELOPMENTAL TOXICITY / TERATOGENICITY

TEST SUBSTANCE

Category Chemical:	No CAS No.
Test Substance:	No CAS No.

Test Substance Purity/Composition and Other Test Substance Comments:	C5-C6 This hydrocarbon mixture is being used to characterize the in developmental toxicity of the C5-C6 fraction of the refinery gas streams. Unleaded baseline gasoline API 99-01 Vapor Condensate Test material is a complex mixture of volatile hydrocarbons. The purity of mixture is 100% and stable based on analysis of chamber atmospheres.
Category Chemical Result Type :	Measured
Unable to Measure or	

Representative Components monitored in Study

Component	Area % range for the three exposure levels
Isobutane	2.0 – 2.9
n-butane	11 – 15
Isopentane	33 - 39
n-pentane	10 - 14
Trans-2-pentene	2.5 – 3.5
2-methyl-2-butene	0.17 – 3.9
2,3,-dimethylbutane	1.5 – 1.9
2-methylpentane	6.8 – 7.9
3-methylpentane	3.9 - 4.5
n-hexane	3.2 – 4.0
methylcyclopentane	1.6 – 1.8
2,4-dimethylpentane	1.1 – 1.4
Benzene	2.2 – 3.4
2-methylhexane	1.1 – 1.5
2,3-dimethylpentane	1.1 – 1.5
3-methylhexane	1.4 – 1.7
Isooctane	1.5 – 1.8
Toluene	2.7 – 4.0

Estimate Justification :	
METHOD	
Route of Administration:	Inhalation
Other Route of Administration:	
Type of Exposure:	Vapor
Species:	Rat (Crl:CD®(SD)IGSBR)
Other Species:	
Mammalian Strain:	Sprague Dawley
Other Strain:	
Gender:	female
Number of Animals per Dose:	25
Concentration:	
Dose:	Target: 0, 2000, 10000, 20000 mg/m^3 Analytical: 0, 1979, 10676, 20638
Year Study Performed :	2008
Method/Guideline Followed:	EPA OPPTS 870.3600
GLP:	yes
Exposure Period:	**Value or** **Lower Exposure Duration :** **Upper Exposure Duration :** 6 hrs/day

Frequency of Treatment:	Gestation Day 5 - 20
Post-Exposure Period:	none
Method / Guideline and Test Condition Remarks:	Baseline Gasoline Vapor Condensate (BGVC) was administered by whole-body inhalation exposure to 25 confirmed-mated Crl:CD®(SD)IGSBR female rats at target doses of 0 (air control) 2000, 10,000, and 20,000 mg/m^3 for six hours (plus the theoretical equilibration time) daily from Gestation Day (GD) 5 through GD 20. The Sponsor selected the exposure levels based upon safety considerations and previously conducted mammalian toxicity studies. The highest exposure level was one-half the lower explosive limit. The concentration of the test atmosphere in each chamber and the chamber room was determined approximately hourly during each exposure by on-line gas chromatography. The chamber concentrations were measured in the breathing zone of the rats. Additionally, a sorbent tube sample of the test atmosphere was collected once during each week of the study. These samples were analyzed by the detailed capillary/GC method used for the initial characterization analysis of the liquid test substance. This analysis was done to determine component proportions of the test material atmosphere compared to the liquid test material. Chamber Homogeneity was evaluated during the validation of the exposure system for this study. Distribution samples were drawn from twelve different points within the chamber at each exposure level. A particle size determination of the aerosol portion of the test atmosphere was conducted three times during the chamber trials from the 20,000 mg/m^3 concentration. The samples were taken using a multistage cascade impactor. Preweighed glass fiber filters were used to collect aerosol on each stage, which are associated with specific cutoff diameters for aerodynamic particle size in microns. Since minimal aerosol was present, no further calculations were performed. Clinical observations were made daily during gestation. Body weight and food consumption measurements were made on GD 0, 5, 8, 11, 14, 17, 20, and 21. On GD 21, animals were sacrificed by CO_2 asphyxiation followed by exsanguination. Cesarean sections were then conducted. The reproductive organs and the abdominal and thoracic cavities were examined grossly. Evaluations of dams during cesarean section were conducted without knowledge of treatment group in order to minimize bias. Uterine weights with ovaries attached were recorded. Uterine contents were examined, and the numbers of live, dead and resorbed fetuses were recorded. Corpora lutea were also counted. All fetuses were weighed, sexed externally, and examined externally for gross malformations. Apparent non-gravid uteri were placed in 10% ammonium sulfide solution for confirmation of non-pregnancy status. The fetuses were placed in a refrigerator to slow down and eventually terminate vital signs after the external examination and weighing. The viscera of approximately one-half of the fetuses of each litter were examined by fresh dissection. After these fetuses were examined, they were decapitated. The heads were preserved in Bouin's solution for at least two weeks, rinsed, and subsequently stored in 70% ethanol. The fetal heads were sectioned and examined with a dissecting microscope for the presence of abnormalities. The remaining fetuses judged to be alive at the C-section were eviscerated, processed for skeletal staining, stained for bone and cartilage, and examined for the presence of skeletal

malformations and variations.

Statistical Analysis: Statistical evaluation of equality of means was done by an appropriate one way analysis of variance and a test for ordered response in the dose groups. First, Bartlett's Test was performed to determine if the dose groups had equal variance (Snedecor and Cochran, 1989). If the variances were equivalent, the hypothesis that there was no difference in response between the groups was tested using a standard one-way analysis of variance (Snedecor and Cochran, 1989). If the variances were equal, the testing was done using parametric methods, otherwise nonparametric techniques were used.

Continuous data will be tested for statistical significance as follows: Where applicable, percentages were calculated and transformed by Cochran's transformation, followed by the arc sine transformation (Snedecor and Cochran, 1989). The raw percentages and the transformed percentages both were tested for statistical significance.

For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used (Snedecor and Cochran, 1989). If significant differences among the means were indicated, Dunnett's Test was used to determine which treatment groups differed significantly from control (Dunnett, 1964). In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model.

For the nonparametric procedures, the test of equality of means was performed using the Kruskal-Wallis Test (Hollander and Wolfe, 1973). If significant differences among the means were indicated, Dunn's Summed Rank Test was used to determine which treatment groups differed significantly from the control (Hollander and Wolfe, 1973). In addition to the Kruskal-Wallis Test, Jonckheere's Test for monotonic trend in the dose response was performed.

Bartlett's Test for equal variance was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of significance.

The following data was not included in the statistical analyses:

- Gestation body weight and body weight change data for females that were not pregnant
- Gestation food consumption for females that were not pregnant

Means and standard deviations were calculated for animal, exposure and chamber environmental data. The coefficient of variation also was calculated when considered relevant for the exposure data.

Fetal body weight was analyzed by a mixed model analysis of variance that provided an accurate statistical model of the biology. The analysis used the litter as the basis for analysis and effectively used the litter size as a covariate. The model considered dose group, litter size, and fetal sex as explanatory variables. If the overall effect of dose, or the dose by sex effect, was statistically significant the dose groups means were tested pairwise vs. the control group using least squares means. The least squares means allowed comparisons that accounted for differences in litter size and sex. The mathematical model was based on a paper by Chen, et al (1996). The analysis was run using SAS with code suggested in Little, et al (1997).

	The analysis of anomalies (malformations or variations) was based on a Generalized Estimating Equation (GEE) application of the linearized model, Ryan (1992). The model used the litter as the basis for analysis and considered correlation among littermates by incorporating an estimated constant correlation and the litter size as a covariate. If the overall effect of dose, or the dose by sex effect, was statistically significant the dose groups were tested pairwise vs. the control group using least squares means. The least squares means allowed comparisons that accounted for differences in litter size. Three categories of anomalies were tested, and within each category specific anomalies also were tested. In addition to the category specific anomalies a series of combined analyses were performed within each category as applicable: Combined Malformations and Variations for All Fetuses Combined Malformations and Variations for Alive Fetuses Malformations for All Fetuses Malformations for Alive Fetuses Variations for All Fetuses Variations for Alive Fetuses

TEST RESULTS

Concentration (LOAEL / LOAEC / NOAEL / NOAEC)

Type	Population:	Value Description:	Value or Lower Concentration:	Upper Concentration:	Units:
NOAEL	Maternal (dams)	≥	20000		mg/m^3
NOAEL	Fetal (F1)	≥	20000		mg/m^3

Results Remarks:	The mean analytical exposure concentrations [± standard deviation (S.D.)] were 1979 ± 98.0, 10676 ± 309.8, and 20638 ± 452.1 for the target concentrations of 2000, 10000, and 20000 mg/m^3, respectively. Chamber uniformity was also within acceptable limits with 12 point sampling means (± S.D.) of 1997 ± 56.4, 10495 ± 195.0, and 19996 ± 275.8 mg/m3 for the respective target concentrations. There was no evidence of maternal toxicity in this study at any concentration tested. All dams survived to scheduled terminal sacrifice on GD 21 and were free of clinical or postmortem effects attributable to treatment with BGVC. However there was a statistically significant linear trend (decrease) in dose response in the GD 5-8 body weight change and a statistically significant linear trend (increase) in dose response in the GD 14-17 body weight change. However, the pairwise analyses of the control data versus each treated group was not statistically significant; mean maternal body weight for the 20,000 mg/m^3 target concentration group on GD 8 was 98.9% of the control mean value. The linear trend for the GD 14-17 body weight change was also not considered biologically significant due to the absence of statistically significant differences between the treated and control groups. There were no statistically significant differences between the control and the BGVC treated groups for uterine implantation data, and external, visceral, and skeletal observations. The most frequently noted observation during fetal examinations was rudimentary lumbar ribs. The incidence of this observation was similar across all groups and was within the historical control range of this laboratory. A statistically significant decrease in mean fetal body weight was evident in all exposed groups. This could be interpreted as an indication of developmental toxicity. However, these decreases are probably neither treatment related nor biologically significant for the following reasons: • The mean fetal weights of the treatment groups were within the historical control range of the laboratory. The mean fetal body weights determined in the control group were greater than this laboratory's historical control mean fetal body weight range and likewise the MARTA historical control data base (mean fetal body weights) for Charles River (Raleigh facility) rat fetuses obtained from dams on GD 21.

	• A comparison of mean litter weights (mean of the sum of all fetus weights/group) revealed that the litter weights of all groups were comparable and the control litter weights were the most variable. • The mean litter size in the control group was smaller than any treated group. Consequently, it must be remembered, however, that among animals which deliver multiple offspring, individual fetal body weights tend to be heavier in smaller litters, as was seen in this study (Romero, 1992). • There was no dose response in the mean fetal weights of the treated groups. The fetal weights of the treated groups were not statistically significantly different from each other. If the lower fetal weights in the treated groups were related to treatment, one would expect that the mean fetal weight of the group exposed to a target concentration of 20,000 mg/m^3 would be at least substantially lower than the mean fetal weight of the group exposed to a target concentration of 2000 mg/m^3. No other observations were evident in the treated groups that were statistically or biologically significantly different from the observations in the control group. In conclusion, administration of the test substance to rats by whole-body inhalation exposure during the period of organogenesis and fetal growth did not result in maternal or developmental toxicity. Therefore, the No Observable Adverse Effect Levels (NOAELs) for maternal and developmental toxicity in this study was established at 20,000 mg/m^3 target concentration.
Conclusion:	BGVC was not a developmental toxicant in Sprague Dawley rats at exposure concentrations up to 20000 mg/m^3. The NOAEL for both maternal and developmental toxicity was ≥ 20000 mg/m^3.
RELIABILITY / DATA QUALITY	
Reliability:	Valid Without Restrictions (KS=1)
Reliability Remarks:	Guideline study
Key Study Sponsor Indicator:	Not a Key Study

REFERENCE	
Reference:	Whole-Body Inhalation Developmental Toxicity Study in Rats with Baseline Gasoline Vapor Condensate (MRD-00-695). Laboratory (EMBSI) study number 169534. ExxonMobil Biomedical Sciences, Inc., Annadale, NJ. Study conducted for the American Petroleum Institute 211(b) Research Group in compliance of the Clean Air Act 211(b) testing requirements. Other references cited in study summary: Dunnett, C., New Tables for Multiple Comparisons with a Control, Biometrics 20, 1964, pp. 482-491. Hollander, M. and Wolfe, D.A. Nonparametric Statistical Methods, John Wiley and Sons, New York, 1973. Little, Milliken, Stroup, and Wolfinger, "SAS System for Mixed Models", SAS Institute, Cary, NC, 1997, section 5.6.2, pg 203. Romero, A., Villamayor, F., Grau, M. T., Sacristan, A., and Ortiz, J. A. "Relationship between Fetal Weight and Litter Size in Rats: Application to Reproductive Toxicology Studies", Reproductive Toxicology 6: 453-456, 1992. Ryan, L., "The use of generalized estimating equations for risk assessment in developmental toxicity", Risk Analysis, 12(3), pg 439-447, 1992. Snedecor, G.W., and Cochran, W.G., Statistical Methods, 8th ed., Iowa State University Press, Ames, Iowa, 1989.

Exposure Experience

Exposure Experience

TEST SUBSTANCE

Category Chemical:	75-28-5
Test Substance:	Isobutane
Test Substance Purity/Composition and Other Test Substance Comments:	No data
Category Chemical Result Type :	Measured

Remark **:** Eight adult volunteers of both sexes were exposed to isobutane in a controlled-environment chamber to monitor their physiological responses to a series of gas concentrations ranging from 250 to 1000 ppm [=594-2377 mg/m3].
Initially, the response to exposure periods of 1 and 2 minutes,
and 1, 2 and 8 hours were studied. Since there were no untoward responses, the eight volunteers were then exposed repetitively to isobutane at a concentration of 500 ppm [=1189 mg/m3] for 1, 2 or 8 hours a day, five days a week for 2 weeks. This was followed by studying the effects of exposure to 2 mixtures of isobutane and propane for 1, 2 or 8 hours a day for 2 days.
During the studies, the subjects were kept under strict medical surveillance.
No untoward subjective or physiological responses were recorded either during or after the exposures.
Special attention was placed on evaluating cardiac and respiratory effects by the use of continual ECG telemetry

and computerised spirometric measurements. Additionally, the following serial laboratory investigations were unaltered by the exposures: complete blood count, urinalysis, serum alkaline phosphatase, SGOT, LDH, serum bilirubin, blood sugar, serum calcium, serum phosphorus, BUN, spontaneous EEG, visual evoked responses, a battery of cognitive tests and an ACTH stimulation test.

27.08.2001 Stewart, R.D. Herrmann, A.A., Baretta, E.D., Forster, H.V, Sikora, J.J., Newton, P.E. and Soto, R. J. (1977) Acute and repetetive human exposure to isobutane. Scand. J. Work Environ. & Health Vol. 3, No. 4, pp 234-243

Exposure Experience

TEST SUBSTANCE

Category Chemical:	74-74-0 (propane), 106-97-8 (butane) [**note** – will need 2 separate entries of same RSS for HPVIS – one for each CASRN]
Test Substance:	Propane and Butane
Test Substance Purity/Composition and Other Test Substance Comments:	Gases in liquid form for bottling; other exposures unknown
Category Chemical Result Type :	Measured
Unable to Measure or Estimate Justification :	No estimated or measured concentrations provided

Remark **:** During laboratory investigations of workers bottling liquefied propane and butane, most of the workers complained of respiratory symptoms, e.g. dry cough and dry throat together with gastrointestinal effects. The

electrocardiographic findings in some workers indicated sinus tachycardia, extrasystole and incomplete right bundle branch block.
Lactic acid production in workers experiencing propane "poisoning" has been reported as slight.

Ikoma records 20 cases of sudden death in which propane and propylene were found in the blood, urine and cerebrospinal fluids of the victims.

27.08.2001

Aviado, D.M., Zakhari, S. and Wanatabe, T. (1977) Propane Chapter 4, pp 49-53 in Non-Fluorinated propellants and Solvents for Aerosols. CRC Press, Cleveland, Ohio.
Ikoma, T. (1972) Nichidai Igaku Zasshi, Vol. 31 No. 2. pp 71-

Additional Remarks

Type Reviews
Remark : There have been several reviews of the information available on various components of the petroleum gas group. These are as follows:

n-Pentane	McKee et al (1998)
1,3-butadiene	EU (1999) and CMA (1999)
9 individual hydrocarbons	Mackerer and Galvin 1999

In addition the report of a safety assessment of isobutane, isopentane, n-butane and propane with respect to usage as aerosol propellants in cosmetics has also been published (Anon 1982).

The FDA has listed Propane, n-Butane and iso-Butane as GRAS (Generally Recognised As Safe) substances (21 CFR 184.1655 and 21 CFR 184.1165)

A scientific literature review of GRAS substances has been published, and this included information on propane, n-butane and iso-butane (NTIS 1974). It should be noted that some of the information included in the scientific literature review has not been included in the robust summary. However, no data have been omitted that are considered to be significant.

21 CFR 184.1165 n-Butane and Iso-Butane

21 CFR 184.1655 Propane

Anon (1982). Final report of the safety assessment of isobutane, isopentane, n-butane and propane..J. Am. College of Toxicol. Vol 1, N0. 4 pp 127-142

CMA (1999). Robust summary of information on Group 1: High butadiene C4

EU (1999). Draft environmental and health risk assessment of butadiene

Mackerer, C. R. and Galvin, J. B. (1999). The toxicology of nine light petroleum hydrocarbons. J. Tox. Env. Health Part A. Volume 58, Number 1-2, September 10-24, 1999

McKee, R., Frank, E., Heath, J., Owen, D., Przygoda, R., Trimmer, G & Whitman, F. (1998). Toxicology of n-Pentane (CAS no. 109-66-0) J. Appl. Toxicol. Volume 18, pages 431-442.

NTIS (1979). Evaluation of the Health Aspects of Nitrogen, Helium, Propane, N-Butane, Iso-Butane and Nitrous oxide as Gases used in Foods. Federation of American Societies for Experimental Biology Bethesda, MD - Life Sciences Research Office, U.S Department of Commerce PB 80112022